PHARMACOGNOSTICAL, PHYTOCHEMICAL INCLUDING ISOLATION OF CRYPTOLEPINE AND IN VITRO ANTIOXIDANT, ALPHA - AMYLASE EFFECT, ANTIMYCOBACTERIAL AND IN VIVO ANTIHISTAMINIC ACTIVITIES OF *Sida acuta* Burm. (Leaves)

A dissertation submitted to
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Chennai – 600 032
In partial fulfillment of the requirements for the award of the degree of
MASTER OF PHARMACY
IN
BRANCH III-PHARMACOGNOSY

Submitted by
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UNDER THE GUIDANCE OF
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COLLEGE OF PHARMACY
MADURAI MEDICAL COLLEGE
MADURAI – 625 020

MAY 2018

CERTIFICATE

This is to certify that the dissertation entitled **"PHARMACOGNOSTICAL, PHYTOCHEMICAL INCLUDING ISOLATION OF CRYPTOLEPINE AND IN VITRO ANTIOXIDANT, ALPHA-AMYLASE EFFECT, ANTIMYCOBACTERIAL AND IN VIVO ANTIHISTAMINIC ACTIVITIES OF *Sida acuta* Burm. (Leaves)"** is a bonafied work done by **Mr.B.EZHILARASAN (261620701), DEPARTMENT OF PHARMACOGNOSY, COLLEGE OF PHARMACY, MADURAI MEDICAL COLLEGE, MADURAI-625020** in partial fulfillment of the Tamil Nadu Dr. M.G.R. Medical University rules and regulations for award of **MASTER OF PHARMACY IN PHARMACOGNOSY** under my guidance and supervision during the academic year 2017-2018.

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CERTIFICATE

This is to certify that the specimen brought by Mr.B.Ezhilarasan, II M.Pharm, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai is identified as *Sida acuta Burm.* belonging to the family Malvaceae.

Station : Madurai.

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HERBARIUM

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NAME : Sida acuta Burm. ✓

FAMILY : Malvaceae

GENUS : Sida

SPECIES : acuta

LOCALITY : Komarampatty (vil) Dindigul (DT)

DATE :

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IAEC – CERTIFICATE

This is to certificate that the project title **ANTI-HISTAMINIC ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF *SIDA ACUTA* BURM** has been approved by the IAEC/B.EZHILARASAN/TNMGRMU/M.Pharm/261620701/KMCP/17/2018.

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(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by office).

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CHAPTER-I

INTRODUCTION

INTRODUCTION

Pharmacognosy the term comes from two Greek words “pharmakon” meaning drug or medicine, and “gnosis” meaning knowledge. Pharmacognosy is “the study of the physical, chemical, biochemical and biological properties of drug, drug substances or potential drug or drug substance of natural origin as well as the search for new drugs from natural sources”.

Pharmacognosy is the study of medicinal uses of various naturally occurring drugs and its history, sources, distributions, method of cultivation, active constituents, medicinal uses, identification test, preservation methods, substituent and adulterants. Plant preparation are said to be medicinal or herbal when they are used to promote health beyond basic nutrition.

The study of drug from plants includes the subjects of botany, chemistry and pharmacology. Botany includes the identification (taxonomy), genetics and cultivation of plants. Chemical characterization of includes the isolation, identification and quantification of constituents in plant materials.

Pharmacology is the study of the biological effects that the chemicals in medicinal plants have on cell cultures, animals and humans practical perspectives as follows

- ✓ Quality control (identity, purity, consistency)
- ✓ Efficacy (therapeutic indications, pharmacological investigations)
- ✓ Safety (adverse reaction, drug interactions, contraindications, precautions)

Herbal medicine

The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Or say, traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts include *Rigveda*, *Atherveda*, *Charak Samhita* and *Sushruta Samhita*. The herbal medicines/traditional medicaments have, therefore, been derived from rich traditions of ancient civilizations and scientific heritage

Medicinal Plant

According to the WHO, ‘‘a medicinal plant is plant which, in one or more of its organs, contains substance that can be used for therapeutic purposes, or which are precursors for chemopharmaceutical semi-synthesis.

Traditional Medicinal Plants

Of the 250000 higher plant species on earth, more than 80000 species are reported to have at least some medicinal value. There are about 400 families in the world of flowering plants, of which at least 315 are represented in India. According to WHO, approximately 21000 plant species have the potential for being used as medicinal plants. Although some of the therapeutic properties attributed to plants have

been proven to be erroneous, the use of traditional medicinal plants for the treatment of various diseases is well known and documented since ancient times.

Importance of plants as a source of new drugs

Herbal medicine is widely practiced in worldwide. For centuries, people have turned to natural remedies to cure common ailments such as colds, allergy, upset stomachs and toothaches and the trend is constantly increasing. Thus, there has been a shift in universal trend from synthetic to herbal medicines, which we can say 'Return to Nature' for the prevention of diseases and ailments. Nature has been a source of medicinal plants. The World Health Organization (WHO) reported that 4 billion people (80% of the world's population) use herbal medicines for some aspect of primary healthcare. Herbal medicine has been recognized by WHO as essential components for primary health care and about 11% of the 252 drugs are derived from plants Since time immemorial, human civilization has been used several plants as food, medicine, clothing and shelter. Vegetarian foods contain high amounts of various "super-nutrients," such as protective antioxidants, phytochemicals, micronutrients, which promote health and protect from diseases. Plants have several pharmacological roles such as antioxidant, antiviral, anticancer, antimicrobial, antifungal and antiparasitic. Plants have free radical scavenging molecules, including flavonoids, phenolics, anthocynins and vitamins, which show antioxidant like activity . It has been reported that the antioxidant property of phytochemicals may be mitigated the oxidative stress in the biological system. Phytochemicals have been reduced the risk of many human diseases include cardiovascular disease, hepato-renal diseases, diabetes, cancers and neurodegenerative disorders. However, several herbal medicines are being derived directly or indirectly from plants that are considered as an important medicine currently in use for curing various human diseases.

Current Regulations for Standardization of Crude Drugs

In recent years there is a spurt in the interest regarding survival of Ayurvedic forms of medication.In the global perspective, there is a shift towards the use of medicine of herbal origin, as the dangers and the shortcoming of modern medicine have started getting more apparent, majority of Ayurvedic formulation are prepared from herbs.It is the cardinal responsibility of the regulatory authorities to ensure that the consumers get the medication, which guarantee with purity, safety, potency and efficacy. The quality control of crude drugs and herbal formulations is of paramount importance in justifying their acceptability in modern system of medicine. But one of the major problems faced by the herbal drug industry is nonavailability of rigid quality control profile for herbal material and their formulations. Internationally several pharmacopoeias have provided monographs stating parameter and standard of many herbs and some product made out of these herbs. Several pharmacopoeias like:

- Pharmacopoeia Committee
- Chinese Herbal Pharmacopoeia
- United States Herbal Pharmacopoeia
- British Herbal Pharmacopoeia
- British Herbal Compendium
- Japanese Standards for Herbal Medicine
- The Ayurvedic Pharmacopoeia of India (API)

These Pharmacopeias lay down monograph for herbs and herbal products to maintain their quality in their respective nations. Government of India too has brought out Ayurvedic Pharmacopoeia India, which recommends basic quality parameters for eighty common Ayurvedic herbal drugs.

Market Potential of Herbal medicines

Herbal medicines continue to be a major market in U.S. pharmaceuticals and contribute a multi-billion dollar business. Approximately 1500 botanicals are sold as dietary supplements; formulations are not subject to food and Drug Administration (FDA) clinical toxicity testing to assure their safety and efficacy. The Indian herbal market size about $1 billon and the export of plant based crude drug is around $ 100 million. The current market potential of herbal medicine is estimated about $ 80-250 billion in Europe and USA. The current market size of the natural health products in china is about USD 650 million, of which imported herbal medicines account for USD 15 million. In response to the expected improvement in modern herbal medicine and reflective of their growing demand for natural medicines, 73% of the respondents to consumer survey indicated they would depend more on herbal medicine in the future.

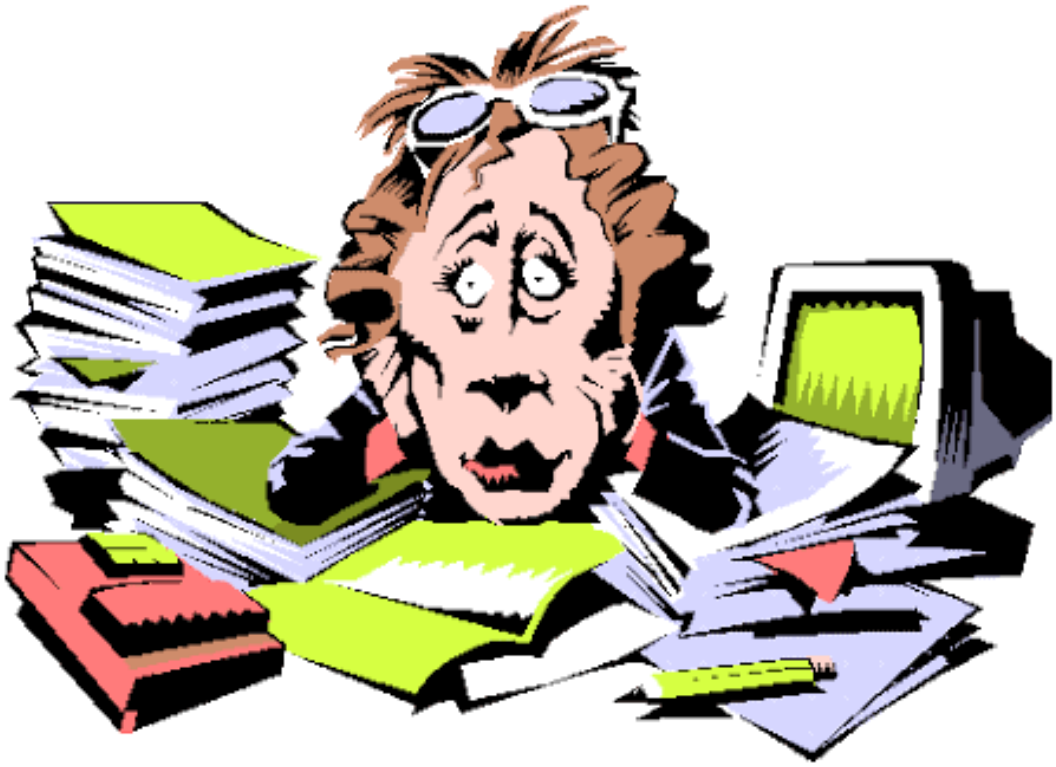
Development of Herbal drug and its challenges

The development of plant drug started when development of chemistry, isolation, purification, characterization of plant active compounds. Herbal medicine is effective, lesser side effect, and affordable than the medicines bought from an allopathic medicine. Herbal medicines include herbs, herbal materials, herbal preparations, and herbal products that contain different parts of plants or other plant materials as active ingredients. It has been well documented that herbal plants and their derivatives play critical roles in modern drug development. Medicinal plants are the natural resources in developing of new drugs.

Future investigation of tribal medicines

Tribal healers in most of the countries, where ethno medical treatment is frequently used to treat cut wounds, skin infection, swelling, aging, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal diseases, snakebite and gastric ulcer, provide instructions to local people as how to prepare medicine from herbal .They keep no records and the information is mainly passed on verbally from generation to generation. World Health Organization (WHO) has shown great interest in documenting the use of medicinal plants used by tribal's from different parts of the world. Many developing countries have intensified their efforts in documenting the ethno medical data on medicinal plants. Research to find out scientific evidence for claims by tribal healers on Indian herbs has been intensified. Once these local ethno medical preparations are scientifically evaluated and disseminated properly, people will be better informed regarding efficacious drug treatment and improved health status.

CHAPTER – II



LITERATURE REVIEW

CHAPTER II

LITERATURE REVIEW

PLANT DESCRIPTION

SCIENTIFIC CLASSIFICATION

Kingdom : Plantae

Class : Angiosperms

Division : Eudiocots

Order : Malvales

Family : Malvaceae

Genus : *Sida*

Species : *acuta*

Synonyms : *Sida carpinifoilia* Linn. ; *Sida lanceolata* Willd.

Common name: Wireweed, Bala, Kurumthotti

VERNACULAR NAME

Bengali : Bala, Brela, Kureta.

English : Wireweed

Gujarat : Beladana

Hindi : Janglimethi, Lahanchikna, Kharenti, Bariara, Paharibariara, karela.

Malayalam : Tsjeruparua, Cheruparua, Malatangi, Kuruntotti.

Marathi : Chikana

Oriya : Badiananla

Sanskrit : Bala, Rajabala, Brihannagahala, Phanijivaka, Pronivika Vatyalika, Vatya.

Tamil : Pazhampasi, Malatangi, Arivalmukham, Malaikurundala, Ponmusattai, Mayir- manikkam, Vathatirippi, Vizhabodhi, Arivalmanai poondu.

Telugu : Cittimu, Cittamutti, Visaboddi, Saha-devi.

DISTRIBUTION AND HABITAT

The plant is distributed throughout the hotter parts of India, chiefly Bombay, Konkan, Deccan, Bengal, Madras and Kerala. It is a fairly common weed of the waste places of the plains districts and often grows gregariously.

DESCRIPTION

Sida acuta Burm., a small, erect, much branched, annual shrub or herb; ranging from 30 to 100 cm in height, with a strong taproot, stem and branches flattened at the extremities, fibrous ,almost woody at times. The weed is frequently found in pastures, wastelands, cultivated lands, roadsides, lawns, and in planted forests. The plant grows well in many soils, including some heavy clay and can tolerate dry as well as high rainfall conditions. **(Chopra et al-1956)**

ETHANOCLAIM USES

LEAVES

Nadkarni KM, 1954 had reported leaves warmed, moistened with a little gingili oil and applied to abscess hasten suppuration; it is used as diuretic in rheumatic affection, demulcent in gonorrhoea and chronic dysentery.

Chopra R N, 1956 leaves made warm and moistened with gingili oil, employed to hasten suppuration.

Ramachandran V S and Nair N C, 1981 had reported Irular tribal people of Tamilnadu used *Sida acuta* leaf juice for vomiting and gastric disorders.

Gill L S, 1982 had identified infusion of the leaves is used to treat for the malarial fever.

Murugesa muthalia, 1988 had identified leaves used for the treatment of rheumatic affection.

Mudaliar M, 1998 had identified leaves are considered to possess demulcent, diuretic, anthelmintic and possess wound healing properties.

Ananil *et al*., 2000 Leaves used for the treatment of eczema, kidney stone, headache

Silja VP *et al*., 2008 had identified leaf juice when boiled with oil and applied to testicular swelling and in elephantiasis and poultice for dandruff.

ROOTS

Nadkarni KM ., I954 had reported roots is used as bitter tonic, stomachic, diaphoretic, antipyretic and useful in the form of decoction or infusion in febrile and some forms of dyspepsia and in mild cases of debility form previous illness and removal of in intestinal worms.

Chopra R N., 1956 had surveyed it is used as astringent, cooling, tonic, nervous and urinary disease and disorders of the blood and bile, bowel complains and as aphrodisiac.

Holdswoth DK., 1974 had identified fresh root is chewed for the treatment of dysentery.

Nadkarni K M., 1976 had surveyed root is used as excellent adaptogenic, immunomodulator, general nutritive tonic and useful in tuberculosis and in disease associated with injury, heart disease, cough and respiratory disease.

Saraswathy *et al*., 1998 had reported root extract is used to treatment of leucorrhoea.

Mohideen S *et al*., 2002 it is used as aphrodisiac, anti-rheumatic, stomachic, diaphoretic, diuretic, anti-pyretic and wound healing properties.

Monika Khare *et al*.,2002 roots are used in stomachic, diaphoretic, antipyretic, cooling, astringent, tonic, nervous and urinary disease and for the disorders of the blood, bile and liver.

Silja VP *et al*., 2008 Root extract is taken in the breathing problem and cough.

WHOLE PLANT

Muthaliar, 1985 had reported the whole plant is used for liver disorders, diuretic, abortifacient in ayurvedic preparations. It also used for treatment of asthma, fever, headache, cough, cold, ulcer, anthelmintic, snake bite, urinary disease, female disorders, antifertility agent and sedative.

Armando Caceres *et al*., 1987 used as asthma, renal inflammation, colds, fever, headache.

Jain SK *et al*., 1991 whole plant has been used as abortifacient, anthelmintic and antiemetic properties.

Barrett B, 1994 had identified decoction of the entire plant is taken orally for asthma, fever, aches and pains, ulcers anti worm medication.

Coee FG and Anderson G, 1996 had reported decoction of the dried entire plant is taken orally for venereal disease.

Nacoulma and Ouedraogo, 1996 had reported whole plant is used for fever, diarrhoea, pulmonary affection, snakebites, insect bites.

Saraswathy A *et al*., 1998 had identified plants are used as rheumatism, facial paralysis, pulmonary tuberculosis, sciatica, haemorrhage, spermatorrhoea, leucorrhoea and gonorrheae.

Otero R *et al*., 2000 had surveyed the whole plants are used to treat snake bite and it lessened the hemorrhagic effect of *Bothrops atrox* venom.

Vasudevan Nair R 2004 had identified it is used for curing neurological disorders, headache, leucorrhoea, tuberculosis, diabetes, fever.

Kayode 2006 had reported plant is used as treatment of malaria, ulcer, fever, gonorrhoea, abortion, breast cancer, poisoning, inflammation, feed for livestock, stops bleeding, treatment of sores wounds antipyretic.

Simplice Damintoti Karou *et al*., 2007 had identified *Sida acuta* whole plant used as fever, headache, skin disease, diarrhoea and dysentery.

Oliver *et al*., 2017 had reported Sida acuta whole plant treatment for rheumatic affections, azoosprmia, oligospermia and spermatorrhea, leucorrhoea, wounds sciatica, nervous and heart diseases, cold, cough, asthma, tuberculosis, and respiratory diseases, disorders of the blood, bile and liver, elephantiasis, hemorrhoids, ulcers, gastric disorders and abdominal pain, headache, fever and malaria, skin diseases, worms, diarrhea and dysentery, venereal disease, renal inflammation, toothache and snake bites.

PHYTOCHEMICAL REVIEW

LEAVES

Adeniyi S A *et al*., 2010 had identified that ethanolic extract of *Sida acuta* showed the presence of alkaloids, flavonoids, saponins, steroids and tanins.

Konate K *et al*., 2010 had reported that aqueous acetone extract of *Sida acuta* showed the presence of saponiside, coumarins, steroids,(ecdysterone, β-sistosterol,

ampesterol), tannins, phenolic compounds (evofolin-A and B, scopoletin, loliolid and 4-ketopinoresinol), polyphenol, sesquiterpene and flavonoids.

Palaksha M N and Ravishankar K 2012 had reported ethanolic and chloroform extract of *Sida acuta* showed the presence of alkaloid, phytosterols, tannins, flavonoids, saponins, fixed oils.

Nawankapa P *et al.*,2015 had identified that ethanolic extract of *Sida acuta* showed phytochemical tannin, alkaloid, saponin, flavonoid, steroid, terpenoids, cardio glycoside and vitamin composition was thiamine, niacin, ascorbic acid, tocopherol, riboflavin and mineral composition calcium, magnesium and zinc.

Raimi *et al.*, 2014 had identified *Sida acuta* contain tannins, saponins, alkaloids, flavonoids, terpenoids, phenolics.

Pooja C *et al*., 2015 had reported *Sida acuta* contain tannins, flavonoids, terpenes, and phenolic compounds.

ROOTS

Pradhan D K *et al.*, 2012 had identified that petroleum ether and methanol extract of *Sida acuta* contain alkaloids, phenols, flavonoids, proteins and amino acids. It also contains carbohydrate and glycosides.

LEAVES AND SEEDS

Marcano L and Hasegawa D.1991 had reported *Sida acuta* contain tannins, saponins, alkaloids, flavonoids, terpenoids and phenolic composition.

WHOLE PLANT

Jank *et al.*, 2003 had reported cryptolepine, quindoline, quindolinone, ecdysterone, beta –sisterol, stigmaterol, ampesterol, evofolin-A and B, scopoletin, vomifoliol, loliolid, and 4- ketopinoresinol

PHARMACOLOGICAL REVIEW

LEAVES

ANTIBACTERIAL SENSITIVITY

Damintoti Karou *et al*., 2005 had identified antibacterial activity of chloroform extract of *Sida acuta*. Antibacterial effect determined by agar well diffusion, broth micro dilution and time-kill assay method. Chloroform extract of *Sida acuta* showed significant antibacterial effect against *Staphylococcus aureus, Enterococcus faecalis, Salmonella thyphi, Shigella boydii, Shigella flexneri, Shigella dysenteriae and Escherchia coli.*

ANTIMICROBIAL SENSITIVITY

Saganuwan *et al* et al., 2006 had investigated antimicrobial activity of methanol, hexane, chloroform and aqueous extraction of *Sida acuta. Sida acuta* extract showed significant antimicrobial effect against *Escherchia coli, Streptococcus pyogenes* and *Salmonella typhimurium.*

ANTIMICROBIAL ACTIVITY

Iroha *et al.*, 2009 had investigated antimicrobial activity of aqueous and ethanol extract of *Sida acuta* against 45 clinical isolates of *Staphylococcus aureus*. The ethanol and aqueous extract of *Sida acuta* showed appreciable antimicrobial activity against *Staphylococcus aureus* isolated from HIV/AIDS Patients. Maximum activity of *Sida acuta* extract was found against gram positive *Staphylococcus aureus* and gram negative *Escherchia coli*.

ANTIMICROBIAL ACTIVITY

Akilandeswari S *et al* 2010 had studied antimicrobial activity of chloroform and ethanol extract of *Sida acuta*. Antimicrobial effect determined by disc diffusion method. Chloroform and ethanol extract of *Sida acuta* showed significant antimicrobial effect *against Staphylococcus auerus, Bacillus subtilis, Escherchia coli, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger* in comparison with gentamycin and Nystatin.

LARVICIDAL ACTIVITY

Niraimathi selvam *et al.*, 2010 had studied the larvicidal activity of ethanolic crude extract of *Sida acuta* against *Anopheles subpictus* and *Culex tritacnior*. The crude extract of Sida acuta showed significant larvicidal properties against *Anopheles subpictus* and *Culex tritaeniorhynchus.*

LARVICIDAL AND REPELLENT ACTIVITY

Marimuthu, 2010 had studied the larvicidal and repellent activities of crude extract of *Sida acuta* against three important mosquitoes. The crude extract of *Sida acuta* showed strong repellent effect100% protection against *Anopheles stephensi, Aedesa egypti, Culex quinquefasiatus.*

GASTRIC ANTIULCER ACTIVITY

Akilandeswari S *et al*., 2010 had studied gastric antiulcer activity of ethanolic extract of *Sida acuta* by aspirin plus pylorus ligation and ethanol induced gastric lesion model in wistar albino rats. Ethanolic extract of *Sida acuta* showed significant antiulcer effect in comparison with famotidine.

INSECTICIDAL ACTIVITY

Adeniyi S A *et al.,* 2010 had investigated the insecticidal activity of ethanolic extract of *Sida acuta* against *Acanthscelides obtectus*. The ethanolic extract of *Sida acuta* showed significant mortality on the target insect.

ANTI-HYPERGLYCEMIC ACTIVITY

Okwuosa C N *et al.,* 2011 had identified anti-hyperglycemic activity on methanolic and ethanolic extract of *Sida acuta*. Anti-hyperglycemic activity determined by alloxan induced diabetic rabbits. The ethanol and methanol extract of *Sida acuta* showed significant hypoglycemic effect in comparison with glibenclamide.

ANTIPYRETIC ACTIVITY

Sharma *et al.,* 2012 had studied the petroleum ether, acetone, ethanol and aqueous extract of *Sida acuta* was evaluated for their antipyretic activity. Acetone extract of *Sida acuta* showed better antipyretic effect amongst other extract.

ANTIBACTERIAL AND ANTHELMINTIC ACTIVITY

Palaksha M N *et al.,* 2012 had identified antibacterial and anthelmintic activities of *Sida acuta*. Ethanolic and chloroform extract of *Sida acuta* different concentration was evaluated anthelmintic activity using pherithema postuma. Ethanolic and chloroform extract of *Sida acuta* significant anthelmintic effect in comparison with piperazine citrate.

ANTIOXIDANT AND THROMBOLYTIC ACTIVITY

Entaz Bahar *et al.,* 2013 had identified in-vitro antioxidant and thrombolytic activity of methanol extract of *Sida acuta.* Antioxidant effect was determined by in-vitro DPPH radical scavenging assay. Methanol extract of *Sida acuta* has moderate

antioxidant effect as well as thrombolytic activity. Methanol extract of *Sida acuta* showed significant activity in comparison with ascorbic acid.

EFFECT ON ELECTROLYTES AND ORGAN FUNCTION PARAMETERS

Enemor V H A *et al.*, 2013 had studied effect of ethanolic extract of *Sida acuta* on some organ function parameters and physiologically important electrolytes in normal wistar albino rats. Ethanol extract of *Sida acuta* showed significant effect in comparison with control.

KIDNEY ELECTROLYE ACTIVITY

Kebe E *et al*., 2013 had identified kidney electrolytic activity of ethanolic extract of *Sida acuta* ethanolic extract of *Sida acuta* possible effect on the kidney electrolytes of adult wistar rat.

ANTIMICROBIAL, ANTIOXIDANT AND CORROSION INHIBITORY ACTIVITY

Johnson AS *et al*., 2013 had studied antimicrobial, antioxidant and corrosion inhibitory activity of green synthesis of silver nanoparticles using *Sida acuta* leaf extract. Antimicrobial activity of *Sida acuta* showed significant inhibitory effect against *Staphylococcus aureus*, *Escherchia coli,* and *Candida albicans* in comparison with gentamysin. Anti-corrosion activity of *Sida acuta* showed good corrosion inhibitory effect. Green synthesis of nanoparticles using *Sida acuta* leaf extract showed significant antioxidant effect in comparison with ascorbic acid.

ANTIBACTERIAL ACTIVITY

Akinnibosun FI and Pela B 2015 had identified antibacterial properties of ethanol and acetone extract of *Sida acuta* against wound bacterial isolates. Antibacterial effect determined by agar well diffusion method. Ethanol and acetone

extract of *Sida acuta* showed significant antimicrobial effect against *Staphylococcus aureus.*

SEXUAL BEHAVIOUR AND APHRODISIAC ACTIVITY

Alok Semwal *et al.*, 2015 had investigated the sexual behavior and aphrodisiac activity of chloroform extract of *Sida acuta*. Chloroform extract of *Sida acuta* showed significant increase in aphrodisiac effect in comparison with sildenafil citrate.

DIURETIC AND ANTI-UROLITHIATIC ACTIVITY

Palaksha MN *et al.*, 2015 had investigated diuretic and anti-urolithiatic activities of ethanolic extract of *Sida acuta* by ethylene glycol-induced hyperoxaluria method in albino rats. Ethanolic extract of *Sida acuta* showed significant diuretic and anti-urolithiatic effect in comparison with cystone.

ANTIOXIDANT ACTIVITY

Palaksha.M N *et al.,* 2016 had investigated in-vitro antioxidant activity on *Melochiacorchorifolia*, *Sida acuta* and *Saccharam officinarum* leaf extract and their phenolic contents. Chloroform and ethanolic leaf extract were subjected to invitro antioxidant activity by various antioxidant assays. The chloroform and ethanolic leaf extract showed significant antioxidant effect in comparison with Gallic acid.

IN-VITRO STABILITY AND AGGREGATORY ACTIVITY

Obioma Benedeth *et al.,* 2016 had identified in-vitro stability and aggregatory effect of ethanol extract of *Sida acuta* in human erythrocyte. Ethanol extract of *Sida acuta* inhibits prostaglandin activity, phospholipase A_2 activity and positive effect on platelet aggregation. Membrane stabilization which showed a

significant inhibition of invitro hemolysis and could have a potential therapeutic effect on disease processes causing destabilization of biological membranes.

ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITY

Obioma Benedeth Eze *et al*., 2016 had studied anti-inflammatory and anti-oxidant activities of ethanolic extract of *Sida acuta* by paw oedema model in rat. Ethanol extract of *Sida acuta* showed significant anti-inflammatory and anti-oxidant effect in comparison with phenylbutazone.

FREE RADICAL SCAVENGING ACTIVITY

Perumalsamy Muneeswari *et al*., 2016 had investigated phytochemical screening and free radical scavenging activity of chloroform extract of *Sida acuta* by DPPH radical scavenging assay, nitric oxide radical scavenging assay, hydroxyl radical scavenging assay, reducing power assay and FRAP assay methods. The chloroform extract of *Sida acuta* showed significant effect in comparison with ascorbic acid.

ALPHA AMYLASE INHIBITORY ACTIVITY

Kemi Feyisayo Akinwunmi *et al*., 2017 had investigated alpha amylase inhibitory activity of cold water, hot water and ethanol extract of *Sida acuta* by alpha amylase inhibitory assay model. Ethanol extract of *Sida acuta* showed more significant alpha amylase inhibitory effect in comparison with acarbose.

BIOINSECTICIDAL ACTIVITY

Manisha G Gadewad *et al.*, 2018 had investigated bio insecticidal activity of methanol and ethyl acetate extract of *Sida acuta* against red cotton bug, *Dysdercus cinegulatus* fab. Methanol and ethyl acetate extract of *Sida acuta* showed potential of

bioinsecticide and crop protecting activity against cotton pest and *Dysdercus cinglatus* fab.

ROOTS

HEPATOPROTECTIVE ACTIVITY

Sridevi *et al.,* 2009 had studied hepatoprotective effects of methanolic extract of *Sida acuta* against liver damage induced by paracetamol overdose as evident from decreased serum levels of glutamide pyruvate transminase, alkaline phosphates and bilirubine in the *Sida acuta* treated groups showed significant hepatoprotective effect compared to the extract of *Sida acuta*.

CALCIUM OXALATE CRYSTAL GROWTH INHIBITORY ACTIVITY

Vimala.T *et al.,* 2012 had investigated the calcium oxalate crystal growth inhibitory effect of the methanolic and aqueous extract of *Sida acuta* by single diffusion method. Aqueous and methanolic extract of *Sida acuta* showed significant activity in comparison with control.

LEAVES AND ROOTS.

CORROSION INHIBITORY ACTIVITY

Umoren S A *et al.,* 2010 had identified corrosion inhibition effect of *Sida acuta* plant extract determined iodide ions on the corrosion inhibition of mild steel in 1m H_2SO_4 by *Sida acuta* extract.

NEUROPHARMACOLOGICAL ACTIVITY

Dora M Benjumea *et al* 2016 had studied neuropharmacological effects of the ethanolic extract of *Sida acuta* determined by sodium pentobarbital-induced sleeping time, anxiolytic activity, test for muscle-effect, pentylenetetrazole (PTZ)-

induced seizures, effect on normal body temperature. Crude extract of *Sida acuta* protects against seizures induced by PTZ and it showed significant anxiolytic effect.

AERIAL PARTS

ANTIPLASMODIAL ACTIVITY

Benzouzi J T *et al.*, 2004 had studied in-vitro antiplasmodial activity of ethanolic and decoction aqueous extract of *Sida acuta* was evaluated on two strain of *Plasmodium falciparum*. Ethanolic extract of *Sida acuta* showed significant antiplasmodial effect than the decoction.

ANTIMICROBIAL ACTIVITY

Dicko *et al.*, 2005 had investigated antimicrobial activity of ethanolic extract of *Sida acuta.* Ethanol extract of *Sida acuta* showed significant inhibitory activity against standard strain and clinical isolates of *Staphylococcus aureus, Bacillus subtilis* and *Streptococcus faccalis*.

ANALGESIS AND ANTI-INFLAMMATORY ACTIVITY

Oboh and Onwukame, 2005 had investigated the analgesic and anti-inflammatory properties crude extract of *Sida acuta* in mice by using tail immersion and mouse ear edema model. Crude extract of *Sida acuta* showed significant analgesic and anti-inflammatory effect. Extract showed significant protection at the highest dose of 500mg/ml.

ANTIMICROBIAL ACTIVITY

Oboh I E *et al*., 2007 had identified antimicrobial activity of ethanol extract of *Sida acuta* by agar well diffusion method. Ethanolic extract of *Sida acuta* showed

significant antimicrobial effect against standard strains and clinical isolates of *Staphylococcus aureus* clinical isolates of *Bacillus subtilis* and *Streptococcus faecalis.*

WHOLE PLANT

ANTI-VENOM ACTIVITY

Otero R *et al.,* 2000 had studied ethanolic extract of *Sida acuta* has a moderate neutralizing activity against the hemorrhagic effect of *Bothrops atrox* venom.

ANTI-MALARIAL ACTIVITY

Karou *et al.,* 2003 had investigated anti malarial activity of *Sida acuta* was evaluated in-vitro on fresh clinical isolates of *Plasmodium falciparum*. *Sida acuta* plant extract showed significant activity and pterocarpus erinaceous has moderate effect.

ANTI-ULCER ACTIVITY

Malairajan *et al.,* 2006 had identified anti-ulcer effect against aspirin plus pylorus ligation gastric ulcer, HCL-ethanol induced ulcer and water immersion stress induced ulcer in rat model. Ethanolic extract of *Sida acuta* showed significant anti-ulcer effect in comparison with omeprazole.

ANTIMICROBIAL ACTIVITY

Karou *et al.,* 2006 had investigated the antimicrobial activity of *Sida acuta* against gram positive and gram negative bacteria .The antibacterial assay performed by agar well diffusion and broth micro dilution method. *Sida acuta* extract showed good antimicrobial activity against several test microorganisms.

Ekpo and Etim., 2009 had studied antimicrobial activity of ethanolic and aqueous extract of *Sida acuta*. Ethanolic extract of *Sida acuta* showed higher significant activity against *Staphylococcus aureus, Bacillus subtilis, Psedomonos aeruginosa, Escherchia coli, Scopulariopsis candida.* Aqueous extract of *Sida acuta* no significant activity.

WOUND HEALING ACTIVITY

Akilandeswari S *et al.,* 2010 had investigated the effect of topical administration of methanolic extract of *Sida acuta* ointment by excision and incision wound model in rats. Methanolic extract of *Sida acuta* showed significant effect in comparison with nitrofurazone.

CYTOTOXICITY AND ANTIOXIDANT ACTIVITY

Pieme *et al.,* 2010 had evaluated in-vitro cytotoxicity and antioxidant activity of *Sida acuta* by MTT assay model. *Sida acuta* extract showed significant anti-proliferative effect and antioxidant effect.

CARDIOVASCULAR ACTIVITY

Kannan *et al.,* 2012 had screened cardiovascular effect of methanolic extract of *Sida acuta* in zebra fish embryos. The methanolic extract of *Sida acuta* showed significant effect in comparison with nebivolol.

ANTIFUNGAL ACTIVITY

Jindal Alka *et al.,* 2012 had identified antifungal activity of *Sida acuta* against *Candida albicans* by microbroth dilution method. Methanol extract of *Sida acuta* showed significant antifungal effect in comparison with terbinafine.

ANTIOXIDANT AND ANTICANCER ACTIVITY

Mahesh Thondawada *et al.,* 2016 had studied in-vitro and in-vivo evaluation of *Sida acuta* for its antioxidant and anticancer effect determined by DPPH and MTT, SRB assay model. Chloroform, toluene and ethyl acetate extract of *Sida acuta* showed significant antioxidant effect in comparison with rutin. *Sida acuta* showed significant anticancer effect in comparison with 5-FU.

ANTIBACTERIAL ACTIVITY

Mathew George *et al.,* 2017 had identified antibacterial activity of *Sida acuta.* Antibacterial effect was determined by agar well diffusion assay method. The ethanolic extract of *Sida acuta* showed significant antibacterial effect in comparison with ciprofloxacin.

CHAPTER- III

AIM AND OBJECTIVE

AIM AND OBJECTIVE

AIM

The aim of the present research is to study the pharmacognostical, phytochemical including isolation of cryptolepin and *in-vitro* antioxidant, alpha-amylase effect, antimycobacterial and *in-vivo* antihistaminic activites of *Sida acuta* Burm (leaves) (Malvaceae)

OBJECTIVE

The present work has been planned to carry out the

PHARMACOGNOSTICAL STUDIES

- Authentication and collection of plan
- Macroscopy of the leaf.
- Powder microscopy includes its identification of characters
- Behavioral characters with different reagents.

PHISICO-CHEMICAL PARAMETERS

- Foreign matter
- Loss on drying (LOD)
- Extractive values with various solvents
- Ash value

PART-B

PHYTOCHEMICAL STUDIES

- Preparation of hydro alcoholic extract
- Qualitative analysis- Preliminary phytochemical screening
- TLC Profile Thin Layer Chromatography

QUATITATIVE ESTIMATION OF PHYTOCONSTITUENTS

- Tannic acid
- Gallic acid
- Rutin in terms of its equivalents
- Identification of R_f value by TLC Method
- Thin Layer Chromatography
- Isolation of cryptolepine by Coloumn Chromatography
- UV-Spectroscopy
- Infrared Spectroscopy
- Nuclear Magnetic Spectroscopy.
- Mass Spectroscopy

PART-C

PHARMACOLOGICAL ACTIVITIES

- ✓ **IN VITRO ANTIOXIDANT ACTIVITY**
    - Hydrogen peroxide scavenging assay
    - Reducing power assay
    - Total Antioxidant Capacity

- ✓ **IN VITRO ALPHA AMYLASE INHIBITORY EFFECT**
- ✓ **IN VITRO ANTIMYCOBACTERIAL ACVTIVITY**
    - Luciferase reporter phage(LRP) assay
- ✓ **ANTI-HISTAMINIC ACTIVITY**

In vivo model

- Isolated Guinea pig ileum preparation.
- Isolated Guinea pig trachea preparation

CHAPTER-IV

MATERIALS AND METHODS

MATERIALS AND METHODS

PLANT COLLECTION & AUTHENTIFICATION

Fresh leaf of *Sida acuta* Burm were collected from Komanampatty,(Village), Dindigul (DT), during the month OF August-2017 was authenticated by Dr. D.Stephen, M.Sc., Ph.D., Assistant Professor, Department of Botany, American College, Madurai-20.The herbarium of this specimen was kept in the department for further references

PART A

PHARMACOGNOSTICAL STUDIES

Morphological and micro morphological examination and characterization of medicinal plants have always been accorded due credentials in the pharmacognostical studies. Botanical identity of the plants is an essential prerequisite for undertaking the analysis of medicinal properties of any plant. A researcher may succeed in getting a new compound or may find many useful pharmacological active properties in the plant. If the botanical identity of the plant happens to be dubious or erratic, the entire work on the plant becomes invalid. Thus it is needless to stress the botanical identity of the crude drug is the threshold in the processes of pharmacological investigations. The researchers should be equipped with all possible diagnostic parameters of the plant on which the researchers plan to work.

MORPHOLOGICAL STUDIES OF *Sida acuta* Burm.

Leaf and petiole were studied seperately for its morphological characters by organoleptic test. The results are tabulated in **Table 1.**

MICROSCOPICAL STUDIES

Freash Leaves Were Selected For The Microscopical Parameters By Using Microscopy.

COLLECTION OF SPECIMEN

The required samples of different organs were cut and removed from the plant and fixed in FAA (Farmalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml).After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary –Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

DEHYDRATION

After 24 hours of fixing, the specimens were dehydrated with graded series of ethyl alcohol and tertiary-butyl alcohol (Sass, 1940). The specimen is kept is in each grade of the fluid for about 6 hrs.Every time the fluid is decanted and immediately the specimen were flooded with next grade of fluid.

INFILTRATION WITH PARAFFIN WAX

After dehydration, the shavings of paraffin wax were added to the vial containing the plant material with pure TBA. The paraffin shavings are added every 30mts at about 40-45ºC four or five times. Then the vials were filled with wax without damaging the tissues. The vial filled with wax is kept open in warm condition to evaporate all TBA, leaving the specimen in pure molten wax. The specimen filled with pure molten wax for 2 or 3 times by decanting the old wax every time.

CASTING TO MOLD

A boat made out of chart board, by folding the margin, is used to prepare a mold of wax containing specimens. The paraffin along with the leaf and petiole specimen was poured into the boat. With the help of heated needles, the specimen were arranged in parallel rows with enough space in between the specimens. The block was then immersed in chilled water and allowed to cool for few hours.

SECTIONING

The paraffin embedded specimens were sectioned with the help of Rotary **Microtome.** The thickness of the sections was 10-12 μm. Dewaxing of the sections was by customary procedure (Johansen, 1940). The sections were stained with **Toluidine blue** as per the method published by O'Brien 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** walls, 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 IKI(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 **clearing** of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with Naoh and mounted in glycerine medium after staining. Different cell component were studied and measured.

PHOTOMICROGRAPHS

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with **Nikon labphoto 2** microscopic Unit. For normal observations **bright field** was used. For the study of **crystals, 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 as given in the standard Anatomy books (**Esau, 1964**).

PREPARATION OF LEAF POWDER

The Leaves were collected and shade dried. It was powdered in a mixer. The course powder was sieved and was stored in a well closed container.

POWDER MICROSCOPY

The course powder was treated with routine reagents to identify the diagnostic features of the plant.

QUANTITATIVE MICROSCOPY OF *Sida acuta* Burm.

Fresh leaves of *Sida acuta* Burm was subjected to microscopical study includes in stomatal number, stomatal index and palisade ratio were determined on fresh leaves using standard procedure. **(Wallis TE. 1953, Wallis TE, 1965).**

VEIN ISLET NUMBER AND VEIN TERMINATION NUMBER

The term vein islet is used to denote the minute area of photosynthetic tissue encircled by the ultimate division of the conducting strands. The number of vein islets per sq.mm. Area is called vein islet number.

Vein terminal number may be defined as the number of vein terminals present in one sq.mm area of the photosynthetic tissue.

DETRMINATION OF VEIN ISLET NUMBER AND VEIN TERMINATION NUMBER

Small square portion from the lamina region of the leaf was cleared in chloral hydrates, stained and mounted on a slide. A camera lucida is set up and by means of a stage micrometer the paper is divided into squares of $1mm^2$ using a 16mm objective. The stage micro meter is then replaced by the cleared preparation and the veins are raced in four continuous squares, either in a square 2mm x 2mm (or) rectangle 1mm x 4mm. When counting, it is convenient to number each vein-islet on the tracing. Each numbered area must be completely enclosed by veins, and those which are incomplete are excluded for the count if cut by top and left-hand sides of the square (or) rectangle but included if cut by the other two sides. Ten readings for vein islet and vein termination number were recorded.

STOMATAL INDEX

It is the percentage, which the numbers of stomata from the total number of epidermal cells, each stoma being counted as one cell.

I. **Stomatal index = S/S+E x 100**

Where, S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area

DETERMINATION OF STOMATAL INDEX

The procedure adopted in the determinations of stomatal number was observed under high power (45 X).The epidermal cells and the stomata were counted. From

these values the stomatal index was calculated using the above formula. The results obtained are presented in **Table:2**

PHYSIO-CHEMICAL PARAMETERS

The powder was subjected to physiochemical parameters such as foreign organic matter, loss on drying, ash values and extractive values with different solvents in increasing order of polarity, volatile oil, and total solids. The procedure was adapted as per **WHO guidelines 1996..**

DETERMINATION OF FOREIGN ORGANIC MATTER

PROCEDURE

An accurately weighed 100g of air dried coarse drug and spread out in a thin layer. The sample drug was inspected with the unaided eye or with the use of 6x lens and the foreign organic matter was separated manually as completely as possible and weighed. The percentage of foreign organic matter was calculated with reference to the weight of the drug taken.

DETERMINATION OF MOISTURE CONTENT (LOSS ON DRYING)

PROCEDURE

An accurately weighed 10 g of coarsely powdered drug was placed in a tarred evaporating dish. Then the dish was dried at 105^{o}C for 5 h and weighed. The drying and weighing was continued at one hour intervals until the difference between the two successive weighing is not more than 0.25 %. The loss on drying was calculated with reference to the amount of powder taken.

DETERMINATION OF SWELLING INDEX

The swelling index is the volume in ml taken up by the swelling of 1g of plant material under specified conditions.

PROCEDURE

About 1g of the crude powder was weighed and transferred to the 25ml of glass stoppered measuring cylinder of 25ml of water and was shaken thoroughly for every10 min for 1 hour. It was allowed to stand at room temperature for 3 hours. The volume in ml occupied by the plant material including and sticky mucilage. The weight was calculated with refer to the dried weight.

DETERMINATION OF EXTRACTIVE VALUES

PROCEDURE

An accurately weighed 5 g of the air dried coarsely powdered drug was macerated with 100mL of various solvents of increasing order of polarity (petroleum ether, benzene, chloroform, ethyl acetate, ethanol, methanol and water) in a closed flask for 24 h, shaking frequently during the first 6 h and allowed to stand for 18 h.

Thereafter filtered rapidly, taking precautions against loss of ethanol. Then evaporate 25 mL of the filtrate to dryness in a tarred flat bottomed shallow dish dry at 105^{o} C and weighed.

DETERMINATION OF ASH VALUES

ASH CONTENT

The residue remaining after incineration is the ash content of crude drug, which simply represents inorganic salts naturally occurring in the drug or adhering to it or deliberately added to it as a form of adulteration.

DETERMINATION OF TOTAL ASH

PROCEDURE

An accurately weighed 3 g of air dried coarsely powdered drug was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450^{o} C, until free from carbon then allowed to cool and weighed. The percentage of ash was calculated with reference to the air dried drug.

DETERMINATION OF ACID INSOLUBLE ASH

PROCEDURE

The total ash obtained from the previous procedure was mixed with 25 ml of 2 M hydrochloric acid and boiled for 5 min in a water bath, and then the insoluble matter was collected in an ash less filter paper and washed with hot water, dried and ignited for 15 min at a temperature not exceeding 450^{o} C, cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

DETERMINATION OF WATER SOLUBLE ASH

PROCEDURE

The total ash obtained from the previous procedure was mixed with 25 ml of water and boiled for 5 min in a water bath, and then the insoluble matter was collected in an ash less filter paper and washed with hot water, dried and ignited for 15 min at a temperature not exceeding 450^{o} C, cooled in desiccators and weighed. The insoluble matter was subtracted from the weight of the total ash; the difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

DETERMINATION OF TOTAL SOLIDS

PROCEDURE

Preparation of evaporating dishes–If volatile solids are to be measured, ignite clean evaporating dishes and watch glasses at 550ºC for 1 hour in a muffle furnace, heat dishes and watch glasses at 103ºC to 105ºC for 1 hour in an oven. Cool and store the dried equipment in a dessicator. Weigh each dish and watch glass prior to use (record combined weight as "W_{dish}").

Total solids = W_{Total}– W_{Dish} / W_{Sample} – W_{Dish} × 100

The results of physio-chemical parameters were tabulated in **Table: 3**

Behavioural characters of the Sida acuta Burm. (Leaf-crude powder) with different reagents

Crude powder when treated with water showed light green colour in visible light. Powder treated with Con HCL showed dark brown colour in visible light. Powder treated with Con.H_2SO_4, aqueous$FeCl_3$ showed black colour in visible light. Powder treated with HNO_3, Water+HNO_3 showed yellow colour in visible light. Powder treated with CH_3COOH, Water+CH_3COOH Showed brown colour in visible light. Powder treated with Water+ Con HCL and aqueous NaOH showed green colour in visible light and powder treated with water+Con H_2SO_4 showed light block colour in visible light. Powder treated with water showed light green colour in UV 254nm.Powder treated with Con HCL showed dark brown colour showed in UV 254nm and powder treated with Con H_2SO_4, aqueous NaOH, aqueous $FeCl_3$ showed black colour in UV 254nm.Powder treated with HNO_3, Water + Con HCL, Water + Con H_2SO_4, Water + HNO_3 showed green colour in UV 254nm.Powder treated with CH_3COOH showed brown colour in UV 254nm and powder treated with Water+CH_3COOH showed dull brown colour in UV 254nm. Powder treated with

water showed dark green colour in UV 365nm. Powder treated with Con HCL, Con H_2SO_4, Water + Con H_2SO_4, Water + CH_3COOH showed green colour in UV 365nm. Powder treated with HNO_3 showed light green colour in UV 365nm and Crude powder treated with CH_3COOH, Water + Con HCL showed brown colour in UV 365nm. Powder treated with Water + Con HCL showed light yellow colour in UV 365nm and powder treated with aqueous $FeCl_3$ showed black colour in UV 365nm. Powder treated with aqueous NaOH did not show any characteristic change in UV 365nm.The results are tabulated in **Table: 4**

PREPARATION OF HYDROALCOHOLIC EXTRACT OF *Sida acuta* Burm.

PROCEDURE

The shade dried and coarsely powdered leaf of *Sida acuta* Burm. (Leaf) was defatted with petroleum ether (60-80˚c). The residue was dried and extracted with hydroalcohol (70%) by Maceration until the complete extract of the material and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark brown).

The above extract was subjected to physical analysis such as colour, consistency, wt/ ml, refractive index and . The results obtained are presented in **Table: 5**

PHYTOCHEMICAL STUDIES

The hydro-alcoholic extract was subjected to qualitative and quantitative analysis. Qualitative analysis includes phytochemical screening of secondary metabolites such as flavonoids, carbohydrates, alkaloids, glycosides, sterols, tannin, protein, aminoacids, carotenoids, volatile oil, quinone, terpenoids, phenolic content

and extract were determined. Quantitative analysis includes estimation of total tannin, total gallic acid, total flavonoid contents in terms of total tannic acid equivalent, total gallic acid equivalent, total flavonoids equivalent (rutin).

PART B

QUALITATIVE ANALYSIS

PRELIMINARY PHYTOCHEMICAL SCREENING

Hydroalcoholic extract of *Sida acuta* Burm. (Leaf) was subjected to qualitative chemical analysis. The various chemical tests were performed on this extract and aqueous extract for the identification of flavonoids, phenolic compounds, alkaloids, glycosides, carbohydrates, carotenoids, proteins, tannin, aminoacids, sterols as per Harborne 1998.

TEST FOR ALKALOIDS

About 2 gm of the powdered material was mixed with 1gm of calcium hydroxide and 5 mL of water into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. To this 200 mL of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was evaporated. To this 5 mL of dilute hydrochloric acid was added followed by 2 mL of each of the following reagents.

MAYER'S TEST:

A small quantity of the extract was treated with Mayer's reagent. Cream colour precipitate indicates the presence of alkaloids.

DRAGENDORFF'S TEST:

A small quantity of the extract was treated with Dragendorff's reagent. Orange brown precipitate indicates the presence of alkaloids.

WAGNER'S TEST:

A small quantity of extract was treated with Wagner's reagent. Reddish brown precipitate indicates the presence of alkaloids.

HAGER'S TEST:

A small quantity of extract was treated with Hager's reagent. Yellow precipitate indicates the presence of alkaloids.

TEST FOR PURINE GROUP (MUREXIDE TEST)

The residue obtained after the evaporation of chloroform was treated with 1mL of hydrochloric acid in a porcelain dish and 0.1 gm of Potassium chlorate was added and evaporated to dryness on water bath. Then the residue was exposed to the vapour of dilute ammonia solution. No purple Colour was obtained indicating the absence of purine group of alkaloids.

TEST FOR INDOLE

To the test solution, add acetic acid and trace amount of anhydrous $FeCl_3$, under – lay /H_2SO_4 intense blue at interface.

TEST FOR QUINOLINE (Thalleioquin Test)

To the extract, add 1 drop of dilute sulphuric acid and 1ml of water. Add bromine water drop wise till the solution acquires permanent yellow colour and add 1ml of dilute ammonia solution, emerald green colour is produced. The powdered drug when heated with glacial acetic acid in dry test tube, evolves red fumes, which

condense in the top portion of the tube. The bark, when moistened with sulphuric acid and observed under ultraviolet light shows a blue fluorescence due to the methoxy group of Quinine and quinidine.

TEST FOR CARBOHYDRATES

MOLISCH'S TEST

The extract of the powdered drug was treated with 2-3 drops of 1% alcoholic αnaphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. A purple colour indicating the presence of carbohydrates.

FEHLING'S TEST

The extract of the powdered leaf was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. Red precipitate was obtained indicating the presence of free reducing sugars.

BENEDICT'S TEST

The extract of the powdered leaf was treated with equal volume of Benedict's reagent. A red precipitate was formed indicating the presence of reducing sugar.

TEST FOR ANTHRAQUINONE GLYCOSIDES

BORNTRAGER'S TEST

The powdered drug was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly. No pink colour was observed in ammoniacal layer showing the presence of anthraquinone glycosides.

MODIFIED BORNTRAGER'S TEST

About 0.1 g of the powdered drug was boiled for 2 minutes with dil.HCl and few drops of $FeCl_3$ solution, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dil.NH_3 solution was added to the benzene extract. No pink colour was observed in ammoniacal layer showing the presence of glycosides.

TEST FOR CARDIAC GLYCOSIDES (FOR DEOXYSUGAR)

KELLER KILIANI TEST

About 1 g of the powdered leaf was boiled with 10 ml of 70 % alcohol for 2 minutes, cooled and filtered. To the filtrate 10 mL of water and 5 drops of solution of lead subacetate were added and filtered, evaporated to dryness. The residue was dissolved in 3 mL of glacial acetic acid. To these 2 drops of ferric chloride solution was added. Then 3 mL of concentrated H_2SO_4 was added to the sides of the test tube carefully and observed. No reddish brown layer was observed indicating the absence of deoxysugars.

RAYMOND TEST

Test solution treated with dinitrobenzene in hot methanolic alkali gives violet colour.

LEGALS TEST'

Test solution when treated with pyridine made alkaline by sodium nitroprusside solution gives pink to red colour.

TEST FOR CYANOGENETIC GLYCOSIDES

Small quantity of the powder was placed in a stoppered conical flask with just sufficient water, to cover it. A sodium picrate paper strip was inserted through the

stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place. Brick red color was produced on the paper indicating the presence of cyanogenetic glycosides.

TEST FOR COUMARIN GLYCOSIDES

WITH AMMONIA

Take a drop of ammonia on a filter paper; to this add a drop of aqueous extract of leaves. Development of fluorescence shows positive test for coumarins.

WITH HYDROXYLAMINE HYDROCHLORIDE

To ethereal extract, added one drop of alcoholic KOH. It was then heated, cooled and acidified with 0.5N hydrochloric acid. Violet colour developed upon addition of a drop of 1 % w/v FeCl3 indicated presence of coumarins.

TEST FOR STEROLS

The powdered drug was first extracted with petroleum ether and evaporated to a residue.

Then the residue was dissolved in chloroform and tested for sterols.

SALKOWSKI'S TEST

A few drops of concentrated sulpuric acid was added to the above solution, shaken well and set aside. The lower chloroform layer of the solution turned red in colour indicatingthe presence of sterols.

TEST FOR LIBBERMANN – BURCHARD'S

To the chloroform solution a few drops of acetic anhydride and 1 ml of concentrated sulphuric acid were added through the sides of the test tube and set aside

for a while. At the junction of two layers a brown ring was formed. The upper layer turned green indicating the presence of sterols.

TEST FOR SAPONINS

FROTH TEST:

0.1g of powder was vigorously shaken with 5ml of distilled water in a test tube for 30 seconds and was left undisturbed for 20 min, persistent froth indicated presence of saponins.

TEST FOR TANNINS

FERRIC CHLORIDE

Small quantity of the powdered drug was extracted with water. To the aqueous extract few drops of ferric chloride solution was added. Bluish black colour was produced indicating the presence of tannins.

GOLD BEATER'S SKIN TEST

Add 2 % hydrochloric acid to all small piece of g old beater's skin, rinses it with distilled water and place in the solution to be tested for five minutes. Then give wash of distilled water and transfer to a 1% ferrous sulphate solution. A brown or black colour on the skin indicates presence of tannin.

TEST FOR PHENOLIC COMPOUNDS

FERRIC CHLORIDE

A small quantity of the powdered drug was extracted with water. To the alcoholic extract few drops of ferric chloride solution was added. Bluish black colour was produced indicating the presence of tannins.

TEST FOR FOLIN COICALTEU REAGENT

To a drop of methanolic extract of a few drop of Folin Coicalteu reagent was added, development of bluish green colour showed presence of phenol.

TEST FOR FLAVONOIDS

SHINODA'S TEST

Little of the powdered drug was heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added. Boiled for five minutes. Red colour was obtained indicating the presence of flavonoids.

ALKALI TEST

To the small quantity of test solution 10% aqueous sodium hydroxide solution was added. Yellow orange color was produced indicating the presence of flavonoids.

LEAD ACETATE

To the test solution add a mixture of 10 % lead acetate in few drops added. It gives white precipitate.

TEST FOR ACID

To the small quantity of test solution, few drops of concentrated sulphuric acid were added. Yellow orange colour was obtained indicates the presence of flavonoids.

TEST FOR PROTEIN AND AMINO ACIDS

MILLON'S TEST

Small quantity of acidulous – alcoholic extract of the powdered drug was heated with Millon's reagent. White precipitate turned red on heating indicate the presence of proteins.

BIURET TEST

To one portion of acidulous – alcoholic extract of the powdered drug one ml of 10% sodium hydroxide solution and one drop of dilute copper sulphate solution were added. Violet colour was obtained indicating the presence of proteins.

NINHYDRIN TEST

To the test solution add Ninhydrin solution, boil, violetcolourindicates presence of amino acid.

TEST FOR SULPHUR CONTAINING AMINO ACID

5 ml test solution is mixed with 2 ml 40 % sodium hydroxide and 2 drops of 10% lead acetate solution. Then boil the solution turned black or brownish due to PLS formation.

TEST FOR TERPENOIDS

Little of the powdered drug was extracted with chloroform and filtered. The filtrate was warmed gently with tin and thionylchloride. Pink color solution appeared which indicated the presence of terpenoids.

TEST FOR VOLATILE OIL

Weighted quantity (250 gm) of fresh leaves were extracted and subjected tohydro distillation using volatile oil estimation apparatus.

TEST FOR FIXED OIL

A small amount of the powder was pressed in between in the filter paper and the paper was heated in an oven at 105^0 C for 10 minutes. A translucent greasy spot appeared indicating the papers.

TEST FOR GUM

The small quantity of extract was added with few drops of alcohol to form white precipitate which indicates the presence of gum.

TEST FOR MUCILAGE

Few ml of aqueous extract was prepared from the powdered crude drug was treated with ruthentium red. Red colour was produced indicating the presence of mucilage.

TEST FORBETACYANINS

To 1 ml of plant extract, 1 ml of 2N NaOH was added and heated for 5 minutes at 100^0C . formation of yellow colour indicated the presence of betacyanins.

TEST FOR ANTHOCYANIN

About 0.2g of plant extract was weighed in separate test tube, 1ml of 2N sodium hydroxide was added, and heated for 5 minutes. Observed for the formation of bluish green colour which indicates the presence of anthocyanin.

TEST FOR LEUCOANTHOCYANINS

To 1 ml of plant extract, 1 ml of isoamyl alcohol was added. formation of red colour indicated the presence of leucoanthocyanins.

TEST FOR QUINONES

To 1 ml of plant extract, 1 ml of conc. H_2SO_4 was added. formation of red colour indicated the presence of quinones.

TEST FOR EMODINS

The dry extract was added to 25% ammonia solution. The formation of a cherry red colour solution indicated the presence of emodins.

TEST FOR COUMARINS

To 1 ml of plant extract, 3 ml of NH_4OH and 2ml of benzene was added. formation of red colour indicated the presence of coumarin.

TEST FOR RESINS

The extracts were treated with acetone. A small amount of water was then added and shaken.Appearence of turbidity indicates the presence of resins.

TEST FOR PHLOBATANNINS

About 2 ml of aqueous extract was added to 2ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was an evidence for the presence of phlobatannins. The results are presented in the **Table**: **6**

QUANTITATIVE ESTIMATION OF PHYTO CONSTITUENTS

DETERMINATION OF TANNIC ACID EQUIVALENT IN (HAESA)

(RabiaNaz and AsghariBano, 2013)

PRINCIPLE

Total tannin content of extract was determined by Folin Denis reagent method. Tannin like compounds reduces phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution. The intensity of which is proportional to the amount of tannins and it was estimated by spectrophotometer at 700 nm.

INSTRUMENT

UV Visible spectrophotometer, Shimadzu (Model 1800).

REAGENT REQUIREMENT

- Folin Denis Reagent
- Sodium carbonate solution (10%)
- Standard tannic acid solution.

PROCEDURE

0.2 mL of (1 mg/ml) hydroalcoholic extract of *Sida acuta* Burm (Leaf)was made up to 1 mL with distilled water. Then add 0.5 ml of Folin Denis reagent and allowed to stand for 15 min, then 1 ml of sodium carbonate solution was added to the mixture and it was made up to 10 mL with distilled water. The mixture was allowed to stand for 30 min at room temperature and the tannin content was determined spectrophotometrically at 700nm.The calibration curve was generated by preparing tannic acid at different concentration (5, 10, 15, 20 and 25µg/mL). The reaction mixture without sample was used as blank. The total tannin content in the leaf extract was expressed as milligrams of tannic acid equivalent per gm of extract. The results are tabulated in **Table: 7** and the calibration graph were presented at **Fig: 22**.

DETERMINATION OF GALLIC ACID EQUIVALENT IN (HAESA)

(Singleton *et al*., 1999)

PRINCIPLE

Total phenolic content of the various concentrations of HAESA was determined by Folin-ciocalteu reagent method. The hydroxyl group (OH) of phenolic compounds reduce the phosphomolybdic acid to molybdenum blue in the presence of alkaline medium (present in Folin reagent). The blue coloured complex was then spectrophotometrically measured at 760nm.

INSTRUMENT

UV Visible spectrophotometer, Shimadzu (Model 1800).

REAGENT REQUIRED

- Folin-Ciocalteu Reagent (1N)
- Sodium carbonate solution (10%)
- Standard Gallic acid solution

PROCEDURE

About 1 mL (1mg/ml and 0.5 mg/mL) of hydroalcoholic extract of *Sida acuta* Burm (Leaf) (HAESA), 0.5 mL of Folin-ciocalteu reagent (1N) were added and allowed to standfor 15 minutes. Then 1 mL of 10% sodium carbonate solution was added to the above solution. Finally the mixtures were made up to 10 mL with distilled water and allowed to stand for 30 minutes at room temperature and total phenolic content was determined spectrophotometrically at 760nm wavelength.

The calibration curve was generated by preparing gallic acid at different concentration (5, 10, 15, 20 and 25 µg/mL). The reaction mixture without sample was used as blank. Total phenolic content of HAESA extract is expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g). The results are tabulated in **Table: 8** and the calibration graph was presented at **Fig:23**

DETERMINATION OF RUTIN (FLAVONOID) EQUIVALENT IN (HAESA)

(Zhishen*et al*., 1999)

PRINCIPLE

Flavonoids present in the extract form, a charge transfer complex with several heavy metals to give a characteristic colour. In this reaction, the high electron positive nature of aluminium attracts the atomic nuclei of the aromatic rings in the flavonoids. Then it will react with potassium acetate in alkaline medium to form a pink coloured complex that is measured spectrophotometrically at 415 nm.

INSTRUMENT

UV Visible spectrophotometer, Shimadzu (Model 1800).

REAGENT REQUIRED

- 10% aluminium chloride
- 1M potassium acetate
- Standard rutin

PROCEDURE

1mL of hydroalcoholic extract of *Sida acuta* Burm (Leaf), 0.1 mL of aluminium chloride solution, 0.1 mL of potassium acetate solution and 2.8 mL of ethanol were added and the final volume was then made up to 5 mL with distilled water. After 20 min the absorbance was measured at 415 nm.

A calibration curve was constructed by plotting absorbance reading of rutin at different concentrations (5, 10, 15, 20 and 25 µg/mL). The sample without aluminium chloride was used as a blank. The total flavonoid content in the extract was expressed as milligrams of rutin equivalent per gram of extract. The results are tabulated in **Table: 9** and the calibration graph is presented at **Fig: 24**

TLC PROFILE OF *Sida acuta* Burm. (leaf)

Thin layer chromatography (TLC) of the hydro alcoholic extract of *Sida acuta* (leaf) showed orange brown spot and Rf value 0.35 may indicate the presence of alkaloid. the solvent system used in **Toluene : ethylacetate : diethylamine (70:20:10),** Rf value 0.37at 365nm and bright blue spot may indicate thepresence of coumarine, the solvent system used in **toluene:ether (1:1)** , Rf value 0.36 at 365nm may indicate the presence of flavonoids.the solvent system used in **Benzene : pyridine: Formic acid (72:18:10).** Rf value 0.33 and deep purple spot may indicate presence of cryptolepine, the solvent system used in **Dichloromethane: Chloroform: Methanol (4:4:1),** Rf value 0.34 and yellow spot may indicate presence of

cryptolepine, the solvent system used in **Ethylacetate: Methanol: Ammonia (80:15:5) .**The results are tabulated in **Table: 10** and **Fig:25.**

Column Chromatography

Column chromatography is an isolation and purification technique. Column dimension and diameter 3cm, leangth 75cm stationary phase aluminium oxide ,the solvent were poured in the following order.

S.No	Sovent elution order	Compound
1	Petroleum ether	-Nil-
2	Petroleum ether and chloroform (50:50)	-Nil-
3	Chloroform and methanol(9:1)	-Nil
4	Methanol	SA-1

ISOLATION

Powdered, oven-dried (60^0 C) leaves of *Sida acuta* 50gm were defatted with 100 ml of petroleum ether for 8 hours.

↓

The marc was extracted by soxhlet with methanol 100 ml Methanol for 8 hours

↓

The extract was evaporated under reduced pressure to give a residue 3.4gm which was thoroughly mixed with AcOH (5% 500ml).

↓

The mixture diluted with water (250ml) and allowed to stand overnight.

↓

The mixture was filtered affording a clear solution and some insoluble material.

↓

The aqueous filtrate was made basic with aqueous ammonia to PH 9.5 and extracted with $CHCl_3$ (500ml).

↓

Extracts were dried over anhydrous $MgSo_4$, filtered and evaporated to afford a dark alkaloidal residue (450mg)

↓

The alkaloidal residue was dissolved in $CHCl_3$ (25ml)

Adsorbed on to Al_2O_3 (20gm) and chromotographed over Al_2O_3 (200gm)

Elution was conducted successively with petroleum ether 40-60 (500ml) followed by petroleum ether-$CHCl_3$ (500ml) and $CHCl_3$ (500ml) $CHCl_3$-MeOH (9:1) 500ml and finally MeOH (500ml)

QUINDOLINE was obtained as yellow solid after column chromatography and preparative tlc on AlO_3 in chloroform.

It gave an orange colouration with Dragendorff's reagent, which is evidence for quindoline alkaloids.

Elution of the column with MeOH (9:1) afforded a violet residue.it crystallized from aqueous ethanol as long violet needles shaped CRYPTOLEPINE

UV Spectral studies of isolated SA-I

The isolated compound crptolepine was dissolved in methanol and transferred to cuvette (quartz) and was scanned under UV range from 200-600 nm in the UV-Visibble spectrophotometer. The isolated compound cryptolepine shows absorbance maxima at λ_{max} 255 λ_{max} 274, λ_{max} 331and compared with that of pure cryptolepine as described by Dwuma-Badu,Ayim et al.1978. The result are tabulated in **Table**:11 and the calibration graph was presented at **Figure**: **26**

Infrared spectral studies of isolated SA-I

The isolated compound cryptolepine was sample handling by liquid membrane method involves dripping several drops of the sample onto an NaCl aperture plate and sandwiching it under another aperture plate, such that no gas bubbles are trapped. The thickness is adjusted and carried out for analysis in infrared spectrophotometer. Group frequency region was 4000cm^{-1} to 1500cm^{-1} & figure print region was 1500cm^{-1} to 600cm^{-1}. The result are tabulated in **Table:12** and the calibration graph was presented in **Figure:27**

NMR Spectral studies of isoloated SA-I

The isolated compound cryptolepine sample 10-50 mg was dissolved in deuterated chloroform, is contained in a 5-mm O.D.glass tube. The sample can absorb electromagnetic radiation in the radiofrequency region at frequencies governed by the characteristics of the sample. Absorption is a function of certain nclei in the molecule.A plot of the frequencies of absorption peaks versus peak intensities constituents the NMR spectrum. The result are tabulated in **Table**:**13,14** and the calibration graph was presented at **Figure: (28,29)**

PART C

PHARMACOLOGICAL STUDIES

ANTIOXIDANT ACTIVITY

Oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals are collectively termed as reactive oxygen species (ROS) and have been implicated in the pathogenesis of various diseases. When generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates and this leads to a number of physiological disorders. The oxidative stress has also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurological disorders, as well as in the process of aging. Many plants contain substantial amounts of antioxidants and can be utilized to scavenge the excess free radicals from human body.

Sida acuta Burm. commonly known as 'Wire weed '. *Sida acuta* is a shrub indigenous to pantropical areas, widely distributed in these regions and widely used in traditional medicine.It is used for various purpose like neurological disorders, headache, leucorrhoea, tuberculosis, diabetics, fever, uterine disorders and as an antirheumatics, and antipyretic agent as described in Ayurveda .Earlier phytochemical studies of *Sida acuta* Burm. revealed the presence of quindoline and cryptolepine. The plant is also reported to contain alkaloids, flavonoids, steroids, terpenoids, cardiac glycoside and phenolic compounds, vitamin C and higher levels of vitamin A and β-carotene. The antioxidant activity DPPH radical scavenging activity of the hydro methanol extract of *Sida acuta* has been reported by (**Raman N.2006**)

Although some ethnobotanical and phytochemical information are available on these plants, their nutraceutical values have not yet been exposed. Therefore the antioxidant potential of these plants was investigated by employing different *in vitro* free radical scavenging assays.

INVITRO ANTIOXIDANT ACTIVITY

DETERMINATION OF HYDROGEN PEROXIDE SCAVENGING ACTIVITY

PEROXIDE MG.Rana et al., (1996)

PRINCIPLE

The principle is based on the capacity of the extract to decompose the hydrogen peroxide to water. H_2O_2 in the presence of $\mathbf{O^{2-}}$ can generate highly reactive hydroxyl radicals via the metal, the scavenging of H_2O_2 in cells is critical to avoid oxidative damage. Thus, the scavenging of hydrogen peroxide is an important antioxidant defence mechanism.

$$\mathbf{Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH + OH^-}$$

The decomposition of hydrogen peroxide to water involves the transfer of electrons as in the equation.

$$\mathbf{H_2O_2 + 2H^+ + 2e^- \longrightarrow 2H_2O}$$

REAGENTS

- 6% hydrogen peroxide diluted with water in the ratio of 1:10
- 0.1 M Phosphate buffer (pH 7.4)

PROCEDURE

To 1 mL of test solutions of different concentrations, 3.8 mL of 0.1 M phosphate buffer solution (pH 7.4) and then 0.2 mL of hydrogen peroxide solution were added. The absorbance of the reaction mixture was measured at 230 nm after 10

min. The reaction mixture without sample was used as blank. Sample blank was also prepared without reagents. Ascorbic acid was used as standard. The percentage inhibition of hydrogen peroxide was calculated using the formula,

% inhibition = [(Control–Test) / Control] × 100

The concentration of the sample required for 50 % reduction in absorbance (IC_{50}) was calculated using linear regression analysis.

The results are tabulated in **Table: 16** and the calibration graph was presented in **Fig 31**.

DETERMINATION OF REDUCING POWER ASSAY Navnath et al,(2010)

Principle

Reducing power assay is a spectrophotometric method and is based on the principle that increases absorbance of the reaction mixture indicates the increase in the reducing power of the sample. Antioxidant activity may be due to a variety of mechanism viz., the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the reducing capacity and free radical scavenging. The assay is based on the reduction of ferric in potassium ferricyanide to ferrous to form potassium ferrocyanide by the sample and the subsequent formation of Prussian blue colour with ferric chloride. The absorbance of the blue complex is measured at 700 nm.

Potassium ferricyanide+ Ferric chloride

↓ **Antioxidant**

Potassium ferrocyanide + Ferrous chloride

INSTRUMENT

UV Visible spectrophotometer, Shimadzu (Model 1800).

MATERIALS REQUIRED

- Ascorbic acid
- 1 % w/v Potassium ferricyanide
- 10 % w/v Trichloro acetic acid
- 0.2 M, Phosphate buffer (pH 6.6)
- 0.1% w/v Ferric chloride

PROCEDURE

The reducing power ability of plant extracts was screened by assessing the ability of the test extract to reduce $FeCl_3$ solution as mentioned by Oyaizu *et al.,* (1986). 0.1 to 0.5 mL of plant extract solution (1 mg/mL) was mixed with 0.75 mL of phosphate buffer and 0.75 mL of 1 % potassium ferricyanide [K_3Fe (CN_6)] and incubated at 50^oC for 20min. About 0.75 mL of 10 % trichloro acetic acid was added to the mixture and allowed to stand for 10min. The whole mixture was then centrifuged at 3000 rpm for 10min. Finally 1.5 mL of the supernatant was removed and mixed with 1.5 mL of distilled water and 0.1mL of 0.1 % ferric chloride solution and the absorbance was measured at 700 nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer was used as blank solution. The results are tabulated in **Table:** 17 and the calibration graph was presented in **Fig:** 32

DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY (Prieto*et al.*, 1999)

Principle

The total antioxidant activity of the extract was evaluated byphosphomolybdenum method. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample and by the subsequent formation of green phosphate Mo (V) complex at acidic pH which has a maximum absorption at 695 nm. This method is routinely used to determine total antioxidant activity of samples.

$Mo^{6+} \rightarrow Mo^{5+}$

Instrument

UV Visible spectrophotometer, Shimadzu (Model) 1800.

Reagents

- 0.6M sulphuric acid
- 28mM sodium phosphate
- 4mM ammonium molybdate

Procedure

An aliquot of 0.3 mL of different concentrations of sample was treated with 2.7 mL of the reagent (H_2SO4, sodium phosphate and ammonium molybdate). In case of blank, 0.3 mL of methanol was used in place of sample. The tubes were incubated in a boiling water bath at 95°C for 90 min. The samples were cooled to room temperature, the absorbance of the aqueous solution of each concentration was measured at 695 nm against blank. The standard vitamin C was treated in a similar manner. The antioxidant activity was expressed as equivalents of Vitamin C (µg/mL). The results are tabulated in **Table:** 18 and the calibration graph was presented in **Fig:** 33.

ALPHA AMYLASE INHIBITORY ACTIVITY

Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis, due to disturbances of carbohydrate, fat and protein metabolism, resulting from defects in insulin production, secretion, insulin action. This can result in long-term damage to organs, such as the kidneys, liver, eyes, nerves, heart and blood vessels. Complications in some of these organs can lead to death.

The pancreas plays a primary role in the metabolism of glucose by producing and secreting the hormones like insulin and glucagon. The islets of Langerhans produce and secrete insulin and glucagon directly into the blood. Insulin is a protein that is essential for proper regulation of glucose and for maintenance of proper blood glucose levels. Glucagon is a hormone that opposes the action of insulin. It is secreted when blood glucose level falls. It increases blood glucose concentration, partly by stimulating the breaking down of stored glycogen in the liver by a pathway known as glycogenolysis. Gluconeogenesis is the production of glucose in the liver from non-carbohydrate precursors such as glycogenic amino acids.

WHO classification of diabetes introduced in 1980 and revised in 1985 was based on clinical characteristics. The two most common types of diabetes were insulin-dependent diabetes mellitus (IDDM) or (type I) and non-insulin-dependent diabetes mellitus (NIDDM) or (type II). WHO classification also recognized malnutrition-related diabetes mellitus and gestational diabetes. Malnutrition-related diabetes was omitted from the new classification because its etiology is uncertain, and it is unclear whether it is a separate type of diabetes.

Many plants have been found to be useful in managing diabetes mellitus. Plants are the major source of drug and are available in the market as extracts directly or indirectly from the plant sources. For the purpose of preventive and curative

reasons plants were used as medicine is various parts of the world. Medicinal herbs were used to treat diabetes in large proportion all over the Globe because of the easy availability and affordability. Plant medicines show a potential hypoglycemic activity in diabetes-induced animals. Further, the metabolites from the plant were formulated and were administered to control diabetes mellitus. Novel compounds with antihyperglycemic potential have to be isolated from the plant sources. Studies reveal the role of crude extracts of plants with potential antidiabetic activity in alloxan and streptozotocin-induced diabetic animals. It is required to isolate, purify and characterize the specific compounds with the efficacy to control by reducing blood glucose level and by means of regenerating the damaged β cells of the pancreatic islets for the normal secretion of insulin. The objective of the review is to collect and elucidate the antidiabetic properties of the medicinal plants used by the traditional healers to manage diabetes mellitus.

IN VITRO ALPHA AMYLASE INHIBITORY ACTIVITY OF *Sida acuta* Burm. (BHUTKAR.M.A and BHISE.S.B, 2012)

PRINCIPLE

The determination of α-amylase inhibition was carried out by quantifying the reducing sugar (maltose equivalent) liberated under the assay conditions. The enzyme inhibitory activity was expressed as a decrease in units of maltose liberated. A modified dinitrosalicylic acid (DNS) method was adopted to estimate the maltose equivalent. The anti-diabetic activity was determined through the inhibition of α-amylase which was expressed as a percentage of inhibition and calculated by the following equations:

% reaction = (maltose) test / (maltose) control × 100

% inhibition = 100% reaction

MATERIALS REQUIRED

- Alpha amylase enzyme,
- 3,5 dinitrosalicylic acid (DNS)
- Acarbose
- Soluble starch
- Sodium potassium tartarate
- 2N Sodium hydroxide
- 20Mm Phosphate buffer(pH 6.9)
- 6.7Mm sodium Chloride

INSTRUMENTS

UV Spectrophotometry

PROCEDURE

A starch solution (1%w/v) was prepared by stirring 1g starch in 100 ml of 20Mm of phosphate buffer (Ph 6.9) containing 6.7mM sodium chloride. The enzyme solution was prepared by mixing 27.5mg of porcine pancreatic α-amylase(PPA)in 100 ml of 20mM of phosphate buffer (PBS,Ph 6.9)containing 6.7mM of sodium chloride.To 100µl of(2,4,8,10,15 µg/ml) plant extract.200 µl (1%) starch solution was added and the mixture was incubated at 37^0c for 20 min. To the reaction mixture 100µl(1% starch solution was added and incubated at 37^0c for 10 min. The reaction was stopped by adding 200µl DNSA (1g of 3, 5 di nitro salicylic acid, 30g of sodium potassium tartarate and 20ml of 2N sodium hydroxide was added and made up to a final volume of 100 ml with distilled water) and kept it in a boiling water bath for 5 minutes. The reaction mixture diluted with 2.2 ml of water and absorbance was read at 540nm. For each concentratrion, blank tubes were prepared by replacing the enzyme solution with 200µl in distilled water. Control, representing 100% enzyme activity was prepared in a similar manner, without extract. The experiments were repeated thrice using the same protocol.

The results are depicted in **Table: 19** and **Fig: 34**

Antimycobacterial activity

Tuberculosis has been present in humans since antiquity, at the latest. The earliest unambiguous detection of *M. tuberculosis* involves evidence of the disease in the remains of bison dated to approximately 17,000 years ago. Skeletal remains show prehistoric humans (4000 BC) had TB and researchers have found tubercular decay in the spines of Egyptian mummies dating from 3000–2400 BC. Tuberculosis (TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis*. It retains certain stains after being treated with acidic solution, so it is classified as an acid-fast bacillus (AFB). *Mycobacterium tuberculosis* is an aerobic, gram positive rod shaped bacteria ranging from 1 to 4 μm in length.Tuberculosis remains the largest cause of death in the world from a single infectious disease and accounts for as much as 40% of deaths in human immunodeficiency virus (HIV) co-infected individuals in some developing countries. Infection with conventional *M. tuberculosis* can effectively be cured with a combination of antitubercular drugs. Ominously, multidrug-resistant tuberculosis (MDR-TB) strains have emerged in several countries, with case fatalities ranging from 40 to 60% in immunocompetent individuals and >80% in immunocompromised individuals.

Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases for many centuries. These have been used extensively as pure compounds or as a crude material. Only a few plant species have been thoroughly investigated for their medicinal properties. India is one of the few countries in the world which has unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for cure of various diseases. The increasing incidence of MDR- and XDR-TB worldwide highlight the urgent need to search for newer anti-tuberculosis compounds/drugs.

It was planned to study the *In vitro* anti-mycobacterial activity of *Sida* acuta Burm. which is commonly used in the treatment of respiratory diseases like bronchitis, asthma, tuberculosis, urogenital diseases and in dysenteries in the indigenous system of medicine.

DETERMINATION OF ANTIMICOBACTERIAL ACTIVITY(PAPITHA.N et al 2013)

PRINCIPLE

Luciferase reporter phage assay(LRP)

Anti-mycobacterial activity of the *Sida acuta* Burm. extracts was evaluated by Luciferase reporter phage (LRP) assay against Standard strain of *M. tuberculosis* H37RV and Clinical isolate of *M.tuberculosis* resistant to Streptomycin, Isoniazid, Rifampicin and Ethambutol (S,H,R & E) at two different concentrations (100 and 500µg/mL). The Luciferase reporter phage assay methodology is rapid, inexpensive and less laborious for high throughput screening of compounds for their anti mycobacterial activity compared to BATEC methodology which is costly, cumbersome and uses radioactive reagents. A compound is considered as an anti-tubercular agent if fifty percent reduction in relative lights units (RLU) is observed when compared to the control using luminometer.

Standard strain of *M.tuberculosis* H37RV and clinical isolate of *M.tuberculosis* resistant to S,H,R & E maintained at National Institute for Research in Tuberculosis, Chennai were used for the anti mycobacterial assay.

INSTRUMENTS

- Luminometer

MATERIALS REQUIRED

- Standard strain H37RV
- Isolate *M.tuberculosis* resistant
- MacFarlands No.2 Standard
- Middlebrook 7H9 Medium
- 0.1M Calcium chloride
- D.Luciferin

PROCEDURE

Standard strain H37RV and a clinical isolate of *M.tuberculosis* resistant to S, H, R & E were grown in Middlebrook 7H9 complete medium 12 with and without extracts of *Sida acuta* Burm. for 3 days at 37°C. Luciferase Reporter Phage Assay10 was done using concentrations of 100 and 500 µg/ml of *Sida acuta* Burm. extracts. Fifty-microliter bacterial suspension equivalent to MacFarlands No.2 standard was added to 400 µl of G7H9 with and without the test compound. For each sample, two drug-free controls and two drug concentrations were prepared and this set up was incubated for 72 h at 37°C. After incubation, 50 µl of the high titer Luciferase reporter phage (phAE129) and 40 µl of 0.1 M CaCl2 were added to all the vials and this setup was incubated at 37°C for 4 h. After incubation, 100 µl of the mixture was taken from each tube into a luminometer cuvette and an equal amount of working D-luciferin (0.3 mM in 0.05 M sodium citrate buffer, pH 4.5) solution was added. The RLU was measured after 10s of integration in the Luminometer. Duplicate readings were recorded for each sample and the mean was calculated. The percentage reduction in the RLU was calculated for each test sample and compared with control. The experiment was repeated when the mean RLU of the control was less than 1000. The results are tabulated in **Table: 20** and the calibration graph was presented in **Figure: 35**

Anti-Histaminic activity

Asthma is a chronic lung disease characterized by recurrent respiratory symptoms such as wheezing, breathlessness, chest tightness, coughing, and variable airflow obstruction that is reversible spontaneously or with treatment. During an asthma attack three things occur: bronchoconstriction, mucous production, and inflammation.

Ayurveda, Siddha, Unani and Folk (Tribal) medicines are the major systems of indigenous medicines. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. Unlike many diseases, which can be attributed to the life style of modern man, asthma is an ancient illness. Mast cells play an important role in some type of allergic reaction because the antibody that causes the allergic reaction that is Ig E have the mast cells which contains about a thousand tiny granules. These granules are loaded with dozens of potent chemicals or mediators, the most powerful in which are histamine and a newly discovered group called leukotrienes. From the present laboratory, there are number of medicinal plants have been reported for antihistaminic/anti-asthmatic activities. Some of them are *Achyranthes aspera*, *Tephrosia purpurea, Dolichos lablab, Eclipta alba, Jasminum sambac, Balanites aegyptiaca, Viscum album, Tridex procumbens, Glycyrrhiza glabra* and *Cassia fistula.* Recently, Soni has reported 100% inhibition of Leukotrienes (which cause asthma) from the EtoAC fraction of *Bacopa monnieri* extract. It is suggested that formulation and patent of the reported medicinal plants is mandatory for further use against asthma and if possible, clinical trials should be done of these plants for their appropriate use.

Management of asthma in traditional medicinal system – Ayurveda

Ayurveda is an example of a long-standing tradition that offers a unique insight onto comprehensive approach to asthma management through proper care of the respiratory tract. This includes maintaining the nourishing functions of the lungs in providing oxygen to the body. Ayurvedic formulations used in the management of asthma therefore judiciously combine herbs for breathing support with anti-oxidant herbs to support digestive, cardiac and nerve functions, expectorant herbs as well as soothing herbs. The following components are normally included in the Ayurvedic approach to the management of asthma.

Essential components

- ✓ Long-term administration of pulmonary tonics to strengthen the lungs.
- ✓ Administration of relaxing expectorants to prevent building up of sputum.
- ✓ Antispasmodic preparations to help/mitigate the effect of the bronchospasm on the pulmonary muscles.

Ancillary components

- ✓ Demulcents could be used to sooth irritation of mucous surfaces.
- ✓ Anti-spasmodic would prevent the over production of sputum in lungs or sinuses.
- ✓ Anti-microbial compounds would prevent secondary infections.
- ✓ Nervine support herbs are needed to enable adaptation to stress, since excessive stress or nervous debility may aggravate the symptoms of asthma.

Herbal drugs used in asthma

Asthma is a global problem. Many synthetic drugs are used to treat acute symptoms of asthma, but they are not completely safe for long term use. Hence search has been started once again to look back to traditional medicine which can be used to treat asthma. The following table 1 gives a brief review of the medicinal plants used as antiasthmatic with their probable mechanism of action.

DETERMINATION OF ANTI-HISTAMINIC ACTIVITY.

Guinea pig ileum preparation (Kulkarni 2003 and Goyal 2007)

MATERIALS REQUIRED

- Kreps solution
- Histamine

INSTRUMENTS

- Mammalian bath

PROCEDURE

Guinea pigs of either sex (1 to 1.5 kg), starved overnight but allowed free access to water, were used .The animals were killed by a blow on the head and were emarginated. A segment of the guinea pig ileum (approximately 2cm long), removed from a freshly killed animal, was tied with a thread to the top and bottom ends without closing the lumens. It was suspended in the same way in a 30 ml organ bath containing tyrode solution maintained at 37 ± 1°C and gassed with air .A tension of 0.5 g was applied and the tissue was allowed to equilibrate for the period of 30 min before adding any extract or drugs to the organ bath. Contractile responses were established for histamine and concentrations were recorded depending on the

responses due to 10 µg/ml histamine using writing lever. The effects of the 500, and 1000µg/ml hydro alcoholic extract of *Sida acuta* on the histamine-induce contraction were investigated. Contact times of 30 s and 5 min time cycle were maintained for proper recording of the responses.

Experimental design

Group 1 received normal saline and Histamine10µg/ml

Group 2 received Histamine 10µg/ml and Hydro alcoholic extract of *Sida acuta* 500µg/ml

Group 3 Histamine 10µg/ml and Hydro-alcoholic extract of *Sida acuta* 1000µg/ml

Group 4 Histamine 10µg/ml and Chlorpheniramine mealete 10µg/ml

Statistical analysis: All the value of *in vivo* anti asthmatic activity were expressed as mean ± standard error of mean (S.E.M) and was examined for significance by ANOVA (analysis of variance) and groups were compared by Dunnett's test for individual comparison of group with control. P Value were measured moderate significant at $P<0.01$, <0.001 level. The results are tabulated in **Table: 21** and the calibration graph was presented in **Figure: 36,37& 38**

Guinea pig tracheal Preparation (Kulkarni 2003 and Goyal 2007)

MATERIALS REQUIRED

- Kreps solution
- Histamine

INSTRUMENTS

- Mammalian bath

Guinea pigs of either sex (1 to 1.5 kg), starved overnight but allowed free access to water, were used. The animals were killed by a blow on the head and were emarginated. After sacrificing the guinea pig, the trachea was dissected out and transferred into a dish containing Krebs solution. Each segment of the tracheal cartilage is cut out to give a number of rings of tracheal muscle. Atleast, 5 to 6 of such rings are tied together by their cartilage portion to give a tracheal chain preparation. It is mounted in the Krebs solution at 35 to 37°C under 0.5 g tension and aerated with air. The tissue was equilibrated for 30 min during which the bath solution was replaced every 10 min). Contractile responses were established for histamine and concentrations were recorded depending on responses due to 10 µg/ml histamine using writing lever. The effects of the 500 and 1000µg hydro-alcoholic extract of *Sida acuta* on the histamine-induced contraction were investigated. Contact times of 90 s and 5 min time cycle were maintained for proper recording of the responses.

Experimental design

Group 1 received normal saline and Histamine10µg/ml

Group 2 received Histamine 10µg/ml and Hydro alcoholic extract of *Sida acuta* 500µg/ml

Group 3 Histamine 10µg/ml and Hydro-alcoholic extract of *Sida acuta* 1000µg/ml

Group 4 Histamine 10µg/ml and Chlorpheniramine mealete 10µg/ml

Statistical analysis: All the value of *in vivo* anti asthmatic activity were expressed as mean ± standard error of mean (S.E.M) and was examined for significance by ANOVA (analysis of variance) and groups were compared by Dunnett's test for individual comparison of group with control. P Value were measured moderate significant at $P<0.01$, <0.001 level. The results are tabulated in **Table: 22** and the calibration graph was presented in **Figure: 39,40&41**

CHAPTER-V

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

MACROSCOPICAL STUDIES

Fresh leaves of *Sida acuta* was subjected to macroscopical studies and the results are presented in table1:

TABLE: 1 Macroscopical studies of *Sida acuta* Burm (Leaf)

S.NO	PARAMETER	OBSERVATION
1	Colour/surface	
	Dorsal Ventral	Dark green Light green
2	Odour	No characteristic
3	Taste	Bitter
4	Leaf Type	Simple
5	Shape	Lanceolate
6	Arrangement	Alternate
7	Apex	Acute
8	Base	equal
9	Stipules	Present
10	Margin	Bi serrate
11	Venation	Reticulate
12	Surface	Hairy
13	Length	4cm to 7.2cm.
14	Width	1.2cm to 2.5cm.
15	Petiole length	Small-short

Macroscopical studies of *Sida acuta* revealed the outer surface showed dark green and the inner surface showed light green with bitter taste and odourless. The leaves are simple, lanceolate shape with acute apex, equal base with stipules, alternate arrangement, bi serrate margin, hairy surface. The leaves are of 4-7cm length and 1.2-2.5 width and its petiole length small-short.

Figure: 1 Habitat *of Sida acuta* Burm (Leaf)

Figure: 2 Diagram of some morphological feature of *Sida acuta* Burm.

Figure: 3 Macroscopy of *Sida acuta* Burm. Leaf (Dorsal view)

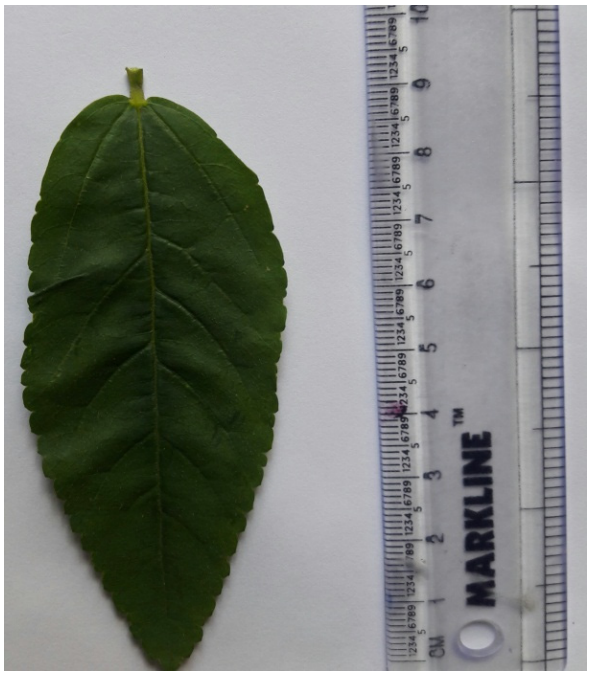


Figure:4 Macroscopy of *Sida acuta* Burm. Leaves (Ventral view)

Figure: 5 Leaf arrangement of *Sida acuta* Burm.

Figure: 6 Flowers of *Sida acuta* Burm.

Figure: 7 Fruits of *Sida acuta* Burm.

Figure: 8 Seeds of *Sida acuta* Burm.

Microscopical Studies of *Sida acuta* Burm (Leaf)

The microscopical section of *Sida acuta* showed the following characters.

Midrib

The midrib has a short pointed, adaxial cone and wide and abaxial semicircular part. The midrib is 550µm in medium vertical plane and 600µm in horizontal plane. There is a wide central vascular bundle which occupies almost major part of the midrib is showed in **Figure: 9**

Similar then midrib in general features. It consists of short adaxial conical part and wide semicircular abaxial part. The vascular bundle consists of three vertical lines of xylem elements and which are of abaxial phloem. The lateral vein is 320µm thick and 350µm wide is showed in **Figure: 10**

The epidermal layer of the consist of wide, uniform squarish cells with prominent cuticle. In the adaxial cone occur a vertical segment of three layers of thick walled cells, the palisade mesophyll extends up to the shoulder of the adaxial cone. The ground tissue which occurs around vascular bundle includes small and large compact parenchyma cells. Some of the parenchyma cells possess druse accumulation of mucilage is showed in **Figure: 11**

Vascular system

The vascular system has wide and thick bowl shaped structure. Xylem occurs in long vertical compact rows. Phloem occurs in thick are beneath the xylem. The xylem elements are in radial multiples and the cells are circular and thick walled. A thick layer of sclerenchyma cells is seen on the lower and upper portions of the vascular system.

Lamina

The lamina is dorsiventral with differentiation of palisade and spongy mesophyll tissues. The lamina is 180µm thick. The adaxial epidermis has their rectangular cells, the abaxial epidermal cells are thin and narrowly rectangular. There are two horizontal rows of thin, loosely arranged columnar palisade cells. The spongy mesophyll cells are spherical and are loosely interconnected with each other forming wide air chambars is showed in **Figure:12**

The marginal part of the lamina is slightly thin and bent down. The epidermal cells are small and thick walled. The mesophyll tissue is undifferentiated and cells compact. Mucilage is secreted in larger quantity at the marginal part of the lamina is showed in **Figure:13**

Petiole

The petiole is flat on the adaxial side and slightly semicircular on the abaxial side. It is 750µm in vertical plane and 950µm in horizontal plane is showed in **Figure: 14**

The epidermal cells of the petiole are square shaped and fairly thick walled inner of the epidermis a continuous zone of five or six layers of parenchyma cells. There is a thin dark line in the adaxial part of the petiole which is formed by crushing of cells due to growth pressure. In the abaxial part of the petiole, parenchymatous ground tissue is wider due to dilated cells is showed in **Figure: 15**

Vascular bundle

The vascular bundle is thick and elliptical in outline, it occupies the adaxial portion of the petiole. The vascular bundle is 750µm in horizontal plane and 370µm in verticle plane. The vascular bundle is surrounded by a thick continuous sclerenchyma

sheath. The sclerenchyma bundle sheath is thicker on the lower part than the upper part. is showed in

The vascular bundle consists of lower part of long, compact xylem elements, xylem elements are circular narrow and thick walled. The proto xylem elements are directed towards the adaxial part. The adaxial band of xylem has proto xylem elements directed towards the abaxial side phloem occurs in thick continuous cylinder around the xylem. Phloem includes sieve elements and companion cells. External the phloem occurs thick cylindary sclerenchyma cells are highly thick walled and lignified is showed in **Figure: 16**

Calcium oxalate crystal

Calcium oxalate crystals are abundant in the midrib is showed in **Figure: 17** Calcium oxalate crystals are present in the petiole. The crystals are druses which are spherical bodies with spiny surface. In the midrib the druses are seen in the phloem parenchyma while in the petiole are located in the ground parenchyma. The druses are up to 40µm in diameter is showed in **Figure: 18**

MICROSCOPICAL STUDIES OF *Sida acuta* Burm(Leaf)

Figure: 9. T.S. of Leaf through Midrib

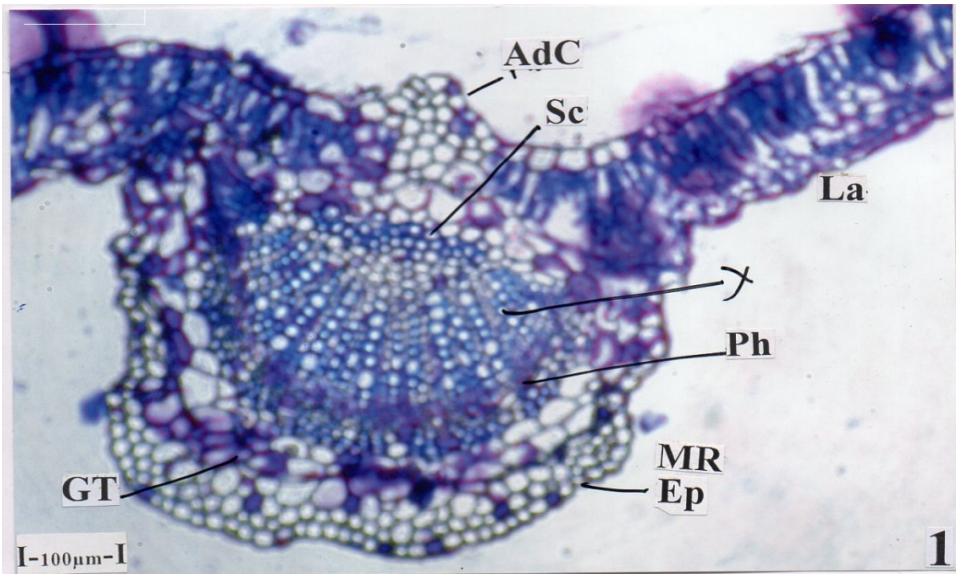


Figure: 9 **Adc** -Adaxial cone, **Sc** -Sclerenchyma, **La-**Lamina, **X-**Xylem, **Ph-**Phloem, **Ep-** Epidermis, **GT-**Ground Tissue.

Figure: 10 T.S of Leaf through Lateral vein

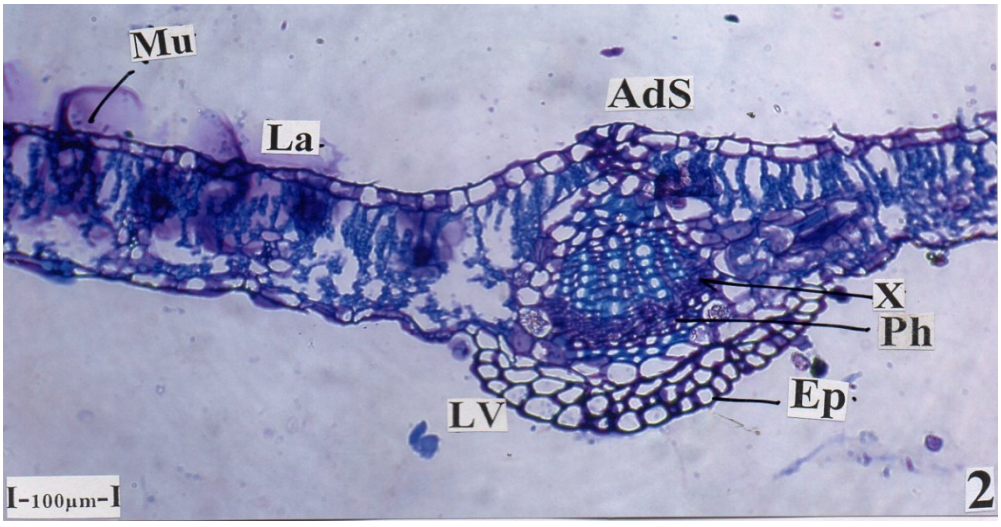


Figure: 10 **Mu-**Mucilage, **La-**Lamina, **AdS-**Adaxial Side, **X-**Xylem, **Ph-**Phloem, **Ep-**Epidermis, **LV-**Lateral vein.

Figure: 11 T.S. of Midrib-Enlarged

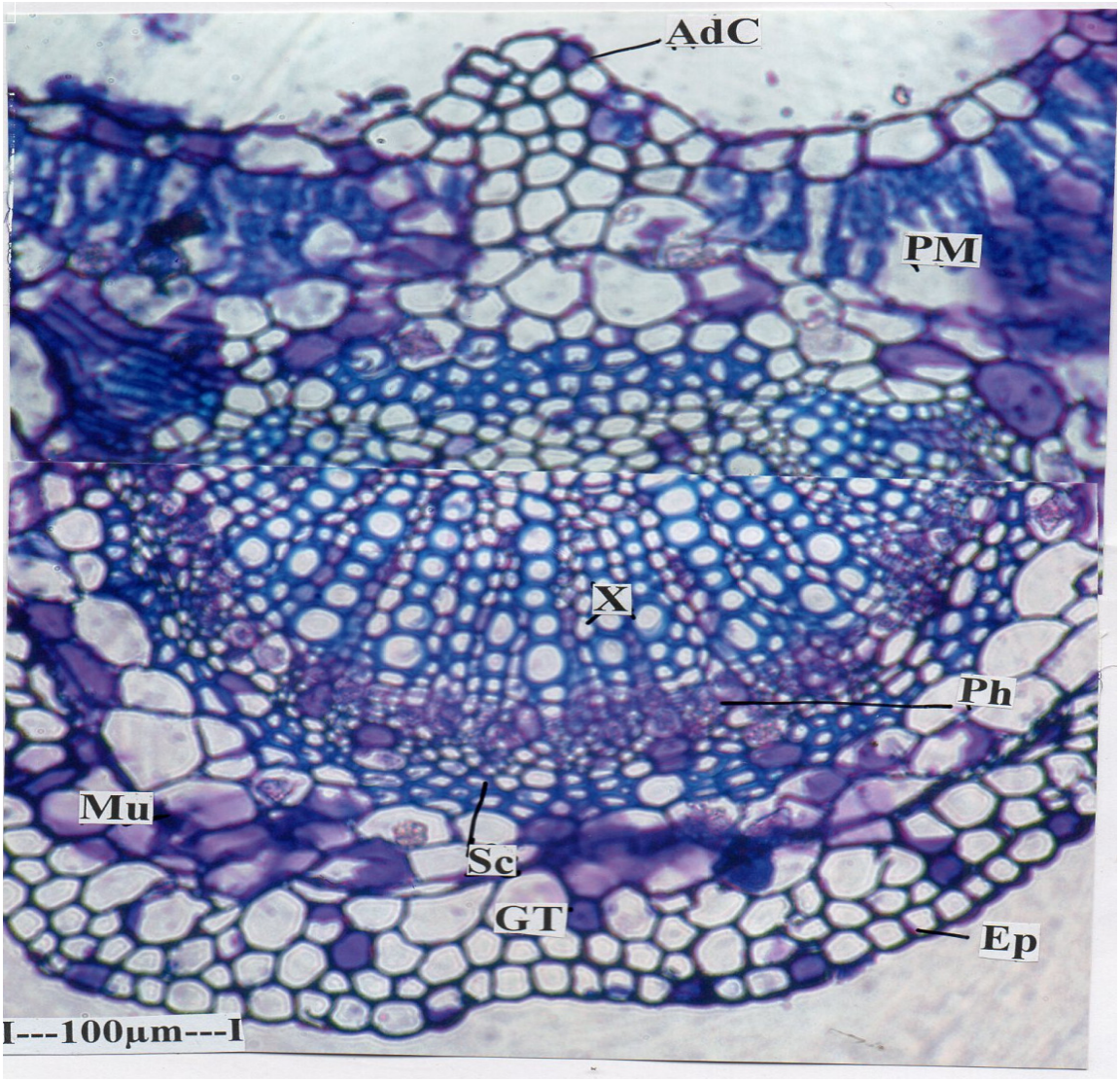


Figure: 11 **AdC**-Adaxial Cone, **PM**-Palisade Mesophyll, **X**-Xylem, **Ph**-Phloem, **Sc**-Sclerenchyma, **Mu**-Mucilage, **GT**-Ground Tissue, **Ep**-Epidermis.

Figure: 12 T.S of Lamina

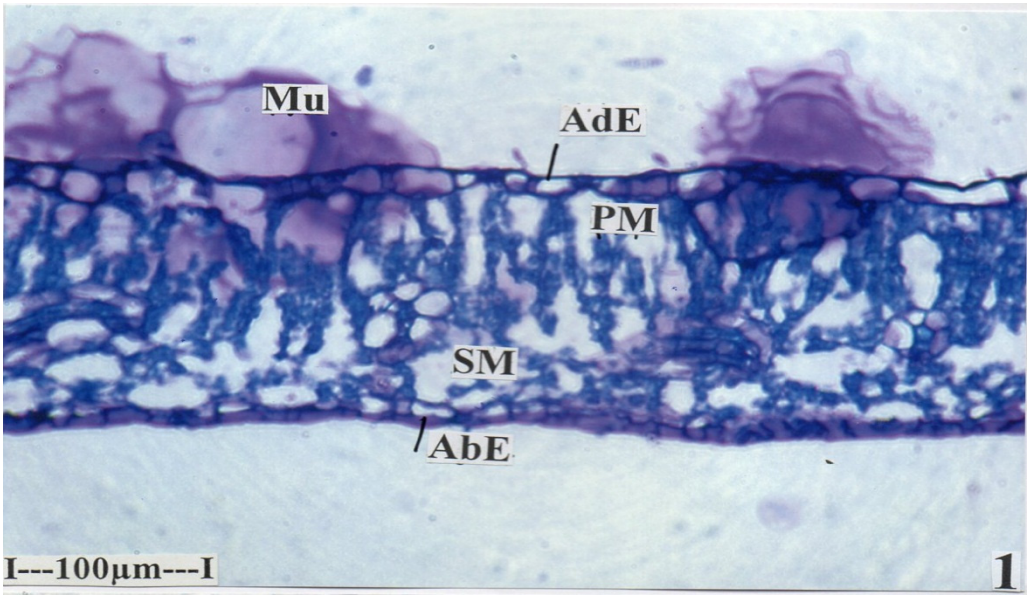


Figure: 12 **Mu-**Mucilage, **AdE**-Adaxial Epidermis, **PM-**Palisade Mesophyll, **SM-**Spongy mesophyll, **AbE-** Abaxial Epidermis.

Figure: 13. T.S of Leaf Margin

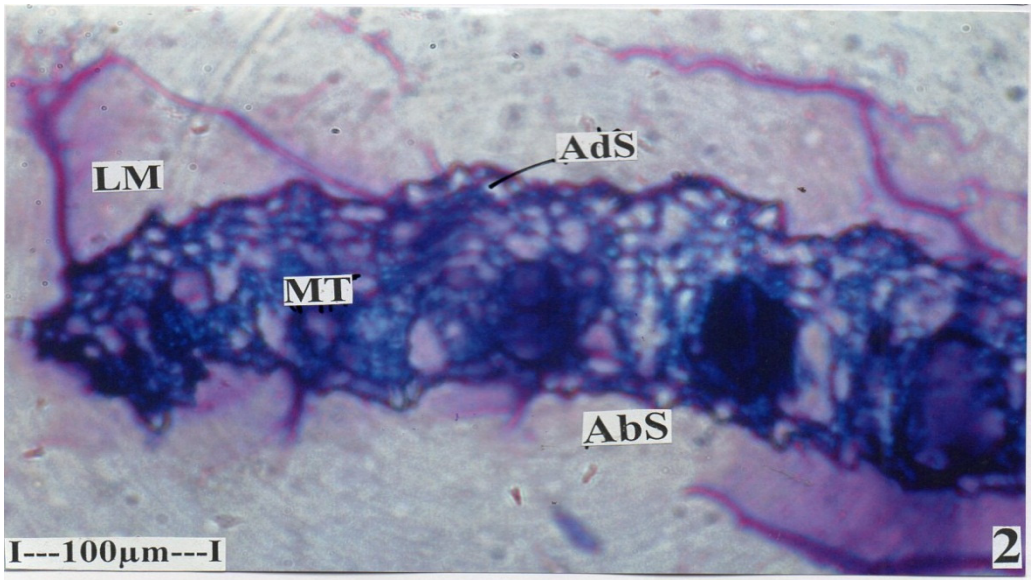


Figure: 13 **LM-**Leaf Margin, **AdS**-Adaxial Side, **MT**-Mesophyll Tissue, **AbS-** Abaxial Side.

Figure: 14 T.S of Petiole-Entire view

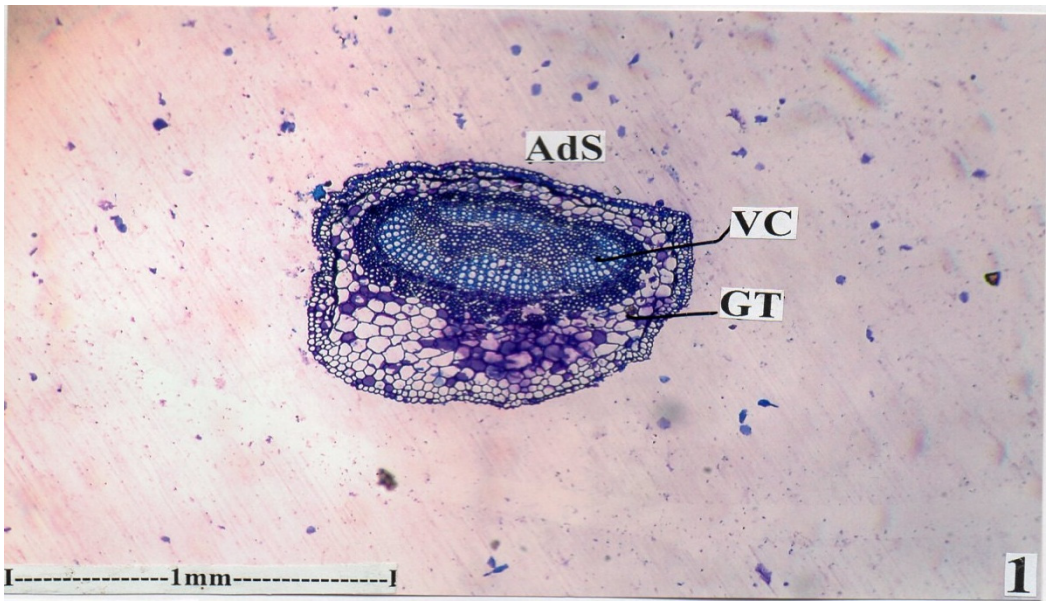


Figure: 14.1 AdS- Adaxial Side, VC-Vascular Cylinder, GT-Ground Tissue.

Figure: 15.T.S of Petiole-Enlarged

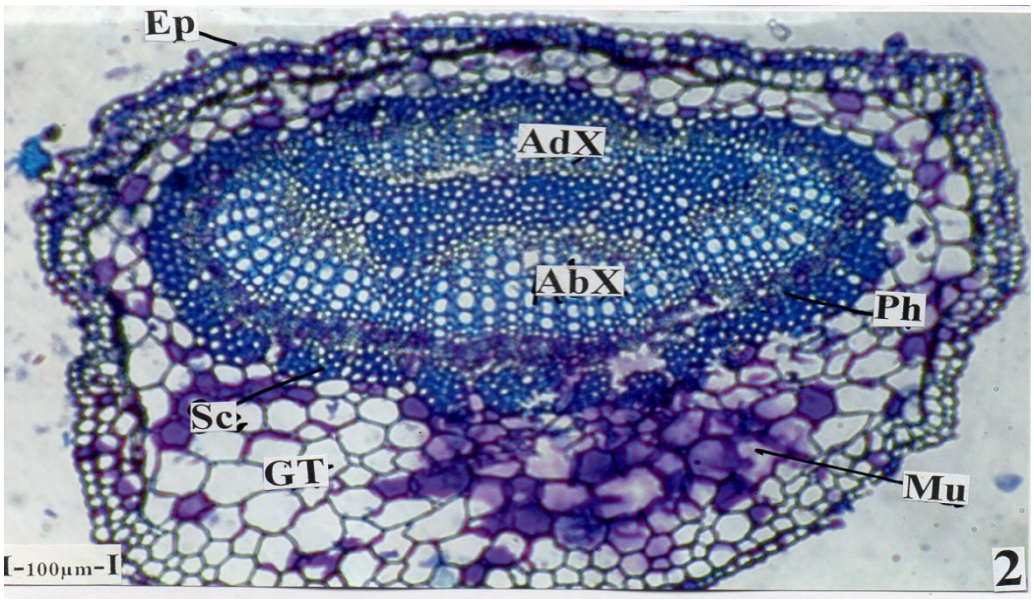


Figure: 15 Ep-Epidermis, AdX- Adaxial Xylem, AbX-Abaxial Xylem, Ph-Phloem, Mu-Mucilage, Sc-Sclerenchyma, GT-Ground Tissue.

Figure: 16 T.S of Petiole-Enlarged view.

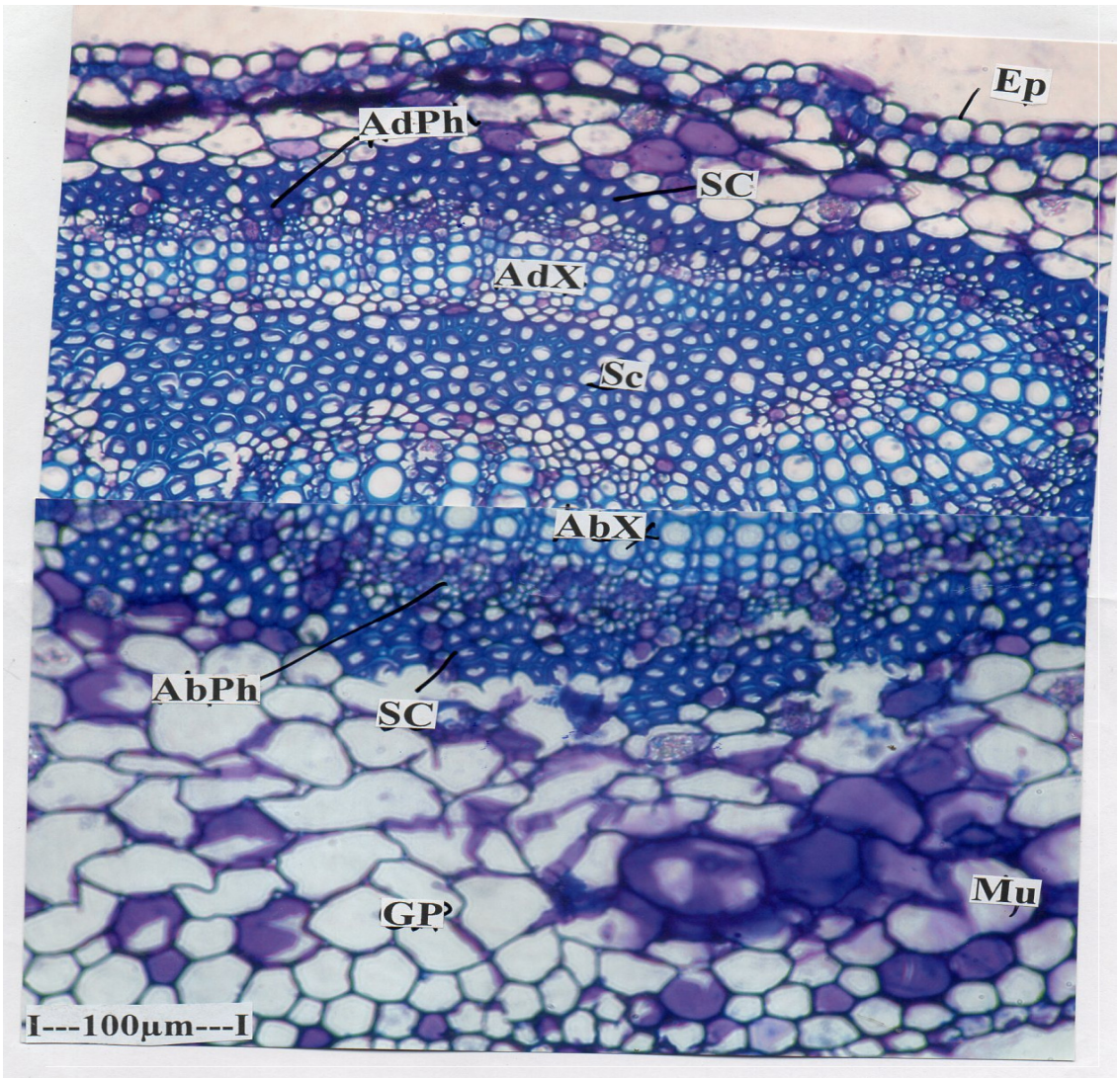


Figure: 16 **Ep**-Epidermis, **AdPh**-Adaxial Phloem, **Sc**-Sclerenchyma, **AdX**-Adaxial Xylem, **AbX**-Abaxial Xylem, **AbPh**-Abaxial Phloem, **GP**-Ground Parenchyma, **Mu**-Mucilage.

Figure: 17 Distribution of Calcium oxalate in the Midrib

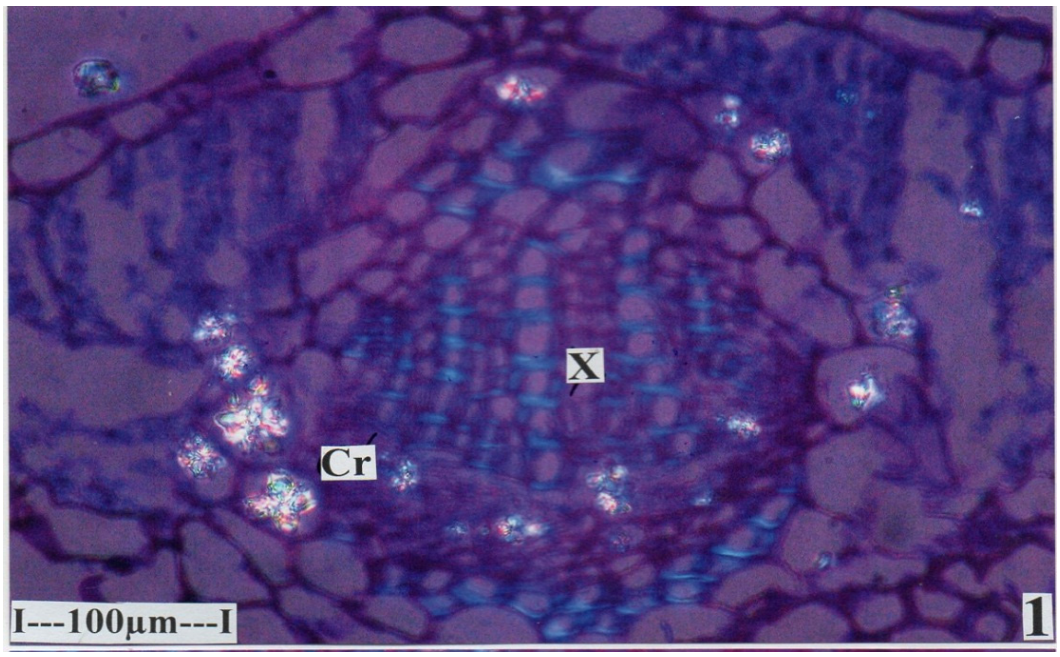


Figure: 17 **Cr**-Crystals, **X**-Xylem.

Figure: 18 Distribution of druses in Petiole

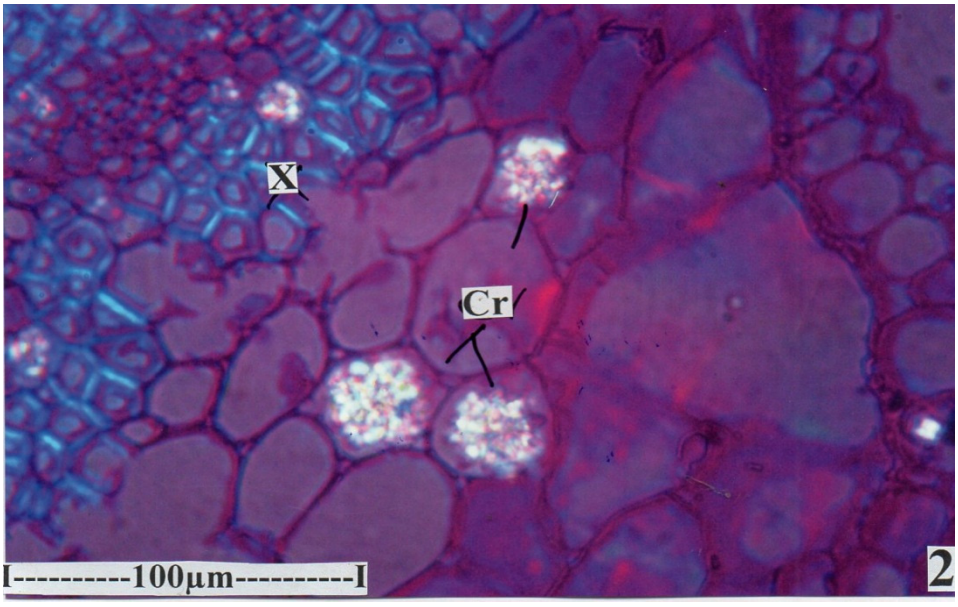


Figure: 18 **Cr**-Crystals, **X**-Xylem.

Figure:19 Vein islet and Vein termination of *Sida acuta* Burm. (Leaf)

Figure:20 Anamocytic stomata of *Sida acuta* Burm. (Leaf)

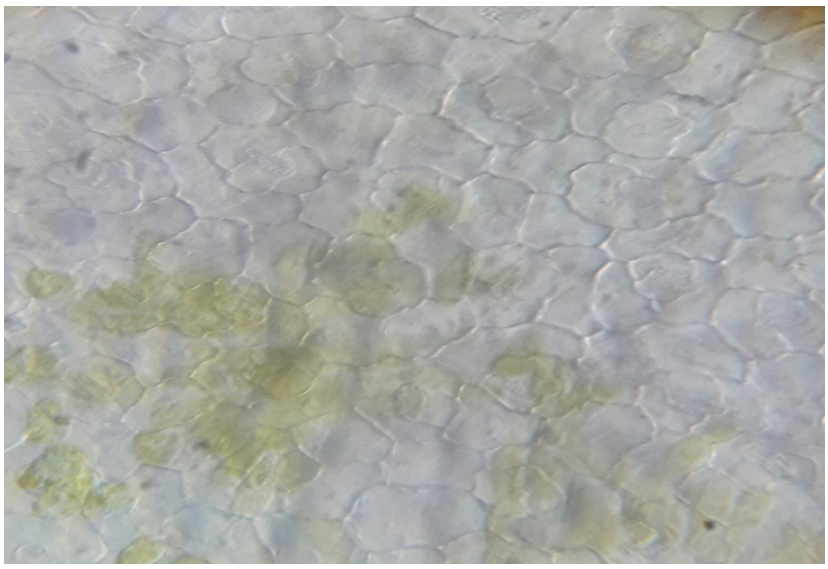


Figure: 21. Powder microscopy of *Sida acuta* Burm (Leaf)

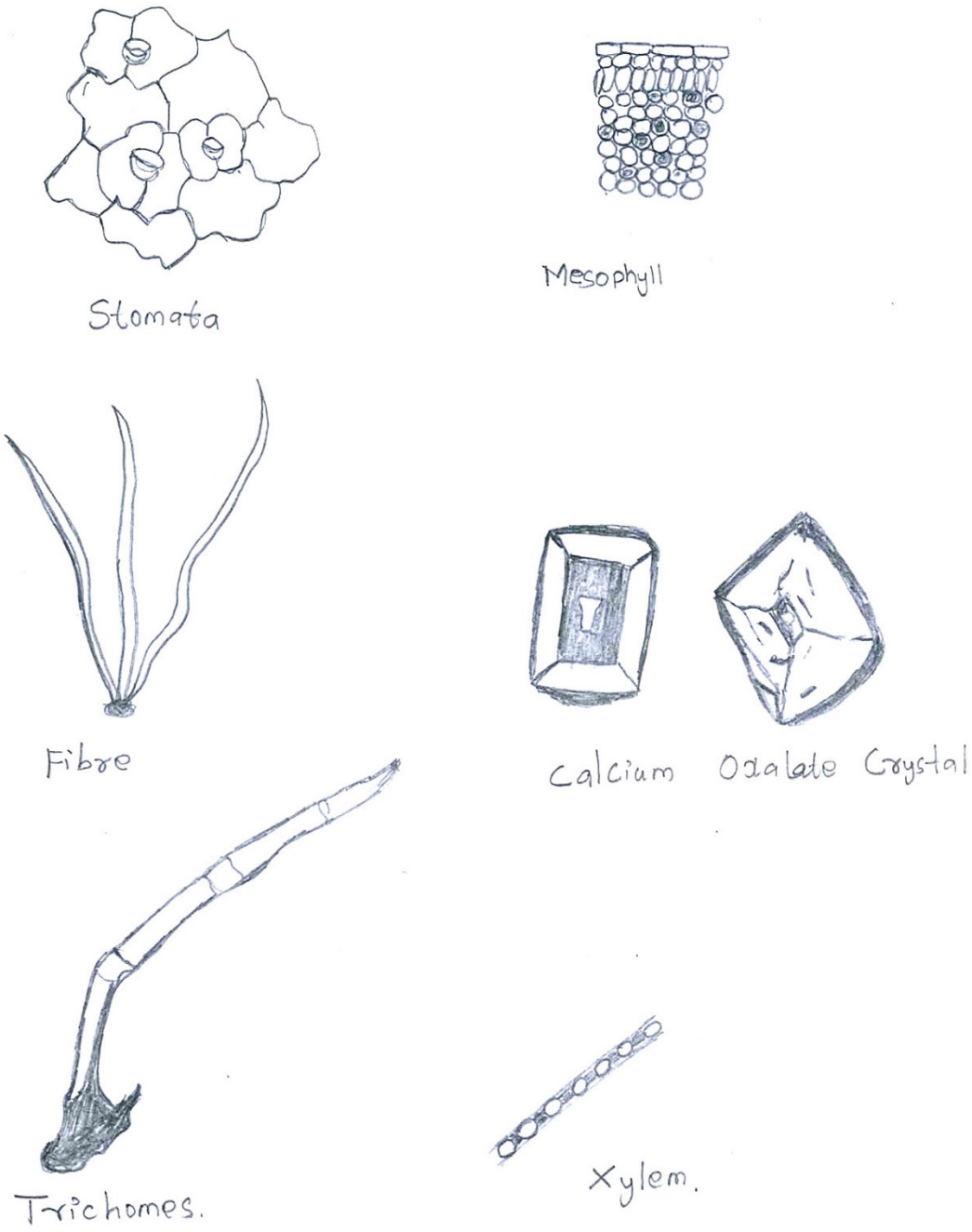


Table: 2 Quantitative Microscopy of *Sida acuta* leaves

Fresh leaves of *Sida acuta* burm. Was subjected to microscopical studies includes in vein islet number, vein termination number stomatal number and stomatal index were determined and the result are showed in Table: 2

S.No	Parameters	Minimum (per mm^2)	Average (per mm^2)	Maximum (per mm^2)
1	Vein Islet Number	24	30	33
2	Vein termination number	17	21	24
3	Stomatal number	26	32`	41
4	Stomatal index	28	34	44

The *Sida acuta* leaf showed vein islet number in the range 24-30-33, vein termination number 17-21-24, stomatal number 26-32-41, stomatal index 28-34-44.

Table: 3 Determination of Physico-Chemical Constituents of *Sida acuta*(Leaf)

The physic chemical constituents of *Sida acuta* powder results are showed in **Table**:3

S.NO	PHYSICO –CHEMICAL CONSTANT	REPORTS
1	Foreign Matter	NIL
2	Loss on Drying	0.93% ± 0.005
4	Total solids	99.06 ± 0.12%
5	Bitterness Value	NIL
6	Volatile oil content	NIL
7	Petroleum ether extractive	0.8 ± 0.005% w/w
8	Ethyl acetate extractive	2.6 ± 0.2% w/w
9	Chloroform extractive	2.6 ± 0.5% w/w
10	Methanol extractive	11.6 ± 2.3% w/w
11	Aqueous extractive	30 ± 3.5% w/w
12	Total ash	3.5 ± 0.1% w/w
13	Water soluble ash	2.0 ± 0.2% w/w
14	Acid soluble ash	1.0 ± 0.1% w/w

Table: 3 Determination of Physico-Chemical Constants of *Sida acuta* (Leaf)

Physio-Chemical Constant of *Sida acuta* (Leaf-Crude-Powder) was found to be, Loss on Drying (0.93 ± 0.005% w/w), Total Solids (99.06 ± 0.12% w/w). The powder did not possess any foreign matter, bitter principle and volatile oil content. The powder also exhibits petroleum ether extractive value (0.8 ± 0.005%w/w) Ethyl acetate extractive value (2.6 ± 0.2% w/w) Chloroform extractive value (2.6 ± 0.5) Methanol extractive value (11.6 ± 2.3%w/w) Aqueous extractive value (30 ±

3.5%w/w), Total ash value (3.5 ± 0.1%w/w), Water soluble ash value (2.0 ± 0.2 %w/w) and Acid insoluble ash value (1.0 ± 0.001%w/w).

Behavioural characters of the *Sida acuta* Burm. (Leaf-crude powder) with different reagents.

Crude leaf powder treated with various reagents and the results are showed in Table:4

Table 4: Behavioural characters of the Sida acuta Burm. (Leaf-crude powder) with different reagents.

Crude powder of *Sida acuta (*Leaf)	**Visible light**	**UV Light (254nm)**	**UV Light (365nm)**
Powder+water	Light Green	Light Green	Dark Green
Powder+Con.HCL	Dark Brown	Dark Brown	Green
Powder+Con.H_2So_4	Black	Black	Green
Powder+HNO_3	Yellow	Green	Light Green
Powder+CH_3COOH	Brown	Brown	Brown
Powder+Con.HCL+Water	Green	Green	Brown
Powder+Con.H_2So_4+Water	Light Block	Green	Green
Powder+Con.HNO_3+Water	Yellow	Green	Light Yellow
Powder+CH_3COOH+Water	Brown	Dull Brown	Green
Powder+aqueous NaOH	Green	Black	No characteristic change
Powder+aqueous $FeCl_3$	Black	Black	Black

Crude powder when treated with water showed light green colour in visible light. Powder treated with Con HCL showed dark brown colour in visible light.

Powder treated with Con.H_2SO_4, aqueous$FeCl_3$ showed black colour in visible light. Powder treated with HNO_3, Water+HNO_3 showed yellow colour in visible light. Powder treated with CH_3COOH, Water+CH_3COOH Showed brown colour in visible light. Powder treated with Water+ Con HCL and aqueous NaOH showed green colour in visible light and powder treated with water+Con H_2SO_4 showed light block colour in visible light. Powder treated with water showed light green colour in UV 254nm.Powder treated with Con HCL showed dark brown colour showed in UV 254nm and powder treated with Con H_2SO_4, aqueous NaOH, aqueous $FeCl_3$ showed black colour in UV 254nm.Powder treated with HNO_3, Water + Con HCL, Water + Con H_2SO_4, Water + HNO_3 showed green colour in UV 254nm.Powder treated with CH_3COOH showed brown colour in UV 254nm and powder treated with Water+CH_3COOH showed dull brown colour in UV 254nm. Powder treated with water showed dark green colour in UV 365nm. Powder treated with Con HCL, Con H_2SO_4, Water + Con H_2SO_4, Water + CH_3COOH showed green colour in UV 365nm. Powder treated with HNO_3 showed light green colour in UV 365nm and Crude powder treated with CH_3COOH, Water + Con HCL showed brown colour in UV 365nm. Powder treated with Water + Con HCL showed light yellow colour in UV 365nm and powder treated with aqueous $FeCl_3$ showed black colour in UV 365nm. Powder treated with aqueous NaOH did not show any characteristic change in UV 365nm.

PREPARATION OF HYDROALCOHOLIC EXTRACT OF *Sida acuta* Burm.

PROCEDURE

The shade dried and coarsely powdered leaf of *Sida acuta* Burm. (Leaf) was defatted with petroleum ether (60-80˚c). The residue was dried and extracted with hydroalcohol (70%) by Maceration until the complete extract of the material and

filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark brown).The above extract was subjected to physical analysis such as colour, consistency, wt/ ml, refractive index. The results obtained are presented in **Table:** 5

Table: 5 Determination of physical parameters of (HAESA)

S.NO	PARAMETERS	REPORTS
1	Refractive index	1.435 ± 0.003
2	Weight per ml	0.890 ± 0.020
3	Consistency	Semi solid
4	Colour	Dark green

The physical parameter of hydro alcoholic extracts of *Sida acuta* Burm. (Leaf) such as refractive index,weight per ml, consistency and colour was determined. It was found to be refractive index (1.435 ± 0.003), weight per ml (0.890 ± 0.020), dark green in colour with semi solid consistency

Preliminary phytochemical screening of hydro-alcoholic extract of *Sida acuta* (Leaf)

Hydroalcoholic extract of *Sida acuta* burm. (Leaf) was subjected to qualitative chemical analysis. The various chemical tests were performed on this extract and aqueous extract for the identification of phytochemicals, secondary metabolites and the results are displayed in Table 6.

Table: 6 Preliminary phytochemical screening of hydro-alcoholic extract of *Sida acuta* (Leaf)

S. No.	Test	Hydroalcoholic extractof *Sida acuta*(Leaf)	Aqueous extract of Sida acuta(Leaf)
1	**Alkaloids**		
	Mayer's Test	Positive	Positive
	Dragendorff's Reagent	Positive	Positive
	Hager's Reagent	Positive	Positive
	Wagner's Reagent	Positive	Positive
	Isoquinoline Test	Positive	Positive
2	**Carbohydrates**		
	Molisch's Test	Positive	Positive
	Fehling's Test	Negative	Negative
	Benedict's Test	Negative	Negative
3	**Anthraquinone Glycoside**		
	Borntrager's Test	Negative	Negative
	Modified Borntrager's Test	Negative	Negative
4	**Cardiac Glycoside**		
	Keller Killiani Test	Positive	Positive
	Raymond Test	Negative	Negative
	Legal Test	Negative	Negative
5	**Cyanogenetic Glycosides**	Negative	Negative
6	**Coumarin Glycosides**	Positive	Positive
7	**Sterols**		
	Salkowshi's Test	Positive	Positive
	Libbermann-Burchard's Test	Positive	Positive
8	**Saponins**	Positive	Positive
9	**Tannin and Phenolic compounds**		
	$FeCl_3$ Test (Alcoholic)	Positive	Positive
	$FeCl_3$ Test (Aqueous)	Positive	Positive
	Gelatin Test(Aqueous)	Positive	Positive
	$Kmno_4$ Test	Positive	Positive
	Gold Beater's Skin Test	Positive	Positive
10	**Flavonoids**		
	Shinoda Test	Positive	Positive
	Lead Acetate Test	Positive	Positive
	Acid Test	Positive	Positive

	Alkali Test	Positive	Positive
11	**Proteins And Free Amino Acids**		
	Millon's Test	Positive	Positive
	Biuret Test	Positive	Positive
	Ninhydrin Test	Positive	Positive
	Sulphur Containing Amino acid	Positive	Negative
12	**Terpenoids**	Positive	Positive
13	**Resins**	Positive	Positive
14	**Fixed Oil**	Positive	Positive
15	**Gum**	Positive	Positive
16	**Mucilage**	Positive	Positive
17	**Quinone**	Positive	Positive
18	**Coumarine**	Positive	Positive
19	**Volatile Oil**	Negative	Negative
20	**Emodine**	Negative	Negative
21	**Betacynins**	Negative	Negative
22	**Anthocynins**	Negative	Negative
23	**Lecothiocyanins**	Negative	Negative
24	**Pholotannins**	Negative	Positive

The phytochemical screening of the hydro-alcoholic extract (70%) *Sida acuta* (Leaf) powder revealed the presence of alkaloids, carbohydrates, cardiac glycosides, coumarine glycosides, sterols, saponins, tannins, phenolic compounds, flavonoids, proteins, amino acids, terpenoids, fixed oils, gum, mucilage, quinone, coumarine, resins,. It shows the absence of anthraquinone glycosides, cyanogenetic glycosides, volatile oils, betacyanins, anthocyanins, lecothiocyanins, emodin, pholotannins.

The aqueous extract of *Sida acuta* (Leaf) revealed the presence of alkaloids, carbohydrates, cardiac glycosides, coumarine glycosides, sterols, saponins, tannins, phenolic compounds, flavonoids, proteins, amino acids, terpenoids, fixed oils, gum, mucilage, quinone, coumarine, resins, pholotannins.It shows the absence of anthraquinone glycosides, cyanogenetic glycosides, volatile oils, betacyanins, anthocyanins, lecothiocyanins, emodin.

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

Hydro alcoholic extract of *Sida acuta* contained **0.086mg/g** of **TAE.** The results of standard curve of tannic acid was showed in **Figure**: 22 and results are displayed in Table:8

FIGURE: 22 CALIBRATION TANNIC ACID

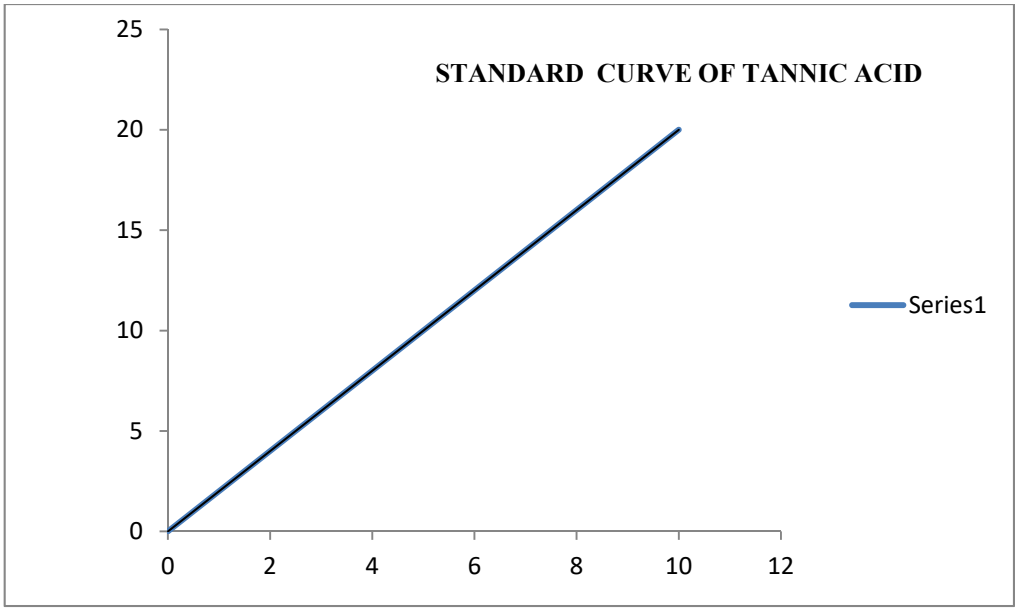


TABLE 7: DETERMINATION OF TANNIC ACID EQUIVALENT IN (HAESA)

S.No	Concentration of tannic acid/(HAESA) (µ/ml)	Absorbance		Amount of Tannic acid equivalent in HAESA (µg/ml)
		Tannic acid	(HAESA)	
		*Mean ± SEM	*Mean±SEM	
1	10	0.054±0.005	0.0016±0.0001	0.0008±0.00001
2	20	0.159±0.004	0.0036±0.0002	0.0018±0.0001
				0.086mg/g

*mean ± SEM

Hydro-alcoholic bextract of Sida acuta contained **417mg/g** of **GAE.** The results of standard curve of Gallic acid was showed in **Figure**: 23 and results are displayed in Table: 9

FIGURE: 23 CALIBRATION OF GALLIC ACID

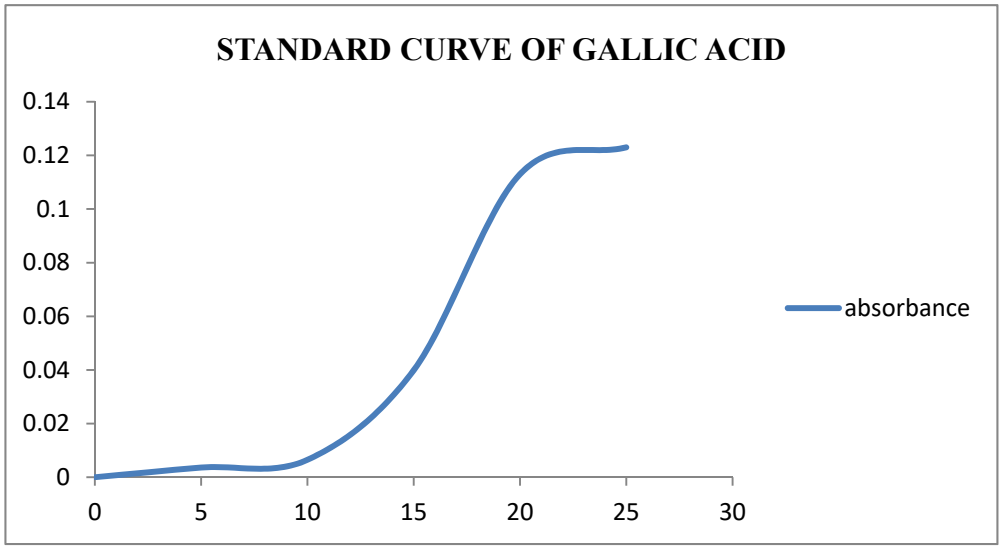


TABLE 8: DETERMINATION OF GALLIC ACID EQUIVALENT IN (HAESA)

S.No	Concentration of Gallic acid/ (HAESA) (µg/ml)	Absorbance		Amount of Gallic acid equivalent in HAESA (µg/ml)
		Gallic acid	(HAESA)	
		*Mean ± SEM	*Mean ± SEM	
1	5	0.058±0.002	0.0036±0.0001	5.22±0.002
2	10	0.095±0.003	0.0065±0.0003	5.82±0.004
3	15	0.168±0.005	0.0399±0.001	6.42±0.002
4	20	0.175±0.003	0.113±0.002	6.82±0.001
5	25	0.301±0.005	0.123±0.003	7.02±0.003
				417mg/g

*mean ± SEM

Hydro alcoholic extract of *Sida acuta* contained **839.6mg/g** of **rutin**. The results of standard curve of Rutin was showed in **Figure**: **24** and results are displayed in **Table: 9**

FIGURE: 24 CALIBRATION CURVE OF RUTIN

STANDARD CURVE OF RUTIN

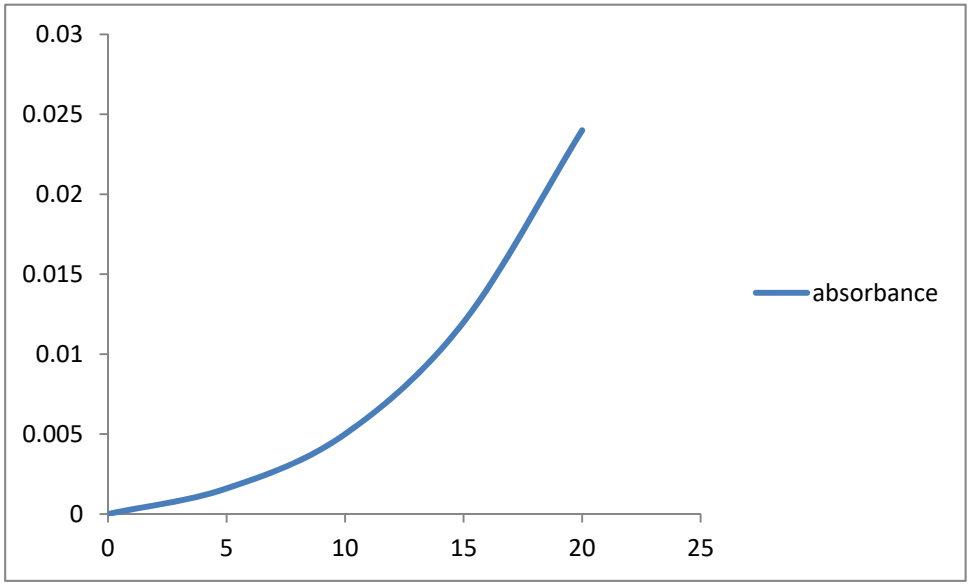


TABLE 9: DETERMINATION OF RUTIN EQUIVALENT IN (HAESA)

S.No	Concentration of rutin (Flavanoid) /(HAESA) (µg/ml)	Absorbance		Amount of rutin equivalent in HAESA (µg/ml)
		Rutin	HAESA	
		*Mean ± SEM	*Mean ± SEM	
1	5	0.018±0.002	0.0016±0.0001	4.33±0.005
2	10	0.029±0.002	0.005±0.0003	7.66±0.003
3	15	0.047±0.001	0.012±0.002	12.66±0.004
4	20	0.055±0.003	0.024±0.001	17.33±0.002
				839.6mg/g

*mean ± SEM

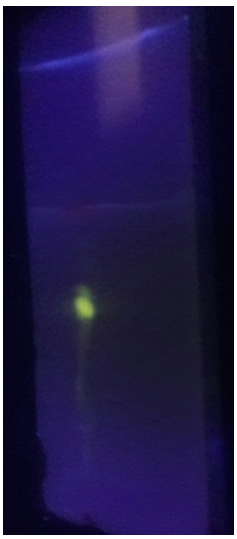
THIN LAYER CHROMATOGRAPHY

Hydro alcoholic extract of *Sida acuta* burm.was subjected to TLC by using different mobile phase to identify the R_f value and the results

TABLE: 10 TLC PROFILE OF HAESA

S.No.	Mobile phase	Rf value	Reports
1.	Toluene:ethyl acetate: diethylamine(70:20:10)	0.35	May indicate the presence of alkaloid.
2.	Toluene: ether(1:1)	0.37	May indicate the presence of flavonoids.
3.	Benzene: Pyridine: Formic acid(72:18:10)	0.36	May indicate the presence of flavonoids.
4.	Dichloromethane: Chloroform: Methanol (4:4:1)	0.33	May indicate the presence of cryptolepine.
5.	Ethyl acetate: Methanol: Ammonia (80:15:5)	0.34	May indicate the presence of cryptolepine.

Figure: 25 Thin Layer Chromatography of isolated SA-I.



Mobile phase : Ethyl acetate: Methanol: Ammonia (80:15:5).

Rf value : 0.34.

UV SPECTROSCOPY

The methnolic(100%) fraction of HAESA was pooled and the named as SA-I.The isolated compound SA-I was dissolved in methanol and was scanned under UV range from 200-600 nm in the UV-Visibble spectrophotometer. The isolated compound SA-I showed absorbance maxima at 255nm, 274nm and 331.

FIGURE : 26 UV SPECTRUM OF SA-I

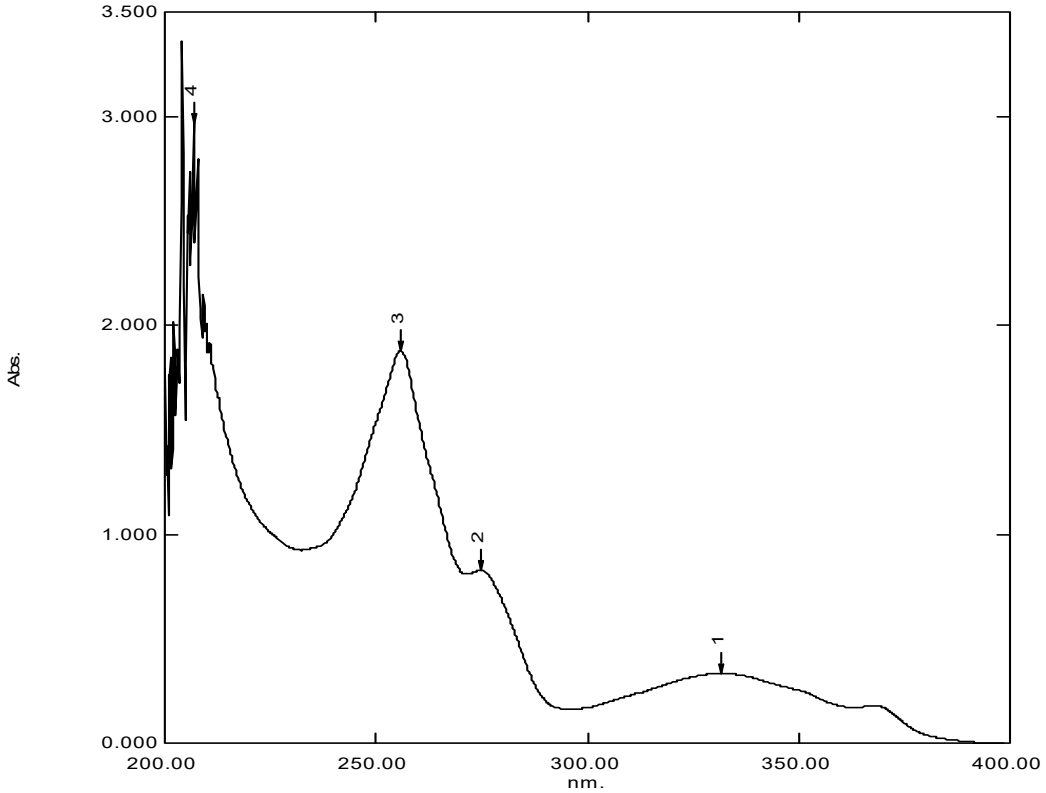
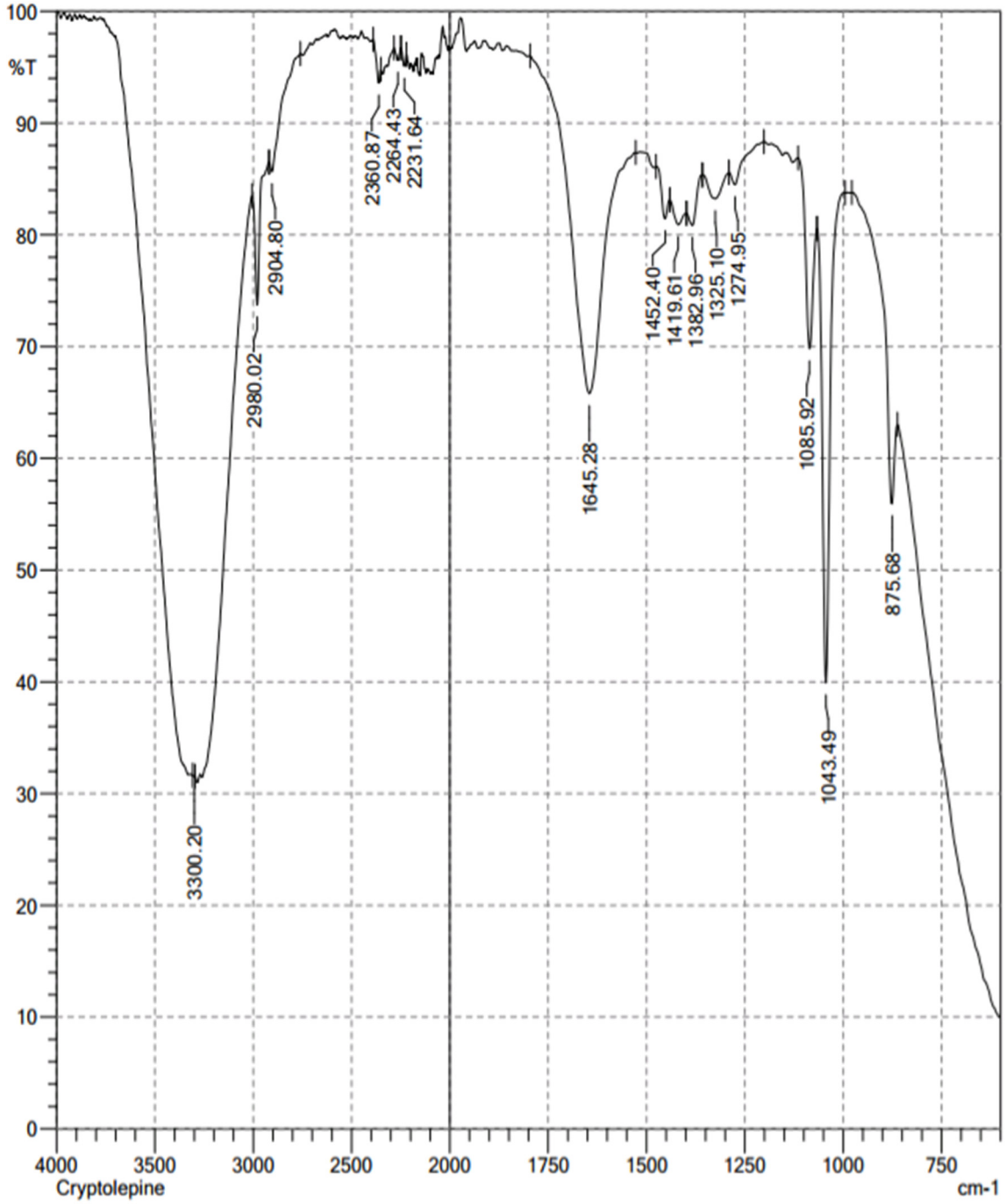


TABLE: 11 UV SPECTRUM OF ISOLATED SA-I

S.No.	Wavelenth(nm)	Absorbance
1	255	1.878
2	274	0.827
3	331	0.333

Figure: 27 INFRARED SPECTROSCOPY OF ISOLATED SA-I



Interpretation of IR spectrum of SA-I

The isolated compound SA-I was sample handling by liquid membrane method involves dripping several drops of the sample onto an NaCl aperture plate and sandwiching it under another aperture plate, such that no gas bubbles are trapped. The thickness is adjusted and carried out for analysis in infrared spectrophotometer. Group frequency region was 4000cm^{-1} to 1500cm^{-1} & figure print region was 1500cm^{-1} to 600cm^{-1}.

Table: 12 Interpretation of Infrared spectrum of SA-I

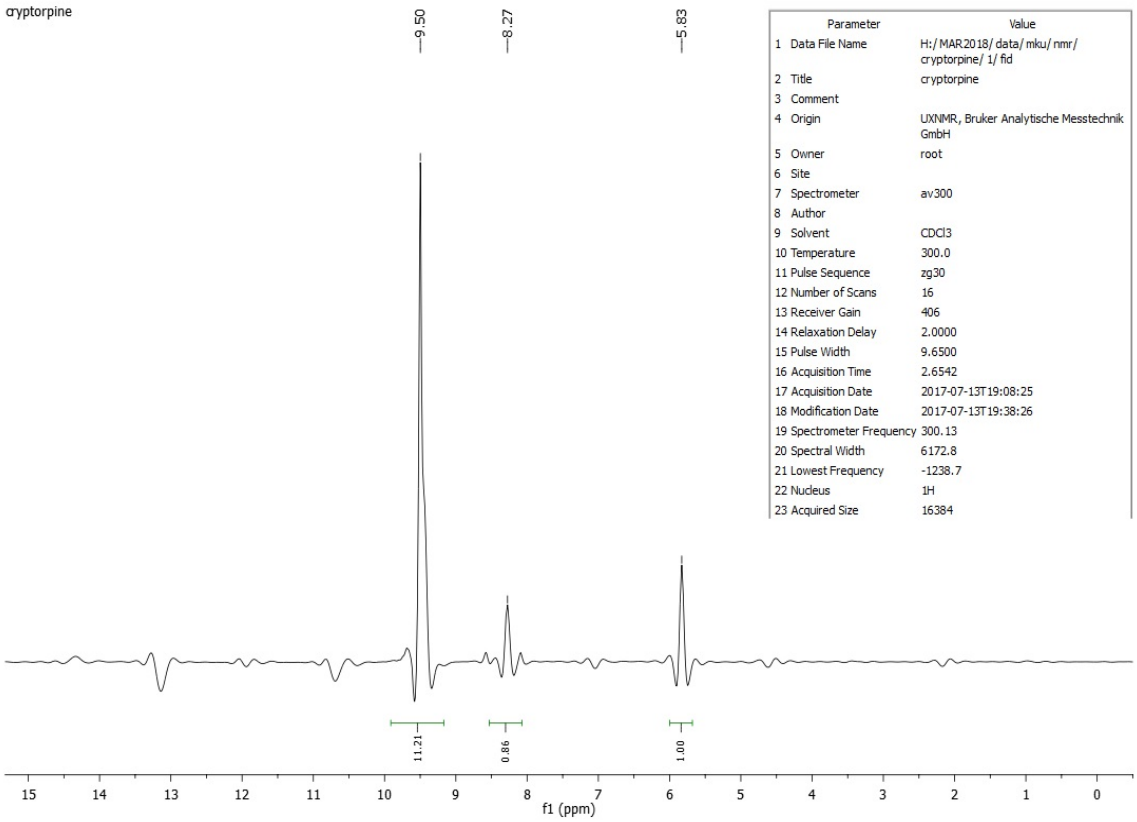
S.no	Standard IR Range (cm^{-1})	Observed IR range(cm^{-1})	Intensity	Types of band	Functional group
1	3200-3600	3300	Strong, Broad	Stretching	OH, Alcohol
2	2850-300	2904, 2980	C-H-, Alkane Strong	Stretching	C-H-, Alkane
3	2100-2270	2264, 2231	Variable not present in symmetrical alkynes	Stretching	-C≡C-, Alkyne
4	1620-1680	1645	Variable	Stretching	-C=C, Alkane
5	1350-1480	1452, 1419	Variable	bending	-C-H, Alkane
6	1345-1385	1382	Strong two bonds	Stretching	N-O, Nitro
7	1080-1360	1325, 1274,1085	Medium	Stretching	C-N, Amine
8	1000-1300	1043	Strong	Stretching	C-O, Ether
9	675-1000	875	Strong	Stretching	=C-H, Alkane

The IR spectrum of isolated SA-I shows a broad absorption band at 3300 cm^{-1} alcoholic –OH, 2904cm^{-1} and 2980cm^{-1} alkane-C-H, 2264 cm^{-1} and 2231cm^{-1} Alkyne-

C≡C 1645cm^{-1} Alkane C-C, 1452cm^{-1} and1419cm^{-1} -C-H alkane, 1382cm^{-1} nitro N-O, 1325cm^{-1},1274cm^{-1} and1085cm^{-1} Amine-C-N ,1043cm^{-1} Ether-C-O, 875cm^{-1} Alkane-C-H.

Figure: 28 H NMR SPECTRAM OF SA-I

Cryptolepine



Interpretation of NMR spectrum of SA-I

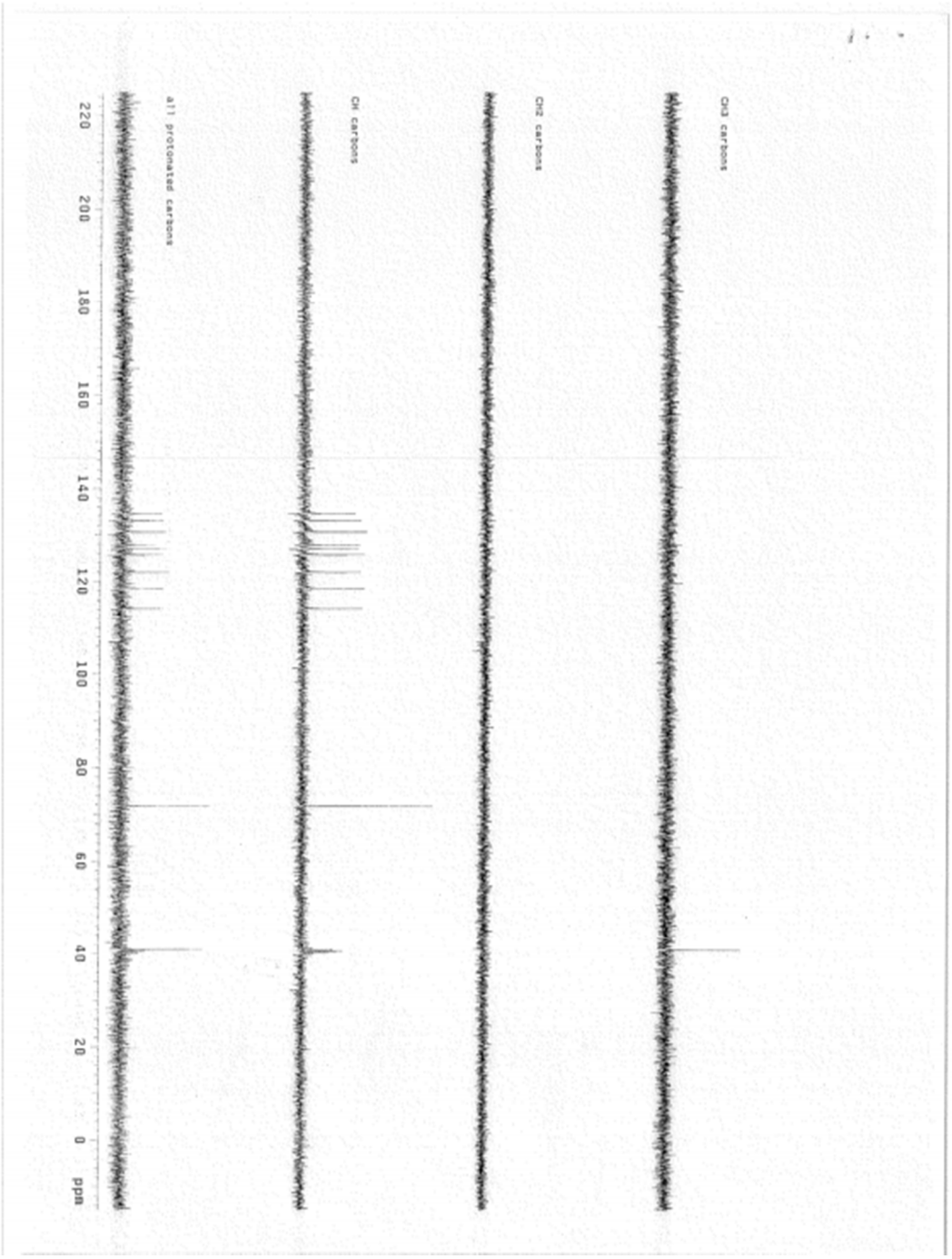
The ^{1}H NMR of isolated SA-I showed an intense singlet at δ 5.83ppm with integral of 36mm corresponding to 3H of the methyl group of SA-I at position 5.Since the methyl group at position 5 of SA-I has no neighbouring protons to couple it. It gave a singlet peak at 5.83ppm typical of signal produced by methyl group attached to tertiary nitrogen.

The proton NMR of isolated SA-I showed aromatic multiples from δ 7.467 to 8.808 ppm aromatic singlet at δ 9.50 ppm corresponding to eight aromatic proton and isolated aromatic hydrogen of SA-I respectively. The aromatic singlet at δ 9.50 ppm from TMS may be due to the hydrogen at position 11 of cryptolepine (H-11) which is the only H without neighbouring hydrogen atoms to split its signal.

Table: 13 Interpretation of NMR spectrum of cryptolepine

S.no	Delta value ppm	Positionof Hydrogen atom	Type of peak
1	δ 5.83	H-5	Singlet
2	δ 8.27	H-6	Aromatic multiplet
3	δ 9.50	H-11	Singlet aromatic proton & isolated aromatic proton.

Figure: 29 ^{13}C NMR SPECTRUM OF ISOLATED SA-I



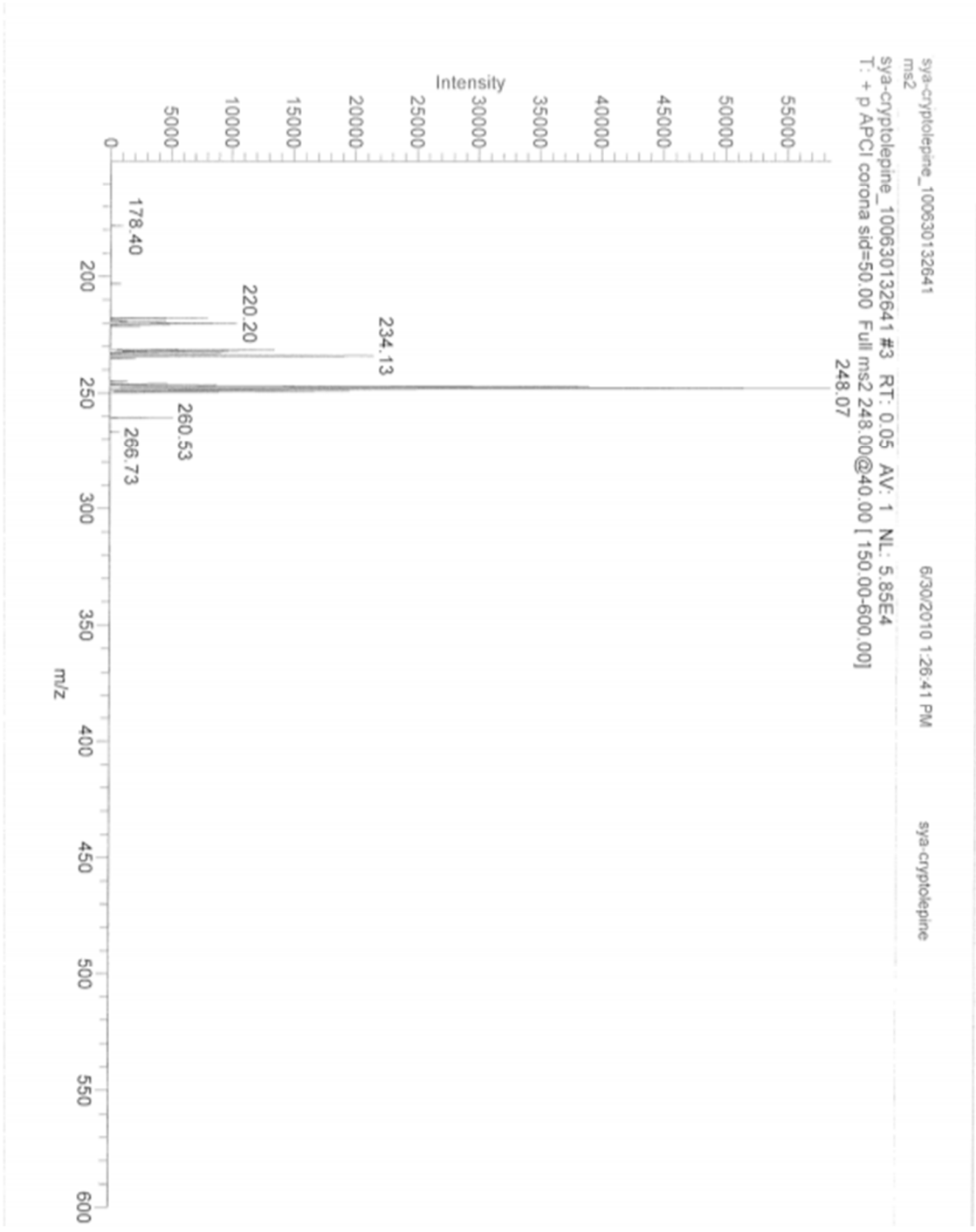
Interpretation of ^{13}C NMR spectrum of isolated SA-I

Hetero aromatic carbons give signals within the range δ 100-165ppm andnine peaks within this range of chemical shift under CH 13C DEPT analysis of isolated SA-I. This confirms the nine protonated hetero aromatic carbons in SA-I: C-1,2,3,4,6,7,8,9, and 11.sp3 hybridised carbons absorb at lowest field (δ 0-50ppm) as compared to sp2(δ100-160ppm aromatic) and sp (δ 65-90ppm) hybridised carbons. Isolated SA-1 showed a singlet at δ 40.3ppm which confirms the presence of the only methyl carbon on the quinoline nitrogen at position 5 of SA-I (>N-CH3).

Table: 14 Interpretation of ^{13}C NMR spectrum of Isolated SA-I

S.No	Delta value ppm	Types of Carbon
1	δ 100-165	Hetero aromatic carbon
2	δ 0-50	Hybridised carbon
3	δ 100-160	Aromatic carbon
4	δ 40.3	Methyl carbon

Figure: 30 MASS SPECTRUM OF ISOLATED SA-I



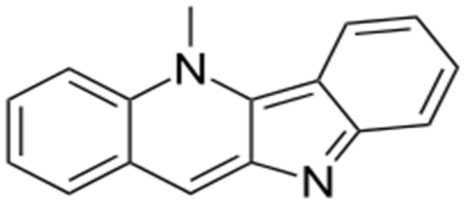
Interpretation of Mass spectrum of isolated SA-I

The base peak of mass spectrometric analysis of isolated SA-I was found to be at *m/z* value of **233.27** corresponding to 100% relative intensity. Hence, the base peak of the mass spectrum of the isolated SA-I which also corresponds to the molecular ion actually occured at *m/z* **232.27**. This value is in agreement with the literature value of the molecular weight of SA-I which was 232g. The peak occuring at *m/z* **219.33**(29.36%). might correspond to N-demethylated fragment of SA-I.

Table: 15 Interpretation of Mass spectrum of SA-I

S.No	Type of ion	m/z Value
1.	Molecular ion	232.27
2	Demethylated fragment ion	219.33

STRUCTURE OF CRYPTOLEPINE



IUPAC NAME : 5-methylindolo [3,2-b]quinoline.

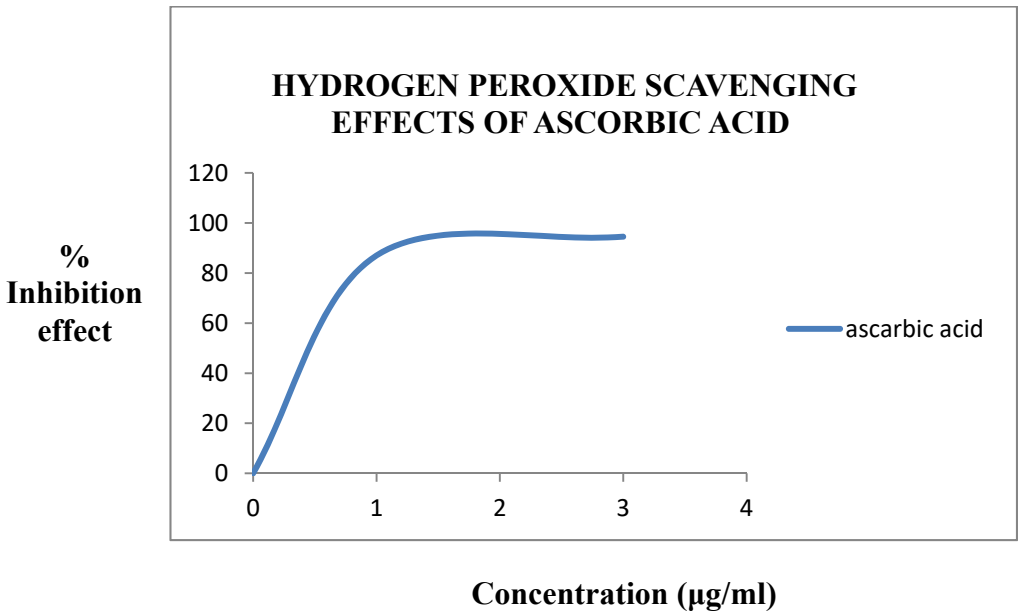
Molecular Formula : $C_{16}H_{12}N_2$

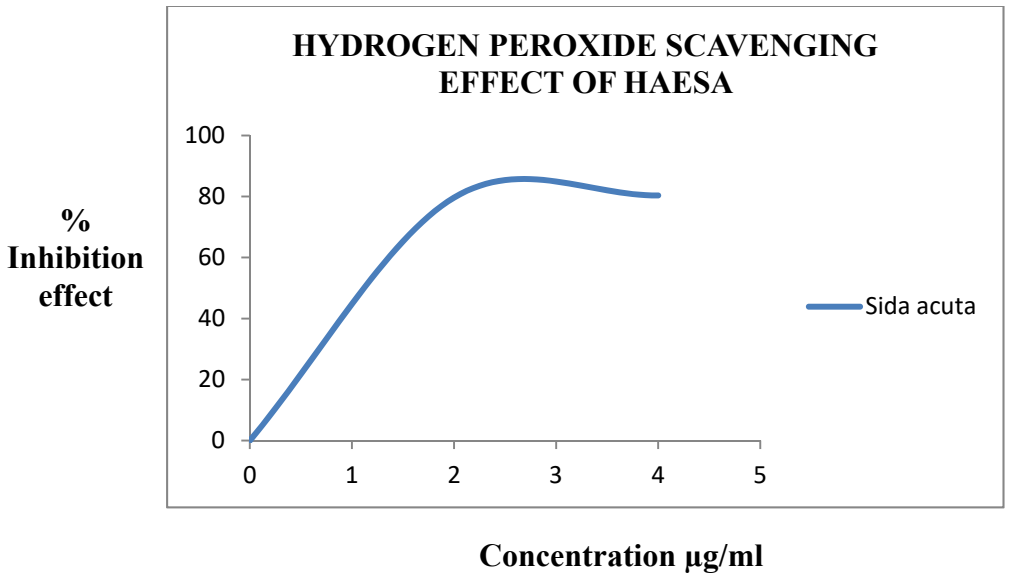
Molecular Weight:232.286g/mol

INVITRO ANTIOXIDANT STUDIES

Hydro-alcoholic extract was subjected to in-vitro antioxidant studies. It includes hydrogen peroxide scavenging activity, total antioxidant capacity and reducing power assay.

Figure: 31 Determination of hydrogen peroxide scavenging activity of *Sida acuta* (leaf) (HAESA)





The inhibitory concentration (IC_{50}) of *sida acuta* (leaf) against hydrogen peroxide scavenging effect is found to be 1.83µg/ml in comparision with ascorbic acid 0.95µg/ml.

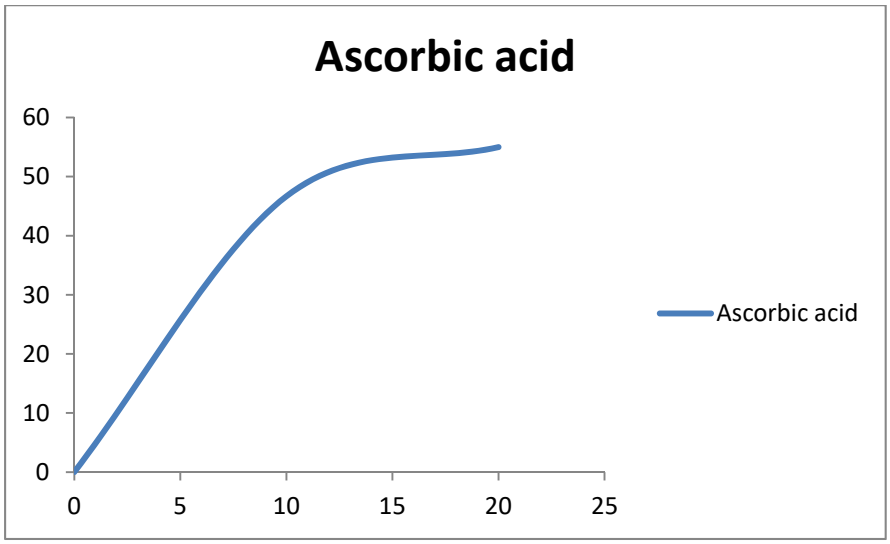
Table: 16 Determination of hydrogen peroxide scavenging activity of *Sida acuta* Burm (leaf) (HAESA)

S.No.	Concentration of ascorbic acid (µg/ml)	Percentege inhibition of Ascorbic acid (Standard) (µg/ml)	Concentration of *Sida acuta* (µg/ml)	Percentege inhibition of *Sida acuta*
1	1	87±1.05	2	79.65
2	3	94±1.50	4	80.35
	IC_{50}	0.95µg/ml		1.83µg/ml

*mean ± SEM, the statistical significance ($p<0.05$)

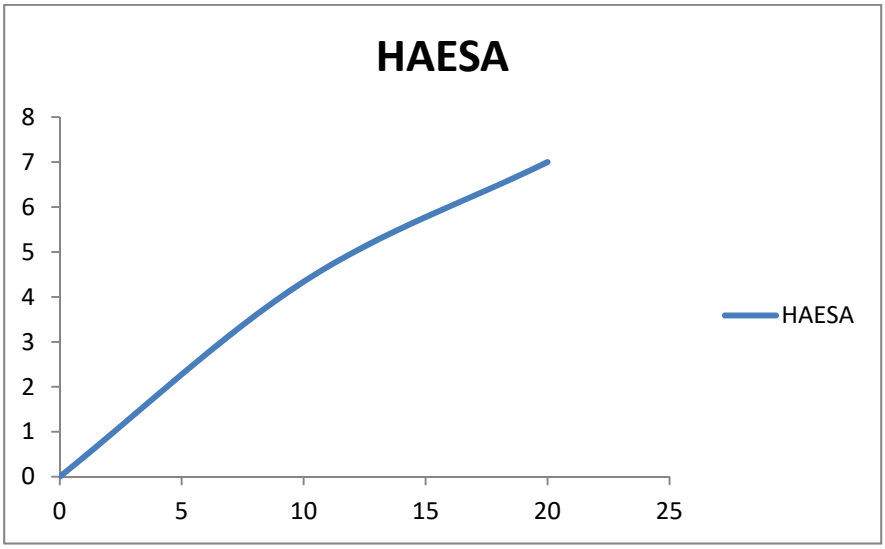
It requires the double the concentration of ascorbic acid to reduce the free radicals. It showed mild antioxidant effect.

Figure: 32 Determination of reducing power assay of *Sida acuta*Burm Leaves (HAESA)



% Inhibition effect

Concentration (µg/ml)



% Inhibition effect

Concentration (µg/ml)

The inhibitory concentration (IC_{50}) of Sidaacuta (leaf) against **reducing power aasy** determined in comparison with ascorbic acid used as a standard. The inhibitory concentration (IC_{50}) of Sidaacuta (leaf) in reducing power assay is 142µg/ml in comparison with ascorbic acid 15µg/ml.

Table: 17 Determination of reducing power assay of *Sida acuta* Burm. (leaf) (HAESA)

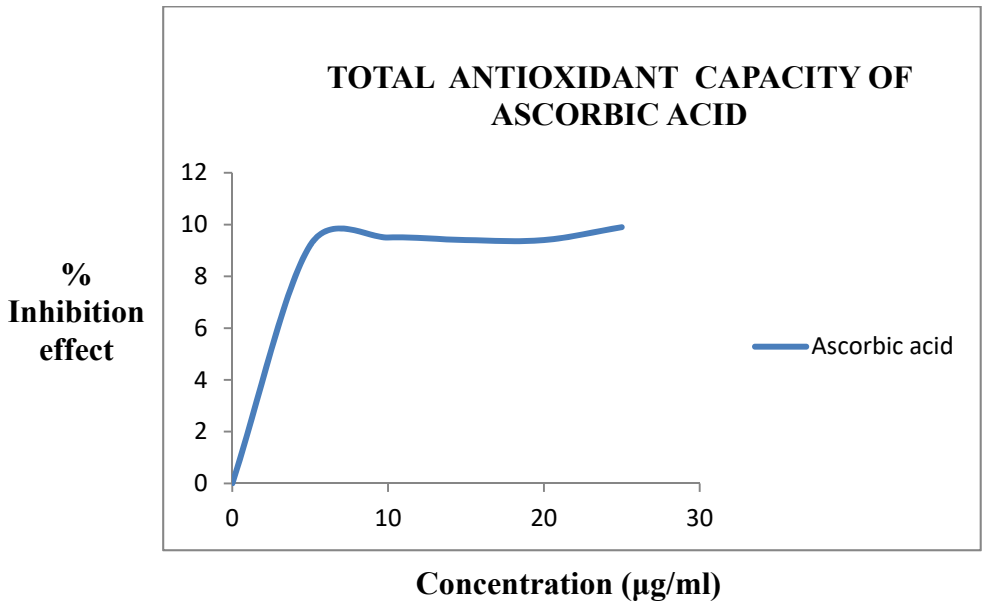
S.No	Concentration of Ascorbic acid/ *Sidaacuta* (µg/ml)	Percentage reduction of ascorbic acid (Standard) (µg/ml)	Percentage reduction of *Sida acuta*(leaf) (µg/ml)
1	10	46.6 ± 2.02	4.3 ± 0.100
2	20	55.0 ± 4.0	7.0 ± 0.02
	IC_{50}	15µg/ml	142µg/ml

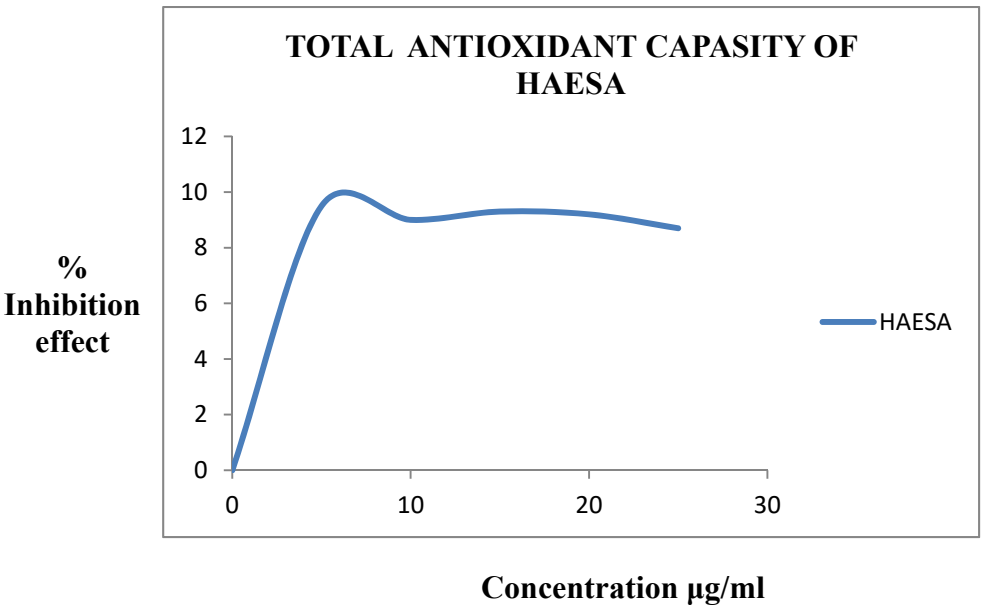
*mean ± SEM

Data was expressed as mean ± SEM, the statistical significance ($p<0.05$)

Concentration of hydro-alcoholic extract of *Sida acuta* requires with 9 times the amount of ascorbic acid.

Figure: 33 Determination of total antioxidant assay of *Sida acuta* Burm (Leaf)





The inhibitory concentration (IC_{50}) of *Sida acuta*(leaf) against **total antioxidant capacity** was determined in comparison with ascorbic acid used as a standard. The total antioxidant capacity is found to be 185.4µg/ml in comparison with ascorbic acid 159.8µg/ml.

Table: 18 Determination of total antioxidant capacity of *Sida acuta* Burm. Leaf (HAESA)

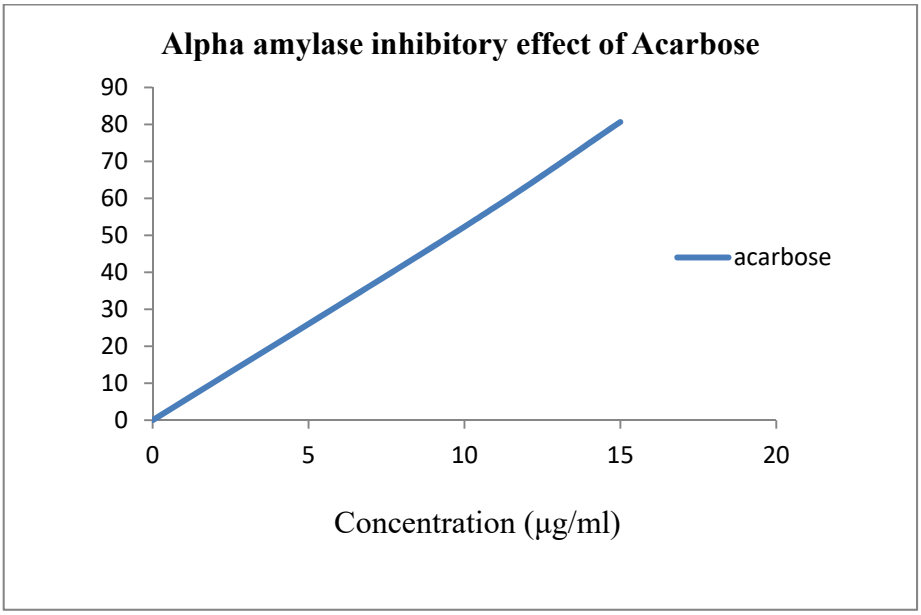
S.no	Concentration of Ascorbic acid/HAESA (µg/ml)	Percentage inhibition of Ascorbic acid (Standard) (µg/ml)	Percentage inhibition of HAESA (leaf) (µg/ml)
1	5	9.2±0.2	8.7±0.05
2	10	9.5±0.5	9.0±0.3
3	15	9.4±0.3	9.2±0.2
4	20	9.4±0.3	9.3±0.20
5	25	9.9±0.4	9.5±0.3
	IC_{50}	160µg/ml	185µg/ml

*mean± SEM

Data was expressed as mean ± SEM, the statistical significance ($p<0.05$) Concentration of hydro alcoholic extract of *Sida acuta* requires with the amount of ascorbic acid.

ALPHA AMYLASE INHIBITORY ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF *Sida acuta* Burm.

Figure: 34 Determination of Alpha amylase inhibitory effect of *Sida acuta* Burm (leaf) HAESA



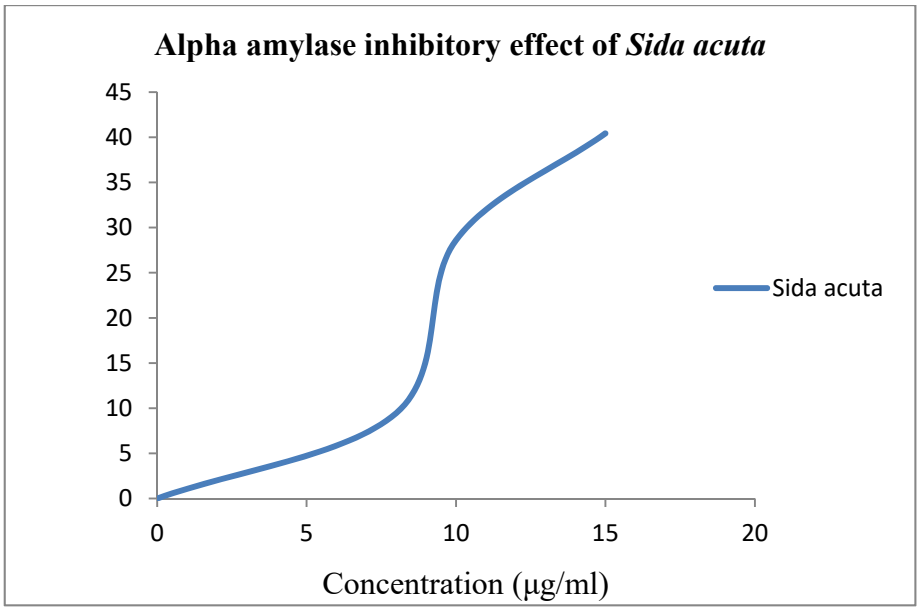


Table: 19. Determination of Alpha amylase inhibitory effect of *Sida acuta* Burm(leaf) (HAESA)

S.No.	Concentration of acarbose (µg/ml)	Percentage inhibition of acarbose(µg/ml)	Concentration of *Sida acuta* (µg/ml)	Percentage inhibition of *Sida acuta* (µg/ml)
1	-	-	8	9.46 ± 0.004
2	10	52.33 ± 0.002	10	28.6 ± 0.02
3	15	80.66. ± 0.003	15	40.43 ± 0.04
		IC_{50}-9.27(µg/ml)		IC-$_{50}$-17.10(µg/ml)

*mean ± SEM, the statistical significance ($p<0.05$)

The inhibitory concentration (IC_{50}) of *Sida acuta* (leaf) against alpha amylase inhibitory effect is found to be 17.10µg/ml in comparison with acarbose 9.27µg/ml.

ANTIMYCOBACTERIAL ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF *Sida acuta* Burm (Leaves) (HAESA)

Figure: 35 Antimycobacterial effect of Hydroalcoholic extract of *Sida acuta* burm (leaves) (HAESA)

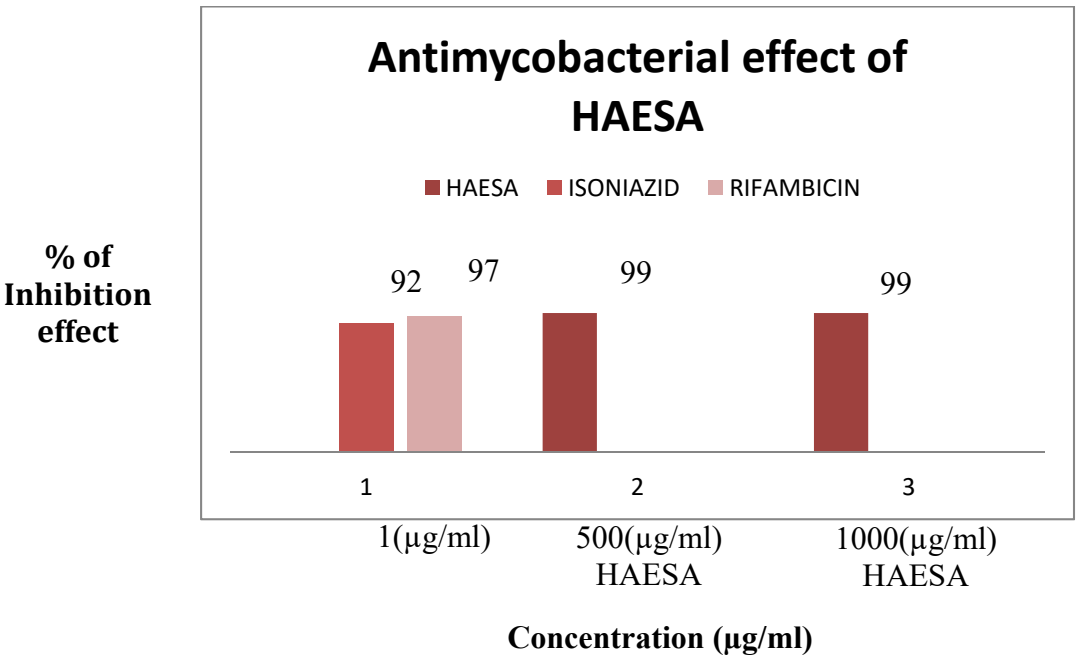


Table: 20 Antimicobacterial activity of Hydro-alcoholic extract of *Sida acuta* Burm leaves against standard strain of Mycobacterium tuberculosis H37Rv.

S.No	Compound	Concentration (µg/ml)	Relative light unit value	% of Inhibition
1	Isolated alkaloid SA-I	500	12715	0%
		1000	12596	0%
2	HAESA	500	110	99%
		1000	75	99%
3	Isoniazid	1	792	92%
4	Rifampicin	1	299	96%

The percentage inhibition of *Sida acuta* Leaf (HAESA) against mycobacterium tuberculosis standard strain of H37Rv. HAESA (500µg/ml and 1000µg/ml) showed anti mycobacterial effect was found to be 99% in comparison with isoniazid (1ug/ml) 92% and rifampicin (1µg/ml) 96%.

ANTI-HISTAMININIC ACTIVITY

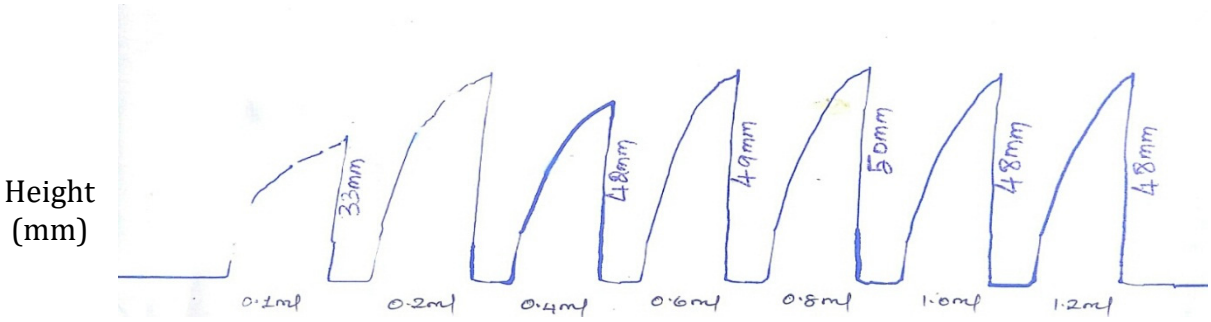


Figure: 36 DRC of histamine in guinea pig ileum

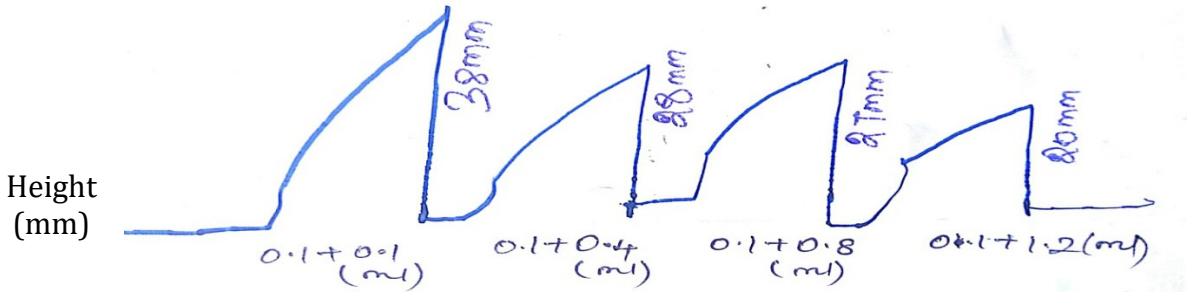


Figure: 37 Effect of the Hydro alcoholic extract of *Sida acuta* against Histamine on guinea pig ileum. (HAESA)

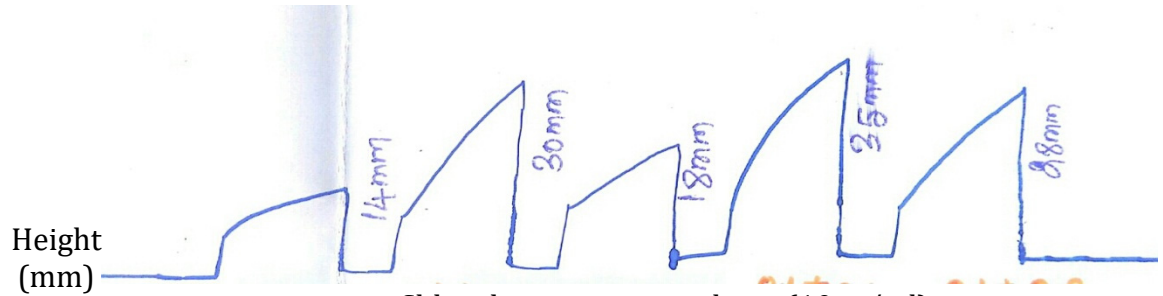
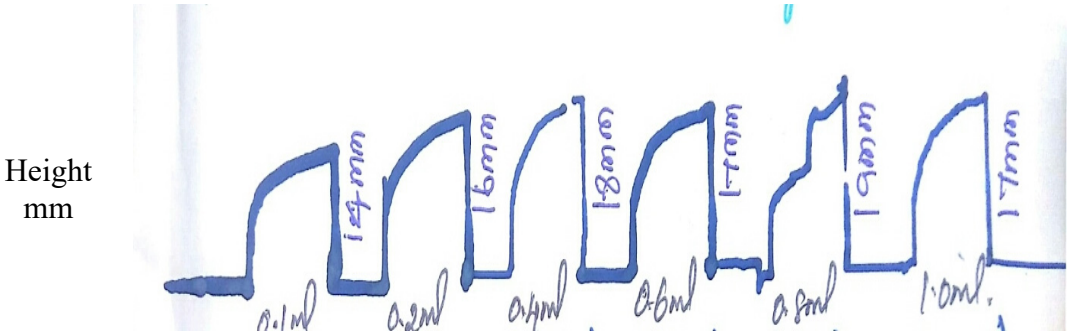


Figure: 38 Effect of the Chlorpheniramine mealeate against Histamine on guinea pig ileum. (HAESA)

Table: 21 Effect of the Hydro alcoholic extract of *Sida acuta* against Histamine on guinea pig ileum. (HAESA)

S.no	Treatement	Dose(10µg/ml)	Height response (h)(mm)	Control height (H) (mm)	% Inhibition of response due to test drug. H-h/H×100
1	Histamine	0.1 ml	33mm	33mm	---------
		0.4 ml	42mm		
		0.6 ml	49mm		
		0.8 ml	50mm		
		1.0 ml	48mm		
		1.2 ml	48mm		
2	HAESA (1000µg/ml) +Histamine	0.1ml+0.1ml	38mm	----------	-0.15%
		0.4ml+0.1ml	28mm		15.15%
		0.8ml+0.1ml	27mm		18.18%
		1.2ml+0.1ml	20mm		39.39%
	Chlorpheniramine mealeate (10µg/ml) +Histamine	0.1ml+0.1ml	14mm		57.57%
		0.1ml+0.2ml	30mm		19.0%
		0.1ml+0.4ml	18mm		45.45%
		0.1ml+0.67ml	35mm		-6.06%
		0.1ml+0.8ml	28mm		15.15%



Histamine (10µg/ml)

Figure: 39 DRC of histamine in guinea pig trachea

Histamine (10µg/ml)+HAESA(1000µg/ml)

Figure: 40 Effect of the Hydro alcoholic extract of *Sida acuta* against Histamine on guinea pig trachea. (HAESA)

Chlorpheniramine mealeate +Histamine
(10µg/ml)

Figure: 41 Effect of the Chlorpheniramine mealeate against Histamine on guinea pig trachea. (HAESA)

Table: 22 Effect of the Hydro alcoholic extract of *Sida acuta* against Histamine on guinea pig ileum. (HAESA)

S.no	Treatement	Dose(10µg/ml)	Height response (h)(mm)	Control height (H) (mm)	% Inhibition of response due to test drug. H-h/H×100
1	Histamine	0.2 ml	33mm	33mm	---------
		0.4 ml	42mm		
		0.6 ml	49mm		
		0.8 ml	50mm		
		1.0 ml	48mm		
		1.2 ml	48mm		
2	HAESA (1000µg/ml) +Histamine	0.1ml+0.1ml	13mm	----------	60.6%
		0.2ml+0.1ml	11mm		66.67%
		0.4ml+0.1ml	11mm		66.67%
		1.6ml+0.1ml	10mm		69.69%
	Chlorpheniramine mealeate (10µg/ml) +Histamine	0.1ml+0.1ml	11mm		66.67%
		0.1ml+0.2ml	10mm		69.69%
		0.1ml+0.4ml	9mm		72.72%
		0.1ml+0.6ml	12mm		63.63%
		0.1ml+	11mm		66.67

CHAPTER- VI

SUMMARY

SUMMARY

Chapter 1

It consists of introduction of the present thesis. It includes the importance of plant and diagnosis, cure & prevent the disease.

Chapter 2

It consists of literature review of the present thesis. It includes ethano claim review, phytochemical review and pharmacological review. Ethano claim review such as plant part used for various disease. Phytochemical review is used for the presence of active constituent detected previously. Pharmacological review helps to identify the activity studied so far.

Chapter 3

It contains aim and objective of the present thesis. The aim of present research is to study the Pharmacological, Phytochemical including isolation of cryptolepin and invitro antioxidant, alpha amylase effect, antimycobacterial and in vivo antihistaminic activity of *Sida acuta* burm leaves.

Chapter 4

It consist of materials and methods of the present thesis. It is divided in to part A, B, C.

Part A includes pharmacognostical studies, macroscopy of *Sida acuta* (Leaf), quantitative microscopy, determination of physio – chemical constants, behavioural characters of the Sida acuta (leaf-crude powder) with different reagent, determination of physical parameters of (HAESA).

Part B includes phytochemical studies, preliminary phytochemical screening of hydro-alcholic extract of *Sida acuta* (leaf), determination of tannic acid equivalent in (HAESA), determination of gallic acid content equivalent in (HAESA), determination of rutin equivalent in (HAESA), TLC profile, UV spectrum ,Infrared spectrum , Nuclear magnatic resonance report of isolated SA-I

Part C includes pharmacological studies, in vitro antioxidant, alpha amylase effect, anti mycobacterial and invivo antihistaminic

Chapter 5

It contains result of the present research thesis. It includes the result for macroscopy of *Sida acuta* (Leaf), quantitative microscopy, Vein islet number-24, 23, 30.Vein termination number-17, 24, 21.Stomatal Number-26, 41, 32.Stomal index-28, 44,34.

Determination of physio-chemical constants, The physio-chemical constants of Sida acuta (leaf-crude powder) was found to be, loss on drying (0.93 ± 0.005), total solids (99.06 ± 0.12%/).the powder did not possess any foreign matter, bitter principle and volatile oil content. The powder also exhibits petroleum ether extractive value (0.8 ± 0.005%w/w), chloroform ethyl acetate extractive value (2.6 ± 0.2%), extractive value (2.6 ± 0.5%w/w), methanol extractive value (11.6 ± 2.3%w/w), aqueous extractive value (30 ± 3.5%w/w), total ash value (3.5 ± 0.1%w/w), water soluble ash (2.0 ± 0.2%w/w), acid soluble ash (1.0 ± 0.1%w/w).

Behavioural characters of the Sida acuta Burm. (Leaf-crude powder) with different reagents, determination of physical parameters of (HAESA), Crude powder when treated with water showed light green colour in visible light. Powder treated with Con HCL showed dark brown colour in visible light. Powder treated with

Con.H_2SO_4, aqueous$FeCl_3$ showed black colour in visible light. Powder treated with HNO_3, Water+HNO_3 showed yellow colour in visible light. Powder treated with CH_3COOH, Water+CH_3COOH Showed brown colour in visible light. Powder treated with Water+ Con HCL and aqueous NaOH showed green colour in visible light and powder treated with water+Con H_2SO_4 showed light block colour in visible light. Powder treated with water showed light green colour in UV 254nm.Powder treated with Con HCL showed dark brown colour showed in UV 254nm and powder treated with Con H_2SO_4, aqueous NaOH, aqueous $FeCl_3$ showed black colour in UV 254nm.Powder treated with HNO_3, Water + Con HCL, Water + Con H_2SO_4, Water + HNO_3 showed green colour in UV 254nm.Powder treated with CH_3COOH showed brown colour in UV 254nm and powder treated with Water+CH_3COOH showed dull brown colour in UV 254nm. Powder treated with water showed dark green colour in UV 365nm. Powder treated with Con HCL, Con H_2SO_4, Water + Con H_2SO_4, Water + CH_3COOH showed green colour in UV 365nm. Powder treated with HNO_3 showed light green colour in UV 365nm and Crude powder treated with CH_3COOH, Water + Con HCL showed brown colour in UV 365nm. Powder treated with Water + Con HCL showed light yellow colour in UV 365nm and powder treated with aqueous $FeCl_3$ showed black colour in UV 365nm. Powder treated with aqueous NaOH did not show any characteristic change in UV 365nm.

Phytochemical screening of the hydro-alcoholic extract (70%) *Sida acuta* (Leaf) powder revealed the presence of alkaloids, carbohydrates, cardiac glycosides, coumarine glycosides, sterols, saponins, tannins, phenolic compounds, flavonoids, proteins, amino acids, terpenoids, fixed oils, gum, mucilage, quinone, coumarine, resins,. It shows the absence of anthraquinone glycosides, cyanogenetic glycosides, volatile oils, betacyanins, anthocyanins, lecothiocyanins, emodin, pholotannins. The

aqueous extract of *Sida acuta* (Leaf) revealed the presence of alkaloids, carbohydrates, cardiac glycosides, coumarine glycosides, sterols, saponins, tannins, phenolic compounds, flavonoids, proteins, amino acids, terpenoids, fixed oils, gum, mucilage, quinone, coumarine, resins, pholotannins.It shows the absence of anthraquinone glycosides, cyanogenetic glycosides, volatile oils, betacyanins, anthocyanins, lecothiocyanins, emodin.

Determination of tannic acid equivalent in (HAESA), Weight of Tannic acid (TAE) equivalent of *Sida acuta* (leaf) 0.086mg/g. Determination of gallic acid content equivalent in (HAESA), Weight of gallic acid (GAE) equivalent of *Sida acuta* (leaf) 417mg/g. Determination of rutin equivalent in (HAESA), Weight of rutin equivalent of *Sida acuta* (leaf) 839.6mg/g.

TLC Profile

Thin layer chromatography (TLC) of the hydro alcoholic extract of *Sida acuta* (leaf) showed orange brown spot and Rf value 0.35 may indicate the presence of alkaloid. the solvent system used in Toluene:ethylacetate:diethylamine(70:20:10), Rf value 0.37at 365nm and bright blue spot may indicate thepresence of coumarine, the solvent system used in toluene:ether(1:1) , Rf value 0.36 at 365nm may indicate the presence of flavonoids.the solvent system used in Benzene : pyridine: Formic acid(72:18:10). Rf value 0.33 and deep purple spot may indicate presence of cryptolepine, the solvent system used in Dichloromethane:Chloroform:Methanol (4:4:1), Rf value 0.34 and yellow spot may indicate presence of cryptolepine, the solvent system used in Ethylacetate: Methanol: Ammonia (80:15:5) .

UV reports of cryptolepine:

CRYYPTOLEPINE ,The isolated compound crptolepine was dissolved in methanol and transferred to cuvette (quartz) and was scanned under UV range from 200-600 nm in the UV-Visibble spectrophotometer. The isolated compound cryptolepine shows absorbance maxima at λ_{max} 255 λ_{max} 274, λ_{max} 331and compared with that of pure cryptolepine as described by Dwuma-Badu,Ayim et al.1978.

Infra red spectrum report:

The IR spectrum of isolated cryptolepine shows a broad absorption band at 3300 cm^{-1} which may be due to alcoholic -OH stretching .It shows a strong absorption band at 2904cm^{-1} and 2980cm^{-1} which may be due to alkane-C-H stretching.it shows variable absorption band at 2264 cm^{-1} and 2231cm^{-1} which may be due to Alkyne-C≡C stretching not present in symmetrical alkynes, variable absorption band at 1645cm^{-1} which may be due to Alkane C-C stretching, Variable absorption band at 1452cm^{-1} and1419cm^{-1} which may be due to-C-H alkane bending, strong two bonds at 1382cm^{-1} which may be due to nitro N-O stretching, Medium absorption band at 1325cm^{-1},1274cm^{-1} and1085cm^{-1} which may be due to Amine-C-N stretching, strong absorption band at1043cm^{-1} which may be due to Ether-C-O stretching and it also show strong absorption band at 875cm^{-1} which may be due to Alkane-C-H stretching.

H NMR spectral report:

The ^{1}H NMR of isolated cryptolepine showed an intense singlet at δ 5.83ppm with integral of 36mm corresponding to 3H of the methyl group of cryptolepine at position 5.Sincethe methyl group at position 5 of cryptolepine has no neighbouring protons to couple it. It gave a singlet peak at 5.83ppm typical of signal produced by methyl group attached to tertiary nitrogen.

13C NMR Hetero aromatic carbons give signals within the range δ 100-165ppm andnine peaks within this range of chemical shift under CH 13C DEPT analysis of isolated SA-I. This confirms the nine protonated hetero aromatic carbons in SA-I: C-1,2,3,4,6,7,8,9, and 11.sp3 hybridised carbons absorb at lowest field (δ 0-50ppm) as compared to sp2(δ100-160ppm aromatic) and sp (δ 65-90ppm) hybridised carbons. Isolated SA-1 showed a singlet at δ 40.3ppm which confirms the presence of the only methyl carbon on the quinoline nitrogen at position 5 of SA-I (>N-CH3).

Mass spectrum report

The base peak of mass spectrometric analysis of isolated SA-I was found to be at *m/z* value of **233.27** corresponding to 100% relative intensity. Hence, the base peak of the mass spectrum of the isolated SA-I which also corresponds to the molecular ion actually occured at *m/z* **232.27**. This value is in agreement with the literature value of the molecular weight of SA-I which was 232g. The peak occuring at *m/z* **219.33**(29.36%). might correspond to N-demethylated fragment of SA-I.

Invitro antioxidant study

It includes three methods. Determination of scavenging activity against hydrogen peroxide, reducing power assay, determination of total antioxidant activity.The inhibitory concentration (IC_{50}) of *Sida acuta* (leaf) against hydrogen peroxide scavenging effect is found to be 1.83µg/ml in comparision with ascorbic acid 0.95µg/ml. The inhibitory concentration (IC_{50}) of *Sida acuta* (leaf) against reducing power assay determined in comparison with ascorbic acid used as standard. The inhibitory concentration (IC_{50}) of *Sida acuta* (leaf) in reducing power assay is found to be 142µg/ml in comparison with ascorbic acid 15µg/ml. The inhibitory concentration (IC_{50}) of *Sida acuta* (leaf) against total antioxidant capacity was

determined in comparison with ascorbic acid used as a standard. The total antioxidant capacity is found to be 185.4µg/ml in comparison with ascorbic acid 159.8µg/ml.

Alpha amylase inhibitory activity

The inhibitory concentration of *Sida acuta* (Leaf) against Alpha amylase inhibitory assay determined in comparison with acarbose used standard. The inhibitory concentration (IC_{50}) OF *Sida acuta* (leaf) in alpha amylase inhibitory effect found to be 17.10µg/ml in comparison with acarbose 9.27µg/ml.

Anti-mycobacterial activity

The percentage inhibition of *Sida acuta* Leaf (HAESA) against mycobacterium tuberculosis standard strain of H37Rv. HAESA (500µg/ml and 1000µg/ml) showed anti mycobacterial effect was found to be 99% in comparison with isoniazid (1ug/ml) 92% and rifampicin (1µg/ml) 96%. There for the extract showed almost similar antimycobacterial effect as that of isoniazid and rifambicin.

Antihistaminic activity

The percentage inhibition of Hydroalcoholic extract of *Sida acuta* (100µg/ml) on histamine induced contraction showed found to be 60.6% in comparision with Chlorpheniramine (1µg/ml) 69.69%. There for the extract showed almost similar anti histaminic effect that of chlorpheniramine maleate

CHAPTER-VII

CONCLUSION

CONCLUSION

- ✓ The present investigations draws the following conclusion (succeeding) inference.
- ✓ Pharmacognostical parameters for the leaves of *Sida acuta* were evaluated with additional scintfic data for the previous work, Multicellular trichomes and anamocytic stomata are affixed information to this plant.
- ✓ Quantitative estimation helps to identify the tannic acid, gallic acid and flavonoids equivalents present in the hydro-alcoholic extract of *Sida acuta*.
- ✓ TLC studies recorded the presence of coumarins, flavonoids,cryptolepine.
- ✓ The phytochemical screening of the hydro-alcoholic extract (70%) *Sida acuta* (Leaf) powder revealed the presence of alkaloids, carbohydrates, cardiac glycosides, coumarine glycosides, sterols, saponins, tannins, phenolic compounds, flavonoids, proteins, amino acids, terpenoids, fixed oils, gum, mucilage, quinone, coumarine, resins.The aqueous extract of *Sida acuta* (Leaf) revealed the presence of alkaloids, carbohydrates, cardiac glycosides, coumarine glycosides, sterols, saponins, tannins, phenolic compounds, flavonoids, proteins, amino acids, terpenoids, fixed oils, gum, mucilage, quinone, coumarine, resins, pholotannins.
- ✓ Isolation of indoloquinoline alkaloids such as cryptolepine.
- ✓ Spectral studies carried out such as UV, IR, H^1 NMR ^{13}C NMR and Mass spectroscopy
- ✓ Pharmacological screening potentiates the biological invitro antioxidant, invitro alpha amylase inhibitory effect, invitro anti micobacterial effect, invivo antihistaminic effect.

- ✓ Further investigation may be carried out for the bio-active fraction for its potential pharmacological effects.
- ✓ Future research studies may be extended to isolate other phyto-constituents present in this plant.

CHAPTER-VIII

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