Phytochemical Investigations and Screening of Antihyperlipidemic and Antioxidant Activities of some Medicinal Plants

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Submitted by
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This is to certify that the thesis entitled **"Phytochemical Investigations and Screening of Antihyperlipidemic and Antioxidant Activities of some Medicinal Plants"** submitted by **Mudduluru Niranjan Babu**, to the The Tamilnadu Dr. M.G.R Medical University, Chennai, for the award of Degree of Doctor of Philosophy in Pharmacy is a record of the independent research work carried out by him at J.S.S. College of Pharmacy, Ootacamund, under my supervision, during 2007-2010. I also certify that the thesis or any part thereof has not formed the basis for the award of any other research degree, of this or any other University, previously.

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Declaration

I hereby declare that the thesis entitled **"Phytochemical Investigations and Screening of Antihyperlipidemic and Antioxidant Activities of some Medicinal Plants"** submitted by me to The Tamilnadu Dr. M.G.R Medical University, Chennai, for the award of Degree of Doctor of Philosophy in Pharmacy, is the result of my original and independent work carried out at J.S.S. College of Pharmacy, Ootacamund, under the supervision of **Dr. K. Elango,** Principal, J.S.S. College of Pharmacy, Ootacamund. The thesis or any part thereof has not formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title, of this or any other university, previously.

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INTRODUCTION

1. Introduction

1.1. Use of medicinal plants in health and disease management

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security.

Food and medicine have been the inseparable companions of mankind from the beginning of man's existence and man had to contend with diseases that affected his life. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. Among the different sources of medicines, plants have been widely used for their healing abilities and have provided mankind with a large variety of potent drugs to alleviate suffering from various diseases. More than 30% of the entire plant species, at one time or other, were used for medicinal purposes. It is estimated that world market for plant derived drugs may account for about Rs.2,00,000 crores. Presently, Indian contribution is less than Rs.5000 crores. Indian export of raw drugs has steadily grown at 26% to Rs.165 crores in 1994-'95 from Rs.130 crores in 1991-'92.

The annual production of medicinal and aromatic plant's raw material is worth about Rs.500 crores. This is likely to touch US $1150 by the year 2010 and US $5 trillion by 2050.

It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine.

1.2. Indian system of medicine.

The Indian subcontinent is endowed with a rich expertise in local health traditions. Alternative medical systems are those that offer independent therapies for the full range of all the disease, as Allopath does. Of the 250000 higher plant species on earth, more than 80,000 are medicinal. India is one of the world's 12 biodiversity centres with the presence of over 45000 different plant species. India's diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Of these, about 15000-20000 plants have good medicinal value. However, only 7000-7500 species are used for their medicinal values by traditional communities. The traditional medicine in India functions through two social streams. One is the local folk stream which is prevalent in rural and tribal villages of India, which is called uncodified system of

medicine. The carriers of these traditions are millions of housewives, thousands of traditional birth attendants, bone setters, practitioners skilled in acupressure, eye treatment or treatment of snakebites or the **vaidyas**, who are the traditional village level herbal physicians. These local health traditions thus represent an autonomous community supported system of healthcare at the village level which runs parallel to state supported system.

A second level of traditional health care system is the academic or classical system. This consists of codified and organized medical wisdom with sophisticated theoretical foundations and philosophical explanations, expressed in thousands of regional manuscripts, covering treatises on all branches of medicine and traditionally systems like Ayurveda, Siddha, Unani, Yoga, Naturopathy and Amchi. Thus the term Indian systems of medicine (ISM) incorporates the systems which originated in India or which originated outside but got adapted in India in the course of time,(Pushpangadan, 1995). The Ayurveda, is predominantly plant based, making use of native plant in spite of powerful contemporary modern medicine. The *Ayurveda* system of medicine uses about 700 species, *Unani* 700, Siddha 600, Amchi 600 and modern medicine around 30 species. The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant product such as gum, resins and latex. Even the Allopathic system of medicine has adopted a number of plant-derived drugs (Table:1-6; medicinal plants used in modern medicine) which form an important segment of the modern pharmacopoeia. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants (Eg. diosgenin, solasodine, β-ionone). Not only,

that plant-derived drug offers a stable market worldwide, but also plants continue to be an important source for new drugs.

Traditional systems of medicine continue to be widely practised on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Global estimates indicate that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material. This fact is well documented in the inventory of medicinal plants, listing over 20,000 species. In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still like drugs from plants. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future.

Among ancient civilisations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About

8,000 herbal remedies have been codified in Ayurveda. The *Rigveda* (5000 BC) has recorded 67 medicinal plants, *Yajurveda* 81 species, *Atharvaveda* (4500-2500 BC) 290 species, *Charak Samhita* (700 BC) and *Sushrut Samhita* (200 BC) had described properties and uses of 1100 and 1270 species respectively, in compounding of drugs and these are still used in the classical formulations, in the Ayurvedic system of medicine. Unfortunately, much of the ancient knowledge and many valuable plants are being lost at an alarming rate. With the rapid depletion of forests, impairing the availability of raw drugs, Ayurveda, like other systems of herbal medicines has reached a very critical phase.

About 50% of the tropical forests, the treasure house of plant and animal diversity have already been destroyed. In India, forest cover is disappearing at an annual rate 1.5 mha/yr. What is left at present is only 8% as against a mandatory 33% of the geographical area. Many valuable medicinal plants are under the verge of extinction. *The Red Data Book of India* has 427 entries of endangered species of which 28 are considered extinct, 124 endangered, 81 vulnerable, 100 rare and 34 insufficiently known species (Thomas, 1997).

Ayurveda, Siddha, Unani and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda is most developed and widely practised in India. Ayurveda dating back to 1500-800 BC has been an integral part of Indian culture. The term comes from the Sanskrit root *Au* (life) and *Veda* (knowledge). As the name implies, it is not only the science of treatment of the ill but covers the whole gamut of happy human life involving the physical, metaphysical and the spiritual

aspects. Ayurveda recognises that besides a balance of body elements one has to have an enlightened state of consciousness, sense organs and mind if one has to be perfectly healthy. Ayurveda by and large is an experience with nature and unlike in Western medicine, many of the concepts elude scientific explanation. Ayurveda is gaining prominence as the natural system of health care all over the world.

Today this system of medicine is being practised in countries like Nepal, Bhutan, Sri Lanka, Bangladesh and Pakistan, while the traditional system of medicine in the other countries like Tibet, Mongolia and Thailand appear to be derived from Ayurveda. Phytomedicines are also being used increasingly in Western Europe. Recently the US Government has established the "Office of Alternative Medicine" at the National Institute of Health at Bethesda and its support to alternative medicine includes basic and applied research in traditional systems of medicines such as Chinese, Ayurvedic, etc. with a view to assess the possible integration of effective treatments with modern medicines. The development of systematic pharmacopoeias dates back to 3000 BC, when the Chinese were already using over 350 herbal remedies. Ayurveda, a system of herbal medicine in India, Sri Lanka and South-East Asia has more than 8000 plant remedies and using around 35,000-70,000 plant species. China has demonstrated the best use of traditional medicine in providing the health care. China has pharmacologically validated and improved many traditional herbal medicines and eventually integrated them in formal health care system.

Green plants synthesise and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. However, a sustained supply of the source material often becomes difficult due to the factors like environmental changes, cultural practices, diverse geographical distribution, labour cost, and selection of the superior plant stock and over exploitation by pharmaceutical industry. Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and /or reduced toxicity. The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants.

Some of the useful plant drugs include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, capscicine, allicin, curcumin, artemesinin and ephedrine among others. In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, works in both mixture of traditional medicine and single active compounds are very important. Where the active molecule cannot be synthesised economically, the product must be obtained from the cultivation of plant material. About 121 (45 tropical and 76 subtropical) major plant drugs have been identified for which no synthetic one is

currently available (table 1). The scientific study of traditional medicines, derivation of drugs through bio-prospecting and systematic conservation of the concerned medicinal plants are thus of great importance.

1.3 Utility of plants in contemporary medicine

A large number of very specific pharmaceutical synthetic products are available to treat almost every disease, but plants still have a definite place in today's therapy. The reasons may be:

- Many of the lead molecules of modern therapy are obtained from the plant sources. The anti-malarial drugs artemisin from ***Artemisia annua,*** quinine from the species of cinchona (Gottlieb, 1982), cardiotonic drug digitoxin fron ***Digitalis purpurea*** and ***Digitalis lanta*** and anticancer drug vincristine from ***Cathranthrus roseus*** have been few of the representatives. Plants would be useful to treat viral diseases as well, example, Neem / Phillanthus.
- A number of plant based drugs such as taxol, reserpine, egotine, opoids, ephedrine, colchicines, rutin, coumarins, anthraquinones, etc., are still a part of standard therapy. Most of these do not have any synthetic substitutes.
- Phytochemicals are a major source of dyes, flavours, sweeteners (Rao, 1993), aromas, perfumes, insecticides (Schmeltz, 1971), antiparasitic drug, and many other substances.
- Though some natural products suffer from certain disadvantages such as their occurrence in minute quantities in the source material, poor solubility, low stability, poor absorption, incorrect distribution, failure to reach the target etc., a structural modification improves the efficiency of these drugs, the modified form being called the 'pro-

drug', example, vindesine, an anticancer alkaloid, is a pro-drug of vinca **(*Cathranthrus roseus*)** alkaloids (Cassady, 1981). Vincristine, the antileukaemia drug which is great demand, suffers from the disadvantage of very low yield from the source material, and so is very expensive.

- Vinblastine, another anticancer drug from the same plant is present at levels of 1,000 times higher than vincristine and the cost is one third of vincristine. Vinblastine is now being used as the parent drug to obtain through structural modifications, the pro-drug vincristine.

Though the usages of plants and plant based products in various contemporary and traditional system of medicine has significantly increased, most of the traditional knowledge about medicinal plants was in the form of oral knowledge that has been lost with persistent invasions and cultural adaptations without any written document and regulations. There is no uniform and standard procedure for the inventory of these plants and the knowledge about their medicinal properties. Therefore, it is essential that such uses of natural products be documented and studied for systemic regulation and wide-spread application.

Table 1. Major plant drugs for which no synthetic one is currently available (Kumar *et al.*,1997*).*

S. No.	Plant name	Source	Use
1	Vinblastine	*Catharanthus roseus*	Anticancer
2	Ajmalacine	*Catharanthus roseus*	Anticancer, hypotensive
3	Rescinnamine	*Rauvolfia serpentina*	Tranquilizer
4	Reserpine	*Rauvolfia serpentina*	Tranquilizer
5	Quinine.	*Cinchona sp*	Antimalarial, amoebic dysentery
6	Pilocarpine	*Pilocarpus jaborandi*	Antiglucoma
7	Cocaine	*Erythroxylum coca*	Topical anaesthetic
8	Morphine	*Papaver somniferum*	Painkiller
9	Codeine	*Papaver somniferum*	Anticough
10	Atropine	*Atropa belladonna*	Spasmolytic, cold
11	Atropine	*Hyoscyamus niger*	Spasmolytic, cold
12	Cardiac glycosides	*Digitalis sp.*	For congestive heart failure
13	Artemisinin	*Artemesia annua*	Antimalarial
14	Taxol	*Taxus baccata*, *T. brevifolia*	Breast and ovary cancer, antitumour
15	Berberine	*Berberis*	For leishmaniasis
16	Pristimerin	*Celastrus paniculata*	Antimalarial
17	Quassinoids	*Ailanthus*	Antiprotozoal

18	Plumbagin	*Plumbago indica*	Antibacterial, antifungal
19	Diospyrin	*Diospyros montana*	
20	Gossypol	*Gossypium sp.*	Antispermatogenic
21	Allicin	*Allium sativum*	Antifungal, amoebiasis
22	Ricin	*Ricinus communis*	Purgative
23	Emetine	*Cephaelis ipecacuanha*	Amoebiasis
24	Glycyrrhizin	*Glycyrrhizia glabra*	Antiulcer
25	Nimbidin	*Azadirachta indica*	Antiulcer
26	Catechin	*Acacia catechu*	Antiulcer
27	Sophoradin	*Sophora subprostrata*	Antiulcer
28	Magnolol	*Magnolia bark*	Peptic ulcer
29	Forskolin	*Coleus forskohlii*	Hypotensive, cardiotonic
30	Digitoxin, Digoxin	*Digitalis, Thevetia*	Cardio tonic
31	Thevenerin,	*Thevetia*	Cardio tonic
32	Nerrifolin	*Thevetia*	Cardio tonic
33	Podophyllin	*Podophyllum emodi*	Anticancer
34	Indicine N-oxide	*Heliotropium indicum*	Anticancer
35	Elipticine	*Ochrosia*	Anticancer
36	Homoharringtonine	*Cephalotaxus*	Anticancer
37	Camptothecine	*Camptotheca acuminata*	Anticancer

A major lacuna in Ayurveda is the lack of drug standardisation, information and quality control. Most of the Ayurvedic medicines are in the form of crude extracts which are a mixture of several ingredients and the active principles when isolated individually fail to give desired activity. This implies that the activity of the extract is the synergistic effect of its various components. In the absence of pharmacopoeia data on the various plant extracts, it is not possible to isolate or standardise the active contents having the desired effects. Ayurvedic pharmacopoeia compiled on modern lines and updated periodically is an urgent requirement. A combination therapy integrating Ayurveda and allopathy whereby the side effects and undesirable reactions could be controlled. Studies can show that the toxic effects of radiations and chemotherapy in cancer treatment could be reduced by Ayurvedic medications and similarly surgical wound healing could be accelerated by Ayurvedic medicines. Modern science and technology have an essential role to play in the process. An integrated approach for the cultivation, conservation and preservation of important plant species through plant molecular biology, plant tissue culture; research on the rationale and methodology of Ayurvedic medical practice; isolation of active constituents and their development into new therapeutics; standardisation and validation of known herbal medicines and other related aspects need to be focussed upon (Sharma *et al.*,1997).

Despite the diverse nature of crops grown in the country and the existence of a fast growing pharmaceutical sector, the share of India in world trade is quite insignificant considering the large geographical area. However, this is bound to rise rapidly with better research inputs and efficient management of the farm sector. So far, India has been involved in the export of only

large volume raw material. To achieve competitive advantage we need to resort to low volume high cost (value) trade through value addition to the raw and unfinished products. It is therefore, necessary to develop genetically superior planting material for assured uniformity and desired quality and resort to organised cultivation to ensure the supply of raw material at grower's end. Post harvest storage and process technologies need to be developed to produce the value added finished products that may be directly utilised by the industry.

Inventorisation of herbal drugs used in traditional and modern medicines for a country like India, appears to be a stupendous task, where a number of well established indigenous or traditional systems, including Ayurveda, Unani, Siddha, Homoeopathy, Tibetan, Amchi, Yoga and Naturopathy are practised along with modern medicine for the management of total health care system.

In all these systems a large number of plant drugs are used, although there may be some common plants. Another problem in correct identification of plants is that the plant drugs in those systems of medicine are known by their classical, *Shastriya* or vernacular names. It is not easy to correlate these names with acceptable scientific names. One plant species can have many vernacular classical names and one name may refer to different plant species. Chinese, Indian, Arabian and other traditional systems of medicines make extensive use of about 5000 plants. India is proud to be rich in biological diversity and tenth among the plant rich countries of Asia, sixth as far as centres of diversity especially agrodiversity are concerned. Nearly three fourth of the drugs and perfumery products used in

the world are available in natural state in the country. India possesses almost 8% of the estimated biodiversity of the world with around 1,26,000 species. It is one of the 12 mega biodiversity centres with 2 hot spots of biodiversity in Western Ghats and north-eastern region. The sacred groves are a miniature ecosystem conserving biodiversity in its pristine form. There are about 400 families in the world of flowering plants, at least 315 are represented in India. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants and about 5000 species have been studied (Tables 2-6). There are at least 121 major plant drugs of known structure, but none of them is currently produced through synthetic means. For developing phytomedicines as a major area of concern, it would be essential to adopt a holistic interdisciplinary approach, have a scientific basis of the understanding of the plant systems, new innovations and their conservation for utilisation in future on a sustainable basis (Sharma, *et al.*,1997). The most urgent need is to rescue and record all traditional knowledge on plants and prepare inventories of medicinal plants along with their traditional uses, also taking into account the tribal knowledge.

An inventory of medicinal plants compiled by the WHO in 1978 covered only ninety member countries and contained 20,000 species, of which only about 250 were of widespread use, and some of which had been analyzed to identify their main active chemical compound. The World Health Assembly – in its resolutions, has emphasized the need to ensure the quality of herbal medicine by using modern control techniques and applying suitable standards (Muttikkal *et al.,* 2006). It is therefore necessary to develop methods for rapid, precise and accurate identification

and estimation of active constituents in order to bring out consistency of important constituents in the formulations. Hence before proceeding to clinical studies, scientist need a tool to authenticate plants and also to detect their potency (Thakkar *et al.*, 2008). In India, the Department of Biotechnology (DBT), Ministry and Technology, Government of India has sponsored a project on inventorizing medicinal plants, wherein literatures on ethnobotanical, chemical, pharmacological and toxicological details of around 1700 plants are being collected (Naranjo,1995).

Table 2. Plant species with therapeutic value under different plant groups (Jiaxiang, 1997).

Plant species	**Therapeutic value**
Thalophytes	230
Bryophytes	39
Pteridophytes	382
Gymnospermae	55
Angiospermae:	
a) Monocotyledones	676
b) Dicotyledones	3495
Total	**4877**

Table 3. Plant families containing over 100 species with therapeutic value (Jiaxiang, 1977).

Family	*Genera*	*Species*
I. Monocots		
Liliaceae	45	165
Orchidaceae	45	135
II. Dicots		
Compositae	89	331
Leguminosae	91	313
Ranunculaceae	31	208
Laminaceae	46	189
Rosaceae	28	146
Umbelliferae	34	123
Rubiaceae	35	118
Euphorbiaceae	30	104
Asclepiadaceae	29	101

Table 4. Major medicinal plants that can be cultivated in India and have established demand for their raw materials(Kumar *et al.*,1977).

S.No	Plant Name
1	*Aconitum sp.*
2	*Adhatoda vasica*
3	*Aloe vera*
4	*Ammi majur*

5	*Atropa acuminata*
6	*Berberis aristata*
7	*Carica papaya*
8	*Catharanthus roseus*
9	*Cassia angustifolia*
10	*Cephaelis ipecacuanha*
11	*Cinchona spp.*
12	*Dioscorea spp.*
13	*Glycyrrhiza glabra*
14	*Hedychium spicatum*
15	*Heracleum candicans*
16	*Hyoscyamus sp.muticus*
17	*Inula racemosa*
18	*Juglans regia*
19	*Juniperus spp.*
20	*Matricaria chamomilla*
21	*Papaver somniferum*
22	*Plantago ovata*
23	*Podophyllum emodi*
24	*Rauwolfia serpentina*
25	*Rheum emodi*
26	*Saussurea lappa*
27	*Swertia chirata*
28	*Urginea indica*
29	*Valeriana wallichii*

Table 5. Medicinal plants on which significant research leads have been obtained with respect to their pharmaceutical potential for which processing and agrotechnology need to be established (Kumar *et al.*,1997).

S.No	Plant name
1	*Andrographis paniculata*
2	*Artemisia annum*
3	*Boswellia serrata*
4	*Centella asiatica*
5	*Coleus forskohlii*
6	*Commiphora weightii*
7	*Curcuma longa*
8	*Phyllanthus amarus*
9	*Picrorhiza kurroa*
10	*Sida rhombifolia*
11	*Taxus baccata*
12	*Withania somnifera*

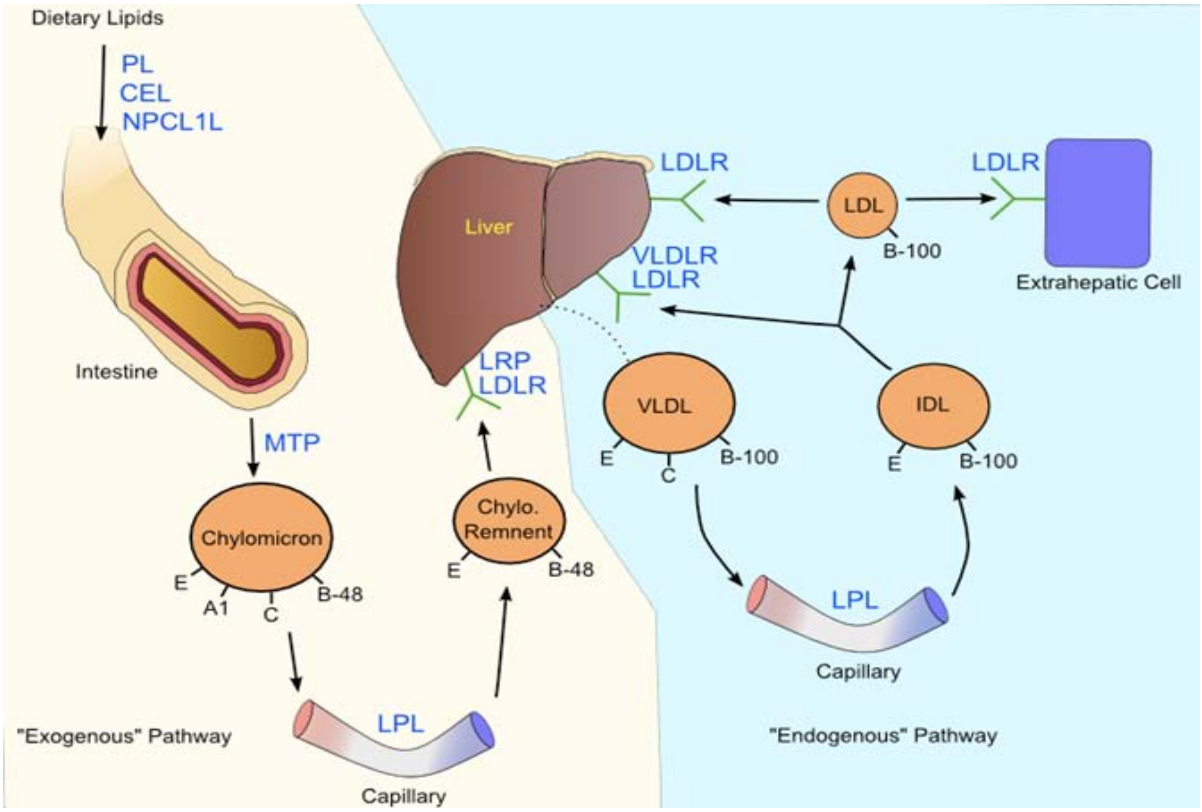
Table 6. Plants which delay ageing process and form health food ingredients in several Ayurvedic formulations (Kumar *et al.*,1997).

S.No	Plant name
1	*Allium sativum*
2	*Aloe barbadensis*
3	*Asparagus racemosus*
4	*Cassia angustifolia*
5	*Curculigo orchioides*
6	*Commiphora weightii*
7	*Centalla asiatica*
8	*Capsicum annum*
9	*Chlorophytum arundinaceum*
10	*Eclipta alba*
11	*Fagopyrum esculentum*
12	*Glycyrrhiza glabra*
13	*Oenothera biennis*
`14	*Panax pseudoginseng*
15	*Plantago ovata*
16	*Withania somnifera*

1.4 Lipids and their role in the body

Major lipids found in blood stream are triglycerides, phospholipids, cholesterol and cholesterol esters and free fatty acids. The function of cholesterol is to help carry fat in the body, because fat being insoluble in water cannot travel on its own in the blood stream. Cholesterol associates with fat and protein and comes out of the liver as lipoprotein. There are several types of lipoproteins for the transport of fatty material in the body such as chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), intermediate density lipoproteins (IDL), high density lipoproteins (HDL). Each has a different function in the transport system. VLDL are responsible to carry endogenous triglycerides from the liver in to the blood stream and to other part of the body. Lipoprotein lipase catalyses triglycerides degradation to generate VLDL remnants which are further degraded by hepatic glyceride hydrolase to generate LDL. It easily adheres along the walls of the arteries and, therefore, called as bad cholesterol. There are different types of HDL like HDL1, HDL2 and HDL3. It is called good cholesterol as it finds and removes stuck LDL of peripheral cell and bring them back to liver. The normal range for total blood cholesterol is between 140 and 200 mg per deciliter (mg/dl) of blood (Petri *et al.*,1975).

1.4.1 Mechanism of lipid transport (Sudhakar *et al.*,2007).



Exogenous Pathway Endogenous pathway

Fig1: Mechanism of lipid transport

Exogenous pathway

- Dietary fats → chylomicrons in intestine
- Chylomicrons → triglycerides and chylomicron remnants by action of lipoprotein lipase (LPL) in adipose tissue of muscle and endothelial cells
- Chylomicron remnants are removed by liver and cleaved resulting in the release of free cholesterol
- Cholesterol can be stored in hepatocytes as esters, released as bile, used to form membranes of lipoproteins

Endogenous pathways

- Transport of TG and cholesterol by VLDL, synthesized in liver.
- FFA from VLDL is deposited in adipose tissue and muscle after lipolysis of TG by LPL.
- The resulting IDL and LDL are taken up by hepatocytes involving high affinity receptor-mediated endocytosis by the LDL receptor.
- LDL constitutes 60 - 70 % of plasma cholesterol levels.
- In lysosomes the esterified cholesterol is hydrolysed and released as free cholesterol for synthesis of cell membranes.
- HDL is involved in the transport of cholesterol from peripheral cells back to the liver.
- When plasma lipoprotein concentrations are high, macrophages and other scavenger cells degrade lipoproteins.
- This leads to cholesterol deposits in macrophages or artial walls (atheroma) and of tendons and skin (xanthomas).

1.4.2 Hyperlipidemia

Hyperlipidemia, the elevation of lipid concentration in plasma, is the manifestation of a disorder in the synthesis and degradation of plasma lipoproteins. Primary type hyperlipidemia can be treated with drugs but the secondary type originating from diabetes, renal lipid necrosis or hypothyroidism demands the treatment of original disease rather than hyperlipidemia (Petri *et al.*,1975).

Levels between 200 and 240 mg/dL indicate moderate risk, and levels surpassing 240 mg/dL indicate high risk. While their role in heart disease is not entirely clear, it appears that as triglyceride levels rise, levels of good cholesterol fall. It is the complex interaction of these three types of lipids that is thrown off when a person has hyperlipidemia. High cholesterol is characterized by elevated levels of LDL cholesterol, normal or low levels of HDL cholesterol, and normal or elevated levels of triglycerides. According to World Health organization (WHO) 2002, almost one fifth (18 %) of global stroke events (mostly nonfatal events) and about 56 % of global heart disease are attributable to total cholesterol levels above 3.2 mmol/l. This amounts to about 4.4 million deaths (7.9 % of the total) and 2.8 % of the global disease burden.

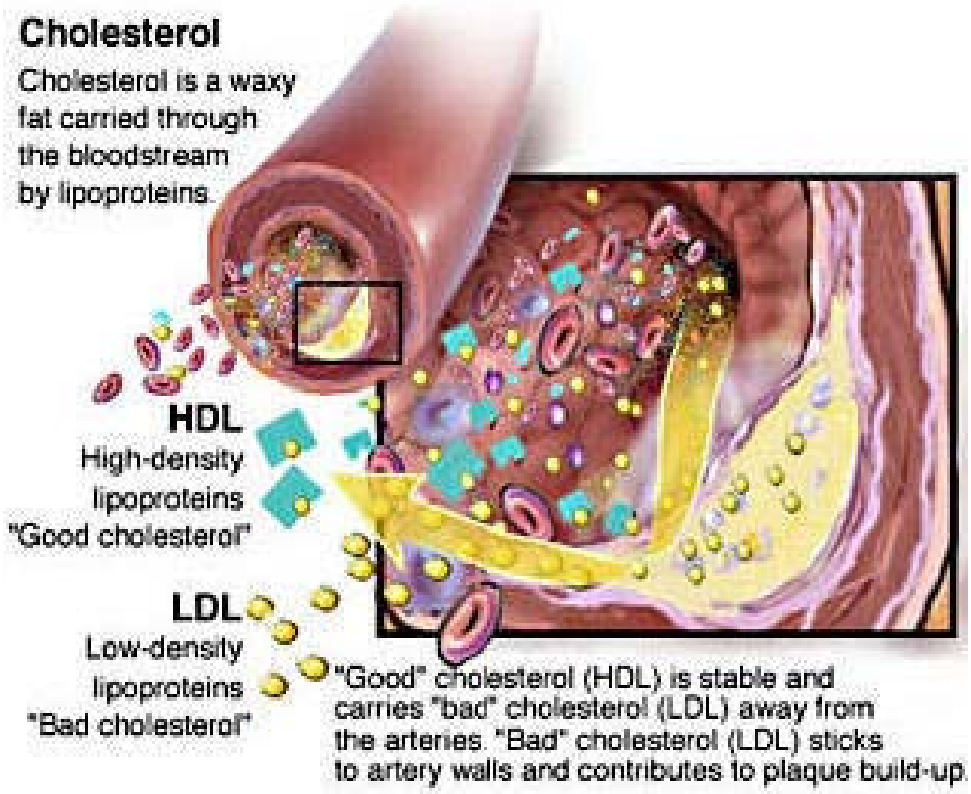


Fig 2: HDL and LDL cholesterol in the arteries

1.4.3 Classification of hyperlipidemia (Petri *et al.*, 1975):

Type I hyperlipidemia: It is characterized by high concentration of blood chylomicrons. Currently there are no drugs available for treating this type.

Type II hyperlipidemia: It is subdivided into type IIA hyperlipidemia and Type IIB hyperlipidemia. Type II A hyperlipidemia is characterized by high LDL and cholesterol levels with a slight increase in blood triglycerides. Type II B hyperlipidemia is characterized by the elevation of triglycerides, serum cholesterol, LDL and VLDL.

Type III hyperlipidemia: It shows elevated levels of triglycerides and IDL. A blockade in the normal conversion of VLDL to LDL results in the accumulation of IDL. Controlled diet is the treatment of this type of hyperlipidemia.

Type IV hyperlipidemia: It is due to high concentration of triglycerides and VLDL and often faulty carbohydrate metabolism. Both diet and drug therapy is recommended for this type of hyperlipidemia.

Type V hyperlipidemia: It shows elevated levels of chylomicrons, VLDL and triglycerides resulting from faulty carbohydrate metabolism. A major concern in patients with hyperlipidemia is the increased risk of atherosclerosis resulting in heart diseases. The aim of treating the patients with hyperlipidemia is to reduce serum cholesterol and / or improve the HDL cholesterol by maintaining a high ratio of HDL to LDL cholesterol level thereby reducing the risk of developing heart disease or the occurrence of further cardiovascular or cerebrovascular events.

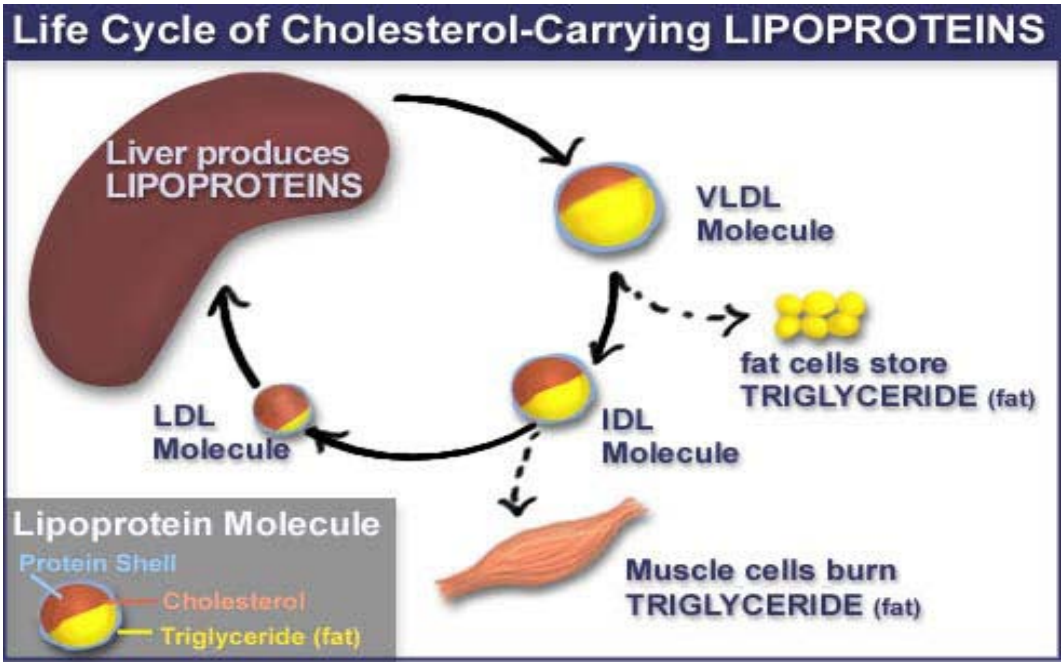


Fig3: Life cycle of cholesterol carrying lipoproteins

1.4.4 Signs and Symptoms (Sudhakar *et al.*,2007)

In its primary stage, high cholesterol generally occurs without any symptoms. For this reason, screening through routine blood tests is crucial for early detection. In its advanced stage, however, high cholesterol may result in any of the following:

- Fat deposits in the tendons and skin (called xanthomas)
- Enlarged liver and spleen (which the health care provider may feel on exam)
- Severe abdominal pain as a result of pancreatitis (happens if triglyceride deposit level in the pancreas is 800 mg/dL or higher)
- Chest pain and even a heart attack (may occur when enough cholesterol has built up in blood vessel walls to block the flow of blood in the heart)

1.4.5 Causes

In some cases, abnormal high cholesterol may be related to an inherited disorder. Certain genetic causes of abnormal cholesterol and triglycerides, known as hereditary hyperlipidemia, are often very difficult to treat. High cholesterol or triglycerides can also be associated with other diseases a person may have, such as diabetes. In most cases, however, elevated cholesterol levels are associated with an overly fatty diet coupled with an inactive life style. It is also more common in those who are obese, a condition that has now reached epidemic proportion in India, affecting as much as half of the adult population.

1.4.6 Causes of high total and LDL cholesterol levels include

- Hereditary hyperlipidemia (types IIa or IIb)
- Diets high in saturated fats and cholesterol
- Liver disease
- Active under thyroid
- Poorly controlled diabetes
- Overactive pituitary gland
- A kidney disorder called nephritic syndrome characterized by elevated cholesterol, loss of protein in the urine leading to low levels of protein in the blood and excessive fluid retention causing swelling
- Anorexia nervosa
- Medications such as progestogens, cyclosporins, and thiazide diuretics

1.4.7 Causes of low HDL cholesterol include

- Malnutrition
- Obesity
- Cigarette smoking
- Certain medications such as beta blockers and anabolic steroids
- Low level of physical activity
- Polycystic ovarian syndrome

1.4.8 Causes of high triglyceride levels include

- Hereditary hyperlipidemia
- Diets high in calories, especially from sugar and refined carbohydrates
- Obesity
- Poorly controlled diabetes
- Insulin resistance
- Alcohol use
- Kidney failure
- Stress
- Pregnancy
- Polycystic ovarian syndrome
- Hepatitis
- Multiple myeloma
- Lymphoma

Certain medications such as estrogens (available in either oral contraceptives or as part of hormone replacement therapy for menopausal women), corticosteroids, a class of cholesterol-lowering medications known as bile acid binding resins and isotretinoin.

1.4.9 Risk factors

There are certain factors that put a person at increased risk of having high cholesterol. While some factors cannot be altered by changes in lifestyle, many can be changed. The most important risk factors for high cholesterol are obesity, diets high in saturated fat and trans fatty acids, low fiber in the diet, physical inactivity, stress, smoking cigarettes, living in an industrialized country, under active thyroid, diabetes, polycystic ovary syndrome

1.5 Current management of hyperlipidemia(Hopper *et al.*,2001).

1.5.1 Diet

Diet modification is always being encouraged in a patient with hyperlipidemia but it alone is rarely successful in bringing about a significant improvement in lipid profile. Randomized control trails of dietary fat reduction or modification have shown variables on cardiovascular morbidity and mortality. There is a common misconception that a healthy diet is one that is low in cholesterol. However, it is the saturated fat contents that are important, although many components of a healthy diet are not related to fat content. For example, the low incidences of coronary heart disease in those who consume Mediterranean diet suggest that increase intake of fruits and vegetables are also important. The typical Mediterranean diet has an abundance of plant food minimally processed, seasonally fresh, and locally grown; fresh fruits as the typical diet dessert, with sweets containing concentrated sugars or honey consumed a few times per week; olive oil as the principle source of fat; dairy products such as cheese and yoghurt, consumed daily in low to moderate amounts; zero to

four eggs consumed weekly; and red meat consumed in a low to moderate amounts.

1.5.2 Salt

Dietary salt (sodium) has an adverse effect on blood pressure and thereby a potential impact on coronary heart disease and stroke. As part of dietary advice the average adult intake of sodium should be reduced from approximately 150 mmol (9 g) to 100 mmol (6 g) of salt. This intake can be reduced by consuming fewer processed food, avoiding many ready meals and not adding salt at the table.

1.6 Lipid lowering therapy (Bennet *et al.*,2003).

1.6.1 Statins

These agents block the rate limiting enzyme for endogenous cholesterol synthesis, hydroxyl-methyl glutaryl coenzyme A reductase (HMG CoA). This results in up-regulation of LDL-receptors in the liver and increased clearance of LDL from the circulation; plasma total cholesterol and LDL-cholesterol fall to attain a maximum effect in 1 month after commencing therapy. All statins cause a dose dependent reduction. LDL-cholesterol falls by an average of 17 % with fluvastatin (20 mg/d), 28 % with simavastatin (10 mg/ d), and 38 % with atorvastatin (10 mg/d). At higher dose a 50 % reduction in LDL-cholesterol is possible. The effect of pravastatin is similar. There is no tolerance to continued administration of the statins, because of a circadian rhythm to LDL-receptor synthesis; statins are a little more effective if given in the evening rather than in the morning. Their efficacy in both primary and secondary prophylaxis of hypercholesterolemia is probably a class effect, although long-term

outcome studies may in time differentiate between the drugs. On current information with no clear advantage or disadvantage between the different statins, the choice of agent to achieve suggested total or LDL-cholesterol levels is heavily influenced by their cost, and the dose likely to achieve the target. Statins are well absorbed after administration orally, and are metabolized in the liver. They are well tolerated, the commonest adverse effect being transient, and usually minor abnormality in liver function tests in some 1% of the patients. Asymptomatic elevation of liver enzymes (creatine phosphokinase, CPK) and myositis occurs more rarely, but is more frequent when statins are combined with other anti-hyperlipidemic drugs such as fibrates and nicotinic acid; patients should be counseled about myositis when these drugs are co-administered. Myositis is also more likely when co-administrated with anti-HIV protease inhibitors, and with drugs that interfere with metabolism of some statins, e.g. cyclosporin.

1.6.2 Fibric acid derivatives

This class include *benzafibrate, ciprofibrate, fenofibrate* and *gemfibrozil.* The drug clorfibrate partly resemble short chain fatty acids and increases the oxidation of these acids in both liver and muscle. In liver, the secretion of triglyceride rich lipoproteins falls, and in muscle the activity of lipoprotein lipase and fatty acid uptake from the plasma are both increased. Fibrates act through a nuclear transcription factor (PPARα) which up-regulates expression of LDL-cholesterol and apolipoprotein A-1 genes, and down-regulates the expression of the apolipoprotein C-11 gene. The result of plasma triglycerides declines by 20-30 % and cholesterol by 10-15%; associated with this a rise in the protective HDL cholesterol. The later effect may be contributed to reduction of nonfatal myocardial infarction

with gemfibrozil in both Helsinki Heart Study and more recent VA-HIT trails. They are the drugs of choice for mixed hyperlipidemia but may be used in hyper cholesterolaemia, along or with anion exchange resins or with statins. There is evidence of verifying efficacy among the drugs both in cholesterol lowering and in additional bifacial effect, such as reduction in blood fibrinogen and urate concentration; the clinical significance of these differences is not yet known.

Fibric acid derivatives are well absorbed from the gastrointestinal tract, extensively bound to plasma proteins and excreted mainly by kidney as unchanged drug or metabolites. They are contraindicated where hepatic and renal function is severely impaired (but gemfibrozil has been used in uremia and nephritic patients without aggravating deterioration of kidney function). Rarely, fibric acid derivatives may include a myositis like syndrome; the risk is greater in patients with poor renal function and in those who are also receiving a statin. Fibrates enhances the effect of co-administrated oral anticoagulants.

1.6.3 Anion-exchange resins

Colestyramine is an oral anionic exchanger resin which binds bile acids in the intestine. Bile acids are formed from cholesterol in the liver, pass into the gut in the bile and are largely reabsorbed at the terminal ileum. The total acid pool in only 3-5 g but, because such enterohepatic recycling takes place 5-10 times a day, on an average 20-30 g of the bile acid are deviated into the intestine every 24 hours. Bile acids bound to colestyramine are lost in the faeces and the depletion of the bile acid pool stimulates conversion of cholesterol to bile acid: the result is a fall in intracellular cholesterol in hepatocytes, and an up-regulation in both LDL-receptors and cholesterol

synthesis. The former has the predominant influence on plasma LDL-cholesterol, which falls by 20-25%. In many patients there is some compensatory increase in hepatic triglyceride output. Anion exchange resins therefore may be used first line for hypercholesterolemia but not when there is significant hypertriglyceridaemia, which may be aggravated in such patients. The resin may be taken mixed with water or orange juice and shaken in a close container.

About half the patients who take colestyramine experience constipation and some complain of anorexia, abdominal fullness and occasionally of diarrhea: these effects are dose related but may limit or prevents its use. Because the drug binds anions, drug such as warfarin, digoxin, thiazide diuretics, phentobarbitone, and thyroid hormones should be taken 1h before and 4h after colestyramine to avoid impairment of their absorption. *Colestipol* is similar to colestyramine.

1.6.4 Nicotinic acid derivatives

Nicotinic acid acts as an antipolytic agent in adipose tissue, reducing the supply of free fatty acids and hence the availability of substrate for hepatic triglyceride synthesis and the secretion of VLDL. Nicotinic acid lowers plasma triglyceride and cholesterol concentrations and rises HDL-cholesterol. Flushing of skin and gastrointestinal upset commonly occurs; the unpleasantness may be diminished by gradually building up the oral dose over 6 weeks and, in time, tolerance develops. Rarely there is major disturbance of the liver function.

Acipimox is better tolerated than nicotinic acid, has a longer duration of action but is less effective. Unlike nicotinic acid, it does not reduce

circulating levels of lipoproteins, the modest reduction of which may contribute to overall protection against the complications of atheroma.

1.6.5 Other drugs

Alpha-tocopherol acetate (vitamin-E): Has no effect on lipid levels but is a powerful antioxidant. Considerable evidence points to oxidation of LDL as an essential step in the development of atheroma, and therefore interest has centered to the role of either endogenous or therapeutic vitamin E in prevention of atheroma. Reduced concentrations of vitamin-E in both blood and fat are found in inhabitants of countries with a high prevalence of ischemic heart disease, and in patient who develop ischemic heart disease. A high dose reduced by half the risk of myocardial infarction in 2000 patients with angina and positive coronary angiogram. However most of the studies have failed to confirm this finding and there is no indication at present for routine prescribing of α-tocopherol in the treatment or prevention of atherosclerosis.

Omega-3 marine triglycerides (maxepa)

Maxepa contains the triglyceride precursors of two polyunsaturated fatty acids (eicosapentaenoic acid and docosahexaenoic acid) derived from oily fish. They have no place in treating hypercholesterolemia. Some patients with moderate to severe hypertriglyceridaemia may respond to oral use, although LDL-cholesterol may rise. There is an associated 90-calorie per day energy load.

Orlistat, a weight reducing agent, lowers the glycaemia of diabetes mellitus to a degree that accords with the weight loss, and improves

hyperlipidaemia to an extent greater than would be expected. Since it is a lipase inhibitor there is a risk of steatorrhoae and malabsorption of fat-soluble vitamins A,D and E.

Ezetimibe(Robert *et al.*,2006), is the first compound approved for lowering total and LDL-C levels that inhibits cholesterol absorption by enterocytes in the small intestine. It lowers LDL-C levels by about 18% and is used primarily as adjective therapy with stains. Outcome studies employing ezetinide with statins are beginning but no results are anticipated.

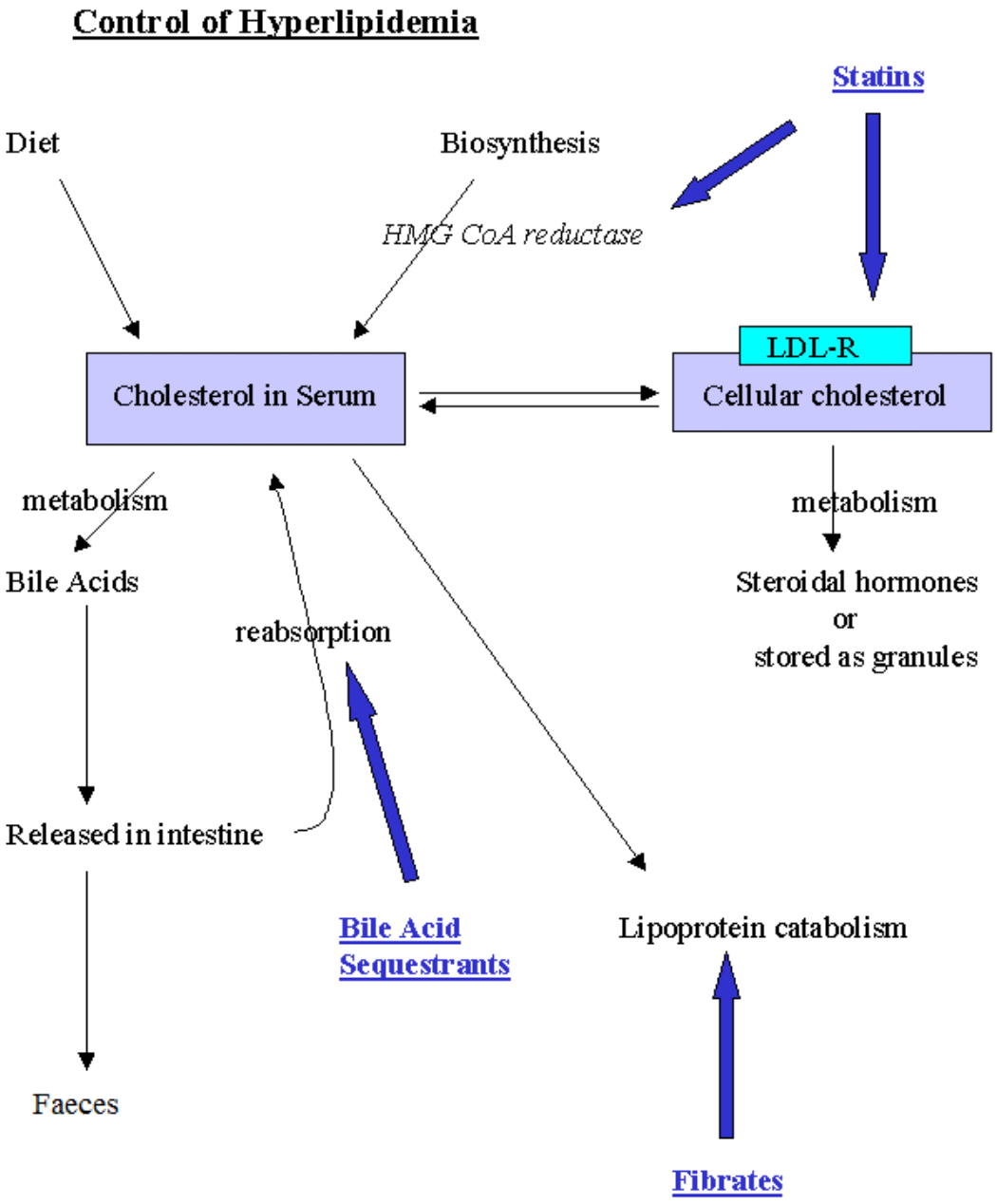


Fig 4. Control of Hyperlipidemia

1.7 Plant remedies for hyperlipidemia (Sharma *et al.*,1997)

A number of plant preparations such as *Allium sativum, Cicer arientinum, Inula recemosa, Terminalia arjuna, trigonella foenum graecum, Commiphora mukul,* green tea and curcumin have been reported to have hyperlipidemic action. Few of these also possess certain other beneficial properties like antianginal and antiplatelet actions. Plant preparations contain many compounds that work synergistically on multiple parts of the body. For example, garlic is not only antibacterial, but antifungal, and helps to lower cholesterol. This synergy of chemicals helps to balance the overall activity of the herb. Since the chemicals in the herbs are nonspecific and unconcentrated, there are generally fewer side-effects from herbs than from manufactured drugs. Further, according to a study published in the April 15, 1998 issue of the Journal of the Americal Medical Association, the fifty leading cause of death in the United States in 1994 was adverse drug reactions of modern medicine, an excess of 100,000 deaths. By contrast, there have been less than 100 adverse reactions and only one death attributed to herbs in Canada Science 1990. Most reactions to herbs have to do with an individual allergic reaction to the herb or to an interaction with prescription drugs.

Table 7. List of plants with antihyperlipidemic activities (Sharma *et al.*,1997)

S. No	Name of plant	Family	Vernacular name	Plant part
1	*Aegle marmelos*	Rutaceae	Bael fruit, Bilwa	Fruits
2	*Agave veracruz*	Amryllidaceae	American aloe, barakhawar	Root, leaves gums
3	*Allium cepa*	Lilliaceae	Onion, piyaj, palandu	Bulbs
4	*Aloe barbadensis*	Lilliaceae	Ghee kumar, gwarpatha	Leaves
5	*Bambusa*	Graminae	Bamboo vamsha	Leaves
6	*Boswellia serrata*	Burserraceae	Salai guggul	Gum
7	*Brassicavercapitata*	Cruciferae	Cabbage	Oil
8	*Cajanus cajan*	Fabaceae	Red gram	Seeds
9	*Capparis decidua*	Capparaceae	Karli, tint	Leaves, fruits and stem
10	*Capsicum capacapiticum*	Solanaceae	Chillies	Fruits
11	*Carum capaticum*	Umbelliferae	Jawan, Ajowan	Fruits, roots
12	*Celastrus paniculatus*	Celestraceae	Khunjri, kusur	Seed oil, barks, roots and fruits
13	*Curcuma amada*	Zingiberaceae	Mango, ginger, harida	rhizomes

14	*Cyamopsis tetragonoloba*	Leguminosae	Guar	Seeds
15	*Emblica officinallis*	Euphorbiaceae	Amla, amalaki	Dried fruits, seeds, leaves
16	*Eugenia cumini*	Myrtaceae	Jamun	Seeds
17	*Inula racemosa*	Compositeae	Pushkarmul	Roots
18	*Juglans regia*	Juglandaceae	Walnut, akhrot	Kerneloil
19	*Medicago sativum*	Papilionaceae	Alfa alfa	Seeds
20	*Memordica charantia*	Cucurbitaceae	Bitter ground	Fruits
21	*Musa saspientum*	Musaceae	Banana, kela	Roots, stems, flowers, fruits
22	*Nepeta hindostana*	Libiateae	Billiola, badranj, boya	Whole plant
23	*Phaseolus aureus*	Fabaceae	Green gram	Seeds
24	*Phaseolu mungo*	Fabaceae	Black gram	Seeds
25	*Picrohiza kurroa*	Schrophulariaceae	Kulki, kataki	Roots
26	*Piper nigrum*	Piperaceae	Golmirch, kalimirch	Leaves
27	*Pisum sativum*	Papilionaceae	Garden pea, matar	Seeds
28	*Pterocarpus marsupium*	Papilionaceae	Indian malabarkino	Gum and leaves
29	*Saussuraea lappa*	Asteraceae	Kustha, kut	Roots
30	*Terminalia arjuna*	Combrereceae	Arjun	Barks

1.8 Reactive oxygen species

Reactive oxygen species (ROS) present a paradox in their biological function. On one hand, they prevent diseases by assisting the immune system, mediating cell signaling and playing an essential role in apoptosis. On the other hand, they can damage important macromolecules in cells and have a role in carcinogenesis and CVD and many other age related diseases. Historically, the generation of ROS was viewed as indiscriminate and random, and their targets as primary determinants of disease and aging. Research also demonstrates that ROS generation is a normal physiological process, particularly for proper immunocompetence and in coordination and activation of numerous signal transduction pathways. The formation of ROS is a natural consequence of aerobic metabolism and is integral for maintaining tissue oxygen homeostasis. Oxygen homeostasis the balance between constitutive oxidants and anti oxidants is maintained through a natural series of reduction – oxidation (redox) reactions involving the transfer of electrons between two electron species: compounds that loose electrons (oxidized) and those that gain electrons (Seifrteda *et al.*,2007), (reduced).

Reactive oxygen species can be classified into oxygen-centered radicals and oxygen-centered non-radicals (Table. 8). **Oxygen centered radicals** are superoxide anion ($^{\bullet}O_2$-), hydroxyl radicals ($^{\bullet}OH$), alkoxyl radicals ($RO^{\bullet}$), and peroxyl radicals ($ROO^{\bullet}$). **Oxygen centered non radicals** are hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). Other reactive species are nitrogen species such as nitric oxide ($NO^{\bullet}$), nitric dioxide ($NO_2^{\bullet}$), and peroxynitrite (Halliwell *et al.*,1995; Halliwell,1996) (OONO-). Reactive oxygen species in biological systems are related to free radicals, even though there are non radical compounds in reactive oxygen species such as

singlet oxygen and hydrogen peroxide. Free radicals exist with one or more unpaired electron in atomic or molecular orbital. Free radicals are generally unstable, highly reactive and energized molecules. When free radicals steal an electron from a surrounding compound or molecule, a new free radical is formed in its place. The newly formed radical then returns to its ground state by stealing electrons from cellular structure of molecules. Thus, the chain reaction continues and can be '**thousands of events long'** (Halliwell *et al.*, 1984) . Reactive oxygen species or free radicals in biological systems can be formed by prooxidative enzyme systems, lipid oxidation, irradiation, inflammation, smoking, air pollutants, and glycoxidation (Stief,2003). Clinical studies reported that ROS are associated with many age related degenerative disease, including atherosclerosis, vasospasms, cancers, trauma, strokes, asthma, hyperoxia, arthritis, heart attack, age pigments, dermatitis, cataractogensesis, retinal damage, hepatitis, liver injury and periodontis (Figure. 5)

Table 8: Antioxidants that affect Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)

	Oxygen- entered radicals	**Oxygen-centered non-radicals**
ROS	Superoxide anion ($^{\bullet}O_2$-) Hydroxyl radicals ($^{\bullet}OH$) Peroxyl redicals ($ROO^{\bullet}$) Alkoxyl radical ($RO^{\bullet}$) Hydroperoxyl ($HO^{\bullet}_2$)	Hydrogen peroxide (H_2O_2) Hypochlorous acid (HOCl) Ozone (O_3) Singlet oxygen $^1O_2$
RNS	Nitric oxide ($NO^{\bullet}$) Nitrogen dioxide ($NO_2^{\bullet}$)	Nitrous acid (HNO_2) Dinitrogen tetroxide (N_2O_4) Dinitrogen trioxide (N_2O_3) Peroxynitrite (OONO-) Peroxynitrous acid (ONOOH) Nitronium cation (NO_2^+) Alkyl peroxynitrites (ROONO)

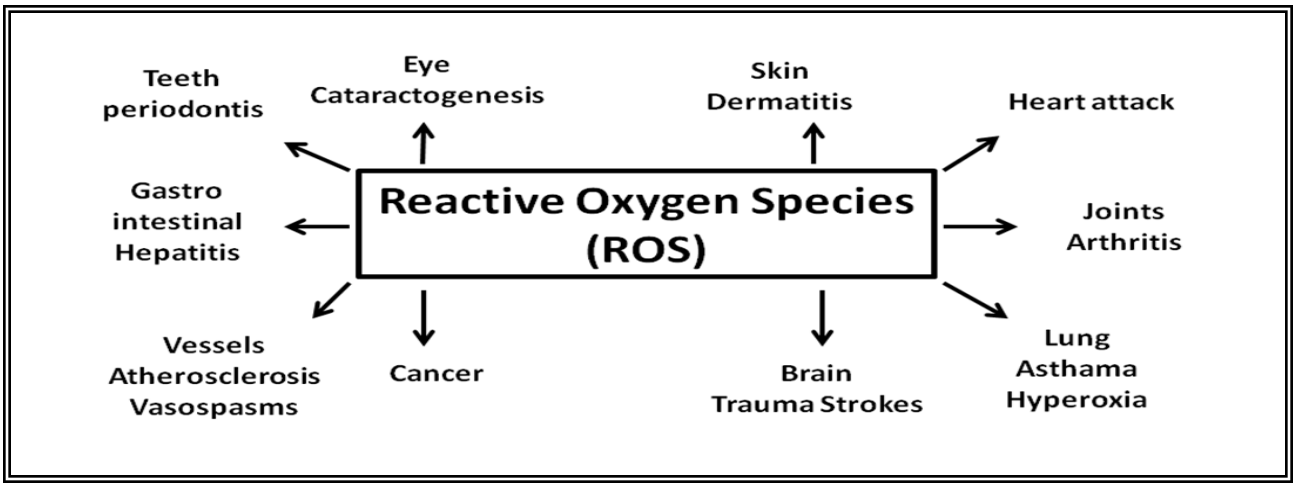


Fig 5: Clinical conditions involving reactive oxygen species (ROS)

1.8.1 Sources of ROS:

Free radicals are continuously formed as a consequence of many oxidative biochemical reactions in the body, which may be biochemical processes in the body or reactions of enzymes or due to external factors like pollution, radiation etc. These can be broadly classified as (Swamy *et al.*,1998; Slater 1984; Lee *et al.*,2004)

a. Endogenous sources
b. Exogenous sources

a. Endogenous sources

These are inbuilt sources of free radicals. These can be any biochemical reaction(s) mediated substances. These also include various electron transport chains, enzymes, various cells components etc. for e.g.

a. Mitochondrial electron transport chain
b. Microsomal electron transport chain
c. Chloroplast electron transport chain

Enzymes:

- Xanthine oxidase
- Indoleamine dioxygenase
- Tryptophan dioxygenase
- Galactose oxidase
- Cyclooxidase
- Mono amino oxidase etc.

b. Exogenous sources

These are also known as external / environmental sources which include atmospheric factors.

- Drug oxidation (paracetamol, antibiotics etc.)
- Cigarette smoke
- Ionizing radiation
- Sunlight
- Heat shock
- Pollution

Physiological factor

These are included under exogenous factors, which include mental status, diseased conditions, etc. e.g.: stress, emotion, etc.

1.8.2 Generation of ROS

When oxygen homeostasis is not maintained, the cellular environment becomes oxidatively stressed. Approximately 1-5% of oxygen consumed by the body is converted into ROS. Three of the major ROS – superoxide radical, hydrogen peroxide and hydroxyl radicals are normal metabolic byproducts that are generated continuously by the mitochondria in growing cells. Other significant intracellular sources of ROS include microsomal cytochrome P_{450} enzymes, flavoprotein oxidases and peroxisomal enzymes involved in fatty acid metabolism and other pro oxidative enzyme systems, lipid oxidation, irradiation, inflammation, smoking, air pollutants, and glycoxidation. Potentially damaging oxidative stress can be generated by excess ROS, which are kept in check by endogenous cellular antioxidant mechanisms. Oxidative stress related enzymes include superoxide

dismutases for eliminating the superoxide radical as well as catalase and glutathione peroxidases for removing hydrogen peroxide and organic peroxides.

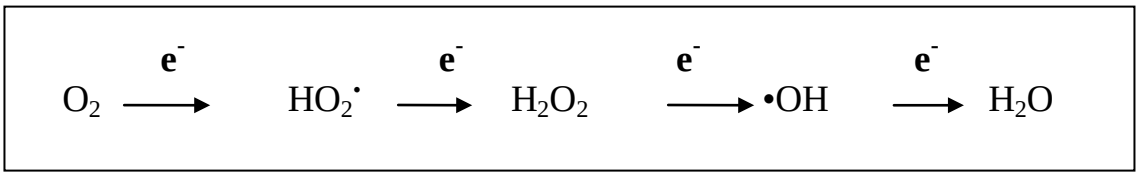


Fig 6: Electron transport chain in mitochondria

Mitochondria, which consume more than 90% of the oxygen in aerobic living organisms, are the main ROS and free radical source. Oxygen in mitochondria is reduced to water by 4 sequential steps (Fig. 6). Perhydroxyl radical ($HO_2^{\bullet}$) or its ionized form, superoxide anion ($^{\bullet}O_2^-$), is the first reduced intermediate of oxygen. Hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$), are inevitable intermediates from oxygen to water reduction steps in body. Approximately 1% to 5% of oxygen consumed by mitochondria is reduced and converted to these reactive oxygen species(Ames *et al.*,1993).

Initially generated superoxide anions and hydrogen peroxide are the main reactive oxygen species causing the oxidation of cells and tissues. Superoxide anion itself is not a strong oxidant, but it react with protons in water solution to form hydrogen peroxide, which can serve as a substrate for the generation of hydroxyl radicals and singlet oxygen. Hydroxyl radicals are strong oxidants and can abstract a hydrogen atom from any carbon-hydrogen bond and oxidized the compound. For example, linoleic acids are mainly located in glycerolipids and phospholipids of cell membranes; therefore, cell membranes are easily oxidized and lose their functionality during the aging process (Haman,2000; Stief,2000).

Pro-oxidative enzymes such as lipoxygenase can generate free radicals30. Lipoxygenase can react with free forms of fatty acids, which can be released from glycerides by membrane bound phospholipase A2.

Environmental sources (Spiteller, 2001; Gutteridge *et al.*,1993), such as ultraviolet (UV) irradiation, and pollutants, also produce reactive oxygen species. Injured cells and tissues can stimulate the generation of free radicals. Reactive oxygen species can be formed in foods through lipid oxidation and photosensitizers exposed to light. Non-enzymatic lipid oxidation requires the presence of free forms of bivalent metal ions such as copper and iron, which are not common for healthy adults. It has been assumed that free forms of iron are generated by the decomposition of iron containing natural sources such as hemoglobin and ferritin.

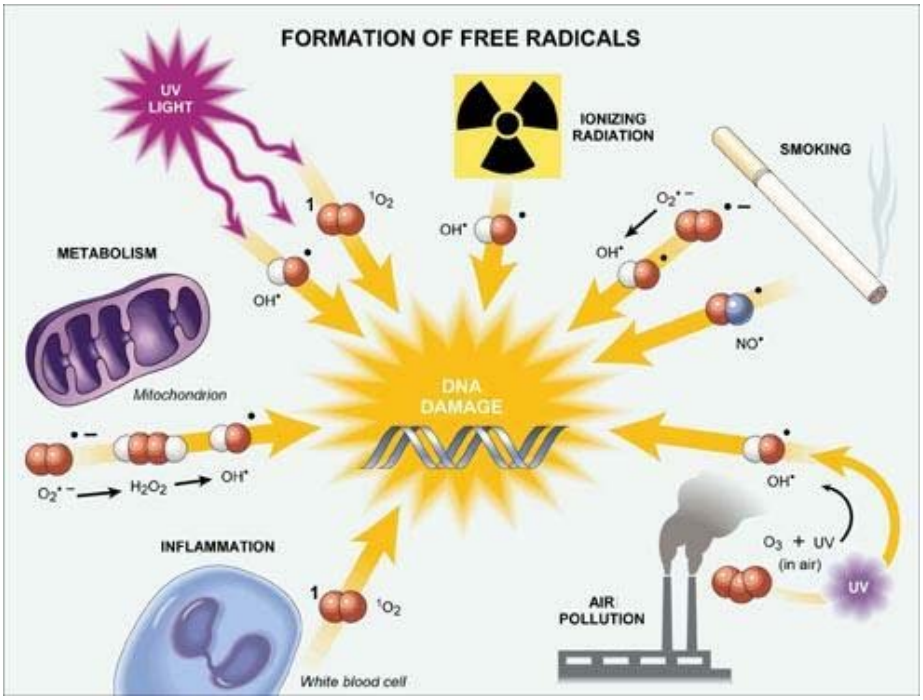


Fig 7 : Formation of free radicals

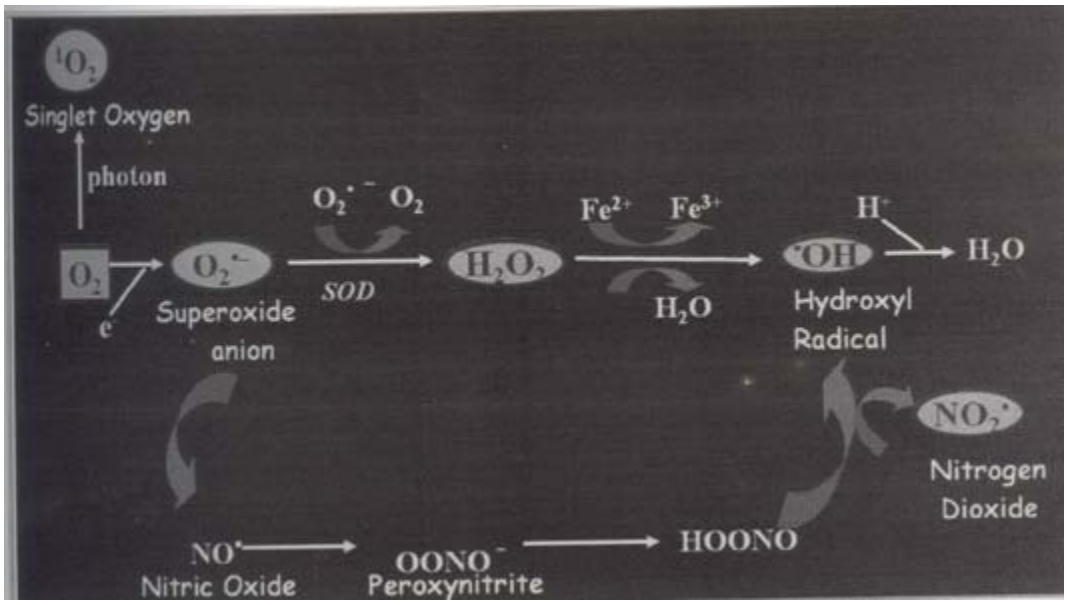


Fig 8. Interconversion of Reactive Oxygen Species (ROS)

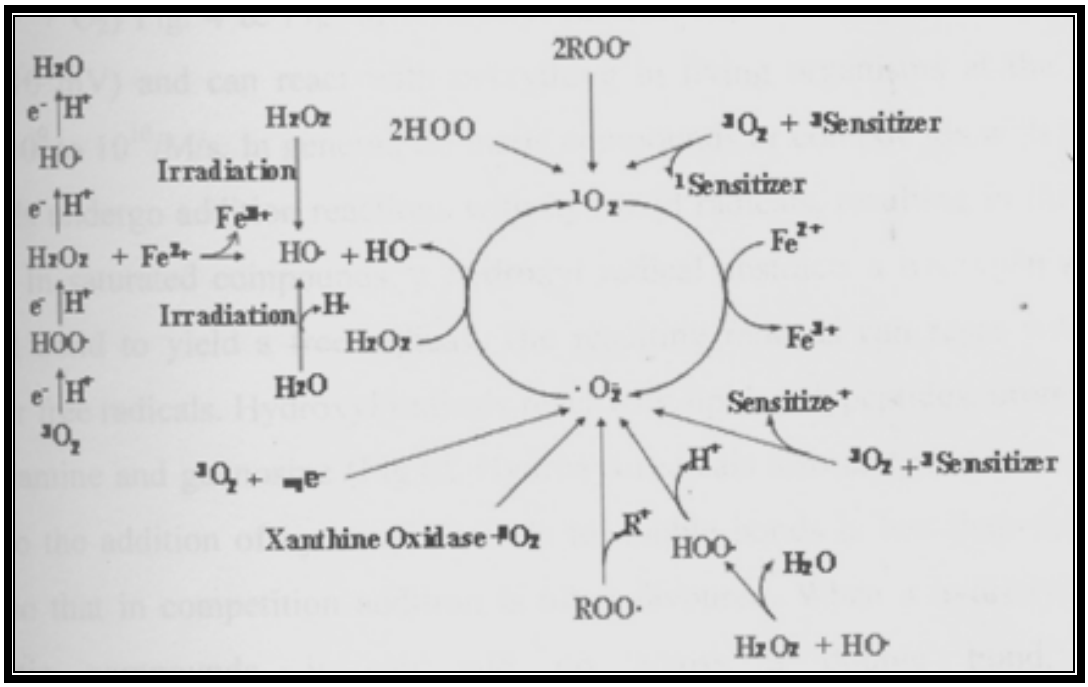


Fig 9. Formation of Reactive Oxygen Species

1.8.3 Reactive oxygen species and their properties

Superoxide anion ($^{\bullet}O_2^-$)

Superoxide anion is a reduced form of molecular oxygen created by receiving one electron (Fig. 8). Superoxide anion is an initial free radical formed from mitochondrial electron transport systems. Mitochondria generate energy using 4 electron chain reactions, reducing oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form superoxide anion (Harman,2000). The superoxide anion plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radicals, or singlet oxygen ($2^{\bullet}O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$) in living systems (Stief, 2000). The superoxide anion can react with nitric oxide (NO•) and form peroxynitrite (ONOO-), which can generate toxic compounds such as hydroxyl radicals and nitric dioxide (Halliwell, 1997).

($ONOO^- + H^+ \longrightarrow {}^{\bullet}OH + {}^{\bullet}NO_2$).

Hydroxyl radicals ($^{\bullet}OH$)

Hydroxyl radicals are the most reactive free radical and can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions such as copper or iron ($^{\bullet}O_2^- + H_2O_2 \longrightarrow {}^{\bullet}OH + OH^- + O_2$) (Fig. 8 & Fig. 9) Hydroxyl radicals have the highest 1- electron reduction potential (2310 mV) and can react with everything in living organisms at the second order rate constant of 10^9 to 10^{10} /M/s. In general aromatic compounds or compounds with carbon - carbon multiple bonds undergo addition reactions with hydroxyl radicals, resulting in the hydroxylated free radicals. In saturated compounds, a hydroxyl radical abstracts a hydrogen atom from

the weakest C-H bond to yield a free radical. The resulting radicals can reacts with oxygen and generate other free radicals. Hydroxyl radicals react with lipid, polypeptides, proteins, and DNA, especially thiamine and guanosine (Fig. 10). Hydroxyl radicals also add readily to double bonds. The barrier to the addition of hydroxyl radicals to double bonds is less than that of hydrogen abstraction, so that in competition addition is often favoured. When a hydroxyl radical reacts with aromatic compounds, it can add on across a double bond, resulting in hydroxycyclohexadienyl radical. The resulting radical can undergo further reactions such as reaction with oxygen, to give peroxyl radical, or decompose to phenoxyl type radicals by water elimination (Halliwell,1996; Slater, 1984; Young *et al.*,2001).

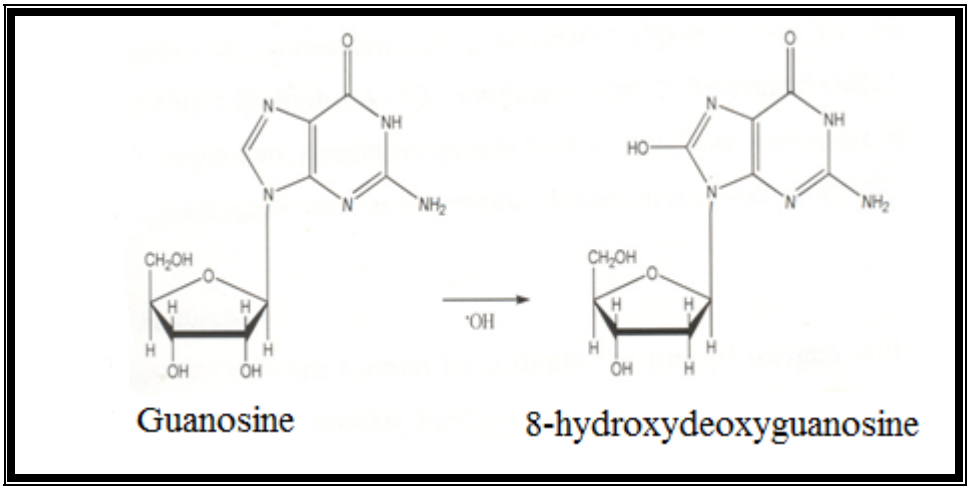


Fig 10. Formation of 8-hydroxydeoxyguanosine from the reaction of guanosine and hydroxyl radical

Hydrogen peroxide

Hydrogen peroxide can be generated through a dismutation from superoxide anion by superoxide dismutase (Fig.8 & Fig.9). Enzymes such as amino acid oxidase and xanthine oxidase also produces hydrogen peroxide from superoxide anion. Hydrogen peroxide is highly diffusible and cross the plasma membrane easily. Hydrogen peroxide is the least reactive molecule among reactive oxygen species and stable under physiological pH and temperature in the absence of metal ions. Hydrogen peroxide is a weak oxidizing and reducing agent and is thus regarded as being poorly reactive. Hydrogen peroxide can generate the hydroxyl radical in the presence of metal ion and superoxide anion

($\bullet O_2^- + H_2O_2 \longrightarrow \bullet OH + OH^- + O_2$).

Hydrogen peroxide can also produce singlet oxygen through reaction with superoxide anion or with HOCl or chloroamines in living systems. Hydrogen peroxide can degrade certain heme proteins, such as hemoglobin, to release iron ions (Halliwell,1997; Stief,2003; Lee *et al.*,2004).

Singlet oxygen

Singlet oxygen is a non radical and in excited state. The electrons in the π antibonding orbitals of singlet oxygen are paired. Metastable phosphatidylcholine hydroperoxides present in the living organism can produce singlet oxygen during their breakdown in the presence of Cu^{2+} in the dark. Singlet oxygen can be formed from hydrogen peroxide, which reacts with superoxide anion, or with HOCl or chloroamines in cells and tissues. Compared with other reactive oxygen species, singlet oxygen is rather mild and non toxic for mammalian tissue. However, singlet oxygen have been known to be involved in the cholesterol oxidation. Oxidation of

cholesterol by singlet oxygen results in formation of 5α – OOH (3β–hydroxyl – 5α – cholest – 6 – ene –5 – hydroperoxide), 5 β – OOH (3β–hydroxy– 5β– cholest– 6–ene– 5– hydroperoxide), 6α–OOH, and 6β–OOH. In the human organism, singlet oxygen is both a signal and a weapon, with therapeutic potency against various pathogens such as microbes, viruses, and cancer cells (Stief, 2003; Lee *et al.*,2004).

Peroxyl and alkoxyl radicals

Peroxyl radicals (ROO•) are formed by a direct reaction of oxygen with alkyl radicals (R), for example, the reaction between lipid radicals and oxygen. Decomposition of alkylperoxides (ROOH) also results in peroxyl (ROO•) and alkoxyl radicals. Irradiation of UV light or the presence of transition metal ions can cause homolysis of peroxides to produce peroxyl and alkoxyl radicals

(ROOH $\longrightarrow$ ROO• + H•, ROOH + $Fe^{3+} \longrightarrow$ ROO• + Fe^{2+} + H^{+})

Peroxyl and alkoxyl radicals are good oxidizing agents, having more than 1000 mV of standard reduction potential. They can abstract hydrogen from other molecules with lower standard reduction potential. This reaction is frequently observed in the propagation stage of lipid peroxidation. Very often the alkyl radical formed from this reaction can react with oxygen to form another peroxyl radical, resulting in chain reaction. Some peroxyl radicals break down to liberate superoxide anion or can react with each other to generate singlet oxygen. Aromatic alkoxyl and peroxyl radicals are less reactive than respective open chain radicals because of the delocalization of elections in the ring (Slater,1984; Halliwell *et al.*,1985).

Nitric oxide and nitric dioxide

Nitric oxide (NO•) is a free radical with a single unpaired electron. Nitric oxide is formed from L-arginine by NO synthase. Nitric oxide itself is not a very reactive free radical, but the overproduction of NO is involved in ischemia reperfusion, neurodegenerative and chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease. Nitric oxide, exposed in human blood plasma, can deplete the concentration of ascorbic acid and uric acid, and initiate lipid peroxidation. Nitric dioxide (NO_2•) is formed from the reaction of peroxyl radical and NO, polluted air and smoking. Nitric dioxide adds to double bonds and abstract labile hydrogen atoms initiating lipid peroxidation and production of free radicals. It also oxidizes ascorbic acid (Halliwell, 1984; Lee *et al.*,2004).

Peroxynitrite

Reaction of NO and superoxide anion can generate peroxynitrite

(O_2^- + NO• ⟶ $OONO^-$) (Fig .8)**.** Peroxynitrite is a cytotoxic species and causes tissue injury and oxidizes low density lipoproteins (LDL). Peroxynitrite appears to be an important tissue – damaging species generated at the site of inflammation and has been shown to be involved in various neurodegenerative disorders and several kidney diseases. Peroxynitrite (OONO-) can cause direct protein oxidation and DNA base oxidation and act as a "hydroxyl radical-like" oxidant. The significance of peroxynitirite as a biological oxidant comes from its high diffusibility across cell membranes. Nitrotyrosine, which can be formed from peroxynitrite-mediated reactions with aminoacids has been found in age-associated tissues (Halliwell,2007; Lee *et al.*,2004).

Enzymatic formation

Pro-oxidative enzymes, including NADPH oxidase, NO synthase or the cytochrome P_{450} chain, can generate ROS. Lipoxygenase generates free radicals. Lipoxygenase needs free PUFA, which are not present in healthy tissue. Membrane-bound phospholipase produces PUFA and lysolecithins. Lysolecithins change the cell membrane structures, and free PUFA are oxidized to form lipid hydroperoxides. Lipoxygenase change the cell membrane structure, and free PUFA are oxidized to form lipid hydroperoxides. There are three major mammalian lipoxygenases: 5-, 12- and 15- lipoxygenases. These enzymes can oxidized arachidonic acid, a PUFA rich in the central nervous system, into hydroperoxy eicosatetraenoic acid. The primary localizations of 5- , 12- and 15- lipoxygenases are in leukocytes and lymphocytes, platelets, leukocytes and airway cells, respectively. 15- lipoxygenase has been identified within atherosclerotic lesions, which suggests that this enzyme may be involved in the *in vivo* formation of oxidized lipids (Stief,2003).

1.8.4 ROS induced damage

Free radicals or reactive oxygen species in the body can cause lipid oxidation, protein oxidation, DNA strand break and base modification, and modulation of gene expression. As the oxidant in the body increases due to the various reasons as stated earlier, oxidized DNA such as 8–hydroxydeoxy guanosine, and oxidative damaged proteins with carbonyl modifications and loss of protein – SH group, increase several times and the ratio of redox couples such as glutathione:oxidized glutathione, NADPH: $NADP^+$ tend to shift to more pro-oxidant values. A serious imbalance between reactive oxygen species and antioxidants causes

oxidative stress. Oxidative stress is caused by antioxidant – deficient diets or by increased production of reactive oxygen species by environmental toxins such as those caused by smoking or by inappropriate activation of phagocytes such as with chronic inflammatory and liver diseases (Halliwell *et al.*,1995,1997; Spiteller, 2001).

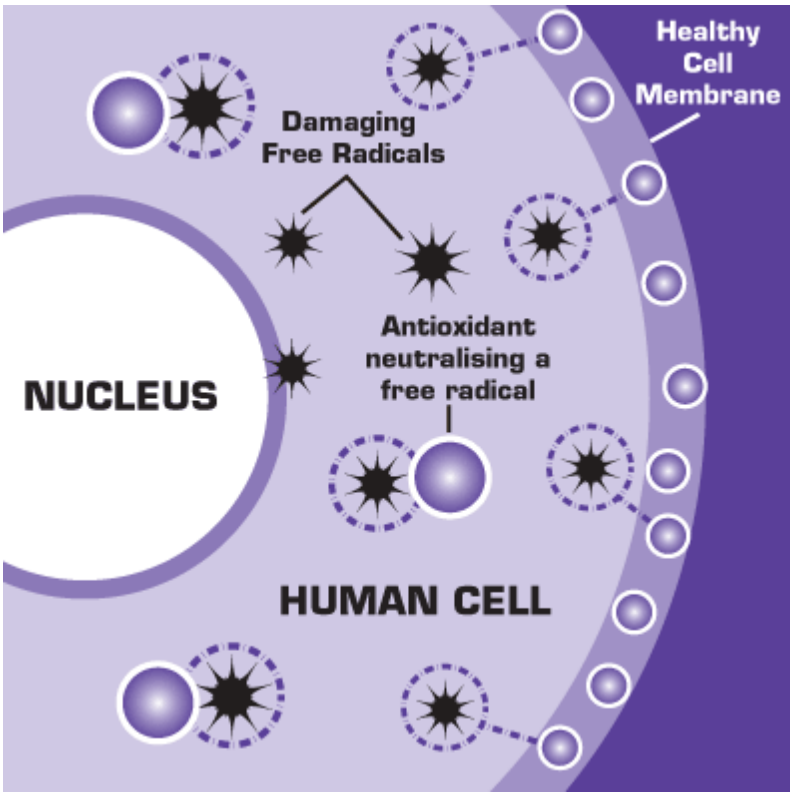


Fig11. Antioxidant neutralizing free radicals.

Lipid oxidation

Lipid oxidation is a free radical chain reaction, and reactive oxygen species can accelerate lipid oxidation. Cell membranes are phospholipids bilayers with extrinsic proteins are the direct target of lipid oxidation. As lipid oxidation of cell membranes increases, the polarity of lipid phase

surface change and formation of protein oligomers increase; and molecular mobility of lipids, number of SH groups, and resistance to thermodenaturation decrease. Malonaldehyde, one of the lipid oxidation products can react with the free amino group of proteins, phospholipids and nucleic acids leading to structural modification, which induce dysfunction of immune system. A high level of lipid oxidation products can be detected in cell degradation after cell injury or disease (liver). The increase of lipid oxidation products are found in liver disease, diabetes, atherosclerosis, apoplexy and inflammation. Low density lipoproteins are complicated structures, and oxidative modification of LDLs has been reported to be involved with the development of atherosclerosis and cardiovascular disease. Oxidized cholesterol or fatty acid moieties in the plasmatic LDL can develop atherosclerosis (Lee *et al.*,2004; Girotti,1997; Boff,2002).

Protein oxidation

Reactive oxygen species can attack proteins and produce carbonyls and other amino acid modifications, including methionine sulfoxide, 2-oxohistidine, and protein peroxides. Modification of protein is mainly initiated by hydroxyl radicals, leading to the oxidation of amino acid side chains, protein-protein cross linkage, and protein fragmentation (Fig. 12a). The availability of oxygen, superoxide anion, and its protonated form (HO_2^-) determines the pathways of protein oxidation processes. 4-hydroxy-2-nonenal from lipid oxidation can react with protein lysine, histidine, and cysteine residues (Fig 12b). Malonaldehyde from lipid oxidation reacts with protein amino groups. NO• , which is an intracellular messenger in the nervous, immune, and cardiovascular systems, is synthesized from L-arginine by NO synthetase in mitochondria and causes oxidation of protein.

Nitrotyrosines, products of reactive nitrogen species on tyrosine, have been detected in atherosclerotic lesions, human urine, and body fluids from patients with chronic inflammatory diseases. Protein oxidation affects the alterations of signal transduction mechanisms, transport systems, enzyme activities, atherosclerosis, and ischemia reperfusion injury. Liver damage is associated at least partly with oxidative modification of proteins. For example, *in vitro* exposure of enzymes to reactive oxygen species induces changes in enzymatic activity heat stability and proteolysis susceptibility similar to those that occur during aging (Lee *et al.*,2004; Berlett,1997).

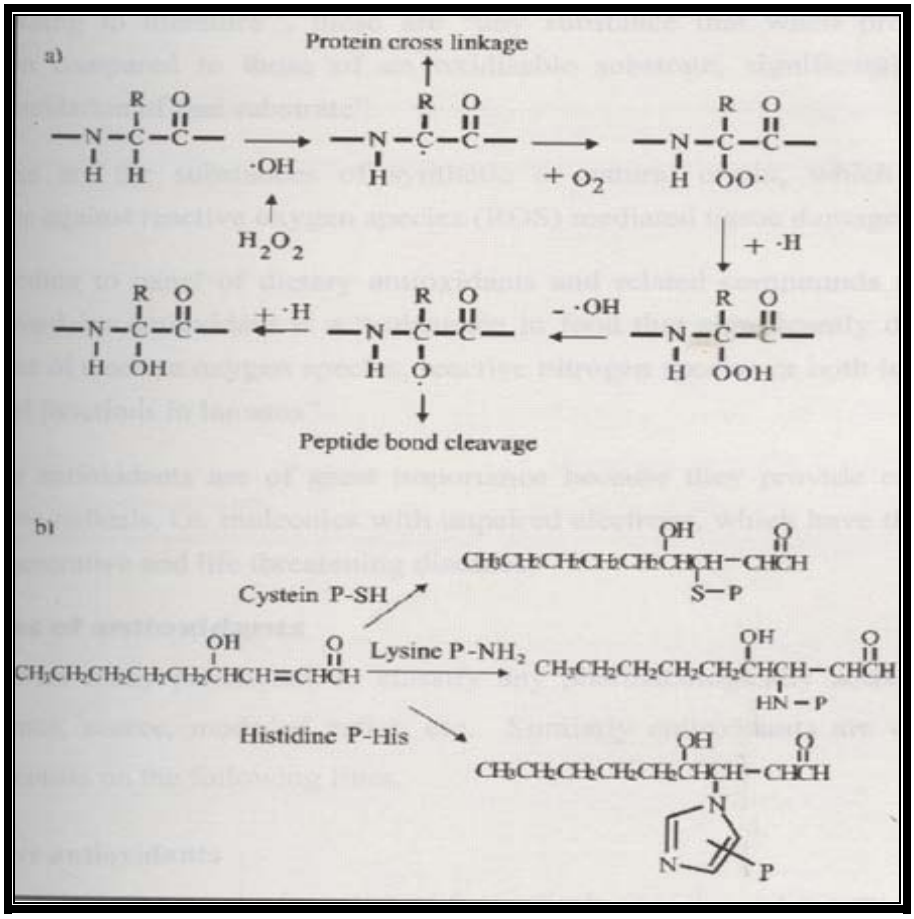


Fig 12. a. Oxidation mechanism of proteins

b. Protein carbonyl formation from aldehydes

DNA strand breaks and modification

Mitochondria and nuclei have their own DNA. Mitochondrial DNA is susceptible to oxidative damages because of the lack of protective protein, histones, and close locations to the reactive oxygen species-producing systems. Hydroxyl radical oxidizes guanosine or thymine to 8-hydroxy-2-deoxyguanosine and thymine glycol, respectively, which changes DNA and leads to mutagenesis and carcinogenesis (Fig. 10). A low level of oxidative base damage in DNA is found in the cells of healthy persons. However, concentration of oxidized DNA base increases in humans with chronic inflammatory diseases such as rheumatoid arthritis or under oxidative stresses such as smoking. If oxidative stress is too great, the DNA repair system using glycosylase is not enough, and mutagenesis and /or carcinogenesis can be induced (Halliwell *et al.*,1984; Lee *et al.*,2004; Ames *et al.*,1993).

1.8.9 Antioxidants

According to literature, these are "any substance that when present in low concentration compared to those of an oxidisable substrate significantly delays or inhibits the oxidation of the substrate" (Scalbert *et al*.2005).

"These are the substances of synthetic or natural origin, which protects the biomembrane against reactive oxygen species (ROS), mediated tissue damage (Chandan K.S. 1995)".

According to panel of dietary antioxidants and related compounds of Food and Nutrition Board, an antioxidant is a "substance in food that significantly decreases the adverse effect of reactive oxygen species,

reactive nitrogen species or both in normal and physiological functions in humans".

These antioxidants are of great importance because they provide electrons that neutralize free radicals, i.e. molecules with unpaired electrons, which have the capability to cause degenerative and life threatening diseases. (Fig. 11)

1.8.10 Types of antioxidants

There are many parameters to classify any pharmacologically active substance, like occurrence, source, mode of action etc. Similarly antioxidants are classified by different scientists on the following lines.

A. Preventive antioxidants

These mainly suppress the formation of free radicals since they act at very early stage of onset of free radicals. These are most valuable and safe ones. Based on different modes of suppression they are:

a. Antioxidative enzymes (Lee *et al.*,2004)

Antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidise/reductase, convert ROS into nonreactive oxygen molecules. Proteins showing antioxidant properties are listed in (Table.9). Superoxide dismutase (SOD) converts superoxide anion into hydrogen peroxide and oxygen. There are 2 types of SOD: a magnesium-containing SOD and a copper-zinc-dependent SOD. Catalase is involved in cellular detoxification and can convert hydrogen peroxide into water and oxygen (Fig. 13). Glutathione peroxidise is the most important hydrogen peroxide-removing enzyme existing in the membrane. Glutathione disulfide

reductase is a flavoprotein that permits the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) by the oxidation of NADH to NAD^+ (Fig. 13).

Table.9. Anti-oxidative enzymes

Proteins	Functions
Superoxide dismutase	Superoxide removal
Catalase	Hydroperoxide removal
Glutathione peroxidase	Hydroperoxide removal
Glutathione disulfide reductase	Oxidized glutathione reduction
Glutathione-S-transferase	Lipid hydroperoxide removal
Methionine sulfoxide reductase	Repair oxidized methionine residues
Peroxidise and lipid hydroperoxide	Decomposition of hydrogen peroxide

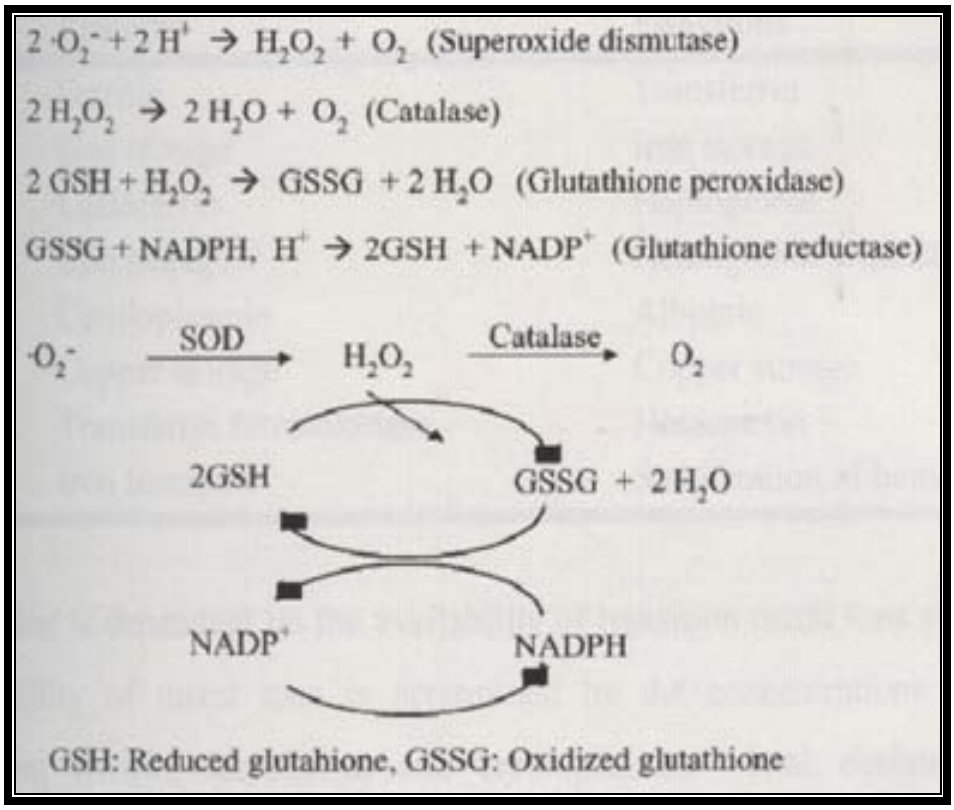


Fig.13. Antioxidant enzymes and their reaction mechanisms.

b. Metal chelating antioxidants

Transition metals such as iron and copper play important role in initiation and propagation steps of lipid oxidation. The initiation step of oxygen oxidation requires removal of a hydrogen atom. The presence of metal can accelerate the initiation step of lipid oxidation by the mechanism of

$$RH + M^{n+} \longrightarrow {}^{+}R\bullet + H^{+} + M^{(n-1)+}.$$

Metals can decompose the hydroperoxide to form peroxyl redical and alkoxyl radical, and accelerate the lipid oxidation at the exponential rate.

$$Fe^{3+}\ (Cu^{2+}) + \longrightarrow ROOH\ Fe^{2+}\ (Cu^{2+}) \longrightarrow ROO\bullet + H^{+}$$

$$Fe^{2+}\ (Cu^{+}) + \longrightarrow ROOH\ \ Fe^{3+}\ (Cu^{2+}) \longrightarrow + RO\bullet + OH^{-}$$

Metals are also involved in the formation of singlet oxygen.

$Fe^{2+} + 2O_2 \longrightarrow Fe^{3+} + \bullet O_2 - {}^1O_2$

Hydrogen peroxide can react with transition metal ions to form hydroxyl radical.

$Mn+ + H_2O_2 \longrightarrow M^{(n+1)+} + \bullet OH + OH^-$

Tabl 10. Metal chelating proteins

Proteins	Functions
Ferritin	Transferring
Iron storage	Iron storage
Lactoferrin	Haptoglobin
Iron storage	Hemoglobin sequestration
Ceruloplasmin	Albumin
Copper storage	Copper storage
Transferring ferro-oxidase	Hemopexin
Iron transport	Stabilization of heme

This reaction is dependent on the availability of transition metal ions such as copper and iron. The availability of metal ions is determined by the concentrations of metal-binding proteins, including ferritin, lactoferrin, and ceruloplasmin. Metal chelators, one type of antioxidative nutraceuticals, form complex ions or coordination compounds with metals by occupying all metal coordination sites and preventing metal redox cycling. Metal chelators can convert metal ions into insoluble metal complexes or generate steric hindrance, which can prevent the interactions between metals and lipid intermediates. Some reported metal-chelating proteins are shown in (Table.10) Metal chelators are phosphoric acid, citric acid, ascorbic acid,

polyphenols such as quercetin, carnosine, some amino acids, peptides, and proteins such as transferrin and ovotransferrin (Seifrieda *et al.*,2007; Lee *et al.*,2004; Ramoon *et al.*,2002).

c. Singlet oxygen-quenching antioxidants

Singlet oxygen is highly reactive toward any molecules with electrons or lone pairs of electrons with low ionization energy. There are 2 types of singlet oxygen-quenching mechanisms: physical and chemical quenching. Physical quenching converts singlet oxygen into triplet oxygen by either energy transfer or charge transfer without generating any intermediates. Chemical quenching is involved with the generation of intermediates, such as oxidized products. Singlet oxygen reactions with compound (A) to form oxidized products (AO_2) are shown in (Fig. 14). Chemical quenching between singlet oxygen ($^1O_2$) and quencher (Q) involves the generation of an oxidized product QO_2. Physical quenching converts singlet oxygen ($^1O_2$) to triplet oxygen ($^3O_2$) without production of oxidized product $–QO_2$. Singlet oxygen quenchers should have electron rich structures such as double bonds in the molecules to react with singlet oxygen. Carotenoids, which have many double bonds, are well-known singlet oxygen quenchers. Uric acid is also a powerful quencher and a hydroxyl radical scavenger, which acts independently of the redox potential (Halliwell *et al.*,1995; Boff *et al.*,2002; Kumuda,2000).

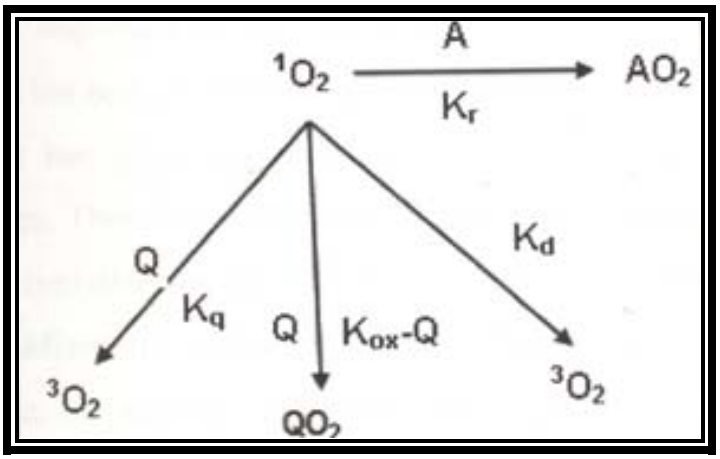


Fig. 14. Singlet oxygen quenching mechanisms.

B. Radical scavenging antioxidants

Antioxidative nutraceuticals, which can donate hydrogen atoms to free radicals, can scavenge free radicals and prevent lipid oxidation (Lee *et al.*, 2004; Chidambara,2001; Hang *et al.*,2005).

a. Hydrophilic: These cannot enter the lipid moiety of LDL. They will be less efficient as they are principally unable to encounter the most of these lipophilic radicals. However, such a compound may act in a synergistic manner with lipophilic antioxidants by regenerating them, e.g. Vitamin C, Albumin etc.

b. Lipophilic: These tend to accumulate in plasma lipoproteins (e.g. LDL) upon supplementation. These groups of antioxidants are supposed to act as highly efficient scavengers against lipid Peroxyl radicals, which are formed within the lipoprotein particles as a consequence of the free radical chain reaction of lipid peroxidation. e.g. vitamin E, carotenoids etc.

c. Repair and denovo enzymes: These mainly act by repairing the damage and reconstituting the membranes.

e.g. Lipase, protease, DNA repair enzymes, transferase etc.

Obesity and hyperlipidemia are among the important disorders affecting mankind and remain one of the serious health problems. No permanent remedy is available to majority of these. In the absence of suitable protective drugs in allopathic medical practices, herbs play important role in the management of various disorders. A number of medicinal plants have been advocated in traditional system of medicine, especially in Ayurveda for treating hyperlipidemia. This usage is in vogue since centuries and are quite often claimed to offer significant relief.

Based on the above facts and ethnomedical claims and the need for the development of newer plant based antihyperlipidemic drugs, it was felt to carry out the antihyperlipidemic and antioxidant activity of the leaf and seeds of ***Achyranthes aspera*** **Linn** and ***Achyranthes bidentata*** **Blume** of Amaranthaceae family .

SCOPE

2. Scope

Many medicinal plants have been found to possess active principles useful for treating diseases. Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic chemicals. Herbal medicines are being used today by about 80% of the world's population mainly in the developing countries for primary health care. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Ancient literature also mentions herbal medicines for age-related diseases, namely, memory loss, osteoporosis, diabetic wounds, heart diseases, immune and liver disorders for which no modern medicine or only palliative therapy is available.

Hyperlipidemia is recognized as one of the great risk factors for coronary artery diseases. Epidemiological studies have clearly pointed out the role of high level serum cholesterol in various pathological conditions of the heart such as coronary artery diseases (CAD). Evidence from studies both in animals and human indicate that progression can be slowed if elevated concentration of the atherogenic lipoprotein and triglycerides are reduced, which in turn prevents coronary heart disease.

Currently available synthetic antihyperlipidemic drugs such as gemfibrozil, clofibrate, probucol, cholestyramine etc., are not totally free from side effect when used for prolonged periods. The current scenario sees the emergence of a number of medicinal plants being evaluated for various

diseases and disorders, although a number of medicinal plants like *Commiphora mukul, Gymnema Silvestre, Pterocarpus marsupium, Trigonella foenum-graceum, Terminallia arjuna* and *Boswellia serata* have been evaluated for their hypolipidemic activity, only *Commiphora mukul* has been well established. (Sharma *et al*,.1997)

The plant material such as leaf and seeds of *Achyranthes aspera* and *Achyranthes bidentata* were selected for present study to screen the antihyperlipidemic effect. It was proposed, therefore, to use 50% alcoholic and aqueous extracts for hyperlipidemia in the present investigation in addition to antioxidant property.

Antioxidant activity was performed by 2,2–diphenyl-1- picryl hydrazyl(DPPH) and nitric oxide radical inhibition activity methods.

In vivo study was performed for screening antihyperlipidemic effect. Triton induced and diet induced hyperlipidemic models have been used for screening antihyperlipidemic activity. Blood cholesterol, triglycerides, LDL, VLDL and HDL cholesterol levels have been estimated. Histopathological changes in aorta was also studied.

PLAN OF WORK

3. Plan of work

The work plan is divided into following phases:

Phase I

The collection of plant material and extraction

- Identification, authentication of the plant material.
- Collection, shade drying and powdering of the leaf and seed.
- Extraction of the powdered material with alcohol and water.

Phase II

Pharmacognostical studies:

The following pharmacognostical parameters were studied.

- Morphological profile of the plant
- Microscopical analysis and anatomical standardization of the plants.
- Determination of physicochemical constants such as total, acid insoluble, water-soluble and sulphated ash values.
- Determination of extractive values.
- Fluorescence analysis.
- Foaming index and mucilage content.

Phase III

Preliminary phytochemical analysis

- Preparation of various extracts.
- Phytochemical tests
- Qualitative analytical tests for detection of various plant constituents namely alkaloids, glycosides, tannins, steroids, flavanoids,

triterpenoids, fixed oils, fats, saponins, carbohydrates, sugars and phenolic compounds in the extracts.

Phase IV

Characterization of active phytoconstituents / chemical components

- Isolation of compounds using column chromatography technique.
- Qualitative chemical test for the indentification of presence of the various phytoconstituents in the fractions.
- Thin layer chromatography (TLC) analysis for the extracted fractions.
- Development of HPTLC profiles for the different extracts.

Phase V

***In vivo* Antihyperlipidemic screening**

- Triton induced hyperlipidemia
- Diet induced hyperlipidemia

***In vitro* antioxidant studies**

- DPPH method
- Nitric oxide radical inhibition assay

PLANT PROFILES AND REVIEW OF LITERATURE

4. Botanical information

4.1. Botanical information of the Genus (Gamble J.S. 1935)

Genus: Achyranthes

Family: Amaranthaceae

The plant family Amaranthaceae consists of approximately 850 species in 65 genera, and is distributed world-wide, in both temperate and tropical regions. Many of these species are commonly used by man as medicinal herbs and as sources of both edible leaves and seeds.

The plant occurs throughout the tropics of Indian sub-continent open place and hilly regions upto an altitude of 4000 ft – 6000 ft.

4.2. The important characters :

Habitat: Herbs, sometimes woody below

Leaves: Opposite, entire, petiolated

Flowers: Hermaphrodite, in slender simple and panicled spikes, soon deflexed, bracts membranous, spinescent, persistent, bracteoles 2, perianth calycine, of 4-5 rigid, lanceolate, aristate segments somewhat connate below, ultimately ribbed and hardened.

Stamens 2-5 filaments filiform, connate at the membranous base and alternating with as many, quardrate. Staminodes which are toothed lacerate or with a toothed scale at the back, anthers 2-celled. Ovary oblong,

subcompressed 1-celled, ovule solitary, pendulous from a long basal funicle, style filiform, stigma apitates.

Fruit: An oblong of ovoid utricle, rounded or areolate at apex, when ripe usually disarticulating above the bract.

Seed: Inverse, oblong, testa coriaceous tegmen membraneous, embryo annular, surrounding the floury albumen, cotyledons oblong, again incurved, radical erect.

Root: Tap root system

The plant *Achyranthes bidentata* and *Achyranthes aspera* have been identified and differentiated by the following characters.

Achyranthes bidentata: Wings of the bracteoles merely auricles at the base, staminodes truncate, toothed but not fimbriate, leaves variable ellipse lanorolare or linear, membranous, glabrous or pubescent.

***Achyranthes aspera* Linn**: Dry ground plants, perianth broad, nearly half as long as spine, staminodes truncate, fimbriate, with or without dorsal appendages, leaves very variable.

4.3 *Achyranthes aspera* Linn

Name: *Achyranthes aspera* Linn

Family: Amaranthaceae

Vernacular Names:

English	: Prickly chaff flower plant
Tamil	: Nayurvi, Katalati
Hindi	: Circita, Cicimda
Malayalam	: Valiyakatarati, Katalati
Sanskrit	: Apamargah, Apangaka
Kannada	: Uttarani
Telugu	: Apamargamu, Uttarani.
Bengali	: Apang, Chirchiti.

Distribution: It is commonly found as a weed on way sides and waste places throughout India upto an altitude of 900m. It is widely distributed in Baluchistan, Ceylon, Africa, America and India.

Botanical Characters

Habitat: An erect or procumbent annual of perennial herb. 1-2m in height, often with a woody base.

Stem: Angular, ribbed, simple or branched from the base often tinged with reddish purple color.

Leaves: Thick, ovate – elliptic or obovate – rounded, but variable in shape and size reaching 4 inch long 3 inch broad.

Flowers: Greenish white numerous in axillary or terminal spikes upto 75 cm long.

Seed: Subcylindric, truncate at the apex, rounded at the base reddish brown.

Root: Tap root system.

Uses: The plant is used as expectorant, revulsive, carminative, digestive, stomachic, laxative, anodyne, depurative, anthelmintic, diuretic, linthontriptic, sudorific, demulcent, haematinic and anti-inflamatory. It is used in caugh, asthma, bronchitis, dyspepsia, flatulence, colic, painful inflammations, dropsy, opthamopathy, vomiting, leprosy, skin disease, pruritus, helminthiasis, strangury, renal and vesical calculi, cardiac disorders, anaemia, vitiated conditions of *kapha* and *vata* and general debility (Singh MP *et al*,.2005).

Fig 15. Fresh plant of *Achyranthes aspera* Linn

Fig 16. *Achyranthes aspera* Linn seeds with husk

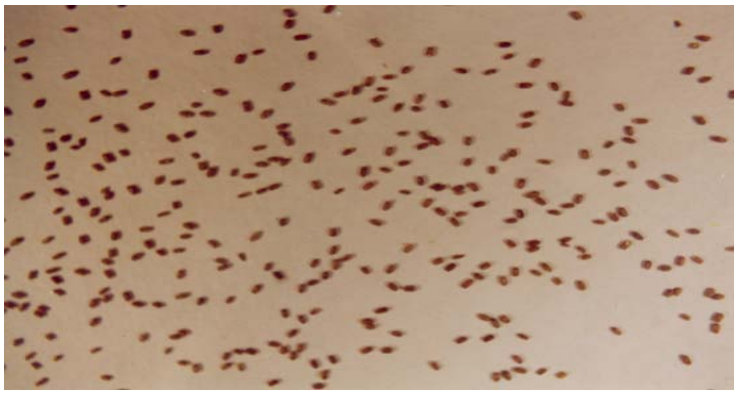


Fig 17. *Achyranthes aspera* Linn seeds without husk

4.4 *Achyranthes bidentata* Blume

ii. *Achyranthes bidentata* Blume

Name: *Achyranthes bidentata* Blume

Family: Amaranthaceae

Vernacular Names : Tamil : Sigappunayurivi

Telugu : Uba

Sanskrit : Milapu

Assam : Bankhar, Apamarga.

Arabic : Iruka

Distribution: It is distributed in the hilly districts throughout India at an altitude of 1200-3200m. It is widely distributed in India, China, Java and Japan.

Botanical Characters

Habitat: An erect or ascending perennial herb or a shrub sometimes with woody root stock.

Stem: Soft, erect and circular at the base, gradually angular towards the tip.

Leaves: Elliptic, oblong or oblong lanceolate, pubescent about 2 inch long to lanceolate and finally linear, in some cases upto 8 inch long, 1.5 inch broad and nearly of quite glabrous.

Flowers: White or green, or red flowers hermaphrodite in axillary terminal spikes. Bracts are memberanous and persistant. Bracteoles two in number and spinecent. Perianth lobes 5 in number. Stamen 5 in number. Filaments are long connate at the memberanous base.

Seed: Inverse, embryo annular surrounding the flourly albumen.
Root: Tap root system.

Uses: The seeds of *Achyranthes bidentata* are a good substitute for cereal grains used in bread making and have been used during the Indian famine. The roots, leaves and stems are widely used as anodyne, anti inflammatory, anti rheumatic, bitter, digestive, diuretic, emmenagogue and vasodilator. They act predominantly on the lower half of the body and are used in the treatment of aching back and knees and asthenia of the lower limbs. Research suggests that they can cause dilation of the cervix and so this herb should not be used when pregnant. The herb is taken internally to treat hypertension, back pains, urine in the blood, menstrual pain, bleeding etc. It lowers blood cholesterol levels and so is used in the treatment of atherosclerosis. The root juice is used in Nepal in the treatment of toothache. This juice is also used in the treatment of indigestion and is considered to be good treatment for asthma. The stem of the plant is used as a toothbrush that is said to be good for the teeth and is also a treatment for pyorrhea. Leaves are used for treating blisters in mouth and for treating cholarae. Root is used for treating scorpion stings and whole plant is used against whooping cough. The plant can be used fresh or dried. (Selvaraj. N *et al*,.2009)

Fig 18. *Achyranthes bidentata* Blume whole plant with spike

Fig 19. *Achyranthes bidentata* Blume seeds with husk

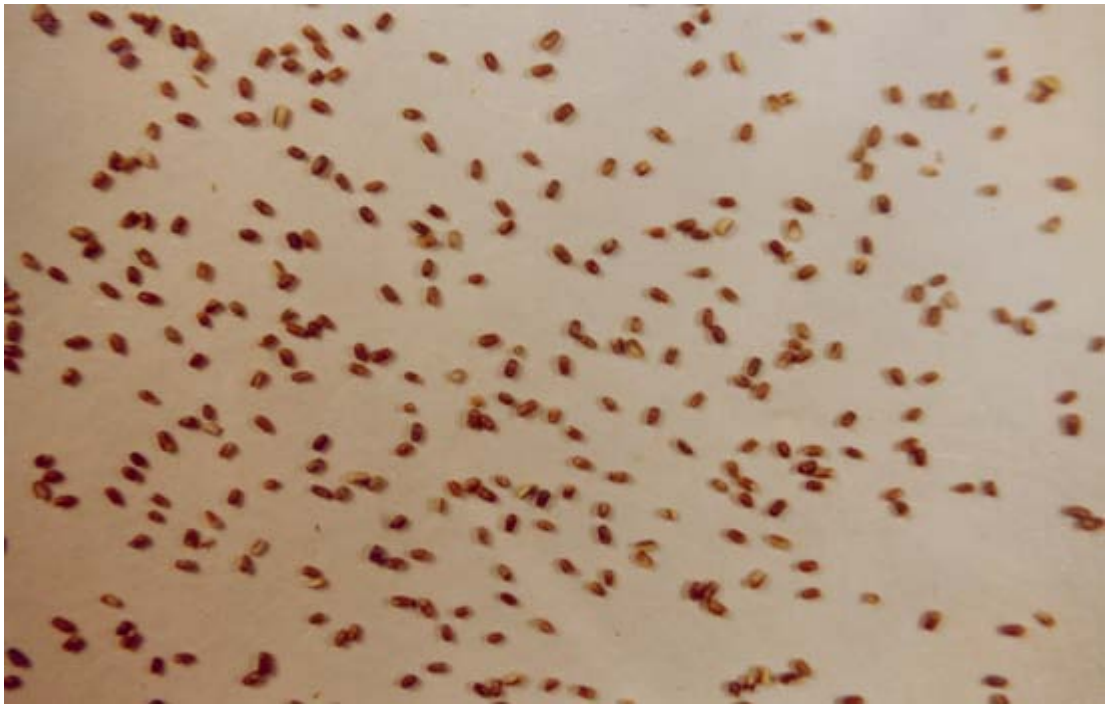


Fig 20. *Achyranthes bidentata* Blume seeds without husk

4.5 LITERATURE REVIEW

4.5.1 *Achyranthes aspera* Linn

Daniel Paul et al., carried out the comparative study on the spermicidal activity of organic solvent fractions from hydroethanolic extracts of *Achyranthes aspera* and *Stephania hernandifolia* in human and rat sperm and found that the n-hexane fraction was most effective, and the chloroform fraction exhibited minimum activity.

Deoran M. **Awari** et al., reported the antilithiatic effect of *Achyranthes aspera* Linnn extract on ethylene glycol induced nephrolithiasis in male albino wister rats and observed that the ethanolic extract of the leaves of the plant shown a reduction in the elevated urinary calcium and oxalate ion concentration in the urine.

Neeru Vasudevan et al., studied the post coital antifertility activity of *Achyranthes aspera* Linn root in proven female albino rats and found that the ethanol extract possessed both anti-implantation and abortificient activity.

Vasudeva Rao Y et al., studied the effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophilla* and the results indicate that *Achyranthes aspera* stimulates immunity and increases resistance to infection in *L.rohita.*

Daniel Paul et al., studied *in vitro* determination of the contraceptive spermicidal activity of a composite extract of *Achyranthes aspera* and *Stephania hernandifolia* on human semen and found that this composite

plant extract in a ratio of 1:3 by weight at different concentrations possessed potential contraceptive spermicidal activity *in vivo*.

Rina Chakrabarti et al., studied the immunity stimulant activity of the *Achyranthes aspera* and found to be active. It also enhances the antigen clearance in Catla catla.

Vasudeva Y et al., studied the potentiation of antibody production in Indian major crap *Labeo rohita,* rohu, by *Achyranthes aspera* as a herbal feed ingredient. The result showed the immunostimulatory activity of the prepared diet containing root extract of *A.aspera.*

Asima Chakraborty et al., studied cancer chemopreventive activity of *Achyranthes aspera* leaves on Epstein-Barr virus activation and two stage mouse skin carcinogenesis. The study suggests that *Achyranthes aspera* leaf extract and the non-alkaloid fraction are valuable antitumor promoters in carcinogenesis.

Pankaj Tahiliani et al., had studied the role of *Achyranthes aspera* on the changes in serum thyroid hormone concentrations and glucose levels in male rats and found that the *Achyranthes aspera* leaf extract is both prothyroidic and antiperoxidative in nature.

Triguna N. Mishra et al., isolated two long chain compounds from shoots of *Achyranthes aspera.*

Triguna N. Mishra et al., isolated and characterized antifungal essential oil and lond chain alcohol from *Achyranthes aspera.*

Muhammad Shoaib Aktar et al., evaluated the hypoglycaemic effect of *Achyranthes aspera* in normal and Alloxan-diabetic rabbits.

Banarji A et al., isolated insect mounting hormone from *Achyranthes aspera.*

4.5.2 *Achyranthes bidentata* Blume

Songlin Zhou et al., prepared aqueous extract of *Achyranthes bidentata* Blume and found that *Achyranthes bidentata* extract promets nerve growth and prevents neuronal apoptosis.

Cui-Cui He et al., studied the osteoprotective effect of extract from *Achyranthes bidentata* in ovariectomized rats and found that the n-butanol soluble fraction of the root of *Achyranthes bidentata* is effective.

Xin Tang et al., prepared aqueous extract of *Achyranthes bidentata* Blume and found that *Achyranthes bidentata* extract promotes neuronal growth in cultured embryonic rat hippocampal neurons.

Hongmei Shen et al., studied the protective effect of *Achyranthes bidentata* polypeptides against NMDA-induced cell apoptosis in cultured hippocampal neurons through differential modulation of NR2A- and NR2B- containing NMDA receptors and found to be active.

Fei Ding et al., studied the rapid effect of *Achyranthes bidentata* extract on the crushed common peroneal nerve of rabbits and found that *Achyranthes bidentata* extract could accelerate peripheral nerve regeneration.

Hongmei Shen et al., studied the protective effect of *Achyranthes bidentata* polypeptides against NMDA-induced cell apoptosis in cultured hippocampal neurons through differential modulation of NR2A- and NR2B- containing NMDA receptors and found to be active.

Fei Ding et al., studied the rapid effect of *Achyranthes bidentata* extract on the crushed common peroneal nerve of rabbits and found that *Achyranthes bidentata* extract could accelerate peripheral nerve regeneration.

Li-Qin Jin et al., investigated the effect of *Achyranthes bidentata* polysaccharides (ABPS) against Lewis lung cancer (LLC) in C57BL/6 mice and postulated that the stimulation of tumor growth by high dose of ABPS is associated with dysfunction of NK cell.

Xian Li et al., isolated a new phytosterone from the roots of *Achyranthes bidentata.*

Hong-Xiang Sun studied the haemolytic activity of *Achyranthes bidentata* saponins (ABS) and its adjuvant potential on the cellular and humoral immune responses of ICR mice against ovalbumin (OVA) and the result suggested that ABS showed a slight haemolytic effect and enhance significantly a specific antibody and cellular response against OVA in mice.

Massimo F Marcone et al., characterized the chemicals of *Achyranthes bidentata* seed.

MATERIALS AND METHODS

5.MATERIALS AND METHODS

5.1 Materials

Plant materials

The following plant materials were selected for the present study.

***Achyranthes aspera* Linn leaves and seeds**

***Achyranthes bidentata* Blume leaves and seeds**

5.2 Instruments

Auto-analyser: Merck Microlab 200 manufactured by M/s Vital scientific N.V., the Netherlands was used to estimate various biochemical parameters viz. cholesterol, triglyceride and HDL cholesterol levels.

Centrifuge: Remi centrifuge, R-8c Laboratory centrifuge and Elvenjan homogenizer, Remi motors Ltd, Mumbai, India were used to separate serum from blood.

Elisa Reader: BioRad Labortories Inc, California, USA, model 550 was used in *in vitro* antioxidant assays.

Grinding mill: Junior Grindwell, Chowthry, J.U.C, Mumbai, India was used for powdering of plant materials.

HPTLC: CAMAG Linomat IV, No-022.78.6, twin trough chamber No-022-5155 and TLC Scanner-3, No.027-6480, were used for HPTLC studies.

Incinerator: Ambassador, model-2265, Matri, Pondichery, India, was used for determination of ash values of plant materials.

Rotary Microtome: Leica Rm 2135, Leica Microsystem GmbH, Germany was used for sectioning of the paraffin embedded specimens of plants and animal tissues.

Rotary evaporator: Superfit, India rotary evaporator was used for concentration of plant extracts.

Spectrophotometer: Shimadzu 160-A UV-VIS Spectrophotometer manufactured by Schimadzu, Japan was used to estimate biochemical parameters.

5.3 Source of chemicals.

Triton WR 1339, was obtained from Sigma USA. Atorvastatin 10 mg tablets (Atorlip), from Cipla. Ascorbic acid was obtained from SD Fine Chemicals Pvt Ltd, Mumbai. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade. 2,2-diphenyl-1-picryl hydrazyl (DPPH) was procured from Sigma Chemical Company, California, USA. Aluminium backed HPTLC plates coated with 0.2mm layers of silica gel 60 F254 plates, serum cholesterol, triglycerides, high density lipoproteins cholesterol were determined using Ecoline diagnostic kits from E-merck Ltd., Mumbai, India.

5.4 Methods

5.4.1 Collection and authentification of plant materials

The plants *Achyranthes aspera* Linn and *Achyranthes bidentata* Blume (fam: Amaranthaceae) are widely found throughout India along road sides and waste places as well as on hill areas upto a height of 1200m. In Tamilnadu, these are found in Nilgiri district and in Erode district. For our work, the leaves and seeds of the plant *Achyranthes aspera* were collected from Kotagiri, the Nilgiri district of Tamilnadu. *Achyranthes bidentata* was collected from Ketty, Emerald and Edakkadu Villages of Nilgiri district of Tamilnadu.

The plants were identified by Prof. Dr. P. Jayaraman, Director, Plant Anatomy Research Center, Chennai, a botanist who authenticated the plant with available literature. The plants were collected by digging the entire plant, cutting and separating the leaves from the plant.

The spikes and the leaves were dried in shade. Then seeds were removed and cleaned to remove any dirt, mud and other plant debris. The seeds were dehusked by means of mechanical stirrer. Then the seeds were separated from the husk by passing through the sieve No. 20. The dried seeds were powdered by means of wood grinder and the powder was passed through the sieve No. 60 for powder analysis and coarse powder was used for phytochemical work.

5.4.2 Extraction of plant material

The term extraction involves separation of therapeutically required portions of the plant material from the inactive components by using selective solvents (menstrum). The extract thus obtained may be ready to use as such in the form of tinctures or fluid extracts or it may be processed to be incorporated in dosage forms like tablets or capsules or may be fractionated to isolate individual chemical compounds which are used as modern drugs.

General methods of extraction

1. Maceration
2. Infusion
3. Digestion
4. Hot extraction (Decoction)
5. Cold extraction (Percolation)
6. Hot continuous extraction (Soxhlet)

Parameters essential for the selection and standardization of the extraction methods

- Authentication of the plant material and removal of foreign matter.
- Use of right plant part, time and place of collection
- Proper drying and powdering of plant materials
- Nature of phytochemicals
    i. Solvent used for extraction depends upon the nature of the active constituents. If the activity lies in the non polar constituents, non polar solvent may be used.
    ii. If the constituents are thermolabile, then cold extraction methods are preferred. For thermostable constituents, Soxhlet extraction can be used.
    iii. In case of hot extraction, higher than required temperature should be avoid.
- Time and number of extraction.
- Proper quantity of the solvent used for extraction.
- Proper concentration and drying of extract to ensure the safety and stability of active constituents.
- Analytical parameters of the final extract e.g. HPTLC fingerprinting and analytical limits should be set to monitor the quality of different batches of the extracts.

Preparation of the extracts

The plant material which was powdered and stored was used for extraction. A weighed quantity of each of the plant powdered material was extracted by cold maceration with 50% ethanol for 72 hr with intermediate heating at 40^{o}C one time in a day. The extract was filtered using Whatmann filter

paper and then the filterate was concentrated under reduced pressure and controlled temperature (40^{o}-50^{o}C). The marc was dried and weighted. The marc was again extracted with water by cold maceration for 72 hrs to yield aqueous extract. (Table. 11)

Table 11. Yield and nature of the extracts

S. No	Plant material	Quantity used forextraction in grams	Type of the extract	Natu of the extract	Yield	
					G	%
1	*Achyranthes aspera* Linn leaf	900	50% ethanol	Dark green	122	13.63
2	*Achyranthes aspera* Linn leaf	760	Aqueous	Dark brown green	147	19.34
3	*Achyranthes aspera* Linn seed	800	50% ethanol	Dark brown green	130	16.25
4	*Achyranthes aspera* Linn seed	620	Aqueous	Dark brown green	126	20.28
5	*Achyranthes bidentata* Blume leaf	900	50% ethanol	Dark green	127	14.11
6	*Achyranthes bidentata* Blume leaf	730	Aqueous	Dark brown green	147	20.13
7	*Achyranthes bidentata* Blume seed	800	50% ethanol	Dark brown green	136	17
8	*Achyranthesbidentata* Blume seed	610	Aqueous	Dark brown green	129	21.14

5.4.3 Pharmacognostical studies

Most of the raw material used by the herbal industry are produced from wild sources and this result in inconsistency in quality, adulteration and substitution. Hence, pharmacognostical evaluation of crude drugs is most important in herbal drug industry. Pharmacognostical studies include authentication, determination of foreign matter, organoleptic evaluation, macroscopic and microscopical examination, saponin content determination and evaluation of ash values and extractive values.

Crude drugs when supplied in intact form can be identified by morphological characteristics. The same can be investigated for histological characteristics to confirm the identity of the supplied drugs. Microscopical techniques provide detailed information about the crude drugs by virtue of their two main analytical uses. Firstly, their property of magnification permits the fine structures of minute objects to be visualized and thereby confirm the structural details of the plant drugs under evaluation. Secondly, these techniques can be used in the determination of the optical as well as the micro-chemical properties of the crude drug specimens under study. The powdered crude drugs can be identified based on the form, the presence or absence of different cell types based on their cytomorphological characters e.g. parenchyma, collenchyma, fibres, stone cells, trichomes, secretary cells, epidermal cells etc. The same may sometimes be achieved by evaluating the cell inclusion characteristics for some unorganized crude drugs like starch grains, aleurone grains, gums, mucilage, calcium oxalate crystals etc. Ash values are helpful in determining the quality and purity of the crude drugs, especially in powdered form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical

determination (Mukherjee, 2002). Extractive values determine the amount of active constituent in a given amount of medicinal plant material when extracted with solvents. Further, these values indicate the nature of the constituents present in a crude drug.

5.4.4 Plant anatomical studies

Preparation of specimen

The fresh healthy samples were cut and removed from the plant and fixed in FAA (formalin 5 ml + acetic acid 5ml + 70% ethyl alcohol 90 ml). After 24 hrs of fixing, the specimens were dehydrated with gradual series of TBA (tertiary butyl alcohol) as per schedule (Sass, 1940). Infiltration of the specimens was carried out by gradual addition of paraffin wax (m.p. 58-60-^{o}C) until TBA solution attained supersaturation. The specimen was cast into paraffin blocks.

Sectioning

The paraffin embedded specimen was sectioned with the help of Rotary **Microtome**. The thickness of the section was 10-12 µm. De-waxing of the sections was done by customary procedure (Johanses, 1940). The sections were stained with **Toluidine Blue** method (O'Brine *et al.*,1964). Since **Toluidine blue** is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the **cellulose** mass, blue to the **lignified cells**, dark green to **suberin**, violet to the **mucilage**, blue to the **protein** bodies etc. Wherever necessary, sections were also stained with **safranin** and **fast-green** and potassium iodide (for starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as cleaning of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid(Sass, 1940) were prepared. Glycerin mounted temporary preparations were made for macerated / cleared materials. Powdered materials of different parts were cleaned with sodium hydroxide and mounted in glycerin medium after staining. Different cell components were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs. Photographs of different magnifications were taken with **Nikon lab photo 2** microscopic unit. For normal observations **bright field** was used. For the study of **crystal, starch grains** and **lignified cells, polarized light** was employed. Since these structures have **birefringent property,** under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are given as in the standard anatomy books (Easu,1964;1979) .

5.4.5 Determination of physicochemical constants

a. Ash values (WHO, 1998)

Ash values are helpful in determining the quality and purity of crude drugs in powdered form. The values vary within fairly wide limits and are therefore an important parameter, for the purpose of evaluation of crude drugs. The total ash usually consists of inorganic radicals like carbonates, phosphates, silicates and silica of sodium, potassium, magnesium and

calcium. Sulphated ash involves the treatment of sample with dilute sulphuric acid before ignition. In this, all oxides and carbonates are converted to sulphates and the ignition is carried out at a higher temperature. Sometimes inorganic variables like calcium oxalate, silica and carbonate contents of crude drugs after total ash values. Such variables are then removed by treating with acid (as they are soluble in HCl) and then, acid insoluble ash value is determined. For the determination of various ash values viz. total ash, acid insoluble ash, sulphated ash and water soluble ash, all the powdered plant materials were passed through sieve no. 40 and used.

b. Determination of total ash

About 3 gm of the powdered material was actually weighed in a silica crucible, which was previously ignited and weighed. The powdered drug was spread as a fine even layer at the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450^{o}C until free from carbon.

The crucible was cooled and weighed and the procedure was repeated to get the constant weight .

c. Determination of acid insoluble ash

The ash obtained as described in the total ash was boiled with 25 ml of 2M hydrochloric acid for 5 min. The insoluble ash was collected on an ashless filter paper and washed with hot water. The residue was transferred into pre-weighed silica crucible, ignited, cooled and weighed and the procedure was repeated to get the constant weight.

d. Determination of water-soluble ash

The ash obtained as described in the total ash was boiled in 25 ml of chloroform water for five min. The insoluble matter was collected in a Gooch crucible or ash less filter paper and washed with hot water. The residue was transferred into pre weighed silica crucible, ignited for 15 min at a temperature not exceeding 450^{o}C, cooled and weighed and the procedure was repeated to get the constant weight. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference of weight was considered as the water soluble ash.

e. Determination of sulphated ash

A silica crucible was heated to redness for about 10 min, allow to cool in a desiccators and weighed. About 1g of powdered sample was accurately weighed and taken in a crucible. The crucible was ignited gently first until the sample was thoroughly charred. The crucible was cooled and the residue was moistened with 1 ml of sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800^{0}C until all black particles disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool, few drops of sulphuric acid was added and again heated. The ignition was carried out as before, allowed to cool and weighed.

5.4.6 Extractive values (WHO, 1998)

The amount of extractive, a sample yield to a given solvent is often an approximate measure of a certain constituents or a group of related constituents the sample contains. In some case, the amount of sample soluble in a given solvent is an index of its purity. The solvent used for

extraction should be in a position to dissolve appreciable quantities of the substances desired.

a. Water soluble extractive

5g of coarsely powdered material was mixed with 100 ml of chloroform water and kept in a closed flask for 24 hr, shaking frequently during the first 6 hr and then allowed to stand for 18 hr. Thereafter, it was filtered rapidly, taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105^0C and weighed.

b. Ethanol soluble extractive

5g of coarsely powdered material was mixed with 100 ml of 95% ethyl alcohol in a closed flask and kept for 24 hr, shaking frequently during the first 6 hr and then allowed to stand for 18 hr. Thereafter, it was filtered rapidly, taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105^0C and weighed.

5.4.7 Fluorescence analysis of the plant powders with various chemical reagents

The organic molecules absorb light usually over a specific range of wavelength, and many of them re-emits such radiations. This phenomenon is called as luminescence. When the re-emission of the absorbed light lasts only whilst the substance is receiving the exciting rays, the phenomenon is known as fluorescence. A very small quantity of the powdered drug was kept in a watch glass in an accumulated form. Then 2-3 drops of respective

reagent was added and the fluorescence character of the plant powder was studied both in day light and UV light as such and after treatment with reagents like sodium hydroxide, picric acid, acetic acid, hydrochloric acid, nitric acid, iodine, ferric chloride etc. Similarly the fluorescence analysis of the plant extracts were observed under visible and UV light (Vijaya Bharathi *et al*,. 2007, Shantha *et al*,.2007).

5.4.8 Determination of foaming index

Many medicinal plant materials contain saponins that can cause a persistent foam when an aqueous decoction is shaken. In order to measure the foaming ability of an aqueous decoction of plant material and their extracts, the foaming index is established.

Foaming index

Weighed accurately about 1 gm of coarsely powdered drug and transferred to 500 ml conical flask containing 100ml of boiling water. Maintained at moderate boiling at 80^0C– 90^0C for about 30 minutes. Cooled and filtered into a volumetric flask and added sufficient water through the filter to make up the volume to 100 ml (V_1). Cleaned 10 stoppered test tubes (ht. 16cm; dia 1.6cm) were taken and marked from 1 to 10. Measured and transferred the successive portions of 1, 2, 3 ml up to 10 ml and adjusted the volume of the liquid in each tube with water to 10 ml. Stoppered the tubes and shaken them in lengthwise motion for 15 seconds uniformly, allowed to stand for 15 minutes and measured the height of the foam. If the height of the foam in every tube is less than 1 cm the foaming is less than 100 (not significant) and if the height of the foam in every tube is more than 1cm, the foaming index is more than 1000 (signifiocant) . The foam was exactly 1cm height

after the dilution of plant material in the first and second tube having *Achyranthes aspara* and *Achyranthes bidentata* respectively. In this case, 10 ml of the first decoction of the plant material is measured and transferred to a 100 ml (V2) volumetric flask and volume is made to 100ml and followed the same procedure.

	V_1	V_2
Foaming index	1000/a	1000/a X 10

a – volume (ml) of decoction used for preparing the dilution in the tube where exactly 1 cm foam was observed.

5.4.9 Determination of mucilage content of the seeds of *Achyranthes aspera* Linn and *Achyranthes bidentata* Blume.

The technique of mucilage determination is useful for determining powdered drugs containing mucilage. The species of Achyranthes viz. *Achyranthes aspera* linn could be conveniently differentiated from *Achyranthes bidentata* blume by determining mucilage content.

1. Extracted about 25 gm of accurately weighed powder drug with solvent ether in a soxhlet apparatus for about 6-8 hours. Filtered and the ether extract was discarded.

2. Dried the marc and macerated overnight with 250 ml of absolute alcohol. Discarded the supernatant liquid after maceration.

3. Macerated the defatted and decolorized powder for about 18-20 hours with 250 ml of 5% aqueous acetic acid solution. Repeated the maceration process at least twice for the complete extraction of mucilage.

4. Combined the acid extract, filtered and concentrated to about 50 ml by evaporation on a water bath.

5. Precipitated the mucilage completely by adding an excess of absolute alcohol. Filtered the entire mixture through a tared filter paper. Washed the residue repeatedly with small volumes of absolute alcohol.

6. Dried the filter paper and the residue to constant weight at 100^0C, cooled in a desiccator and weighed.

7. Calculated the percentage of mucilage with reference to air dried drug.

5.4.10 Phytochemical studies

The therapeutic potentials of plant and animal origin are being used from the ancient times in the form of crude drugs or galenicals prepared from them without the isolation of the pure compounds. The pharmacological action of crude drug is determined by the nature of its constituents. To obtain these pharmacological effects, the plant materials are used as such in their crude form or they are extracted with suitable solvent to take out the desired components and the resulting principles employed as therapeutic agents. The development of phytochemistry took its turn, when modern isolation methods for herbal ingredients found their way. Percolation process was used for the extraction of crude drugs since 1817.

Qualitative phytochemical analysis

General screening of the raw plant powder, alcoholic and aqueous extracts of the plant material is carried out for qualitative determination of the nature of organic compounds present in them (Rastogi *et al*,.2003, Ramman 2006).

Test for alkaloids

(a) **Dragendorff's test:** Dissolve a few mg of alcoholic or aqueous extract of the drug in 5 ml of distilled water, add 2 M hydrochloric acid until an acid reaction occurs, then add 1ml of Dragendorff's reagent, an orange or orange-red ppt. is produced immediately.

(b) Hager's test: To 1 ml of alcoholic extract of the drug taken in a test tube, add a few drops of Hager's reagent. Formation of yellow ppt. confirms the presence of alkaloids

(c) **Wagner's test:** Acidify 1ml of alcoholic extract of the drug with 1.5% v/v of hydrochloric acid and a few drops of Wagner's reagent. A yellow or brown ppt. is formed.

(d) **Mayer's test:** Add a few drops of Mayer's reagent to 1ml of acidic aqueous extract of the drug. White or pale yellow ppt. is formed.

Test for carbohydrates

(a) Benedict's test: To 0.5 ml of aqueous extract of the drug add 5 ml of Benedict's solution and boil for 5 min. Formation of a red coloured ppt is due to the presence of carbohydrates.

(b) Fehling's tests: To 2 ml of aqueous extract of the drug add 1 ml of a mixture of equal parts of Fehling's solution 'A' and Fehling's solution 'B' and boil the contents of the test tube for few min. A red or brick red ppt is formed.

(c) Molisch's test: In a test tube containing 2 ml of aqueous extract of the drug add 2 drops of a freshly prepared 20 % alcoholic solution of α-naphthol and mix, pour 2 ml concentrated sulphuric acid through the side so as to form a layer below the mixture. Carbohydrates, if present , produce a red-violet ring which disappears on the addition of an excess of alkali solution.

(d) Barfoed's test: To 2 ml of extract solution, add 2 ml of Barfoed's reagent. Mix well. Heat for 1-2 minute in boiling water bath and cool. Formation of a red precipitate indicates the presence of carbohydrate.

(e) Anthrone test: To 2 ml of anthrone test solution, add 0.5 ml of aqueous extract of the drug. A green or blue colour indicates the presence of carbohydrates.

Test of glycosides

(a) Legal's test: Dissolve the extract in pyridine and add sodium nitroprusside solution and then make it alkaline by adding 10% solution of sodium hydroxide. The formation of pink red colour shows the presence of glycosides.

(b) Baljet test: To 1 ml of the test extract add 1 ml of sodium picrate solution, an yellow to orange colour reveals the presence of glycosides.

(c) Borntrager's test: Add a few ml of dilute sulphuric acid to 1 ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer is treated with 1 ml of ammonia. The formation of red colour shows the presence of anthraquinone glycosides.

(d) Keller Kiliani test: Dissolve the extract in acetic acid containing traces of ferric chloride and transfer to a test tube containing sulphuric acid. At the junction, formation of a reddish brown colour, which gradually becomes blue, confirms the presence of glycosides.

Test for saponins

(a) In a test tube containing about 5 ml of an aqueous extract of the drug add drop of sodium bicarbonate solution, shake the mixture vigorously and leave for 3 mins. Honey comb like froth is formed.

(b) About 1 ml of extract is diluted separately with distilled water to 20 ml, and shaken in a graduated cylinder for 15 minutes, a 1 cm layer of foam indicates the presence of saponins.

Test for proteins and amino acids

(a) Biuret's test: To 1 ml of hot aqueous extract of the drug add 5-8 drops of 10% sodium hydroxide solution followed by 1 or 2 drops of 3 % w/v copper sulphate solution. A red or violet colour is obtained.

(b) Millon's test: Dissolve a small quality of aqueous extract of the drug in 1 ml of distilled water and 5-6 drops of Millon's reagent. A white ppt is formed which turns red on heating.

(c) Ninhydrin test: Add 2 drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) to 2 ml of the aqueous filterate. A characteristic purple colour indicates the presence of amino acids.

Test for flavanoids

Shinoda test / Magnesium and hydrochloric acid reduction:

In a test tube containing 0.5 ml alcoholic extract of the drug, add 5-10 drops of dil hydrochloric acid followed by a small piece of magnesium. In the presence of flavanoids, a pink and reddish pink or brown colour is produced.

Test for steroids

(a) Libermann-Burchard's test: Add 2 ml of acetic anhydride solution to 1 ml of petroleum ether extract of the drug in chloroform followed by 1 ml of concentrated sulphuric acid. A greenish colour is developed which turns to blue.

(b) Salkowski reaction: Add 1ml of concentrated sulphuric acid to 2 ml of chloroform extract of the drug carefully, through the side of the test tube. A red colour is produced in the chloroform layer.

(c) Libermann's reaction: Mix 3 ml of extract with 3 ml of acetic anhydride, heat and cool, add few drops of concentrated sulphuric acid. Appearance of blue colour indicate the presence of steroids.

Test for tannins and phenolics

(a) Ferric chloride test: To 1-2 ml of extract of the drug add few drops of 5% $FeCl_3$ solution. A green colour indicates the presence of gallotannins while a brown colour indicates the presence of tannins.

(b) To the extract add potassium dichromate solution, formation of a precipitate shows the presence of tannins and phenolics.

(c) Gelatin test: The extract (50 mg) is dissolved in 5 ml of distilled water and 2 ml of 1% solution of gelatin in 10% sodium chloride is added to it. White precipitate indicates the presence of phenolic compounds.

(d) Lead acetate test: The extract (50mg) is dissolved in distilled water and to this, 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

(e) Alkaline reagent test: An aqueous solution of the extract is treated with 10 % ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavanoids.

Test for resins

Dissolve the extract in acetone and pour the solution into distilled water. Turbidity indicates the presence of resins.

Test for starch

Dissolve 0.015g of iodine and 0.075g of potassium iodide in 5ml of distilled water and add 2-3 ml of an aqueous extract. A blue colour is produced.

Test for gums and mucilages

a. The extract (100 mg) is dissolved in 10 ml of distilled water and to this add 25 ml of absolute alcohol with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilages.

b. Hydrolyse the test solution using dilute HCl. Perform Fehling's or Benedict's test. Red colour is developed.
c. Powdered drug material shows red colour with ruthenium red.
d. Powdered drug swells in water.

Test for fixed oils

a. Spot test: Press a small quantity of extract between two filter papers. Oil stains on paper indicate the presence of fixed oils.
b. Saponification test: To 1 ml of the extract add few drops of 0.5 N alcoholic potassium hydroxide along with drops of phenolphthalein. Heat the mixture on a water bath for 1 -2 hrs. The formation of soap or partial neutralization indicates the presence of fixed oils.

Estimation of total phenolic content

Total soluble phenolic of the extract were determined with Folin-Ciocalteu reagent using pyrocatechol as the standard . This test is based on the oxidation of phenolic groups with phosphomolybdic andphosphotungstic acids. After oxidation a green blue complex is formed and its absorbance is measured at 765 nm.

Chemical and reagents used

1. Folin-Ciocalteu reagent: Folin-Ciocalteu reagent was diluted (1:10) with distilled water and used.
2. Sodium carbonate: 202.5 g of sodium carbonate was dissolved in 1 ml of distilled water and used (0.7 M).

Procedure

An aliquot of 0.1 ml suspension of 1 mg of the extracts in water was totally transferred to a 100 ml Erlenmeyer flask and the final volume was adjusted to 46 ml by the addition of distilled water. Folin-Ciocalteu reagent (1 ml) was added to this mixture, followed by 3 ml of 2 % sodium carbonate 3 min later. Subsequently, the mixture was shaken for 2 hrs at room temperature and the absorbance was measured at 760 nm. The concentration of total phenolic compounds in the extracts was determined as µg pyrocathecol equivalent by using the standard pyrocathecol graph.

Estimation of flavanoid content

Total soluble flavanoid content of the extracts was determined with aluminium nitrate using quercetin as the standard.

Chemical and reagents used

1. Aluminium chloride-10%

   10 gm of $AlCl_3$ is dissolved in 100ml of distilled water, filtered and used.

2. Potassium acetate – 1m

   98.1 g of Potassium acetate was dissolved in 1 litreof distilled water and used.

3. Methanol – Distilled

Procedure

One mg of the extract was added to 1 ml of 80 % ethanol. An aliquot of 0.5 ml was added to test tubes containing 0.1 ml of 10 % aluminium nitrate, 0.1 ml of 1 M potassium acetate and 4.3 ml of 80 % ethanol. The

absorbance of the supernatant was measured at 415 nm after incubation at room temperature for 40 min. The total flavanoid content in the extracts was determined as µg quercetin equivalent by using the standard quercetin graph.

5.4.11 Chromatography analysis

Chromatography is a physical method of separation in which the components to be separated are distributed between the two phases; one of these is a stationary phase bed and the another is a mobile phase which percolates through this bed. It occurs as a result of repeated sorption/desorption during the movement of the sample components along the stationary bed, and the separation is due to differences in distribution constants of the individual sample components. The stationary phase includes solid, liquid coated on a solid support. The mobile phase includes liquid and gas.

Based on the stationary and mobile phase, the chromatographic technique can be of the following types:

1. **Gas chromatography**: The mobile phase is a inert gas and the stationary phase is either an adsorbent or a liquid distributed over the surface of a porous, inert support.
2. **Liquid chromatography**: The mobile phase is a liquid of low viscosity that is caused to flow through a bed of sorbent. The sorbent may be immiscible liquid coated on to a porous support, or an inert sorbent of controlled pore size.
3. **Thin Layer Chromatography(TLC)** : A liquid mobile phase moves through a layer of sorbent by the action of capillary forces.

TLC is a open bed technique as pressure is not required for the movement of the mobile phase.

GC and LC are closed bed techniques as pressure gradient is used in the movement of the mobile phase through the stationary phase.

The information obtained by the chromatographic experiment is called the chromatogram, a record of the concentration or the mass profile of the sample components as a function of the movement of the mobile phase. Information that can be extracted from a chromatogram include

a. An indication of the sample complexity or the number of components present based on the number of peaks.
b. Qualitative identification of the sample based on the accurate measurement of the peak positions.
c. Quantitative assessment of the relative concentration or the amount of substance present based on the peak size.

The common chromatographic techniques used are column chromatography, paper chromatography, thin layer chromatography, gas chromatography and high performance liquid chromatography.

a. Column chromatography

The principle underlying the separation of the compounds is their adsorption at the solid-liquid interface. For successful separation the compounds of the mixture should show different degrees of affinity for the solid support (or adsorbent) and the interaction between adsorbent and the component must be reversible.

As the adsorbent is washed with the fresh solvent, the various components move down the column and arrange themselves in the order of affinity to the adsorbent. Those with the least affinity move down the column at a faster rate than those with grater affinity. 5g of the extract of *Achyranthes aspera* was subjected for separating using petroleum ether, chloroform, ethylacetate, acetone and 50% ethanol. The same procedure was followed for *Achyranthes bidentata* also.

b. Thin layer chromatography analysis

Thin layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be effected by observation of spots of identical R_f value and about equal magnitude obtained, respectively with a unknown and reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serve for semi quantitative estimation.

TLC is used for the separation of simple mixtures where speed, low cost, simplicity are required (Harborne. 1984, Wagner *et al*,.1996).

Preparation of plate

30g of silica gel GF was weighed out and made in to a homogenous suspension with 60 ml of distilled water to form slurry. The suspension was poured into a TLC applicator, which was adjusted to 0.25 mm thickness on a flat glass plate (20 X 10 cm and 20 X 5 cm) and coated on plates. The coated plates were allowed to dry in air, followed by heating at 100-105^0 C for 1 hr, cooled and protected from moisture. The plates were stored in a dry atmosphere. Whenever required the plates were dried in a hot air oven at 100^0 C for 30 min for activation.

Application

The extracts were dissolved in methanol (0.1-1%). They were sucked within capillary tubes and spotted on the prepared TLC plate 2 cm above its bottom end. The spots were equally sized as far as possible and had a diameter ranging from 2-5 mm.

Selection of mobile phase

The solvent system were selected based on the chemical constituents identified in the qualitative phytochemical analysis. The solvent or mobile phase used depends upon various factors mentioned below.

a. Nature of the substance to be separated
b. Nature of the stationary phase
c. Mode of chromatography (i.e; normal phase and reversed phase)
d. Separation to be achieved (i.e; analytical or preparative)

Pure solvents or mixture of solvents were used as mobile phase for separation of various plant constituents from respective plants. The better solvent system, which gave good separation and more number of spots was selected for each extract. The R_f values were noted for each of the selected

plant extracts, using different detecting agents viz., daylight, UV light, iodine vapour, with Dragendorff's reagent, vanillin sulphuric acid and ninhydrin reagent.

c. High performance thin layer chromatography (HPTLC)

HPTLC is a advanced versatile chromatographic technique for quantitative analysis with high sample throughput and is complementary to HPLC/GLC. It provides a chromatographic drug finger print. It is therefore suitable for monitoring the identity and purity of drugs. In HPTLC, the various steps involved are:

i. Application of sample
ii. Chromatographic development
iii. Detection of spots
iv. Quantification
v. Documentation

Application of sample

An automatic applicator (Linomat) is used for sample application. A known quantity of sample is dissolved in known volume of solvent and the sample applied on prepared TLC plate either in the form of a spot or a band. However a band form is preferred because:

- Larger quantities of sample can be handled for application.
- Better separation because of rectangular area in which compounds are present in the plate.
- Response of densitometry is better due to variable concentration of substances in a spot.

Chromatographic development (separation)

Development of the chromatogram is affected after the solvent of the applied sample is completely evaporated. Rectangular glass chambers or twin trough chambers are commonly used for TLC development.

Detection of spots

For densitometric scanning, detection under UV light is generally preferred. But post chromatographic derivatisation reactions are essentially required for detection when individual compounds does not respond to UV light or do not have intense fluorescence.

Quantification and Documentation

Densitometry is *in situ* instrumental measurement of visible, UV absorbance and fluorescence quenching. The scanner converts the spot/band on the layer in to a chromatogram consisting of peaks similar in appearance to HPLC.

The portion of the scanned peaks on the recorder chart is related to R_f values of the spots on the layer and the peak height or area is related to the concentration of the substance on the spot.

Application of sample

Commercially available pre coated plates of silica gel GF_{254} were used for the study. The different fractions were applied on plates with band width of 6mm. Application rate was maintained at 5 µl/ml, using Linomat IV applicator (automatic TLC applicator, Camag, Switzerland). A sample volume of 5 µl was applied.

Chromatogram development

The plates were developed in twin trough chamber using the solvent system as used in the TLC of different fractions. After developing, the plates were air dried and observed under UV light chamber (camag UV chamber-3, model no: 002.9120).

Densitometric Scanning

The developed plates were scanned using densitometer at 254 and 366 nm (Camag TLC Scanner – 3, model no: 0227.6480, combined with integration software, CATS 4.06, switzerland).

5.4.12 Antihyperlipidemic screening

Animals

Healthy adult male albino rats of Wistar strain weighing between 180-220g were obtained from the animal house, J.S.S. College of Pharmacy, Ootacamund, India for the screening of antihyperlipidemic activity of the plant extracts. The animal were housed in polypropylene cages in adequately, well ventilated room and maintained under standard environment conditions (22-28^{0}C, 60-70% relative humidity, 12h dark/light cycle).

The animals were fed with standard rat feed pellets (Amurth Rat Feed, Nav Maharashtra Chakan Oil Mills Ltd., Pune) and water ad libitum (Aquaguard filter water). The study was approved by the institutional animal ethics committee; approval no: JSSCP/IAEC/Ph.D/Ph.Cology/ 02/2008-09.

5.4.13 Acute toxicity studies:

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=3) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for a overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. It mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight.

Preparation of test solution

The aqueous and ethanolic extracts of Achyranthes *aspera* Linn and *Achyranthes bidentata* Bl leaf and seeds were suspended in 0.3% CMC separately, and administered orally at 100 mg/kg and 200 mg/kg body weight.

Standard drug: Atorvastatin (2mg/kg) was suspended in 0.3% CMC and administered orally to rats.

5.4.14 Triton induced hyperlipidemia (Frantz *et al.*, 1955)

Grouping of animals

The experimental design of the investigation was carried out in 19 groups with six animal in each group in the following regimen;

Group I — Received 0.3% w/v carboxy methyl cellulose (CMC) orally for one week.

Group II — Received triton (250 mg/kg b.w) i.p route

Group III — Received atorvastatin (2 mg/kg) for 7 days in 0.3% CMC, Orally

Group IV — Received aqueous leaf extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally

Group V — Received aqueous leaf extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally

Group VI — Received ethanolic leaf extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally

Group VII — Received ethanolic leaf extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally

Group VIII Received aqueous seed extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally

Group IX Received aqueous seed extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally

Group X Received ethanolic seed extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally

Group XI Received ethanolic seed extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally

Group XII Received aqueous leaf extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally

Group XIII Received aqueous leaf extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally

Group XIV Received ethanolic leaf extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally

Group XV Received ethanolic leaf extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally

Group XVI Received aqueous seed extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally

Group XVII Received aqueous seed extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally

Group XVII Received ethanolic seed extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally

Group XIX Received ethanolic seed extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally

On 8th day for the overnight fasted rats, triton was administered at 250 mg/ kg, by intraperitoneal route. The blood samples were collected after triton administration at 0h and 24h. These were centrifuged for 15 minutes at 3000 rpm and plasma was separated. Plasma samples were used for the estimation of cholesterol, triglyceride and HDL cholesterol, using Merck kits in autoanalyser (Microlab 200, Merck).

5.4.15 High fat diet induced hyperlipidemia (Filliois *et al.*,1956)

Grouping of animals

The experimental design of the investigation was carried out in 18 groups with six animals in each group and carried out in the following regimen;

Group I Received normal diet

Group II Received cholesterol rich diet for 28 days

Group III Received aqueous leaf extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally

Group IV	Received aqueous leaf extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally
Group V	Received ethanolic leaf extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally
Group VI	Received ethanolic leaf extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally
Group VII	Received aqueous seed extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally
Group VIII	Received aqueous seed extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally
Group IX	Received ethanolic seed extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally
Group X	Received ethanolic seed extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally
Group XI	Received aqueous leaf extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally
Group XII	Received aqueous leaf extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally

Group XIII Received ethanolic leaf extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally

Group XIV Received ethanolic leaf extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally

Group XV Received aqueous seed extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally

Group XVI Received aqueous seed extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally

Group XVII Received ethanolic seed extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally

Group XVIII Received ethanolic seed extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally

The animals in group II to XVIII were fed with atherogenic diet (high fat diet) consisting of rat chow (67g), cholesterol (1.5g), milk powder (8g), salt (2g), coconut oil (5ml) and multiple vitamin (0.5g). The control animals (group I) was fed with normal diet for 28 days.

Blood sample were collected and plasma was separated. This was used for estimation of cholesterol, triglyceride and HDL cholesterol, LDL and VLDL cholesterol. The rats were sacrificed after the collection of blood

samples and the aorta and liver were excised immediately for histopathological examination.

5.4.16 Estimation of biochemical parameters (Friedewalde *et al*,.1992)

1) Total cholesterol (TC)

Cholesterol in plasma was estimated by CHOD-PAP method using an Ecoline Diagnostic kit.

Cholesterol and its esters were released from lipoproteins by detergents. Cholesterol esterase hydrolysis the esters. In the subsequent enzymatic oxidation by cholesterol oxidase, H_2O_2 was formed. This was converted into a coloured quinonimine in a reaction with 4-aminoantipyrine and phenol catalyzed by peroxidase. The absorbance of the sample and of the standard was measured against the reagent blank value at 546 nm. Cholesterol level in plasma was expressed as mg/dl.

2) HDL cholesterol

The HDL cholesterol was separated from plasma after precipitation of LDL and VLDL cholesterol by phosphotungstic acid precipitating reagent. The supernatant after centrifugation was estimated using Ecoline diagnostic kit by CHOD-PAP method. The absorbance of the sample and of the standard was measured against the reagent blank value at 546 nm. HDL cholesterol level in plasma was expressed as mg/dl.

3) LDL cholesterol

LDL cholesterol was calculated by using the formula

$$\text{LDL cholesterol} = \text{Total Cholesterol} - \left[\text{HDL Cholesterol} - \frac{\text{Triglyceride}}{5}\right]$$

LDL cholesterol level in plasma was expressed as mg/dl.

4) VLDL cholesterol

VLDL cholesterol was calculated by the formula

$$\text{VLDL cholesterol} = \frac{\text{Triglyceride}}{5}$$

VLDL cholesterol level in serum / plasma was expressed as mg/dl.

5) Triglyceride (TG)

Triglyceride level in plasma was estimated using an Ecoline diagnostic Kit.

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol + Fatty acid}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow{\text{GPO}} \text{Dihydroxy-acetone-phosphate + } H_2O_2$$

$$2H_2O_2 + \text{Aminoantipyrine + 4 cholesterol} \xrightarrow{\text{POD}} \text{Quinonimine + } 4H_2O_2$$

The absorbance of the sample and of the standard was measured against the reagent blank value at 546 nm. The triglyceride level in plasma was expressed as mg/ dl.

5.4.17 Antioxidant activity

Free radicals are chemical species possessing an unpaired electron that can be considered as fragments of molecules and which generally are extremely reactive and short lived. They are continuously produced during the body's normal functions such as respiratory and some cell-mediated immune functions. They are also found or generated through environmental pollution, cigarette smoke, automobile exhaust fumes, radiation, air pollutants, pesticides etc. Naturally, there is a dynamic balance between the amount of free radicals generated in the body and antioxidants to quench and / or scavenge them and protect the body against their deleterious effects. Any additional burden of free radicals either from the environment or produced within the body, can impair the balance between free-radicals (Pro-oxidants) and anti-free-radicals (antioxidants) leading to oxidative stress, which may result in tissue injury and subsequent diseases (Tiwari, 2001).

There is an increasing evidence to support the involvement of free radicals in several human diseases. In recent years, it has become increasingly apparent that in man, free radicals play a role in a variety of normal regulatory systems.

A majority of disease conditions like atherosclerosis, hypertension, ischaemic disease, alzheimer's disease, Parkinson's diseases, cancer and inflammatory (Alho *et al,*.1999, Tanizawa H *et al,*.1992, Rao *et al,*.2006)

are being caused primarily due to the imbalance between pro-oxidant and antioxidant homeostasis.

Anti-oxidant principles from natural resources possess multi face tenderness in their multitude and magnitude of activities and provide enormous scope in correcting the imbalance (Tiwari, 2001). Active oxygen species and other free radicals have long been known to be mutagenic.

These agents have more recently emerged as mediators of the other phenotypic and genotypic changes that lead from mutation to neoplasia. Therefore, free radicals may contribute widely to cancer development in humans. More interestingly, free oxygen radicals are increasingly discussed as important factors involved in the phenomenon of biological aging (Cheeseman KH *et al*,.1993). From the above discussions, it is proven that the free radical production in animal cell is inevitable and because they can be damaging and causative for a variety of diseases or disorders, they should be eliminated from the body.

Anti-oxidants are supposed to reduce the risk of cancer and other diseases by helping the body to getrid of oxygen free radicals, which are thought to contribute to cancer development by damaging the DNA. Many plants and their extracts are rich sources of agents such as anti-oxidants, which can prevent the occurrence of cancer by reducing free-radical induced cell damage (Cheeseman KH *et al*,.1993). In the present study, the antioxidant and antihyperlipidemic activities of the extract were carried out, based on the literature survey and the potential of the leaf and seed of these two plants.

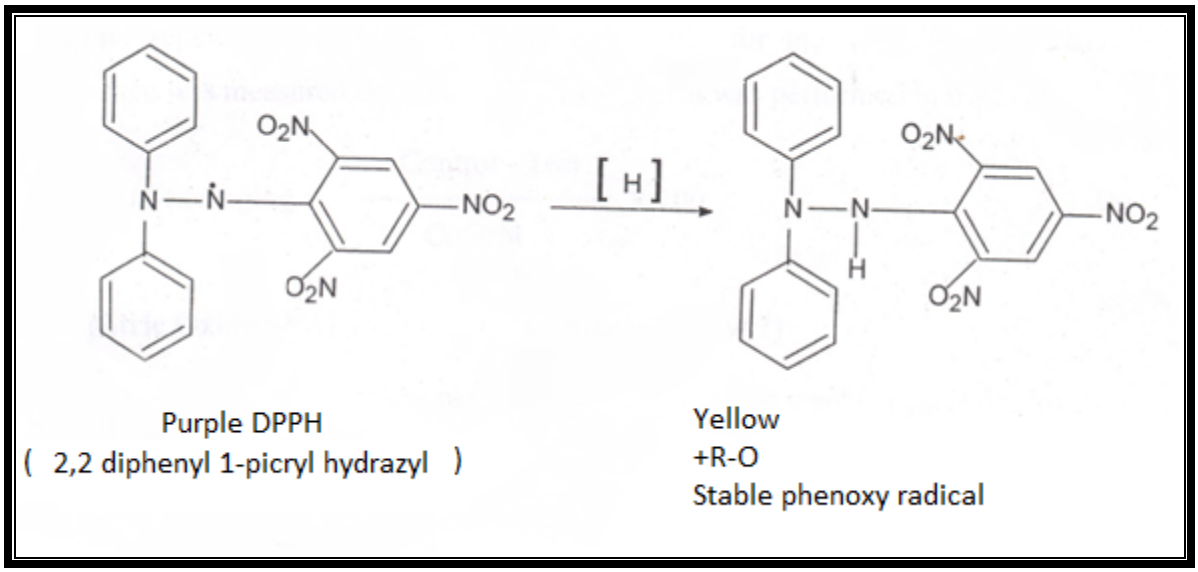


Fig 21. Reduction of DPPH free radical

***In vitro* antioxidant studies**

a. Diphenyl-1-picryl hydrazyl (DPPH) method (Mensor *et al*,.2001)

The antioxidant activity of various extracts of the leaf and seed of the two plants was assessed on the basis of the radical scavenging effect of the stable 1, 1- diphenyl-2-picryl hydrazyl (DPPH) free radical. The DPPH free radical is reduced to a corresponding hydrazine when it reacts with a hydrogen donor.

The DPPH radical is purple in colour and upon reaction with hydrogen donor, it changes to yellow in colour. It is a discoloration assay, which is evaluated by the addition of antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured.

Preparation of DPPH solution

22 mg of DPPH (2,2-diphenyl-1-picryl hydrazyl) was dissolved in 100 ml of methanol. From this, 18 ml was taken and diluted to 100 ml using methanol to obtain 100 µM DPPH solutions.

Preparation of test solution

105 mg of leaf and seed extracts *Acharanthes asperia* Linn and *Achyranthes bidentata* Bl extracts were dissolved in dimethyl sulfoxide (DMSO) separately. Each of these solutions was serially diluted separately to obtain concentration of 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml.

Preparation of standard solution

1 mg/ ml solution of ascorbic acid in DMSO was prepared. This was serially diluted to obtain the concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml.

Procedure

The assay was carried out in a 96 well microtiter plate. To 200µl of DPPH solution, 10 µl of each of the test sample or the standard solution was added separately in microtiter plates. The plates were incubated at 37^0 C for 30 minutes and absorbance of each solution was measured at 490 nm using microtiter plate reader (ELISA) against the corresponding test and standard blanks.

The remaining DPPH was calculated. IC_{50} values was calculated for the test solution and also compared with the IC_{50} value of standard (ascorbic

acid). IC_{50} value is the concentration of the sample required to scavange 50% DPPH free radicals.

b. Nitric oxide radical inhibition activity

Nitric oxide is a very unstable species under the aerobic condition. It reacts with O_2 to produce stable products, nitrates and nitrite through intermediates NO_2, N_2O_2 and N_3O_4. It is estimated by using the Griess reagent. In the presence of test compound, which is a scavenger; the amount of nitrous acid will decrease. The extent of decrease will reflect the extent of scavenging, which is measured at 546 nm (Rastogi *et al*,.2003).

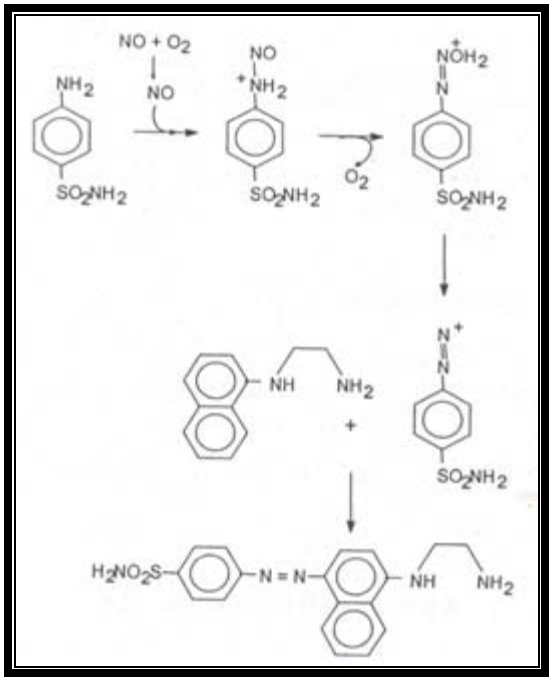


Fig.22 Principle involved in NO˙ scavenging activity

Preparation of reagents

Sodium nitroprusside solution: 0.2998 g of sodium nitroprusside was dissolved in 100 ml of distilled water.

Naphthyl ethylene diamine dihydrochloride (NEDD) solution: 0.1 g of NEDD was dissolved in 60 ml of 50% glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask with distilled water. Sulphanalic acid solution: 0.33 g of sulphanalic acid was dissolved in 100 ml of 20% glacial acetic acid by heating.

Preparation of test solutions

84 mg of each of the leaf and seed extracts *Achyranthes aspera* Linn and *Achyranthes bidentata* Bl extracts were dissolved in dimethyl sulfoxide (DMSO) separately. Each of these solutions was serially diluted separately to obtain concentration of 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml.

Preparation of standard solution

1 mg/ ml solution of ascorbic acid was prepared, separately in DMSO. This was serially diluted with DMSO to obtain the concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml.

Method

The reaction mixture (6ml) containing sodium nitropreside (4 ml), phosphate buffer saline (PBS, 1 ml) and extracts in DMSO was incubated at 25^0C for 15 minutes. After incubation, 0.5 ml of the reaction mixture containing nitrate was removed, 1 ml of sulphanalic acid reagent (0.33 g in 20% glacial acetic acid) was added, mixed well and allowed to stand for 5 minutes for completion of diazotization and then 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 minutes in diffused light. A pink coloured chromophore was formed. The

absorbance of these solutions, were measured at 540 nm against corresponding blank solution. LC_{50} value was calculated.

5.4.18 Statistical analysis

Statistical analysis was carried out using GraphPad software (GraphPad Prism) by one –way Analysis of Variance (ANOVA) followed by Turkey's multiple comparison test. Results are expressed as mean ± SD from six rats in each group. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

6. RESULTS AND DISCUSSION

Medicinal plants play a key role in the human health care as they have been used as medicines over the centuries. All through human history, there has been a noticeable concern for health care and the cure of diseases, a logical approach to the study of drugs and their activities is the recognition of the basic principles behind the biochemical events leading to drug actions.

The World Health Organization estimates that 65-80% of the world's population use traditional medicine as their primary form of the health care. The use of herbal medicine, the dominant form of the medical treatment in developing countries, has been increasing in developed countries also. Many people believed that because herbal remedies are 'natural', they are entirely 'safe' (Shrikumar *et al,*.2007). There is a great demand for herbal medicines because of their wide biological activities, higher safety margin and lesser costs than the synthetic drugs (Thakkar *et al,*.2008).

In the dawn of human culture evolution, the art of curing was essentially magical and was based on logic than on scientific evidence. There was no uniform or standard procedure for maintaining the inventing of these plants and the knowledge about their medicinal properties without any written documentation or regulation. Therefore, it is essential that such uses of natural products be documented and studied for systematic regulation and wide spread application.

The World Health Organization has recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. Materials of plant origin are prone to contamination, deterioration and variation in composition. Hence it is necessary to develop methods for rapid, precise and accurate identification and estimation of

active constituents in order to bring out consistency of important constituents in the formulations (Thakkar *et al,*.2008).

In this connection, the present study is an attempt two standardize to traditional medicinal plants with respect to their pharmacological, phytochemical and biological activity.

6.1 Pharmacognostical studies

Pharmacognostical studies play an important role in the standardization of plant material. In the present study, two plants were selected based on their ethnomedical uses and was authenticated. Their morphological and microscopical characters were determined. A detailed study of microscopic characters of the two plants along with the photographs serves in identifying the special characters of the plants.

6.1.1 *Achyranthes aspera* Linn Amaranthaceae

The plant is a weed of dry waste places. It is a tall herb growing up to 1 m in height. The leaves are opposite petiolate, spathulate, and hairy inflorescence is long terminal or axillary spike. Flowers bisexual, bracteate and bracteolate, hard and hyaline. Perianth numbers five, free, membranous, 3-nerved. Stamens are five alternating with five staminodes. Ovary superior, one celled with one ovule, on pendulous placentation. Fruit: oblong, utricle; Seeds: pericarp and seed coat fused into a membrane. Embryo curved. Endosperm is cellular and abundant.

Fig 23. Morphological features showing the leafs and inflorescence of *Achyranthes aspera* Linn

Microscope studies

Leaf (Fig 24.1,2)

The leaf has quite prominently projecting midrib and well differentiated lamina. The midrib consists of a tall, conical adaxial part and wide abaxial part (Fig 24.1). It is 700µm longitudinally; and the adaxial cone is 250 µm thick; the abaxial expanded portion is 700 µm wide.

The midrib has thin epidermal layer of small cells with thick walls. One or two sub-epidermal layer of cells and a wide portion in the adaxial hump have small thick walled collenchyma cells. Remaining ground tissue has wide, thin walled compact parenchyma cells (Fig. 24.1).

The vascular system includes an adaxial accessory strand and three abaxial strands forming an arc (Fig.25.1,2). The adaxial strand is collateral. It has broadly conical central strand and two small, less prominent lateral strands. The central larger strand has wide, horizontally, flat phloem and large cluster of wide, thick walled, circular xylem elements. The small lateral strands have a few xylem elements and small nest of phloem elements (Fig. 25.1).

The three abaxial strands are top-shaped and collateral. They have wide, angular thick walled xylem elements, either in rows or in clusters; phloem occurs in flat band on the outer part of the strand. A thin layer of fibres (sclerenchyma) occur attached with phloem band (Fig. 25.2). The xylem elements are up to 20 µm in diameter.

Lamina (Fig. 24.2). The lamina is 110 µm thick. The lateral veins do not project beyond the surface of the leaf-blade. The adaxial epidermis consists of large, circular, thin walled cells. The adaxial epidermis is 20 µm thick. The abaxial epidermis is narrow with small elliptical thin walled cells. The mesophyll tissue consists of two layers of narrow, cylindrical, loosely arranged cells. The palisade zone is 30 µm in height. The spongy mesophyll onsists of four or five layers of lobed aerenchyma cells. The lateral vein has small collateral vein with a layer of parenchymatous bundle which extends adaxially up to the epidermis (Fig. 24.2).

Crystals (Fig 26.1, 2). Calcium oxalate druses are abundant in the leaf. In the midrib they are found in the ground parenchyma cells and are diffuse in

distribution. In the mesophyll cells of the lamina, they occur in dilated, circular idioblasts (Fig. 26.2). The druses are 20 µm in diameter.

Venation of the lamina (Fig.27.1, 2). The lateral veins and veinlets are thin and less prominent. They form wide reticulations with large, vein-islets bordered by thin vein boundaries. The islets are polygonal and random orientation. The vein-terminations are present in most of the islets. They are simple or forked. They are long, slender, straight or curved (Fig. 27.2).

Fruit. The fruit is achene, where the seed and pericarp are fused to form a single unit (Fig. 28.1,2).

Pericarp is fairly thick comprising of epicarp, mesocarp and endocarp. The epicarp is a membranous epidermis with narrow layer of cells and thick cuticle. The mesocarp is fairly wide and has thin walled delicate cells. The endocarp is unistratose and the cells are wide, tabular and fairly thick walled. The entire pericarp is about 100 µm thick (Fig. 28.1,2).

Seed coat (fig. 28.3). The seed coat has a wide layer of unistratose cells. The cells are wide tabular and thickened along the outer walls. The layer is nearly 30 µm thick.

Endosperm (Fig 28.2). The endosperm is copious and fills the entire seed. It is cellular type. The endosperm cells are polyhedral thin walled and compact (Fig. 29.1). The cells have granular content.

Embryo (Fig. 29.1, 2). The embryo is deeply curved and horse-shoe shaped. It has two flat thick cotyledons and cylindrical radical. The cells of the embryo are darkly stained and meristematic.

Perianth members (Fig.30.1, 2). The perianth members of the fruit are thin and membranous. They are boat shaped in sectional view and three nerved. The median nerve has prominent vascular bundle while lateral nerves has smaller bundles (Fig. 30.1). The perianth has 2-5 layers of adaxial thick walled fibrous and one or two layered abaxial fibres. In between the fibre-layers are parenchymatous ground tissue comprising of compact thick walled cells. The vascular strand has a small group of xylem and a few phloem elements. The vascular strand is surrounded all around by a thick sheath of 2-4 layers of thick walled, lignified fibres (Fig. 30.2).

Powder microscopic observation

The powdered preparation shows the following components.

1. **Epidermal trichomes**. Non-glandular covering types of epidermal tichomes are abundant in the powder. The trichome has a membranous spherical basal cell, a short, vertically oblong sub-basal cell and long, pointed terminal cells. The trichomes are unbranched thick walled with narrow lumen (Fig.31.1, 2). The surface of the trichome is surrounded by clusters of calcium oxalate crystalline bodies (Fig. 31.3). The trichomes are 200 – 300 µm long and 10 µm thick.
2. **Cylindrical or spindle shaped cells** are abundant in the powder. They are the isolated endosperm cells. They have dense reticulate pits (Fig. 32.1, 2). The cells are 100-120 µm long and 40 µm wide.

3. **Pollen grains (Fig 33.1).** Circular pollen grains are often seen in the powder. They have reticular exine and echinate outer wall. The pollen grains are 10 – 12 µm in diameter.
4. **Perianth fragments (Fig.33.1).** Broken pieces of perianth are often seen in the powder. They have longitudinally oriented, long thick walled cells. Stomata are also seen on the perianth. The stomata are anomocytic type (without subsidiary cells).
5. **Masses of endosperm cells** as well as **isolated cells** (Fig 34.1,2) are seen in the powder. When the cells are viewed under polarized light microscope, they exhibit granular starch grains which are bright and distinct (Fig. 34.1).
6. **Isolated fibres** also common in the powder. They are spindle shaped needles, with thick liquefied walls and reduced lumen. The fibers are bundle sheath fibers of the leaf vascular strands. They are 250-320 µm long and 15 µm thick.

Microscopy profiles of *Achyranthes aspera* Linn leaf

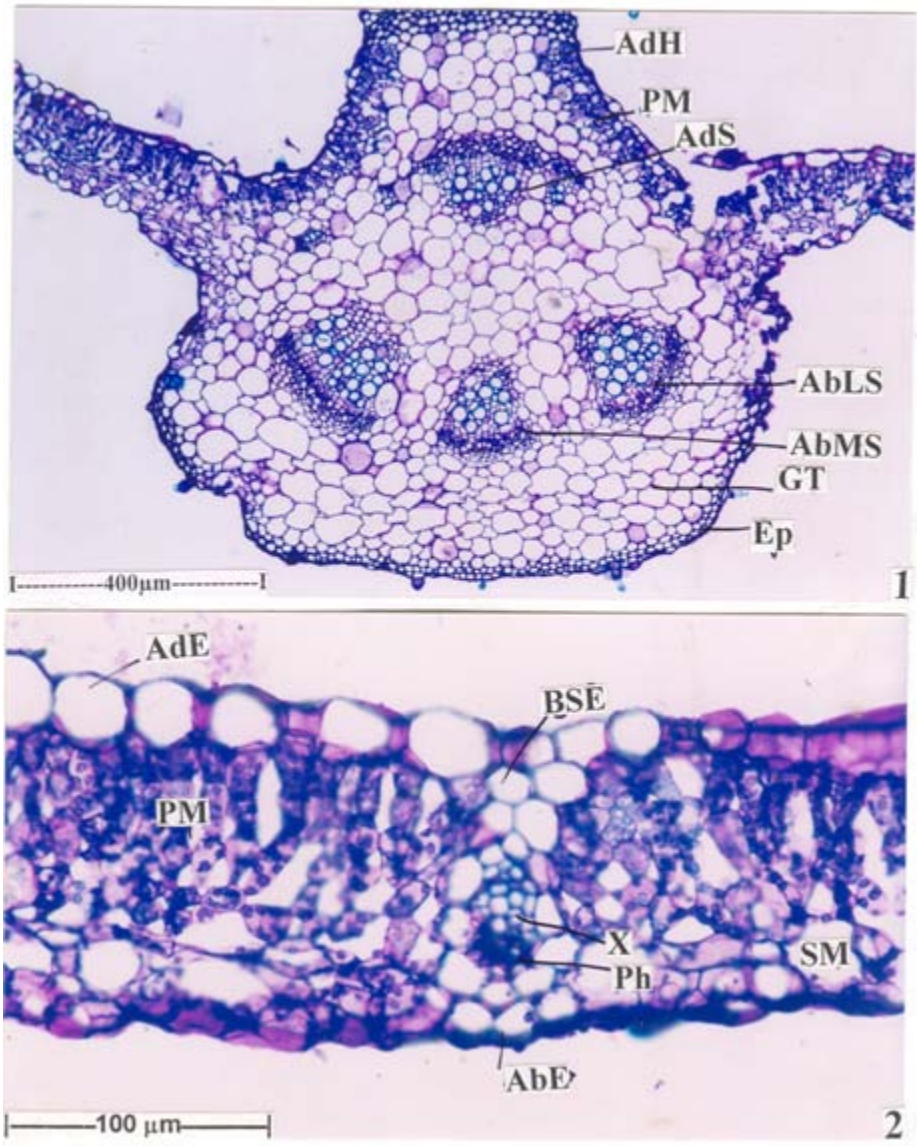


Fig 24. Anatomy of the leaf

1. **T.S. of leaf through midrib with lamina**
2. **T.S of lamina with lateral vein**

(AbE – Abaxial epidermis; AbMS – Abaxial median strand; AbLS – Abaxial lateral strand; AdE – Adaxial epidermis; AdH – Adaxial hump; AdS – Adaxial strand; BSE – Bundle sheath extension; Ep – epidermis; GT – Ground tissue; Ph – Phloem; PM – padisade mesophyll; SM – spongy mesophyll; X-Xylem)

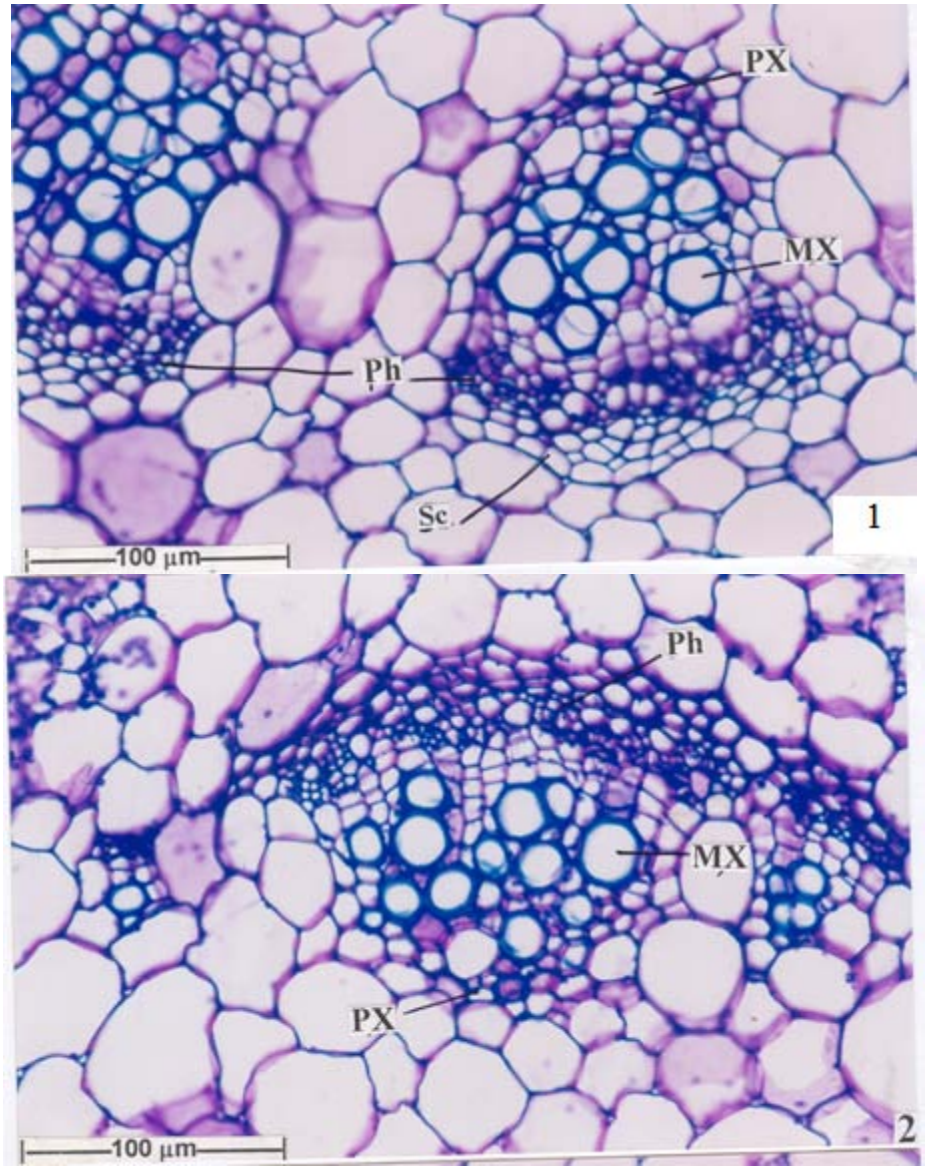


Fig 25. Structure of the midrib vascular bundles
1.Adaxial vascular bundles
2.Abaxial vascular bundles
(MX – Metaxylem; Ph – Phloem; PX – Protoxylem; Sc – Sclerenchyma)

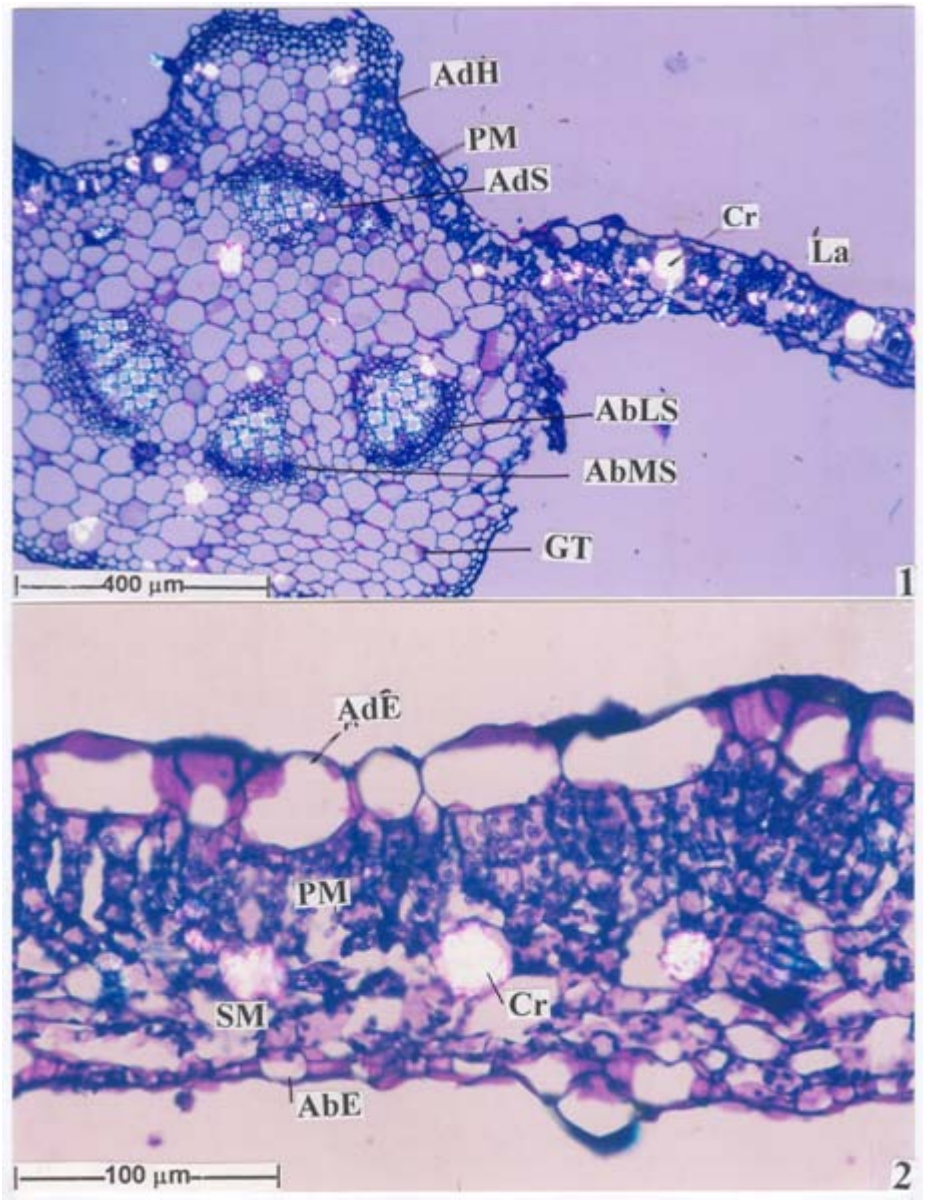


Fig 26. Crystal distribution in the leaf
(as seen under polarized light microscope)

1. **T.S. of leaf showing crystals in the ground tissue and mesophyll tissue**
2. **T.S. of lamina showing crystals in the mesophyll tissue**
   ((AbE – Abaxial epidermis; AbMS – Abaxial median strand; AbLS – Abaxial lateral strand; AdE – Adaxial epidermis; AdH – Adaxial hump; AdS – Adaxial strand; Cr – Crystal; GT – Ground tissue; La – Lamina; PM – padisade mesophyll; SM – spongy mesophyll)

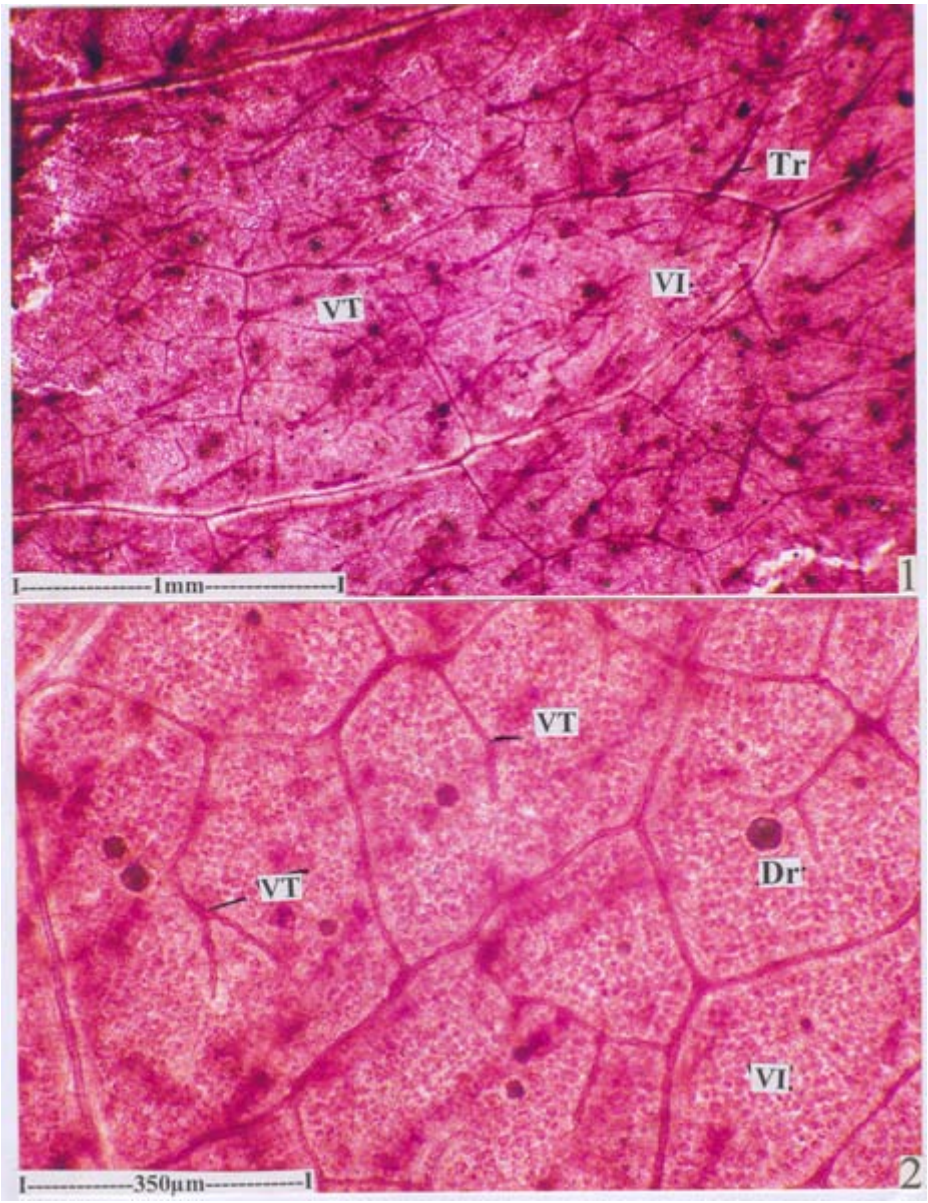


Fig 27. Venation pettern

1. **Cleared leaf showing vein-islets and vein-termination**
2. **Cleared leaf showing vein-islets and vein-terminations (Enlarged)**
   (Dr – Druses; Tr – Trichome; VI – Vein-islets; VT – Vein-termination)

Anatomy of the fruit of *Achyranthes aspera* Linn

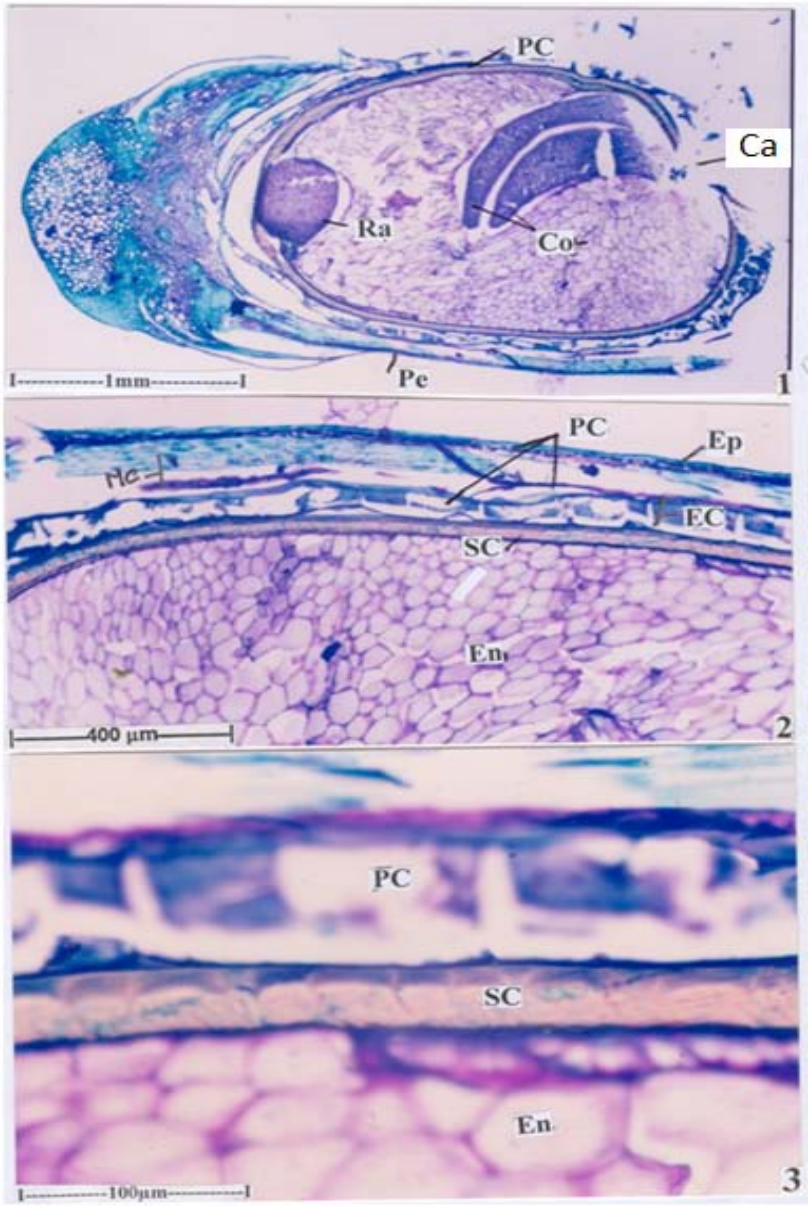


Fig 28. Anatomy of the fruit

1. **L.S of fruit – entire view**
2. **Pericarp and endosperm enlarged**
3. **Endocarp, seed coat and endosperm magnified**
   (Ca – Caryopsis; Cot – Cotyledon, EC – Endocarp; EP – Epicarp; En – Endosperm; MC- Mesocarp; PC – Pericarp; Pe – Perianth; Ra- Radicle; SC – Seed Coat)

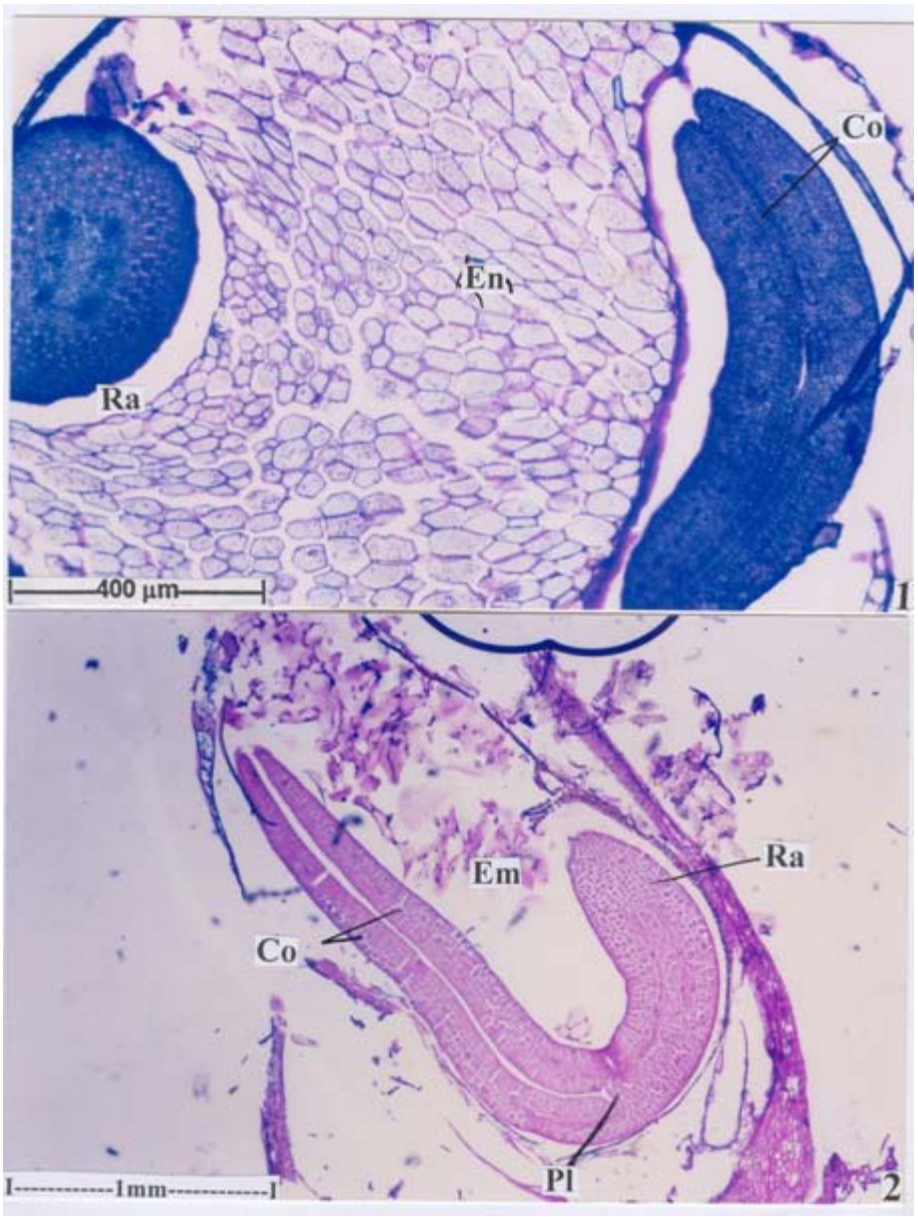


Fig 29. Structure of the cotyledons and radicle

1. **L.S. of fruit showing cotyledons and radicle**
2. **L.S. of cotyledons – magnified**
   (Co: Cotyledon; Em – Embryo; En – Endosperm; PI – Plumule; Ra – Radicle)

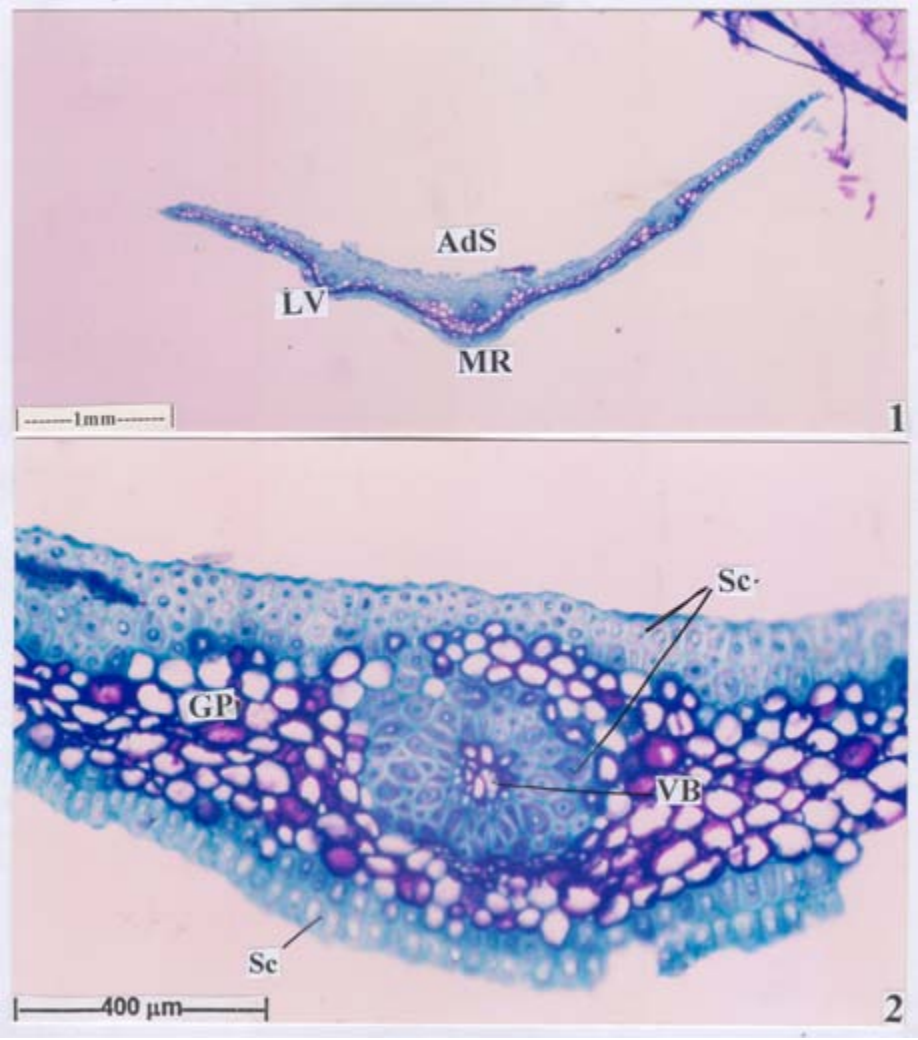


Fig 30. Anatomy of the perianth

1. **T.S. of perianth – entire view**
2. **T.S. of perianth midrib portion – enlarged**
   (AdS – Adaxial side; GP – Ground parenchyma; LV – Lateral vein; MR – Midrib; Sc – Scleroids; VB – Vascular bundle)

Powder microscopy of leaf of *Achyranthes aspera* Linn

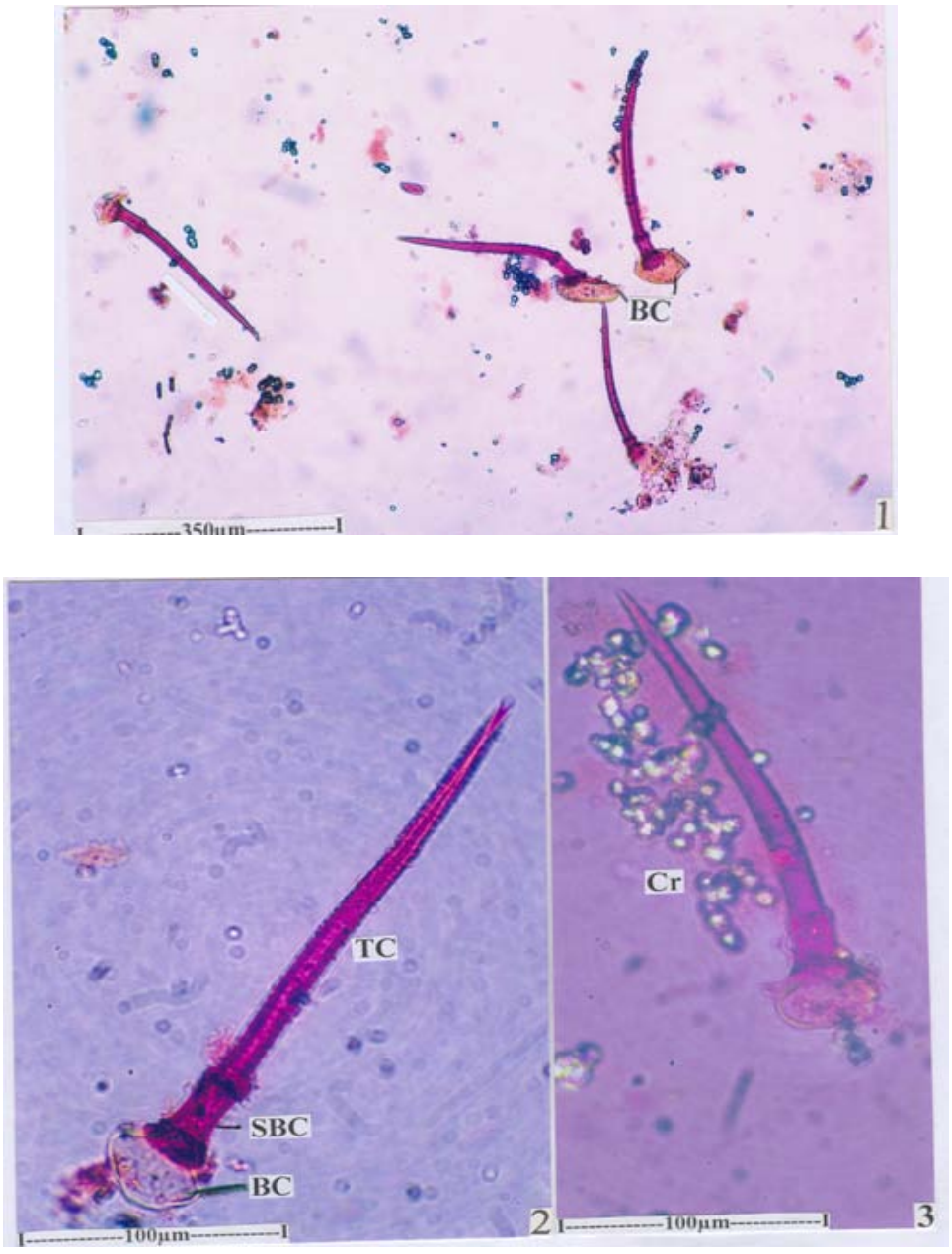


Fig 31. Powder microscopy of the leaf

1. **Leaf powder showing covering non-glandular trichomes**
2. **A trichome under bright field microscope**
3. **A trichome under polarized light microscope showing crystal in the leaf powder.**

(BC – Basal cell; Cr – Crystal; SBC – Sub basal cell; TC – Terminnal cell)

Powder microscopy of fruit of *Achyranthes aspera* Linn

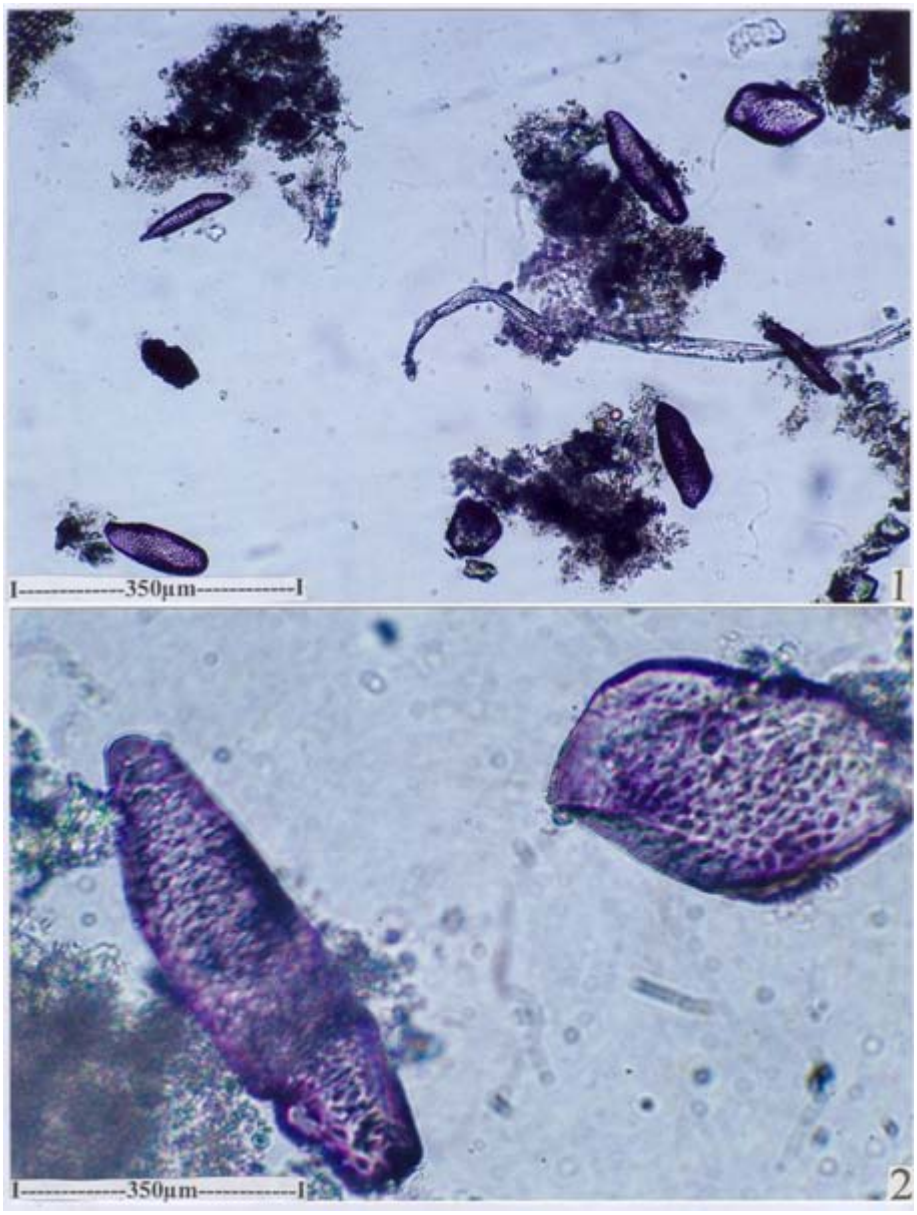


Fig 32. Powder microscope of the fruit

1. **Fruit powder showing the endosperm cells**
2. **Two endosperm cells magnified**

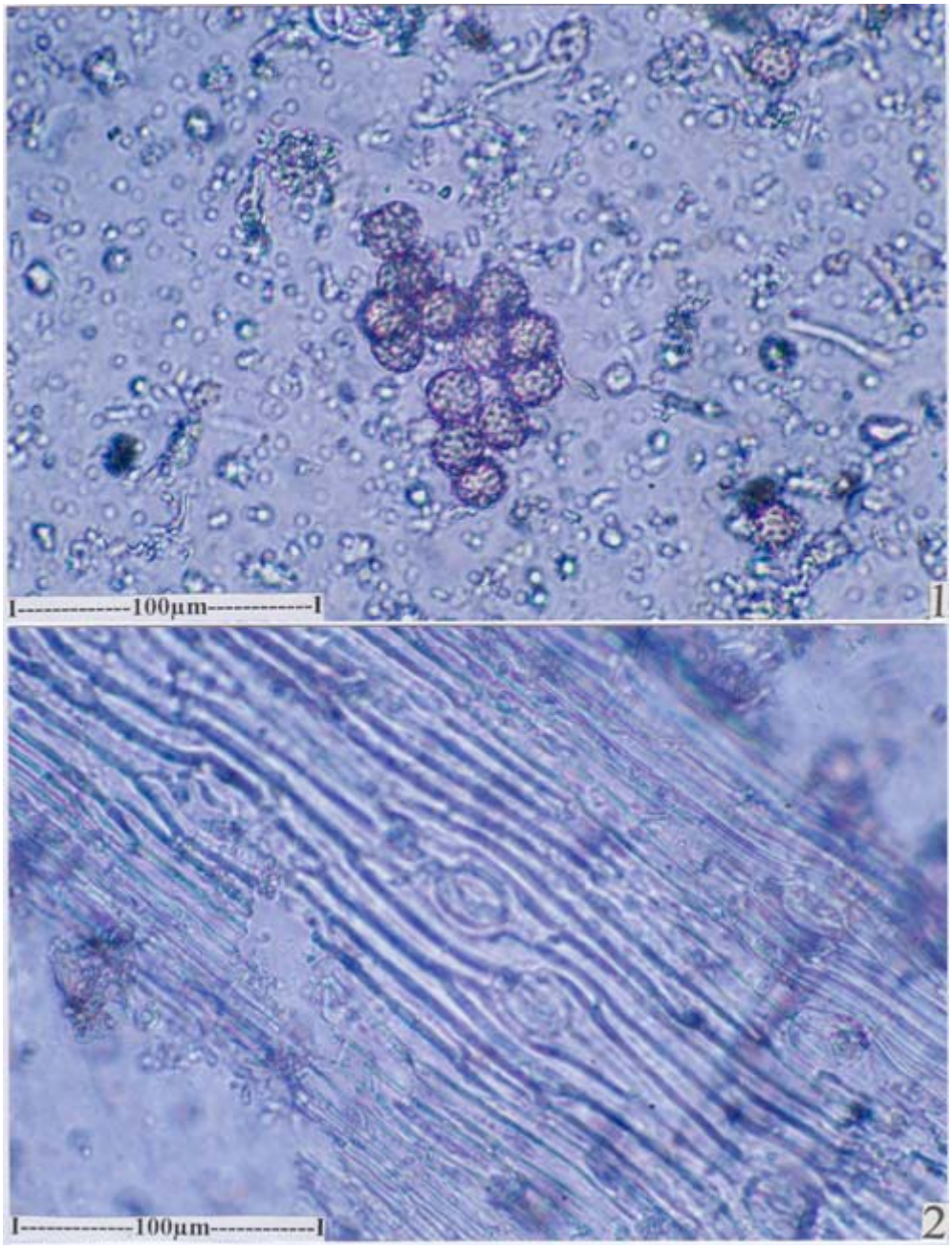


Fig 33. Powder analysis

1. **Fruit powder showing pollen grains**
2. **Fruit powder showing stomata in the perianth lobe**

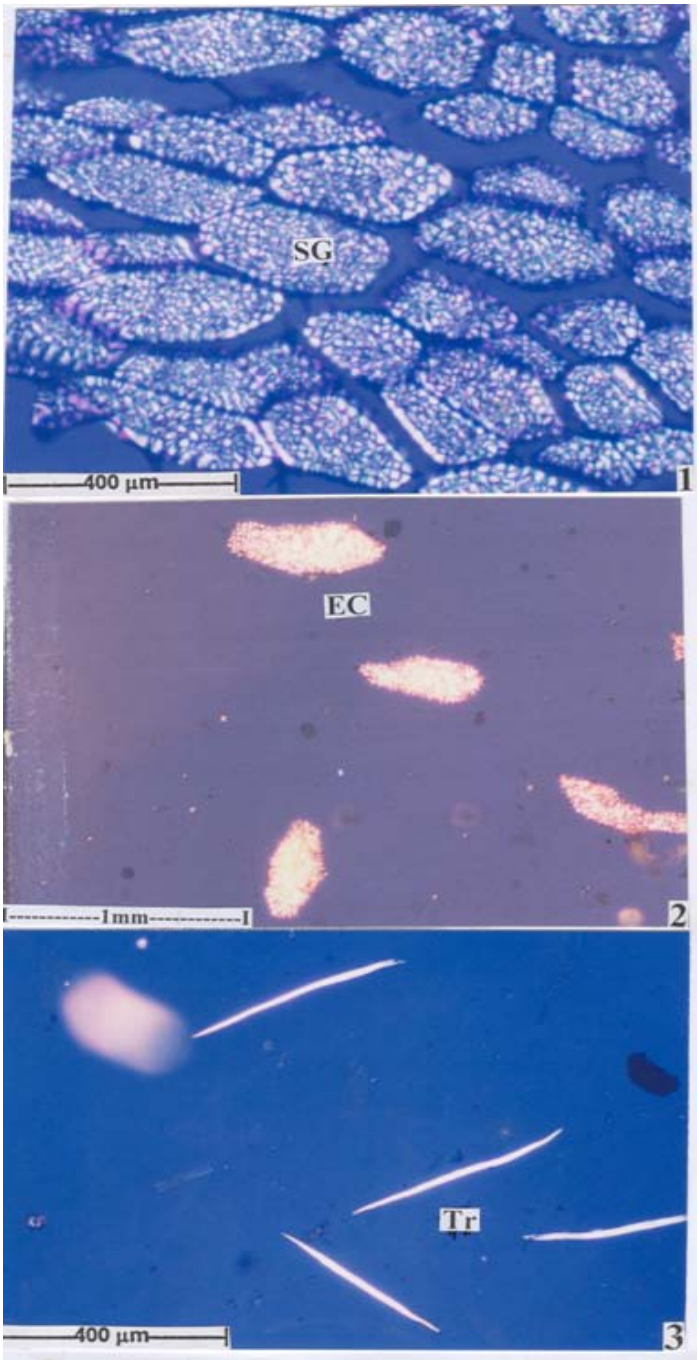


Fig 34. Fruit powder under polarized light microscope

1. **Endosperm cells filled with starch grains**
2. **Some endosperm cells magnified**
3. **Fruit trichomes**

(EC – Endosperm cell; SG – Starch grains; Tr – Trichome)

6.1.2 *Achyranthes bidentata* Blume

Amaranthaceae

The plant is a sub shrub, growing up to 1.5 m in height. It is found in the hills above 200 m. The leaves are simple, opposite, elliptic-ovate to oblanceolate; pubescent below. Inflorescence is spike, terminal. Bracts and bracteoles pointed and hard. Perianth – 5, free; Stamens 5 with 5 fimbriate staminodes. Ovule with one ovule attached on coiled cunicle. Fruit is one seeded utricle. Seed is endospermous, folded embryo, thin testa, fused with the pericarp.

Fig 35. Morphological features showing the leafs and inflorescence of *Achyranthes bidentata* Blume

Microscopic studies

Leaf:

The leaf has prominent midrib as well as lateral veins and dorsiventral lamina (Fig36.1). The midrib has wide, short adaxial cone and semicircular wide abaxial part (Fig36.2). It is 580 µm long vertical axis and 500 µm along horizontal axis. The epidermal layer of the midrib is thin with small squarish thick walled cells. A small mass of cells beneath the epidermis of adaxial cone are collenchymatous. One or two layers of subepidermal cells in the abaxial part are also collenchymatous. Remaining ground tissue consists of fairly wide, thin walled compact parenchyma cells.

The vascular system consists of an abaxial arc three discrete strands and adazial single strand (Fig.36.2). All the strands are collateral with inner xylem elements and outer phloem elements (Fig . 37.1,2).

The abaxial strand is smaller than the abaxial strands. It has only three or four groups of angular thick walled xylem elements and wide mass of phloem elements (Fig 37.1).

The abaxial arc of strands are vertically elongated and possess wider, thick walled angular xylem elements and thick mass of phloem (Fig. 37.2). Two or three layers of sclerenchyma cells form a thin cap on the outer part of the phloem.

Lamina (Fig. 38.1,2): The lamina has thick blunt leaf margin and uniformly thick leaf-blade. The leaf blade has large, adaxial epidermal cells with papillate outer tangential walls. The cells are 30 µm in height. The abaxial epidermis is comparatively narrow, the cells being circular or squarish; the cells are 20 µm in diameter.

The mesophyll tissue is differentiated into upper palisade zone, comprising of two or three layers cylindrical compact cells; the lower zone consists of four of five layers large lobed loosely arranged cells.

The leaf margin is 200 µm thick. The epidermis and mesophyll tissue are not modified in the marginal part. They are similar to those of the middle part of the lamina (Fig. 38.1).

Crystal distribution (Fig. 39.1,2): Calcium oxalate crystals quite abundant in the lamina. They are predominantly druses, i.e., spherical spiny bodies. They occur both in the mesophyll cells and in the parenchyma cells which ensheath veins. In the mesophyll cells the druses occur inside dilated, circular modified cells. (Fig. 39.1, 2). These modified cells possessing the druses are called idioblasts (Fig. 39.2). The crystals are also located all along the veins (Fig. 39.1). These crystals are mostly prismatic type. The druses are 30 µm in diameter. The crystals along the veins are 10 µm wide.

Fruit and seed: The fruit is achene, where the seed, seed coat and pericarp are fused to form a single structure. The bracts, bracteolates and the tapels (perianth) are persistent with the fruit (Fig. 40).

The fruit has a thin pericarp comprising of outer wide, radially oblong, palisade like epicarp and inner radially rectangular thick walled mesocarp and a membranous endocarp.

The seed coat and the endosperm occur as a prominent bulge on one side of the fruit; the perisperm is in the form of wide circular sac (Fig. 41.2). Initially the perisperm is bilobed (Fig 41.1); later it bulges into a spherical body. The seed coat is unistratose and consists of a single vertically elongated, thick walled lignified cells (Fig 42.1, 2; 43.2). It is nearly 20 µm thick. The pericarp which is fused with the seed coat is 30 µm wide. The seed coat encloses free nuclear endosperm (Fig 43.2).

Powder microscopy

The powder of the leaf and fruit shows the following inclusions.

(i) Leaf-epidermal fragments (Fig 44.1).

The epidermal cells are small, polyhedral with thick, straight and smooth walls. The epidermis has stomata which are either paracytic or anomocytic type. The guard cells are 15x20 µm in size.

(ii) Fragments of lamina (Fig 44.2, 3)

Fragments of lamina with venation pattern and dense distribution of the druses are seen in the powder. The venation is reticulate and veins are thin and distinct. The vein-islets are wide and distinct. They are variable in shape and

size. Vein-terminations are distinct. They are short or long and straight. Some of the terminations are branched (Fig 44.3). The druses occur inside wide circular mesophyll idioblasts, randomly distributed in the islets.

(iii) Epidermal trichomes (Fig.45.1,2)

Two celled, uniseriate, unbranched trichomes are abundant in the powder. They are dead, covering type. They have thick walls and narrow lumen. The trichome arises from a circular epidermal cell. The trichomes are 150 – 300 µm thick.

(iv) Calcium oxalate crystals are abundant in the powder (Fig. 46.1, 2). The prismatic crystals occur in vertical strand in the pericarp of the fruit (Fig. 46.1) or in small or large groups in the perisperm (Fig 46.2).

(v) Tapels or perianth segments (Fig 47.1)

Broken pieces of perianth segments are seen in the powder. They consists of vertically elongated, narrow, wavy cells with thick walls. Stomata are also seen in the segments. They are anomocytic type. The guard cells are elliptical and have stomatal pores (Fig 47.1). The guard cells are 12 x 20 µm in size.

(vi) **Grains** (Fig 47.2) are occasionally seen in the powder. They are circular with pitted exine. They are 20 µm in diameter.

(vii) **Pollengrains** (Fig. 47.3) of oval, circular or elliptical shape are common in the powder. They are concentric with central hilum. The grains are 40x50 in size.

Microscopical profiles of
***Achyranthes bidentata* Blume leaf**

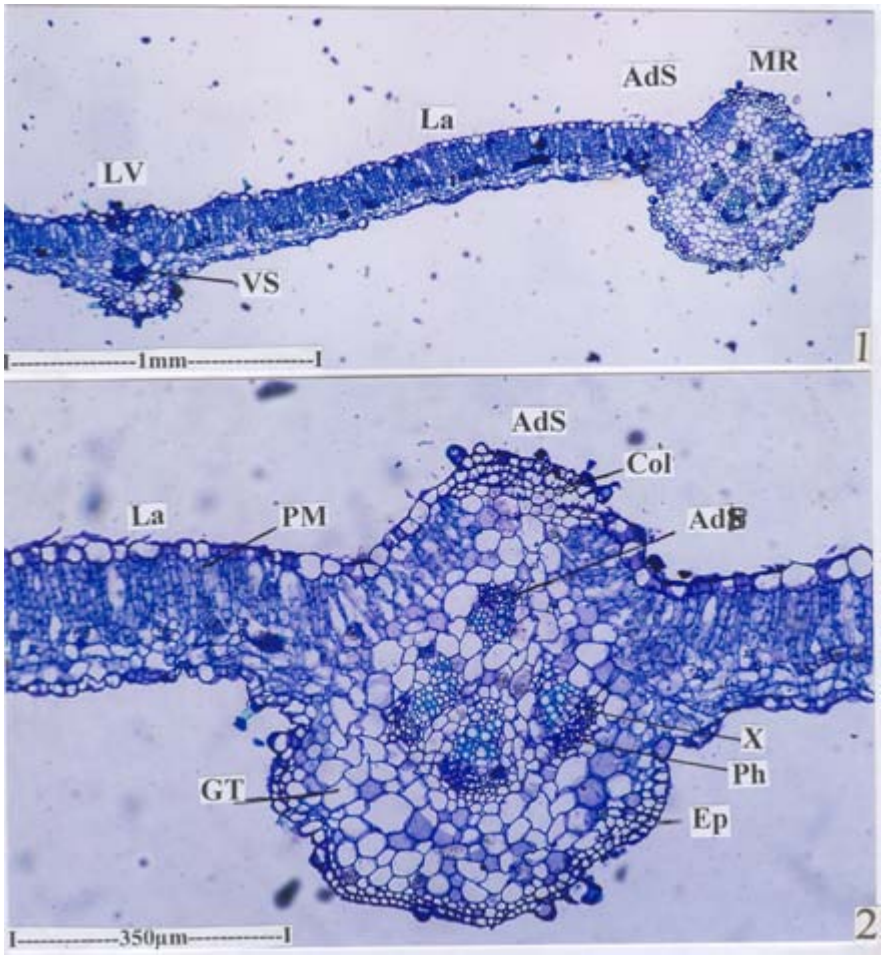


Fig 36.

1. **T.S. of leaf through midrib and lateral vein**
2. **T.S of leaf through midrib**

(AdS – Adaxial side; AdB – Adaxial strand; Col – Collenchyma;
Ep – epidermis; GT – Ground tissue; La – Lamina; LV – Lateral vein; Ph – Phloem; PM – Padisade mesophyll; VS- Vascular straind; X-Xylem)

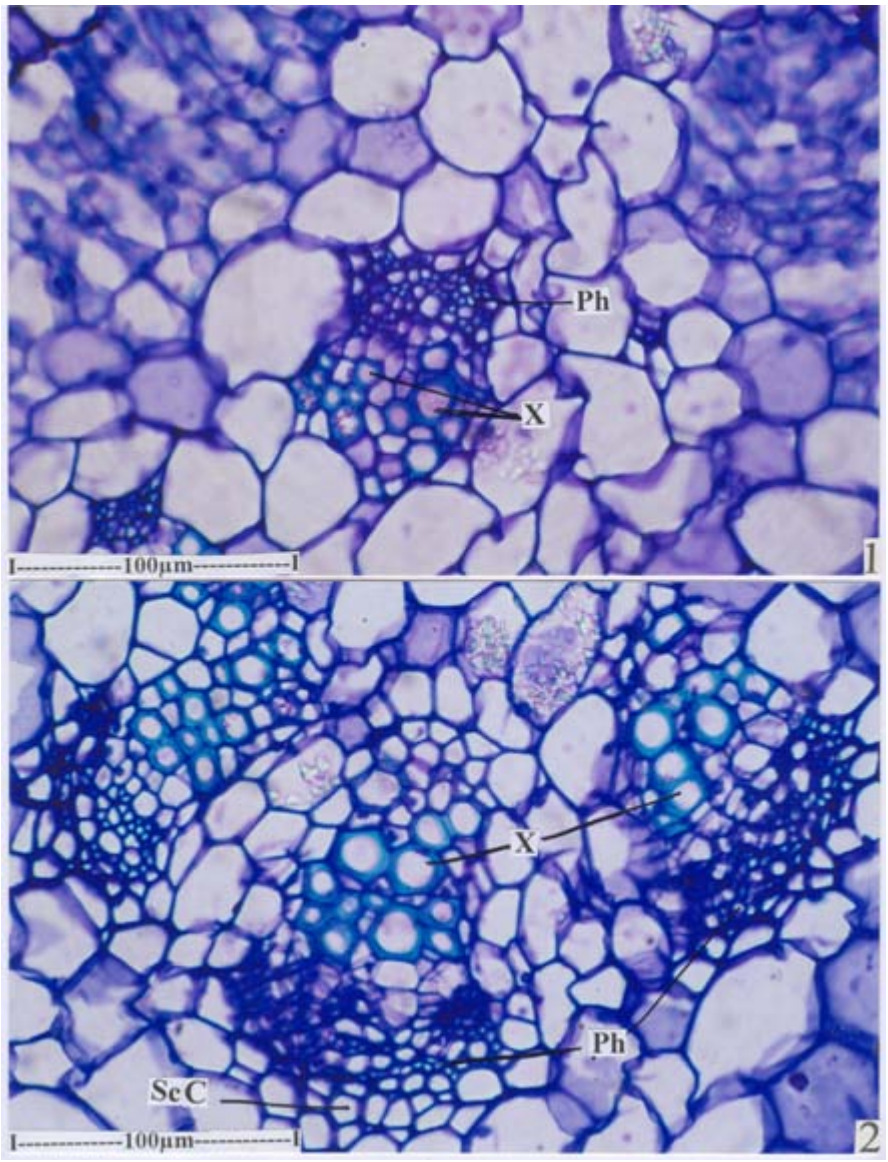


Fig 37.
1. Adaxial vascular bundles of the midrib
2. Abaxial median and lateral bundles of the midrib
(Ph – Phloem; ScC – Sclerenchyma cap; X – xylem)

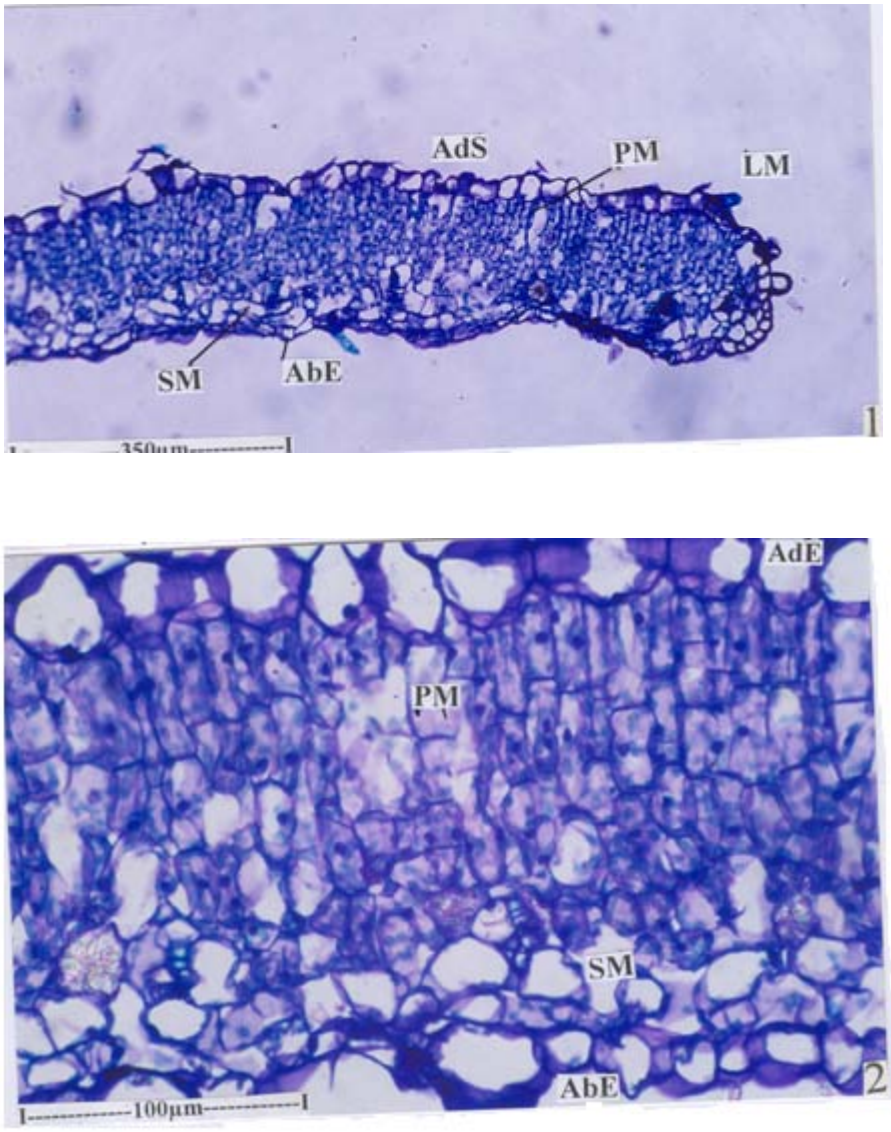


Fig 38.

1. **T.S. of leaf margin**
2. **T.S. of middle part of lamina**
   (AbE – Abaxial epidermis; AdE – Adaxial epidermis; AdS – Adaxial side; LM – Leaf margin; PM – Padisade mesophyll; SM – Spongy mesophyll)

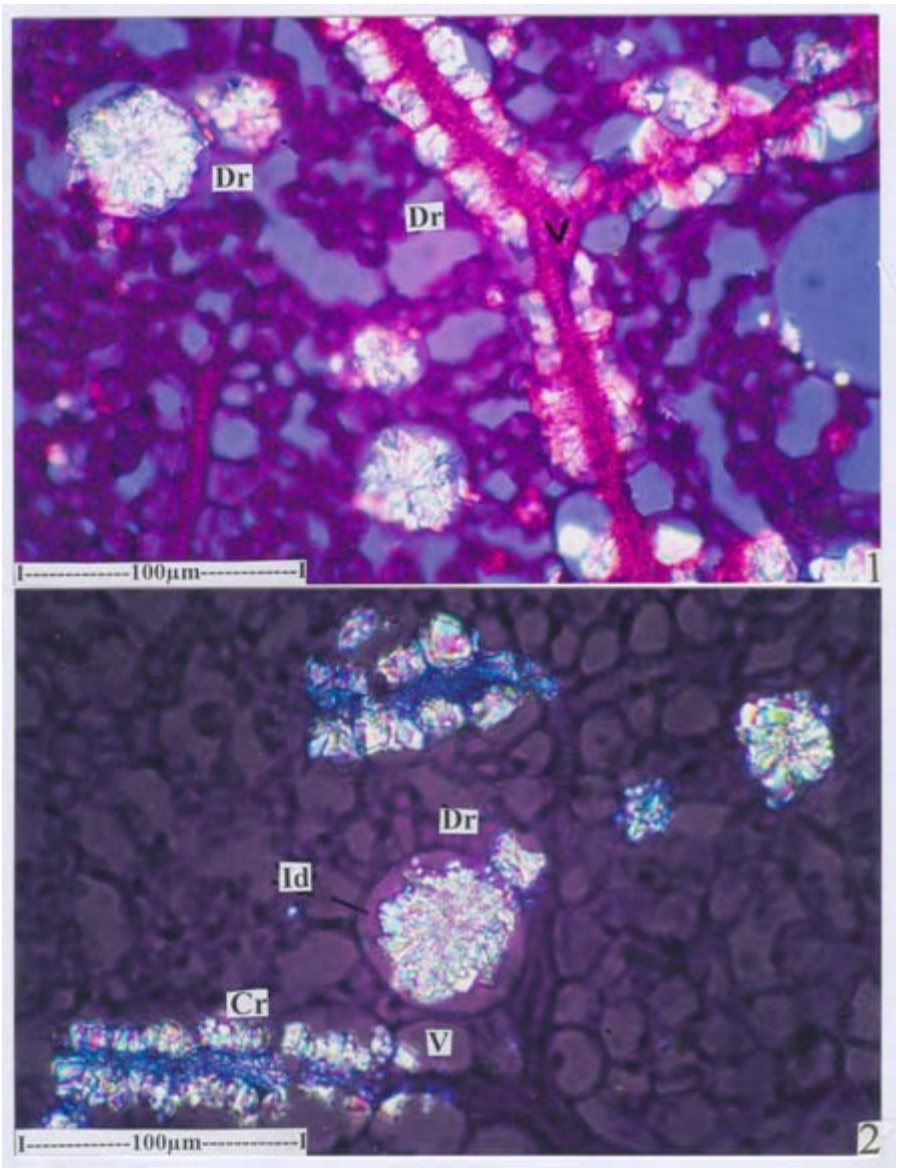


Fig39.

1. Crystal distribution in the lamina

2. Druses in the mesophyll and crystals of prismatic type along the veins.

(Cr – Crystals; Dr- Druses; Id – Idioblast; V – Vein)

Anatomy of fruit and seed of
***Achyranthes bidentata* Blume**

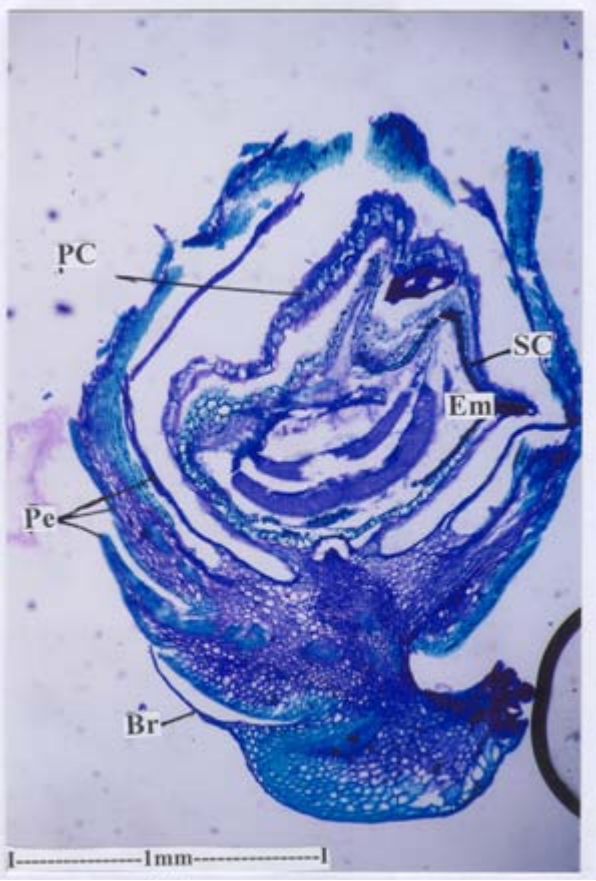


Fig 40.
Vertical section of the fruit and seed

(Br – Bract; Em- Embryo; Pc – Pericarp; Pe- Perianth; SC – Seed coat)

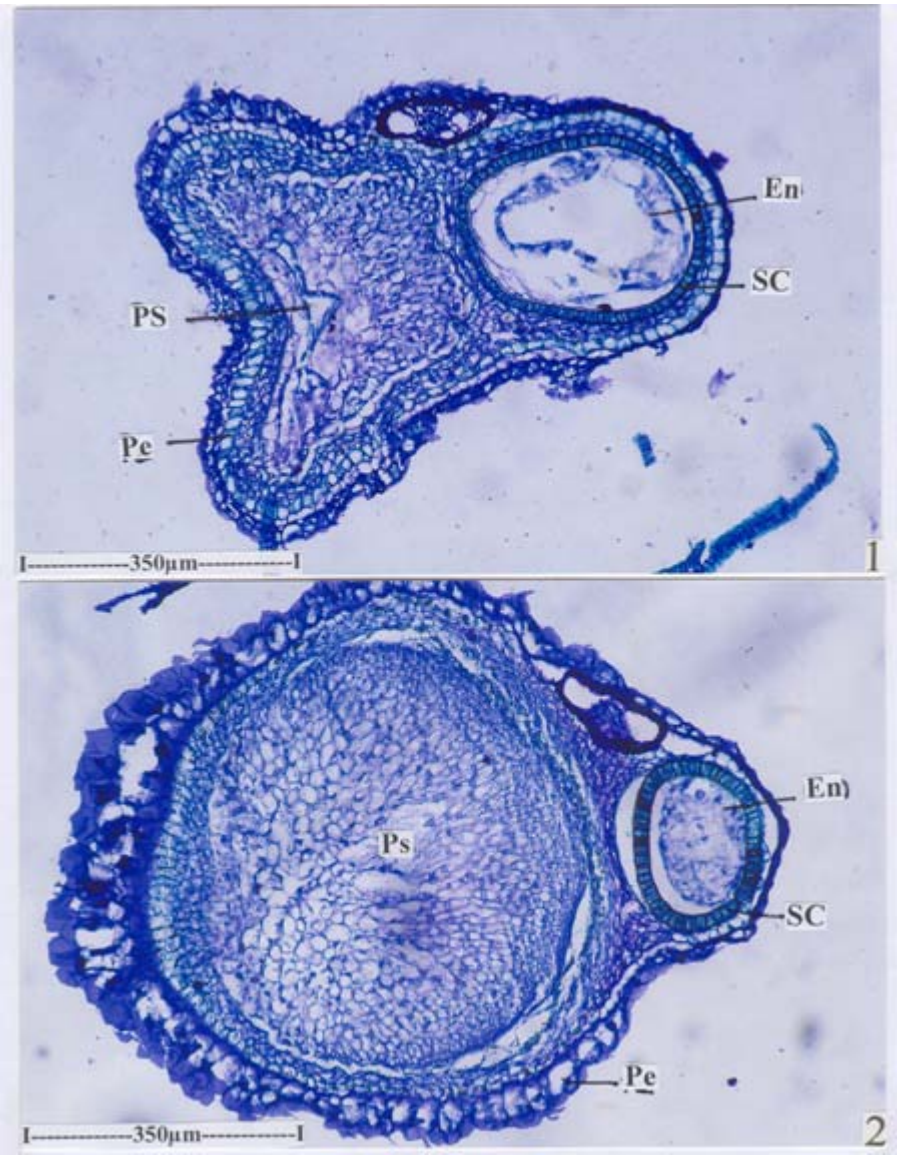


Fig 41.

1. T.S of young lobed fruit
2. T.S. of mature circular fruit
(En – Endosperm; Pe – Pericarp; PS – Perisperm; SC – Seed coat)

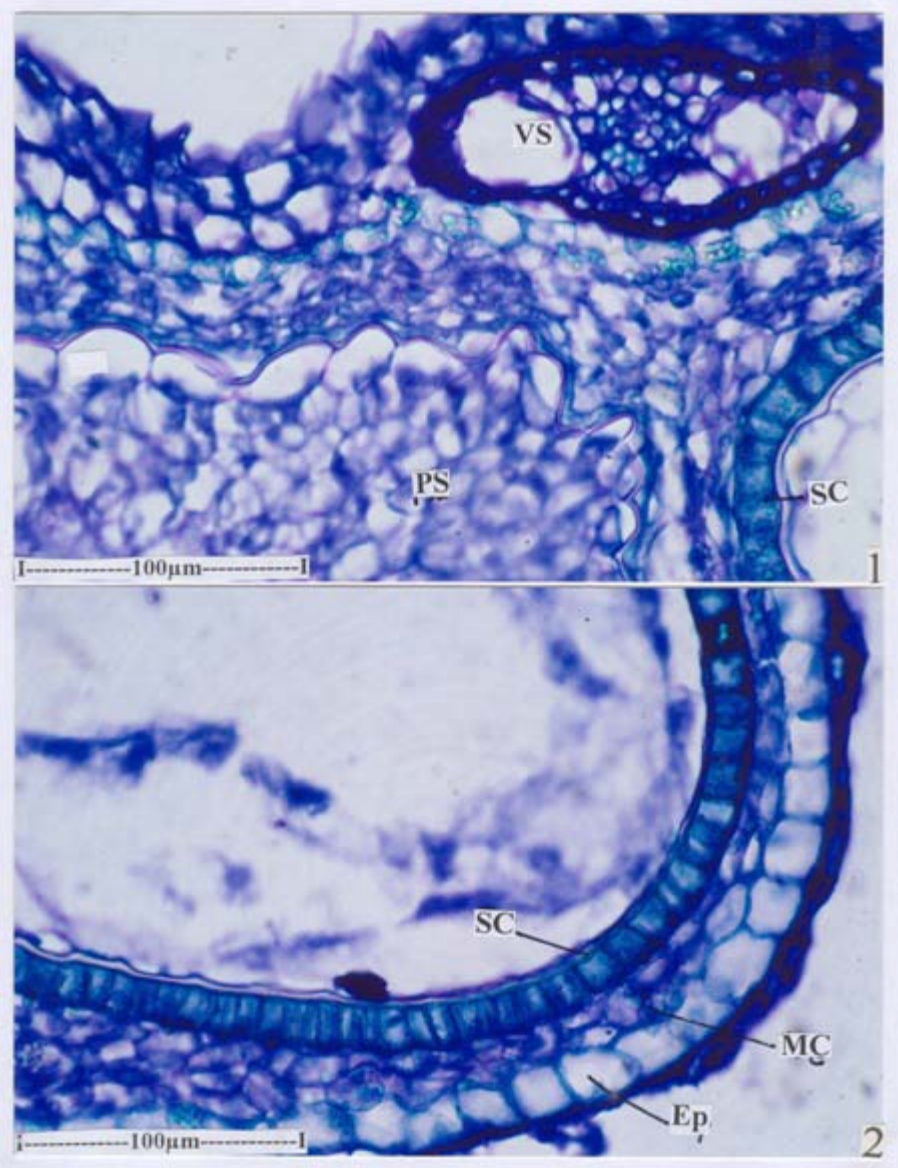


Fig 42.

1. Pericarp – a portion enlarged

2. Seed coat and pericarp – fused portion

(Ep – Epidermis (epicarp); MC – Mesocarp; PS – Perisperm; SC – Seed coat; VS – Vascular strand)

Microscopical profile of *Achyranthes bidentata* Blume seed

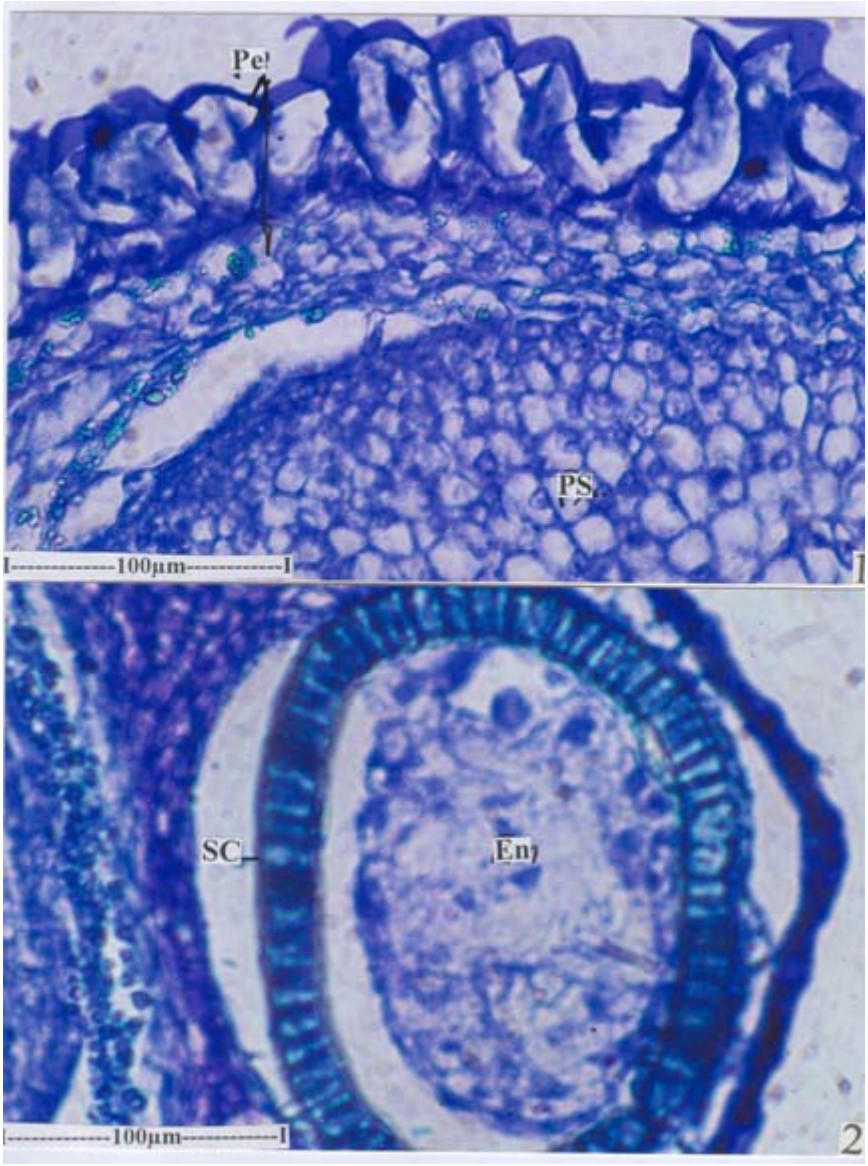


Fig 43.

1. Pericarp and perisperm
2. Seed coat and endosperm

(En – Endosperm; Pe – Pericarp; PS – Perisperm; SC – Seed coat)

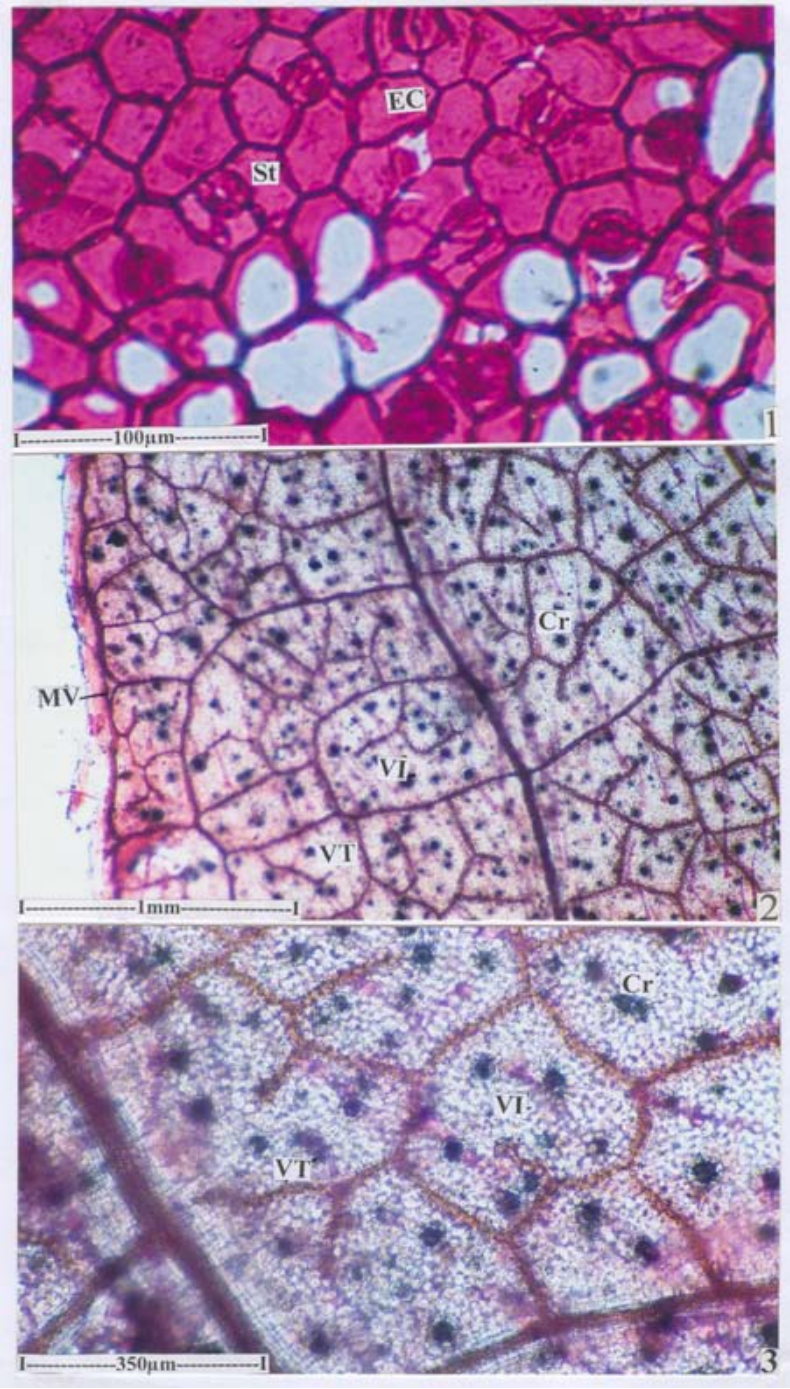


Fig 44.

1. Epidermal fragment in the powder
2. Leaf fragment showing venation
3. Vein-islets and vein terminations with crystal distribution

(EC – Epidermal cells; St – Stomata; VI – Vein islet; VT – Vein termination)

Powder microscopy of leaf of *Achyranthes bidentata* Blume

`Fig 45.
1. Epidermal trichomes
2. Single trichome enlarged

(BC – Basal Cell; Tr – Trichome)

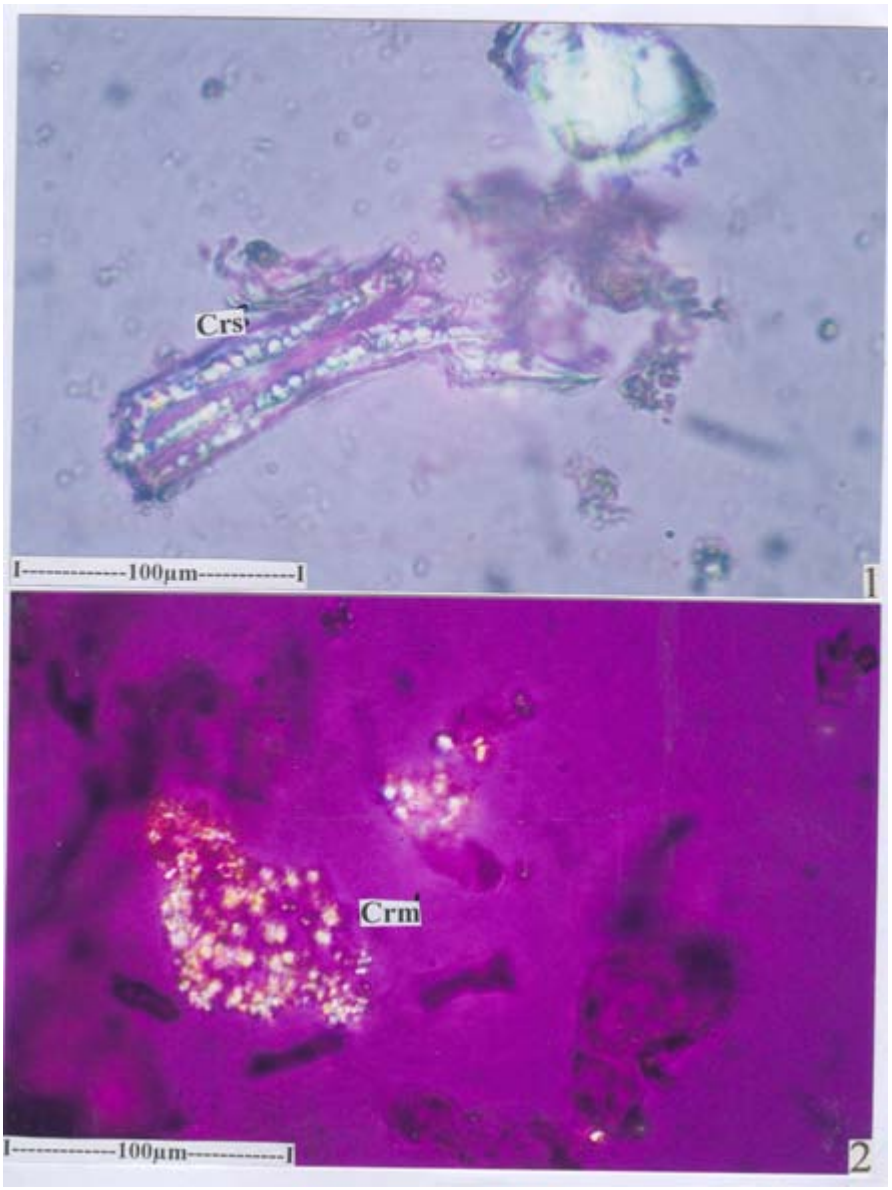


Fig 46.

1. Vertical row of crystals in a cell of the seed

2. Mass crystals in the endosperm

(Crs – Crystal Strand; Crm – Crystal mass)

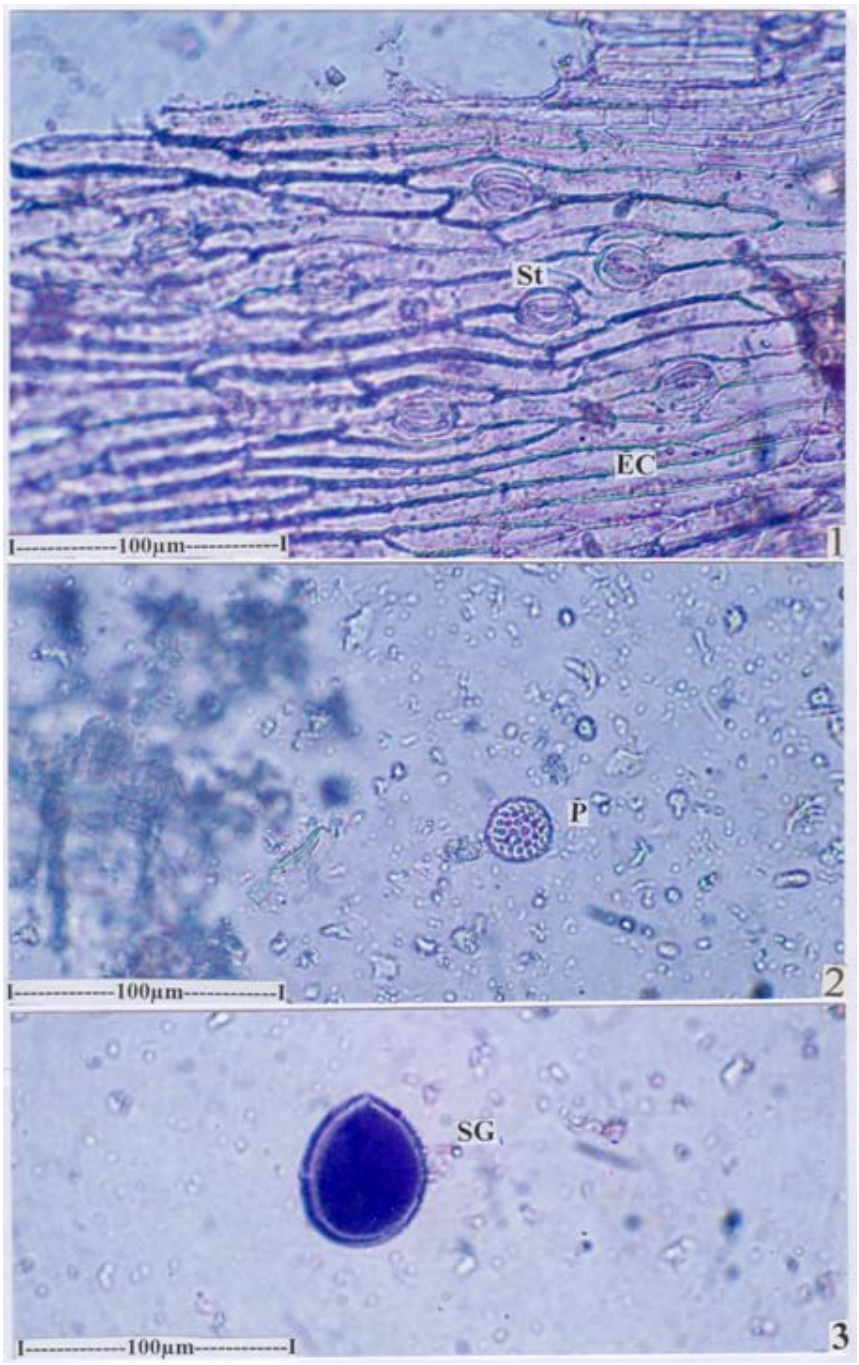


Fig 47.

1. Surface view of the perianth (Tapel)
2. A pollen
3. A starch grain (IKI Stained)

(EC – Epidermal Cells; P – Pollen; SG: Starch grains; St – Stomata)

6.2 Physiochemical studies

Determination of physiochemical constants is important for the purpose of evaluation of crude drugs. The quality parameters of the crude drugs as raw materials were established with the help of several official determinations based on physical and physiochemical studies. These studies were aimed at ensuring standardization of herbal drugs under investigation. Several physiochemical parameters were established for the two plants.

Ash values

The total ash and sulphated ash values for *Achyranthes bidentata* leaf were found to be higher than *Achyranthes aspera* leaf and were 13.984 and 9.803 w/w respectively. The acid insoluble and water soluble ash values were more for *Achyranthes aspera* leaf and were 5.694 and 3.466 w/w respectively. The total ash, acid insoluble ash, water soluble ash and sulphated ash values of *Achyranthes aspera* seed were higher than *Achyranthes bidentata* seed and were 3.712, 1.266, 0.566 and 3.545 respectively. The ash values of the leaf and seed were shown in Table 12 and 13.

Ash values are helpful in determining the quality and purity of crude drugs in powdered form according to the standard procedure. Ash values of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug.

Extractive values

The leaf powder of *Achyranthes aspera* and *Achyranthes bidentata* showed alcoholic and water soluble extractive values as 4.92, 7.66 and 4.10, 6.92% respectively. Whereas the seed powder of *Achyranthes aspera* and

Achyranthes bidentata showed alcoholic and water soluble extractive values as 7.36, 12.32 and 7.182, 10.80% respectively. (Table 14 and 15)

Extractive values are useful for evaluation of crude drugs and gives idea about the nature of chemical constituents present in them. The amount of extractive, a drug yield to a given solvent, is often an approximate measure of a certain constituent of group of related constituents the drug contains. In some cases the amount of drug soluble in a given solvent is an index of its purity. Extractive values are primarily useful, for the determination of exhausted or adultrated drugs.

Fluorescence analysis

The fluorescence analysis of the raw plant powders on treatment with various reagents showed the presence of various constituents and is shown in Table 16-19. The powders showing blue colour with iodine and brown colour with ferric chloride indicates the presence of starch (carbohydrate) and phenolic compounds like tannins flavanoids, etc. The formation of green colour with nitric acid and yellow colour in alcohol indicates the presence of flavanoids and phenolic compounds.

The fluorescence behavior of the powdered drug in different solution towards ordinary light and ultraviolet light (both long and short wavelengths) gives an idea about various phytoconstituents present in the plant drugs. These results indicate the presence of some particular phytoconstituents in the respective plant powders and extracts which was later, confirmed by the phytochemical tests.

Table 12. Leaf ash values

Plant name	Part used	Total ash (% W/W)	Acid insoluble ash (% W/W)	Water soluble ash (% W/W)	Sulphated ash (% W/W)
Achyranthes aspera Linn	Leaf	12.171	5.694	3.466	9.210
Achyranthes bidentata Blume	Leaf	13.984	5.266	3.112	9.803

All the values are average of four determinations

Table 13. Seed ash values

Plant name	Part used	Total ash (% W/W)	Acid insoluble ash (% W/W)	Water soluble ash (% W/W)	Sulphated ash (% W/W)
Achyranthes aspera Linn	Seed	3.712	1.266	0.566	3.545
Achyranthes bidentata Blume	Seed	3.100	1.125	0.446	2.950

All the values are average of four determinations

Table 14. Extractive values of leaf

Plant name	Part used	Alcohol soluble extractive (%)	Water soluble extractive (%)
Achyranthes aspera Linn	Leaf	4.92	7.66
Achyranthes bidentata Blume	Leaf	4.10	6.92

Table 15. Extractive values of seed

Plant name	Part used	Alcohol soluble extractive (%)	Water soluble extractive (%)
Achyranthes aspera Linn	Seed	7.36	12.32
Achyranthes bidentata Blume	Seed	7.182	10.80

Table 16. Fluoresence analysis of raw powder of *Achyranthes aspera* leaf

S. No	Sample + Reagents	UV Light		Visible Light
		Short 254 nm	Long 366 nm	
1	Drug powder	Pale green	Bluish green	Pale green
2	Powder + aqueous sodium hydroxide	Pale green	Blue	Pale green
3	Powder + alkaline sodium hydroxide	Green	Pale green	Green
4	Powder + 1 N hydrochloric acid	Green	Dark green	Green
5	Powder + 50 % sulphuric acid	Pale green	Green	Green
6	Powder + 50 % nitric acid	Green	Bluish green	Dark green
7	Powder + picric acid	Yellowish green	Yellowish green	Green
8	Powder + acetic acid	Dark green	Dark green	Green
9	Powder + ferric chloride	Green	Green	Pale green
10	Powder + nitric acid + ammonia	Dark green	Dark green	Green

Table 17. Fluoresence analysis of raw powder of *Achyranthes aspera* seed

S. No	Sample + Reagents	UV Light		Visible Light
		Short 254 nm	Long 366 nm	
1	Drug powder	Pale green	Pale green	Blue green
2	Powder + aqueous sodium hydroxide	Pale green	Pale green	Pale green
3	Powder + alkaline sodium hydroxide	Pale bluish green	Pale green	Pale green
4	Powder + 1 N hydrochloric acid	Bluish green	Dark green	Green
5	Powder + 50 % sulphuric acid	Emerald green	Green	Green
6	Powder + 50 % nitric acid	Pale green	Green	Dark green
7	Powder + picric acid	Brownish yellow	Brownish yellow	Pale green
8	Powder + acetic acid	Dark green	Dark green	Green
9	Powder + ferric chloride	Brownish green	Brownish green	Green
10	Powder + nitric acid + ammonia	Dark green	Dark brown	Dark green

Table 18. Fluoresence analysis of raw powder of *Achyranthes bidentata* leaf

S. No	Sample + Reagents	UV Light		Visible Light
		Short 254 nm	Long 366 nm	
1	Drug powder	Pale green	Pale green	Pale brown
2	Powder + aqueous sodium hydroxide	Pale green	Pale green	Light blue
3	Powder + alkaline sodium hydroxide	Bluish green	Pale green	Pale green
4	Powder + 1 N hydrochloric acid	Green	Green	Green
5	Powder + 50 % sulphuric acid	Green	Green	Dark green
6	Poeder + 50 % nitric acid	Dark green	Pale green	Green
7	Powder + picric acid	Brownish yellow	Green	Pale yellow green
8	Powder + acetic acid	Dark green	Green	Green
9	Powder + ferric chloride	Green	Pale green	Green
10	Powder + nitric acid + ammonia	Green	Dark brown	Dark green

Table 19. Fluoresence analysis of raw powder of *Achyranthes bidentata* blume seed

S. No	Sample + Reagents	UV Light		Visible Light
		Short 254 nm	Long 366 nm	
1	Drug powder	Green	Green	Brownish green
2	Powder + aqueous sodium hydroxide	Pale green	Dark green	Pale green
3	Powder + alkaline sodium hydroxide	Pale bluish green	Pale green	Pale green
4	Powder + 1 N hydrochloric acid	Green	Dark green	Green
5	Powder + 50 % sulphuric acid	Dark green	Green	Green
6	Poeder + 50 % nitric acid	Dark green	Green	Dark green
7	Powder + picric acid	Brownish green	Green	Yellowish green
8	Powder + acetic acid	Dark green	Green	Green
9	Powder + ferric chloride	Brownish green	Brownish green	Green
10	Powder + nitric acid + ammonia	Dark green	Green	Dark green

Table 20. Foaming index of *Achyranthes aspera* Linn and *Achyranthes bidentata* Blume

S.No	Test tube No.	Height of foam (in cm)	
		***Achyranthes aspara* Linn**	***Achyranthes bidentata* Blume**
1	1	1	1
2	2	2	1.9
3	3	2.2	2.1
4	4	2.4	2.1
5	5	2.6	2.4
6	6	3.2	3.2
7	7	3.4	3.5
8	8	3.6	3.9
9	9	3.9	4.2
10	10	4.6	4.7

Foaming index

i. The foaming index of *Achyranthes aspara* = 1000
ii. The foaming index of *Achyranthes bidentata* = 1000

Table 21. Mucilage content of the seeds of *Achyranthes aspera* Linn and *Achyranthes bidentata* Blume

S. No	Plant	Part used	Mucilage content (%)
1	*Achyranthes aspera* linn	Seed	2.89
2	*Achyranthes bidentata* blume	Seed	3.56

6.3 Phytochemical studies

Organoleptic characters of *Achyranthes aspera* and *Achyranthes bidentata* leaf

The leaf powder of *Achyranthes aspera* and *Achyranthes bidentata* is pale green in colour with characteristic odour and no characteristic taste. The powder was coarse in appearance and when triturated with water it was non sticky in nature. The powder on shaking with water gave foam like froth and no oil stain was found when the powder was pressed between filter papers for 24 hours.

Organoleptic characters of *Achyranthes aspera* and *Achyranthes bidentata* seed

The seed powder of *Achyranthes aspera* and *Achyranthes bidentata* is pale brown in colour with characteristic odour and mucilaginous taste. The powder was fine in appearance and when triturated with water it was sticky in nature. The powder on shaking with water gave foam like froth and no oil stain was found when the powder was pressed between filter papers for 24 hours.

A preliminary organoleptic character of both the powdered plant material was studied and the results are shown in Table 22-25.

Qualitative phytochemical analysis

In the qualitative phytochemical analysis of *Achyranthes aspera* and *Achyranthes bidentata* leaf raw powder in 50% alcohol were found to show positive results for the presence of alkaloids, terpenoids, steroids, saponin, glycoside and mucilage wherein the raw powder in water showed the presence of alkaloids, terpenoids, steroids, saponins, glycosides and

mucilage. The 50% ethanolic and water extract of *Achyranthes aspera* and *Achyranthes bidentata* leaf showed the presence of alkaloids, terpenoids, steroids, saponins, phenols and glycosides. Qualitative phytochemical analysis of *Achyranthes aspera* and *Achyranthes bidentata* seed raw powder in 50% alcohol were found to show positive results for the presence of alkaloids, terpenoids, steroids, saponin, glycoside and mucilage wherein the raw powder in water showed the presence of alkaloids, terpenoids, steroids, saponins, glycosides, fixed oils and mucilage. The 50% ethanolic and water extract of *Achyranthes aspera* and *Achyranthes bidentata* seed showed the presence of alkaloids, terpenoids, steroids, saponins and glycosides and mucilage.

The details of the results are summarized in Table 30 and 31.

Phytochemical evaluation helps in laying down the pharmacopoeial standards.

6.4 HPTLC finger print analysis

HPTLC finger print analysis is a useful to identify phytoconstituents. The chromatogram was developed for each extracts by using different mobile phases. The Rf values with their corresponding peak area at 254 nm were recorded in the table no 32-36.

Table 22. Organoleptic character of *Achyranthes aspera* leaf raw powdered material.

Powder character	
Colour	Pale green
Appearance	Coarse powder
Odour	Characteristics
Taste	No Characteristics

Treatment	Observation
Powder triturate with water	Non sticky
Powder shaken with water	Foam like froth
Powder pressed between filter paper for 24 hrs	No oil stain

Table 23. Organoleptic character of *Achyranthes aspera* seed raw powdered material.

Powder character	
Colour	Pale brown
Appearance	Fine powder
Odour	Characteristic
Taste	Mucilaginous

Treatment	Observation
Powder triturate with water	Sticky
Powder shaken with water	Foam like froth
Powder pressed between filter paper for 24 hrs	No oil stain

Table 24. Organoleptic character of *Achyranthes bidentata* leaf raw powdered material.

Powder character	
Colour	Pale green
Appearance	Coarse powder
Odour	Characteristics
Taste	No Characteristics

Treatment	Observation
Powder triturate with water	Non sticky
Powder shaken with water	Foam like froth
Powder pressed between filter paper for 24 hrs	No oil stain

Table 25. Organoleptic character of *Achyranthes bidentata* seed raw powdered material.

Powder character	
Colour	Pale brown
Appearance	Fine powder
Odour	Characteristic
Taste	Mucilaginous

Treatment	Observation
Powder triturate with water	Sticky
Powder shaken with water	Foam like froth
Powder pressed between filter paper for 24 hrs	No oil stain

Table 26. The colour and consistency of various extracts of *Achyranthes aspera* linn leaf

S. No	Name of the extract	Colour	Consistency
1	Petroleum ether	Light blue	Greasy
2	Chloroform	Pale green	Sticky mass
3	Ethyl acetate	Dark green	Viscous mass
4	Acetone	Green	Viscous mass
5	50% ethanol	Dark green	Viscous mass
6	Aqueous	Green	Viscous mass

Table 27. The colour and consistency of various extracts of *Achyranthes aspera* linn seed

S. No	Name of the extract	Colour	Consistency
1	Petroleum ether	Light brown	Greasy
2	Chloroform	Dark brown	Viscous mass
3	Ethyl acetate	Pale green	Viscous mass
4	Acetone	Brownish green	Sticky mass
5	50% ethanol	Dark green	Sticky mass
6	Aqueous	Blackish green	Sticky mass

Table 28. The colour and consistency of various extracts of *Achyranthes bidentata* Blume leaf

S. No	Name of the extract	Colour	Consistency
1	Petroleum ether	Green	Greasy
2	Chloroform	Dark green	Sticky mass
3	Ethyl acetate	Dark green	Viscous mass
4	Acetone	Green	Viscous mass
5	50% ethanol	Dark green	Viscous mass
6	Aqueous	Blackish green	Sticky mass

Table 29. The colour and consistency of various extracts of *Achyranthes bidentata* Blume seed

S. No	Name of the extract	Colour	Consistency
1	Petroleum ether	Light brown	Greasy
2	Chloroform	Dark brown	Viscous mass
3	Ethyl acetate	Pale green	Viscous mass
4	Acetone	Brownish green	Sticky mass
5	50% ethanol	Dark green	Sticky mass
6	Aqueous	Blackish green	Sticky mass

Table 30. Qualitative phytochemical analysis of raw powder and extract of plant leaf

S. No	Constitients	Raw powder				50 % Ethanol extracts of *Achyranthes aspera* leaf	Aqueous extract of *Achyranthes aspera* leaf	50 % Ethanol extracts of *Achyranthes bidentata* leaf	Aqueous extract of *Achyranthes Bidentata* leaf
		Achyranthes Aspera leaf		*Achyranthes bidentata* leaf					
		Alc	Aq	Alc	Aq				
1	Alkaloids	+	+	+	+	+	+	+	+
2	Terpenoids	+	+	+	+	+	+	+	+
3	Steroids	+	+	+	+	+	+	+	+
4	Tannins	-	-	-	-	-	-	-	-
5	Saponins	+	+	+	+	+	+	+	+
6	Flavonoids	+	+	+	+	+	+	+	+
7	Phenols	+	+	+	+	+	+	+	+
8	Proteins	-	-	-	-	-	-	-	-
9	Carbohydrates	-	-	-	-	-	-	-	-
10	Glycosides	+	+	+	+	+	+	+	+
11	Gum	-	-	-	-	-	-	-	-
12	Fixed oils	-	-	-	-	-	-	-	-
13	Mucilage	+	+	+	+	+	+	+	+

Table 31. Qualitative phytochemical analysis of raw powder and extract of plant seed

S. No	Constitients	Raw powder				50 % Ethanol extracts of *Achyranthes aspera* seed	Aqueous extract of *Achyranthes aspera* seed	50 % Ethanol extracts of *Achyranthes bidentata* seed	Aqueous extract of *Achyranthes Bidentata* seed
		Achyranthes Aspera seed		*Achyranthes bidentata* seed					
		Alc	Aq	Alc	Aq				
1	Alkaloids	+	+	+	+	+	+	+	+
2	Terpenoids	+	+	+	+	+	+	+	+
3	Steroids	+	+	+	+	+	+	+	+
4	Tannins	-	-	-	-	-	-	-	-
5	Saponins	+	+	+	+	+	+	+	+
6	Flavonoids	+	+	+	+	+	+	+	+
7	Phenols	+	+	+	+	+	+	+	+
8	Proteins	-	-	-	-	-	-	-	-
9	Carbohydrates	-	-	-	-	-	-	-	-
10	Glycosides	+	+	+	+	+	+	+	+
11	Gum	-	-	-	-	-	-	-	-
12	Fixed oils	+	-	+	-	+	+	+	-
13	Mucilage	+	+	+	+	+	+	+	+

Table 32. HPTLC finger print analysis of petroleum ether extract of *Achyranthes aspera* Linn and *Achyranthes bidentata* Blume (leaf and seed)
Mobile phase - Hexane:ethyl acetate (2:1)

Sample Name / Track No	Quantity of the sample applied (µl)	No. of spots	Rf value	Peak Area	Area %
AA.leaf Track -1	5	9	0.09 0.24 0.28 0.34 0.55 0.68 0.72 0.85 0.88	5800.9 2670.4 3013.2 2028.4 1447.4 3257.3 2647.4 4123.4 3256.6	20.54 9.45 10.67 7.18 5.12 11.53 9.37 14.60 11.53
AB.leaf Track-2	5	8	0.09 0.46 0.50 0.55 0.60 0.68 0.72 0.85	6648.6 1777.8 1514.9 2821.4 2665.8 5950.3 5595.6 2466.8	22.58 6.04 5.15 9.58 9.05 20.21 19.01 8.38
AA.seed Track-3	5	7	0.09 0.24 0.29 0.69 0.73 0.86 0.89	5848.8 2761.7 3121.4 3794.0 3022.9 4598.9 3863.1	21.65 10.22 11.56 14.05 11.19 17.03 14.30
AB.seed Track - 4	5	6	0.09 0.54 0.58 0.66 0.71 0.83	6660.1 2913.3 2715.6 6020.3 5666.3 2619.3	25.04 10.95 10.21 22.64 21.31 9.85

Table 33. HPTLC finger print analysis of chloroform extract of *Achyranthes aspera* Linn and *Achyranthes bidentata* Blume (leaf and seed)
Mobile phase – Hexane:ethyl acetate (2:1)

Sample Name / Track No	Quantity of the sample applied (µl)	No. of spots	Rf value	Peak Area	Area %
AA.leaf Track -1	5	11	0.07 0.23 0.26 0.30 0.38 0.49 0.54 0.67 0.71 0.86 0.90	45115.3 2558.4 3046.2 4625.2 3255.4 1681.6 1963.4 1500.2 538.0 1852.4 3941.5	64.38 3.65 4.35 6.60 4.65 2.40 2.80 2.14 0.77 2.64 5.62
AB.leaf Track-2	5	10	0.07 0.26 0.35 0.46 0.49 0.54 0.58 0.67 0.71 0.86	46363.6 5010.3 3113.9 2425.2 2790.0 3631.9 2357.6 4244.8 2617.7 669.6	63.32 6.84 4.25 3.31 3.81 4.96 3.22 5.80 3.57 0.91
AA.seed Track-3	5	7	0.07 0.47 0.54 0.67 0.71 0.79 0.86	1486.3 286.0 173.9 1442.5 937.7 594.2 2211.0	20.84 4.01 2.44 20.23 13.15 8.33 31.00
AB.seed Track - 4	5	5	0.07 0.67 0.71 0.79 0.86	1402.6 1442.5 937.7 594.2 2211.0	21.29 21.90 14.23 9.02 33.56

Table 34. HPTLC finger print analysis of ethyl acetate extract of *Achyranthes aspera* Linn and *Achyranthes bidentata* Blume (leaf and seed)

Mobile phase - Ethyl acetate:glacial acetic acid:formic acid:water (100:11:11:26)

Sample Name / Track No	Quantity of the sample applied (µl)	No. of spots	Rf value	Peak Area	Area %
AA.leaf Track -1	5	9	0.08 0.40 0.43 0.48 0.58 0.66 0.71 0.84 0.94	56.6 136.6 321.1 647.0 2692.1 2917.5 7108.9 26028.3 23540.9	0.09 0.22 0.51 1.02 4.24 4.60 11.20 41.02 37.10
AB.leaf Track-2	5	6	0.30 0.42 0.57 0.64 0.80 0.94	102.1 496.4 1012.5 2057.4 2938.3 29622.4	0.28 1.37 2.79 5.68 8.11 81.76
AA.seed Track-3	5	5	0.48 0.62 0.71 0.84 0.94	2018.2 2787.9 8919.5 31324.6 18118.4	3.20 4.41 14.12 49.59 28.68
AB.seed Track - 4	5	5	0.44 0.59 0.69 0.83 0.93	2047.5 2834.6 8976.2 31423.1 18198.9	3.23 4.47 14.14 49.50 28.67

Table 35. HPTLC finger print analysis of acetone extract of *Achyranthes aspera* Linn and *Achyranthes bidentata* Blume (leaf and seed)
Mobile phase - toluene: ethyl acetate: formic acid (4:5:1)

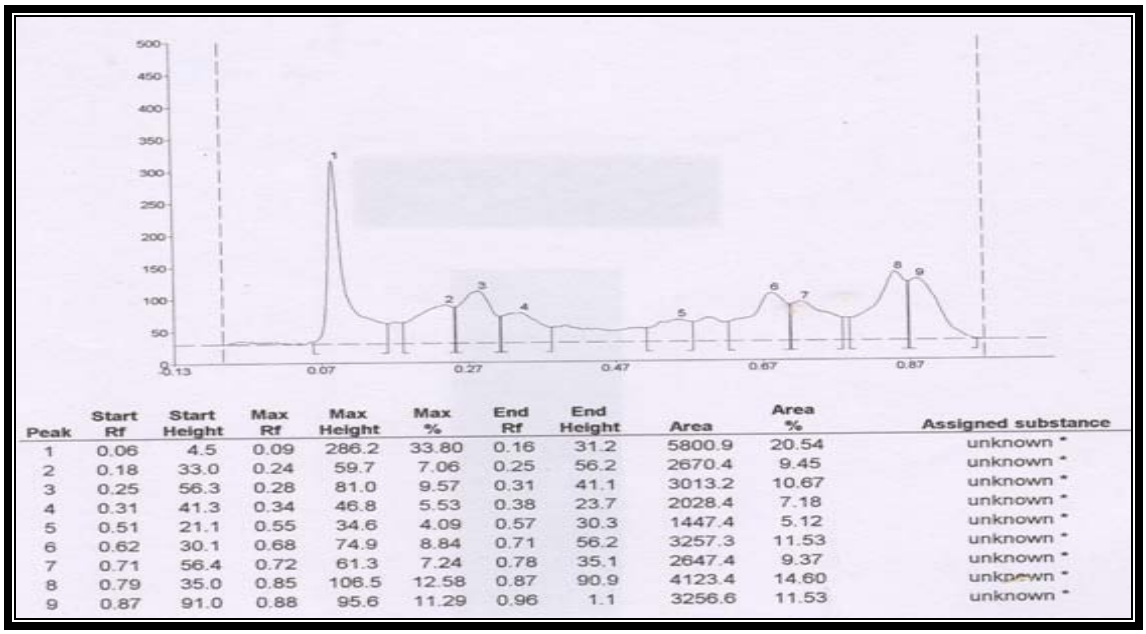
Sample Name / Track No	Quantity of the sample applied (µl)	No. of spots	Rf value	Peak Area	Area %
AA.leaf Track -1	5	8	0.14 0.22 0.27 0.29 0.31 0.37 0.52 0.64	240.4 499.9 211.4 90.0 135.5 361.6 263.8 209.2	11.95 24.85 10.51 4.48 6.73 17.97 13.11 10.40
AB.leaf Track-2	5	11	0.12 0.15 0.20 0.37 0.49 0.60 0.64 0.68 0.71 0.83 0.93	2689.2 2911.1 1460.7 5590.7 594.8 771.6 717.8 413.1 268.1 5320.8 14155.6	7.71 8.34 4.19 16.02 1.70 2.21 2.06 1.18 0.77 15.25 40.57
AA.seed Track-3	5	10	0.14 0.19 0.27 0.36 0.41 0.52 0.61 0.64 0.82 0.92	2100.0 3923.5 285.1 1399.9 212.7 174.7 219.8 745.0 3264.3 4855.7	12.22 22.84 1.66 8.15 1.24 1.02 1.28 4.34 19.00 28.26
AB.seed Track - 4	5	8	0.12 0.20 0.35 0.49 0.62 0.66 0.81 0.93	1489.4 792.0 1323.2 430.5 330.0 429.0 580.2 2661.7	18.53 9.86 16.47 5.36 4.11 3.34 7.22 33.12

Table 36. HPTLC finger print analysis of ethanol extract of *Achyranthes aspera* Linn and *Achyranthes bidentata* Blume (leaf and seed)
Mobile phase - n butanol:glacial acetic acid:water (4:1:1)

Sample Name / Track No	Quantity of the sample applied (µl)	No. of spots	Rf value	Peak Area	Area %
AA.leaf Track -1	5	12	-0.10	2713.1	4.41
			0.01	11527.3	18.73
			0.14	4250.2	6.90
			0.22	1176.0	1.91
			0.35	5482.6	8.91
			0.38	3058.9	4.97
			0.47	5978.5	9.71
			0.61	7118.4	11.56
			0.68	4582.2	7.44
			0.82	4842,9	7.87
			0.90	8629.3	14.02
			0.94	2994.7	3.57
AB.leaf Track-2	5	11	-0.10	2426.8	1.65
			0.01	19723.7	13.38
			0.13	8173.5	5.54
			0.17	4542.1	3.08
			0.22	4397.7	2.98
			0.34	27244.7	18.48
			0.45	9207.1	6.25
			0.58	10296.6	6.98
			0.60	7854.8	5.33
			0.67	12452.9	8.45
			0.89	41098.9	27.88

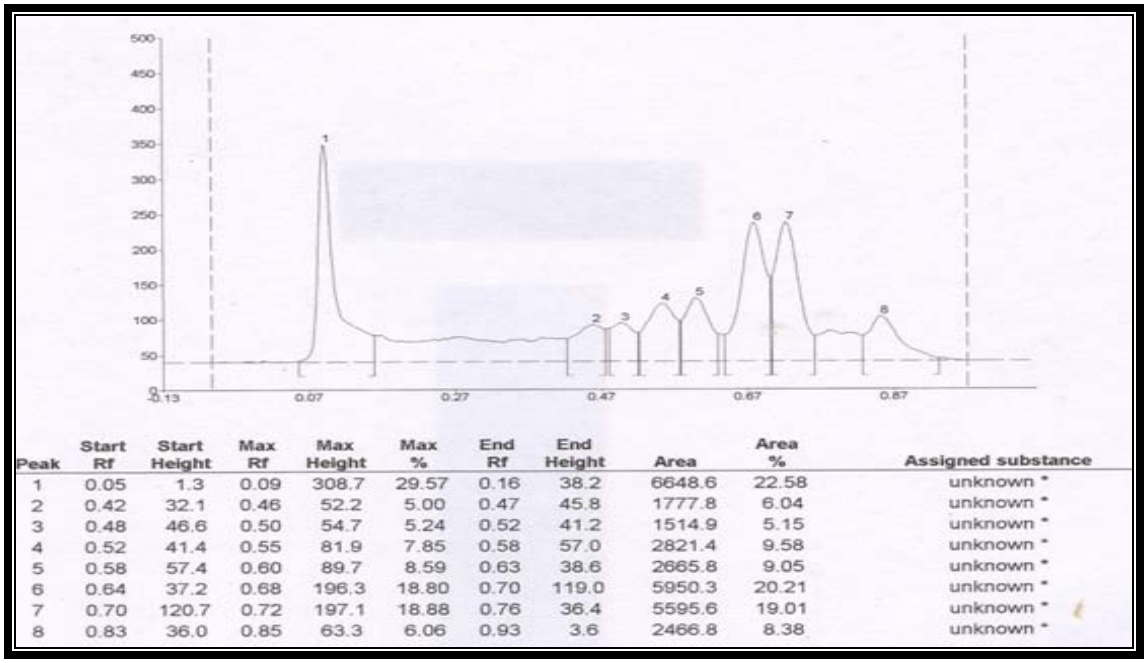
Sample Name / Track No	Quantity of the sample applied (µl)	No. of spots	Rf value	Peak Area	Area %
AA.seed Track-3	5	12	-0.10 0.02 0.14 0.17 0.21 0.31 0.38 0.47 0.61 0.68 0.91 0.94	2782.5 13422.1 2383.3 1511.1 1384.6 2546.5 2084.2 4066.1 4739.3 2116.0 5853.0 2306.2	6.16 29.70 5.27 3.34 3.06 5.63 4.61 9.00 10.49 4.68 12.95 5.10
AB.seed Track - 4	5	8	-0.10 0.01 0.12 0.36 0.45 0.57 0.67 0.88	2123.0 18077.5 5744.2 19123.6 12712.1 21027.9 11862.9 54328.4	1.46 12.47 3.96 13.19 8.77 14.50 8.18 37.47

Fig 48. Track 1. ID: *Achyranthes aspera* Linn leaf-Petroleum ether extract.



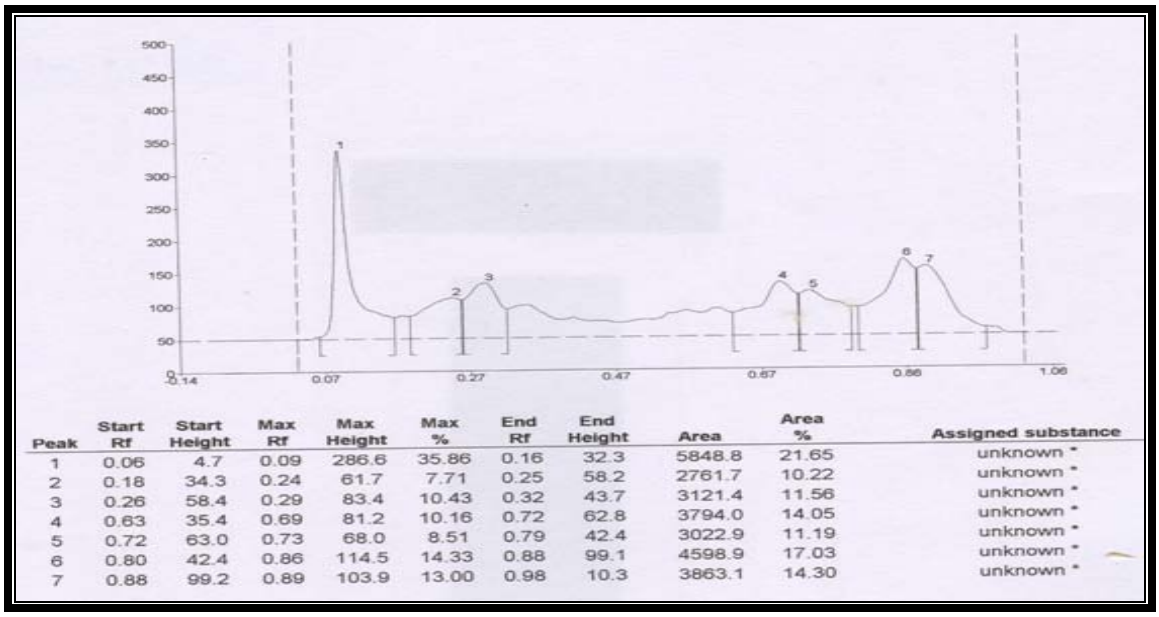
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.06	4.5	0.09	286.2	33.80	0.16	31.2	5800.9	20.54	unknown *
2	0.18	33.0	0.24	59.7	7.06	0.25	56.2	2670.4	9.45	unknown *
3	0.25	56.3	0.28	81.0	9.57	0.31	41.1	3013.2	10.67	unknown *
4	0.31	41.3	0.34	46.8	5.53	0.38	23.7	2028.4	7.18	unknown *
5	0.51	21.1	0.55	34.6	4.09	0.57	30.3	1447.4	5.12	unknown *
6	0.62	30.1	0.68	74.9	8.84	0.71	56.2	3257.3	11.53	unknown *
7	0.71	56.4	0.72	61.3	7.24	0.78	35.1	2647.4	9.37	unknown *
8	0.79	35.0	0.85	106.5	12.58	0.87	90.9	4123.4	14.60	unknown *
9	0.87	91.0	0.88	95.6	11.29	0.96	1.1	3256.6	11.53	unknown *

Fig 49. Track 2. ID: *Achyranthes bidentata* Blume leaf-Petroleum ether extract



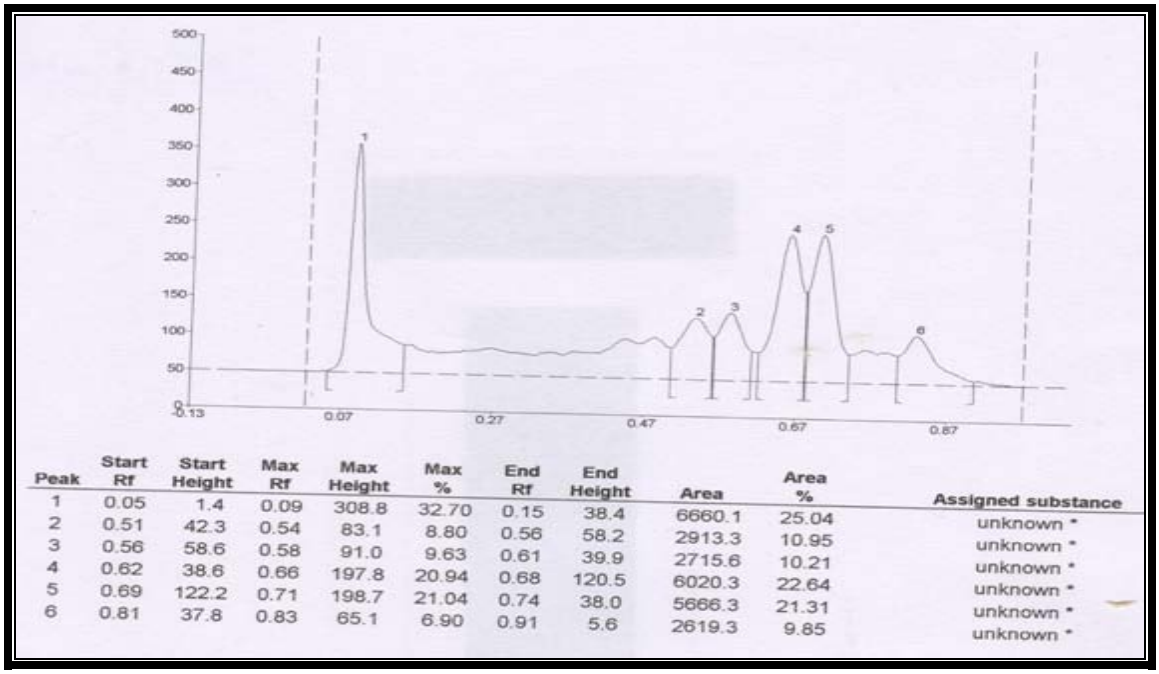
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	1.3	0.09	308.7	29.57	0.16	38.2	6648.6	22.58	unknown *
2	0.42	32.1	0.46	52.2	5.00	0.47	45.8	1777.8	6.04	unknown *
3	0.48	46.6	0.50	54.7	5.24	0.52	41.2	1514.9	5.15	unknown *
4	0.52	41.4	0.55	81.9	7.85	0.58	57.0	2821.4	9.58	unknown *
5	0.58	57.4	0.60	89.7	8.59	0.63	38.6	2665.8	9.05	unknown *
6	0.64	37.2	0.68	196.3	18.80	0.70	119.0	5950.3	20.21	unknown *
7	0.70	120.7	0.72	197.1	18.88	0.76	36.4	5595.6	19.01	unknown *
8	0.83	36.0	0.85	63.3	6.06	0.93	3.6	2466.8	8.38	unknown *

Fig 50. Track 3. ID: *Achyranthes aspera* Linn seed-Petroleum ether extract



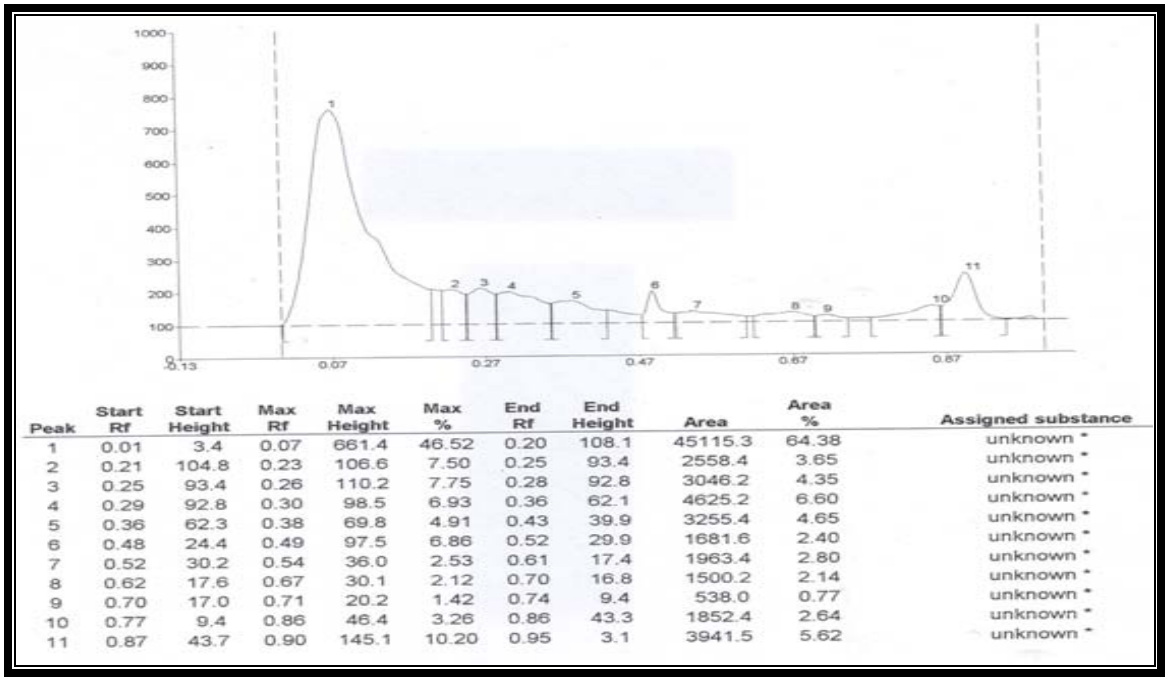
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.06	4.7	0.09	286.6	35.86	0.16	32.3	5848.8	21.65	unknown *
2	0.18	34.3	0.24	61.7	7.71	0.25	58.2	2761.7	10.22	unknown *
3	0.26	58.4	0.29	83.4	10.43	0.32	43.7	3121.4	11.56	unknown *
4	0.63	35.4	0.69	81.2	10.16	0.72	62.8	3794.0	14.05	unknown *
5	0.72	63.0	0.73	68.0	8.51	0.79	42.4	3022.9	11.19	unknown *
6	0.80	42.4	0.86	114.5	14.33	0.88	99.1	4598.9	17.03	unknown *
7	0.88	99.2	0.89	103.9	13.00	0.98	10.3	3863.1	14.30	unknown *

Fig 51. Track4. ID: *Achyranthes bidentata* Blume seed-Petroleum ether extract



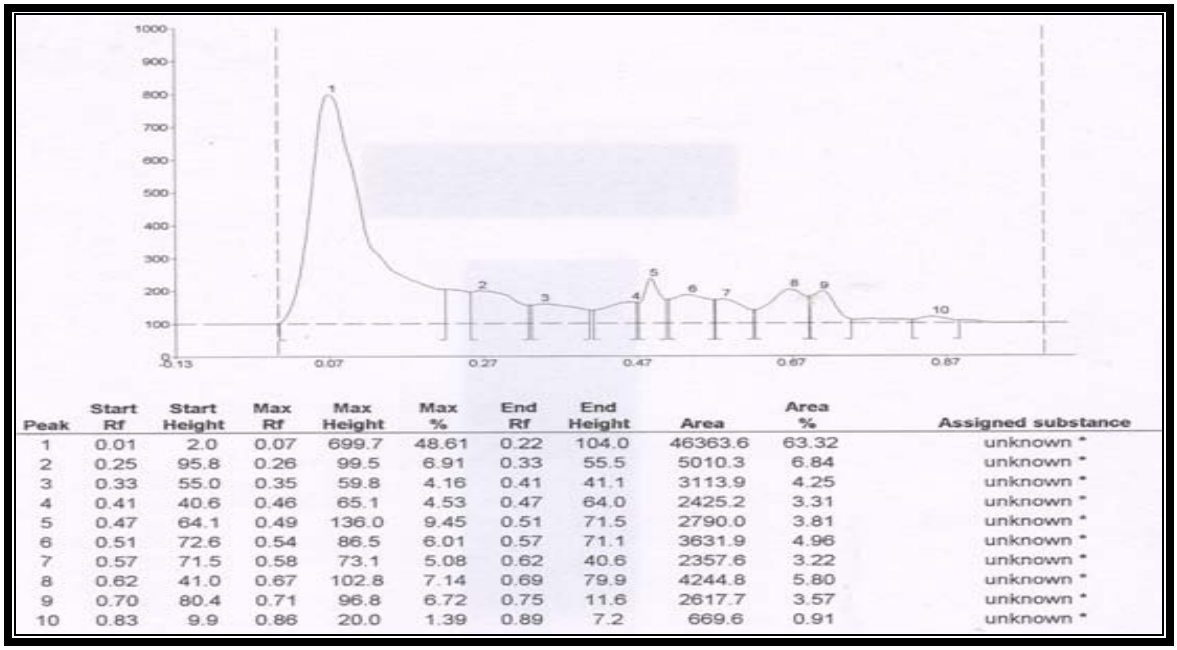
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	1.4	0.09	308.8	32.70	0.15	38.4	6660.1	25.04	unknown *
2	0.51	42.3	0.54	83.1	8.80	0.56	58.2	2913.3	10.95	unknown *
3	0.56	58.6	0.58	91.0	9.63	0.61	39.9	2715.6	10.21	unknown *
4	0.62	38.6	0.66	197.8	20.94	0.68	120.5	6020.3	22.64	unknown *
5	0.69	122.2	0.71	198.7	21.04	0.74	38.0	5666.3	21.31	unknown *
6	0.81	37.8	0.83	65.1	6.90	0.91	5.6	2619.3	9.85	unknown *

Fig 52. Track 1. ID: *Achyranthes aspera* Linn leaf-Chloroform extract



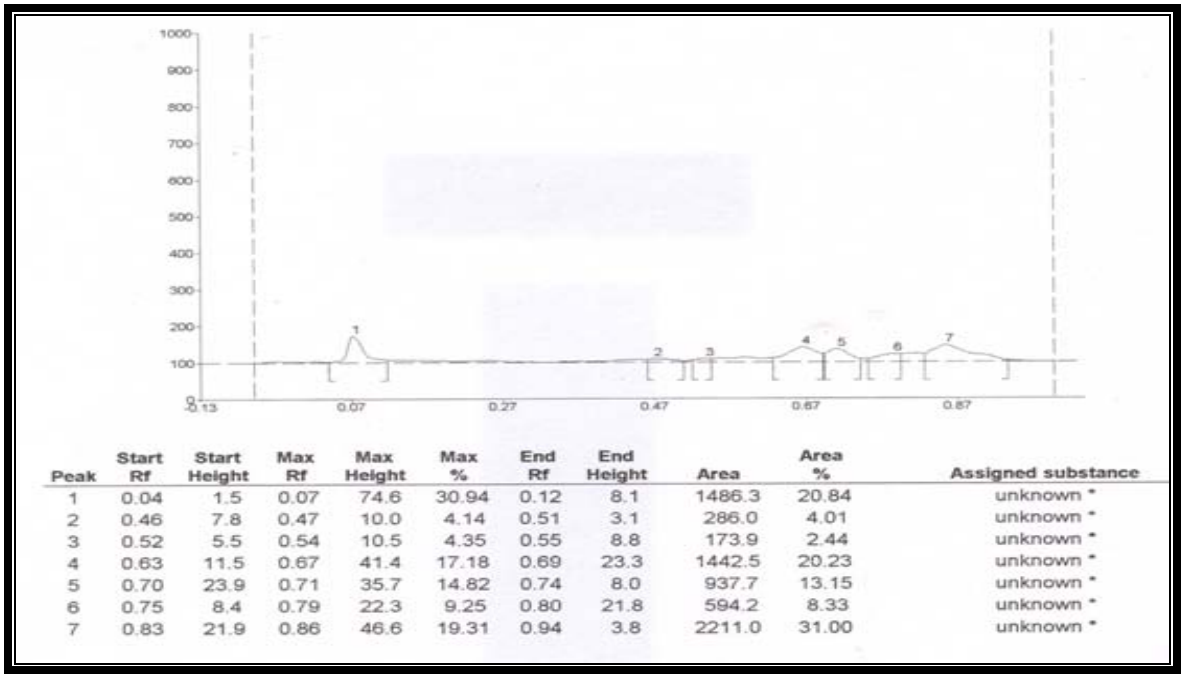
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	3.4	0.07	661.4	46.52	0.20	108.1	45115.3	64.38	unknown *
2	0.21	104.8	0.23	106.6	7.50	0.25	93.4	2558.4	3.65	unknown *
3	0.25	93.4	0.26	110.2	7.75	0.28	92.8	3046.2	4.35	unknown *
4	0.29	92.8	0.30	98.5	6.93	0.36	62.1	4625.2	6.60	unknown *
5	0.36	62.3	0.38	69.8	4.91	0.43	39.9	3255.4	4.65	unknown *
6	0.48	24.4	0.49	97.5	6.86	0.52	29.9	1681.6	2.40	unknown *
7	0.52	30.2	0.54	36.0	2.53	0.61	17.4	1963.4	2.80	unknown *
8	0.62	17.6	0.67	30.1	2.12	0.70	16.8	1500.2	2.14	unknown *
9	0.70	17.0	0.71	20.2	1.42	0.74	9.4	538.0	0.77	unknown *
10	0.77	9.4	0.86	46.4	3.26	0.86	43.3	1852.4	2.64	unknown *
11	0.87	43.7	0.90	145.1	10.20	0.95	3.1	3941.5	5.62	unknown *

Fig 53 . Track 2. ID: *Achyranthes bidentata* Blume leaf-Chloroform extract



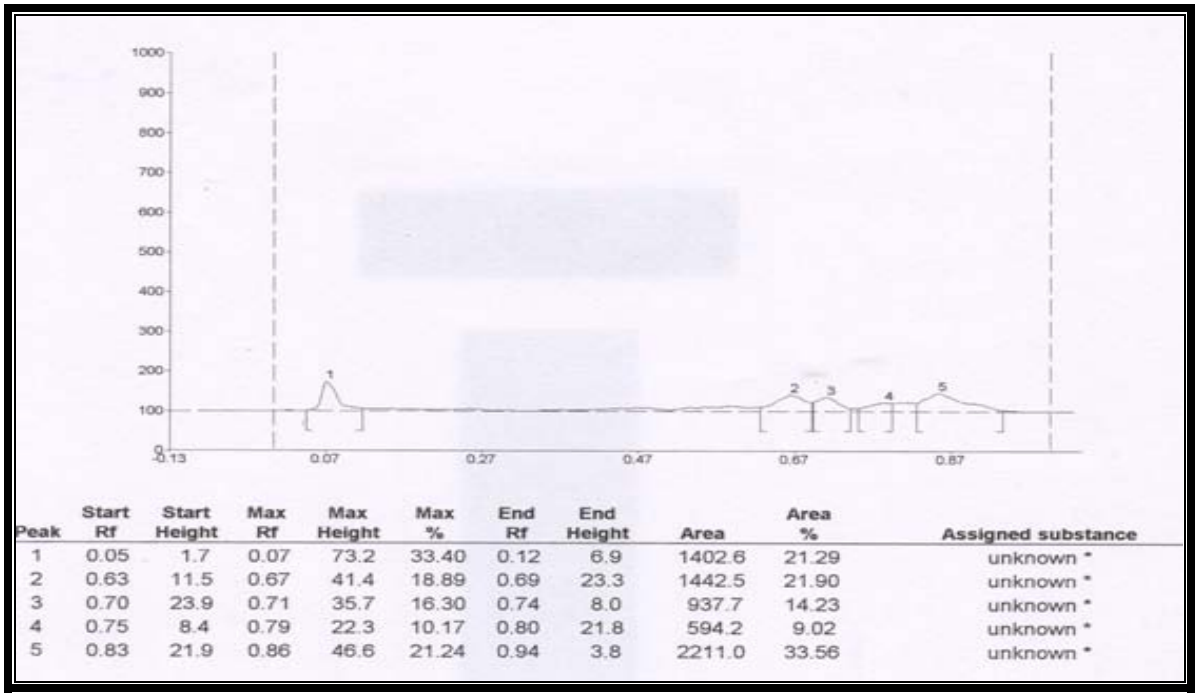
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	2.0	0.07	699.7	48.61	0.22	104.0	46363.6	63.32	unknown *
2	0.25	95.8	0.26	99.5	6.91	0.33	55.5	5010.3	6.84	unknown *
3	0.33	55.0	0.35	59.8	4.16	0.41	41.1	3113.9	4.25	unknown *
4	0.41	40.6	0.46	65.1	4.53	0.47	64.0	2425.2	3.31	unknown *
5	0.47	64.1	0.49	136.0	9.45	0.51	71.5	2790.0	3.81	unknown *
6	0.51	72.6	0.54	86.5	6.01	0.57	71.1	3631.9	4.96	unknown *
7	0.57	71.5	0.58	73.1	5.08	0.62	40.6	2357.6	3.22	unknown *
8	0.62	41.0	0.67	102.8	7.14	0.69	79.9	4244.8	5.80	unknown *
9	0.70	80.4	0.71	96.8	6.72	0.75	11.6	2617.7	3.57	unknown *
10	0.83	9.9	0.86	20.0	1.39	0.89	7.2	669.6	0.91	unknown *

Fig 54. Track 3. ID: *Achyranthes aspera* Linn seed-Chloroform extract



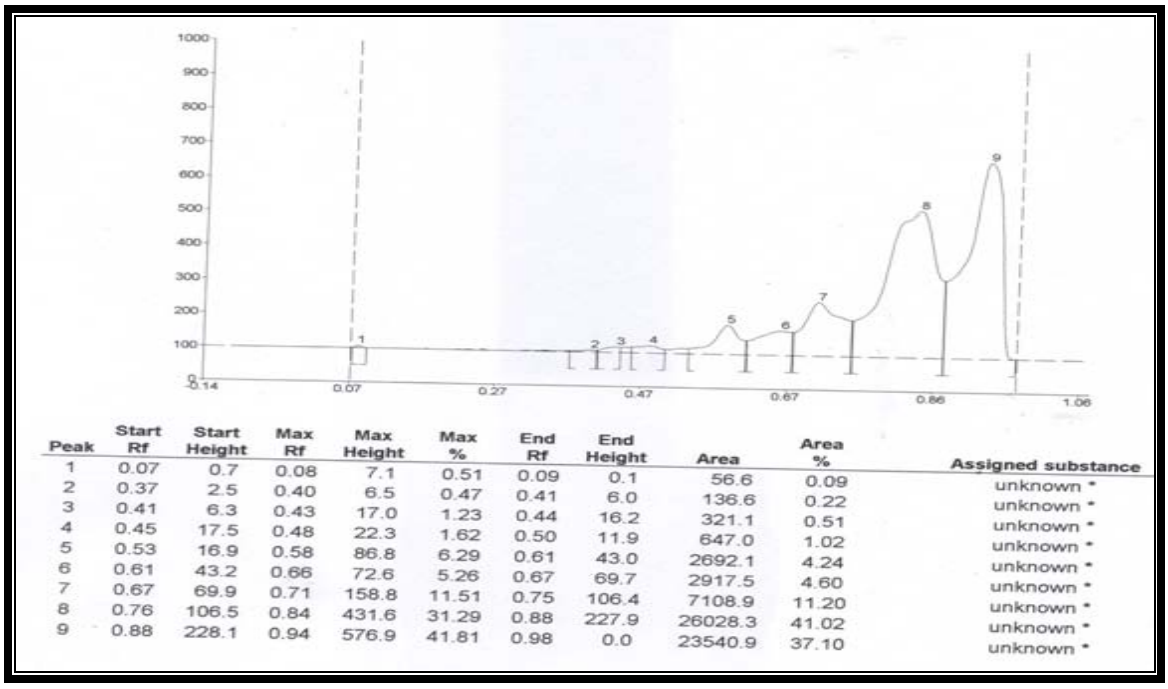
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	1.5	0.07	74.6	30.94	0.12	8.1	1486.3	20.84	unknown *
2	0.46	7.8	0.47	10.0	4.14	0.51	3.1	286.0	4.01	unknown *
3	0.52	5.5	0.54	10.5	4.35	0.55	8.8	173.9	2.44	unknown *
4	0.63	11.5	0.67	41.4	17.18	0.69	23.3	1442.5	20.23	unknown *
5	0.70	23.9	0.71	35.7	14.82	0.74	8.0	937.7	13.15	unknown *
6	0.75	8.4	0.79	22.3	9.25	0.80	21.8	594.2	8.33	unknown *
7	0.83	21.9	0.86	46.6	19.31	0.94	3.8	2211.0	31.00	unknown *

Fig 55. Track 4. ID: *Achyranthes bidentata* Blume seed-Chloroform extract



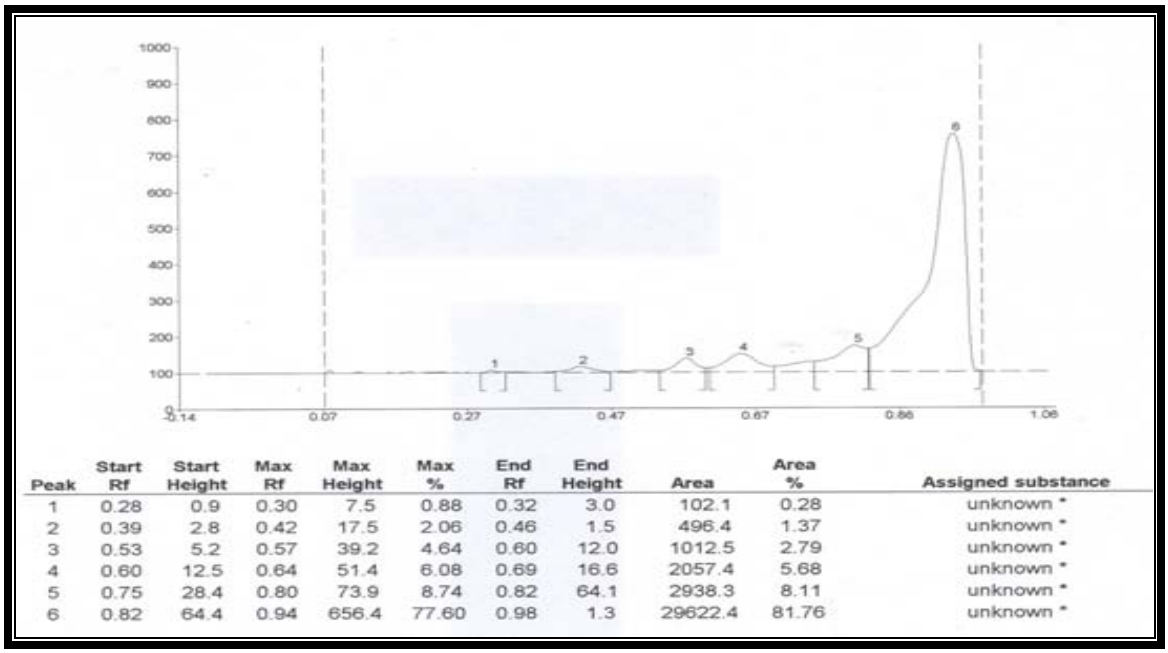
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	1.7	0.07	73.2	33.40	0.12	6.9	1402.6	21.29	unknown *
2	0.63	11.5	0.67	41.4	18.89	0.69	23.3	1442.5	21.90	unknown *
3	0.70	23.9	0.71	35.7	16.30	0.74	8.0	937.7	14.23	unknown *
4	0.75	8.4	0.79	22.3	10.17	0.80	21.8	594.2	9.02	unknown *
5	0.83	21.9	0.86	46.6	21.24	0.94	3.8	2211.0	33.56	unknown *

Fig 56. Track 1. ID: *Achyranthes aspera* Linn leaf-ethyl acetate extract



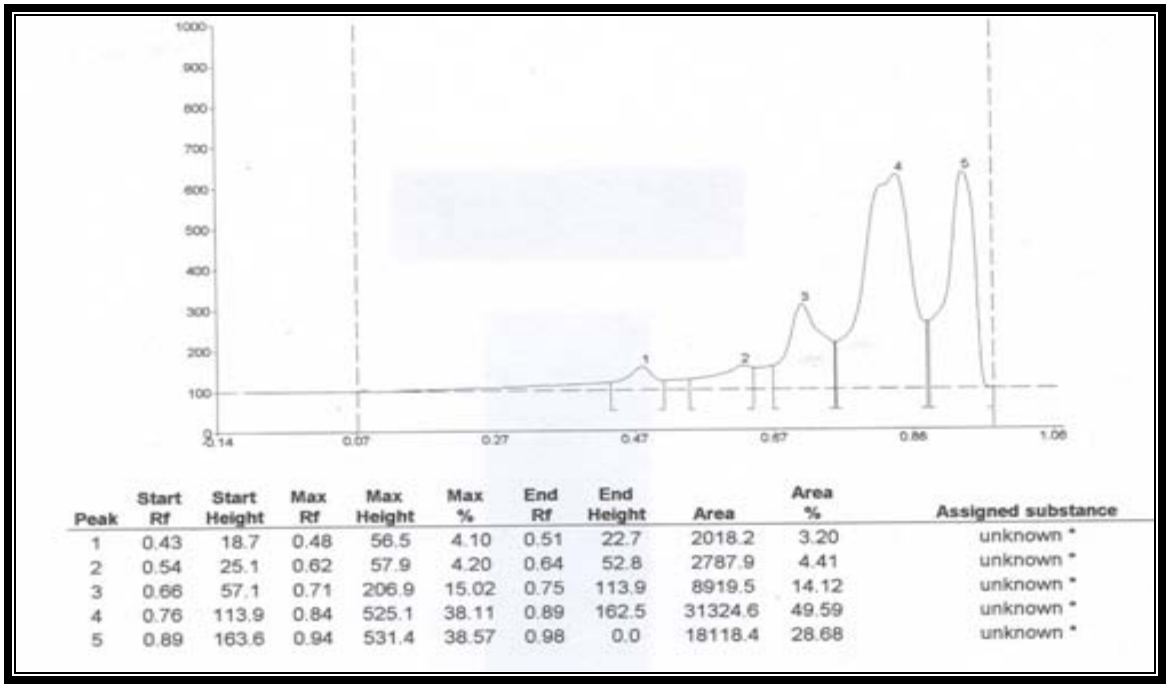
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	0.7	0.08	7.1	0.51	0.09	0.1	56.6	0.09	unknown *
2	0.37	2.5	0.40	6.5	0.47	0.41	6.0	136.6	0.22	unknown *
3	0.41	6.3	0.43	17.0	1.23	0.44	16.2	321.1	0.51	unknown *
4	0.45	17.5	0.48	22.3	1.62	0.50	11.9	647.0	1.02	unknown *
5	0.53	16.9	0.58	86.8	6.29	0.61	43.0	2692.1	4.24	unknown *
6	0.61	43.2	0.66	72.6	5.26	0.67	69.7	2917.5	4.60	unknown *
7	0.67	69.9	0.71	158.8	11.51	0.75	106.4	7108.9	11.20	unknown *
8	0.76	106.5	0.84	431.6	31.29	0.88	227.9	26028.3	41.02	unknown *
9	0.88	228.1	0.94	576.9	41.81	0.98	0.0	23540.9	37.10	unknown *

Fig 57. Track 2. ID: *Achyranthes bidentata* Blume leaf-ethyl acetate extract



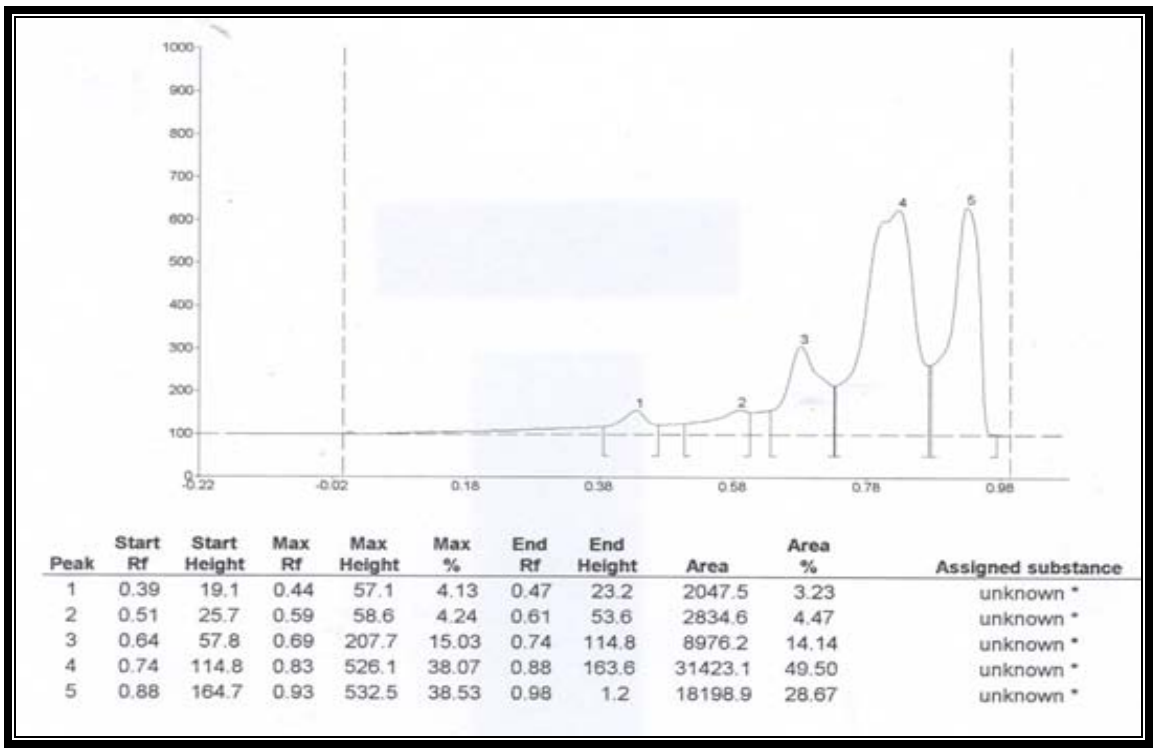
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.28	0.9	0.30	7.5	0.88	0.32	3.0	102.1	0.28	unknown *
2	0.39	2.8	0.42	17.5	2.06	0.46	1.5	496.4	1.37	unknown *
3	0.53	5.2	0.57	39.2	4.64	0.60	12.0	1012.5	2.79	unknown *
4	0.60	12.5	0.64	51.4	6.08	0.69	16.6	2057.4	5.68	unknown *
5	0.75	28.4	0.80	73.9	8.74	0.82	64.1	2938.3	8.11	unknown *
6	0.82	64.4	0.94	656.4	77.60	0.98	1.3	29622.4	81.76	unknown *

Fig58. Track 3. ID: *Achyranthes aspera* Linn seed-ethyl acetate extract



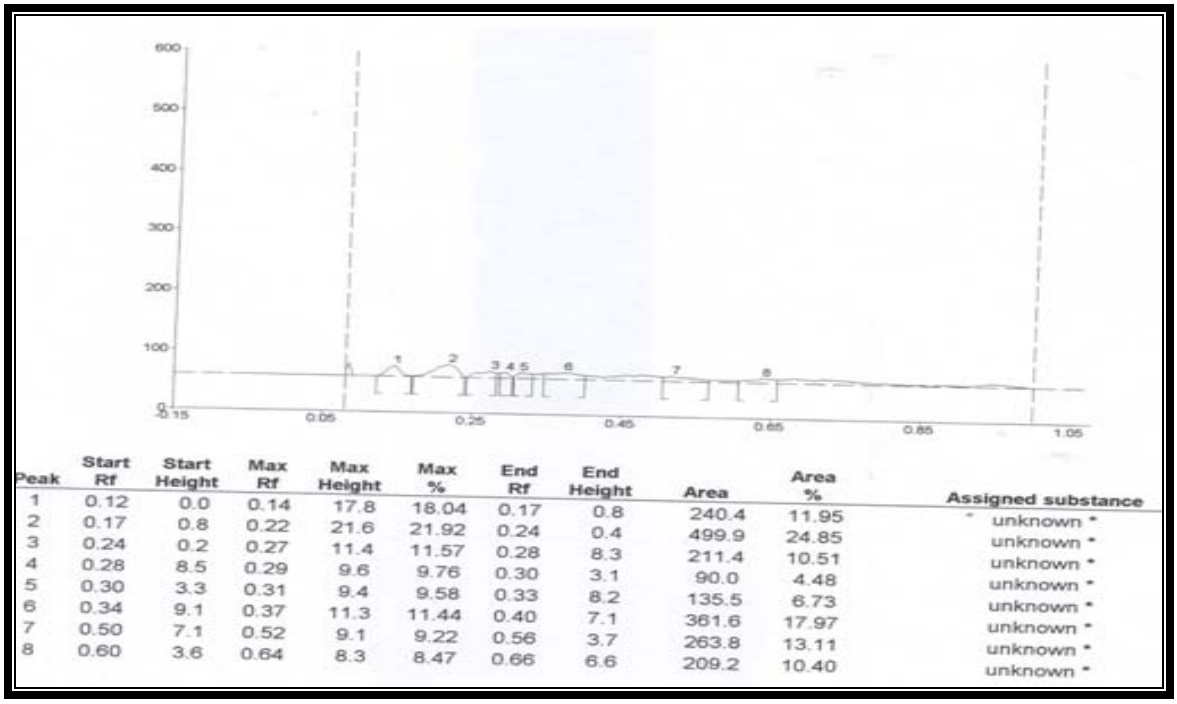
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.43	18.7	0.48	56.5	4.10	0.51	22.7	2018.2	3.20	unknown *
2	0.54	25.1	0.62	57.9	4.20	0.64	52.8	2787.9	4.41	unknown *
3	0.66	57.1	0.71	206.9	15.02	0.75	113.9	8919.5	14.12	unknown *
4	0.76	113.9	0.84	525.1	38.11	0.89	162.5	31324.6	49.59	unknown *
5	0.89	163.6	0.94	531.4	38.57	0.98	0.0	18118.4	28.68	unknown *

Fig 59. Track 4. ID: *Achyranthes bidentata* Blume seed-ethyl acetate extract



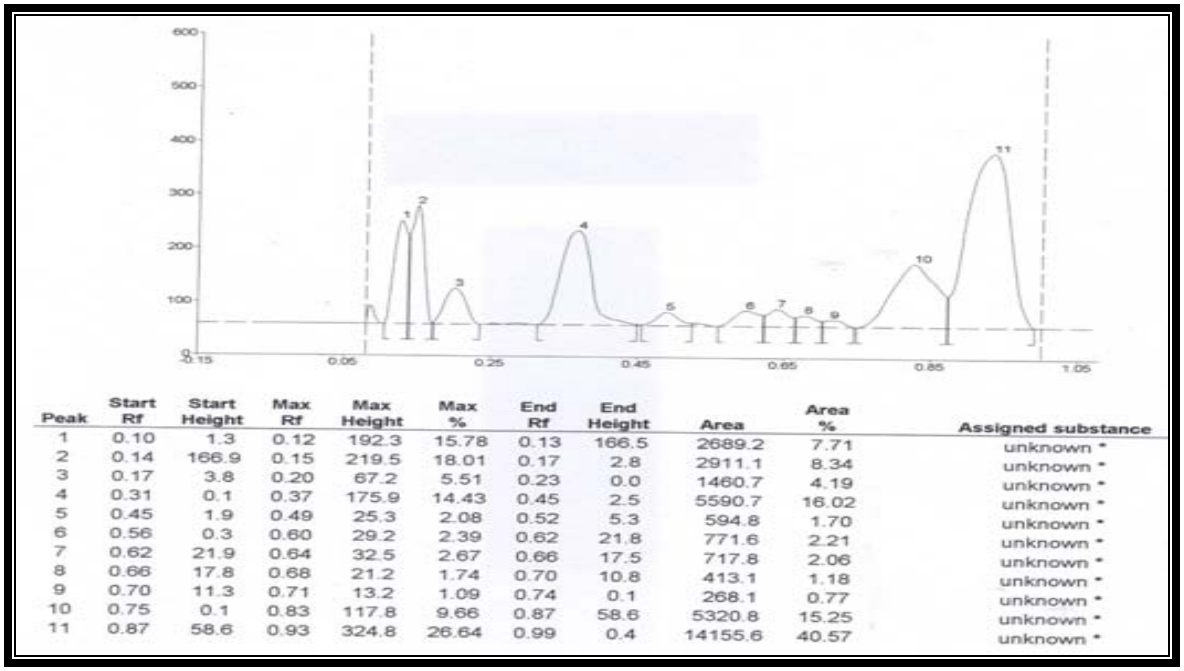
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.39	19.1	0.44	57.1	4.13	0.47	23.2	2047.5	3.23	unknown *
2	0.51	25.7	0.59	58.6	4.24	0.61	53.6	2834.6	4.47	unknown *
3	0.64	57.8	0.69	207.7	15.03	0.74	114.8	8976.2	14.14	unknown *
4	0.74	114.8	0.83	526.1	38.07	0.88	163.6	31423.1	49.50	unknown *
5	0.88	164.7	0.93	532.5	38.53	0.98	1.2	18198.9	28.67	unknown *

Fig60. Track 1. ID: *Achyranthes aspera* Linn leaf-Acetone extract



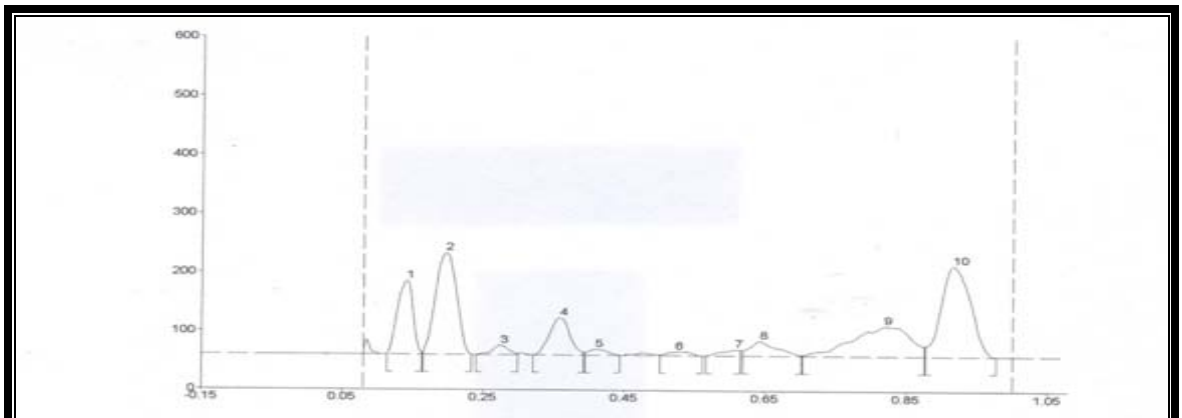
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.12	0.0	0.14	17.8	18.04	0.17	0.8	240.4	11.95	unknown *
2	0.17	0.8	0.22	21.6	21.92	0.24	0.4	499.9	24.85	unknown *
3	0.24	0.2	0.27	11.4	11.57	0.28	8.3	211.4	10.51	unknown *
4	0.28	8.5	0.29	9.6	9.76	0.30	3.1	90.0	4.48	unknown *
5	0.30	3.3	0.31	9.4	9.58	0.33	8.2	135.5	6.73	unknown *
6	0.34	9.1	0.37	11.3	11.44	0.40	7.1	361.6	17.97	unknown *
7	0.50	7.1	0.52	9.1	9.22	0.56	3.7	263.8	13.11	unknown *
8	0.60	3.6	0.64	8.3	8.47	0.66	6.6	209.2	10.40	unknown *

Fig 61. Track 2. ID: *Achyranthes bidentata* Blume leaf-Acetone extract



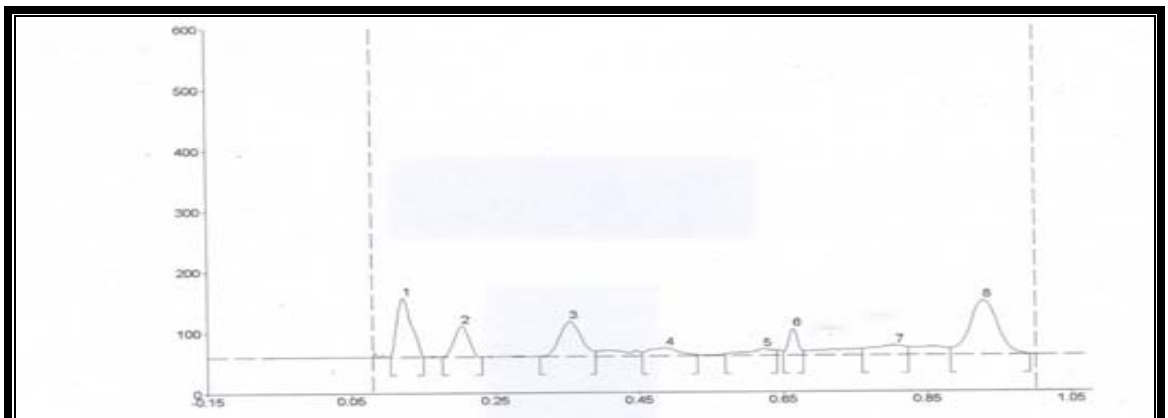
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.10	1.3	0.12	192.3	15.78	0.13	166.5	2689.2	7.71	unknown *
2	0.14	166.9	0.15	219.5	18.01	0.17	2.8	2911.1	8.34	unknown *
3	0.17	3.8	0.20	67.2	5.51	0.23	0.0	1460.7	4.19	unknown *
4	0.31	0.1	0.37	175.9	14.43	0.45	2.5	5590.7	16.02	unknown *
5	0.45	1.9	0.49	25.3	2.08	0.52	5.3	594.8	1.70	unknown *
6	0.56	0.3	0.60	29.2	2.39	0.62	21.8	771.6	2.21	unknown *
7	0.62	21.9	0.64	32.5	2.67	0.66	17.5	717.8	2.06	unknown *
8	0.66	17.8	0.68	21.2	1.74	0.70	10.8	413.1	1.18	unknown *
9	0.70	11.3	0.71	13.2	1.09	0.74	0.1	268.1	0.77	unknown *
10	0.75	0.1	0.83	117.8	9.66	0.87	58.6	5320.8	15.25	unknown *
11	0.87	58.6	0.93	324.8	26.64	0.99	0.4	14155.6	40.57	unknown *

Fig 62. Track 3. ID: *Achyranthes aspera* Linn seed-Acetone extract



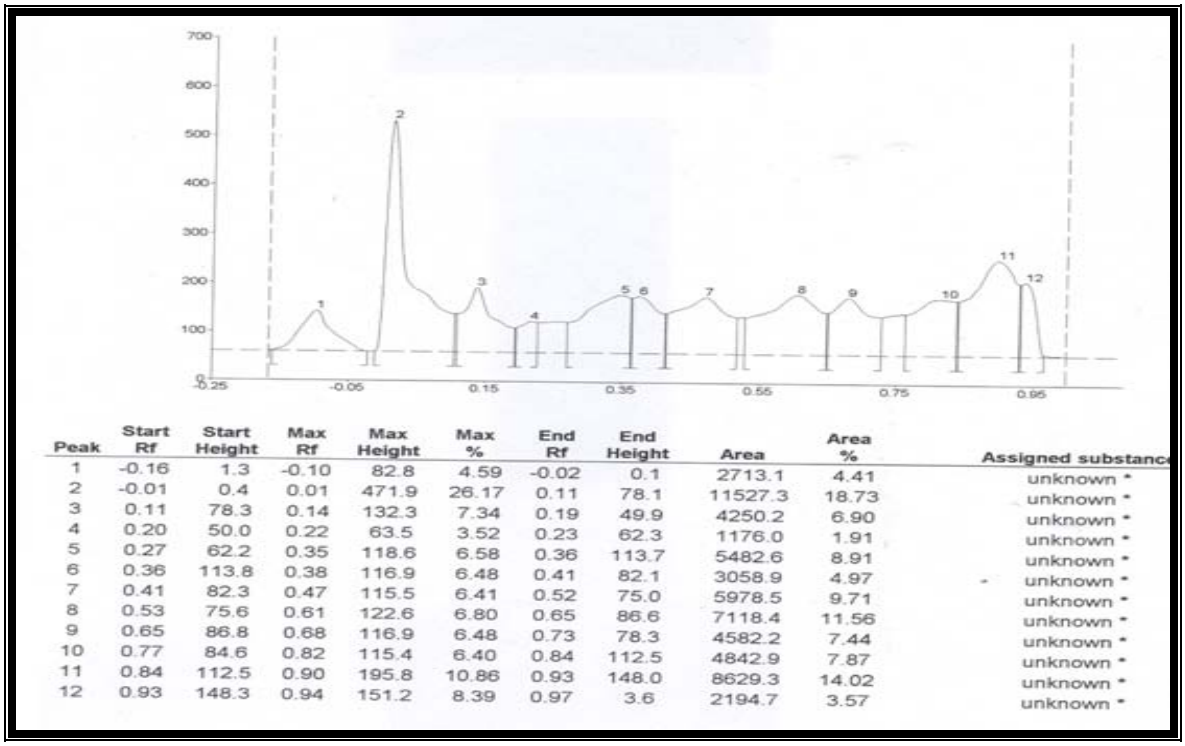
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.11	0.8	0.14	125.9	19.74	0.16	4.8	2100.0	12.22	unknown *
2	0.16	6.0	0.19	173.3	27.17	0.23	0.2	3923.5	22.84	unknown *
3	0.24	0.1	0.27	16.0	2.51	0.30	2.7	285.1	1.66	unknown *
4	0.32	0.2	0.36	63.7	9.99	0.39	5.0	1399.9	8.15	unknown *
5	0.39	5.0	0.41	10.3	1.61	0.44	0.5	212.7	1.24	unknown *
6	0.50	1.5	0.52	6.6	1.03	0.56	0.2	174.7	1.02	unknown *
7	0.56	0.1	0.61	10.6	1.66	0.61	10.3	219.8	1.28	unknown *
8	0.62	10.1	0.64	25.4	3.99	0.70	1.9	745.0	4.34	unknown *
9	0.70	2.1	0.82	51.6	8.10	0.88	18.5	3264.3	19.00	unknown *
10	0.88	18.6	0.92	154.3	24.19	0.98	0.1	4855.7	28.26	unknown *

Fig 63. Track 4. ID: *Achyranthes bidentata* Blume seed-Acetone extract



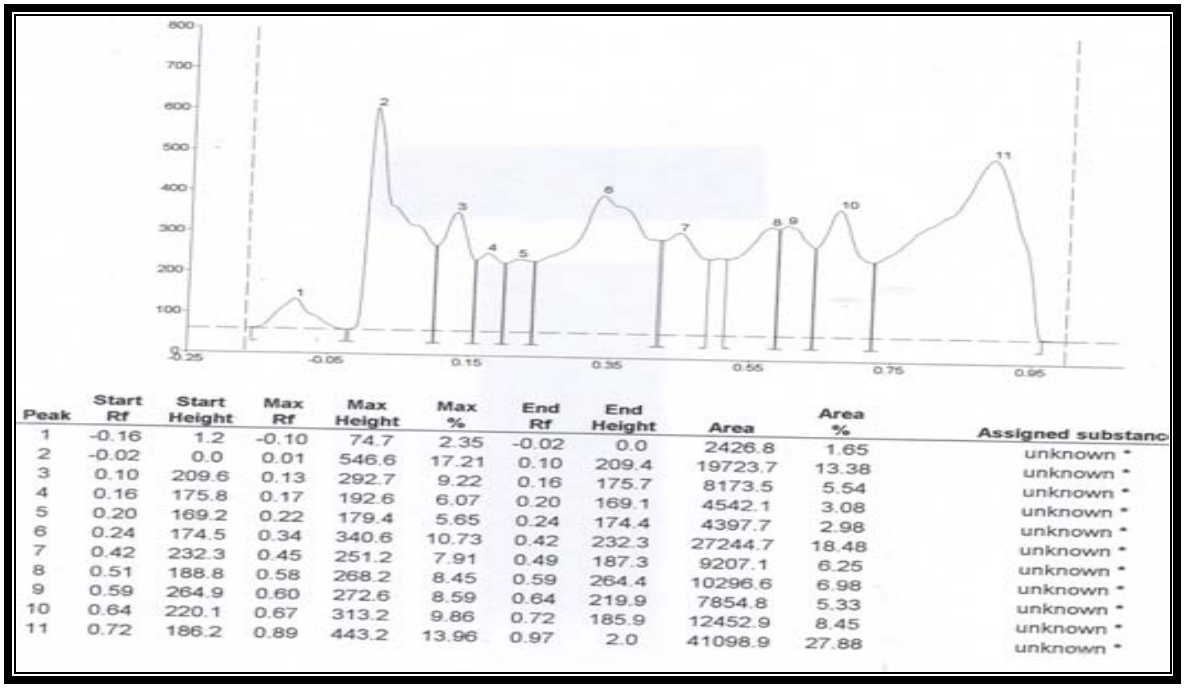
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.10	1.8	0.12	97.1	25.61	0.15	1.0	1489.4	18.53	unknown *
2	0.17	0.2	0.20	50.9	13.42	0.23	0.0	792.0	9.86	unknown *
3	0.31	0.6	0.35	57.9	15.28	0.39	8.6	1323.2	16.47	unknown *
4	0.45	6.1	0.49	13.4	3.53	0.53	1.6	430.5	5.36	unknown *
5	0.57	2.7	0.62	11.1	2.93	0.64	8.7	330.0	4.11	unknown *
6	0.65	7.1	0.66	44.0	11.61	0.68	7.2	429.0	5.34	unknown *
7	0.76	10.3	0.81	15.7	4.14	0.82	13.0	580.2	7.22	unknown *
8	0.88	11.0	0.93	89.0	23.49	0.99	0.2	2661.7	33.12	unknown *

Fig 64. Track 1. ID: *Achyranthes aspera* Linn leaf-Ethanolic extract



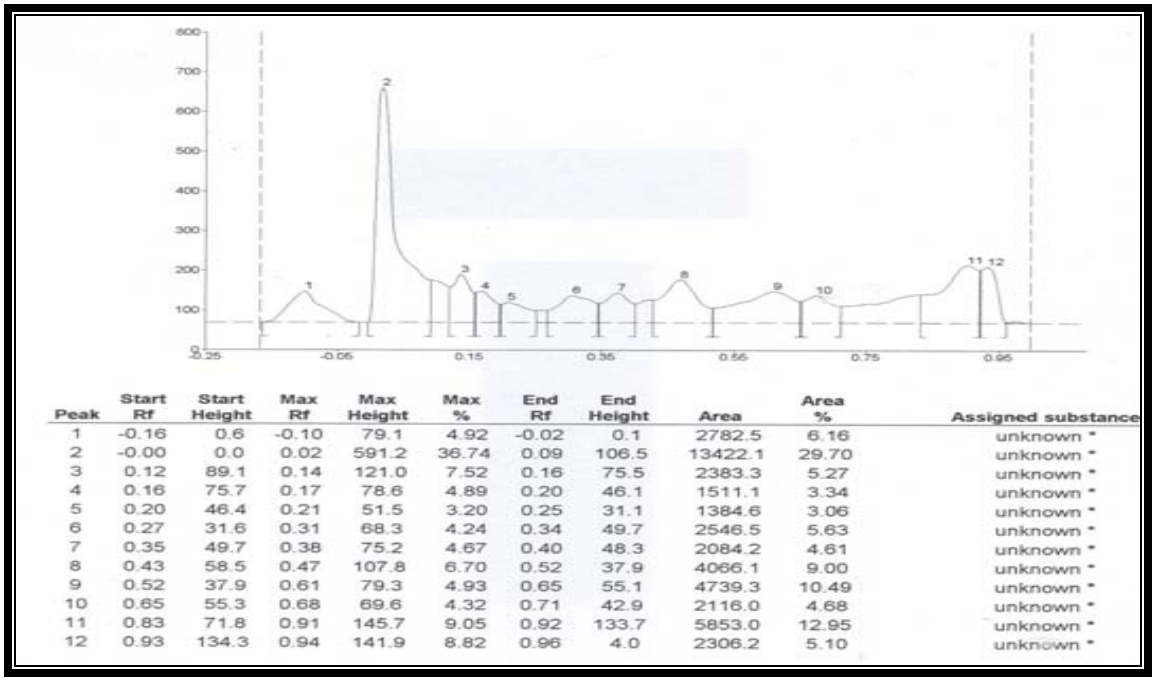
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substanc
1	-0.16	1.3	-0.10	82.8	4.59	-0.02	0.1	2713.1	4.41	unknown *
2	-0.01	0.4	0.01	471.9	26.17	0.11	78.1	11527.3	18.73	unknown *
3	0.11	78.3	0.14	132.3	7.34	0.19	49.9	4250.2	6.90	unknown *
4	0.20	50.0	0.22	63.5	3.52	0.23	62.3	1176.0	1.91	unknown *
5	0.27	62.2	0.35	118.6	6.58	0.36	113.7	5482.6	8.91	unknown *
6	0.36	113.8	0.38	116.9	6.48	0.41	82.1	3058.9	4.97	unknown *
7	0.41	82.3	0.47	115.5	6.41	0.52	75.0	5978.5	9.71	unknown *
8	0.53	75.6	0.61	122.6	6.80	0.65	86.6	7118.4	11.56	unknown *
9	0.65	86.8	0.68	116.9	6.48	0.73	78.3	4582.2	7.44	unknown *
10	0.77	84.6	0.82	115.4	6.40	0.84	112.5	4842.9	7.87	unknown *
11	0.84	112.5	0.90	195.8	10.86	0.93	148.0	8629.3	14.02	unknown *
12	0.93	148.3	0.94	151.2	8.39	0.97	3.6	2194.7	3.57	unknown *

Fig 65. Track 2. ID: *Achyranthes bidentata* Blume leaf-Ethanolic extract



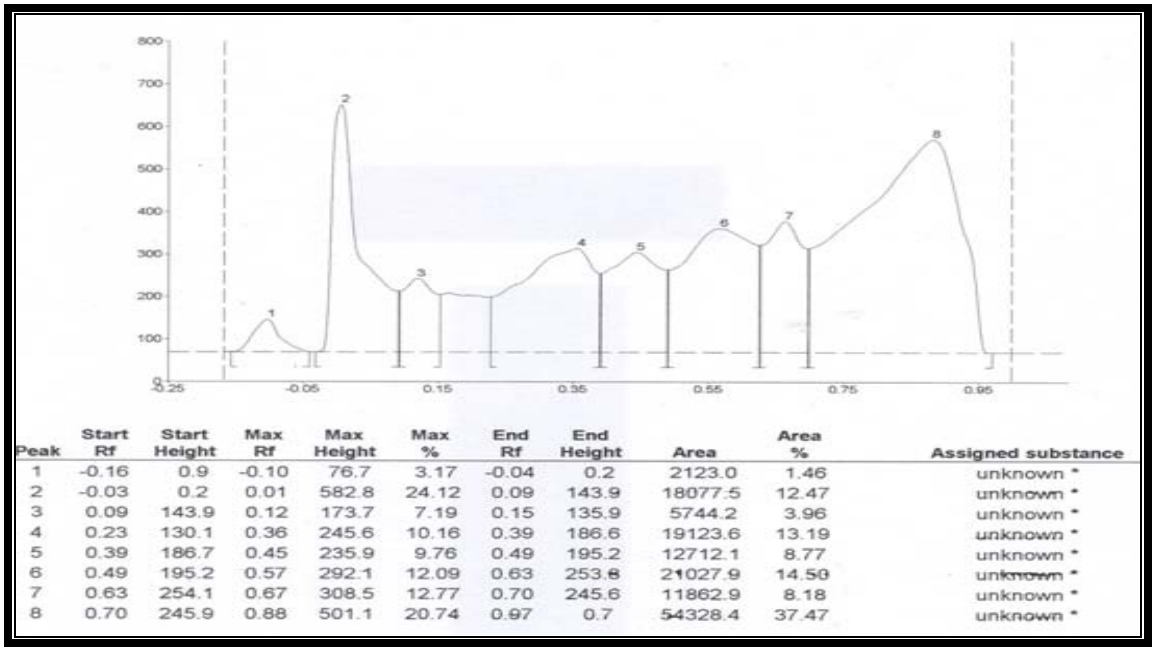
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substanc
1	-0.16	1.2	-0.10	74.7	2.35	-0.02	0.0	2426.8	1.65	unknown *
2	-0.02	0.0	0.01	546.6	17.21	0.10	209.4	19723.7	13.38	unknown *
3	0.10	209.6	0.13	292.7	9.22	0.16	175.7	8173.5	5.54	unknown *
4	0.16	175.8	0.17	192.6	6.07	0.20	169.1	4542.1	3.08	unknown *
5	0.20	169.2	0.22	179.4	5.65	0.24	174.4	4397.7	2.98	unknown *
6	0.24	174.5	0.34	340.6	10.73	0.42	232.3	27244.7	18.48	unknown *
7	0.42	232.3	0.45	251.2	7.91	0.49	187.3	9207.1	6.25	unknown *
8	0.51	188.8	0.58	268.2	8.45	0.59	264.4	10296.6	6.98	unknown *
9	0.59	264.9	0.60	272.6	8.59	0.64	219.9	7854.8	5.33	unknown *
10	0.64	220.1	0.67	313.2	9.86	0.72	185.9	12452.9	8.45	unknown *
11	0.72	186.2	0.89	443.2	13.96	0.97	2.0	41098.9	27.88	unknown *

Fig 66. Track 3. ID: *Achyranthes aspera* Linn seed-Ethanolic extract



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.16	0.6	-0.10	79.1	4.92	-0.02	0.1	2782.5	6.16	unknown *
2	-0.00	0.0	0.02	591.2	36.74	0.09	106.5	13422.1	29.70	unknown *
3	0.12	89.1	0.14	121.0	7.52	0.16	75.5	2383.3	5.27	unknown *
4	0.16	75.7	0.17	78.6	4.89	0.20	46.1	1511.1	3.34	unknown *
5	0.20	46.4	0.21	51.5	3.20	0.25	31.1	1384.6	3.06	unknown *
6	0.27	31.6	0.31	68.3	4.24	0.34	49.7	2546.5	5.63	unknown *
7	0.35	49.7	0.38	75.2	4.67	0.40	48.3	2084.2	4.61	unknown *
8	0.43	58.5	0.47	107.8	6.70	0.52	37.9	4066.1	9.00	unknown *
9	0.52	37.9	0.61	79.3	4.93	0.65	55.1	4739.3	10.49	unknown *
10	0.65	55.3	0.68	69.6	4.32	0.71	42.9	2116.0	4.68	unknown *
11	0.83	71.8	0.91	145.7	9.05	0.92	133.7	5853.0	12.95	unknown *
12	0.93	134.3	0.94	141.9	8.82	0.96	4.0	2306.2	5.10	unknown *

Fig 67. Track 4. ID: *Achyranthes bidentata* Blume seed-Ethanolic extract



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.16	0.9	-0.10	76.7	3.17	-0.04	0.2	2123.0	1.46	unknown *
2	-0.03	0.2	0.01	582.8	24.12	0.09	143.9	18077.5	12.47	unknown *
3	0.09	143.9	0.12	173.7	7.19	0.15	135.9	5744.2	3.96	unknown *
4	0.23	130.1	0.36	245.6	10.16	0.39	186.6	19123.6	13.19	unknown *
5	0.39	186.7	0.45	235.9	9.76	0.49	195.2	12712.1	8.77	unknown *
6	0.49	195.2	0.57	292.1	12.09	0.63	253.8	21027.9	14.50	unknown *
7	0.63	254.1	0.67	308.5	12.77	0.70	245.6	11862.9	8.18	unknown *
8	0.70	245.9	0.88	501.1	20.74	0.97	0.7	54328.4	37.47	unknown *

6.5 Antihyperlipidemic screening

Coronary heart disease and its complications like acute myocardial infection and sudden cardiac death is a sequel to competitive life styles, which in turn pose, a challenge to the human ability to withstand emotional stress and strain, mental tension and pressure. The etiology and pathogenicity of coronary disease lie embedded in the casual relationship between the development of atherosclerosis, elevated plasma lipid levels, high levels, high levels of cholesterol in blood and plasma, genetic makeup, endocrinological aberration, immunologic and autonomic factors, blood flow and coagulation. Treatments designed to diminish the clinical impact of coronary artery disease (CAD) have two fundamental goals. The first goal is to reduce the symptomatic limitations imposed by an obstructed arterial blood supply which is unable to meet the peak oxygen demands of the myocardium. The second goal, namely lipid lowering treatment, is to prevent clinical events by causing selective regression of the lipid rich subgroup of plaques which are vulnerable to fissuring, ulceration and hemorrhage.

It is generally agreed that diet and weight control are the first line treatment of CAD patients with high cholesterol or triglyceride blood levels. However, there are patients who do not respond adequately to non-drug management, and in such cases, the use of lipid lowering agent is considered necessary. The choice of lipid reducing agent is usually related to the dominant abnormality (elevated plasma concentrations of cholesterol or triglyceride, or both).

In the present study, leaf and seed extracts of *Achyranthes aspera* and *Achyranthes bidentata* were administered to rats for screening triton induced hyperlipidemia and diet induced hyperlipidemia.

6.5.1 Triton induced hyperlipidemia

6.5.1.1. Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes aspera* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

The study revealed that triton induced group showed significant ($P<0.001$) increase in the levels of cholesterol, triglyceride, LDL, VLDL cholesterol and decrease in the levels of HDL cholesterol. Treatment with aqueous extract of AA leaf (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AA leaf (100 mg/kg and 200 mg/kg) showed significant activity ($P<0.001$) in reducing the levels of cholesterol. 50% ethanol extract of AA leaf (200 mg/kg) significantly decreased the levels of triglycerides when compared to negative control and 50% ethanol extract of AA leaf (100 mg/kg) showing that the extract is possessing dose dependent activity.

Controversially, treatment with aqueous extract of AA leaf (100 mg/kg) produced significant ($P<0.05$) activity in increasing the levels of HDL cholesterol whereas such effect was not observed aqueous extract of AA (200 mg/kg). Aqueous extract of AA leaf (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AA leaf (100 mg/kg and 200 mg/kg) significantly decreased the levels of LDL cholesterol. Treatment with aqueous extract of AA leaf (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AA leaf (100 mg/kg and 200 mg/kg) showed significant activity in reducing the levels of VLDL cholesterol.The 50% ethanol extract of AA leaf at a dose of 200 mg/kg produced significant inhibition of cholesterol, VLDL ($P<0.001$) and LDL ($P<0.01$) whereas such effect was not observed in the case of HDL showing that the levels of HDL are maintained. Further, it possessed the equipotent activity with atorvastatin. (Table 37; Figure 68)

Table 37. Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes aspera* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 h	24h	0 h	24h	0 h	24h	0 h	24h	0 h	24h
Control(G1)	47.8 ±3.6	47.8 ±3.6	56.4 ±1.78	56.4 ±1.7	34.2 ±1.04	34.2 ±1.04	20.65 ±3.54	20.65 ±3.54	10.8 ±0.35	10.8 ±0.3
Triton(G2)	62.54 ±4.59	108.82 ±9.70$^{###}$	44.53 ±2.16	74.62 ±6.7$^{###}$	35.4 ±2.32	26.36 ±1.57$^{###}$	22.30 ±3.87	35.87 ±8.36$^{###}$	7.56 ±2.97	20.5 ±4.7$^{###}$
Atorvastatin (G3)	53.52 ±4.4	63.24 ±2.30***	35.76 ±4.7	53.86 ±3.2***	31.13 ±3.6	27.53 ±2.14	15.92 ±0.95	25.64 ±2.30**	7.99 ±1.98	11.4 ±3.5***
AA aqueous leaf extract (100)(G4)	64.76 ±3.1	68.63 ±4.32***	55.34 ±1.43	71.37 ±3.3	34.86 ±1.23	31.38 ±3.65*	24.72 ±1.69	28.63 ±1.92*	12.34 ±1.94	14.7 ±3.3*
AA aqueous leaf extract (200)(G5)	53.76 ±4.86	66.89 ±5.76***	44.65 ±3.39	70.75 ±2.5	33.34 ±2.34	30.25 ±2.55	21.94 ±1.86	28.89 ±1.76*	9.65 ±1.39	13.75 ±1.5**
AA 50% ethanol leaf extract (100)(G6)	57.87 ±5.5	65.46 ±6.6***	39.54 ±3.54	68.86 ±2.54	33.24 ±1.76	29.39 ±1.32	27.87 ±2.15	27.46 ±2.86*	9.54 ±2.32	14.8 ±1.2*
AA 50% ethanol leaf extract (200)(G7)	60.76 ±7.52	64.96 ±5.7***	38.66 ±5.85	55.86 ±2.73*** €€€	32.56 ±2.93	28.24 ±3.00	19.75 ±2.58	26.96 ±1.76**	8.66 ±1.06	12.6 ±2.7***

Values are expressed as mean±S.D; n=6.
$^{###}$-P<0.001 when compared G1 vs G2
*-P<0.05, **-P<0.01, ***-P<0.001 when compared G2 vs G3, G4, G5, G6 and G7
$^{€€€}$-P<0.001 when compared G6 vs G7

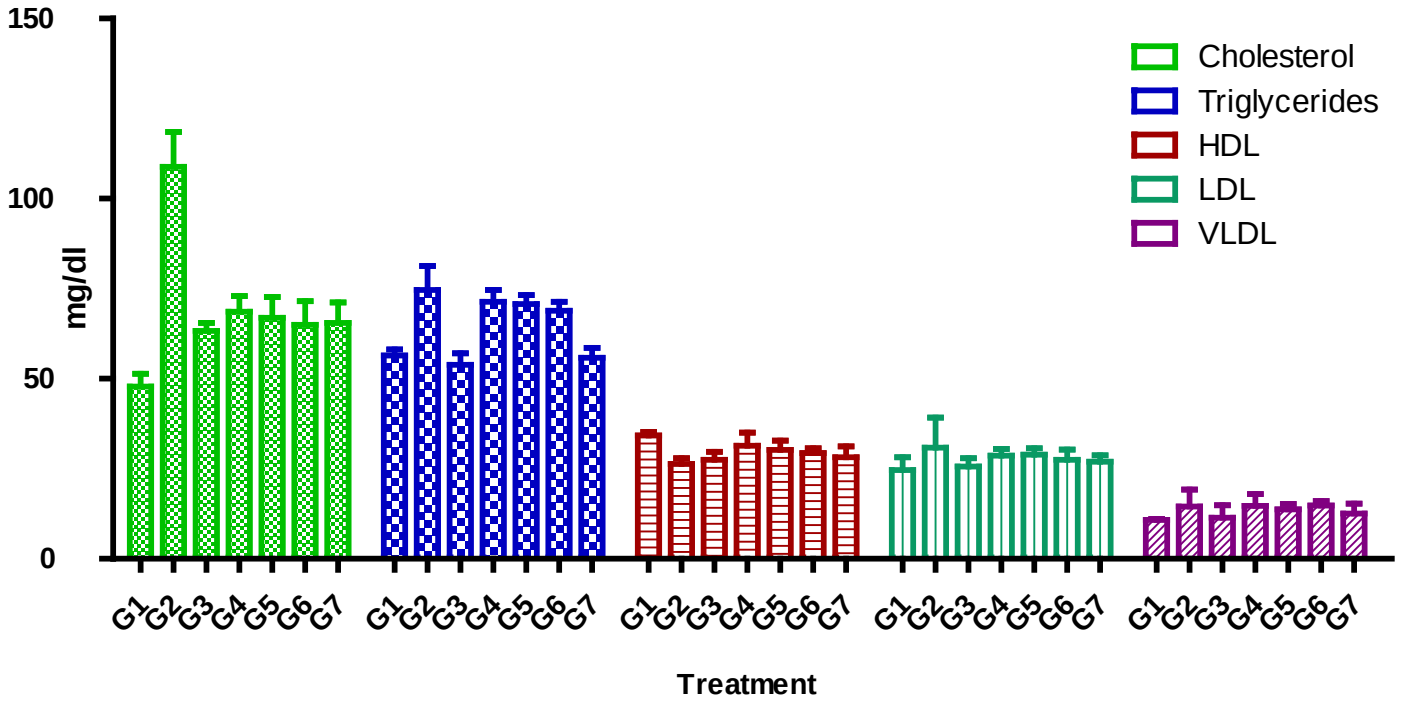


Fig 68. Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes aspera* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

G1=Control, G2=Triton, G3=Atorvastatin, G4=AA aqueous leaf extract(100), G5=AA aqueous leaf extract(200), G6= AA 50% ethanol leaf extract(100), G7= AA 50% ethanol leaf extract(200)

6.5.1.2. Effect of aqueous and 50% ethanol seed extracts of *Achyranthes aspera* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

The study revealed that triton induced group showed significant (P<0.001) increase in the levels of cholesterol, triglyceride, LDL, VLDL cholesterol and decrease in the levels of HDL cholesterol. Treatment with aqueous extract of AA seed (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AA seed (100 mg/kg and 200 mg/kg) showed significant activity (P<0.001) in reducing the levels of cholesterol. Aqueous extract of AA seed (200 mg/kg) showed significantly decreased (P<0.01) the levels of triglycerides. 50% ethanol extract of AA seed (100 mg/kg and 200 mg/kg) significantly (P<0.001) decreased the levels of triglycerides.

50% ethanol extract of AA seed (200 mg/kg) showed significantly (P<0.05) reduced the levels of LDL cholesterol. Treatment with Aqueous extract of AA seed (100 mg/kg and 200 mg/kg) showed significant activity (P<0.01) in reducing the levels of VLDL cholesterol. 50% ethanol extract of AA seed (100 mg/kg and 200 mg/kg) significantly (P<0.001) decreased the levels of VLDL cholesterol and showed equipotent activity with atorvastatin.

Both doses of 50% ethanol extract of AA seed significantly (P<0.001) reduced the levels of cholesterol, triglycerides and VLDL whereas only 200 mg/kg of 50% ethanol extract of AA seed produced a significant activity (P<0.05) in reducing the levels of LDL. However the levels of HDL were unchanged and maintained when compared to negative control. (Table 38; Figure 69)

Table 38: Effect of aqueous and 50% ethanol seed extracts of *Achyranthes aspera* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 h	24h	0 h	24h	0 h	24h	0 h	24h	0 h	24h
Control(G1)	47.8 ±3.6	47.8 ±3.6	56.4 ±1.78	56.4 ±1.7	34.2 ±1.04	34.2 ±1.04	20.65 ±3.54	20.65 ±3.54	10.8 ±0.35	10.8 ±0.3
Triton(G2)	62.54 ±4.59	108.82 ±9.70$^{###}$	44.53 ±2.16	74.62 ±6.7$^{###}$	35.4 ±2.32	26.36 ±1.57$^{###}$	22.30 ±3.87	35.87 ±8.36$^{###}$	12.56 ±2.97	14.5 ±4.7$^{###}$
Atorvastatin(G3)	53.52 ±4.4	63.24 ±2.30***	35.76 ±4.7	53.86 ±3.1***	31.13 ±3.65	27.53 ±2.14	15.92 ±0.95	25.64 ±2.30**	7.99 ±1.98	11.4 ±3.1***
AA aqueous seed extract(100)(G8)	62.75 ±2.89	76.68 ± 4.53***	58.53 ±1.46	68.93 ±3.1	44.78 ±1.53	28.69 ±2.97	26.67 ±1.57	32.47 ± 1.96	11.25 ±1.84	13.8 ±3.2**
AA aqueous seed extract(200)(G9)	61.67 ±4.86	68.45 ±4.96***	46.79 ±3.34	66.68** ±2.5	38.46 ±2.33	28.15 ±2.63	22.26 ±1.97	34.99 ±1.83	10.43 ±1.29	13.2 ±1.8**
AA 50% ethanol seed extract(100)(G10)	58.43 ±5.5	68.48 ±8.6***	43.44 ±3.83	63.96 ±2.2***	35.76 ±5.74	27.88 ±1.35	22.87 ±2.86	33.59 ±2.64	9.34 ±2.3	12.8 ±1.2***
AA 50% ethanol seed extract(200)(G11)	56.74 ±6.22	64.46 ±6.82***	45.73 ±6.85	59.53 ±2.3***	33.92 ±2.71	26.96 ±2.56	18.57 ±2.88	28.86 ±1.86*	8.69 ±1.3	12.2 ±2.7***

Values are expressed as mean±S.D; n=6.
$^{###}$-P<0.001 when compared G1 vs G2
*-P<0.05, **-P<0.01, ***-P<0.001 when compared G2 vs G3, G8, G9, G10 and G11

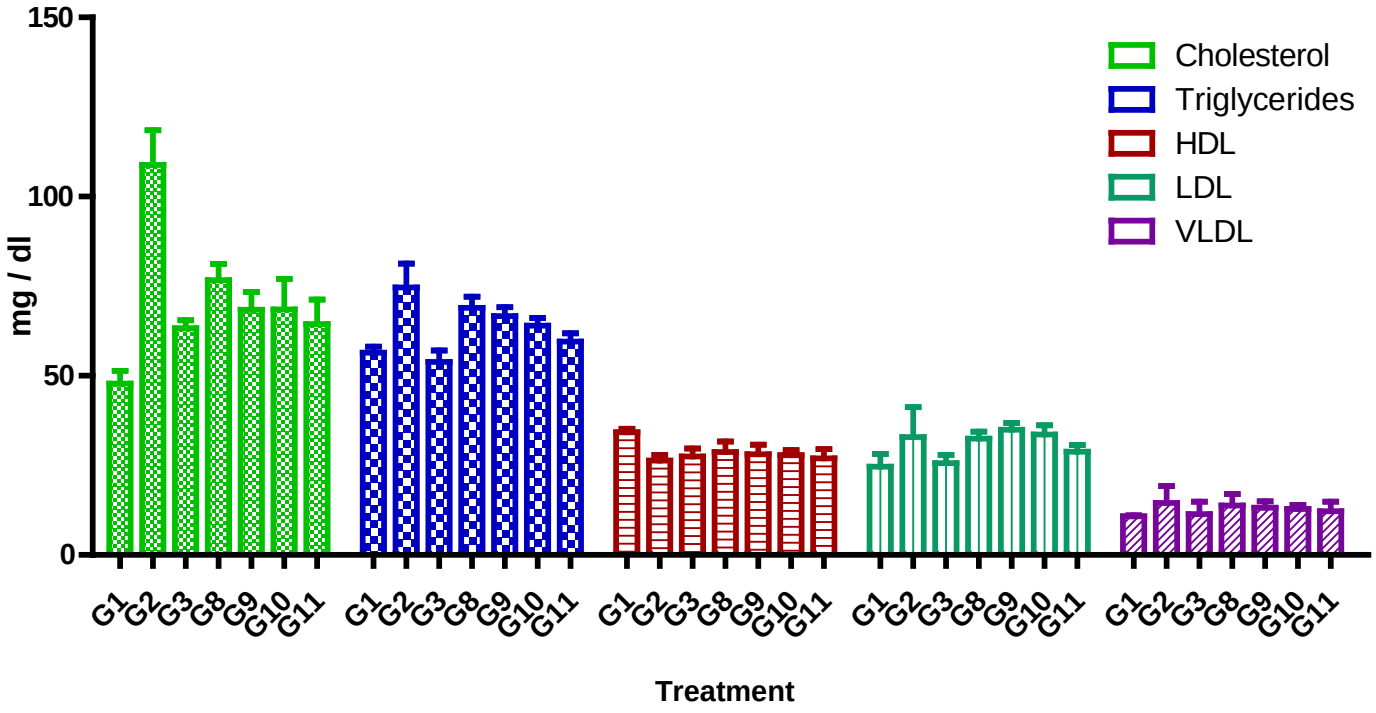


Fig 69. Effect of aqueous and 50% ethanol seed extracts of *Achyranthes aspera* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

G1=Control, G2=Triton, G3=Atorvastatin, G8=AA aqueous seed extract(100), G9=AA aqueous seed extract(200), G10= AA 50% ethanol seed extract(100), G11= AA 50% ethanol seed extract(200)

6.5.1.3. Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes bidentata* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

The study revealed that triton induced group showed significant (P<0.001) increase in the levels of cholesterol, triglyceride, LDL, VLDL cholesterol and decrease in the levels of HDL cholesterol. Treatment with aqueous extract of AB leaf (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AB leaf (100 mg/kg and 200 mg/kg) showed significant activity (P<0.001) in reducing the levels of cholesterol. Aqueous extract of AB leaf (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AB leaf (100 mg/kg and 200 mg/kg) significantly (P<0.001) decreased the levels of cholesterol.

Treatment with aqueous extract of AB leaf (200 mg/kg) and 50% ethanol extract of AB leaf (100 mg/kg and 200 mg/kg) showed significant activity in reducing the levels of LDL cholesterol. Additionally, aqueous extract of AB leaf (200 mg/kg) treated group shown significant activity (P<0.01) in reducing the levels of LDL cholesterol when compared to aqueous extract of AB leaf (100 mg/kg) treated group. Aqueous extract of AB leaf (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AB leaf (100 mg/kg and 200 mg/kg) significantly decreased the levels of VLDL cholesterol.

The levels of HDL were unchanged and maintained when compared to negative control. The aqueous extract of AB leaf (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AB leaf (100 mg/kg and 200 mg/kg) produced significant (P<0.001) effect in reducing the levels of cholesterol, triglycerides and VLDL where as only 50% ethanol extract of AB leaf (200 mg/kg) produced such significant (P<0.001) effect in reducing the levels of LDL cholesterol. (Table 39; Figure 70)

39. Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes bidentata* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 h	24h	0 h	24h	0 h	24h	0 h	24h	0 h	24h
Control(G1)	47.8 ±3.6	47.8 ±3.6	56.4 ±1.78	56.4 ±1.7	34.2 ±1.04	34.2 ±1.04	20.65 ±3.54	20.65 ±3.54	10.8 ±0.35	10.8 ±0.3
Triton(G2)	62.54 ±4.59	108.82 ±9.70$^{###}$	44.53 ±2.16	74.62 ±6.7$^{###}$	35.4 ±2.32	26.36 ±1.57$^{###}$	22.30 ±3.87	35.87 ±8.36$^{###}$	7.56 ±2.97	14.5 ±4.7$^{###}$
Atorvastatin(G3)	53.52 ±4.4	63.24 ±2.30***	35.76 ±4.7	53.86 ±3.1***	31.13 ±3.65	27.53 ±2.14	15.92 ±0.95	25.64 ±2.30**	4.99 ±1.98	9.4 ±3.1***
AB aqueous leaf extract(100)(G12)	61.76 ±2.15	89.63 ±4.3***	42.34 ±2.13	68.37 ±3.2***	33.86 ±1.86	29.66 ±2.96	20.76 ±1.86	38.89 ±1.76	6.68 ±1.35	13.4 ±1.8**
AB aqueous leaf extract(200)(G13)	59.76 ±5.01	76.89 ±5.04***	40.65 ±3.17	64.75 ±2.9***	38.67 ±2.39	29.25 ±2.68	18.84 ±2.23	28.56 ±3.08*††	5.89 ±1.85	12.8 ±1.2***
AB 50% ethanol leaf extract(100)(G14)	56.87 ±5.5	72.46 ±8.4***	38.54 ±3.95	58.86 ±2.7***	33.63 ±1.76	28.88 ±1.56	18.57 ±1.67	27.57 ±2.65*	5.75 ±1.23	11.7 ±2.3***
AB 50% ethanol leaf extract(200)(G15)	54.76 ±6.22	64.06 ±6.94***	36.66 ±5.05	55.86 ±3.0***	34.9 ±2.6	28.76 ±2.95	16.92 ±0.95	24.64 ±2.30***	5.21 ±1.98	11.4 ±3.1***

Values are expressed as mean±S.D; n=6.
$^{###}$-P<0.001 when compared G1 vs G2
*-P<0.05, **-P<0.01, ***-P<0.001 when compared G2 vs G3, G12, G13, G14 and G15
††-P<0.01 when compared G12 vs G13

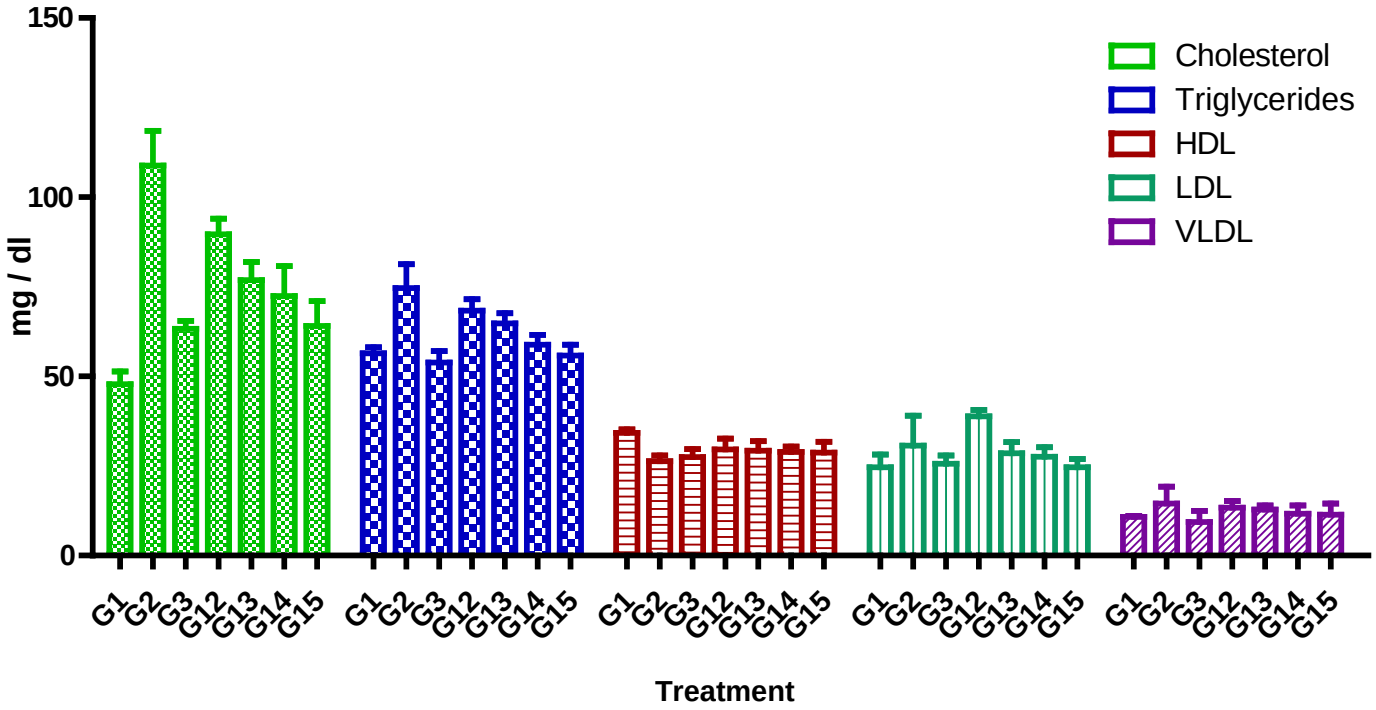


Fig 70. Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes bidentata* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

G1=Control, G2=Triton, G3=Atorvastatin, G12=AB aqueous leaf extract(100), G13=AB aqueous leaf extract(200), G14= AB 50% ethanol leaf extract(100), G15= AB 50% ethanol leaf extract(200)

6.5.1.4. Effect of aqueous and 50% ethanol seed extracts of *Achyranthes bidentata* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

The study revealed that triton induced group showed significant (P<0.001) increase in the levels of cholesterol, triglyceride, LDL, VLDL cholesterol and decrease in the levels of HDL cholesterol. Treatment with aqueous extract of AB seed (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AB seed (100 mg/kg and 200 mg/kg) showed significant activity (P<0.001) in reducing the levels of cholesterol. 50% ethanol extract of AB seed (100 mg/kg and 200 mg/kg) significantly (P<0.001) decreased the levels of triglycerides. Treatment with aqueous extract of AB seed (200 mg/kg) showed significant activity (P<0.01) in reducing the levels of triglycerides whereas such activity was not observed on treatment with aqueous extract of AB seed (100 mg/kg).

Treatment with aqueous extract of AB seed (200 mg/kg) and 50% ethanol extract of AB seed (100 mg/kg and 200 mg/kg) showed significant activity (P<0.001) in reducing the levels of cholesterol. Aqueous extract of AB seed (200 mg/kg) was also produced significant activity with a significance P<0.01. At a dose of 100 mg/kg, both aqueous and 50% ethanol extract of AB seed showed significant (P<0.01) reduction in the levels of VLDL cholesterol. 200 mg/kg of both aqueous and 50% ethanol extract of AB seed significantly (P<0.001) reduced the levels VLDL cholesterol showing the dose dependent activity.The levels of HDL were unchanged and maintained when compared to negative control. Both doses of 50% ethanol extract and 200 mg/kg of aqueous extract of AB seed significantly (P<0.001) decreased the levels of cholesterol, triglycerides, LDL and VLDL where as 100 mg/kg of aqueous extract of AB seed significantly decreased the cholesterol, LDL, and VLDL levels. (Table 40; Figure 71)

40. Effect of aqueous and 50% ethanol seed extracts of *Achyranthes bidentata* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 h	24h	0 h	24h	0 h	24h	0 h	24h	0 h	24h
Control(G1)	47.8 ±3.6	47.8 ±3.6	56.4 ±1.78	56.4 ±1.7	34.2 ±1.04	34.2 ±1.04	20.65 ±3.54	20.65 ±3.54	10.8 ±0.35	10.8 ±0.3
Triton(G2)	62.54 ±4.59	108.82 ±9.70###	44.53 ±2.16	74.62 ±6.7###	35.4 ±2.32	28.36 ±1.57###	22.30 ±3.87	35.87 ±8.36###	26.56 ±2.97	14.5 ±4.7###
Atorvastatin(G3)	53.52 ±4.4	63.24 ±2.30***	35.76 ±4.7	53.86 ±3.1***	31.13 ±3.65	29.53 ±2.14	15.92 ±0.95	25.64 ±2.30***	7.99 ±1.98	11.4 ±3.1***
AB aqueous seed extract(100)(G16)	58.72 ±1.45	78.64 ±4.57***	42.53 ±1.46	68.93 ±3.7	34.78 ±1.8	40.46 ±2.52	28.67 ±1.45	28.47 ±1.48**	23.25 ±1.2	13.2 ±3.7**
AB aqueous seed extract(200)(G17)	58.67 ±3.86	75.35 ±4.96***	40.79 ±3.34	66.68 ±2.5**	34.46 ±2.33	37.86 ±2.55	18.26 ±1.43	22.99 ±1.24***	21.43 ±1.48	12.2 ±1.8***
AB 50% ethanol seed extract(100)(G18)	57.43 ±5.5	67.84 ±8.63***	40.44 ±3.83	60.96 ±2.2***	33.86 ±5.47	31.46 ±1.78	31.87 ±2.96	23.59 ±2.96***	18.34 ±2.46	12.8 ±1.5**
A B50% ethanol seed extract(200)(G19)	55.74 ±5.22	65.89 ±5.5***	38.73 ±6.85	59.53 ±2.3***	32.92 ±2.65	30.98 ±2.58	35.57 ±2.88	27.86 ±1.86***	14.69 ±1.37	11.8 ±2.7***

Values are expressed as mean±S.D; n=6.
###-P<0.001 when compared G1 vs G2
-P<0.01, *-P<0.001 when compared G2 vs G3, G16, G17, G18 and G19

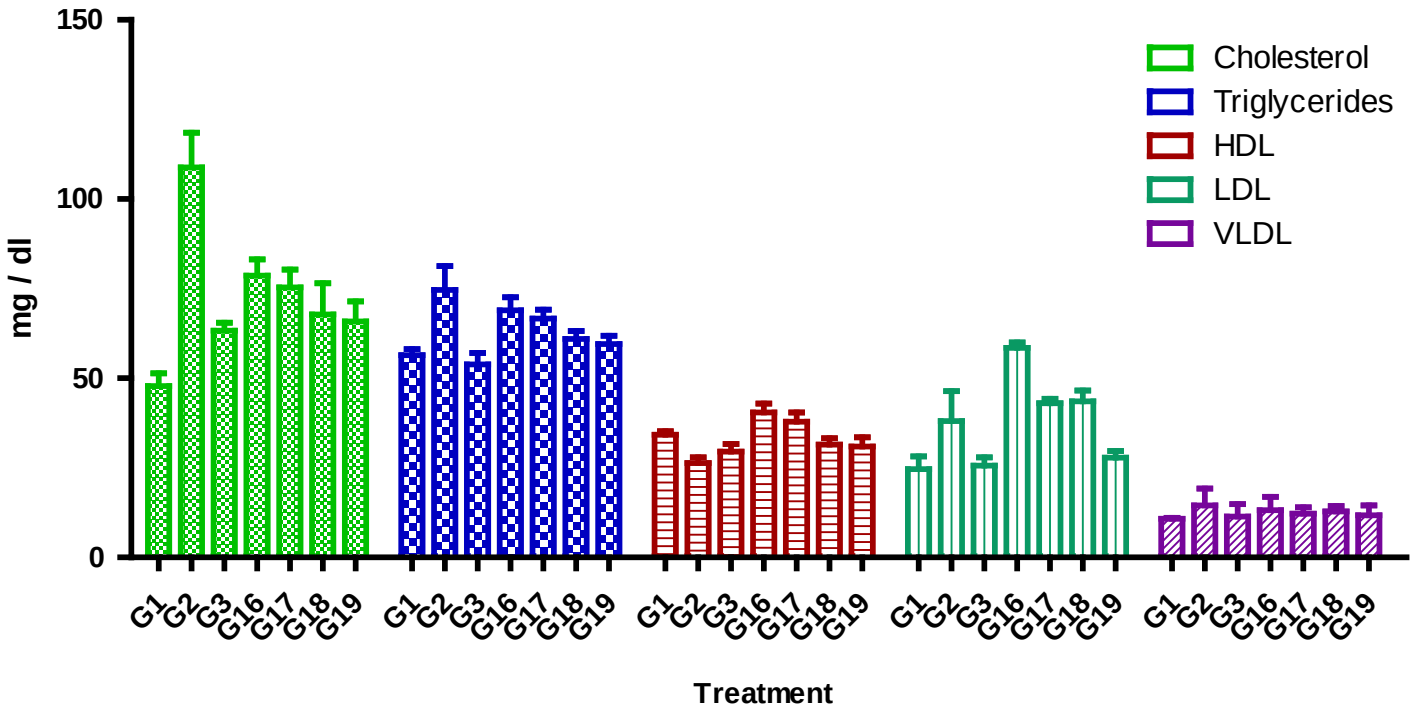


Fig 71. Effect of aqueous and 50% ethanol seed extracts of *Achyranthes bidentata* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

G1=Control, G2=Triton, G3=Atorvastatin, G16=AB aqueous seed extract(100), G17=AB aqueous seed extract(200), G18= AB 50% ethanol seed extract(100), G19= AB 50% ethanol seed extract(200)

6.5.2 Diet induced hyperlipidemia

6.5.2.1. Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes aspera* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

The study revealed that high fat diet induced group showed significant increase in the levels of body weight ($P<0.05$), cholesterol, triglyceride, LDL, VLDL cholesterol and decrease in the levels of HDL cholesterol ($P<0.001$). All the treatment groups showed the similar body weights and there is no significant change when compared to negative control. Treatment with aqueous extract of AA leaf (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AA leaf (100 mg/kg and 200 mg/kg) showed significant activity ($P<0.001$) in reducing the levels of cholesterol. Aqueous extract of AA leaf (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AA leaf (100 mg/kg and 200 mg/kg) significantly decreased ($P<0.001$) the levels of triglycerides when compared to negative control. Further, treatment with 200 mg/kg of 50% ethanol extract of AA leaf showed much significant activity in reducing the cholesterol ($P<0.001$) and triglycerides ($P<0.01$) when compared to 100 mg/kg treated group.

The levels of HDL were unchanged and maintained when compared to negative control. Treatment with aqueous extract of AA leaf (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AA leaf (100 mg/kg and 200 mg/kg) showed significant ($P<0.001$) activity in reducing the levels of both LDL and VLDL cholesterol and the activity was equipotent to the treatment with atorvastatin. (Table 41; Figure 72)

Table 41. Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes aspera* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Body weight		Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week
Normal diet(G1)	154.43 ±3.54	243.75 ±9.57	47.8 ±3.6	58.8 ±3.6	56.4 ±1.78	60.4 ±1.7	34.2 ±1.04	37.2 ±1.04	60.80 ±3.54	68.80 ±3.54	10.8 ±0.35	15.8 ±0.3
Atherogenic diet (G2)	156.67 ±6.87	268.63 ±14.7$^{\#}$	85.37 ±1.43	455.7 ±2.4$^{\#\#\#}$	55.97 ±1.86	180.8 ±2.96$^{\#\#\#}$	37.86 ±1.86	36.82 ±2.75$^{\#\#\#}$	139.52 ±4.13	386.21 ±5.3$^{\#\#\#}$	11.86 ±1.86	36.65 ±2.75$^{\#\#\#}$
AA aqueous leaf extract(100)(G3)	155.35 ±8.65	262.63 ±14.2	75.33 ±2.43	347.1 ±5.6***	59.55 ±1.24	135.2 ±1.24***	34.62 ±6.76	36.36 ±1.57	135.55 ±1.2	256.53 ±3.0***	10.87 ±15.6	22.36 ±1.57***
AA aqueous leaf extract (200)(G4)	154.86 ±7.86	260.87 ±15.6	74.87 ±2.1	336.3 ±1.3*** ††	58.67 ±1.45	128.25 ±12.3***	35.37 ±3.25	38.66 ±2.96	134.67 ±1.45	252.76 ±4.7***	11.78 ±9.56	20.98 ±2.96***
AA 50% ethanol leaf extract (100)(G5)	153.24 ±7.67	258.78 ±9.56	74.35 ±7.34	349.3 ±6.5***	56.57 ±2.88	109.6 ±1.37***	34.34 ±9.59	35.45 ±2.16	134.57 ±2.88	236.88 ±5.04***	11.34 ±9.59	18.45 ±2.16***
AA 50% ethanol leaf extract (200)(G6)	156.48 ±5.5	255.46 ±9.56	70.87 ±4.3	296.7 ±6.5*** €€€	56.20 ±3.86	96.79 ±3.34*** €€	32.76 ±8.18	34.37 ±1.43	133.67 ±3.86	234.89 ±5.8***	13.97 ±1.07	15.35 ±2.96***

Values are expressed as mean±S.D; n=6.
$^{\#}$-P<0.05,$^{\#\#\#}$-P<0.001 when compared G1 vs G2
***-P<0.001 when compared G2 vs G3, G4, G5 and G6
$^{€€}$-P<0.01, $^{€€€}$-P<0.001 when compared G5 vs G6

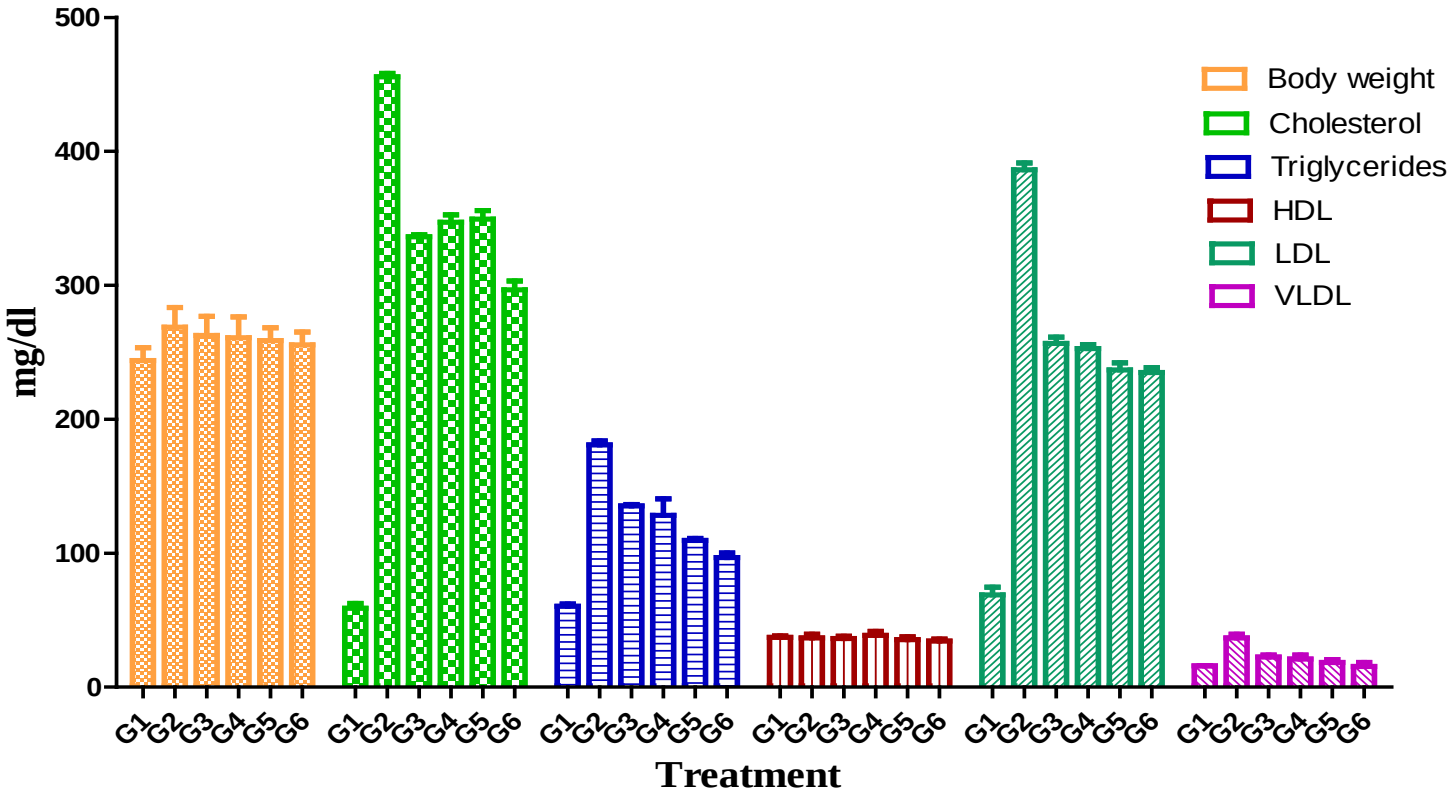


Fig 72. Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes aspera* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

G1= Normal diet, G2= Atherogenic diet, G3=AA aqueous leaf extract(100), G4=AA aqueous leaf extract(200), G5= AA 50% ethanol leaf extract(100), G6= AA 50% ethanol leaf extract(200)

6.5.2.2. Effect of aqueous and 50% ethanol seed extracts of *Achyranthes aspera* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

The study revealed that high fat diet induced group showed significant ($P<0.001$) increase in the levels of body weight, cholesterol, triglyceride, LDL, VLDL cholesterol and decrease in the levels of HDL cholesterol. Treatment with aqueous extract of AA seed (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AA seed (100 mg/kg and 200 mg/kg) showed significant activity ($P<0.001$) in reducing the body weight, cholesterol and triglycerides. Further, treatment with 200 mg/kg of aqueous and 50% ethanol extract of AA seed showed much significant activity ($P<0.001$) when compared to 100 mg/kg treated group.

Treatment with aqueous extract of AA seed (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AA seed (100 mg/kg and 200 mg/kg) showed significant activity ($P<0.001$) in reducing the levels of LDL and VLDL cholesterol and the activity was equipotent to the treatment with atorvastatin. Further, 50% ethanol extract of AA seed (200 mg/kg) produced a better effect ($P<0.001$) in reducing the levels of VLDL cholesterol when compared to 50% ethanol extract of AA seed (100 mg/kg) showing the dose dependent activity. However the levels of HDL were unchanged and maintained when compared to negative control. (Table 42; Figure 73)

Table 42. Effect of aqueous and 50% ethanol seed extracts of *Achyranthes aspera* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Body weight		Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week
Normal diet(G1)	154.43 ±3.54	243.75 ±9.57	47.8 ±3.6	58.8 ±3.5	56.4 ±1.78	60.4 ±1.73	34.2 ±1.04	37.2 ±1.04	60.80 ±3.54	68.80 ±3.54	10.8 ±0.35	15.8 ±0.3
Atherogenic diet(G2)	156.67 ±6.87	268.63 ±14.7$^{###}$	85.37 ±1.43	455.7 ±2.4$^{###}$	55.97 ±1.86	180.8 ±2.96$^{###}$	37.86 ±1.86	36.82 ±2.75	139.5 ±4.13	386.2 ±5.3$^{###}$	11.86 ±1.86	36.65 ±2.75$^{###}$
AA aqueous seed extract(100)(G7)	156.35 ±8.65	253.86 ±14.1***	81.06 ±1.2	352.3 ±5.3***	51.30 ±5.54	135.8 ±1.8***	31.59 ±2.96	35.86 ±4.54	70.57 ±2.88	293.7 ±1.36***	10.37 ±5.34	25.53 ±0.51***
AA aqueous seed extract (200)(G8)	159.55 ±1.24	255.25 ±1.24***	85.56 ±12.3	312.0 ±9.7*** †††	48.28 ±4.22	131.6 ±12.8***	31.86 ±1.86	38.65 ±2.75	68.92 ±0.95	252.5 ±1.9***	11.09 ±9.79	24.84 ±2.76***
AA 50% ethanol seed extract (100)(G9)	155.65 ±3.65	256.76 ±3.85***	75.12 ±8.71	314.8 ±2.7***	56.67 ±6.8	106.3 ±7.34***	32.43 ±3.67	35.86 ±14.84	64.57 ±2.88	248.3 ±2.4***	9.45 ±5.43	24.45 ±2.16***
AA 50% ethanol seed extract(200)(G10)	158.45 ±5.37	252.43 ±3.67***	72.54 ±5.32	287.3 ±7.3*** €€€	53.35 ±8.65	97.30 ±6.7***	31.47 ±8.65	32.30 ±6.76	72.67 ±3.86	245.9 ±1.3***	13.84 ±2.1	18.37 ±1.43*** €€€

Values are expressed as mean±S.D; n=6.
$^{###}$-P<0.001 when compared G1 vs G2
***-P<0.001 when compared G2 vs G7, G8, G9 and G10
†††-P<0.001 when compared G7 vs G8
$^{€€€}$-P<0.001 when compared G9 vs G10

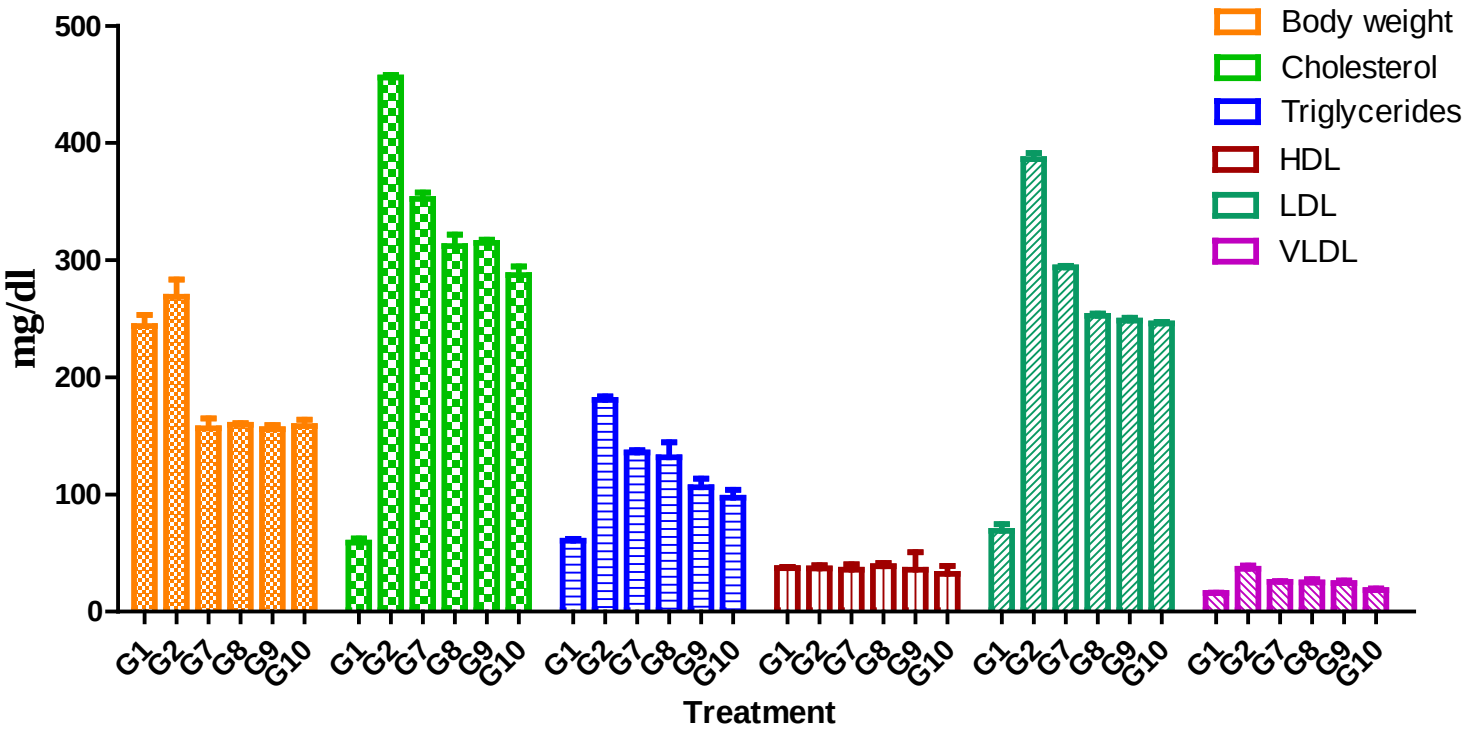


Fig 73. Effect of aqueous and 50% ethanol seed extracts of *Achyranthes aspera* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

G1= Normal diet, G2= Atherogenic diet, G7=AA aqueous seed extract(100), G8=AA aqueous seed extract(200), G9= AA 50% ethanol seed extract(100), G10= AA 50% ethanol seed extract(200)

6.5.2.3. Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes bidentata* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

The study revealed that high fat diet induced group showed significant increase in the levels of body weight ($P<0.01$), cholesterol, triglyceride, LDL, VLDL cholesterol and decrease in the levels of HDL cholesterol ($P<0.001$). All the treatment groups showed the similar body weights and there is no significant change when compared to negative control. Treatment with aqueous extract of AB leaf (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AB leaf (100 mg/kg and 200 mg/kg) showed significant activity ($P<0.001$) in reducing the levels of both cholesterol and triglycerides. Further, treatment with 200 mg/kg of aqueous and 50% ethanol extract of AB leaf showed much significant activity ($P<0.001$) in reducing the cholesterol levels when compared to 100 mg/kg treated group.

The levels of HDL were unchanged and maintained when compared to negative control. Both aqueous and 50% ethanol extract of AB leaf (100 mg/kg and 200 mg/kg) significantly ($P<0.001$) decreased the levels of LDL and VLDL cholesterol. Further, 50% ethanol extract of AB leaf (200 mg/kg) showed much significant activity ($P<0.001$) in reducing the LDL cholesterol levels when compared to 100 mg/kg treated group. (Table 43; Figure 74)

Table 43. Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes bidentata* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Body weight		Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week
Normal diet(G1)	154.43 ±3.54	243.75 ±9.57	47.8 ±3.6	58.8 ±3.6	56.4 ±1.78	60.4 ±1.73	34.2 ±1.04	37.2 ±1.04	60.80 ±3.54	68.80 ±3.54	10.8 ±0.3	15.8 ±0.3
Atherogenic diet(G2)	156.67 ±6.87	268.63 ±14.7$^{\#\#}$	85.37 ±1.43	455.7 ±2.2$^{\#\#\#}$	55.97 ±1.83	180.8 ±2.96$^{\#\#\#}$	37.86 ±1.86	36.82 ±2.75	139.5 ±4.13	386.2 ±5.3$^{\#\#\#}$	11.86 ±1.8	36.65 ±2.75$^{\#\#\#}$
AB aqueous leaf extract(100)(G11)	166.35 ±8.65	270.86 ±14.8	81.06 ±12.9	355.3 ±5.6***	56.30 ±5.54	125.8 ±1.2***	31.59 ±2.96	35.86 ±4.5	138.5 ±2.88	290.7 ±1.3***	11.37 ±5.3	25.16 ±2.54***
AB aqueous leaf extract (200)(G12)	159.55 ±1.24	265.85 ±1.24	80.56 ±12.3	336.0 ±9.1*** †††	55.28 ±4.22	119.6 ±2.4***	28.86 ±1.86	33.65 ±2.75	132.9 ±0.95	286.4 ±1.9***	12.21 ±9.7	24.84 ±2.65***
AB 50% ethanol leaf extract (100)(G13)	163.65 ±3.65	266.76 ±3.85	75.12 ±8.76	348.8 ±2.7***	63.67 ±6.87	96.35 ±7.34***	29.43 ±3.6	35.86 ±4.84	127.5 ±2.82	281.1 ±2.4***	12.45 ±5.4	19.67 ±2.16***
AB 50% ethanol leaf extract (200)(G14)	168.45 ±5.3	253.43 ±3.67	65.54 ±5.32	312.3 ±7.3*** €€€	59.35 ±8.65	92.76 ±6.76***	27.47 ±8.65	31.30 ±6.76	118.6 ±3.86	225.3 ±1.6*** €€€	11.04 ±2.4	18.37 ±1.43***

Values are expressed as mean±S.D; n=6.
$^{\#\#}$-P<0.01,$^{\#\#\#}$-P<0.001 when compared G1 vs G2
***-P<0.001 when compared G2 vs G11, G12, G13 and G14
†††-P<0.001 when compared G11 vs G12
$^{€€€}$-P<0.001 when compared G13 vs G14

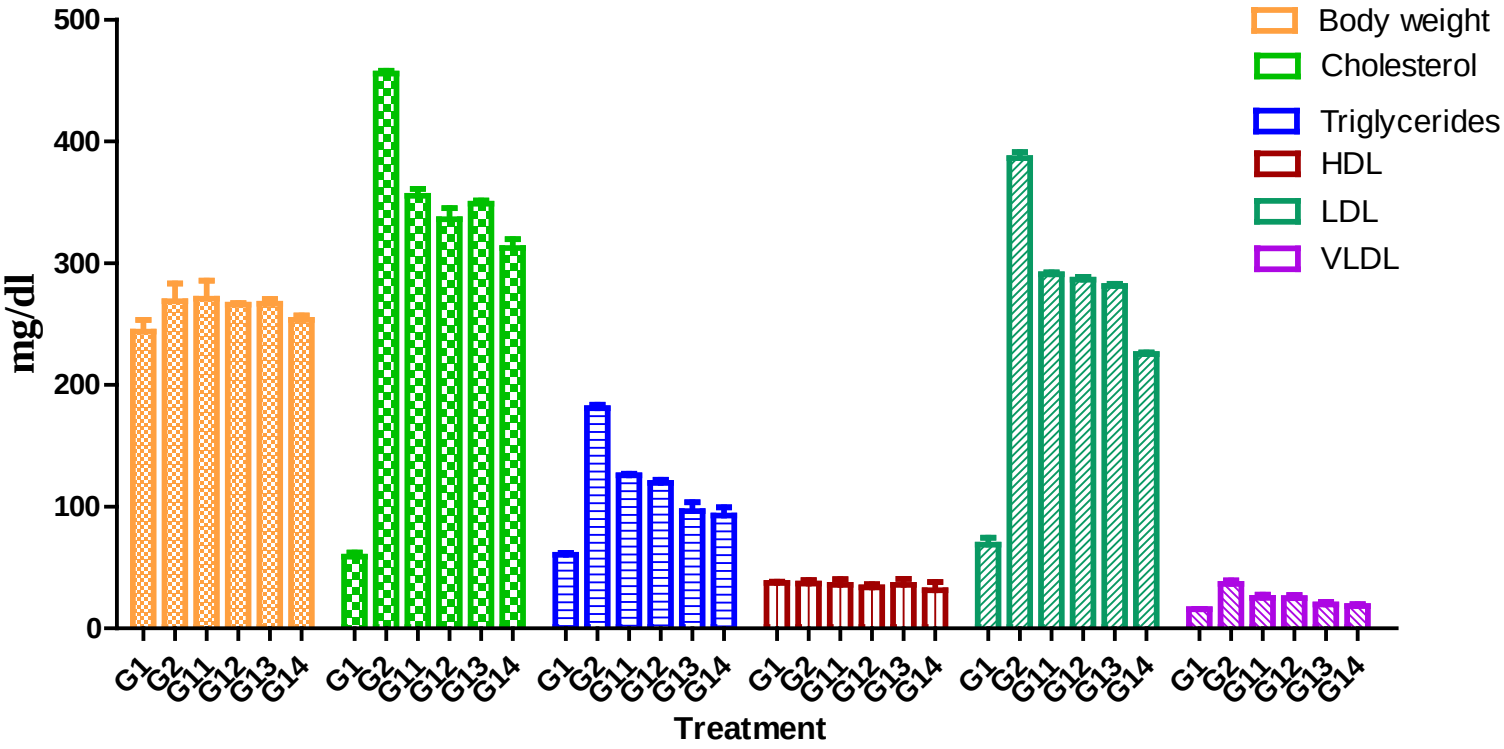


Fig 74. Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes bidentata* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

G1= Normal diet, G2= Atherogenic diet, G11=AB aqueous leaf extract(100), G12=AB aqueous leaf extract(200), G13= AB 50% ethanol leaf extract(100), G14= AB 50% ethanol leaf extract(200)

6.5.2.4. Effect of aqueous and 50% ethanol seed extracts of *Achyranthes bidentata* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

The study revealed that high fat diet induced group showed significant ($P<0.001$) increase in the levels of body weight, cholesterol, triglyceride, LDL, VLDL cholesterol and decrease in the levels of HDL cholesterol. Treatment with aqueous extract of AB seed (200 mg/kg) showed significantly ($P<0.05$) reduced the body weight. Aqueous extract of AB seed (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AB seed (100 mg/kg and 200 mg/kg) significantly decreased ($P<0.001$) the levels of cholesterol and triglycerides.

Treatment with aqueous extract of AB seed (100 mg/kg and 200 mg/kg) and 50% ethanol extract of AB seed (100 mg/kg and 200 mg/kg) showed significant activity ($P<0.001$) in reducing the levels of LDL and VLDL cholesterol. Further, 50% ethanol extract of AB leaf (200 mg/kg) showed much significant activity ($P<0.001$) in reducing the LDL cholesterol levels when compared to 100 mg/kg treated group. However the levels of HDL were unchanged and maintained when compared to negative control. (Table 44; Figure 75)

Table 44. Effect of aqueous and 50% ethanol seed extracts of *Achyranthes bidentata* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Body weight		Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week
Normal diet(G1)	154.43 ±3.54	243.75 ±9.57	47.8 ±3.6	58.8 ±3.6	56.4 ±1.78	60.4 ±1.73	34.2 ±1.04	37.2 ±1.04	60.80 ±3.54	68.80 ±3.54	10.8 ±0.3	15.8 ±0.3
Atherogenic diet(G2)	156.67 ±6.87	268.63 ±14.7###	85.37 ±1.43	455.7 ±2.4###	55.97 ±1.86	180.8 ±2.96###	37.86 ±1.86	36.82 ±2.75	139.5 ±4.13	386.8 ±5.3###	11.6 ±1.8	36.65 ±2.75###
AB aqueous seed extract(100)(G15)	168.37 ±10.6	264.66 ±5.5	80.86 ±14.8	331.5 ±2.9***	55.30 ±5.54	128.7 ±6.5***	34.21 ±3.33	37.86 ±1.86	135.5 ±2.88	269.5 ±4.8***	10.7 ±3.2	30.16 ±2.54***
AB aqueous seed extract (200)(G16)	165.86 ±1.34	248.86 ±1.84**	79.82 ±2.9	318.8 ±3.1***	52.28 ±4.22	125.3 ±5.72***	36.23 ±1.86	34.65 ±12.87	133.9 ±2.9	269.8 ±2.5***	11.4 ±5.7	27.84 ±2.65***
AB 50% ethanol seed extract (100)(G17)	169.76 ±7.18	255.43 ±3.67	74.46 ±1.18	310.4 ±3.5***	55.67 ±6.72	116.3 ±7.34***	35.43 ±3.67	35.35 ±7.34	125.5 ±3.54	234.5 ±4.8***	11.7 ±9.7	22.67 ±2.16***
AB 50% ethanol seed extract (200)(G18)	165.77 ±8.36	247.86 ±1.7	75.37 ±8.34	304.4 ±8.6***	52.30 ±5.5	95.76 ±6.76***	35.02 ±8.63	30.86 ±1.86	126.6 ±3.86	218.7 ±2.3***€€€	12.8 ±5.3	19.54 ±1.43***

Values are expressed as mean±S.D; n=6.
###-P<0.001 when compared G1 vs G2
-P<0.01, *-P<0.001 when compared G2 vs G15, G16, G17 and G18
€€€-P<0.001 when compared G17 vs G18

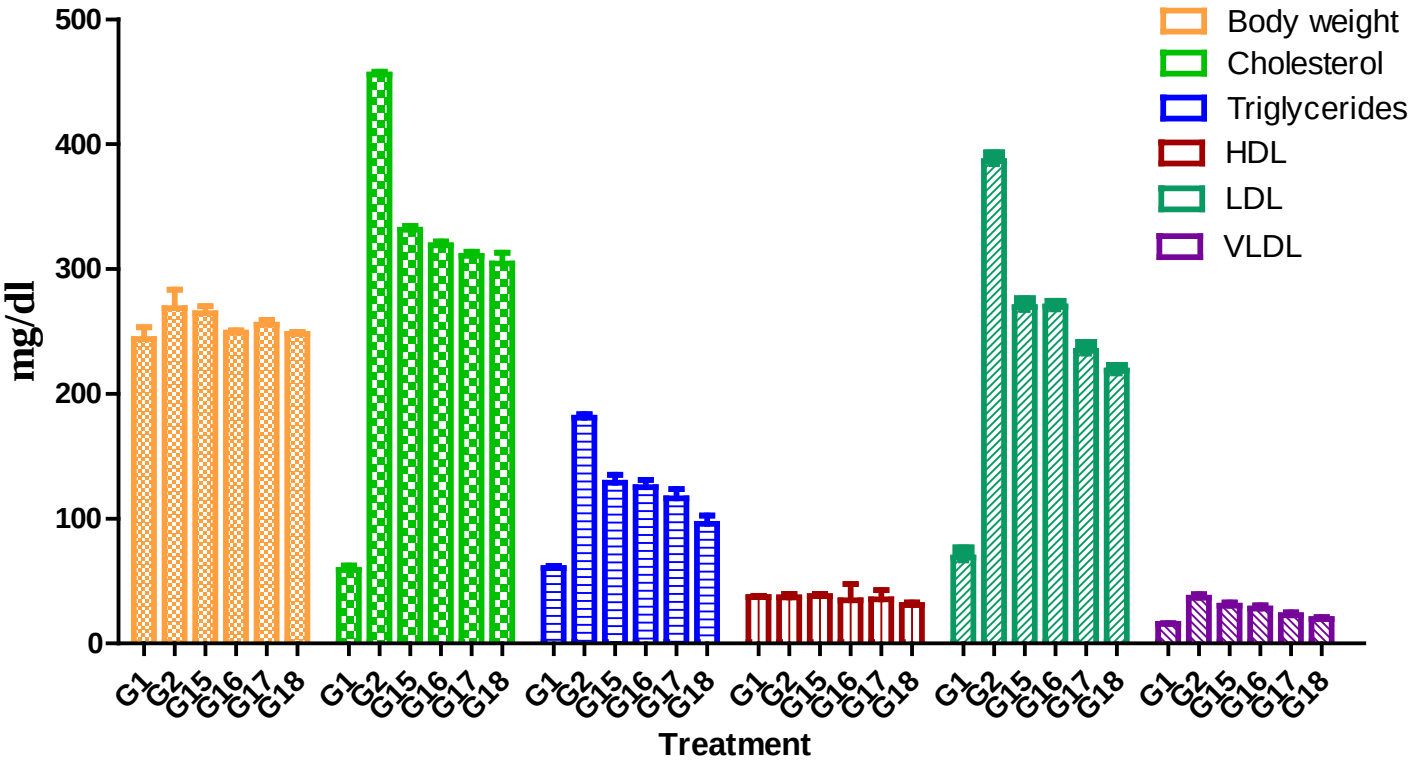


Fig 75. Effect of aqueous and 50% ethanol seed extracts of *Achyranthes bidentata* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

G1= Normal diet, G2= Atherogenic diet, G15=AB aqueous seed extract(100), G16=AB aqueous seed extract(200), G17= AB 50% ethanol seed extract(100), G18= AB 50% ethanol seed extract(200)

6.6 Histopathology studies

Histopathology of aorta of rats treated with atherogenic diet shows presence of numerous foam cells which confirmed the induction of hyperlipidemia. While groups treated with 50% ethanol leaf and seed extract of *Achyranthes aspera* and *Achyranthes bidentata* (200 mg/kg b.w) (group VI, X, XIV and XVIII) showed absence of foam cells. Few foam cells were found in case of groups tretated with 50% ethanol leaf and seed extract of *Achyranthes aspera* and *Achyranthes bidentata* (100 mg/kg b.w) (group V, IX, XIII and XVII). Histopathology of arota and are recorded in Figure 72-78.

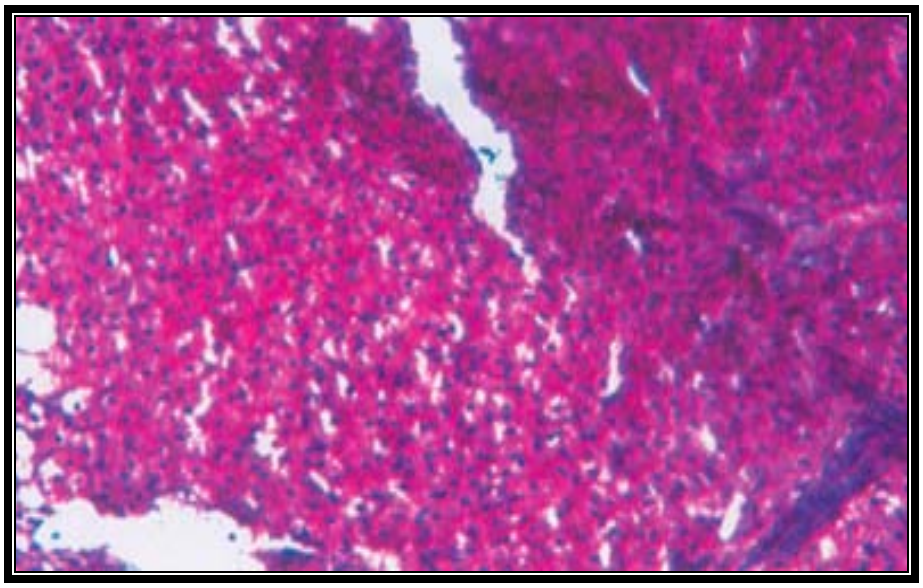


Fig 76. Section of the aorta from hyperlipidemic rat with numerous foam cells in media (high fat diet)

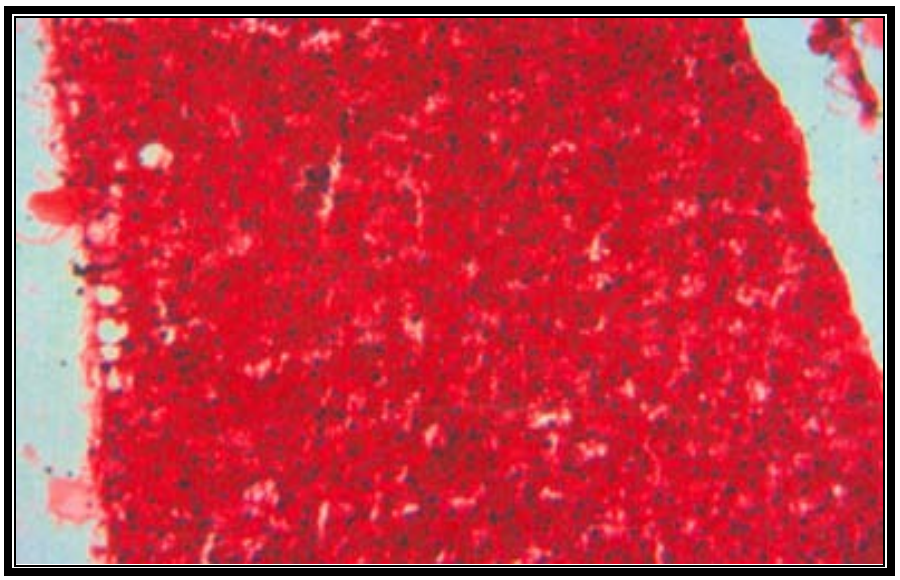


Fig 77. Section of the aorta showed no foam cells; only edema in the media.
(50% ethanol leaf extract of *Achyranthes aspera,* 200 mg/kg b.w)

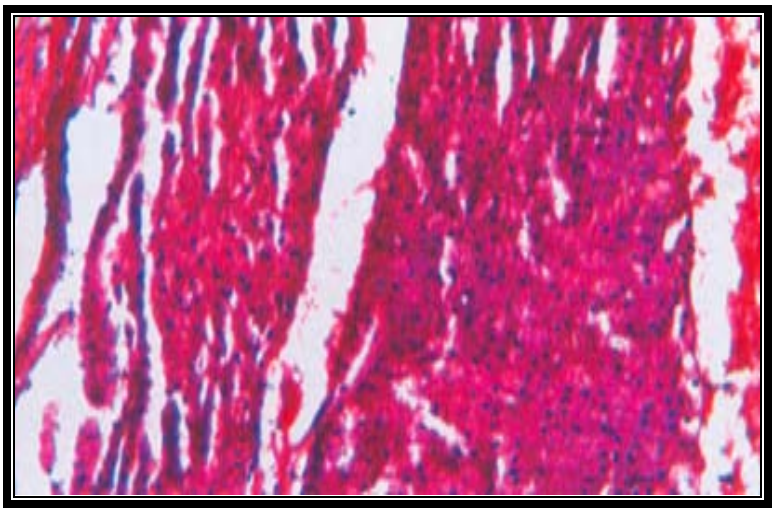


Fig 78. Section of the aorta showed no foam cells in the media (50% ethanol seed extract of *Achyranthes aspera,* 200 mg/kg b.w)

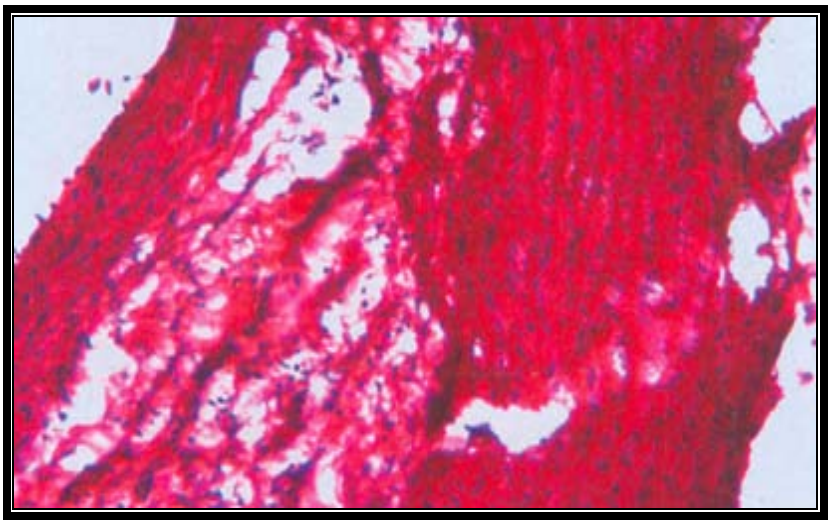


Fig 79. Section of the aorta showed no foam cells in the media (50% ethanol leaf extract of *Achyranthes bidentata,* 200 mg/kg b.w)

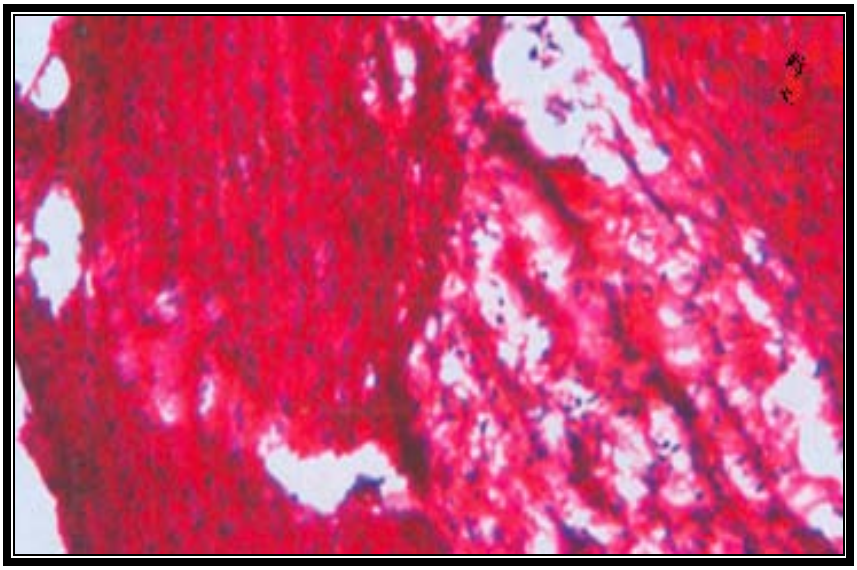


Fig 80. Section of the aorta showed no foam cells in the media (50% ethanol seed extract of *Achyranthes bidentata,* 200 mg/kg b.w)

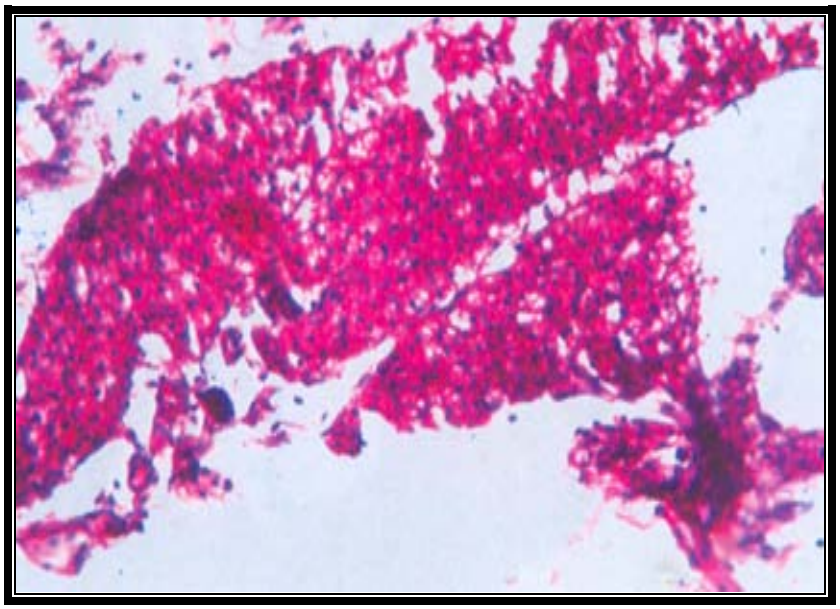


Fig 81. Section of the aorta showed few foam cells in the media (50% ethanol leaf extract of *Achyranthes bidentata,* 100 mg/kg b.w)

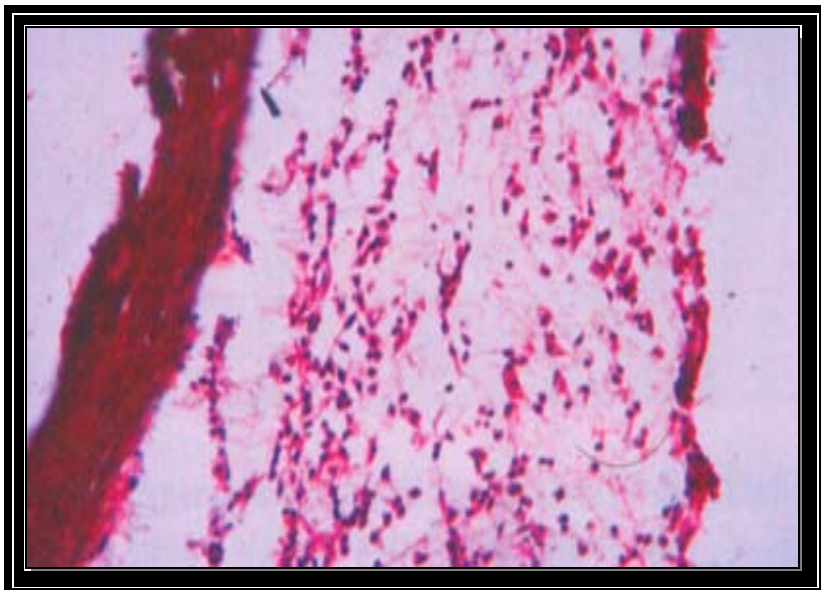


Fig 82. Section of the aorta showed few foam cells in the media (50% ethanol leaf extract of *Achyranthes aspera*, 100 mg/kg b.w)

6.7 Antioxidant activity

IC_{50} values of the ethanol and aqueous leaf and seed extract of *Achyranthes aspera* and *Achyranthes bidentata* by DPPH and nitric oxide method are given in Tabl 45. In DPPH method, IC_{50} values of *Achyranthes bidentata* 50% ethanol seed and leaf extract (200 mg/kg b.w) are found to be 18.56±2.12 and 19.79±2.31 respectively. IC_{50} values of *Achyranthes aspera* 50% ethanol seed and leaf extract (200 mg/kg b.w) are found to be 20.35±3.12 and 22.34±3.50 respectively. The standard drug ascorbic acid shows an IC_{50} value of 4.3±0.6. All other extracts show higher IC_{50} values.

These results show that the *Achyranthes bidentata* 50% ethanol seed and leaf extract (200 mg/kg b.w) has a higher antioxidant potential in scavenging free radicals.

In nitric oxide method, *Achyranthes bidentata* 50% ethanol seed and leaf extract (200 mg/kg b.w) is found to have an IC_{50} value of 210.9±2.3 whereas the other extracts shown higher IC_{50} values. The standard drug, ascorbic acid, shows an IC_{50} value of 85.43±2.1. These results show that the *Achyranthes bidentata* 50% ethanol seed and leaf extract (200 mg/kg b.w) has a higher antioxidant potential.

Table 45. Antioxidant activity of the aqueous and ethanol leaf and seed extracts by DPPH and nitric oxide methods

S.No.	Test Compounds mg/kg	IC_{50} values ± SEM (µg/ml)	
		DPPH	Nitric Oxide
1	AA aqueous leaf extract 100	124.76±6.89	389.4±4.53
2	AA aqueous leaf extract 200	115.97±6.20	415.12±2.36
3	AA 50% ethanol leaf extract 100	30.59±3.65	453.68±1.32
4	AA 50% ethanol leaf extract 200	22.34±3.50	394.5±1.2
5	AA aqueous seed extract 100	69.56±4.8	418.52±2.87
6	AA aqueous seed extract 200	76.65±4.87	450.3±5.4
7	AA 50% ethanol seed extract 100	25.87±2.65	339.32±3.57
8	AA 50% ethanol seed extract 200	20.35±3.12	258.65±3.21
9	AB aqueous leaf extract 100	72.64±383	436.3±4.21
10	AB aqueous leaf extract 200	65.3±3.06	520.36±6.5
11	AB 50% ethanol leaf extract 100	32.39±2.65	411.23±1.5
12	AB 50% ethanol leaf extract 200	19.79±2.31	246.14±3.43
13	AB aqueous seed extract 100	55.86±7.65	409.35±3.1
14	AB aqueous seed extract 200	53.79±6.3	473.97±3.43
15	AB 50% ethanol seed extract 100	26.45±3.2	357.67±3.7
16	AB 50% ethanol seed extract 200	18.56±2.12	210.9±2.3
17	Ascorbic acid (Standard)	4.3±0.6	85.43±2.1

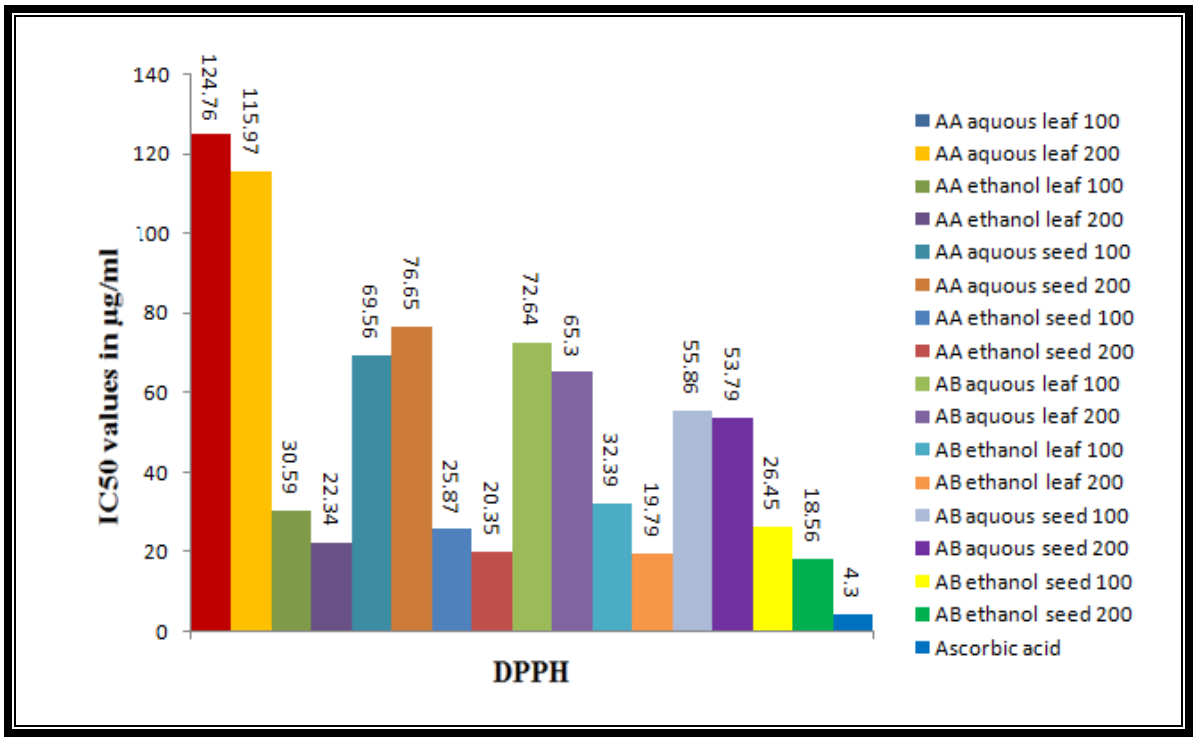


Fig 83. Antioxidant activity of the aqueous and ethanol leaf and seed extracts by DPPH method

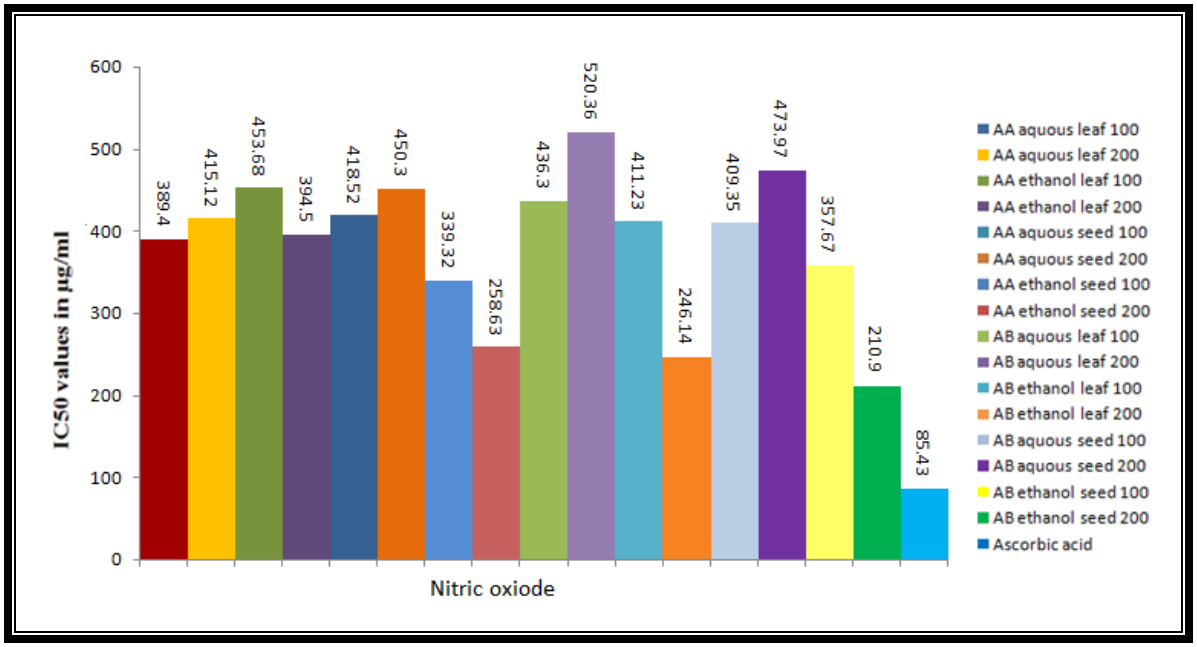


Fig 84. Antioxidant activity of the aqueous and ethanol leaf and seed extracts by nitric oxide methods

Discussion

The current anti-hyperlipidemic therapy includes principally statins and fibrates; the former corrects the altered blood lipid profile by inhibiting the biosynthesis of cholesterol and the latter acts by enhancing the clearance of triglyceride rich lipoproteins. The investigation on plant drugs will be a useful strategy in the discovery of new lead molecules eliciting improved activity by regulating the different mechanisms maintaining the lipid metabolism and thus can be used in treating hyperlipidemia of varied etiology (Robert et al., 2006).

Achyranthus aspera is already proved for its spermicidal, antilithiatic, antifertility, immunostimulatory, cancer chemopreventive activity (Daniel et al., 2006; Deorao et al., 2009; Neeru et al., 2006; Vasudeva et al., 2006; Asima et al., 2002). *Achyranthus bidentata* is already proved for its cancer chemoprotective, osteoprotective, neuroprotective, and immunostimulatory (Cui-Cui et al., 2009; Hong-Xiang et al., 2006; Li-Qin et al., 2007; Songlin et al., 2009). But these plant species were also demonstrated to reduce the serum cholesterol levels. The anti-hyperlipidemic activity of *A.aspera* and *A.bidentata* had not been elucidated in an controlled hyperlipidemic animal models, the present study has been designed to evaluate the lipid – controlling activity of *A.aspera* and *A.bidentata* against triton-induced hyperlipidemia and high fat diet-induced hyperlipidemia, in male Wistar albino rats.

On the induction with triton, hypercholesterolemia occurs through the mobilization of cholesterol from the liver into plasma compartment initially (first phase) and that subsequent to such mobilization increased synthesis

of hepatic cholesterol occurs (second phase) which is accompanied by the elevation of 3-hydrocy -3-methylglutatyl-CoA reductase activity (Kuroda et al., 1977). Intra peritoneal administration of resulted in an enormous elevation of serum cholesterol, triglycerides, LDL, and lowering of VLDL cholesterol levels at 24 hrs and the observations are on par with the previous studies (Vijaya C et al., 2009).

Treatment with aqueous and 50% ethanol extracts of AA leaf and AA seed at two dose levels 100 mg/kg and 200 mg/kg showed significant activity in lowering the levels of cholesterol and VLDL in triton induced hyperlipidemic rats. The 50% ethanol extract of AA leaf and AA seed at a dose level of 200 mg/kg produced better activity in reducing the levels of triglycerides, LDL and VLDL showing the significant dose dependent activity. Regression of these markers is strongly supporting the antihyperlipidemic activity of 50% ethanol extract of AA leaf and AA seed (200 mg/kg).

Aqueous and 50% ethanol extracts of AB leaf and AB seed at two dose levels 100 mg/kg and 200 mg/kg significantly lowered the levels of cholesterol, triglycerides, LDL, and VLDL. Further, 200 mg/kg of both aqueous and 50% ethanol extract of AB leaf and AB seed produced a better activity when compared to lower dose showing the significant dose dependent activity. The results revealing that the AB leaf and seed extract possessed the better activity than AA leaf and seed extracts showing that the *A.bidentata* is having high potential in lowering the hypercholesterolemia. These results may indicate that the leaf and seed extracts of *A.aspera* and *A.bidentata* may interfere with cholesterol-

biosynthesis as Triton accelerated the hepatic synthesis of cholesterol (Vogel et al., 1997).

A high-cholesterol diet regarded as an important factor in the development of cardiac diseases, since it leads to the development of hyperlipidemia, atherosclerosis, and ischemic heart disease. A variety of mechanisms, i.e. inhibition of mevalonate pathway, decrease in NO bioavailability and cGMP metabolism, increase in free radical and peroxynitrite formation, inhibition of heat shock response, and expression of oxidized low density lipoprotein receptors which induces apoptosis, have been shown to play a role in cardiac effects of hyperlipidemia (Puskas et al., 2004).

Triton induced hypercholesterolemia, though simple and rapid for evaluating hypolipidemic compounds, is rather artificial. Hence the lipid controlling potential of *A.aspera* and *A.bidentata* leaves and seeds were further evaluated in high-fat diet induced hyperlipidemic rat model. When male Wistar albino rats were kept on cholesterol-rich diet supplemented for 4 weeks and body weight, serum cholesterol, trigylcerides, LDL, VLDL levels were elevated where as HDL levels were significantly reduced. Elevated circulating lipid levels may be outcome of inhibitory effect of high fat dietary intake on lipogenesis (Rothwell et al., 1983).

The treatment of high-fat diet induced hyperlipidemic rats with aqueous and 50% ethanol extracts of AA leaf and AA seed at two dose levels 100 mg/kg and 200 mg/kg showed significant activity in lowering the levels of cholesterol and VLDL. Though the levels of HDL were not increased, they are maintained as that of control rats. The extracts also possessed the significant activity in lowering the body weights. Further, the higher dose

of aqueous and 50% ethanol extract of AA leaf and AA seed produced better activity in reducing the levels of cholesterol, triglycerides, and VLDL showing the significant dose dependent activity.

Aqueous and 50% ethanol extracts of AB leaf and AB seed at two dose levels 100 mg/kg and 200 mg/kg significantly lowered the levels of cholesterol, triglycerides, LDL, and VLDL. The levels of HDL were not reduced and body weights are not increased, they are maintained as that of control rats. Further, the higher dose of aqueous and 50% ethanol extract of AB leaf and AB seed produced better activity in reducing the levels of cholesterol and LDL showing the significant dose dependent activity.

The presence of foam cells resulted due to the proliferation of stromal cells is a characteristic feature of lipid deposition and atherosclerosis (Fillios et al., 1967). Histopathology of aorta of rats treated with atherogenic diet shows presence of numerous foam cells which confirmed the induction of hyperlipidemia. Groups treated with 50% ethanol extract of leaf and seed of *A. aspera* and *A. bidentata* at a dose of 200 mg/kg showed absence of foam cells. Whereas few foam cells were found in case of groups treated with 100 mg/kg of 50% ethanol extract of leaf and seed of *A. aspera* and *A. bidentata*, supporting the dose dependent activity.

Presence of saponins, flavonoids, and triterpenoids in *A. aspera* and *A. bidentata* was already reported (Deorao et al., 2009; Li et al., 2007). Saponins derived from the *Acorus calamus* were reported to reduce the serum cholesterol levels by preventing the cholesterol absorption, interfering with entero-hepatic circulation and increasing its fecal excretion (Parab et al., 2002). Flavonoids present in the *Pterocapus marsupium* were

observed to be responsible for lowering the serum cholesterol, LDL and VLDL cholesterol levels (Farboonlay et al., 1993). Lupeol, a triterpenoid was reported for its cardioprotective activity against experimental hypercholesterolemia (Sudhahar et al., 2007). The lipid lowering activity of *A. aspera* and *A. bidentata* leaf and seed extract may be attributed to the presence of phytoconstituents such as saponins, flavonoids, and triterpenoids as reported for other plant extracts.

Recent studies have demonstrated that increased formation of free radicals or reactive oxygen species (ROS) contribute to cardiovascular disease progression (Kaliora et al., 2006). The generation of large amounts of reactive oxygen species can overwhelm the intracellular antioxidant defense, causing activation of lipid peroxidation, protein modification and DNA breaks. ROS induced depletion of antioxidants is a key factor for initiation of atherosclerosis (Hiroi et al., 1999).

DPPH is a free radical, which produces a violet solution in ethanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncolored ethanol solutions. The use of DPPH provides an easy and rapid way to evaluate antioxidants. 50% ethanol of AB seed and leaf extract (200 mg/kg) has showed highest free radical scavenging activity against DPPH and NO· free radicals. Whereas 200 mg/kg dose of 50% ethanol of AA seed and leaf extract was also produced the better activity.

It is strongly suggested that DPPH free radical abstracts the phenolic hydrogen of the electron – donating molecule and this could be the general mechanism of the scavenging action of antiperoxidative flavonols (Ratty et al., 1988). Triterpenoid rich species are already proved for their antioxidant

and antiatherosclerotic activity (Somova et al., 2003). The antioxidant activity could be due to the presence of flavonoids and triterpenoids.

The anti hyperlipidemic activity may be attributed to the inhibition of ROS by the flavonoids and triterpenoids present in the extracts. All the beneficial effects of the extracts may be due to their antioxidant and antihyperlipidemic effects carried out by saponins, flavonoids, and triterpenoids present in them.

SUMMARY AND CONCLUSION

7. SUMMARY AND CONCLUSION

Achyranthes aspera Linn and *Achyranthes bidentata* Blume are two perennial herbs with slender and rambling branches. The two plant materials were collected from different parts of Nilgiri district of Tamilnadu and authenticated. Physicochemical parameters like total ash, acid insoluble ash, water soluble ash, and sulphated ash values were determined. Fluorescence analysis was carried out for the plant powder and their extracts.

The dried and powdered leaves and seeds of plants were extracted with water and 50% ethanol through cold maceration. Alcohol soluble extractive and water soluble extractive values were determined. Phytochemical studies from the plant extracts using column chromatography by gradient elution method was carried out for the TLC and HPTLC analysis and R_f values were recorded.

The phytochemical studies of the plant extracts showed the presence of alkaloids, glycosides, triterpenoids, saponins, flavonoids and mucilage. These results gave clues regarding the presence of some particular phytoconstituents in the respective plant extracts.

The *in vitro* antioxidant assays of the plant extract exhibited potent antioxidant activity with low IC_{50} values in DPPH reducing power ability and Nitric oxide scavenging activity. 50% ethanol of both plants at a dose level of 200 mg/kg showed better antioxidant activity.

The *in vivo* screening for antihyperlipidemic activity with triton induced hyperlipidemia and high fat diet induced hyperlipidemia model showed

that both aqueous and 50% ethanol extracts of plants at a dose of 200 mg/kg exhibited significant activity against hyperlipidemia.

Antihyperlipidemic activity and *in vitro* antioxidant activity of the various extracts may be attributed to the presence of triterpenoids, saponins, and flavonoids. From these studies, it can be concluded that both the plants are endowed with significant antihyperlipidemic and antioxidant activity.

However, the future studies need to be carried out to isolate and screen the phytoprinciples responsible for the anti-hyperlipidemic and antioxidant activity. More wide-cut understanding of mechanism needs further studies with more animal model viz. isoproterenol induced myocardial infarction for their anti-hyperlipidemic activity and SOD, GSH, and lipid peroxidation inhibition assays for antioxidant activity. Works in this direction are in progress in the institution.

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ANNEXURES

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AUTHENTICATION CERTIFICATE

Based upon the organoleptic /macroscopic / microscopic examination of fresh / ~~market~~ sample, it is certified that the specimen given by Mr. M. Nivanjan Babu, Ph.D Scholar, JSS College of Pharmacy, Ooty. is identified as below :

Binomial: ① *Achyranthes aspera* L. var. *aspera* L. Leaf & Seed
② *Achyranthes bidentata* Bl. —— Leaf & Seed.

Family: A. maranthaceae

Synonym(s): —

Regional names: ① Nayuruvi ; ② Sivappu Nayuruvi

Reg.No of the certificate: PARC/2010/525

References : Nair, N.C & Henry, A.N. Flora of Tamilnadu, India 1: —— .1983.
Henry, A.N. *et al.* Ibid. II: p.189 .1987.
Ibid. —— III: —— .1989.

Date: 02-05-2008

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Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA)

Institutional Animal Ethics committee (IAEC)

CERTIFICATE

Title of the Project: **Phytochemical investigation and screening of anti-hyperlipidemic activity and antioxidant activity of some medicinal plants**

Proposal Number: JSSCP/IAEC/Ph.D/Ph.Cology/02/2008-09

Date received after modification (if any):

Date received after second modification:

Approval date: 22.10.08

Animals: **Wistar rats**/ Albino mice
Rabbits / Guinea pigs

No. of animals sanctioned: 208

Male/Female

Expiry date (Termination of the Project):

Name of IAEC/CPCSEA chairperson: **Dr.K.Elango**

Signature of Chairperson

Date: 22.10.08

Chairperson
Institutional Animal Ethics Committee
JSS College of Pharmacy
Rocklands, Ooty-643 001