Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(4); 456-465
DOI number: 10.25258/phyto.v9i2.8115
ISSN: 0975-4873

Research Article

Evaluation of Antioxidant and Antihyperhipidemic Activity of *Pterospermum acerifolium* (Sterculiaceae) *In Vitro*

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Received: 13th Jan, 17; Revised 16th March, 17, Accepted: 10th April, 17; Available Online:25th April, 2017

ABSTRACT
The objective of present research work was to determine the antioxidant and antihyperlipidemic activity in the leaves of *Pterospermum acerifolium* belonging to family Sterculiaceae. The phytochemical analysis of leaves of *Pterospermum acerifolium* showed the presence of anthraquinones, cardiac glycosides, flavonoids, saponins and tannins etc. Oxidative compounds are responsible for prognosis of many diseases like Alzheimer's disease, ischemic heart disease, Parkinson disease, hyperlipidemia and atherosclerosis. Antioxidants are the compounds responsible for curing such ailments that are formed by oxidative stress to the cells. Antioxidant activity of bark of *Pterospermum acerifolium* was determined by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH). Ascorbic acid and finofibrate was used as standard drug and antihyperlipidemic activity was determine by computer-assisted high-performance liquid chromatography and determine the various types of lipoprotein like LDL, VLDL and HDL.

Keywords: Hyperlipidemia, Antioxidant, *Pterospermum acerifolium*.

INTRODUCTION

Cholesterol levels have become the source of health concerns, even though cholesterol is one of the most important substances in the human body. In past, the amount of cholesterol information and dietary advice bombarding the public has grown exponentially[1].It is a highly prognostic risk factor for atherosclerosis, coronary artery disease, and cerebral vascular diseases[2]. hyperlipidemia is usually defined as conditions in which the concentration of triglyceride or cholesterol-carrying lipoproteins in plasma exceeds an random normal limit[3]. Lipids have been considered as "fats" in the bloodstream, which is commonly divided into cholesterol and triglycerides[4]. These lipoproteins deposit in the interstitial space of arteries arising from aorta, restricting the blood supply to the heart. This phenomenon is known as atherosclerosis. Atherosclerosis (sclero-hardening) of arteries is a disease of the arterial network known as a progressive and still killer disease characterized by the development of lesions called atherosclerosis plaques in the walls of coronary arteries and which reduces blood flow to the myocardium known as coronary artery disease (CAD)[5]. Many factors contributing to its etiology, for example diabetes, glucocorticoids, diet, psychological factors are the major one[6].

It is characterized by elevated serum total cholesterol and low density lipoprotein and very low density lipoprotein cholesterol and reduce high density lipoprotein are the risk factor for coronary heart diseases. Hyperlipidemia - primary and a secondary type indicate the complexities related with disease. Primary disease may be treated with anti-lipidemic drugs but the secondary type originating from diabetes, renal lipid nephrosis or hypothyroidism demands the treatment of original disease rather than hyperlipidemia.

Utilization of much fat may lead to the production of large amount of VLDL, resulting in the formation of excess amounts of LDL which may stick to the walls of the blood vessels if the quantity of HDL is inadequate, causing blockages for the ordinary flow of blood. Therefore, it is perfection in human diet is highly recommended for disease prevention[6].The major aim of treatment in patients with hyperlipidemia is to reduce the risk of rising ischemic heart disease or cardiovascular or cerebrovascular disease[7].

Oxygen is essential for life. Human body consume oxygen for the production of energy by metabolism of biomolecules. The metabolic pathways also produce reactive oxygen species (ROS) like superoxide (O3-), hydrogen peroxide and hydroxyl radical (OHº). ROS have been reported to act as second messengers causing signal transduction in many systems[8]. These free radicals and ROS produced in sufficient amount in the body. An redundant production of these ROS leads to oxidative stress and cause cellular and sub cellular damage by peroxidation of lipid membranes, denaturing of proteins, opening of DNA strands and unruly of cellular functions. Endogenous antioxidants such as glutathione peroxidase, α- tochopherol, superoxide dismutase and melatonin are synthesized within human body to defend the cells from oxidative stress induced by ROS and free radicals. As antioxidant is a molecule capable of slowing or p reventing the oxidation of other molecules[9]. In pathological conditions endogenous antioxidants are

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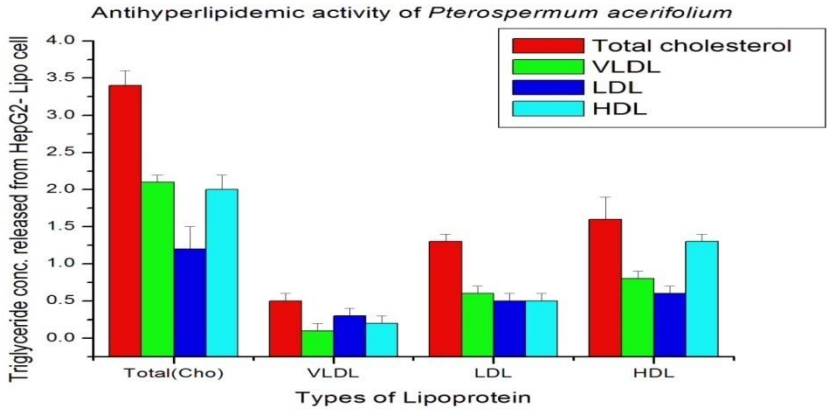


Figure 1: Time course of Total triglyceride release from HepG2-Lipo cells. Following HepG2-Lipo cell preculture in DMEM containing 10% FCS, the cells were cultured in serum-free DMEM containing 0.1% BSA, and the total triglyceride levels, VLDL, LDL and HDL were assessed. Data represent mean ± SD (n = 4).

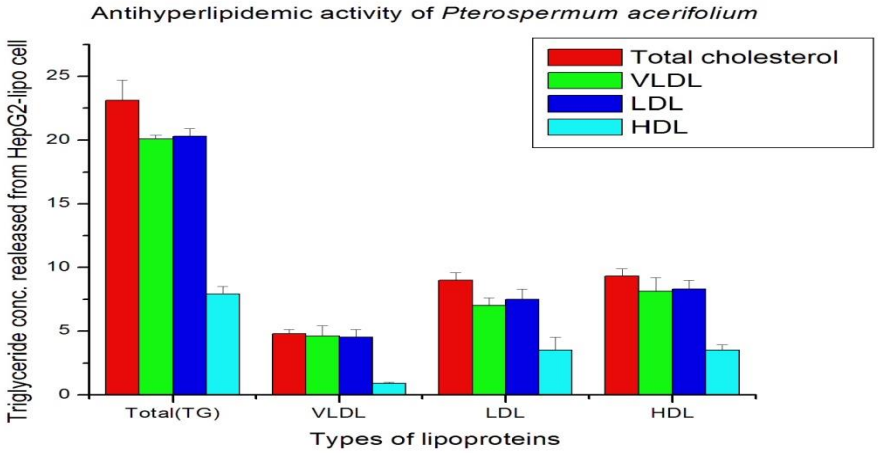


Figure 2: Time course of Total cholesterol release from HepG2-Lipo cells. Following HepG2-Lipo cell preculture in DMEM containing 10% FCS, the cells were cultured in DMEM containing 0.1% BSA, and the total cholesterol levels, VLDL, LDL and HDL were assessed. Data represent mean ± SD (n = 4).

inadequate to fight with ROS. This lead to the way that disease progression and oxidative damage to cells may be retarded by supplement endogenous antioxidant by exogenous antioxidants obtain from plants. In plants, antioxidants are phenolic compounds that vary from simple phenolic acids to highly polymerized compounds like tannins. These compounds help in continued existence of plants and also encourage health in humans[10].

Endogenous and exogenous antioxidants function as free radical scavengers and improve human health. Antioxidant inhibit the production of free radicals involved in pathogenesis of many diseases like Parkinson's disease, Alzheimer's disease, atherosclerosis, ischemic heart disease and aging process.

Many medicinal plants contains large number of antioxidants that play a vital role in absorbing and neutralizing free radicals, quenching super oxides or decomposing peroxides[10].

MATERIALS AND METHODS

Collection and Authentication of plant

Fresh leaves of *Pterospermum acerifolium* (Sterculiaceae family) collected in the month of May 2016 from Sec-12, town park, Faridabad, Haryana. The leaves were dried in shade to avoid the deterioration of phytoconstituents and formed a coarse powder by using a grinder The powdered leaves were packed in air bags and stored in air tight containers until use.

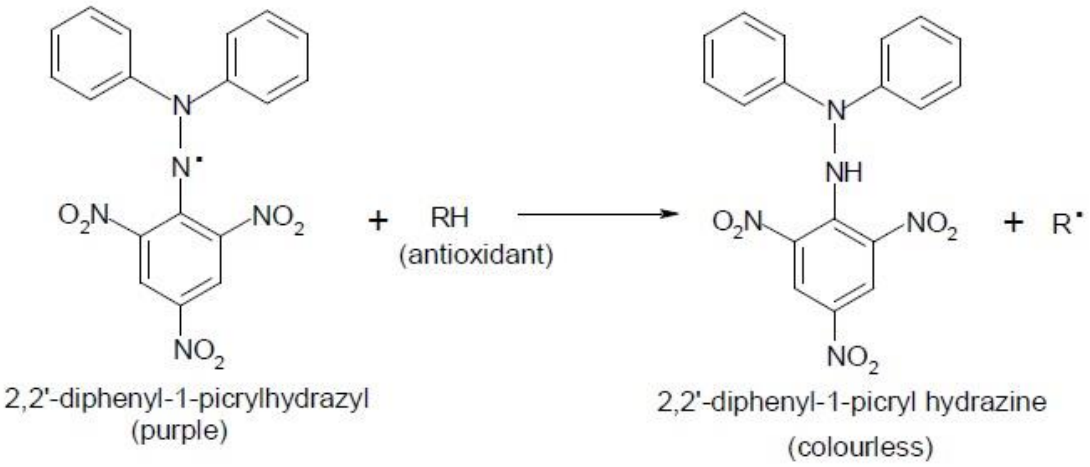
The botanical identity was confirmed by department of herbarium, University of Rajathan, Jaipur, Rajasthan.

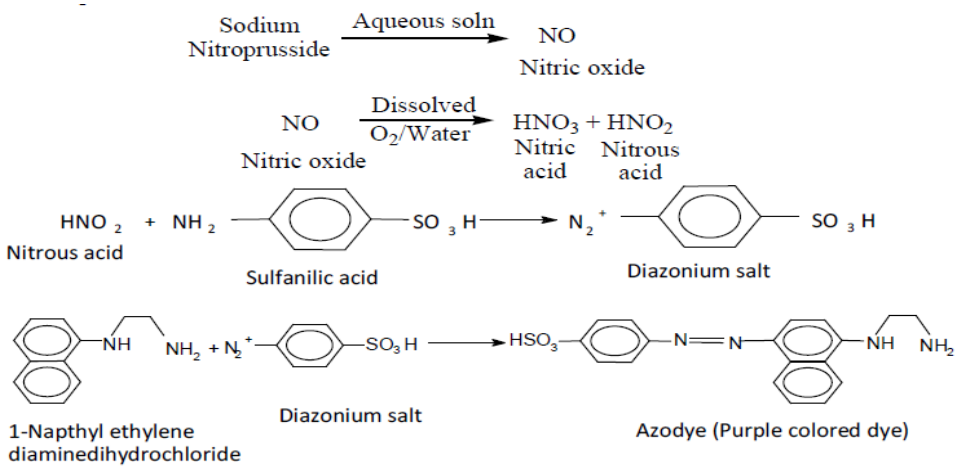
Extraction of Plant Material

About 1.5 kg of shade dried powder of leaves of *Pterospermum acerifolium* was extracte out with petroleum ether, Chloroform, Ethyl acetate and ethanol to obtain the petroleum ether, Chloroform, Ethyl acetate and ethanol extract. The crude ethanol extract, after removal of the solvent, was dissolved in 10% sulfuric acid solution and partitioned with chloroform, ethyl acetate, and water successively to give chloroform, ethyl acetate, and water soluble fractions respectively.

Preliminary Physio-chemical and Phytochemical screening

physio chemical tests like total ash value, acid insoluble ash, water soluble ash and sulphate ash were determined Preliminary tests were done for the absence or presences





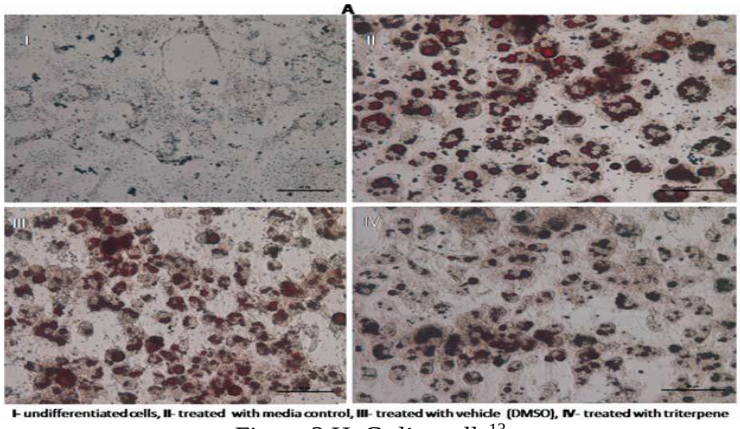


Figure 3 HpG_2 lipocells[13]

of phytoconstituents like Alkaloids, Flavonoids, Glycosides, Saponins, Sterols, Terpenes and Tannins[11].

In-Vitro Antioxidant activity

Chemicals and Reagents

All chemicals and reagents were used of analytical grade includes DPPH (diphenyl picryl hydrazyl), petroleum ether, methanol, choloroform, ethyl acetate, n-butanol , water, and sodium carbonate, Ascorbic acid, Deoxy-D-ribose, thiobarbutyric acid (TBA), nicotinamide adenine dinucleotide (NADH), nitrobluetetrazolium (NBT),

Table 1: Extractive value of Pterospermum acerifolium

S. no	Parameters	% Yield
1	Total ash	4.67
2	Acid insoluble ash	0.33
3	Water soluble ash	2.67
4	Sulphate ash	1.50
5	Moisture content	8.44

butylated hydroxyl anisole (BHA). nitrogen and N_2O were used for degasing the samples. Water was used for the

Table 2: Percentage yield of Pterospermum acerifolium i.e Leaves.

S. no	Extracts	Wt. of Extract (gm)	Wt. of crude drugs(gm)	% Yield
1	Ethanol	32	300	10.6%
2	Petroleum ether	28	300	9.33%
3	Chloroform	27	300	9.00%
4	Ethyl acetate	26	300	8.66%

Table 3: Phytochemical screening of Pterospermum acerifolium leaves.

Test for	Petroleum ether extract	Cholorofo rm Extract	Ethyl acetate extract	Ethanol
Alkaloids	+	+	-	+
Carbohydrates	-	-	-	-
Glycosides	-		+	+
Tannins phenolic compound	_	_	+	+
Test for triterpenoid	_	_	_	_
Gum and mucilage	_	_	_	+
Flavonoids	-	-	+	+
Saponin	-	-	+	-
Steroids	-	-	-	-

preparation of solutions and all the solutions were prepared fresh[12].

DPPH Method DPPH scavenging activity was calculated by the spectrophotometric method. A stock solution of DPPH (1.5 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml of methanol. Decrease in the absorbance at different concentration (10-125 µg/ml) of sample extract after 15 min. From % inhibition IC50 was calculated [10,12]

Procedure for DPPH Free Radical Scavenging Activity

Preparation of stock solution of sample:-

10 mg of extract was dissolved in 10 ml of methanol to obtain 1000 µg/ml solution.

(1) Dilution of test solution: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml solution of test were prepared from stock solution.

(2) Preparation of DPPH solution: 15 mg for DPPH was dissolved in 10 ml of methanol. Then solution was covered with aluminium foil to protect from light.

(3) Estimation of DPPH scavenging activity: 75 µl of DPPH solution was in use and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75 µl of DPPH and 100 µl of the test sample of different concentration were placed in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. At zero time absorbance was taken in UV-Visible at 517 nm for each concentration. Decrease in absorbance of DPPH with sample of different concentration was measured after 15 minute at 517 nm[10].

% inhibitions of DPPH radical by test compound were calculated by the following formula:-

% Reduction = Control absorbance – Test absorbance/ Control absorbance*100

Hydrogen peroxide scavenging assay

Hydrogen peroxide is generated by several oxidase enzymes. There is facts that hydrogen peroxide, either directly or indirectly via its reduction produces hydroxyl radical cause severe damage to biological systems. when a scavenger is incubated with hydrogen peroxide, the loss of hydrogen peroxide can be determined spectrophotometrically at 230 nm[12].

Preparation of phosphate buffer saline (pH 7.4)

Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8 g of sodium chloride in adequate water to produce 1 l, PH was adjusted if necessary with 0.1N HCl/NaOH.

Preparation of Hydrogen peroxide (20 mM)

Hydrogen peroxide (20 mM) was prepared by mixing 3.4 ml H_20_2 with phosphate buffer (pH 7.4) and made up to 100 ml.

Preparation of extract and standard solutions

Accurately weighed 30 mg of the extracts and standard, dissolved separately in 10 ml of methanol. These solutions were diluted with methanol to obtain the lower dilutions.

Procedure

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various

Table 4: IC_{50} of ascorbic acid against DPPH.

S. no	Group	Concentration (µg/ml)	Absorbance (700nm)	% Inhibition
	Control STD (Ascorbic acid)		0.9873±0.002	-
1.		5	0.8362±0.001	15.30
2.		10	0.7457±0.001	24.47
3.		20	0.6747±0.001	31.66
4.		30	0.4583±0.001	53.58
5.		50	0.2077±0.003	78.96
	TEST (PAET)			
1.		5	0.8460±0.001	14.31
2.		10	0.7747±0.002	21.53
3.		20	0.6457±0.001	34.60
4.		30	0.4898±0.002	50.39
5.		50	0.2861±0.001	71.02
	TEST (PAEA)			
1.		5	0.8761±0.006	11.26
2.		10	0.8113±0.004	17.82
3.		20	0.6876±0.001	30.36
4.		30	0.5487±0.001	44.42
5.		50	0.3155±0.001	68.04

concentrations (100 to 800 ug/ml) of 1 ml of the extract or standard in methanol were added to 2 ml of hydrogen peroxide solution in phosphate buffer saline. After 10 min, absorbance was measured at 230 nm[10,12].

Nitric oxide radical inhibition assay

Sodium nitroprusside spontaneously generates nitric oxide at physiological pH, in aqueous solutions. Nitric oxide, generated in this manner it is converted into nitrous and nitric acids on contact with dissolved oxygen and water. The liberated nitrous acid is determined by using a modified Griess-Illosvoy method. Nitrous acid reacts with Griess reagent, which forms a purple azo dye. In the presence of test components, likely to be scavengers, the amount of nitrous acid will decrease. Decrease in the formation of purple azo dye will imitate the extent of scavenging. The absorbance of the chromophore formed is measured at 540 nm[12].

Preparation of Sodium nitroprusside (10 mM)

Weighed accurately 0.3 g of sodium nitroprusside and dissolved in distilled water to make up the volume upto 100 ml.

Preparation of Naphthyl ethylene diaminedihydrochloride (NEDD, 0.1%)

Weighed 0.1 g of NEDD and dissolved in 60 ml of 50% glacial acetic acid by heating and make up the volume upto 100 ml in a volumetric flask with water.

Preparation of Sulphanilic acid (0.33%) reagent

Accurately weighed 0.33 g of sulphanilic acid and dissolved in 20% glacial acetic acid by heating and make the volume upto 100 ml in a volumetric flask.

Preparation of extract and standard solutions

The extracts and the standard (21 mg each) were separately dissolved in 1 ml of freshly distilled DMSO. These solutions were diluted separately to obtain the lower concentrations (range: 20 to 1000 µg/ml) to find out 50% NO scavenging activity (IC50).

Procedure

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), PBS (pH 7.4, 1 ml) and for 150 min, extract or standard solution were incubated at room temperature. After incubation, remove 0.5 ml of the reaction mixture containing nitrite ion and add 1 ml of sulphanilic acid reagent, mixed well and allow to stand for 5 min for end of diazotization. Then, 1 ml of NEDD was added, mixed and permissible to stand for 30 min in diffused light. A pink colored chromophore was produced. The absorbance was measured at 540 nm[10,12].

Antihyperlipidemic activity

Chemicals

fenofibrate was purchased from Hema Pharmaceuticals Pvt. Ltd. Gujrat.

Cell culture

A human hepatoma cell line, HepG2, was obtained from RIKEN Cell Bank. The HepG2-derived subclone (HepG2-Lipo) which secretes high levels of cholesterol and TG, was selected from HepG2 cells by measure the amounts of cholesterol and triglycerides in the culture medium as an indicator. Maintain the cells in Dulbecco's modified Eagle's medium containing 10% (v/v) FCS and antibiotics[13].

Lipoproteins secreted from hepatoma cells

Hepatoma cells (105 cells) were seeded and precultured in DMEM containing 10% (v/v) FCS in 24-well plates for 2 days. Washed the cells twice with PBS, and incubated in

Table 5: IC_{50} of ascorbic acid against H_2O_2.

S.no.	Group	Concentration (µg/ml)	Absorbance (700 nm)
	STD (Ascorbic acid)		
1.		10	0.0464±0.001
2.		20	0.1670±0.002
3.		30	0.2722±0.008
4.		40	0.3665±0.001
5.		50	0.5205±0.002
	TEST (PAET)		
1.		50	0.0078±0.001
2.		100	0.1514±0.001
3.		200	0.2529±0.003
4.		300	0.4846±0.001
5.		500	0.9764±0.003
	TEST (PAEA)		
1.		50	0.0088±0.001
2.		100	0.099±0.003
3.		200	0.2156±0.004
4.		300	0.3459±0.002
5.		500	0.5044±0.002

Data presented in (Mean± SD), n=3

Table 6: IC_{50} of ascorbic acid against NO.

S.no.	Group	Concentration (µg/ml)	Absorbance (700 nm)
	STD (Ascorbic acid)		
1.		5	0.03470±0.001
2.		10	0.0683±0.001
3.		20	0.0960±0.001
4.		30	0.126±0.001
5.		50	0.1686±0.002
	TEST (PAET)		
1.		5	0.0485±0.001
2.		10	0.0698±0.001
3.		20	0.0960±0.001
4.		30	0.1230±0.001
5.		50	0.1448±0.001
	TEST (PAEA)		
1.		5	0.0271±0.001
2.		10	0.0553±0.002
3.		20	0.0827±0.002
4.		30	0.1052±0.001
5.		50	0.1366±0.003

Data presented in (Mean± SD), n=3

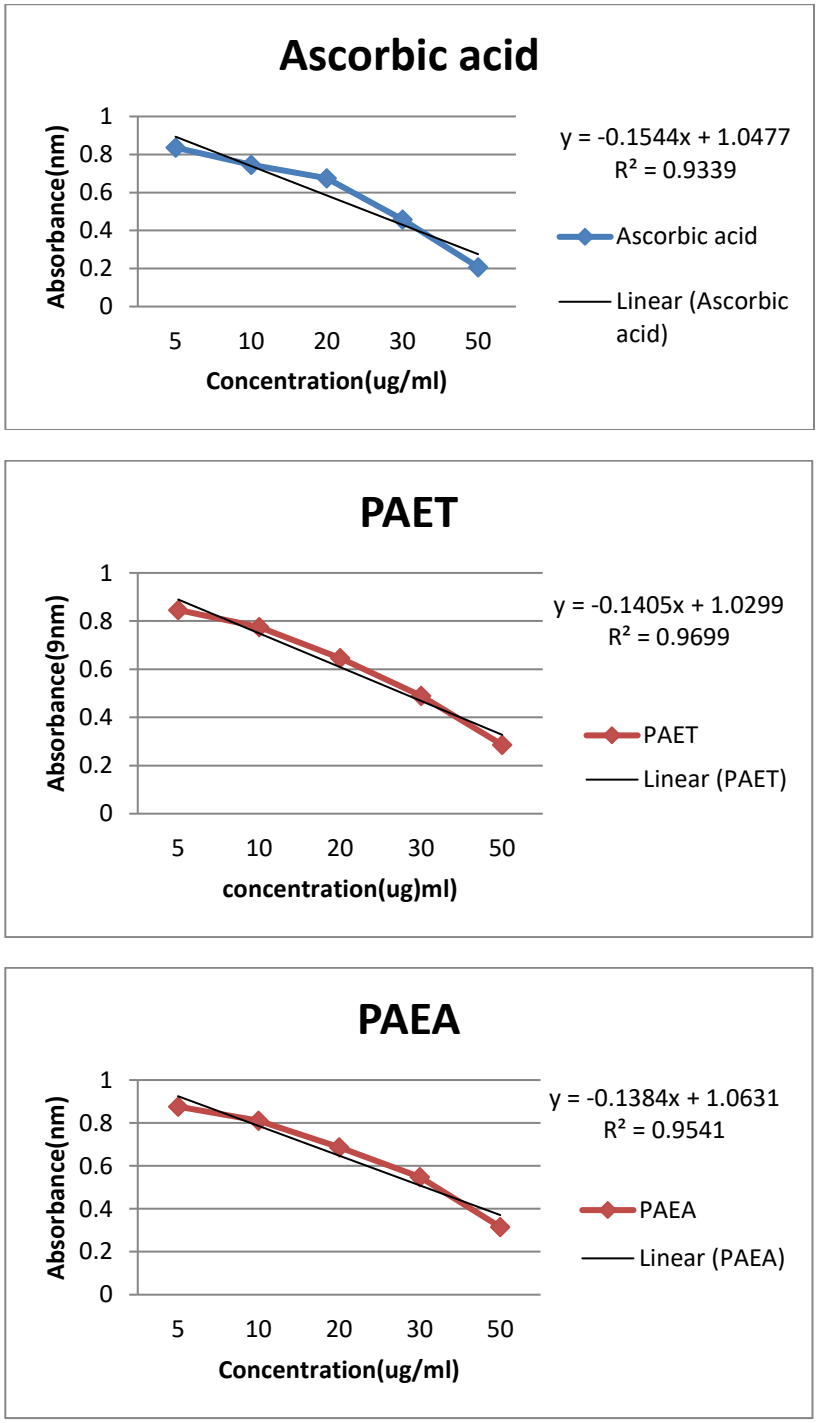


Figure 4: PAET and PAEA free radical scavenging activity are given in the fig 1 and IC_{50} of Ascorbic acid against DPPH is 29.43 µg/ml, IC_{50} of PAEA against DPPH is 32.16 µg/ml, IC_{50} of PAET against DPPH is 35.33 µg/ ml.

FCS-free DMEM containing 0.1% (w/v) BSA for suitable periods with or without agents. The culture media were subjected for the analysis of lipoprotein after centrifugation at 15,0009g for 5 min[14].

Cholesterol and TG levels in culture medium

The distribution and determination of three major classes of lipoprotein were performed as earlier described (Okazaki et al. 2005). Briefly, lipoproteins from 80 ll culture media were separated using a gel permeation HPLC system (LipoSEARCH). Continously effluents were monitor at 550 nm after enzymatic reaction using diacolor liquid TG-S (Toyobo Co. Ltd) for triglycerides and Cholescolor liquid kit for cholesterol (Toyobo Co. Ltd). Triglycerides and Cholesterol concentrations in the three major lipoproteins were calculated using frozen sample-based standard material (Kyowa Kirin Co. Ltd) as a standard and in computer program (Usui et al. 2002; Okazaki et al. 2005)[14].

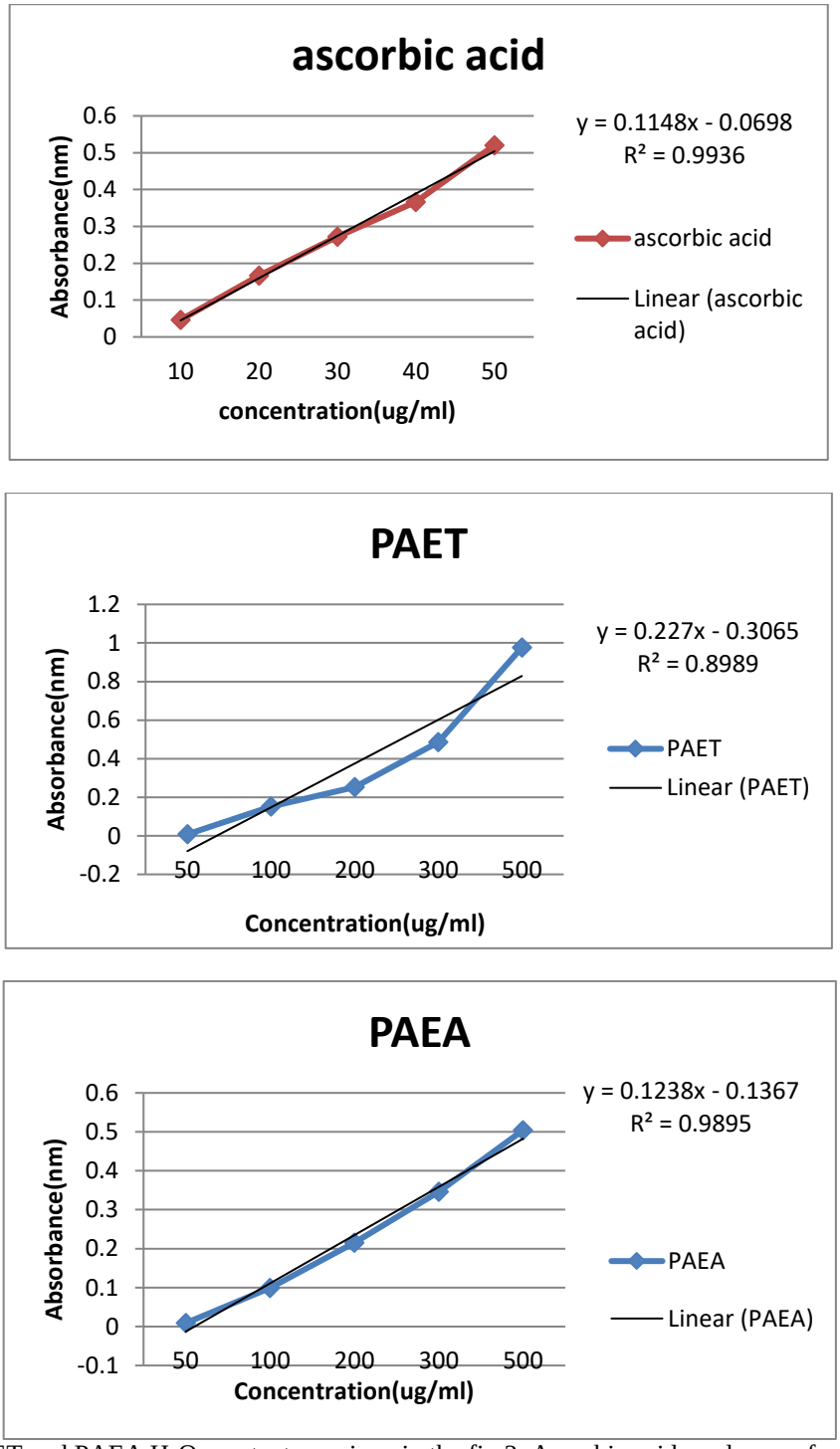


Figure 5: PAET and PAEA H_2O_2 content are given in the fig 2, Ascorbic acid used as a reference drug and approximately 20 µg/ml Ascorbic acid = 20 µg/ml PAET = 25 µg/ml PAEA.

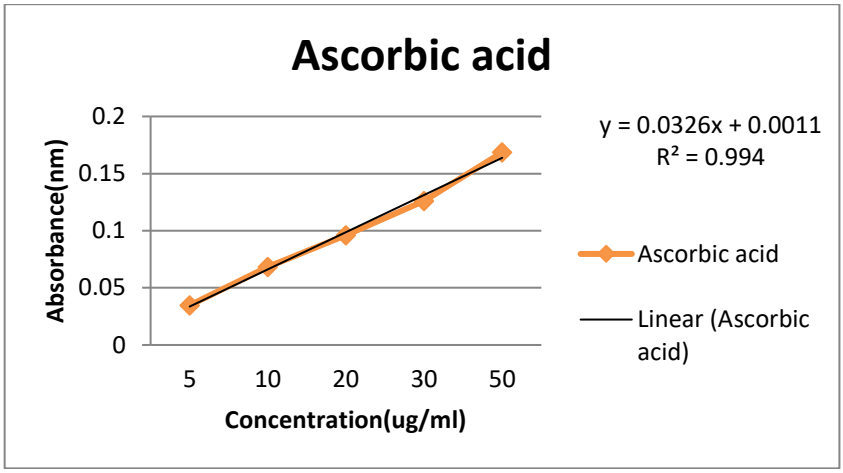
RESULTS

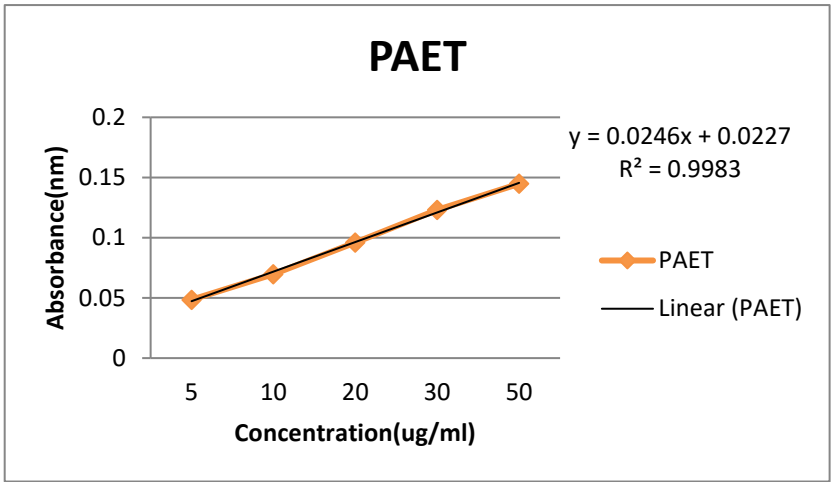
Evaluation parameters of leaf of Pterospermum acerifolium

Physiochemical evaluation of *Pterospermum acerifolium* was perform and determine the ash value 4.67%, Acid soluble ash 0.33%, water soluble ash 2.67%, sulphate ash1.50% and moisture content 8.44%.

Extract of *Pterospermum acerifolium* i.e leaves was extracted by using ethanol, chloroform, petroleum ether and ethyl acetate and determine the % yield which is 10.6%,9.00%,9.33% and 8.66%. Ethanol consist maximum amount of % yield so we used ethanol to determine the antioxidant and antihyperlipidemic activity of *Pterospermum acerifolium* i.e leaves.

Various Phytochemical test i.e alkaloid, flavonoid, triterpenoids, saponin, steroids and glycosides of leaves of pterospermum acerifolium were performed by using four extract i.e Petroleum ether, chloroform, ethyl acetate and ethanol. Ethanol and ethyl acetate consist more chemical





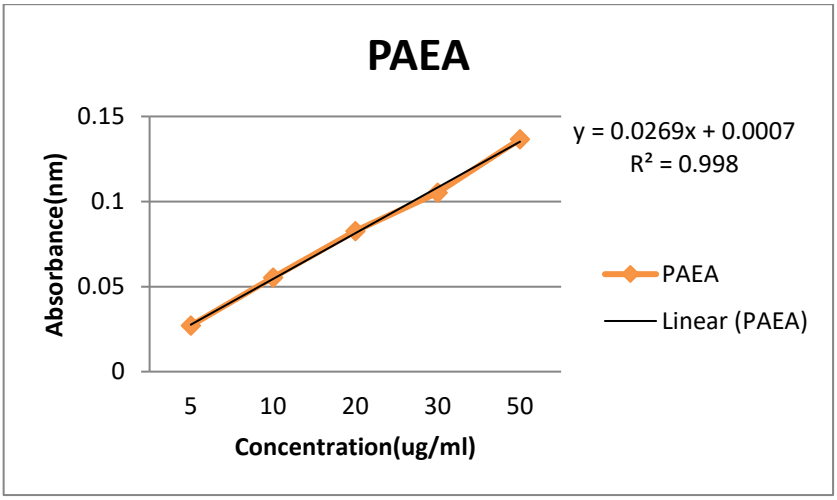


Figure 6: PAET and PAEA Nitric oxide content are given in the fig 3, Ascorbic acid used as a reference drug and approximately 20 µg/ml Ascorbic acid = 20 µg/ml PAET = 25 µg/ml PAEA.

constituent as compared to chloroform and petroleum ether. Flavonoids, saponin and phenolic compound which are responsible for antioxidant activity are present in ethanol and ethyl acetate and flavonids which are responsible for hyperlipidemic activity are present in ethanol and ethyl acetate. So, we prefer ethanol for the evaluation of antihyperlipidemic activity.

PAET and PAEA free radical scavenging activity are given in the table 1 and IC_{50} of Ascorbic acid against DPPH is 29.43 µg/ml, IC_{50} of PAEA against DPPH is 32.16 µg/ml, IC_{50} of PA

Table 7: $HepG_2$ Lipocells.

Group	Control	Test 1	Test 2	Standard
Cell no.	0.19±0.01	0.16±0.02	0.17±0.02	0.18±0.01

Table 8: Determination of TG, LDL, VLDL and HDL.

Triglycerides	Control	Test 1	Test 2	Standard
Total	23.1 ± 1.6	20.1± 2	20.3±1.9	7.9±0.6
VLDL	4.8±0.3	4.6±0.8	4.5±0.6	0.9±0.1
LDL	9±0.6	7±0.6	7.5±0.8	3.5±1
HDL	9.3±0.6	8.1±1.1	8.3±0.7	3.5±0.4

Table 9: Determination of Total Cholestrol, TG, VLDL and HDL.

Cholesterol	Control	Test 1	Test 2	Standard
Total	3.4±0.2	2.1±0.1	1.2±0.3	2.0±00.2
VLDL	0.5±0.1	0.1±0.1	0.3±0.1	0.2±0.1
LDL	1.3±0.1	0.6±0.1	0.5±0.1	0.5±0.1
HDL	1.6±0.3	0.8±0.1	0.6±0.1	1.3±0.1

ET against DPPH is 35.33 µg/ ml.

PAET and PAEA H_2O_2 content are given in the Table 2, Ascorbic acid used as a reference drug and approximately 20 µg/ml Ascorbic acid = 20 µg/ml PAET = 25 µg/ml PAEA.

PAET and PAEA Nitric oxide content are given in the Table 3, Ascorbic acid used as a reference drug and approximately 20 µg/ml Ascorbic acid = 20 µg/ml PAET = 25 µg/ml PAEA.

Following HepG2 and HepG2-Lipo cell preculture in DMEM containing 10% (v/v) FCS, the cells were cultured in DMEM containing 0.1% BSA with or without 500 lM fenofibrate, and the levels of triglycerides and cholesterol were determine.

Determine the various types of lipoprotein i.e Total triglyceride, VLDL, LDL, HDL by comparing with a standard drug i.e finofibrate by using gel permeation HPLC method and determine that methanol extract of leaves of *Pterospermum acerifolium* decrease the level of Total triglyceride, VLDL, LDL and HDL due to the presences of flavonoids i.e quirecetin.

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CONCLUSION

The data presented here shows that leaves of *P. acerifolium* extracts, have great significant antioxidant activity almost equal to that of standards and may be used as an alternative to the synthetic antioxidants. Thus, this study gives a strong impact for expanding the investigations of natural antioxidants for use in health care and food industry and Hyperlipidemia is also a crtical condition of elevated lipid levels in the body that ultimately lead to the development and progression of various CVDs. The link between hyperlipidemia and occurance of CVDs has already been established, the problem of enchanced cholesterol levels in blood is still prevailing and is being a cause for many coronary disorders. Studies reveal that an increase in HDL cholesterol and decrease in TC, LDL cholesterol and TG is associated with a decrease in the risk of ischemic heart diseases.Though many drugs are available to treat Hyperlipidemia. The antihyperlipidaemic activity of plants plays an important role in the reduction of CVD. Plant parts or plant extract are sometimes even more potent than known hypolipidemic drugs. Currently used hypolipidemic drugs are associated with so many adverse effects and withdrawal is associated with rebound phenomenon which is not seen with herbal preparations. The data presented here shows that leaves of *P. acerifolium* extracts, have great significant antioxidant activity almost equal to that of standards and may be used as an alternative to the synthetic antioxidants. Thus, this study gives a strong impact for expanding the investigations of natural antioxidants for use in health care and food industry.

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