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Antioxidant and iron chelating potential of *Pongammia pinnata* and its role in preventing free radical induced oxidative damage in plasmid DNA

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Abstract

Reactive oxygen species (ROS) and free radical-mediated reactions are involved in degenerative or pathological processes. Antioxidants are believed to play an important role in preventing chronic diseases by reducing the oxidative damage to cellular components caused by ROS.

In the present study, *Pongamia pinnata* leaf (PPL), seed (PPS), and flower (PPF) were investigated for their total phenolic and flavonoid contents, antioxidant activity by ABTS and DPPH method, scavenging activities for different free radicals such as hydroxyl, superoxide, nitric oxide, hydrogen peroxide, peroxynitrite, singlet oxygen, hypochlorous acid, the inhibition of lipid peroxidation in mice brain homogenate, reducing power, iron chelating and protection of DNA damage caused by free radicals.

PPL showed the best antioxidant activity compared to both PPS and PPF. The extract of PPL possessed most potent activity compared to other extracts in scavenging assay for singlet oxygen, hydroxyl radical, superoxide radical and nitric oxide radical. PPF exhibited strongest inhibitory activity against hypochlorous acid and peroxynitrite anion among these three extracts. PPL was the best amongst three to inhibit lipid peroxidation and Fe^{2+}-ferrozine complex formation. PPL was also found effective in protecting plasmid DNA nicking at lower concentration while both PPS and PPF did the same at higher concentration. PPL presented highest content of phenolics and flavonoids among these three extracts.

The present results show that *Pongammia pinnta* acts as an antioxidant, iron chelator and protector of oxidative DNA damage.

Keywords: Oxidative stress, DNA protection, Reactive oxygen species, lipid peroxidation, phenolic content.

Introduction

Oxidative stress is defined in general as excess formation or incomplete removal of highly reactive molecules such as reactive oxygen species (ROS). ROS are produced in the cells by cellular metabolism and other exogenous environmental agents. In vivo, some of the ROS play a vital role in energy production, phagocytosis, regulation of cell growth and intracellular signalling [1]. On the other hand, they can damage a wide range of essential biomolecules such as proteins, DNA and lipids [2], resulting in the development of a variety of diseases such as cancer, coronary heart disease, diabetes, liver injury, atherosclerosis, neurodegenerative disorders and cardiovascular

diseases [3,4]. Living systems have several antioxidant defense mechanisms that help to prevent the destructive effects of ROS by reacting with them, chelating catalytic metals, decomposing peroxides and also by acting as oxygen scavengers [5]. But, sometimes these repair mechanisms fail to keep pace with such deleterious effects. Antioxidants are the compounds that can alleviate the damage caused by ROS. Many synthetic antioxidants have shown toxic or mutagenic effects, which have shifted the attention on to the naturally occurring antioxidants [6]. Several studies have already shown that plants are immense source of natural antioxidants as they contains a wide variety of free radical scavenging molecules such as phenolics, flavonoids, vitamins terpenoids that are rich in antioxidant activity [7,8].

Pongammia pinnata (L) Pierre (Leguminosae, Syn. P. glabra Vent), popularly known as karanj in Hindi, and karanja, maktamala or gaura in Sanskrit and Indian beech in English, is a medium sized glabrous, perennial tree that grows in moist environment along rivers or sea coast all over India and further distributed eastwards mainly in the littoral regions of South Eastern Asia and Australia [9]. Historically, this plant has long been used in India and neighbouring regions as a source of traditional medicines, animal fodder, green manure, timber and fish poison [10]. In Ayurveda, different parts of this plant have been widely used for the treatment of leprosy, piles, ulcers, chronic fever, liver pain, bronchitis, whooping cough, diarrhea, dyspepsia, flatulence, gonorrhea, inflammations, pectoral diseases, hemorrhoids and anemia [11,12]. During past several years, Pongamia pinnata is gaining lot of interest in the scientific community for its antidiabetic [13], antihyperglycaemic and antilipidperoxidative [14], anti-inflammatory [15], antiplasmodial [16], antibacterial [17], antiviral [18], ulcerprotective [19], gastroprotective [20], antihyperammonemic and antioxidant [21], hepatoprotective [22], antinociceptive and antipyrectic activity [23]. Pongamia pinnata has also been reported to contain a large number of furanoflavonoids, e.g. karanjin, pongapin, kanjone, pongamol and pongaglabrone, along with a number of simpler flavonoids and lipid like arachidonic acid [24-26]. Moreover, it is called as "Biodiesel plant" as the seed oil of this plant has been well recognized as the source of fuel for the biodiesel industry [27].

The present work is focussed on studying; the scavenging potential of methanolic extracts of PPL, PPS and PPF on different free radicals; the antioxidant activity by ABTS and DPPH method; the determination of phenolic and flavonoid contents as well as the attempts have also been made to assess the iron chelating and DNA protective property of these extracts.

Material and Methods

Chemicals

2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was obtained from Roche diagnostics, Mannheim, Germany. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Fluka, Buchs, Switzerland. Potassium persulfate ($K_2S_2O_8$), 2-deoxy-2-ribose, ethylenediamminetetraacetic acid (EDTA), mannitol, nitro blue tetrazolium (NBT), sodium nitroprusside (SNP), lipoic acid, quercetin and ferrozine were obtained from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Folin-ciocalteu reagent, xylenol orange, butylated hydroxytoluene (BHT) and N,N-dimethyl-4-nitrosoaniline were obtained from Merck, Mumbai, India. 1,1-diphenyl-2-picrylhydrazyl (DPPH), Gallic acid and curcumin were obtained from MP Biomedicals, France. Catalase was obtained from HiMedia Laboratories Pvt. Ltd, Mumbai, India. Evans blue was purchased from BDH, England. Diethylene-triamine-pentaacetic acid (DTPA) was obtained from Spectrochem Pvt. Ltd, Mumbai, India. Thiobarbituric acid (TBA) was obtained from Loba Chemie, Mumbai, India.

Plant material

The leaf, seed and flower of *P. pinnata* were collected along the banks of river Ganges near Uttarpara, West Bengal, India. It was identified and authenticated through the Botanical Survey of India (BSI), Kolkata, India and a voucher specimen (NM-7) was submitted there.

Animals

Male Swiss albino mice (20±2 g) were purchased from Chittaranjan National Cancer Institute (CNCI), Kolkata, India and were maintained under a constant 12-h dark/ light cycle at an environmental temperature of 22±2°C. The animals were provided with normal laboratory pellet diet and water ad libitum. All experiments were performed after obtaining approval from the institutional animal ethics committee.

Preparation of plant extract

The powder (100 g) of the dried leaves, seeds and flowers of *P. pinnata* was stirred using a magnetic stirrer with 500 ml mixture of methanol : water (7:3) for 15 h; then the mixture was centrifuged at 2850 g and the supernatant decanted. The process was repeated again with the precipitated pellet. The supernatants were collected, concentrated in a rotary evaporator and lyophilized. The residue was stored at -20°C until use.

Antioxidant activity

ABTS method

Antioxidant capacity was evaluated by an improved $ABTS^{\cdot+}$ radical cation decolorisation assay in comparison to trolox standard [28]. The $ABTS^{\cdot+}$ radical cation was generated from the overnight mixture of ABTS solution with potassium persulfate. Then 10 µl of all extracts (1-10 mg/ml) were added into 1 ml $ABTS^{\cdot+}$ solution and the decrease in absorbance was measured at 734 nm. All experiments were repeated six times. The trolox equivalent antioxidant capacity (TEAC) was expressed as trolox equivalent and plotted against concentration of samples.

DPPH method

The complementary study for the antioxidant capacity of the plant extract was confirmed by the DPPH (1, 1 –diphenyl -2 -picrylhydrazyl) scavenging assay [29]. Different concentrations (0-100 µg/ml) of these extracts and the standard ascorbic acid were mixed with equal volume of ethanol. Then 50 µl of DPPH solution (1 mM) was pipetted into the previous mixture and stirred thoroughly. The resulting solution was kept standing for 10 minutes at room temperature before the O.D. was measured at 517 nm. The measurement was repeated with six sets. The percentage of scavenging was calculated from the values of the control and the test samples.

Free radical scavenging activity

Hydroxyl radical scavenging

The Fenton inhibition assay was carried out to measure the hydroxyl radical scavenging activity of these extracts (0-200 µg/ml). The assay was performed by a previously described method [28]. All tests were carried out six times. Mannitol was used as a positive control.

Superoxide radical scavenging

Measurements of superoxide anion scavenging activities of the samples (0-120 µg/ml) and standard quercetin were done based on the reduction of NBT according to a previously described method [28]. All tests were performed six times.

Scavenging of nitric oxide radical

Nitric oxide generated from sodium nitroprusside (SNP) interacts with oxygen and produce nitrite ions which forms a pink colored chromophore by Griess Illosvoy reaction [28]. The chromophore generated was spectrophotometrically measured at 540 nm against blank and samples (0-70 µg/ml). All tests were performed six times. Curcumin was used as a standard.

Assay of peroxynitrite scavenging

Evans blue bleaching assay was used to measure the peroxynitrite scavenging activity [28]. The percentage of scavenging of $ONOO^{-}$ was

calculated by comparing the results of the test (0-200 µg/ml) and blank sample. All tests were performed six times. Gallic acid was used as reference compound.

Hydrogen peroxide scavenging

FOX-reagent method was used to determine this activity of these samples (0-2 mg/ml) and the reference compound sodium pyruvate, as previously described [28]. All tests were carried out six times.

Determination of the Effects on singlet oxygen

The production of singlet oxygen ($^1O_2$) was determined by monitoring N,N-dimethyl-4-nitrosoaniline (RNO) bleaching, using earlier reported method [28]. The scavenging activities of samples (0-200 µg/ml) were compared with lipoic acid, used as a reference compound. All tests were performed for six times.

Scavenging assay of HOCl

According to a previously described method [28], hypochlorous acid (HOCl) scavenging activities of all the extracts (0-100 µg/ml) were evaluated by measuring the decrease in the absorbance of catalase at 404 nm. Ascorbic acid was used as standard. All tests were performed six times.

Inhibition of lipid peroxidation

The inhibition of lipid peroxidation activities of three extracts were (0-25 µg/ml) determined by quantification of thiobarbituric acid-reactive substances (TBARS) using a previously reported method [29]. trolox was used as standard. All tests were repeated six times.

Fe^{2+} ion chelating

The chelating activity of these extracts (0-120 µg/ml) and standard EDTA was carried out according to an earlier depicted method [28]. All tests were performed six times.

Measurement of reducing power

The Fe^{3+}-reducing power of three extracts (0-1 mg/ml) was determined by a standard method described before [28]. All tests were performed six times. Ascorbic acid was used as a reference compound.

DNA protection

The protection of the pUC-18 plasmid DNA damaged by Fenton reaction generated $^{\cdot}OH$ radicals was studied by quantifying the decrease of supercoiled DNA after oxidative attack, following an earlier reported method [29]. After migration in 1% agarose gel, it was stained with ethidium bromide and visualized in a UV transilluminator. The DNA bands were quantified through densitometry and the following formulae were used to calculate the percentage of protection.

% SC = [1.4 X SC/(OC+(1.4 X SC))] X 100

where, SC = supercoiled; OC = open circular; 1.4 = correction factor

% protection = 100 X [(control SC – chelator SC)/(control SC – no chelator SC) – 1]

The ability of the plant extract to protect the DNA supercoil can be expressed by the concentration of sample required for 50% protection, designated as the $[P]_{50}$ value.

Determination of Total Phenolic and flavonoid Content

The amount of total phenolics present in the plant seed extract was determined using Folin-Ciocalteu (FC) reagent by a formerly reported method [28]. A gallic acid standard curve (R^2 = 0.9468) was used to measure the phenolic content. The amount of total flavonoids was determined with aluminium chloride ($AlCl_3$) according to a known method [28]. The flavonoid content was calculated from quercetin standard curve (R^2 = 0.9947).

Statistical analysis

All data were reported as the mean ± SD of six measurements. The statistical analysis was performed by KyPlot version 2.0 beta 15 (32 bit). The IC_{50} values were calculated by the formula, Y = 100*A1/(X + A1) where A1 = IC_{50}, Y =

response (Y = 100% when X = 0), X = inhibitory concentration. The IC_{50} values were compared by paired t test. $p < 0.05$ was considered significant.

Results and Discussion

Antioxidants are deployed to prevent action of ROS by scavenging those formed. Thus, oxidatively induced tissue damage is minimized. A number of natural antioxidants, from different kinds of plant materials [30], have received much attention for their antioxidant activities. In the present study, three parts (PPL, PPS and PPF) of important medicinal plant, P pinnata, were investigated for their antioxidant activities.

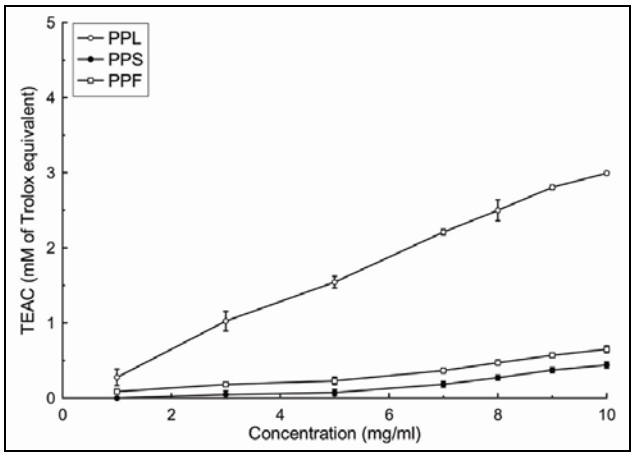

Fig. 1: Total Antioxidant Activity.

Total antioxidant activity of PPL, PPS and PPF. The TEAC values were determined from trolox standard curve and plotted against concentration of samples. All data are expressed as mean ± S.D. (n=6).

The ABTS and DPPH systems have both been commonly used to measure the total antioxidant status of the sample because of their good reproducibility. Although these assays are not biologically relevant, both assays are performed as a preliminary study to estimate the direct free radical scavenging abilities. In terms of TEAC, PPL showed highest activity among them, while the activity of PPF was higher than PPS (Figure 1). In DPPH system (Figure 2), the best scavenging was shown by PPL (IC_{50} 53.04 ± 2.84 µg/ml) followed by PPF (IC_{50} 528.46 ± 47.95 µg/ml) and PPS (IC_{50} 1022.46 ± 26.29 µg/ml). The standard compound ascorbic acid also revealed scavenging activity on DPPH radicals with IC_{50} value of 5.29 ± 0.28 µg/ml (Table 1). Both the data demonstrate that the antioxidant activity of PPL lead PPF and PPS respectively.

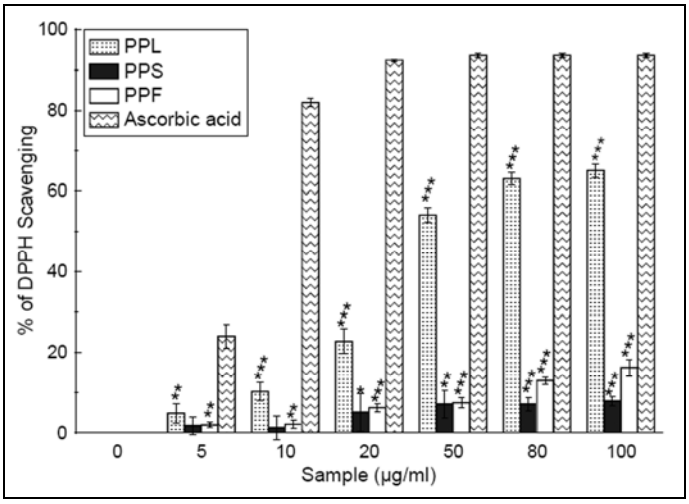

Fig. 2: DPPH Radical Scavenging.

DPPH radical scavenging activities of plant extracts and the reference compound ascorbic acid. The data represent the percentage of scavenging of DPPH radical. The results are mean ± S.D. of six parallel measurements. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. 0 µg/ml.

Hydroxyl radicals are the major active species causing lipid oxidation and enormous biological damage. Hydroxyl radicals were detected by their ability to degrade 2-deoxy-2-ribose in to fragments, malondialdehyde (MDA), that on heating with TBA at low pH form a pink chromogen. PPL, PPS and PPF significantly inhibited ·OH induced deoxyribose degradation in a concentration dependent manner with IC_{50} value of 670.58 ± 52.56 µg/ml, 1017.87 ± 206.77 µg/ml and 798.39 ± 39.58 µg/ml respectively (Figure 3). The reference compound, mannitol, also suppressed deoxyribose degradation with an IC_{50} value of 571.45 ± 20.12 µg/ml (Table 1). The observed IC_{50} values indicate that PPL is the best scavenger with the order PPL>PPF>PPS.

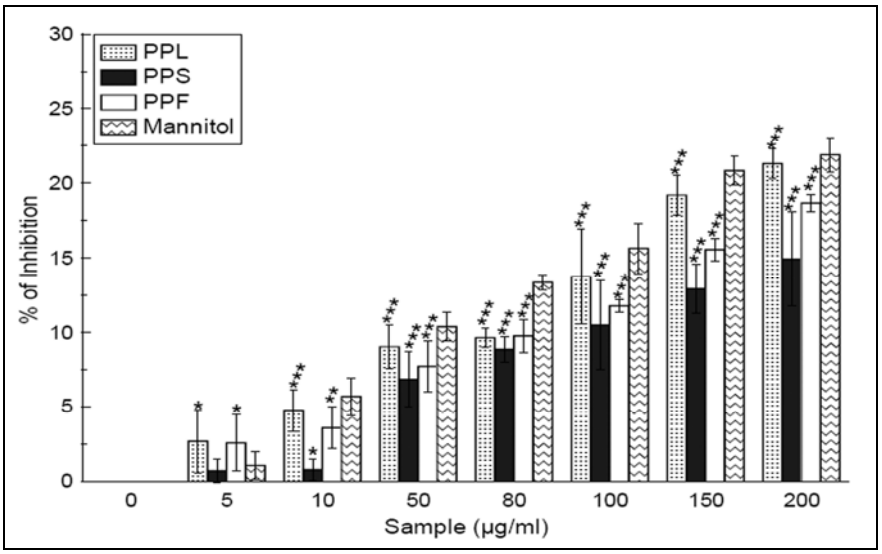

Figure 3: Hydroxyl radical scavenging activity.

Hydroxyl radical scavenging activities of PPL, PPS, PPF and the reference compound mannitol. The data represent the percentage of inhibition of deoxyribose degradation. All data are expressed as mean ± S.D. (n=6). *p < 0.05, **p < 0.01 and ***p < 0.001 vs. 0 µg/ml.

Superoxide anion is also another harmful reactive oxygen species as it damages cellular components in biological systems [31]. As shown in Figure 4, the extracts also effectively scavenge the superoxide radicals as revealed by the NBT reduction assay. The positive control quercetin showed scavenging activity with IC_{50} value of 60.5 ± 2.09 µg/ml. In comparison, the IC_{50} values of PPL, PPS and PPF were 22.57 ± 1.17 µg/ml, 140.62 ± 7.24 µg/ml and 106.24 ± 10.12 µg/ml respectively (Table 1). PPL exhibits the highest superoxide scavenging activity with the order PPL>PPF>PPS.

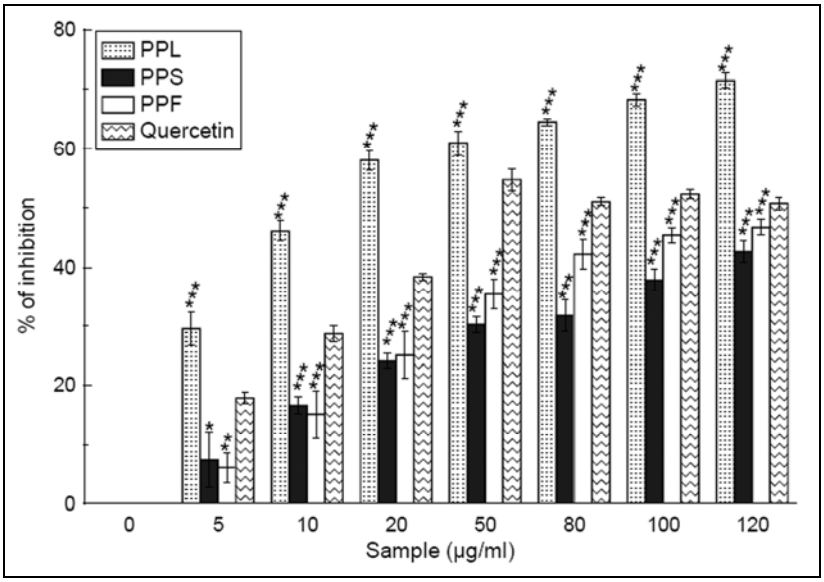

Fig. 4: Superoxide Radical Scavenging.

Scavenging effect of PPL, PPS, PPF and standard quercetin on superoxide radical. The data represents the percentage of superoxide radical inhibition. All data are expressed as mean ± S.D. (n=6). *p < 0.05, **p < 0.01 and ***p < 0.001 vs. 0 µg/ml.

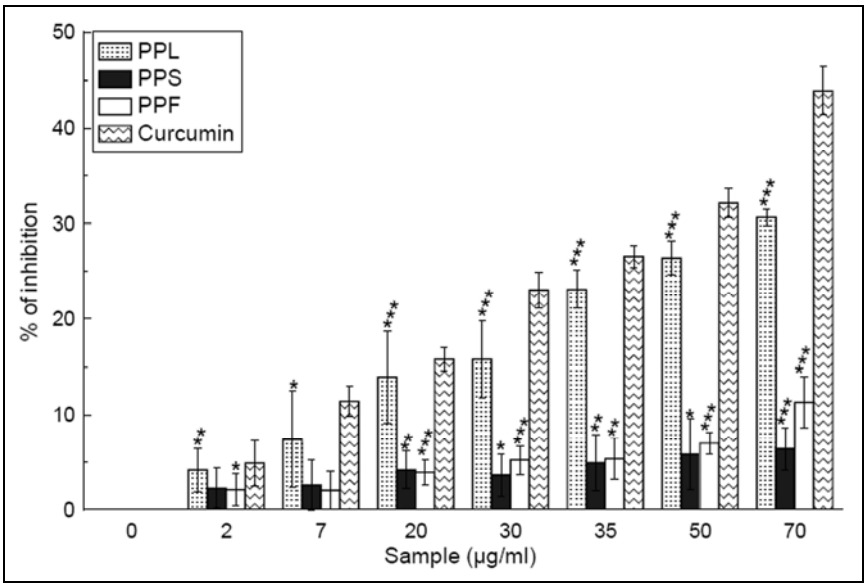

Fig. 5: Nitric oxide Radical Scavenging.

Nitric oxide radical scavenging activities of the extracts (PPL, PPS, PPF) and standard curcumin. The data represent the % of nitric oxide inhibition. Each value represents mean ± S.D. (n=6). *p < 0.05, **p < 0.01 and ***p < 0.001 vs. 0 µg/ml.

Nitric oxide (NO), a short-lived free radical generated endogenously and overproduction of NO can mediate toxic effects, e.g. DNA fragmentation, cell damage and neuronal cell death [32]. Figure 5 shows the percentage inhibition of nitric oxide radical generation by three extracts compared to standard curcumin. The 50% inhibition (IC_{50}) by PPL, PPS and PPF were found as 140.63 ± 9.46 µg/ml, 958.51 ± 47.61 µg/ml and 595.26 ± 12.28 µg/ml respectively, whereas curcumin showed 90.82 ± 4.75 µg/ml as IC_{50} (Table 1).The present results indicate that PPL exerts best activity among the three extracts. According to the IC_{50} values, the order is in the fashion of PPL>PPF>PPS.

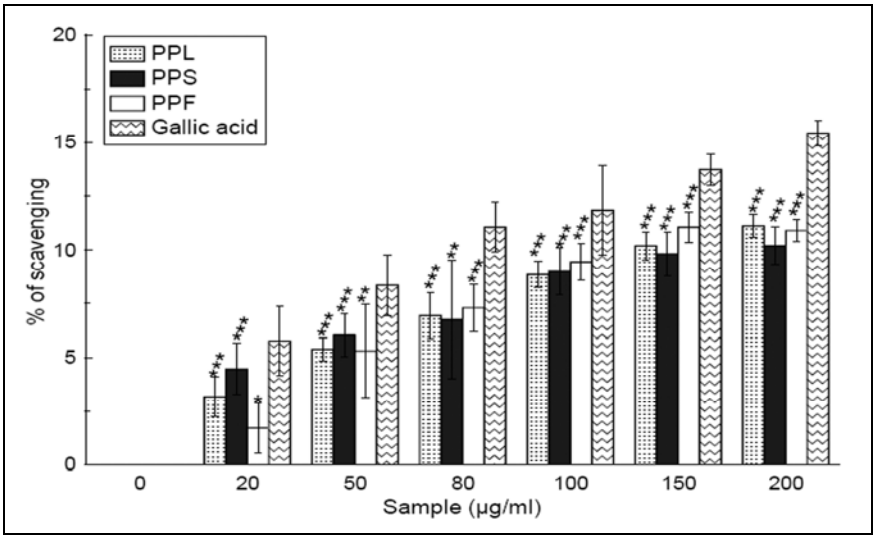

Fig. 6: Peroxynitrite Anion Scavenging.

The peroxynitrite anion scavenging activities of PPL, PPS, PPF and standard gallic acid. Each value represents mean ± S.D. (n=6). *p < 0.05, **p < 0.01 and ***p < 0.001 vs. 0 µg/ml.

Another highly reactive free radical is peroxynitrite anion which has been implicated in the pathogenesis of various diseases such as heart disease, Alzheimer's disease, and atherosclerosis [33]. The generation of peroxynitrite anions was markedly inhibited by PPL, PPS and PPF with an IC_{50} value of 1304.75 ± 43.47 µg/ml, 1366.57 ± 28.90 µg/ml and 1271.74 ± 99.89 µg/ml respectively (Figure 6). The standard gallic acid also inhibited peroxynitrite formation with an IC_{50} value of 876.25 ± 56.96 µg/ml (Table 1). PPF shows the strongest scavenging activity for peroxynitrite with the decreasing order of PPF>PPL>PPS as it is evident from IC_{50} values.

The scavenging activity for hydrogen peroxide compared to standard sodium pyruvate (IC_{50} = 3.24 ± 0.30 mg/ml) were not significant. So, the data are not shown.

Singlet oxygen is generated in the skin upon UV-irradiation and induces hyperoxidation, oxygen cytotxicity and decreases the antioxidative activity [34]. Figure 7 illustrates the percentage of scavenging of singlet oxygen by all the three extracts and standard lipoic acid. The IC_{50} value of PPL, PPS and PPF were found to be 212.24 ± 17.34 µg/mL, 945.40 ± 38.32 µg/mL and 1155.36 ± 19.50 µg/mL respectively, whereas, the IC_{50} value of lipoic acid was found to be 46.16 ± 1.16 µg/mL (Table 1).From the IC_{50} values it is anticipated that PPL is more efficient scavenger than PPS and PPF.

Hypochlorous acid (HOCl), another harmful ROS, is produced through the oxidation of Cl^- ions by the neutrophil myeloperoxidase at the sites of inflammation [35]. PPL, PPS and PPF scavenged HOCl with the IC_{50} being 833.46 ± 112.81 µg/mL, 929.73 ± 36.46 µg/mL and 349.88 ± 90.66 µg/mL, respectively (Figure 8). Standard ascorbic acid was found to have 50% inhibitory activity at 235.96 ± 5.75 µg/mL (Table 1).From the results obtained, it can be concluded that PPF is the most potent HOCl scavenger with the order PPF>PPL>PPS.

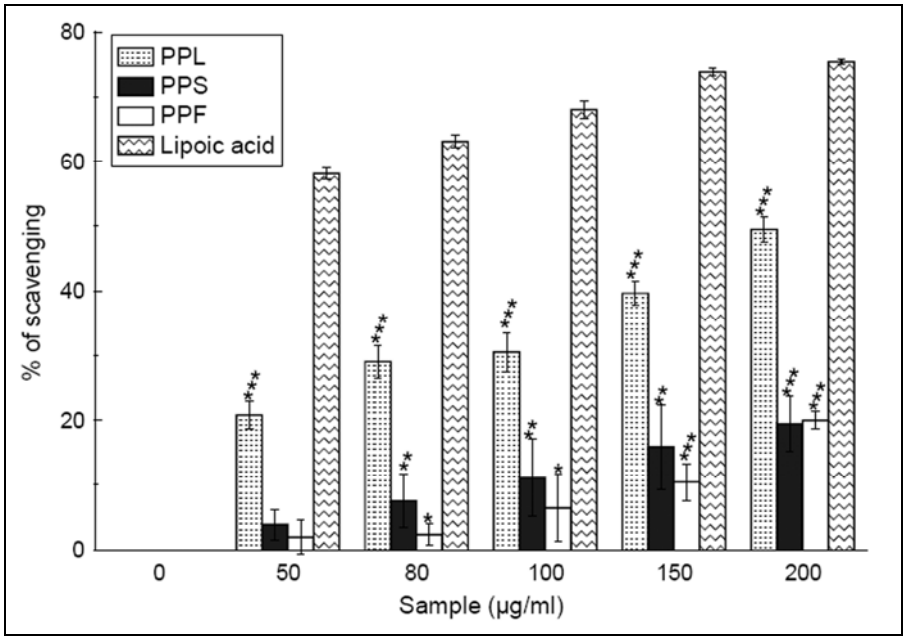

Fig. 7: Singlet Oxygen Scavenging.

Effects of PPL, PPS, PPF and standard lipoic acid on the scavenging of singlet oxygen. The results are mean ± S.D. of six parallel measurements. *p < 0.05, **p < 0.01 and ***p < 0.001 vs. 0 µg/ml.

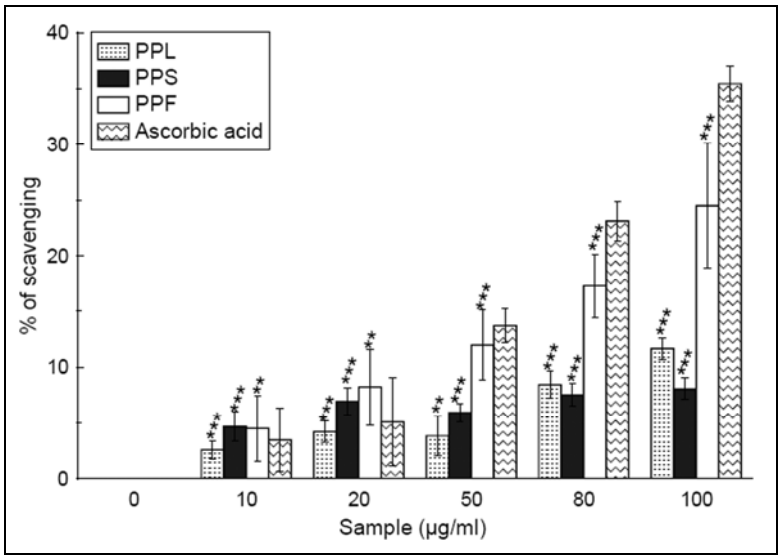

Fig. 8: HOCl Scavenging.

Hypochlorous acid scavenging activity of PPL, PPS and PPF and standard ascorbic acid. All data are expressed as mean ± S.D. (n=6). ***p < 0.001 vs 0 µg/ml.

Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acids in the cell membranes that generates a number of degradation products. The inhibition of $FeSO_4$-ascorbic acid induced TBARS formation in brain homogenate by the extracts, indicating their anti-lipid peroxidation activities. PPL, PPS and PPF exhibited suppressive effect on the lipid peroxidation with an IC_{50} value of 119.83 ± 14.59 µg/mL, 479.16 ± 56.42 µg/mL and 219.36 ± 73.35 µg/mL respectively (Figure 9). The reference compound trolox also showed inhibitory effect with an IC_{50} value of 6.75 ± 0.16 µg/mL (Table 1). PPL represents best inhibitory activity to lipid peroxidation followed by PPF and PPS.

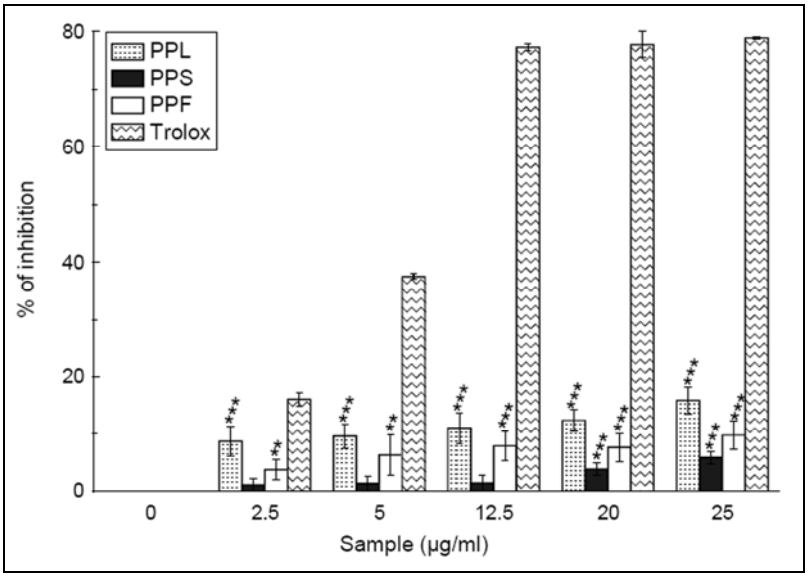

Fig. 9: Inhibition of Lipid Peroxidation.

Lipid peroxidation inhibiting capacity of the PPL, PPS and PPF extracts and the standard trolox. The data is expressed as the % of lipid peroxidation inhibition of brain homogenate, induced by Fe^{2+}/ascorbic acid. Each value represents mean ± S.D. (n=6). **p < 0.01 and *** p < 0.001 vs. 0 µg/ml.

Iron is an essential mineral for normal physiology, but in excess, they undergo the Fenton reaction and form highly reactive hydroxyl radicals and thereby contribute to oxidative stress [36]. As illustrated in Figure 10(a), PPL, PPS and PPF moderately suppressed Fe^{2+}-ferrozine complex formation with an IC_{50} value of 334.60 ± 32.21 µg/mL, 1146.70 ± 88.31µg/mL and 719.73 ± 64.94 µg/mL respectively compared to standard EDTA that exhibited potent activity (Table 1) with an IC_{50} value of 1.27 ± 0.05 µg/ml [Figure 10(b)]. Among the three extracts, PPL is the most potent iron chelator, whereas, PPS and PPF showing moderate activity.

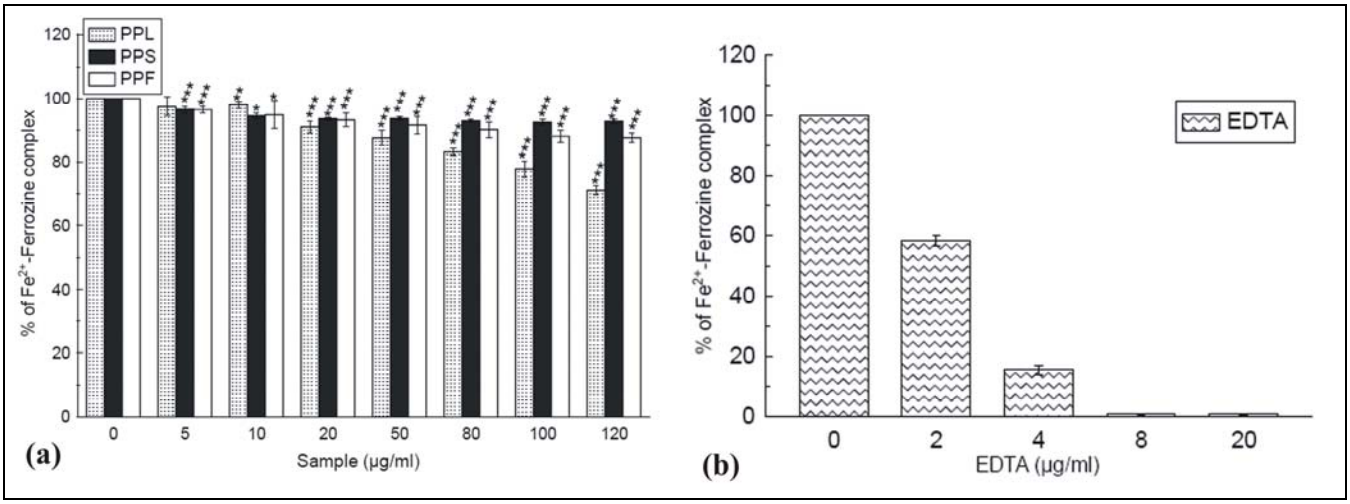

Fig. 10: Iron Chelation.

Effect of (a) PPL, PPS and PPF and (b) standard EDTA on ferrozine-Fe^{2+} complex formation. The data expressed as % inhibition of chromogen formation. The results are mean ± S.D. of six parallel measurements. *p < 0.05, **p < 0.01 and ***p < 0.001 vs. 0 µg/ml.

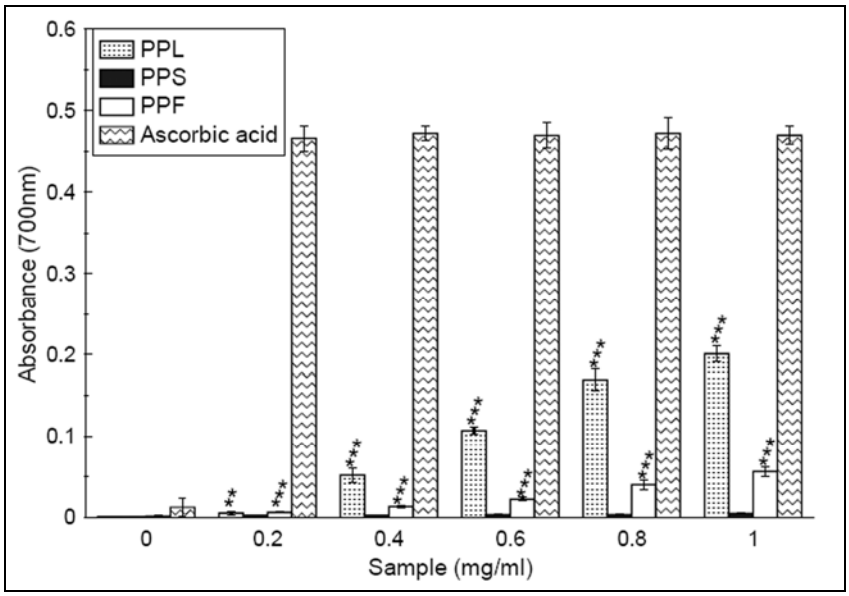

Fig. 11: Reducing Power.

The reductive ability of PPL,PPS, PPF extract and standard ascorbic acid. The absorbance (A_{700}) was plotted against concentration of sample. Each value represents mean ± S.D. (n=6). **p < 0.01 and ***p < 0.001 vs 0 mg/ml.

The reducing capacity of compound may serve as a significant indicator of its potential antioxidant activity. The ferric-reducing antioxidant power of the extracts was found to increase in a concentration-dependent manner (Figure 11). At 1mg/ml, the absorbances of the PPL, PPS and PPF were 0.2, 0.004 and 0.056 while that of standard ascorbic acid was 0.47. PPL is found most efficient with the order PPL>PPF>PPS.

Oxidative DNA damage has been implicated to be involved in various degenerative diseases. The effect of all the extracts on Fe^{2+}-dependent hydroxyl radical induced DNA damage of pUC18 plasmid was studied. The treatment of supercoiled (SC) DNA with Fenton's reagent directed to the alteration of DNA to open circular form (OC). The addition of extracts to the reaction mixture substantially decreased the DNA strand scission and retained the SC form, thus effectively protect DNA, in a dose dependent manner (Figure 12). The $[P]_{50}$ value of PPL,PPS and PPF were found to be 2.58 ± 0.04 µg/ml, 23.40 ± 3.58 µg/ml and 9.76 ± 0.35 µg/ml respectively. Among the three extracts, PPL inhibits DNA damage most significantly relative to PPF and PPS as it is evident from $[P]_{50}$ values.

It is well known that phenolic and flavonoids are constituents of many plants and they exhibit considerable free radical scavenging activities, through their reactivity as hydrogen or electron donating agents, and metal ion chelating properties [37]. The total phenolic content per 100 mg extracts of PPL, PPS and PPF were 409.68 ± 0.01, 13.27 ± 0.004 and 83.93 ± 0.004 mg/ml gallic acid equivalent. The flavonoid contents were found to be 245.19 ± 0.003, 191.53 ± 0.001 and 227.70 ± 0.001 mg/ml quercetin equivalent per 100 mg extracts of PPL, PPS and PPF. The phytochemical investigations of *P. pinnata* demonstrate that PPL is the most potent source of phenolics and flavonoids compared to PPS and PPF.

Table 1: Comparison of the free radical scavenging and lipid peroxidation inhibiting properties of 70% methanolic extracts of PPL, PPS and PPF.

Name of Assay	70% methanolic crudes of				
	PPL	PPS	PPF	Standard	Value
DPPH	53.04 ± 2.84***	1022.46±26.29***	528.46±47.95***	Ascorbic acid	5.29 ± 0.28
Hydroxyl ($OH^{\cdot}$)	670.58±52.56**	1017.87±206.77**	798.39±39.58***	Mannitol	571.45±20.12
Superoxide ($O_2^{\cdot-}$)	22.57±1.17***	140.62 ±7.24***	106.24±10.12***	Quercetin	60.5 ± 2.09
Nitric oxide ($NO^{\cdot}$)	140.63±9.46***	958.51 ±47.61**	595.26±12.28***	Curcumin	90.82 ± 4.75
Peroxynitrite ($ONOO^{-}$)	1304.75±43.47***	1366.57±28.90**	1271.74±99.89***	Gallic acid	876.25±56.96
Singlet oxygen ($^1O_2$)	212.24 ± 17.34***	945.40 ± 38.32**	1155.36±19.50***	Lipoic acid	46.15 ± 1.16
Hypochlorous acid (HOCl)	833.46±112.81***	929.73±36.46***	349.88±90.66*	Ascorbic acid	235.96 ± 5.75
Lipid peroxidation	119.83 ± 14.59***	479.16±56.42***	219.36±73.35***	Trolox	6.75 ± 0.16

IC_{50} values of the extracts of PPL, PPS and PPF for free radical scavenging and lipid peroxidation inhibition. Each value represent mean ± SD (n=6)
*** p< 0.05; ** p< 0.01;*** p< 0.001; NS = Non significant.**

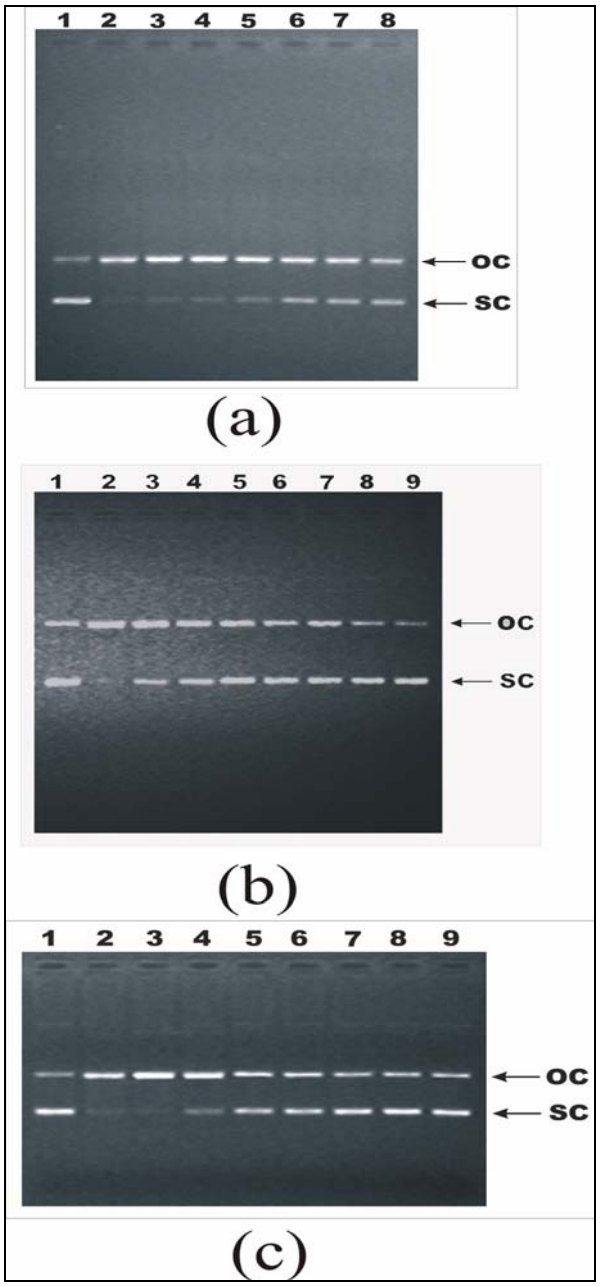

Fig. 12: DNA Strand Scission Assay

Protection effect of PPL, PPS and PPF extracts on DNA strand scission induced by H_2O_2 and Fe^{2+}. Lane 1, native pUC18 plasmid DNA as control; Lane 2, DNA + Fenton's reagent; Lane 3-8, DNA + Fenton's reagent + PPL (a) with increasing concentrations (0.5-3 µg/ml); Lane 3-9, DNA + Fenton's reagent + PPS (b) and PPF (c) with increasing concentrations (3-30 µg/ml).

Conclusions

Medicinal herbs are known to contain a variety of antioxidants. Numerous in vitro studies have shown that some phytochemicals are potent antioxidant, metal chelators or free radical scavengers which may account for their health-promoting properties. The present study aimed to evaluate the possible antioxidant activity of the P pinnata used widely in Indian indigenous systems of medicine. The present results confirm the antioxidant properties of these extracts and these effects were attributed to their phenolic and

flavonoid constituents. Overall, PPL showed more potent activity than PPF and PPS. From these results it can be suggested that PPL plays significant role in the antioxidant activity of Pongammia pinnata. Further phytochemical work need to be done on these extracts including fractionation to isolate active constituent and subsequent pharmacological evaluation.

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