"EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF LEAVES OF *PUTRANJIVA ROXBURGHII* USED AGAINST TRITON INDUCED HYPERLIPIDEMIC RATS"

The Tamil Nadu Dr.M.G.R Medical University Chennai

In partial fulfilment of the degree of

MASTER OF PHARMACY

(PHARMACOLOGY)

Submitted by

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Under the guidance of

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CERTIFICATE

This is to certify that this dissertation entitled **"EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF LEAVES OF *PUTRANJIVA ROXBURGHII* USED AGAINST TRITON INDUCED HYPERLIPIDEMIC RATS"** Submitted by **MANGALASELVAN S (Reg No: 261725656)** to The Tamil Nadu Dr.M.G.R Medical University, Chennai in partial fulfillment for the degree of **MASTER OF PHARMACY IN PHARMACOLOGY** is a bonafied work carried out by the candidate under my guidance and supervision in the **DEPARTMENT OF PHARMACOLOGY**, RVS College of Pharmaceutical Sciences, Coimbatore-02.

I have fully satisfied with his performance and work. I have forward this dissertation work for evaluation.

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I have fully satisfied with his performance and work. I have forward this dissertation work for evaluation.

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DECLARATION

I hereby declare that this dissertation entitled **"EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF LEAVES OF *PUTRANJIVA ROXBURGHII* USED AGAINST TRITON INDUCED HYPERLIPIDEMIC RATS"** submitted by me, in partial fulfillment of the requirements for the degree of **MASTER OF PHARMACY IN PHARMACOLOGY** to The Tamil Nadu Dr.M.G.R Medical university, Chennai is the result of my original and independent research work carried out under the guidance **Dr. BENITO JOHNSON, M.Pharm.,Ph.D.,** Professor & Head, **DEPARTMENT OF PHARMACOLOGY**, RVS College of Pharmaceutical Sciences, Coimbatore-02, & Co-Guide **Mr. Rajesh, SURAS LAB,** during the academic year 2018- 2019.

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EVALUATION CERTIFICATE

This is to certify that the dissertation work **"EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF LEAVES OF *PUTRANJIVA ROXBURGHII* USED AGAINST TRITON INDUCED HYPERLIPIDEMIC RATS"** submitted by, **MANGALASELVAN S (Reg No: 261725656)** to The Tamil Nadu Dr. M. G. R Medical University, Chennai in partial fulfillment for the Degree of **MASTER OF PHARMACY IN PHARMACOLOGY** is a bonafied work carried out during the academic year 2019-2020 by the candidate at **DEPARTMENT OF PHARMACOLOGY**, RVS College of Pharmaceutical Sciences, Coimbatore and was evaluated by us.

Examination centre:

Date:

Internal Examiner

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ABSTRACT

Hyperlipidemia is a disorder of lipid metabolism manifested by increase of plasma concentrations of the various lipid and lipoprotein fractions such as increase of serum total cholesterol (TC), low-density lipoprotein (LDL), triglyceride (TG) concentrations, and a decrease in the high-density lipoprotein (HDL) concentration. Hyperlipidemia is the key risk factor for cardiovascular disorders and has been reported as the most common cause of death in developed as well as developing nations. And hence only in this approach, intention has been to evaluate the anti-hyperlipidemic and antioxidant activity of *Putranjiva roxburghii* leaf extract on High fat diet-induced hypercholesterolemia and triton induced hyperlipidemia models. . It has been observed from our experimental research, there has been a remarkable and significant activity observed, against the anti-hyperlipidemic activity. Futuristic scope of this study will be extended to the investigation and isolation of the specific components responsible for antihyperlipidemic trait.

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1. INTRODUCTION

1.1 Hyperlipidemia

Hyperlipidemia prevalence continued to increase annually, requiring the development of drugs capable of lowering blood lipids to reduce mortality and morbidity due to cardiovascular complications. Although synthetic lipid-lowering drugs are useful in treating hyperlipidemia, there are number of adverse effects. So, the cur-rent interest has stimulated the search for new lipid-lowering agents with minimal side effects from natural sources. Hyperlipidemia is a disorder of lipid metabolism manifested by increase of plasma concentrations of the various lipid and lipoprotein fractions such as increase of serum total cholesterol (TC), low-density lipoprotein (LDL), triglyceride (TG) concentrations, and a decrease in the high-density lipoprotein (HDL) concentration. Hyperlipidemia is the key risk factor for cardiovascular disorders and has been reported as the most common cause of death in developed as well as developing nations. Hyperlipidemia may be caused by specific genetic abnormalities called primary hyperlipidemia or may be idiopathic caused by lifestyle habits or medical diseases such as diabetes, kidney disease, pregnancy, hypothyroidism and heart disease.

Herbal medicines are the oldest remedies known to mankind. Herbs had been used by all cultures throughout history. In the last few years, there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects when comparing other leaf of medicine. India being the botanical garden of the world with more than 2400 medicinal plants out of 21000 species being listed by WHO, is the largest producer of medicinal plants around the globe.

Putranjiva roxburghii is a large, evergreen tree, 10-15m in height, indigenous to the evergreen forests at altitude of 450-1,200m and cultivated throughout the hotter parts of India. Leafof this plant is straight rough whereas bark is green or black, 1.25cm thick, exuding milky latex, leaves broad obovate, elliptic, decurrent, glabrous, entire inflorescence solitary axillaries, cauliforous and ramflours on short leafy shoots. Male head is sessile or on short peduncles receptacles, sometimes born on the ultimate twing, Female head are oblong ovoid receptacle, syncarpus, cylindrics. Seeds are separated horny endocarpus enclosed by sub-gelatinous exocarpus (1mm thick)

oblong ellipsoid in nature. The sweet yellow sheaths around the seeds are about 3-5 mm thick and have a taste similar to that of pineapple, but milder and less juicy. Even though it is well known for its antibacterial, anti-inflammatory, anti-diabetic, antioxidant and immunomodulatory properties there are no evidences regarding the anti-hyperlipidemic effect of the leafhence our study has its relevance

The biggest organ in the body is the "LIVER" and it is likewise fills in as the essential metabolic organ of the body. In spite of the fact that the liver is comprised of various cells like hepatocytes, endothelial, kupffer and stellate cells are the most dominating with critical capacities. Another most essential one of a kind component of the liver is its capacity to recover. Well grown-up liver (i.e. Grown-up) is the standard organ accountable for detoxifying and metabolizing, exogeneous/endogenous mixes, rendering them more hydrophilic, which as often as possible impact their force and action.

Liver infections are the genuine restorative issues went up against by the people wherever all through the world. The epidemiological review demonstrates that around 20,000 passings happen reliably in light of liver issue. In Africa and Asia, the major drivers of liver maladies are contaminations by infection and parasite, while in Europe and in North America, a vital reason is liquor manhandle. Liver ailments are primarily realized by deadly chemicals, over the top affirmation of ceaseless liquor, diseases and immune system. Hepatic harm by over measurements of drug appears, from every angle, to be a run of the mill contributing component. Liver is required to do physiological limits and additionally to guarantee against the perilous of dangerous drugs and chemicals. Prescription impelled substance damage is accountable for 5% of each mending focus attestation and half of all serious liver disappointment. Over 75% of episodes of specific prescription reactions achieve liver transplantation or death.

1.2 Prevalence of hyperlipidemia

Raised cholesterol increases the risks of heart disease and stroke. Globally, a third of ischaemic heart disease is attributable to high cholesterol. Overall, raised cholesterol is estimated to cause 2.6 million deaths (4.5% of total) and 29.7 million disability adjusted life years (DALYS), or 2.0% of total DALYS. Raised total cholesterol is a major cause of disease burden in both the developed

and developing world as a risk factor for Ischemic heart disease and stroke. A 10% reduction in serum cholesterol in men aged 40 has been reported to result in a 50% reduction in heart disease within 5 years; the same serum cholesterol reduction for men aged 70 years can result in an average 20% reduction in heart disease occurrence in the next 5 years. In Ireland, a 30% reduction in the heart disease death rate has been attributed to 4.6% reduction of the population mean for total cholesterol. In Finland, 50% of the decline in IHD mortality has been explained by the reduction of population blood cholesterol level. In 2008 the global prevalence of raised total cholesterol among adults (≥ 5.0 mmol/l) was 39% (37% for males and 40% for females). Globally, mean total cholesterol changed little between 1980 and 2008, falling by less than 0.1 mmol/L per decade in men and women.

The prevalence of elevated total cholesterol was highest in the WHO Region of Europe (54% for both sexes), followed by the WHO Region of the Americas (48% for both sexes). The WHO African Region and the WHO South East Asian Region showed the lowest percentages (22.6% for AFR and 29.0% for SEAR). The prevalence of raised total cholesterol increased noticeably according to the income level of the country. In low income countries around a quarter of adults had raised total cholesterol, in lower middle income countries this rose to around a third of the population for both sexes. In high-income countries, over 50% of adults had raised total cholesterol; more than double the level of the low-income countries.

Interruption of transport protein:

Bile stream might be hindered by meds that impact transport proteins at canalicular film. Loss of villous strategies and interruption of transport pumps, for instance, multidrug resistance-related protein 3 hinder release of bilirubin realizing cholestasis

Cytolytic T-cell actuation: Co-valent binds of pharmaceutical to Cytochrom P-450 compound goes about as an immunogen enacting T-cells and cytokines and energizing multifaceted safe responses.

Apoptosis of hepatocytes: Enhancement of apoptotic pathways by tumor rot calculate alpha receptor of Fas may trigger the course of intercellular caspases, which achieve altered cell passing.

Mitochondrial disturbance: A couple of meds limit mitochondrial limit by twofold effect on both beta-oxidation vitality creations by frustrating the union of nicotinamide adenine dinucleotide and flavin adenine dinucleotide, realizing decreased ATP era. Bile pipe damage: Dangerous metabolites discarded in bile may achieve mischief to bile course epithelium[1-3].

Solution/sedate incited liver harm is a prosperity issue, and is depended upon to increase as the amount of drugs being eaten up augmentations, both remedy and non-solution, and in view of the present example of usage of pharmacologically dynamic substances in correlative and option prescription. Prescription/tranquilize incited hepatotoxicity is the most surely understood reason alluded for withdrawal of authoritatively endorsed meds from the business. It also speaks to more than 50 percent of occasions of serious liver disappointment in the United States. The positive recurrence of solution/medication incited liver harm is difficult to gage, and all things considered, concentrates going to measuring its event encounter the evil impacts of drawbacks, for instance, under-detailing and that data by expansive start from audit thinks about. Frequently, there is in like manner a nonattendance of information about self-arrangement and use of home grown item that may associate with prescription and non-doctor embraced medications[4].

Despite the repeat of solution actuated liver harm being low, data from the Centers for Disease Control and Prevention in the U.S. report pretty much 1600 new extraordinary examples of liver disappointment yearly, of which Paracetamol hepatotoxicity speaks to plus or minus 41%. Exactly when taking a sexual orientation at hospitalized patients, the rate of hostile solution reactions is assessed to be 6.7%, and deadly disagreeable medicine reactions mean 0.32%, as controlled by a meta-investigation of around 40 imminent reviews. Amid the period 1995 to 2005, the reports of horrible prescription reactions and also passings related to these, have drastically increased. Various examples of pharmaceutical activated liver harm are particular, i.e. the reaction is whimsical considering the known pharmacological properties of the medication, and from this time forward is scarcely perceptible amid preclinical periods of change. There are however studies to demonstrate that these reactions might be liable to an extended affectability of the patient to the medicine being alluded to, dependent upon such segments as other going with contaminations or other relating prescriptions. Certain innate factors, for instance, HLA-sort, can once

in a while add to the affectability of a man to opposing pharmaceutical reactions. Commonly, clinically clear hostile drug reactions happen when some season of inertness, wherever in the compass going from one to 12 months (most by and large within 90 days), and about constantly vanish after departure of the solution. Pharmaceutical researched liver damage may give a couple of unmistakable clinical segments; hepatitic/hepatocellular, cholestatic or mixed.

Regardless of their etiology, solution/tranquilize prompted hepatotoxicity remains a significant issue amid medicine advancement in the pharmaceutical business, both concerning extended threat for patients encountering clinical trials, besides tolerant hazard after the acquaintance of new drug with the treatment. Moreover, because of the extended costs that takes after disappointment of a prescription to-be at a late stage in medicine improvement or after its launch.

1.3 Hepatoprotective Allopathic Treatment

Couple of present day drugs are accessible for treating liver illnesses that incorporates:

Ursodeoxycholic corrosive (Ursodiol): Ursodiol diminishes intestinal retention and stifles hepatic union and capacity of cholesterol. It is predominantly utilized as a part of administration of constant hepatic ailments in people.

Penicillamine: Penicillamine chelates a few metals like copper, iron, lead and mercury shaping stable water dissolvable edifices which are renally discharged.

1.19 Role of Herbal antioxidants in ROS

The damaging effects of ROS is tackled effectively by antioxidants, normally superoxide and hydrogen peroxide are produced in the body. If excess quantities of generation leads to pathological ROS production. Many herbs has the potential to compromise ROS such as green tea, grape seed, ginseng and *Scutellaria baicalensis.* Long while herbal medicines used for the diabetes has been in existence. Current pre-clinical and clinical studies have demonstrated that many of them exhibit potent anti-inflammatory and anti-oxidative properties, and have also identified the active phytochemicals responsible for their activities[22]. The herbal medicines and nutraceuticals, as well as their bioactive components, which exhibit anti-inflammatory and anti-oxidative properties, provide a promising approach for the prevention and

treatment of diabetic complications. The etiology of diabetes and its complications are because of free radicals and for the reason herbs with antioxidant properties are believed to possess faith in controlling and minimizing the damage due the reactions. The list of some herbs used for diabetes and its complications are given in Nearly 400 herbs are accounting for diabetes treatment worldwide.

Acacia arabica (Babhul) has got anti-diabetic agent shown to have hypoglycemic effect. *Aegle marmelos* (Bengal Quince) which improves digestion and reduces blood glucose, urea and serum cholesterol level. *Allium cepa* (Onion) is a potential antioxidant, anti hyperglycemic and anti hyperlipedemic activity. *Allium sativum* (Garlic) has been used to increases insulin secretion and controls lipid peroxidation. Aloe vera stimulates β cell to secrete insulin, Anti-inflammatory and wound healing. *Azadirachta indica* (Neem) evidenced using anti-hyperglycemic, hepatoprotective and antioxidant activities. *Eugenia jambolana* (Jamun) is a viable anti-hyperglycemic agent. *Mangifera indica* (Mango) is a anti-diabetic agent, reduces intestinal glucose uptake. *Momordica charantia* (Bitter gourd) is utilized as antidiabetic and antihyperglycemic Agent. *Ocimum Sanctum* (Holy basil) cause glucose level decline in fasting condition, triglyceride and total lipid content. *Phyllanthus amarus* (Bhuiawala) is a antinflammatory, anticancer, antioxidant and antidiarrhoeal.

Icariin a flavonoid of *Epimedium pubescens* known to have considerable antioxidant activity. They demonstrated cardiac functions and mitochondrial oxidative stress in streptomycin induced diabetic rats. The observations are in favor of controlling oxidative stress of cardiac complications in diabetes induced animal. An 8 weeks of administration markedly improved cardiac function and ROS has been proved effectively.

The nanotechnology is facing expansions in all dimensions for serving mankind, that almost all the countries are striving to explore for the social well being and economy of the country[23]. Nanoparticles are known to have tremendous applications in the field of diagnosis and theraphy. Such imperative nanoparticles have very great trait to carry and serve like an antioxidant, antihyperglycemic and ROS interfering action. Treatment of antidiabetic potent nanoparticle with plants would have therapeutic value do create a new platform for herbal medicines in nanoscience for drug delivery.

1.19 High antioxidant activity of *Dalbergia sissoo* (Indian Rosewood)

Extracts of the plant leafbark, they intervened to extend they work towards *invitro* antioxidant determination by chemical method, using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. In their experimentation of aqueous and methanolic extract they found aqueous extact has greater activity[24-26]. They concluded that plant has high antioxidant activity and it may find it very useful in the treatment of diseases and complications caused by oxidative stress.

1.20 Nanoencapsulation of *Albizia chinensis*

Nanoencapsulation of this herb having potential antioxidant activity of its content quercitrin. The polymer poly-D,L-lactide (PLA) is used to encapsulate the material and solvent evaporation technique was deployed to prepare the nanodimensions of the drug. The drug quercitin was made to encapsulate to increase the solubility, permeability and stability of the molecule[27]. Moreover, the properties of nanomedcine has provided a new potential use of less useful highly active antioxidant molecule towards the development of oxidative stress related inflammation and its related complication profiles.

1.21 Antioxidant enriched Silymarin Nanoparticles

Porous silica nanoparticles of silymarin to increase the solubility as it has the considerable antioxidant activity. The silymarin nanoparitcles were prepared by porous microemulsion and ultrasonic corrosion methods. The results are bioavailability of *silymarin* was considerably increased despite the drugs basic poor solubility nature[28]. The evidences are strong that herbal components are appropriate option for oxidative stress management in excessive ROS generation.

1.22 Metal antioxidant nanoparticle

As antioxidants have significant role in influencing ROS, such antioxidant nanoparticles are prepared from metals such as gold, silver and so on. These methods of producing metal antioxidant nanoparticle using plant extracts are extremely biosynthesized.The prepared nanoparticles are characterized by UV-vis spectroscopy, XRD, TEM and SAED analyses.

This method utilizes cheap production of nanoparticles with non toxic nature They got nanoparticles of size range 5 to 15 nm, *and Zingiber officinale* as stabilizing and reducing agent which is more potent than asprin. Characterization was done by Dynamic Light scattering (DLS), TEM and UV-Vis Spectroscopy[29]. The produced nanopartcles are biocompatible with the blood has been observed.

Diabetic treatment channelizing to the effective control of glucose level and specific strategy to target the ROS generating pathway curbing, do produce better results and compliments each other beneficially. A biological antioxidants capable of restraining oxidative stress mediated diabetic complication in due course of hyperglycemia is still mandatory to foresee better clinical improvements. The antioxidant enriched herbal components is the viable tool to cope with oxidative stress condition in diseased condition especially, the diabetes[30-32]. Secondly, evidences are there that such components of antioxidant, antidiabetic and hypoglycemic herbs are tailored to nanotization for the maximum benefit. Provided with the strong scientific back up evidences, the clinical implications of nanotechnology based herbal constituents such as antioxidants are in great need to the mankind, to fight with oxidative stress related complications in diabetes and related ailments.

Table 1: List of some herbs for diabetes and its complications

S.No	Botanical name	Common/Vernacular Name
1.	*Eugenia Jambolana*	Indian Gooseberry
2.	*Momordica charantia*	Bitter gourd
3.	*Ocimum sanctum*	Holy Basil
4.	*Phyllanthus amarus*	Bhuiawala
5.	*Pterocarpus marsupium*	Benga
6.	*Tinospora cordifolia*	Guduchi
7.	*Trigonella foenum*	Fenu greek
8.	*Withania somnifera*	Ashwagandha

Table 2: List of herbs and its intention to intend

Name of Herb	Common/Vernacular	Intention/purpose
Acacia Arabica	Babhul	Anti-diabetic agent shown to have hypoglycemic effect.
Aegle marmelos	Bengal Quince	Improves digestion and reduces blood glucose, urea and serum cholesterol level
Allium cepa	Onion	Antioxidant, anti hyperglycemic and anti hyperlipedemic activity
Allium sativum	Garlic	Increases insulin secretion and controls lipid peroxidation
Aloe vera	Kathalai	Stimulates β cell to secrete insulin, Anti-inflammatory and wound healing
Azadirachta indica	Neem	Anti-hyperglycemic, hepatoprotective and antioxidant activity
Eugenia jambolana	Jamun	Anti-hyperglycemic
Mangifera indica	Mango	Anti-diabetic agent, reduces intestinal glucose uptake.
Momordica charantia	Bitter gourd	Antidiabetic and Antihyperglycemic Agent
Ocimum Sanctum	Holy basil	Glucose level decline

		in fasting condition, triglyceride and total lipid content
Phyllanthus amarus	Bhuiawala	Antiinflammatory, anticancer, antioxidant and antidiarrhoeal

2. LITERATURE REVIEW

Wadood, et al., (2015)[56]studied in his study investigates the hypoglycemic and hypolipidemic effects of an ethylacetate (EA) fraction of the mature leaves of *A. heterophyllus* in a streptozotocin (STZ) induced diabetic rat model. In normoglycemic rats, administration of a single dose (20 mg/kg) of the EA fraction resulted in a significant ($P < 0.05$) reduction in the fasting blood glucose concentration and a significant improvement in glucose tolerance ($P < 0.05$), compared to the controls. In STZ-induced diabetic rats, chronic administration of the EA fraction of *A. heterophyllus* leaves daily for 5 weeks resulted in a significant lowering of serum glucose, cholesterol and triglyceride (TG) levels. Compared to control diabetic rats, the extract-treated rats had 39% less serum glucose, 23% lower serum total cholesterol and 40% lower serum TG levels and 11% higher body weight at the end of the fifth week.

Karunanayake, EH et al., (1999)[57] examined the antioxidative, hypoglycemic, and hypolipidemic activities of *Putranjiva roxburghii* leaf extracts. Various extracts like 70% ethanol n-butanol, water, chloroform, and ethyl acetate extracts are examined. The administration of 70% ethanol extract or n-butanol extract to streptozotocin (STZ)-diabetic rats significantly reduced fasting blood glucose (FBG) from 200 to 56 and 79 mg%, respectively; elevated insulin from 10.8 to 19.5 and 15.1 µU/ml, respectively; decreased lipid peroxides from 7.3 to 5.4 and 5.9 nmol/ml, respectively; decreased %glycosylated hemoglobin A1C (%HbA1C) from 6.8 to 4.5 and 5.0%, respectively; and increased total protein content from 2.5 to 6.3 and 5.7 mg%, respectively. Triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), VLDL-C, and LDL/HDL ratio significantly declined by -37, -19, -23, -37, and -39%, respectively, in the case of 70% ethanol extract; and by -31, -14, -17, -31, and -25%, respectively, in the case of n-butanol extract; as compared to diabetic rats. HDL-C increased by +37% (70% ethanol extract) and by +11% (n-butanol extract). Both JFEE and JFBE have shown appreciable results in decreasing FBG, lipid peroxides, %HbA1C, TC, LDL-C, and TG levels, and increasing insulin, HDL-C, and protein content.

Tangijahe, et al., (2010)[59] studied the wound healing activity of the leaves of *Putranjiva roxburghii lam*. (moraceae) on ex-vivo porcine skin wound healing model and found that that the ethyl acetate extract of the leaves possesses potential wound healing activity.

Bao, H et al. (2011)[60] investigated and evaluated the antimicrobial and phytochemical properties of *Putranjiva roxburghii* in leaf extracts Hexane, dichloromethane and ethanol were used as extraction solvents and test organisms were *Escherichia coli, Micrococcus luteus, Aspergillusniger and Trichoderma sp.* A disc diffusion test was adopted to test the susceptibility of the selected microbes to the extracts while Minimum inhibitory concentration (MIC) was determined using serial dilution of extracts. Ethanolic leafbark extracts (30mg/ml) of A.heterophyllusexibits significant antibacterial activity against Escherichia coli with 9.50 ± 0.44 inhibition zone radii. Dichloromethane extracts of leaf and leafbark showed lesser antibacterial activity against both of the bacteria with inhibition zones of 3.00 ± 0.34 mm to 5.66 ± 0.16 mm while hexane extracts did not show any antibacterial activity. Antifungal activity on the other hand was not detected in any of the extracts. Phytochemical screening confirmed the presence of phytosterols, anthraquinone, terpenoids, phenols, glycosides, flavonoids and diterpenes.

Selvaraj B, et al., (2010)[61] in this study "Antidiabetic activity of *Putranjiva roxburghii rag* extract studied in high fat fed- low dose STZ induced experimental type 2 diabetic rats" reports that *Putranjiva roxburghii rag* possess antibacterial, anti-inflammatory, antioxidant and immunomodulatory properties. In the study Diabetic rats were treated with Putranjiva roxburghii rag extract at a dosage of 300 mg/kg b.w daily for 30 days. Metformin (200 mg/kg. b.w) was used as a reference drug and fasting blood glucose, plasma insulin and HbA1c were the parameters under consideration. The extract supplementation attenuated the elevated levels of glucose, glycosylated hemoglobin, AST, ALT and ALP. The insulin level was improved with an improvement in hepatic glycogen content of insulin resistant diabetic rats. The altered activities of glycogen metabolizing enzymes were normalized upon extract treatment. Also the extract improves insulin sensitivity which is evident from intraperitoneal insulin tolerance test. The results show that the rags of Putranjiva roxburghii is non toxic and possess significant antidiabetic properties.

Ganeshkumar, V et al., (2011)[62] explained herbal remedies have evolved with enormous impending of alleviate. Herbal medicine progress against the non-communicable disease like diabetes is one of the propel area of research in the field of worldwide medicine. Hyperlipidemia is a disorder of lipid metabolism manifested by increase of plasma concentrations of the assortment of lipid and lipoprotein fractions. Hyperlipidemia has been one of the maximum risk factors contributing to the occurrence and relentlessness of coronary heart diseases. HMG Co A reductase is a key enzyme involving in rate limiting step of cholesterol biosynthesis. Conservative anti-hyperlipidemic drugs have restricted efficacies and vital side effects, so that alternative lipid lowering agents are required. This review explains the plants possessing significant anti-hyperlipidemic activity with their botanical name, family, part used, extract used and inducing agent of hyperlipidemia

Feng Lin yen., et al., (2008)[63] explained Increasing drug resistance of pathogens and negative consequences of antibiotic usage has led to the search for alternative medicines from nature. Many plants have been exploited to cure infectious diseases from time immemorial. The present investigation evaluated the antimicrobial and phytochemical properties of *Putranjiva roxburghii i.e.* Jack fruit (Kos in Sinhala) and *Lantana altilis i.e.* Bread fruit (Dhel in Sinhala) leaf and leafbark extracts. Hexane, dichloromethane and ethanol were used as extraction solvents and test organisms were *Escherichia coli*, *Micrococcus luteus*, *Aspergillus niger* and *Trichoderma* sp. A disc diffusion test was adopted to test the susceptibility of the selected microbes to the extracts while Minimum inhibitory concentration (MIC) was determined using serial dilution of extracts. Phytochemical screening was carried out by specific chemical identification tests. Bioassay data were statistically analyzed using two-way ANOVA (SPSS 20 at 95% confidence level). Ethanolic leafbark extracts (30mg/ml) of *A.heterophyllus* and *A.altilis* possessed significant antibacterial activity against *Escherichia coli* with 9.50 ± 0.44 mm and 7.49 ± 0.28 mm inhibition zone radii respectively. Dichloromethane extracts of leaf and leafbark showed lesser antibacterial activity against both of the bacteria with inhibition zones of 3.00 ± 0.34 mm to 5.66 ± 0.16 mm while hexane extracts did not show any antibacterial activity. Antifungal activity on the other hand was not detected in any of the extracts. Bacterial antibiotic tetracycline and fungal antibiotic ketoconazole which were used as positive controls were more effective even at 1/10 concentration compared to all the plant

extracts tested. Phytochemical screening confirmed the presence of phytosterols, anthraquinone, terpenoids, phenols, glycosides, flavonoids and diterpenes in both of the trees. These results confirm the potential antibacterial activity of *A.heterophyllus* and *A.altilis*

Xia cao et al., (2012)[66] demonstrated the anti-hyperlipidemic effect of methanolic extract of whole plant of *Rhinacanthus nasutus* ((RNM) was tested in Triton and fat diet induced hyperlipidemic rat models. Here, Acute hyperlipidemia was induced by administration of single dose of Triton X 100 (400 mg/kg,i.p) and Chronic hyperlipidemia was induced by feeding fat diet for 21 days to rats. Treatment with RNM (200 and 400 mg/kg, p.o) significantly reduced the hyperlipidemia i.e., decreased levels of serum Total Cholesterol, Triglycerides, Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C) , and increase of serum High Density Lipoprotein Cholesterol (HDL-C) when compared to vehicle control and standard drug Atorvastatin (10 mg/kg). The results demonstrated that methanolic extract of whole plant of *Rhinacanthus nasutus* possessed significant antihyperlipidemic activity.

Kannan BN, et al., (2010)[67] designed to perform preliminary phytochemical screening, acute oral toxicity and to evaluate antihyperglycemic activity of whole plant of *Glycosmis pentaphylla* ethanolic extract. .*Glycosmis pentaphylla,*whole plant was extracted using ethanol as solvent by soxhlet apparatus. The extract was subjected to preliminary phytochemical screening. Acute oral toxicity studies were performed to determine test dose . The evaluation of antihyperlipidemic activity was done using Triton X 100 and High Fat Diet induced hyperlipidemia models in Wistar albino rats. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, flavonoids, proteins, and amino acids. Doses up to 2000mg/kg were found to be safe after acute toxicity tests. Cholesterol, triglycerides, HDL, LDL, VLDL, SGOT, SGPT, Total protein and glucose were measured. The results suggested that EGP (ethanolic extract.*Glycosmis pentaphylla*) possess antihyperlipidemic activity against hyperlipidemia induced by Triton X 100 and also High Fat Diet induced experimental models.

Praveen kumar, K et al., (2011)[6I]illustrated the greatest risk factor of coronary heart disease. Currently available hypolipidaemic drugs have been associated with number

of side effects. Herbal treatment for hyperlipidaemia has no side effects and is relatively cheap and locally available. Literature claims that Saponins are able to reduce hyperlipidemia. Based on high saponin content in herbal plants, *Spermacoce hispida* (*S. hispida*) was selected and the present study focus on the antihyperlipidaemic activity of ethanolic seed extract of *S. hispida* against triton-WR-1339 induced hyperlipidaemia in rats. Hyperlipidaemia was induced in Wistar rats by intraperitoneal (i.p) injections of Triton WR-1339 at a dose of 400 mg/kg body weight. *S. hispida* was administered orally at a dose of 200 mg/kg to triton WR-1339 induced hyperlipidaemic rats. After administration of *S. hispida* shows a significant decrease in the levels of cholesterol, phospholipids, triglycerides, LDL, VLDL and significant increase in the level of HDL in serum and liver tissues against triton induced hyperlipidaemic in rats. Therefore it effectively suppressed the triton induced hyperlipidemia in rats, suggesting the potential protective role in Coronary heart disease.

De Goot H et al., (1998)[69] Phytochemical investigation of the leaves of *Putranjiva roxburghii* furnished six compounds from different combinations of petroleum ether, chloroform and methanol. Structures of these compounds were elucidated and established by standard spectroscopic methods. Isolated compounds are n-Octadec-9-enoyl α-L-rhamnopyranoside(1), n-octadec-9,12-dienoyl-α-L-rhamnopyranoside (2), n-octadec-9,12-dienoyl-β-D-glucopyranoside (3), n-octadec-9-enoyl-β-D-glucopyranoside (4), n-octadec-9-enoyl-β-D-arabinopyranoside (5) and n-octadec-9-enoyl-α-D-xylopyranoside (6) respectively. The structures of all the phytoconstituents are elucidated on the basis of spectral data analyses and chemical reactions.

Fernandez SP et al., (2006)[70] studied *Mangifera indica* L., known as mango (Family; Anacardiaceae), commonly used herb in ayurvedic medicine, traditionally used for their antidiabetic, anti-oxidant, anti-viral, cardiotonic, hypotensive, anti-inflammatory properties, antibacterial, anti fungal, anthelmintic, anti parasitic, anti tumor, anti HIV, antibone resorption, antispasmodic, antipyretic, antidiarrhoeal, antiallergic, immunomodulation, hypolipidemic, anti microbial, hepatoprotective, gastroprotective effects.To investigate effect of aqueous extract *of Mangifera indica* L.leaf on high cholesterol fed diet rats. High cholesterol fed diet rats exhibited significant increase in total serum cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and significant decrease in high density lipoproteins. Treatment with

aqueous extract of *Mangifera indica* leaves significantly decreased total serum cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and increased in high density lipoproteins rats. Hypolipidemic activity of M. indica may be attributed due to presence of flavonoids, Saponins, glycosides, tannins, phenolics.

Heim KE et al., (2002)[71] examined that the hyperlipidemia plays an important role in the development of atherosclerosis, the main cause of death in the world. In this study, the lipid-lowering effect of *Carica papaya* leaf in rats fed with a high cholesterol diet was evaluated. Daily doses of *C. papaya* extract 0, 31, 62 or 125 mg/kg body weight were orally administered in 300 µl polyethylene glycol to hypercholesterolemic rats; it was also administered 62 mg/kg body weight of the extract to rats with normal diet. After a 20-day treatment, the animals were sacrificed; blood and liver were analyzed. Hypercholesterolemic rats showed an increased serum and liver cholesterol, triacylglycerols, and atherogenic index. The *C. papaya* extract produced a significant decrease of serum and liver cholesterol concentrations in hypercholesterolemic rats, but did not modify serum or liver triacylglycerols; however, the extract reduced the atherogenic index in a dose-dependent manner. *C. papaya* treatment decreased LDL-C and increased HDL-C in serum significantly. When the oxygen consumption was evaluated in phosphorylating and resting states, the respiratory control in hypercholesterolemic rats mitochondria was lower than in normal diet rats. However, a higher respiratory control in hypercho- lesterolemic rats mitochondria was observed after C papaya treatment. The liver morphological data are in accordance with serum and liver biochemical values. Our data support that *C. papaya* has a significant hypocholesterolemic action and HDL-C raising effect on rats fed with a cholesterol- rich diet.

3. AIM & OBJECTIVE

AIM

To successfully evaluate the anti-hyperlipidemic and antioxidant activity of *Putranjiva roxburghii leaf extract on* High fat diet-induced hypercholesterolemia and triton induced hyperlipidaemia models.

OBJECTIVE

To conduct a literature survey for establishing the relevance of the study

To collect and authenticate *Putranjiva roxburghii* leaves

To characterize the antioxidant property of the extract

4. PLAN OF THE WORK

- Plant Collection
- Plant Authenication
- Solvent Extraction
- Phytochemical Screening
- Acute Toxicological Studies
- Pharmacological Screening For Antihyperlipidaemic Activity
    - High-cholesterol diet induced rat model.
    - Triton induced rat model.
    - Effect on Normocholesteremic rats
- Pharmacological Screening For Antioxidant Activity
    1. Superoxide radical scavenging activity
    2. DPPH radical reducing activity
    3. Lipid peroxidation assay
    4. Nitric oxide scavenging assay
    5. Hydrogen per oxide assay

- Statistical analysis, compilation of Results &Tabulation of data.

4.1 Plant Profile

Putranjiva roxburghii belongs to the family Euphorbiaceae (spurge family). It is a moderate sized evergreen tree which grows in moist evergreen forests. This tree has antiinflammatory, anti-oxidant, anti-microbial, anti-nociceptive, anti-pyretic, anti-emetic, cytotoxic, hypoglycemic, larvicidal, cytogenetic toxicity, aphrodisiac, anthelmintic, radioprotective, CNS depressant and analgesic activities, and contains terpenoids, mustard oils, flavonoids, tannins, alkaloids, glycosides and phenolic compounds. Owing to the diverse biological significance and chemical constituents of Putranjiva roxburghii, this tree is regarded as highly beneficial and useful and further study on this tree is urgently needed. This critical review summarizes the literature dealing with ethnomedical uses, chemical constituents, and pharmacological profile of the leaf, stem bark and seed of *putranjiva roxburghii.* Particular attention is given to its analgesic, anti-inflammatory, anti-hyperlipidemic, diuretic, hepatoprotective, anthelmintic, and anti-bacterial effects so that its potential uses in pharmaceutics can be better evaluated[72-77]. We hope that adequate amount of information in this review endorses the importance of this tree and attracts the attention of readers to further explore biological diversity.

Common Name: *Putranjiva;* Lucky bean tree

Hindi: Putijia

Marathi: Jivanputra, Patravanti

Tamil: Irukolli, Karupala

Malayalam: Pongalam

Telgu: Kuduru, Putrajivika

Kannada: Amani Putrajiva

Bengali: Putranjiva

Table 3: Scientific Classification

Scientific classification	
Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Malpighiales
Family:	Putranjivaceae
Genus:	*Putranjiva*

Figure 1: Trunk and leaves of *Putranjiva roxburghii*

Figure 2: Leaves of *Putranjiva roxburghii*

Figure 3: Leaves and fruits of *Putranjiva roxburghii*

Figure 4: Trees and banches of *Putranjiva roxburghii*

Figure 5: Fruits and leaves of *Putranjiva roxburghii-I*

Figure 6: Fruits and leaves of *Putranjiva roxburghii-II*

Figure 7: Fruits and leaves of *Putranjiva roxburghii-III*

Figure 8: Seeds of *Putranjiva roxburghii*

General Information

Putranjiva roxburghii is an evergreen tree growing up to several metres tall. The tree is harvested from the wild for local use as a medicine and source of beads, oil and wood. An attractive tree with pendant branches, it is grown as an ornamental in gardens, especially in India. This species is usually dioecious, though occasional monoecious forms are found. Generally, both male and female forms need to be grown if fruit and seed are required. A decoction of leaves and fruit is taken for the treatment of liver complaints, colds, fevers and rheumatism. The hard, white seeds of the fruit are threaded into necklaces and also used for rosaries .Necklaces made from the seeds are traditionally given to children as a protection from disease. An olive-brown, fixed oil is obtained from the seed. It is used for burning. An essential oil is obtained from the seed[78]. The grey wood is close-grained, moderately hard. The wood is sometimes used for construction, turnery, tool handles.

Putranjiva is a plant genus of the family Putranjivaceae, first described as a genus in 1826. It is native to Southeast Asia, the Indian Subcontinent, Japan, southern China, and New Guinea.

Along with *Drypetes* (of the same family), it contains mustard oils as a chemical defense against herbivores.[3] The ability to produce glucosinolates is believed to have evolved only twice, in the Putranjivaceae and the Brassicales

Putrajivaka, Sutajva, Putrakamanjari are few common names of the tree *Putranjiva roxburghii*. It is known as Karupali or Irukolli in Siddha system of medicine. It is native to India and found wild or cultivated in almost all parts of country. In Delhi, Putrajivaka tree can be seen at Race course Road and many other roads of Lutyens' Delhi.].

Tree Description

Moderate sized evergreen tree;height is up to 12m with pendent branches and dark grey bark having horizontal lenticels, Leaves simple, alternate, distichous;stipule triangular, acute, caducous;petiole 0.4-1 cm long, nearly glabrous, planoconvex in cross section;lamina 5- 9.5 x 1.5-3.5 cm, elliptic-oblong to elliptic-ovate, apex acute or slightly acuminate with blunt tip, base asymmetric, margin serrate, chartaceous, dark green and shining, glabrous, midrib nearly flat above, secondary nerves 9-12 pairs, slender, tertiary nerves reticulate, Flower unisexual, male flowers inaxillary clusters, female flowers axillary, solitary, Drupe, ellipsoid, with persistent style, seeds.

General Habitat

Tree is generally grows on alluvial soil along the rivers, swamp, or evergreen forests. The tree prefers moist evergreen forests[79]. In drier places, tree has stunted growth. The germination of seeds takes place during rainy season.

Flowering and fruiting: March-August

Parts used for medicinal purpose: Leaves, seeds and fruits

Constituents:

Ethanol extraction of the fresh leaves of *P. roxburghii* proved convenient for the isolation of polyphenolic compounds. In addition to triterpenoids this extract yielded ellagic acid, gallic acid, gallocatechin, ellagi-and gallo-tannins and saponins.

Leaves contain fatty oil and kernel contains an essential oil with mustard smell, isothiocyanate yielding glycosides, glucoputranjivin, glucocochlearin, glucojiaputin and glucocleomin.

The essential oil contains isopropyl and 2-butyl isothiocyanates as the main constituents and 2-methylbutyl isothiocyanate as a minor component.

Medicinal Uses of *Putrajiva*

- Putra jivak tree is a medicinal tree. Its leaves, fruits and stone/seeds are used sfor medicinal purpose.
- The leaves of the tree are refrigerant, analgesic, antipyretic and anti-inflammatory and used to treat fever, catarrh and sterility.
- The leaves are also used to treat allergic red pimples on the body.
- Decoction of leaves is used for treating cold, fever, and rheumatism. For swollen and inflamed joints, the leaves of the tree are used externally.
- The crushed leaves are applied to swollen throat of animals.
- The seeds are sweet, acrid, refrigerant, laxative, anti-inflammatory, aphrodisiac, and diuretic.
- The seed paste is useful against headache and powdered seed used for knee pain.
- The seeds are also known as Putrajivak beej and are procreant. These are used to treat sterility, azoospermia, and habitual abortions.
- The other conditions in which seeds are useful are diseases due to vitiation of Vata and Pitta, burning sensation, constipation, inflammations, and infertility.
- Necklace prepared from stones/nuts of fruit are wore to prevent harm. Religiously, it is believed it ward of evil forces[80-82]. Fruits as neckless are also used by pregnant women to prevent miscarriage.

5. MATERIALS AND METHODS

5.1. Solvent Extraction and Phytochemical Screening

Plant collection and extraction *Putranjiva roxburghii* were collected from the regions of ananthagiri hills vikarabad, Telengana. After that the plant parts such as leaf and bark were coarsely powdered and subjected to successive solvent extraction using soxhlet apparatus.

Phytochemical screening Qualitative phytochemical screening with the extract of both the plants *Putranjiva roxburghii* was determined as follows: Carbohydrate (Anthrone method), Alkaloids (200 mg plant material in 10 ml methanol ,filtered); a 2ml filtrate + 1%HCL + steam,1 ml filtrate+6 drops of Mayor, s reagent/Wagner, s reagent/Dragendroff reagent, creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids. Flavanoids (200 mg plant material in 10 ml ethanol, a 2 ml filtrate + conc. HCL+ magnesium ribbon pink tomato red colour indicated the presence of flavonoids. Tannins, (200 mg plant material in 10 ml distilled water, filtered): a 2ml filtrate + 2 ml FeCl3, blue black precipitate indicated the presence of tannins. Glycosides (Keller-Killani test: 2 ml filtrate+ 1 ml glacial acetic acid + FeCl3 + conc.H2SO4); green – blue colour indicted the presence of glycosides. steroids(Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered);a 2ml filtrate +2 ml acetic anhydride +conc.H2SO4. blue ring indicated the presence of terpenoids, Saponins(frothing test: 0.5 ml filtrate+ 5 ml distilled water); frothing persistence indicated presence of saponins. Anthraquinones- 2 ml of plant extracts were treated with 1 ml of dilute ammonia and shaken vigorously. Pink red colour in the ammonical layer indicates the presence of anthraquinones[83-87]. Cardiac glycosides (Keller-Killani test) were analysed. Anti microbial screening were carried out in nutrient agar media.

5.2. Phytochemical Test

Chemical tests performed in the screening and identification of phytochemical constituents in the tested medicinal plants was carried out in extracts as well as powder

5.2.1. Maeyer's reagent

0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0g of Potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

5.2.2. Dragendorff's reagent

Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions (A and B) were mixed in1:1 ratio.

5.2.3. Test for alkaloids

About 0.5 to 0.6 g of the plant extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer's and Dragendorff's).

5.2.4. Test for steroids

About 0.5 g of the extract was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid.

5.2.5. Test for terpenoids

An aliquot 0.5 ml of methanolic extract was mixed with 2 ml of CHCl3 in a test tube. 3ml of concentrated H2SO4 was carefully added to the mixture to form a layer.

5.2.6. Test for flavonoids

To the substance in alcohol, a few magnesium turnings and few drops of concentrated Hydrochloric acid were added and boiled for five minutes.

5.2.7. Test for tannins

The 0.5 g of powdered sample of each medicinal plant leaves was boiled in 20 ml of distilled water in a test tube and then filtered. The filtration method used here was the normal.

5.2.8. Test for Phytosterol

The extract (2 mg) was dissolved in 2 ml of acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulfuric acid was added along the side of the test tube.

5.2.9. Test for Phytosterol

1. Foam Test: 5 ml of the test solution taken in a test tube was shaken well for five minutes.

2. Olive oil test: - Added a few drops of olive oil to 2ml of the test solution and shaken well.

5.2.10. Test for glycosides

1.Keller -Killiani test: Added 0.4 ml of glacial acetic acid and a few drops of 5% ferric chloride solution to a little of dry extract. Further 0.5 ml of concentrated sulfuric acid was added along the side of the test tube carefully.

2. Hydroxyanthraquinone Test; To 1 ml of the extract, added a few drops of 10% potassium hydroxide solution.

5.3 Spectrophotometric Phytochemical Estimation

5.3.1 Determination of Total Alkaloid

To 1mg/ml of APDM was added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel and 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100μg/ml) were prepared in the same manner as described for the fraction (APDM). The absorbance for test and standard solutions were determined against the reagent blank at 460 nm with an UV/Visible spectrophotometer 75-80 . The total alkaloid content was expressed as mg of AE/g of extract.

5.3.2 Total flavonoids Content

A total of 1 ml of APDM was diluted with 200 µl of distilled water separately followed by the addition of 150 µl of sodium nitrite (5%) solution. This mixture was incubated for 5 minutes and then 150 µl of aluminium chloride (10%) solution was added and allowed to stand for 6 minutes. Then 2 ml of sodium hydroxide (4%) solution was added and made up to 5 ml with distilled water. The mixture was shaken well and left it for 15 minutes at room temperature. The absorbance was measured at 510 nm. Appearance of pink colour showed the presence of flavonoids content. The total flavonoids content was expressed as gallic acid equivalent mg GAE/g extract on a dry weight basis using the standard curve.

5.3.3 Total Phenolics Content

The total phenolics content of APDM was estimated using Folin-Ciocalteau reagent.About 20 µg of APDM was taken separately and it was made up to 1 ml with distilledwater. Then 500 µl of diluted Folins-phenol reagent (1:1 ratio with water) and 2.5 ml of sodium carbonate Na2CO3 (20%) were added. The mixture was shaken well and incubated in the dark for 40 minutes for the development of colour. After incubation, the absorbance was measured at 720 nm. A calibration curve of gallic acid was constructed.

5.3.4 Atomic Absorption Spectrometer (AAS) Measurement

APDM (0.5 g) was weighed using analytical weighing balance into digesting flask, 5 ml of the mixture of nitric and perchloric acid in ratio (4:1) was added, it was heated at 100°C until the solution get discoloured. On cooling, it was made up to the mark of 50 ml in volumetric flask with distilled water. The dilute filtrate solution was used for analysis of elements of interest (K, Na, Mg, Ca, Cu, Fe, Mn, Zn, Ni, Cd, Cr and Pb) by AAS (Perkin Elmer Analyst 400 Atomic Absorption Spectrometer) using suitable hollow cathode lamps 81-83 . The concentration of various elements was determined by relative method using A.R. grade solutions of elements of interest.

5.3.5 Spectroscopic Analysis

Fourier Transform Infrared (FTIR), spectra were obtained with the aid of a diamond attenuated total reflectance (ATR) accessory on an Agilent Cary 630 spectrophotometer within scanning range of 4000 to 650 cm -1.

Table 4. Photochemical test

S.No	PHYTOCHEMICAL TEST	EXTRACTS	
		Aqueous	Ethanolic
1	Alkaloids	-	-
2	Tannins	-	+
3	Flavonoids	-	-
4	Steroids	-	+
5	Phenols	+	+
6	Glycosides	+	+
7	Terpenoids	+	+
8	Anthraquinones	-	+
9	Saponins	+	-
10	Caridiac glycosides	-	-

Preparation of *Putranjiva roxburghii* leaf extract

The leaves were initially separated from the main tree and rinsed with distilled water and shade dried and then homogenized into fine powder and stored in air tight bottles. Calculated amount of powder in contrasting amount of organic solvents in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 h. And then it was filtered with the help of muslin cloth and centrifuged. The supernatant was collected and the solvent was evaporated by solvent distillation apparatus to make the final volume of one-fourth of the original volume[88-92]. It was stored at 40 °C in air tight bottles for further studies.

Qualitative Phytochemical Screening of Plant Extracts

The crude extracts of the plant were subjected to chemical tests for the identification of various active constituents as described below.

Table 4: Qualitative test protocol

S. No	Test	Methods Used
1	Alkaloids	Dragendroff's test, Wagner's test, Mayer's test
2	Flavanoid	Sulphuric acid test
3	Steroids	Liebermann Burchard test, Salkowaski test
4	Saponins	Foam Test
5	Phenolic Compounds & Tannins	Bromine Water Test
6	Fats &Oils	Sudan Red III reagent Test
7	Glycosides	Keller-killiani Test
8	Protein	Biuret test, Xanthophoretic test, Lead acetate test
9	Carbohydrates	Molisch test, Fehling test, Benedict's test
10	Amino Acids	Ninhydrin test

Animals

Healthy albino rats of either sex of 2-2½-months-old of body weight 125-150 g were housed in polypropylene cages at 25±2°C with light dark cycle of 12 h in the Animal House of the study centre are to be used for the study. It should be acclimatized for seven days. All animals are to be given with standard rat feed and water ad libitum. The experiments were performed after approval of the protocol by the minute of Institutional Animal Ethics Committee (IAEC) and animal care wastaken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute Toxicity Studies

Acute toxicity studies for the extracts were conducted as per OECD guidelines 423 using female Albino rat. Each animal was administered the extracts by oral route.

The animals were observed for mortality up to 72 hrs[93-99]. The ethanolic and aqueous extract was found to be safe up to 2000 mg/kg body weight.

High – Cholesterol Diet Model

Swiss albino rats weighing (200 – 250gm) were used for a standard experimental method as high cholesterol diet consisting of Cholesterol (1%), sodium cholate (0.5%), sucrose (30%), casein(10%), butter (5%) and standard chow diet (53.5%) for 7 days. The animals divided into three groups of control, test and standard drug treated animals. The studies conducted in two stages. In the preliminary stage effective hypolipedemic doses of test and standard drugs are worked out and in the final stage the effect of test and standard drugs are studied. The lipid profile includes total cholesterol LDL, HDL, VLDL and triglycerides were studied. The blood samples were collected after 6, 24 and 48hour of drug administration.

Group 1: Control

Group 2: Ethanolic extract

Group 3: Aqueous extract

Group 4: Standard Fenofibrate

Triton Induced Hyperlipidemic Rats

The antihyperlipidemic effects of the above extracts were evaluated in 45 triton induced hyperlipidemic rats starved for 18hours. The rats were divided into 4 Groups of 8 animals each. and then injected with Triton at a dose of 100mg/kg body weight except rats of group 1 which served as a normal vehicle treated group 2 and 3 were treated daily with a dose of 200 and 400mg/kg ethanolic and aqueous extracts respectively immediately after the Triton injection by i.p. administration[100-105]. Blood samples were collected after 6, 24 and 48 hour of Triton injection evaluates the lipid profiles.

Group 1: Control

Group 2: Ethanolic extract

Group 3: Aqueous extract

Group 4: Standard Fenofibrate

Effect on Normocholesteremic Rats

The hypolipedemic effects of the extracts were evaluated in 4 groups of Normocholesteremic rats fasted for 18hour and these studies were carried out as described for antihyperlipidemic effects the rats were treated orally for 7 days with the divided doses of 200mg/kg and 400mg/kg p.o. After the end of the stipulated period of drug treatment, all animals were starved for 20hour and blood samples were collected from the puncture of retro-orbital plexus and analysed for blood lipid profile.

Group 1: Control

Group 2: Ethanolic extract

Group 4: Standard Fenofibrate

Biochemical Analysis of Serum

Serum samples were analysed for total cholesterol, High density lipoproteins, Low density lipoproteins and very low density lipoproteins using standard enzymatic assay kit.

6. RESULTS AND DISCUSSION

The toxicity for the aqueous and ethanolic extracts leaf of *Putranjiva roxburghii* was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline No. 420) method of CPCSEA was adopted for toxicity studies. There were no sign of toxicity for first 48 hours and no animal died on 14 day of study at a dose of 2000 mg/kg.

In Vitro Antioxidant Activities

A. Superoxide Radical Scavenging Activity

Principle

The assay is based on the ability of drug to inhibit the reduction of nitro blue tetrazolium (NBT) by Superoxide, which is generated by the reaction of photo reduction of riboflavin within the system. The superoxide radical thus generated reduce the NBT to a blue colored complex.

Reagents

- Nitro blue tetrazolium (NBT) - 1.5nm (12.3mg/10ml)
- Riboflavin - 0.12µm (4.5mg/100ml)
- NaCN/EDTA - 0.0015% NaCN in 0.1M EDTA
- Phosphate buffer - 0.06M (pH 7.8)

Procedure

The reaction mixture contained EDTA (0.1 M), 0.3mM NaCN, Riboflavin (0.12mM), NBT (1.5 n moles), Phosphate buffer (67mM, pH 7.8) and various concentrations of the flower extract in a final volume of 3ml. The tubes were illuminated under incandescent lamp for 15min. The optical density at 560 nm was measured before and after illumination. The inhibition of superoxide radical generation was determined by comparing the absorbance values of the control with that of seed oil extract and fraction-IV. Vitamin C was used as positive control. The concentration of fraction-IV required to scavenge 50% superoxide anion (IC_{50} value) was then calculated.

Calculation

$$\textbf{\% inhibition} = \frac{OD\ of\ control - OD\ of\ sample}{OD\ of\ control} \times 100$$

B. DPPH Radical Reducing Activity

Principle

It is a rapid and simple method to measure antioxidant capacity. It involves the use of free radical, DPPH (2, 2- Diphenyl - 1- picrylhydrazyl) (Aquino et al, 2001). The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and is purple in color. The color turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolourisation is stochiometric with respect to the number of electrons captured.

Reagents:

- DPPH - 3mg in 25ml methanol (stored in dark bottle)
- Methanol

Procedure

Freshly prepared DPPH (187 µl) was taken in different test tubes protected from sunlight. To this solution added different concentrations (0, 25, 50, 75,100,150,200µg/ml) of seed oil extract and fraction-IV. The volume was made up to 1ml with methanol. Keep the tubes in dark and after 20 min absorbance was measured at 515nm. Methanol was used as blank and vitamin C was used as positive control. The concentration of test materials to scavenge 50% DPPH radical (IC_{50} value) was calculated from the graph plotted with % inhibition against Concentration.

Lipid Peroxidation Activity

- The stocking solutions, various working conc. were produced to get concentration of 25, 50, 75, 100, 150 & 200 µg/ml with distilled water.
- The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 25, 50, 75, 100, 150 & 200 µg/ml were prepared by distilled water.

- Potassium hydrogen phosphate (0.19 gram) was mixed with 8 gm of sodium chloride. To this 2.38 gm of disodium hydrogen phosphate was dissolved and made up to 1000 milliliter alongside DM H_2O and pH was adjusted to 7.4
- To a set of eight clean dry test tubes, 2 ml of 0.25Mm HCL containing 15% trichloroacetic acid and 0.38% thiobarbituric acid were added and to this 1 ml of different concentrations of the test extracts were added. For five minutes the sample was kept. Centrifugation was done and absorbance of the upper layer was measured at 538 nm and the lipid peroxide content was found. All experiments were performed in triplicate.

. Nitric Oxide Scavenging Method

- The stocking solutions, various working conc. were produced to avail concentration of 25, 50, 75, 100, 150 & 200 µg/ml with distilled water.
- The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 25, 50, 75, 100, 150 & 200 µg/ml were prepared by distilled water.
- Sodium nitroprusside 5mM in phosphate buffer at pH 7.4 saline was added with a range of concentrations of the test sample or standard and incubated at 25^{o}C for 150 minutes. At regular intervals, 1.5 ml of samples (incubated test sample) were taken off and a poured with 1.5 ml Griess reagent (1% Sulphanilamide, phosphoric acid (2 percent), and 0.1 percent NEDA 2.HCL.
- The absorbance was read at 540 nm. The difference in the absorbance between test and control on nitric oxide was determined and depicted as percent scavenging of NO radical.
- Capability to scavenge the NO radical was designed by using standard formula .All experiments were performed in triplicate.

Hydrogen Peroxide Method

- The stocking solutions, various working conc. were produced to get concentration of 25, 50, 75, 100, 150 & 200 µg/ml with distilled water.

- The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 25, 50, 75, 100, 150 & 200 µg/ml were prepared by distilled water.
- 1 ml of standard and test solution was added to 0.6 ml hydrogen peroxide solution. After 10 minutes the reading of the solution was read at 230 nanometer using UV/VIS spectrophotometer alongside a blank containing PBS without H_2O_2.
- The percentage scavenging of hydrogen peroxide of both plant fraction and standard compound were determined.
- The percentage inhibition was calculated to tests & standard making usage of the following formula. All experiments were performed in triplicate.

Calculation

$$\textbf{\% inhibition} = \frac{OD\ of\ control - OD\ of\ sample}{OD\ of\ control} \times 100$$

High-Cholesterol Diet Induced Rat Model

Group 1: Animals were fed with a standard diet and was given 0.9% saline once daily for 8 weeks with the aid of oropharyngeal cannula.

Groups 2: animals served as hypercholesterolemic (fed with 2% w/w pure cholesterol enriched diet) negative control[105-110].

The animals in group 3, 4 and 5 were fed with 2% w/w pure cholesterol enriched diet supplemented orally with 1 ml of the extract corresponding to 250, 500, and 1000 mg/kg per bwt (LD_{50}>2500., respectively, once daily for 8 weeks.

Group 6 were fed with 2% w/w pure cholesterol enriched diet and supplemented orally with 1 ml of gemfibrozil (100 mg/kg per bwt) once daily for 8 weeks.

Table 5. Cholesterol induced diet model

Sample	**Treatment (mg/dl)**				
Serum	Control	HC	HC+ Ethanolic Extract	HC+ Aqueous Extract	HC+ Standard (Fenofibrate)
TC	152.6 ± 2.0	219.0 ± 9.0	179.86±0.98	165.3±0.94	156.6 ± 5.2ab
TG	138.3 ± 23.0	225.0 ± 8.0	165.0 ± 7.0	142.9 ± 6.5	139.0 ± 7.0
LDL	80.9±0.27	136.±0.63	107.05±0.82	94.51±0.37	84.59±0.56
HDL	45.0 ± 2.4	38.0 ± 4.4	39.0 ± 3.1	42.3±0.56	44.2±0.17

HC- High Cholesterol; GMF- Gemfibrozil; TC-total cholesterol; TG-triacylglycerol; LDL- low density lipoprotein; HDL- High density lipoprotein

Table 6. Blood parameters

Sample	**Treatment (mg/kg/b.wt)**				
Serum	Control	HC	HC+ Ethanolic Extract	HC+ Aqueous Extract	Standard (GMF)
RBC (x 10^6/Ml	7.56±0.33	5.64±0.17	6.11±0.34	6.89±0.45	7.34±0.48
Hb (g/dl)	12.6±0.46	8.56±1.76	10.55±0.58	13.22±0.78	13.03±0.56
PCV (%)	38±1.56	29.33±0.77	36.3±1.43	35.89±0.77	36.77±0.34
MCV (pg)	91±0.34	70.33±0.87	90.4±0.47	89.83±0.29	92.38±0.58
MCH (pg)	29.30±0.56	25.14±0.46	26.31±1.87	27.37±0.63	30.62±1.20
MCHC (g/dL)	30.34±0.87	26.95±1.56	30.34±0.56	29.57±0.33	31.56±0.45
WBC (x 10^3/ µL	6.97±1.56	9.97±0.04	9.22±0.56	9.12±0.54	7.59±0.53
Neutrophils (%)	54.36±1.20	34.70±0.42	40.89±0.76	45.35±0.78	50.64±0.74

Monocytes (%)	6.00±2.03	4.99±0.13	5.68±0.57	6.07±0.67	5.43±0.66
Lymphocytes (%)	29.30±0.78	54.0±0.56	48.87±0.56	39.40±0.41	32.67±0.62
Eosinophils (%)	1.90±0.03	2.03±0.32	1.67±0.79	2.05±0.55	1.78±0.21
Basophils (%)	0.24±0.04	0.35±0.23	0.29±0.13	0.25±0.54	0.23±0.50
Platelets ($x10^3/\mu L$	325±1.56	647.0±0.45	415.0±0.58	392.0±0.82	341.4±0.16

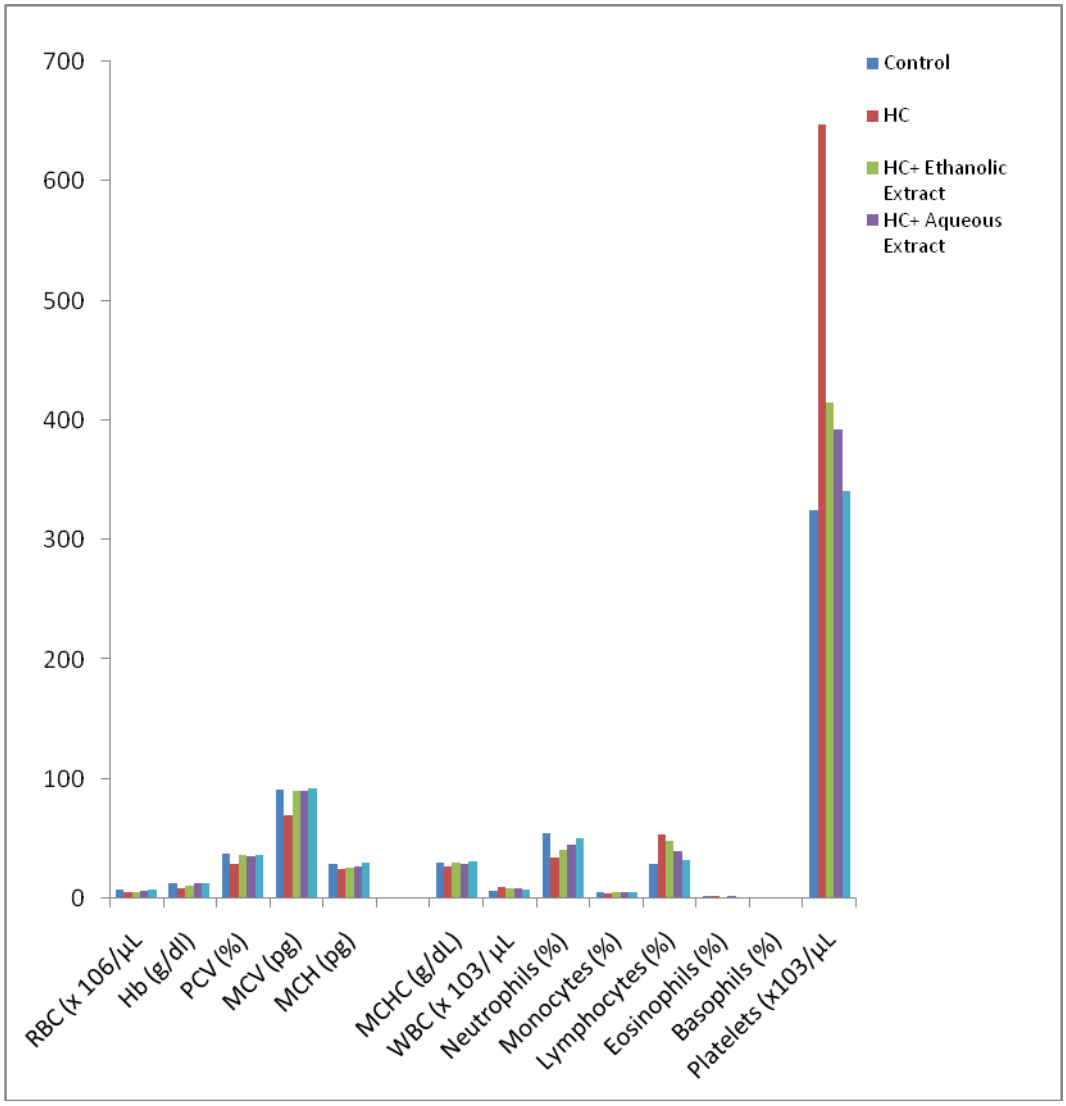

Figure 9: Haematological Parameters

Triton Induced Rat Model

Chemicals

Triton WR-1339 (A non-ionic detergent, Isooctyl poly oxyethylene phenol) was obtained from Sigma Chemicals Co, Mumbai.

Procedure

Eight week old adult male albino rats of *Wistar* strain, weighing approximately 150 to 200 g, were acclimatized for 7 days at room temperature (22±2ºC) and humidity of 45-64% in a 12- hour light/dark cycle in a room under hygienic condition. They were given access to water and a commercial diet *ad libitum*. The experiments were carried out in the Suran labs, Hyderabad, as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (IAEC).

Induction of hyperlipidaemia

Hyperlipidaemia was induced in *Wistar* rats by intraperitoneal (i.p) injections of Triton WR-1339 at a dose of 400 mg/kg body weight. After 72 hours of triton injection received a daily dose of 5% CMC in 5ml/kg body weight for 7 days.

Experimental design

In the experiment, the rats were divided into three groups of eight rats each. Group I rats received 5% CMC and considered as controls, Group II rats were treated with Triton WR-1339 (400 mg/kg body weight with Ethanolic extract) and Group III rats were treated with Triton WR-1339 (400 mg/kg body weight with aqueous extract) and ethanolic extract of *Putranjiva roxburghii* leaf (200mg/kg body weight) and Standard fenobirate (100mg/kg body weight).

At the end of 8th day, rats were fasted overnight and sacrificed by cervical dislocation. Blood was collected, and serums were separated by centrifugation. Liver tissues were excised immediately and rinsed in ice-chilled normal saline, 500mg of the tissues were homogenized in 5.0 ml of 0.1 M Tris–HCl buffer (pH, 7.4). Biochemical estimations were carried out in serum and liver tissues, parameters such as cholesterol (Zak's, 1977), phospholipids (Rouser *et al*., 1970), triglycerides (Rice,

1970), LDL (Friedwald Levy and Frederickson, 1972), VLDL (Henry *et al*., 1998) and HDL (Varley *et al*., 1980) were analyzed.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnnet t test using Graph Pad prism software package 9.05. Results were expressed as mean ± SD from 8 rats in each group. *P* values <0.05 were considered as significant.

Table 7: Effect of *Putranjiva roxburghii* on changes in the levels of cholesterol and phospholipids in serum and liver tissue of control and experimental animal

Groups	Cholesterol		Phospholipids	
	Serum	Liver	Serum	Liver
Control	078±2.12	071±1.85	052±0.45	083±1.87
Ethanolic extract+Triton (200 mg/Kg)	098±0.33	097±0.54	064±0.58	092±0.33
Aqueous extract+ Triton(400 mg/Kg)	084±0.87	077±0.33	056±0.67	088±0.76
Standard Fenofibrate	078±0.23	072±1.59	051±1.62	085±1.62

Each value is mean ± SD for eight rats in each group, one way ANOVA followed by Dunnett's test.

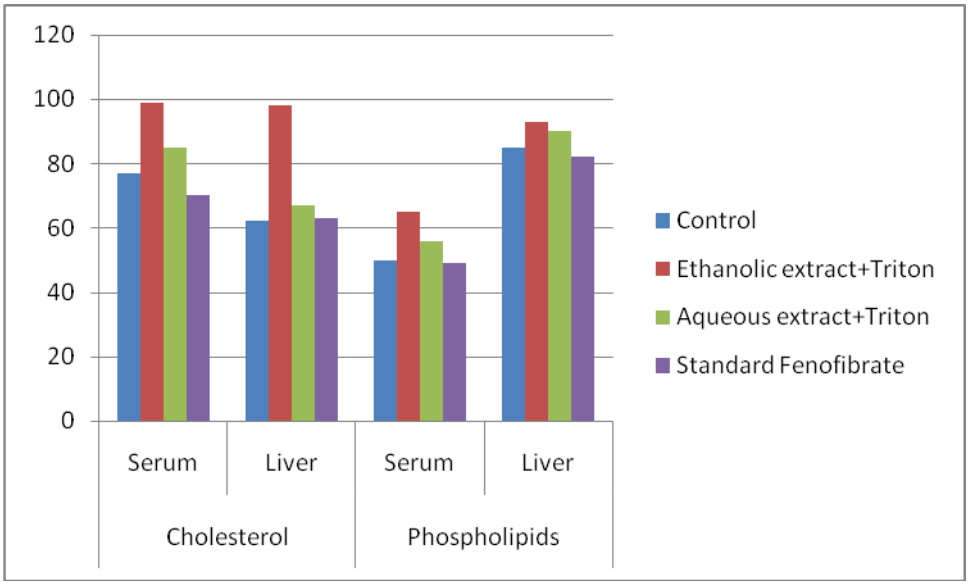

Figure 10: Scheme of Effects of Cholesterol and Phospholipids

Table 8: Effect of *Putranjiva roxburghii* on changes in the levels of triglycerides and LDL in serum and liver tissue of control and experimental animal

Groups	Triglycerides		LDL	
	Serum	Liver	Serum	Liver
Control	075±1.98	061±2.00	038±1.44	021±0.76
Ethanolic extract+Triton	096±0.65	093±0.78	065±0.39	035±0.58
Aqueous extract+Triton	088±0.57	064±1.98	060±1.52	030±1.77
Standard Fenofibrate	069±2.01	058±0.73	035±0.42	018±0.36

Each value is mean ± SD for eight rats in each group, one way ANOVA followed by Dunnet t test.

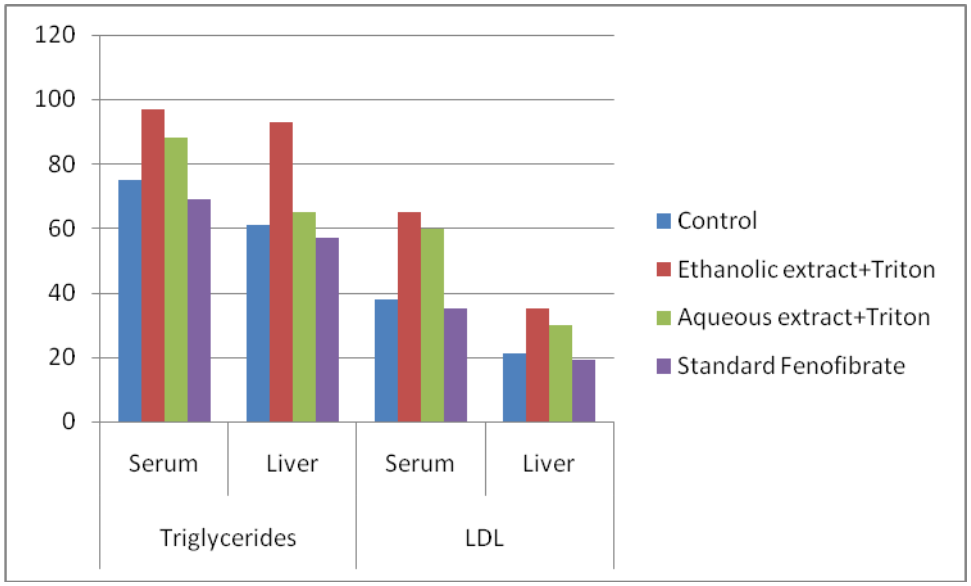

Figure 11: Scheme of Effects of triglycerides and LDL

Table 9: Effect of *Putranjiva roxburghii* on changes in the levels of VLDL and HDL in serum and liver tissue of control and experimental animal

Groups	VLDL		HDL	
	Serum	Liver	Serum	Liver
Control	016±2.60	013±2.05	039±1.62	030±0.24
Ethanolic extract+Triton	015±0.55	014±0.73	045±1.56	036±0.56
Aqueous extract+Triton	014±1.83	011±0.56	055±0.47	030±0.35
Standard Fenofibrate	012±0.92	010±0.37	057±1.83	047±0.28

Each value is mean ± SD for eight rats in each group, one way ANOVA followed by Dunnett's test.

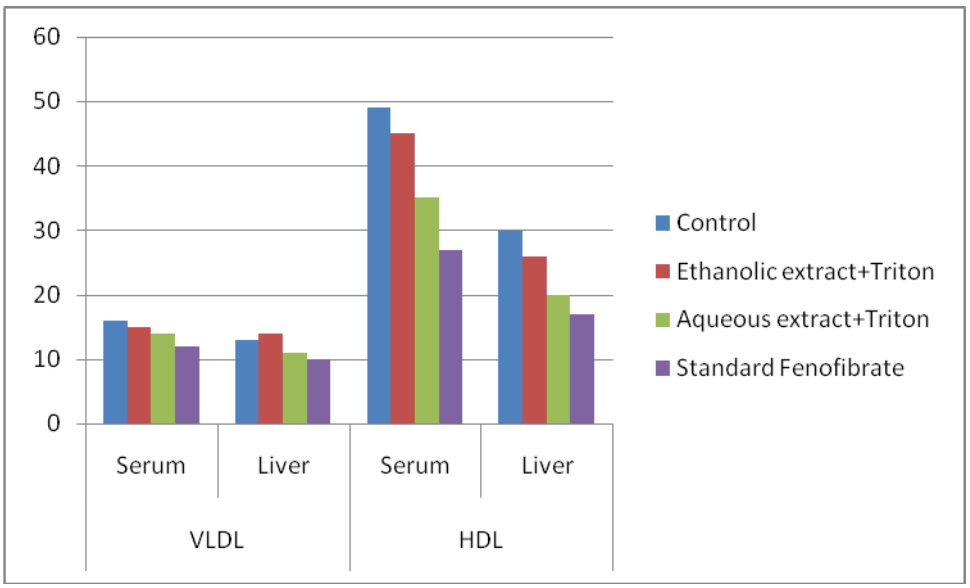

Figure 12: Scheme of Effects of VLDL and HDL

EFFECT ON NORMOCHOLESTEREMIC RATS:

The hypolipidemic effects of the extracts were evaluated in 4 groups fasted for 18 hours and these studies were carried out as described for antihyperlipidemic effects. The rats were treated orally for 7 days. After the end of the stipulated period of drug treatment, all the animals were starved for 20 hours and blood samples were collected from the puncture of retro-orbital plexus and analyzed for blood lipid profile.

Table 10. Effect of the extracts on blood lipid profile

Groups	Blood lipid profile			
	Cholesterol	**Triglycerides**	**HDL**	**LDL**
Control	149.6 ± 2.0	221.0 ± 9.0	79.86±0.98	85.3±0.94
Ethanolic extract	136.3 ± 23.0	227.0 ± 8.0	87.0 ± 7.0	122.9 ± 6.5
Aqueous extract	81.9±0.27	137.±0.63	104.05±0.82	94.51±0.37
Standard Fenofibrate	46.0 ± 2.4	39.0 ± 4.4	129.0 ± 3.1	86.3±0.56

In statistical analysis the extract treated groups have been compared with their respective control. P<0.01 (ANOVA followed by Dunnett's t-test)

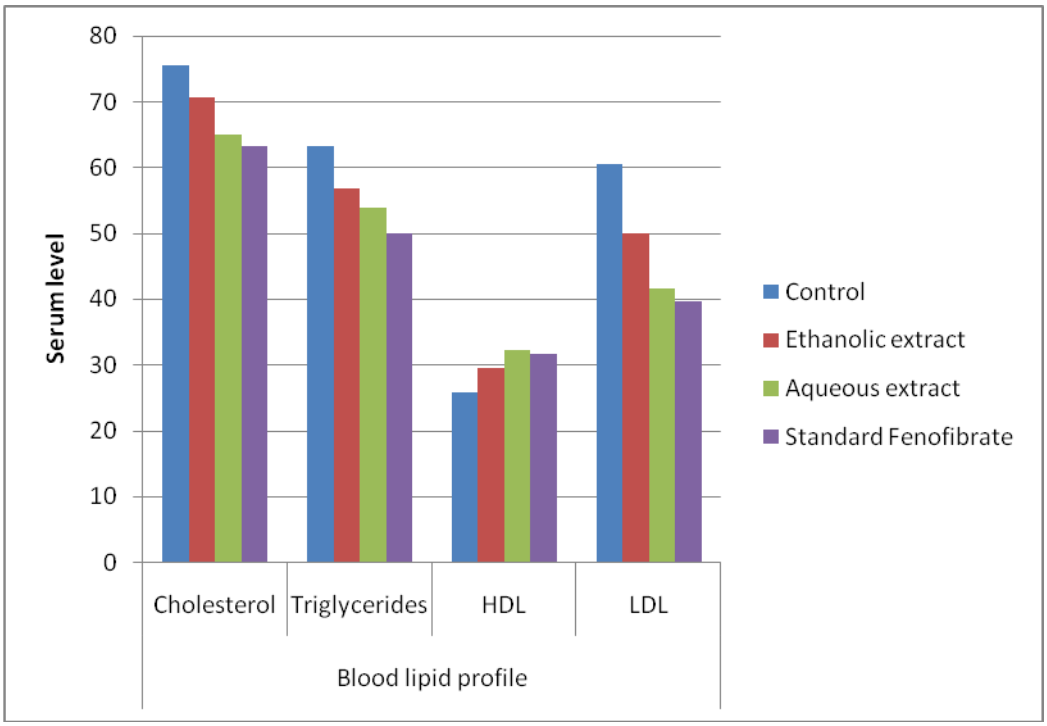

Figure 13: Scheme of Blood lipid profiles

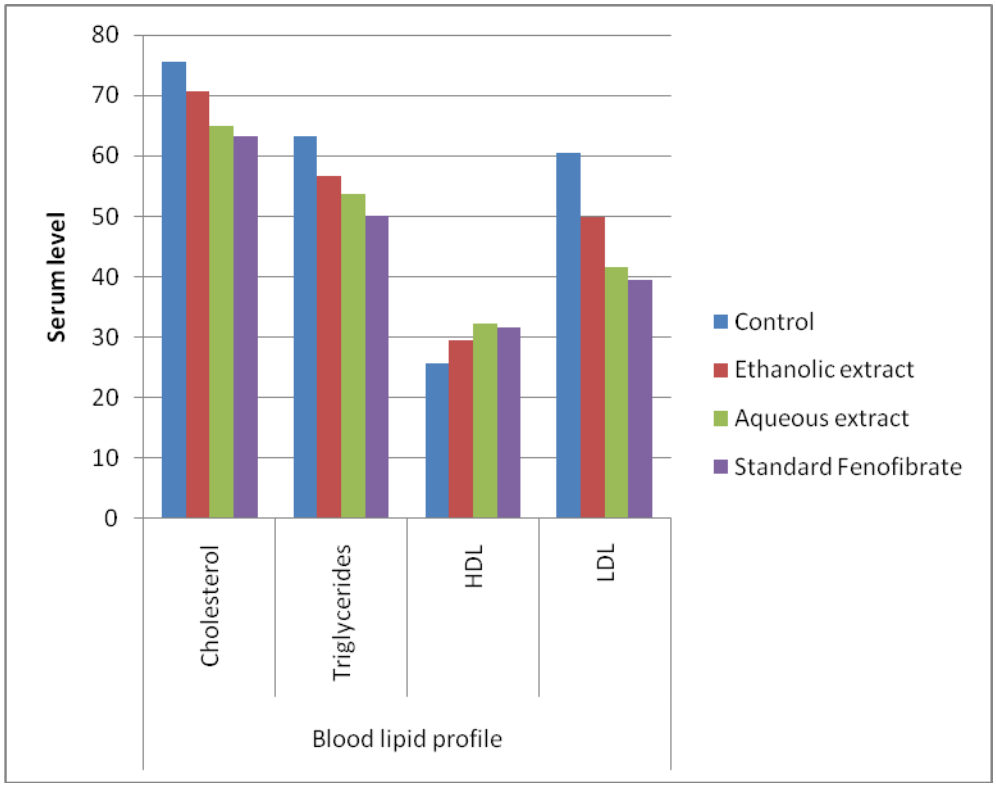

Figure 14: Scheme of blood lipid profiles

OUTCOMES

The antihyperlipidaemic and antioxidant activity of the plant leafextract is studied and the significance is evaluated.

Aqueous Extract of the taken sample was found to be best.

Table 11. Superoxide Radical Scavenging activity

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	73.50±0.70	46.46±0.34	24.23±1.45
50	77.63±3.12	56.47±0.86	38.01±0.34
75	82.33±0.96	65.00±0.87	48.44±0.14
100	85.93±0.79	70.23±1.46	59.36±1.11
150	93.30±0.02	73.56±1.76	68.36±1.08
200	94.54±0.06	75.22±0.60	70.57±0.59

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibition

All the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples.

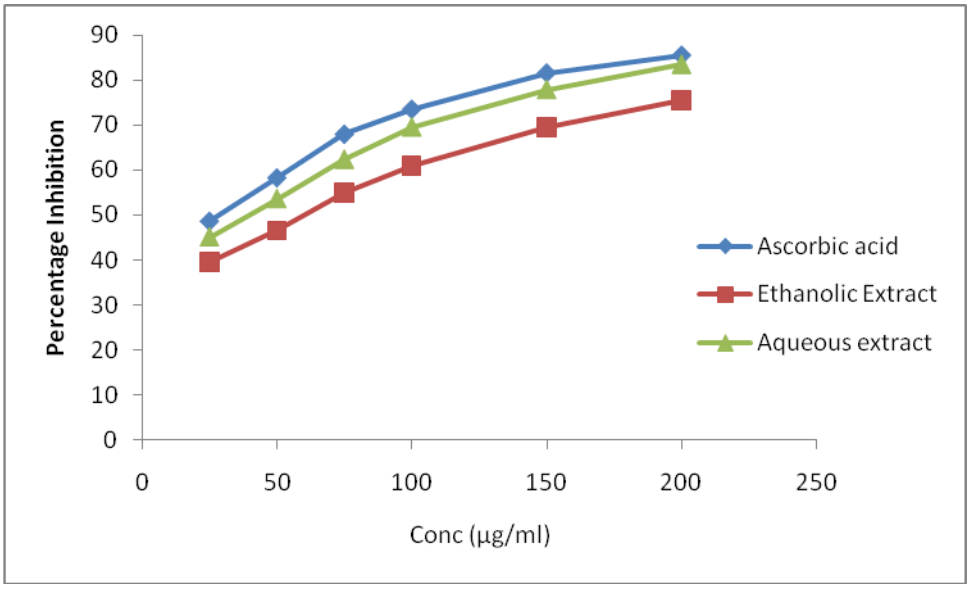

Figure 15:. Schematic representation of superoxide radical scavenging activity of all the extracts

Table 12. DPPH Assay

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	67.20±0.22	62.25±0.29	66.24±0.90
50	71.44±0.57	63.54±0.62	68.52±0.13
75	77.22±0.67	64.22±0.56	72.44±2.76
100	79.44±0.29	68.77±0.56	77.34±1.56
150	88.47±1.05	75.24±2.33	85.59±1.99
200	93.02±0.02	77.23±0.24	88.33±1.97

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibition

All the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples.

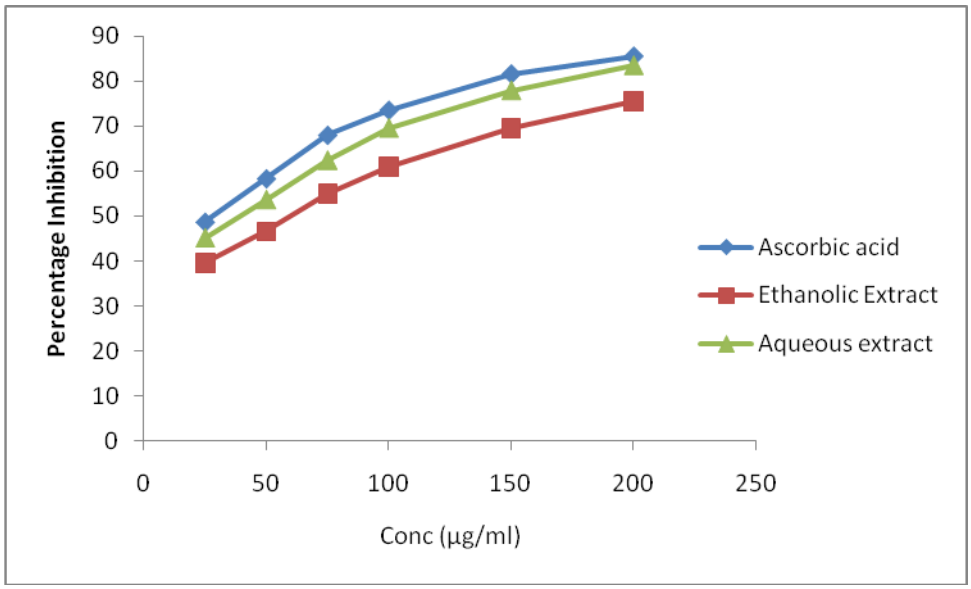

Figure 16: Schematic representation of DPPH activity of all the extracts

Table 13. Lipid per oxidation Assay

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	69.52±2.97	45.33±3.45	54.55±1.36
50	74.56±1.93	53.34±1.25	61.50±1.50
75	81.47±0.48	61.45±1.34	69.45±1.78
100	84.32±1.62	67.77±1.36	74.38±0.56
150	88.52±0.35	74.28±3.46	83.67±1.91
200	89.67±0.46	78.54±0.87	86.33±0.73

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibition

All the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples.

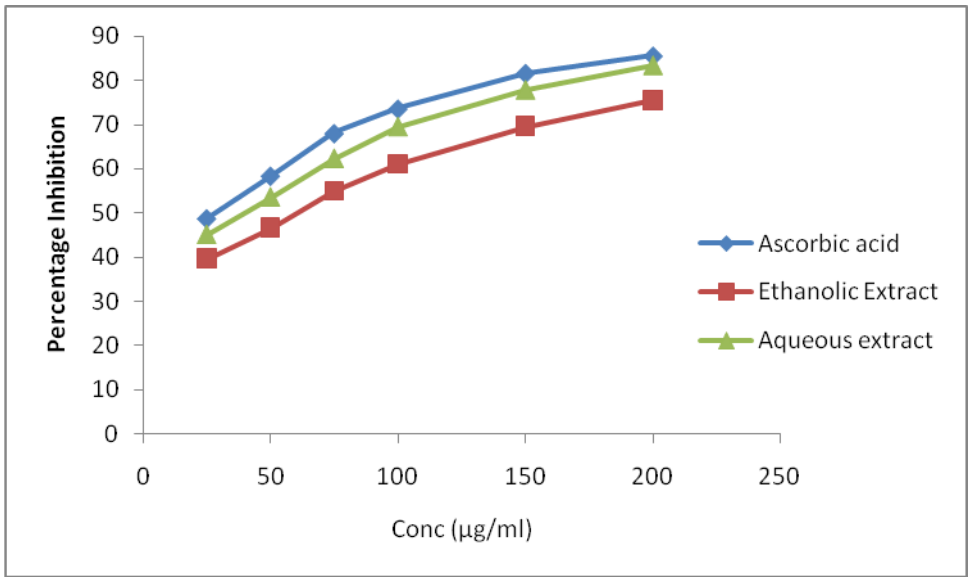

Figure 17: Schematic representation of Lipid per oxidation Assay of all the extracts

Table 14. Nitric oxide scavenging assay

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	43.50±0.62	30.62±0.56	35.33±2.56
50	55.36±1.57	36.57±0.33	49.44±0.45
75	68.22±0.32	42.67±1.56	60.67±0.77
100	80.12±0.66	50.28±1.36	72.53±1.32
150	91.56±0.23	60.12±0.38	87.62±1.66
200	95.33±2.34	69.88±0.34	93.44±0.65

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibition

All the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples.

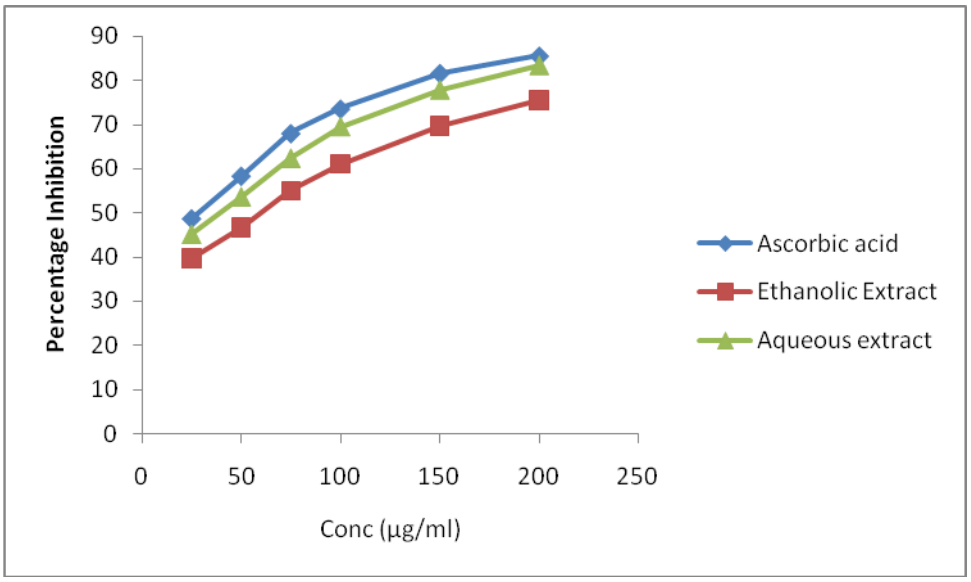

Figure 18: Schematic representation of Nitric oxide scavenging assay of all the extra

Table 15. Hydrogen per oxide assay

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	48.66±0.22	39.56±0.34	45.09±0.42
50	58.32±0.68	47.58±1.26	54.60±1.52
75	67.99±1.66	55.88±1.62	62.36±1.67
100	74.56±0.12	60.97±1.42	69.56±0.79
150	81.62±0.26	69.49±1.31	76.93±1.74
200	85.57±0.12	75.45±0.38	84.49±0.57

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibition

All the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples.

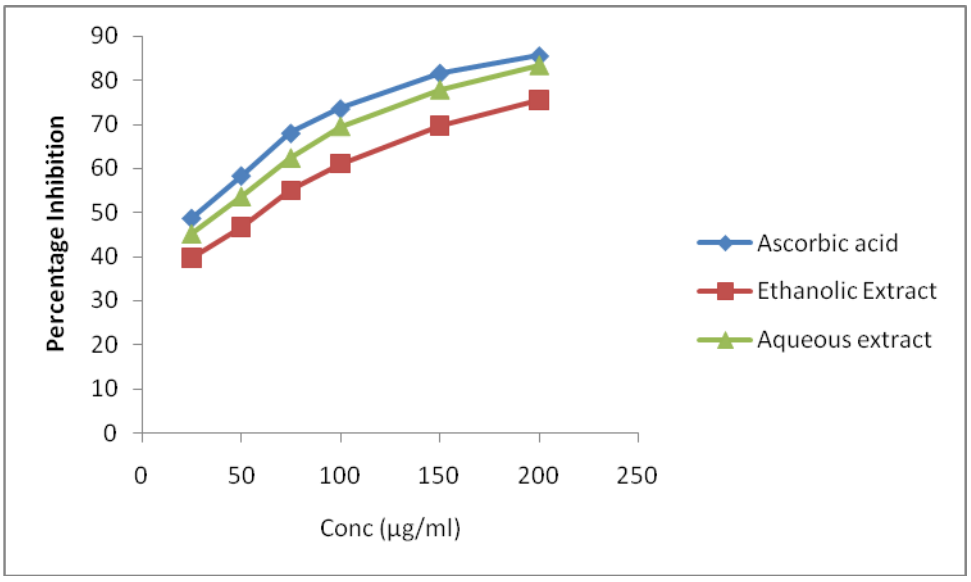

Figure 19: Schematic representation of Hydrogen peroxide assay of all the extracts

7. CONCLUSION

In this current approach, we have selected *Putranjiva roxburghii*, where its different part of plants has been in exploitation since ancient times for various benefits, including inflammation, constipation, and it has been that, in our current approach we have been investigating on the aspect and perspective of hypolididemic activity. It has been observed from our experimental research, there has been a remarkable and significant activity observed, against the anti-hyperlipidemic activity. Futuristic scope of this study will be extended to investigation and isolation of the specific components responsible for antihyperlipidemic activity.

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