Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 4, Suppl 2, 2011 ISSN - 0974-2441

Research Article

ANTIOXIDANT, DNA DAMAGE PROTECTION AND ANTIBACTERIAL EFFECT OF P*SORALEA CORYLIFOLIA*

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Received: 6 September 2011, Revised and Accepted: 5 October 2011

ABSTRACT

Psoralea corylifolia is widely used in the Chinese and Indian system of medicine for the treatment of anxiety, mood elevation and varied diseases, the antioxidant potential, DNA damage protection and its antibacterial effects was evaluated. The seed of the plant was studied for its antioxidant and phytochemical constituents. Sequential extraction was carried with different solvents with increasing polarity. The antioxidant activity was evaluated for free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, metal chelating activity and reducing power. The phytochemical constituents, proximate compositional analysis, DNA nicking assay using pBR322 plasmid DNA and antibacterial using Gram +ve and –ve strains were carried out. The results indicated that ethanol, methanol and water extracts showed significant antioxidant potency compared to other solvent extracts and also possess metal chelation and reducing power activity, protection against DNA damage and possess antibacterial activity. Hence it is suggested that seeds of *Psoralea corylifolia* has potential application in food systems as an antioxidant and probably in biological systems as a nutraceutical.

Key words: *Psoralea corylifolia,* DNA damage, antioxidant activity, anti-bacterial.

INTRODUCTION

Natural antioxidants can act on scavenging reactive oxygen species (ROS) which is preferred over synthetic antioxidants which can do so but some risks are involved/their safety is doubtful over the natural one. ROS exacerbate factors in cell injury and ageing process [1]. It is believed that higher intake of antioxidant rich foods is associated with decreased risk of degenerative diseases [2]. Natural antioxidants like phenolic compounds, flavonoids which are secondary plant metabolites present in food products of plant origin [3] can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes and also exhibit a wide range of biological effects, including anti-ageing, anti-mutagenicity, and protective effects on oxidative stress. Several studies have shown that plant derived antioxidant nutraceuticals, scavenge free radicals and modulate oxidative stress related degenerative diseases [4]. Apart from this, studies reveal that vegetables/plant sources contain several antioxidant nutrients in addition to vitamins C, E and carotenoids which contribute to their antioxidant capacity [5, 6].

Thus, efforts have been made to search for novel natural antioxidants from tea, fruits, vegetables, herbs and spices. *Psoralea corylifolia L.* (Fabaceae) has been used in Chinese and Indian traditional medicine for the treatment of various inflammatory diseases of the skin and to improve vitality. The seeds of this plant have been used as a tonic, to treat uterine hemorrhage, and as a coronary vasodilatory agent in Chinese traditional medicine. Several terpenoids and flavonoid constituents were isolated from this natural medicine [7]. *Psoralea corylifolia* seed extract possess immunomodulatory and anti-tumor properties [8]. Psoralidin and bavachalcone were isolated from *Psoralea corylifolia*, and its effects on osteoclast differentiation were evaluated with primary cultures of osteoclast precursor cells [9]. However, studies on the antioxidant potency of this herb are sparse. Hence the aim of the present study was to investigate the *in vitro* antioxidant, DNA damage protection and antibacterial effect of ethanol extract of *Psoralea corylifolia* seed (PCE).

MATERIALS AND METHODS

Chemicals

Butylated hydroxyanisole (BHA), nitroblue tetrazolium (NBT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide reduced (NADH), deoxyribose 4-dimethyl amino cinnamaldehyde, Catechin were purchased from M/s Sigma chemicals Co. (St. Louis, MO). Thiobarbituric acid (TBA), gallic acid, trichloroacetic acid (TCA), ethylenediamine tetra-acetic acid (EDTA), ascorbic acid and other chemicals were purchased from M/s Sisco Research Laboratories, Mumbai, India, pBR322 plasmid was purchased from Genei, Bangalore. All reagents procured were analytical grade.

Preparation of the powder and Extraction

Psoralea corylifolia seeds were purchased from reliable local suppliers. The plant was identified and authenticated by Dr. M.S Sudarshana (Associate Professor) at Department of Botany, University of Mysore, with the reference specimen and the voucher number was obtained (BOT-01-2010). The seed material were washed, dried in hot air oven at 40^0C and then finely powdered. The powder was used for extraction. Sequential extraction of the powder was done with different solvents with increasing polarity i.e. hexane, chloroform, ethyl acetate, acetone, methanol, ethanol and water. 30g of the powder was extracted in 300ml of the solvent in glass conical flask on a shaker for 24h at room temperature. The extract was filtered with Whatmann filter paper no. 1 and dried by flash evaporation/lyophilisation.

Preparation of the powder and Extraction

The proximate composition of *Psoralea corylifolia* was determined using the Association of Official Chemists (AOAC) method [10].The mineral content of the stem was analyzed by an atomic absorption spectrophotometer (model AAS Vario 6, Shimadzu, Japan). The vitamin C content was estimated by titrimetric method using 1, 2-dicholorophenol indophenol dye. Vitamin E and total carotenoids in the powdered extract of *Psoralea corylifolia* were estimated as prescribed by Sadasivam and Manickam [11].

Total phenolic contents

Total phenolic content was estimated spectrophotometrically by Folin-Ciocalteau method [12] with some modifications. To 0.1ml extract of different concentration was made up to 3ml with double distilled water, to this 0.5ml of folin-Ciocalteau reagent was added and allowed to stand at room temperature for 10min, to the mixture 2ml of 7% sodium carbonate was added and kept in boiling water bath for 1min, cooled, after which the absorbance was read at 650nm against blank. The concentration was calculated using gallic acid as standard, and the results were expressed as milligram gallic acid equivalents per gram extract.

Flavonoids

The determination of flavonoids was carried out according to Delcour and Varebeke [13]. To 1ml of different concentration of the extract, 5ml chromogen reagent (1 g 4-dimethyl amino cinnamaldehyde dissolved in a cooled mixture of 250ml of concentrated HCl and 750ml of methanol, made upto 1L methanol) was added, after 10min, the absorbance was measured at 640nm against a blank consisting of water instead of extract

and the flavonoids content was calculated with (±) catechin and the concentration was expressed as (±) catechin equivalents.

Tannins

A 0.5g sample was weighed and boiled with 75ml of water for 30min. The contents were centrifuged at 800 X g for 20min and the supernatant was made up to 100ml in a volumetric flask using water. Aliquots were treated with Folin-Denis reagent and absorbance was measured at 700nm [11].

Antioxidant activity by free radical scavenging method

2,2 -diphenyl 2-picrylhydrazyl (DPPH) radical was used for the determination of the antioxidant activity of *Psoralea corylifolia* extract. The method described by Hatano et al., [14] was used with slight modifications. Briefly, 3ml of the reaction mixture contained 0.1ml of different concentration of the extract (1-10mg/ml) and aliquot of DPPH solution (0.5ml, 50mg/100ml) in methanol. Reaction mixture without the extract was used as control. The mixture was shaken vigorously and allowed to stand in dark for 45min. The antioxidant scavenging activity was measured as the decrease in absorbance of DPPH at 515nm against a blank and calculated using the following formula.

$$\text{Scavenging Effect (\%)} = [(A0 - A_1) / A_{0]} \text{ X } 100$$

Where, A_0 is the absorbance of control and A_1 is the absorbance of sample. All the values expressed are the mean values carried out in duplicates. BHA was used as a positive control.

Superoxide anion scavenging activity

Measurement of superoxide radical scavenging activity of the PC extracts was done based on the method described by Liu et al. [15].The superoxide is generated in 3ml of tris HCl (16mM pH8.0) containing 1ml of NBT (50µm) solution, 1ml NADH (78µM) solution and sample solution of different extracts in a concentration of 1-10µg/ml .The reaction was started by adding 1ml of PMS solution (10um) to the mixture. The reaction mixture was incubated at 25°C for 5 min. and the absorbance at 560nm was measured against the blank samples. L-ascorbic acid was used as a control and BHA was used as positive control.

$$\text{\% Inhibition} = [(A_{control} - A_{sample}) / A_{control}) \text{ x100}]$$

Where $A_{control}$ is the absorbance of the L-ascorbic acid and A_{sample} the absorbance of *Psoralea corylifolia* or standards. The data at each point were the average of two measurements.

Hydroxyl radical scavenging activity

Non-site specific hydroxyl radical mediated 2-deoxy-D-ribose degradation

The assay was performed as described by Halliwell et. al. [16] with slight modification. All solutions were freshly prepared. The reaction mixture contained 100µl of 28mM 2-deoxy-D-ribose (dissolved in KH_2PO_4- K_2HPO_4) phosphate buffer pH 7.4, 500µl solution of various concentration of the extracts (100µg-1000µg/ml), 200µl of 200µM $FeCl_3$ and 1.04mM EDTA (1:1 v/v), 100µl of H_2O_2 (1.0 mM) and 100µl ascorbic acid (1.0mM). After an incubation period of 1hr. at 37°C the extent of deoxy ribose degradation was measured by the TBA reaction. 1ml of TBA (1% in 50mM NaOH) and 1ml of TCA were added to the reaction mixture, tubes were heated at 100° C for 20 min. After cooling the absorbance was read at 532 nm against a blank (containing only buffer and deoxy ribose). The % inhibition was calculated by the formula

$$\text{Inhibition (\%)} = [(A_0 - A_1) / A_{0]} \text{ X } 100$$

Where, A_0 is the absorbance of control and A_1 is the absorbance of sample. All the values expressed are the mean values carried out in duplicates. BHA was used as a positive control.

Site-specific hydroxyl radical mediated 2-deoxy-D-ribose degradation

The ability of the extracts to inhibit site specific hydroxyl radical mediated degradation was also carried out to understand its role as a metal chelator. The method was the same as described above except that buffer replaced EDTA.

Reducing Power

The reducing power of the prepared PC extract was determined according to method of Oyaizu [17]. Briefly various concentration of the extract (0-5mg/ml) and the standard compound were mixed with PO_4 buffer (2.5ml, 0.2mol/L, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$], (2.5ml, 1%). The mixture was incubated at 50°Cfor 20min. To the mixture 10% TCA was added to the mixture, which was then centrifuged at 3000rpm for 10min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml), $FeCl_3$ (0.5ml, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. α- tocopherol was used as a positive control.

Metal chelating activity

The assay was determined as described by Dinis, Madeira & Almeida [18]. Briefly the extracts were added to a solution of 2mmol/L $FeCl_2$ (0.05ml). The reaction mixture was initiated by the addition of 5mmol/L ferrozine (0.2ml), the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562nm. The % of Inhibition of ferrozine-Fe^{2+} complex was given below formula

$$\text{\% Inhibition} = [(A_0 - A_1) / A_0] \text{ X } 100$$

Where, A_0 was the absorbance of the control & A_1 was the absorbance in the presence of the samples of PC extracts and standards. The ability of the extract to chelate ferrous ion was compared with the standard BHA.

In vitro DNA damage protection assay: Plasmid DNA nicking assay
DNA nicking assay was performed using pBR322 plasmid DNA as per the method described by Lee *et al* [19]. A mixture of 10µl of PC extract of different concentration (0.125-2µg) and plasmid DNA (0.5µg) was incubated at room temperature followed by the addition of 10µl of Fenton's reagent (30mM H_2O_2, 50µM ascorbic acid and 80µM $FeCl_3$). The final volume of the mixture was made up to 20µl and incubated for 30 min at 37°C. The DNA samples was electrophoresed on 1% agarose gel and band intensities were measured using Easy win 32 software from Herolab (Germany).

Antibacterial Assay

The methanol extract of PC was assayed for five strains of Gram +ve bacterial strains viz. *Bacillus cereus* ATCC1457, *Bacillus subtilis* DFR13, *Enterococcus faecalis* (lab culture), *Listeria monocytogenes* ATCC13932, *Staphylococcus aureus* and Gram negative bacterial strains viz. *Aeromonas hydrophius* MTCC646 *Escherichia coli* ATCC14028, *Psudomonas aeroginosa* DFR219, *Salmonella enteritidis* ATCC14028, *Shigella boydii* MTCC1457, *Vibrio parahaemolyticus* ATCC01: K25. Antibacterial activity was determined by agar-well diffusion method as described by Booth [20]. All the microbes were inoculated in nutrient broth and were incubated at 37°C for 24 hours. Ethanol, methanol and water extract was dissolved in dimethyl sulphoxide (DMSO) to a final concentration of 100µg. Further sterilized by filtration through a 0.22 µm membrane filter. Hundred micro litre of suspension containing 10^8 colony forming units ml^{-1} of bacteria spread on nutrient agar medium and wells (8 mm) were punched out using sterile cork borer. 100 µL of different concentrations of the extracts were filled into each well. Petri dishes were incubated at 37°C for 24 hrs. At the end of the incubation period, inhibition zone formed on the medium were measured in millimetre (mm). The assays were performed in duplicate and inhibition zone was also compared with ampicillin 10µg/disc as a reference standard.

Statistical analysis

The statistical processing of the data obtained from all studies is expressed as means ± standard deviation (SD) of two separate experiments using the computer programme Excel.

RESULTS AND DISCUSSION

In the present study different solvents viz. hexane, chloroform, ethyl acetate, acetone, methanol, ethanol, and water were used sequentially for extracting antioxidant compounds from the seed of PC. Among the various seed extracts obtained, the yield of ethanol extract was found to be high as compared to other extracts (Table 1). The proximate composition of the seed is shown in Table. 2. Protein content of this was found to be 29.1%, which is higher than almost all grams and pulses (moth beans 23.6%) but low in comparison with soyabean (43.2%). The potassium content was 440 mg/100g which is quiet low in comparison with lotus stem (dry) (3007mg/ 100g). However, it is higher than that of lettuce (34mg/100g) [21], a leafy vegetable with lowest content of potassium.

Table 1: Percentage yield of Psoralea corylifolia in different solvents

Sample Extracts	% yield	Total Polyphenols mg GAE/g extract	Flavonoids mg CE/g extract	Tannin mg/g extract
Hexane	3.4±0.21	6.25±0.91	1.01±0.12	2.21±0.31
Chloroform	8.3±0.51	12.93±0.66	3.90±0.24	10.24±1.54
Ethyl acetate	6.9±0.91	24.74±1.24	6.41±0.87	16.63±3.24
Acetone	4.7±0.75	16.15±2.35	4.24±0.52	12.32±2.47
Methanol	9.8±0.62	36.24±1.32	9.25±1.02	30.25±1.24
Ethanol	14.2±0.43	41.51±3.21	10.34±2.36	36.14±1.58
Water	7.6±0.37	29.62±1.54	7.13±0.78	21.17±1.10

Table 2: Proximate composition, vitamins and mineral content of *Psoralea corylifolia* seeds

Moisture	(%)	5.10±0.06
Protein	(%)	29.10±2.50
Fat	(%)	7.50±1.40
Carbohydrates	(%)	32.43±2.45
Ash	(%)	8.96±0.70
Crude Fibre	(%)	16.42±3.20
Vitamin C	(mg/100g)	18.15±0.08
Vitamin E	(mg/100g)	155.50±0.23
Total Carotenoids	(mg/100g)	223.12±7.78
Iron	(mg/100g)	69.68±1.32
Zinc	(mg/100g)	1.41±0.02
Sodium	(mg/100g)	38.01±2.31
Potassium	(mg/100g)	440.24±9.21

Table 3: Antibacterial activity of *Psoralea corylifolia* extracts against Gram (+) ve and Gram (-) ve bacterial strains

Microorganisms	*Psoralea corylifolia* extract - Inhibition zone (mm)			Antibiotics Inhibition zone (mm)
	Ethanol 100 µg	Methanol 100 µg	Water 100 µg	Amphicillin (10 µg/disc)
*Bacillus cereus (*ATCC1457)	19.0±0.2	15.0±0.3	18.0±0.2	15.5±0.2
*Bacillus subtilis (*DFR 13)	18.0±0.1	13.0±0.2	0.00	17.5±0.1
Enterococcus faecalis (lab culture)	16.0 ±0.2	16.0±0.2	0.00	21.0±0.2
*Listeria monocytogenes (*ATCC1393)	23.0 ±0.5	19.0±0.2	09.0 ±0.5	23.5±0.1
Staphylococcus aureus (lab culture)	16.0 ±0.5	17.0±0.5	09.0 ±0.5	22.5±0.1
Aeromonas hydrophius (MTCC646)	25.0 ±0.3	22.0±0.2	0.00	06.0±0.1
*Escherichia coli (*ATCC14028)	11.0 ±1.0	10.0±0.2	00.0	02.5±0.1
Psudomonas aeroginosa (DFR219)	0.00	0.00	0.00	08.0 ±0.1
*Salmonella enteritidis (*ATCC14028)	0.00	0.00	0.00	01.0±0.2
*Shigella boydii (*MTCC1457)	0.00	09.0±0.5	0.00	09.0±0.2
Vibrio parahaemolyticus (ATCC01:K25)	12.0 ±0.5	10.0 ±0.2	15.0±0.2	21.0±0.1

Polyphenols, flavonoids and Tannins

Natural antioxidants not only protect lipids from oxidation, but also provide health benefits associated with preventing damage due to biological degeneration and it is well-known that plant phenolics are highly effective free radical scavengers and antioxidants [22] due to their hydrogen donating ability. In general, antioxidant activities of plant are often explained with respect to their total phenols, flavonoids tannin content and antioxidant vitamins. Table 1gives the total polyphenols, flavonoids, tannins from different solvent extracts, which followed the order: ethanol > methanol > water > acetone > ethyl acetate > chloroform > hexane. Similarly flavonoids and tannin content in the extracts was found to be highest in ethanol followed by methanol, water, acetone, ethyl acetate, chloroform and hexane. Hence ethanol extract contained high levels of polyphenols, flavonoids and tannins.

DPPH free radical scavenging activity

The models of scavenging the stable DPPH radical of widely used method to evaluate the free radical scavenging ability of various samples [23]. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. Hence the DPPH-scavenging activities of the extract were taken as the parameter to check their antioxidant potential. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517nm induced by antioxidants. BHA is a food grade antioxidant, used here as reference standard. The extracts were able to reduce the stable radical DPPH to the yellow-coloured di-phenyl picryl hydrazine. The scavenging effect of the PCE and standards with the DPPH radical was found to be in the following order: BHA (95.6%) > ethanol (91.0%) > methanol (76.42%) > water (40.42%) > ethyl acetate (61.27%) > chloroform (46.24%) > hexane (65.93%) > acetone (31.05%) as given in Fig.1. The IC_{50} values (concentration of sample required to scavenge 50% free radical were calculated from regression equation. Regression equations were prepared from the concentrations of the extracts and percentage inhibition of free radical formation). IC_{50} values of the PCE were ethanol: 3.264µg/ml, methanol: 4.679 µg/ml, water: 5.194 µg /ml, ethyl acetate: 5.302 µg, chloroform 7.021 µg, hexane: 5.391 µg/ml, and acetone: 5.462 µg/ml. The data reveal that all these extracts possess the effect of scavenging free radical properties. However, the ethanol extract showed better radical scavenging activity than the other extracts.

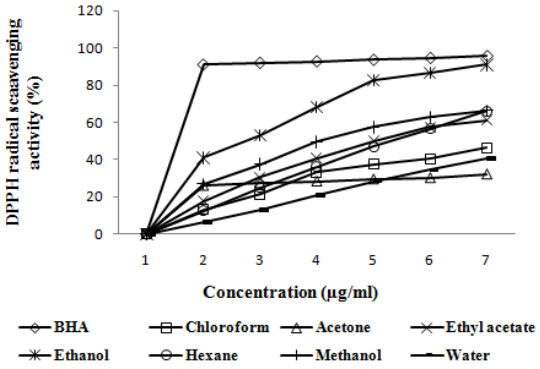

Figure 1: DPPH radical scavenging activity of *Psoralea corylifolia* extracts

Superoxide radical ($O_2^{\cdot-}$) scavenging activity

Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species. The superoxide radical is known to be produced *in vivo* and can result in the formation of H_2O_2 via dismutation reaction. Moreover, the conversion of superoxide and H_2O_2 into more reactive species, e.g., the hydroxyl radical, has been thought to be one of the unfavourable effects caused by superoxide radicals [24]. The scavenging activity of PCE on superoxide radicals enhanced with increase in concentration as given in Fig 2. The scavenging effect of the PCE and standards with the superoxide radical was found to follow the order: BHA (84.68%) > ethanol (81.78%) > methanol (80.88%) > water (80.09%) > ethyl acetate (70.40%) > chloroform (47.27%) > hexane (57.25%) > acetone (74.65%). IC_{50} values of the PCE were ethanol (1.97µg/ml), methanol (2.13µg/ml), water (2.24µg/ml), ethyl acetate (2.84µg/ml), chloroform (5.27µg/ml), hexane (3.93µg/ml), and acetone (2.51µg/ml). Ethanol extract was markedly a more potent scavenger of superoxide anion than other PCE's. However, the reference compound, BHA exhibited higher superoxide scavenging activity than the extracts.

Hydroxyl ($OH^{\cdot}$) radical-scavenging activity

Figs 3 and 4 show the effect of PCE's in deoxyribose scavenging assays (non-site and site-specific, respectively). It was observed that all the extracts were effective in scavenging the hydroxyl radicals in non-site specific assay as well as in site specific assay. However, the change was comparatively greater in the non-site specific than in the site-specific assay indicating their weak scavenging effect. However, the PCE-ethanol showed higher effectiveness in the non-site specific assay at the concentration of 500µg/ml (94.3%) than that of methanol (93.29 %) > water (41.03%) > ethyl acetate (87.54%) > chloroform (88.24%) > hexane (10.02%) > acetone (90.64%). IC_{50} values of the PCE were ethanol (1.69µg/ml), methanol (2.13µg/ml), water (2.24µg/ml), ethyl acetate (2.84µg/ml), chloroform (5.27µg/ml), hexane (3.93µg/ml), and acetone (2.51µg/ml). The site-specific assay of PCE showed that methanol at the concentration of 5µg/ml (85.79%), is comparatively higher than that of ethanol (75.84%), water (66.87%), ethyl acetate (68.04%), chloroform (59.42%), hexane (4.15%) and acetone (68.35%). IC_{50} values of the PCE were ethanol (1.97µg/ml), methanol (2.13µg/ml), water (2.24µg/ml), ethyl acetate (2.84µg/ml), chloroform (5.27µg/ml), hexane (3.93µg/ml) and acetone (2.51µg/ml).

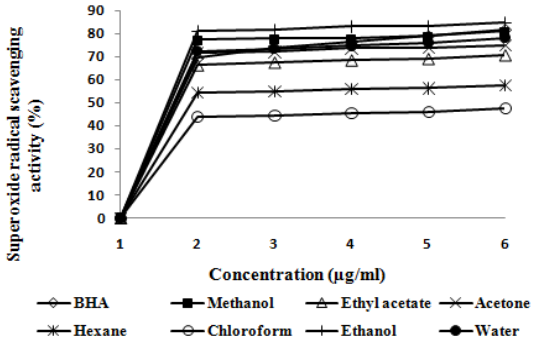

Figure 2: Superoxide radical scavenging activity of *Psoralea corylifolia* extracts

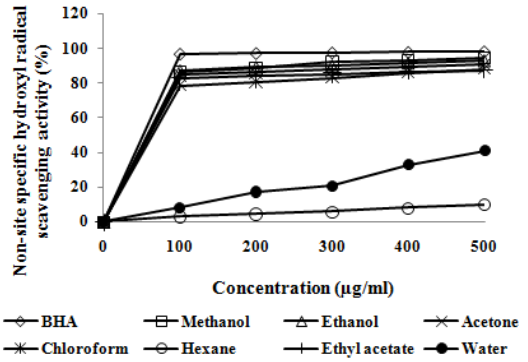

Figure 3: Hydroxyl radical scavenging activity (Non-site specific) of *Psoralea corylifolia* extracts

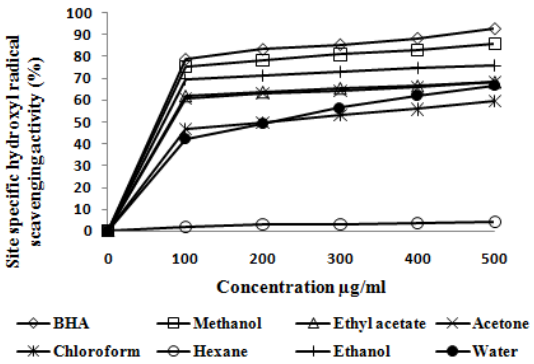

Figure 4: Hydroxyl radical scavenging activity (Site- specific) of *Psoralea corylifolia* extracts

Therefore the extracts were efficient in competing with deoxyribose for $OH^{\cdot}$ radicals produced free in solution from a Fe^{2+} - EDTA chelate [25]. Among the oxygen radicals specifically, the hydroxyl radical is the

most reactive which severely damages adjacent bio-molecules such as proteins, DNA, PUFA, nucleic acid and almost any biological molecule it touches. This damage causes aging, cancer and several diseases [26]. In addition, this radical species is considered as one of the quick initiators of the lipid oxidation process, abstracting hydrogen atoms from unsaturated fatty acids [27]. Therefore, the removal of hydroxyl radical is probably one of the most effective defenses of a living body against various diseases. Several workers [28] have employed this system to assess the biological activity of various natural plant- derived bio-molecules. IC_{50} values of the PCE were ethanol (1.97µg/ml), methanol (2.13µg/ml), water (2.24µg/ml), ethyl acetate (2.84µg/ml), chloroform (5.27µg/ml), hexane (3.93µg/ml) and acetone (2.51µg/ml).

Metal chelating activity

The method of metal chelating activity is based on chelating of Fe^{2+} ions by the reagent ferrozine which is a quantitative formation of a complex with Fe^{2+} ions [18]. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of the rate of colour of reduction therefore allows estimation of the chelating activity of the coexisting chelator [29]. Fig. 5 shows that the formation of the Ferrozine- Fe^{2+} complex is not complete in the presence of PCE-ethanol, methanol and water, indicating their capacity in chelating iron. The absorbance of Ferrozine- Fe^{2+} complex decreased linearly in a dose dependent manner. However, ethanol extract showed more chelating ability than methanol and water extracts. Still, the chelating ability was relatively lower than that of EDTA. In this assay, all these three extracts in comparison with standard EDTA interfered with the formation of ferrous complex with the reagent ferrozine, suggesting that they have chelating activity and capture the ferrous ion before ferrozine. EDTA is used for comparison as it is a known metal ion chelator. Metal chelating capacity of the extracts was significant as they reduced the concentration of the catalysing transition metal in lipid peroxidation [30].

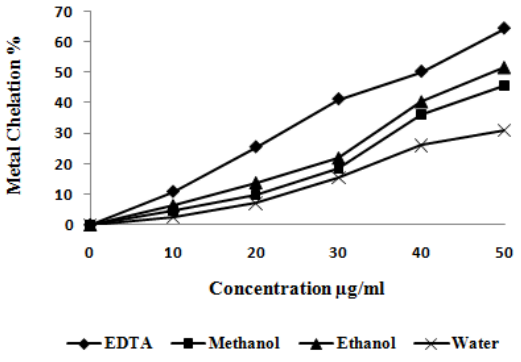

Figure 5: Metal chelation activity of *Psoralea corylifolia* extracts.

Reducing power

In the reducing power assay, the presence of reductants (antioxidants) in the sample would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's blue at 700nm. Fig.6. shows the reducing power of PCE-ethanol, methanol and water and the reference compound, α-tocopherol. Here, the ethanol was shown more reducing power than methanol and water. However, the reducing power of α-tocopherol was relatively more pronounced than that of sample extracts. Earlier authors [31, 32] have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating hydrogen atom [33]. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Our data on the reducing power of the extracts suggest that it is likely to contribute significantly towards the observed antioxidant effect. The reducing power of bioactive compounds has been reported to be associated with their antioxidant activity [34, 35].

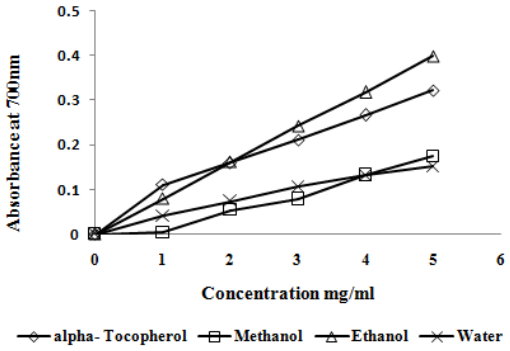

Figure 6: Reducing power activity of *Psoralea corylifolia* extracts.

In vitro DNA damage protection assay

The protective effect of extracts on oxidative DNA strand breakage was evaluated with pBR322 plasmid DNA. Kumar et al., [36] reported that oxidative modification of DNA has been suggested to contribute to aging and various diseases including cancer and chronic inflammation. The plasmid DNA was mainly of the super coiled form (band at the bottom) as in lane 1- DNA (pBR322 plasmid alone) and open circular form (band at the top) as in lane 2 – DNA and Fenton's reagent, where nicking was caused by hydroxyl radicals (Fig.7). Inhibitory effect of PC extracts on plasmid DNA nicking caused by hydroxyl radicals was seen with different concentration of extracts as in subsequent lane along with SOD. In presence of Fenton's reagent, the super coiled form decreased and converted into open circular form. Addition of extract significantly inhibited the formation of open circular form compared to positive control. The results revealed that ethanol and methanol extracts at 2µg level was potent in preventing the oxidative damage than water extract.

Antibacterial Activity

Since microorganisms become resistant over the period of time against antibiotics. Hence there is a need to explore the natural antibiotic sources for the prevention and treatment of several diseases. The present study represents the antimicrobial properties of ethanol, methanol and water. The PCE had inhibitory activity against the listed Gram +ve bacteria *Enterococcus faecalis, Listeria monocytogenes, Staphylococcus aureus* except *Bacillus cereus and Bacillus subtilis*. It also had inhibitory activity against the Gram –ve bacteria, *Aeromonas hydrophius, Escherichia coli, Psudomonas aeroginosa, Shigella boydii,* and *Vibrio parahaemolyticus* except *Salmonella enteritidis as* shown in Table 3.

CONCLUSION

For the purpose of characterizing antioxidant activity of plant extracts, it is desirable to subject it to tests that evaluates the range of activities such as 2, 2-diphenyl-2-picrylhydrazyl (DPPH), superoxide anion radicals scavenging, metal ion chelation, reducing power, hydroxyl radicals scavenging activity. Antioxidant- rich plant extracts serve as nutraceuticals that alleviate the oxidative stress and therefore slow down the degenerative diseases. This present study reports the antioxidant activity, DNA damage protection and antibacterial activities of *Psoralea corylifolia* extract. These activities of the extracts indicate as a potential source of natural antioxidants or nutraceuticals with potential application to reduce oxidative stress with consequent health benefits.

ACKNOWLEDGEMENT

The authors would like to thank Dr (Mrs.) Farhath Khanum, Head, Biochemistry and Nutrition Discipline and Dr A.S. Bawa, Director, DFRL, Mysore for their support and encouragement in carrying out the work.

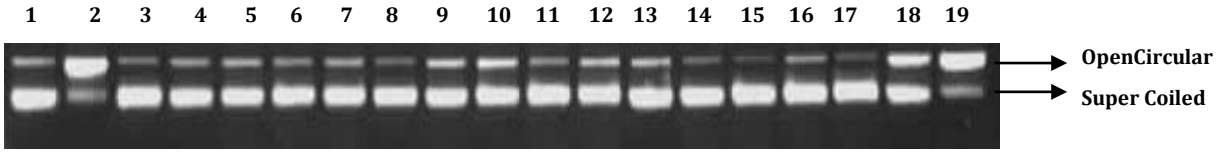

Fig.7: Inhibitory effect of *P.corylifolia* extracts on plasmid DNA nicking caused by hydroxyl radicals.

Lane 1- DNA (pBR322 plasmid alone); lane 2 – DNA and Fenton reagent ; Lane 3- DNA, Fenton reagent and 0.125µg methanol extract(ME); lane 4- DNA, Fenton reagent and 0.5µg ME; lane 5- DNA, Fenton reagent and 1µg ME; lane 6- DNA, Fenton reagent and 1.5µg ME; lane 7- DNA, Fenton reagent and 2µg ME; Lane 8- DNA,Fenton reagent and 0.125µg Ethanol extract(EE); lane 9- DNA, Fenton reagent and 0.5µg EE ; lane 10- DNA, Fenton reagent and 1µg EE; lane 11- DNA, Fenton reagent and 1.5µg EE; lane 12- DNA, Fenton reagent and 2µg EE; Lane 13- DNA,Fenton reagent and 0.125µg Water extract(WE); lane 14- DNA, Fenton reagent and 0.5µg WE; lane 15- DNA, Fenton reagent and 1µg WE; lane 16- DNA, Fenton reagent and 1.5µg WE; lane 17- DNA, Fenton reagent and 2µg WE; lane 18- DNA, Fenton reagent and SOD 2U; lane 19- DNA

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