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Evaluation of *in vitro* Antioxidant and Lipid Peroxidation Inhibition Potential of Leaves of *Pterospermum acerifolium* Willd

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Abstract: Objective: The study was designed to investigate the antioxidant activity along with lipid peroxidation inhibition potential of leaves of *Pterospermum acerifolium* (L.) Willd. **Materials and Methods:** The antioxidant activity of petroleum ether (PAEE) and chloroform (PACE) extract of leaves was studied by 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, hydroxyl radical scavenging activity (site specific and non site specific) and Total Antioxidant Capacity (TAO%). Lipid Peroxidation Inhibition (LPO) potential of the extracts was also studied in the rat liver homogenate. **Results:** Results showed, 1000 μg mL^{-1} of PAEE showed moderate free radical scavenging activity in all the models. Subsequently, 1000 μg mL^{-1} of PACE showed moderate activity. In LPO assay, 1000 μg mL^{-1} of PAEE showed moderate inhibition percentage. **Conclusion:** Hence, it was evident from the results that, PAEE and PACE both at concentration of 1000 μg mL^{-1} showed moderate free radical scavenging activity.

Key words: *Pterospermum acerifolium*, lipid peroxidation assay, site specific and non site specific hydroxyl radical scavenging assay

INTRODUCTION

Free radicals are chemical moieties, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability (Ali *et al*., 2008). Reactive Oxygen Species (ROS) formed *in vivo*, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical entities (Ali *et al*., 2008). They are an important part of the defence mechanisms against infection, but excessive generation of these free radicals on unsaturated fatty acids has been implicated in the pathogenesis of vascular disease (Manonmani *et al*., 2002) and progression of many disease like hyperlipidemia, diabetes mellitus and its complications such as atherosclerosis, myocardial infarction, neuropathy, nephropathy and retinopathy (Mazumder *et al*., 2011). The concentration of ROS is maintained by antioxidant enzymes and non enzymatic scavengers, normal levels of antioxidant enzymes are not sufficient for the eradication of the free radical injury (Paramaguru *et al*., 2012). Synthetic antioxidants like Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA) commonly used in processed foods have side effects and are carcinogenic, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value (Ali *et al*., 2008). Plants often contain substantial amounts of antioxidants, including tocopherols (vitamin E), carotenoids, ascorbic acid, flavonoids and tannins (McCune and Johns, 2002).

Pterospermum acerifolium (L.) Willd (Family: Sterculiaceae) is an evergreen large tree up to 24 m in height and 2.5 m in girth with smooth bark, greyish brown in colour and found in the Sub-Himalayan tract and outer valleys from Yamuna eastwards to West Bengal and in Assam and Manipur, up to an altitude of 1200 m (Mazumder *et al*., 2011). It has been traditionally used for blood troubles, inflammation, ulcer, tumors, leprosy and for small pox eruptions (Kirtikar and Basu, 1998). Flowers are used as a general tonic, antitumor agent, analgesic and for the treatment of diabetes, gastrointestinal disorder, leprosy, blood troubles, bronchitis, cough, cephalic pain, migraine and inflammation (Chatterjee and Pakrashi, 1997). The leaves are used as haemostatic and antimicrobial agent (Kirtikar and Basu, 1935). The present study was undertaken to establish the antioxidant and lipid peroxidation potential of petroleum ether and chloroform extracts of leaves of *Pterospermum acerifolium* (L.) Willd.

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MATERIALS AND METHODS

Plant collection: Leaves of *Pterospermum acerifolium* was collected from the campus of BIT-Mesra, Ranchi, Jharkhand, India in the month of August 2011. The plant material had been identified and authenticated from taxonomy department of National Botanical Research Institute (NBRI), Lucknow. The voucher specimens were retained in the Department of Pharm. Sciences, BIT-Mesra, Ranchi for future reference.

Extraction: Air dried powder *Pterospermum acerifolium* leaves (5 kg) was exhaustively extracted with Petroleum ether and Chloroform successively for 48 h each, filtered, concentrated on rotavapour (Buchi, USA) to obtain a extract of *Pterospermum acerifolium* Petroleum ether extract (PAEE) and *Pterospermum acerifolium* Chloroform extract (PACE). The yield for PAEE and PACE was found to be 2.4 and 1.45% w/w, respectively.

Preliminary phytochemical analysis: The preliminary phytochemical investigation was done by the standard chemical tests of Evans and Brain and Turner (Brain and Turner, 1975).

***In vitro* antioxidant activity**

Free radical scavenging activity using DPPH: The ability of the samples to scavenge the free radicals was estimated by *in vitro* method using a stable nitrogen centered radical viz., DPPH. Scavenging of DPPH free radical determines the free radical scavenging capacity or antioxidant potential of the test sample, which shows its effectiveness, prevention, interception and repair mechanism against injury in a biological system. Extract 0.05 mL dissolved in methanol was added to a methanolic solution of DPPH (100 μM, 2.95 mL) at different concentration (200-1000 μg mL^{-1}) and the absorbance was recorded at 517 nm (Paramaguru *et al*., 2012):

$$\text{DPPH scavenging activity (\%)} = \frac{\text{AC-AS}}{\text{AC}} \times 100$$

where, AC is the absorbance value of the control and AS is the absorbance value of the added test samples solution.

Non-site-specific OH radical scavenging activity mediated 2-deoxy-D-ribose degradation: The deoxy ribose method was used for determining the scavenging effect on OH as describe by Halliwell (Halliwell and Gutteridge, 1989; Verma *et al*., 2010). The reaction mixture contained ascorbic acid (50 μM), $FeCl_3$ (20 μM), EDTA (2 μM), H_2O_2 (1.42 mm), deoxyribose (2.8 mm), with different concentrations of samples in a final volume of 1 mL in potassium phosphate buffer (10 mM, pH 7.4). It was incubated at 37°C for 1 h and then 1 mL of 2.8% TCA and 1 mL of 1% TBA were added. The mixture was heated in boiling water bath for 15 min. It was cooled and absorbance was measured at 532 nm with the spectrophotometer (Shimadzu 1700).

Site-specific OH radical scavenging activity mediated 2-deoxy-D-ribose degradation: The ability of the extract to inhibit site-specific OH mediated degradation was also carried out to understand its role as a metal chelator. The reaction mixture contained ascorbic acid (50 μM), $FeCl_3$ (20 μM), Buffer (2 μM), H_2O_2 (1.42 mM), deoxyribose (2.8 mM), with different concentrations of samples in a final volume of 1 mL in potassium phosphate buffer (10 mM, pH 7.4). It was incubated at 37°C for 1 h and then 1 mL of 2.8% TCA and 1 mL of 1% TBA were added. The mixture was heated in boiling water bath for 15 min. It was cooled and absorbance was taken at 532 nm in a spectrophotometer (Shimadzu 1700) (Halliwell and Gutteridge, 1989; Verma *et al*., 2010).

Total antioxidant capacity: Total antioxidant capacity was measured according to spectrophotometric method. 0.1 mL of the extract (10 mg mL^{-1}) dissolved in water was added to an Eppendorf tube with combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mm sodium phosphate and 4 mm ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as the standard (Paramaguru *et al*., 2012).

Assay of lipid peroxidation: Male inbred Wistar rats (160-180 g) were procured from the animal house of BIT-Mesra, Ranchi, India. All animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24-27°C and humidity 60-65% with 12: 12 light: dark cycles). Food was provided in the form of dry pellets and water *ad libitum.* The animals were allowed to get acclimatized to the laboratory conditions for 7 days before the commencement of the experiment. All experiments involving animals complied with the ethical standards of animal handling and approved by Institutional Animal Ethics Committee (BIT/PH/IAEC/34/2011).

Randomly selected rats were fasted overnight and were sacrificed by cervical dislocation, dissected and abdominal cavity was perfused with 0.9% w/v saline. Whole liver was taken out and visible clots were removed

and weighed amount of liver was processed to get a clear homogenate in cold phosphate buffered saline, pH 7.4 using glass Teflon homogenizer and filtered to get a clear homogenate. The degree of lipid peroxidation was assayed by estimating the Thiobarbituric Acid Reactive Substances (TBARS) by using the standard method with minor modifications (Govindarajan *et al*., 2003). Briefly, different concentrations of samples (200-1000 μg mL^{-1}) were added to the liver homogenate. Lipid peroxidation was initiated by adding 100 μL of this reaction mixture was taken in a tube containing 1.5 mL of 0.67% TBA in 50% acetic acid. The mixture was heated in a hot water bath at 85°C for 30 min and in a boiling water bath to complete the reaction. The intensity of the pink coloured complex formed was measured at 535 nm in a spectrophotometer (Shimadzu 1700). The percentage of inhibition of lipid peroxidation was calculated by comparing the results of test with those of controls not treated with the extracts.

RESULTS AND DISCUSSION

Characteristic phytochemical studies showed the presence of triterpenes, steroids and saponins in petroleum ether extract (PAEE) and the presence of steroids and saponins in chloroform extract (PACE) (Table 1).

PAEE and PACE scavenged the DPPH radical in a dose dependent and in a time dependent manner (Fig. 1, 2). The 1000 μg mL^{-1} of the PAEE showed the higher percentage of inhibition 42.46% followed by 800 μg mL^{-1} (36.43%), 600 μg mL^{-1} (24.91%), 400 μg mL^{-1} (18.87%), 200 μg mL^{-1} (15.14%) (Table 2). In PACE, 1000 μg mL^{-1} showed higher percentage inhibition of about 37.91% followed by 800 μg mL^{-1} (26.64%), 600 μg mL^{-1} (19.51%), 400 μg mL^{-1} (15.32%), 200 μg mL^{-1} (11.32%) (Table 3). The DPPH assay measured hydrogen atom (or one electron) donating activity and hence provided an evaluation of antioxidant activity due to free radical scavenging. DPPH, a purple colored stable free radical, was reduced into the yellow-colored diphenyl-picrylhydrazine which is measured spectrophotometrically at 517 nm, The degree of discoloration indicates the scavenging potentials of the antioxidant (Blois, 1958).

Hydroxyl radical can abstract hydrogen atoms from biological molecules, including thiols, leading to the formation of sulphur radicals capable to combine with oxygen to generate oxysulphur radicals, a number of which damage biological molecules (Verma *et al*., 2010). Reducing agents such as ascorbic acid can accelerate OH formation by reducing Fe^{3+} ions to Fe^{2+} and were assessed by monitoring the degraded fragments of deoxyribose, through malondialdehyde (MDA) formation. Hydroxyl radical scavenging activity can be done under two conditions to derive two separate inferences, role on hydroxyl trapping ('non-site-specific assay', where EDTA is added) and role of metal chelation ('site-specific assay', where no EDTA is added) (Puppo, 1992; Verma *et al*., 2010). In the case of non site specific OH radical inhibition scavenging activity, 1000 μg mL^{-1} of the PAEE showed

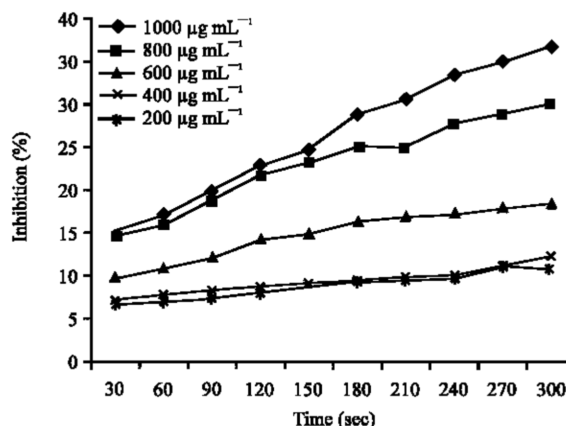

Fig. 1: Time dependent DPPH scavenging activity of *Pterospermum acerifolium* petroleum ether extract

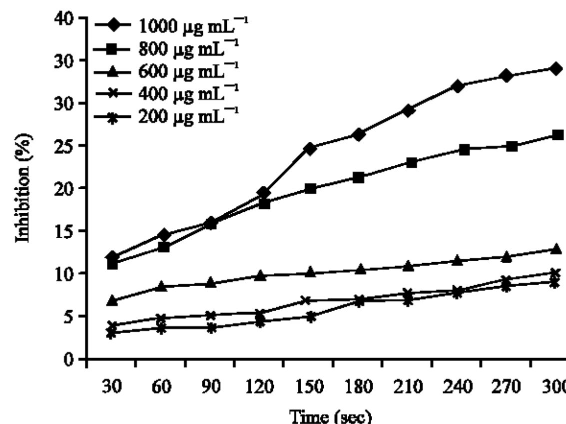

Fig. 2: Time dependent DPPH scavenging activity of *Pterospermum acerifolium* chloroform extract

Table 1: Preliminary phytochemical tests of *Pterospermum acerifolium* (L.) Willd

Tests for constituents	Petroleum ether extract	Chloroform extract
Alkaloids	-	-
Flavonoids	-	-
Tannins	-	-
Glycosides	-	-
Proteins	-	-
Carbohydrates	-	-
Triterpenes	+	-
Steroids	+	+
Terpenoids	-	-
Saponins	+	+
Reducing sugars	-	-

+: Present, -: Absent

Table 2: *In vitro* antioxidant activity of the petroleum ether extract (PAEE) of the leaves of *Pterospermum acerifolium*

	Inhibition (%)			
Concentration (μg mL^{-1})	DPPH activity	Non site specific OH radical	Site specific OH radical	LPO
1000	42.46	39.12	35.23	65.52
800	36.43	35.57	31.47	59.32
600	24.91	30.86	27.63	53.21
400	18.87	26.14	21.06	48.21
200	15.14	22.95	17.28	41.14
Ascorbic acid	91.62	84.45	79.16	-
Quercitin	-	-	-	86.48

Table 3: *In vitro* antioxidant activity of the chloroform extract (PACE) of the leaves of *Pterospermum acerifolium*

	Inhibition (%)			
Concentration (μg mL^{-1})	DPPH activity	Non site specific OH radical	Site specific OH radical	LPO
1000	37.91	35.71	29.13	40.31
800	26.64	31.34	25.97	37.03
600	19.51	26.96	19.82	32.16
400	15.32	21.39	15.93	25.41
200	11.32	17.96	12.60	21.19
Ascorbic acid	91.62	84.45	79.16	-
Quercitin	-	-	-	86.48

Table 4: Total antioxidant activity of *Pterospermum acerifolium* (L.) Willd

	Total antioxidant activity (%)	
Concentration (μg mL^{-1})	Petroleum ether extract	Chloroform extract
1000	45.51	53.31
800	41.14	47.76
600	35.56	41.82
400	29.74	37.73
200	25.55	32.24
Ascorbic acid (Std)	92.46	92.46

the higher percentage of inhibition 39.12% followed by 800 μg mL^{-1} (35.57%), 600 μg mL^{-1} (30.86%), 400 μg mL^{-1} (26.14%), 200 μg mL^{-1} (22.95%) (Table 2). In PACE, 1000 μg mL^{-1} showed higher percentage inhibition of about 35.71% followed by 800 μg mL^{-1} (31.34%), 600 μg mL^{-1} (26.96%), 400 μg mL^{-1} (21.39%), 200 μg mL^{-1} (17.96%) (Table 3). In the case of site specific OH radical inhibition scavenging activity, 1000 μg mL^{-1} of the PAEE showed the higher percentage of inhibition 35.23% followed by 800 μg mL^{-1} (31.47%), 600 μg mL^{-1} (27.63%), 400 μg mL^{-1} (21.06%), 200 μg mL^{-1} (17.28%) (Table 2). In PACE, 1000 μg mL^{-1} showed higher percentage inhibition of about 29.13% followed by 800 μg mL^{-1} (25.97%), 600 μg mL^{-1} (19.82%), 400 μg mL^{-1} (15.93%), 200 μg mL^{-1} (12.60%) (Table 3).

The total antioxidant activity of the samples was calculated on the basis of formation of phosphomolybdenum complex which was measured spectrophotometrically at 695 nm (Paramaguru *et al*., 2012). 1000 μg mL^{-1} of the PAEE showed the higher percentage of TAO 45.51% followed by 800 μg mL^{-1} (41.14%), 600 μg mL^{-1} (35.56%), 400 μg mL^{-1} (29.74%), 200 μg mL^{-1} (25.55%) (Table 4). In PACE, 1000 μg mL^{-1} showed higher percentage of TAO about 53.31% followed by 800 μg mL^{-1} (47.76%), 600 μg mL^{-1} (41.82%), 400 μg mL^{-1} (37.73%), 200 μg mL^{-1} (32.24%) (Table 4).

Initiation of the LPO by Ferrous sulphate takes place either through ferryl-perferryl complex (Gutteridge 1985) or through hydroxyl radical by Fenton's reaction (Halliwell and Gutteridge, 1989). The different concentrations of samples (200-1000 μg mL^{-1}) were studied for lipid peroxidation inhibition by using Thiobarbituric acid reactive substances (TBARS) in rat liver homogenate. PAEE at a concentration of 1000 μg mL^{-1} showed maximum lipid peroxidation inhibition percentage of 65.52% followed by 800, 600, 400 and 200 μg mL^{-1} which exhibited lipid peroxidation inhibition percentages of 59.32, 53.21, 48.21 and 41.14%, respectively. In the case of PACE, 1000 μg mL^{-1} showed maximum lipid peroxidation inhibition percentage of 40.31% followed by 800, 600, 400 and 200 μg mL^{-1} which exhibited lipid peroxidation inhibition percentages of 37.03, 32.16, 25.41 and 21.19%, respectively.

CONCLUSION

The present study demonstrated that, PAEE and PACE possesses moderate free radical scavenging activity and lipid peroxidation inhibition potential. Preliminary phytochemical tests showed the absence of flavonoids and phenols in the extracts which may be have the reason for that extracts possessing moderate antioxidant activity. Thus subsequent study with other solvents is required to bring out the complete antioxidant potential of the plant.

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