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PHYTOCHEMICAL, ANTIBACTERIAL AND ANTIOXIDANT STUDIES ON SEED EXTRACTS OF *PSORALEA CORYLIFOLIA* LINN.

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ABSTRACT

Plant derived molecules are emerging as an important viable component to control plant and human diseases. The preparation of extracts of various parts of plants and development of suitable formulations for testing their antioxidant potency is being encouraged throughout the world. Higher plants have the capacity to produce a large number of phytochemicals with complex structural diversity known as secondary metabolites. Among the solvent extracts methanol and ethyl acetate extract showed activity against all the tested bacteria. The zone of inhibition for ethyl acetate extract ranged from 16-25mm and methanol extract ranged from 13-19mm. DPPH radical scavenging capacity of P. corylifolea showed that all the extracts have higher scavenging efficacy in the order petroleum ether (20µg/ml) > methanol (23µg/ml) > chloroform (25µg/ml) > ethyl acetate (29µg/ml) indicating higher activity compared to standard ascorbic acid (35µg/ml). The nitric oxide scavenging efficacy was strongest only in methanol extract with an IC_{50} value 27µg/ml. The promising antibacterial activity and antioxidant activity of P. corylifolea extracts as led to scientific validation of its medicinal properties.

KEY WORDS

Antibacterial activity; Antioxidant activity; Phytochemicals; P. corylifolea

INTRODUCTION

Plants are basis of sophisticated traditional medicine system that has been in existence for thousands of years. They provide a good source of starting material for the discovery of biologically active molecules that could be developed into new drugs. The plant chemicals involved in these purposes are largely the secondary metabolites which are derived biosynthetically from plant primary metabolites. Ethno medicine has been a very important source of drugs and many plant species contain substances with therapeutic activity. All drugs and medicinal agents were derived from natural substances and most of these remedies were obtained from higher plants. Ethno pharmacology investigations classically involved traditional healers, botanists, anthropologists, chemists and pharmacologists.

The non-nutritional exogenous biochemicals (alkaloids, flavonoids, andterpenoids) have therapeutic potentials. Most of the secondary plant compounds employed in modern medicine were first discovered through ethno botanical investigation [1].

Psoralea corylifolia Linn. grows throughout the plains of India, especially in the semi-arid regions. This plant is also widely distributed in the tropical and subtropical regions of the world, especially China and southern Africa [2].

It is useful in treatment of vomiting, piles, bronchitis, inflammation, anemia etc. It improves hair growth and complexion. It is used to treat vitiligo, leprosy and leucoderma internally. It also used for scorpion sting

and snake- bite. It is an effective invigorant against impotence, menstruation disorder, and uterine hemorrhage. The crude drug has been used for the treatment of enuresis, pollakiuria, painful feeling of cold in the waist and knees, and weak kidney. It is used in the treatment of debility and other problems related to kidney inefficiency, such as febrile disorders, low back pains, frequent urination, incontinence, and bed wetting. Seeds are sweet, bitter, acrid, and astringent. They impart vigor and vitality; improve digestive power and receptive power of mind. Seeds are antipyretic and alexiteric. *P. corylifolia* is a well-known nervine tonic in vata diseases [3].

MATERIALS AND METHODS

Plant material

Psoralea corylifolia plant seeds was collected from herbal shop in Mysore, Karnataka and used for the preparation of different solvent extracts. Fig .1

Test pathogens

Authentic cultures of human pathogenic bacteria viz., *Escherichia coli* (MTCC 7410), *Bacillus subtilis* (MTCC 121), *Bacillus cereus* (MTCC 1272), *Salmonella typhi* (MTCC 733), *Enterobacter aerogenes* (MTCC 7325) and *Staphylococcus aureus* (MTCC 7443) which served as test bacteria were obtained from Microbial Type Culture Collection, Chandigarh, India.

Extraction

Thoroughly washed samples of the test plant were dried in shade and powered using a warring blender. Solvent extraction was carried out using soxhlet extraction apparatus. Powdered leaf material (100 g) was placed in a porous thimble in the upper chamber. In the lower boiling flask, the extracting solvent (200 ml) was added. The flask was heated by thermostat-controlled heating mantle. The round bottom flask was filled successively with different solvents in the following order petroleum ether, chloroform, ethyl acetate and methanol based on polarity from low to high. The temperature was adjusted based on the boiling point of the solvents. The solvent was heated to reflux and extracted. The sample filled in the thimble was extracted with the non-polar to polar solvents successively till colorless extract was obtained on the top of the extractor. The solvent extract was concentrated separately under reduced pressure. After complete evaporation of the solvent, each of these solvent extracts was weighed and preserved in brown air tight bottle at 5 ^{o}C until further use.

Phytochemical analysis

Phytochemical analysis of petroleum ether, chloroform, ethyl acetate and methanol extracts was carried out for the detection of active secondary metabolite or different constituents such as tannins, alkaloids, flavonoids, terpenoids, steroids, carbohydrates, proteins and saponins. The dried extracts obtained by soxhlet extraction were reconstituted in methanol and 1 ml of each extract was subjected to standard phytochemical analysis according to the procedure described by Harborne [4].

Antibacterial activity

Antibacterial activity of the fractions and compounds was determined by agar cup diffusion method [5]. On NA medium, cups were made using 7 mm cork borer A 50 µl of 24 h bacterial culture containing 106 CFU/ ml of bacteria was spread on the solid media with a sterile swab moistened with the bacterial suspension. The fractions and compounds were reconstituted in methanol to a concentration of 100 mg/ ml. The 100 µl of fractions and compounds were placed in individual cup and methanol (100 µl) was placed in the central cup, which served as negative control. All the plates were incubated for 24 h at 37 ^{o}C and zone of inhibition if any around the cups were measured in millimeter. For each treatment, three replicates were maintained and all assays were repeated

Antioxidant activity

(A) DPPH Radical Scavenging Assay

Free radical- scavenging activity of the different extracts was measured in terms of hydrogen donating or radical-scavenging ability using stable radical DPPH as described by Blois method [6]. The stock solutions were prepared by dissolving 0.001 g of the extracts in one ml of DMSO. Different concentrations (20, 40, 60, 80 and 100 µg) of stock solution were made up to 2 ml with methanol. Solution of DPPH (0.1 mmol) in methanol was prepared and 1 ml of this solution was added to each of the above test solutions. The mixture was shaken vigorously and incubated for 30 min and then the absorbance was measured at 517 nm. Each experiment was run in triplicates and values are expressed as the mean ± Standard Deviation (SD). Ascorbic acid (AA) was used as standard parallel to the test compound and DMSO served as negative control. The capacity of the extracts to scavenge the DPPH radical was calculated using the following equation:

$DPPH^{\cdot}$ scavenging effect (%) = $[(A_c - A_b)/A_c \times 100]$

where, A_c is the absorbance of the negative control *i.e.* without sample; A_b is the absorbance of the sample.

(B) Hydroxyl Radical Scavenging Assay

Ability of the compounds to effectively scavenge hydrogen peroxide was determined according to the method of Ruch *et al*. [7], (1989) where it is compared with that of butylated hydroxyanisole (BHA) as standard. The hydroxyl radical ($OH^{\cdot}$) in aqueous media was generated through Fenton system**.** The stock solutions were prepared by dissolving 0.001 g of the extracts in one ml of DMSO. The assay mixture (5 ml) contained following reagents: safranin (11.4 µmol), EDTA–Fe (II) (40 µmol), H_2O_2 (1.76 µmol), the extract solution (4, 8, 12, 16 and 20 µl) and a phosphate buffer (0.067 mol, pH 7.4). The assay mixtures were incubated at 37 °C for 30 min in a water bath and the absorbance was measured at 520 nm. BHA was used as a standard compound for suppression of hydroxyl radical. Each experiment was run in triplicates and the values are expressed as the mean ± Standard Deviation (SD). The suppression ratio for $OH^{\cdot}$ was calculated using the following formula:

Suppression ratio (%) = $[(A_o - A_i)/A_o \times 100]$

Where A_0 = Absorbance of the control; A_i = Absorbance of the test compound/ extract.

(C) Nitric oxide scavenging assay

Test of nitric oxide radical scavenging capacity was determined according to the method of Green *et al.* [8]. The assay is based on generation of nitric oxide (NO) from sodium nitroprusside (SNP) and was measured by Griess reagent. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions which can be quantified by Griess Reagent. Sodium nitroprusside 10 mM (1.5 ml) in phosphate buffer (pH 7.4) was mixed with various concentrations (20, 40, 60, 80 and 100 µg) of 1 ml extract and the mixture was incubated at 25 ºC for 150 min during which sodium nitroprusside spontaneously generates nitric oxide. After incubation, 1.5 ml Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride) were added. The reaction mixture was incubated again at room temperature for 30 min. The final volume (4 ml) of the test solution was made-up with the phosphate buffer (pH 7.4). The absorbance of the solution was measured at 546 nm against the blank and IC_{50} values were calculated. AA was used as a standard compound in this experiment. Each experiment was run in triplicates and values are expressed as the mean ± Standard Deviation (SD). The scavenging per cent was calculated using the following formula:

Nitric oxide$^{\cdot}$ scavenging effect (%) = $[(A_c - A_t)/A_c \times 100]$

A_c= absorbance of control

A_t= absorbance in presence of the sample of extract

RESULTS AND DISSCUSSION

Antibacterial activity of solvent extracts of *Psoralea corylifolia*

The four solvents *viz.* petroleum ether, chloroform, ethyl acetate and methanol were used for extraction. The *Psoralea corylifolia* plant screened for antibacterial activity against human pathogenic bacteria. Among the solvent extracts methanol and ethyl acetate extract recorded activity against all the tested bacteria. The zone of inhibition for ethyl acetate extract ranged from 16-25 mm and methanol extract ranged from 13-19 mm. chloroform extract showed least activity. While petroleum ether extract did not show activity against any of the tested bacteria. Methanol and ethyl acetate extract showed maximum activity against *Bacillus cereus, Bacillus Subtilis, E. coli*, *Enterobacter, salmonella typhi, staphylococcus aureus* and chloroform showed least activity against *Enterobacter*.

Antibiotic streptomycin recorded a zone of inhibition in the range of 23 mm to 30 mm against *Bacillus cereus, Bacillus subtilis, E.coli, Enterobacter, Salmonella typhi, staphylococcus aureus*. The activity recorded by the extracts is slightly lesser than the antibiotics indicating the potency of the extract.

Antioxidant activity of *Psoralea corylifolea*

DPPH radical scavenging capacity of *P. corylifolea* showed that all the extracts have higher scavenging efficacy in the order petroleum ether (20 µg/ml) > methanol (23 µg/ml) > chloroform (25 µg/ml) > ethyl acetate (29 µg/ml) indicating higher activity compared to standard ascorbic acid (35 µg/ml). Results of hydroxyl scavenging assay of *P. corylifolea* showed that petroleum ether extract were most active with IC_{50} values 20µg/ml and 27 µg/ml, respectively. Methanol extract showed moderate potency of scavenging activity at 37µg/ml, but chloroform has very least activity at 84 µg/ml which is less than that of standard ascorbic acid (39 µg/ml). So, all the plant extracts except chloroform has better scavenging activity compared to

ascorbic acid. The nitric oxide scavenging efficacy was strongest only in methanol extract with an IC_{50} value 27 µg/ml. Both petroleum ether and ethyl acetate extracts showed moderate scavenging activity at 54 µg/ml. Chloroform extract showed least activity (75 µg/ml) compared to ascorbic acid (31µg/ml) (Table 2).

Pytochemical analysis of solvent extracts of *Psoralea corylifolia*

The result of phytochemical analysis of *Psoralea corylifolia* revealed the presence of alkaloids, flavonoids, terpenoids, tannins, steroids and proteins in methanol extracts alkaloids, flavonoids and proteins were present in petroleum ether and ethyl acetate extract. Alkaloids are present in chloroform extract. The results of phytochemical analysis of *Psoralea corylifolia* are given in Table 3.

Table 1. Antibacterial activity of solvent extract of *Psoralea corylifolia*

Bacteria	Solvent control	PE	CHL	EA	ME	Streptomycin
Bacillus cereus	-	-	-	20.0±1.1	13.3±0.57	29.6±0.57
Bacillus subtilis	-	-	-	22.6±1.1	13.6±0.57	25.3±0.57
Escherichia coli	-	-	-	16.3±0.57	12.6±0.57	26.6±0.57
Enterobacter aerogens		-	14.6±0.5	18.3±0.57	17.6±0.57	-
Salmonella typhi	-	-	-	19.6±0.57	14.6±0.57	-
Salmonella aureus	-	-	-	19.6±0.57	14.6±1.1	30.3±1.15

PE: petroleum ether, CHL: chloroform, EA: ethyl acetate, ME: methanol extracts

Table 2: The IC_{50} values of DPPH radical, hydrogen peroxide and nitric oxide radical scavenging by different extracts of *Psoralea corylifolea*

Solvent extract	IC_{50} (µg/ml)		
	DPPH	H_2O_2	NO
Petroleum ether	20±0.27	20±0.05	54±0.70
Chloroform	25±0.14	84±0.98	75±0.33
Ethyl acetate	29±0.49	27±0.34	54±0.70
Methanol	23±0.80	37±0.34	27±0.23
Ascorbic acid	35±0.23	39±0.96	31±0.97

Table 3. Phytochemical analysis of solvent extracts of *Psoralea corylifolia*

Phytochemical compounds	Extracts			
	PE	CF	EA	ME
Alkaloids	+	+	+	+
Flavonoids	+	-	+	+
Terpenoids	-	-	-	+
Tannins	-	-	-	+
Steroids	-	-	-	+
Carbohydrates	-	-	-	-
Proteins	+	-	+	+
Saponins	-	-	-	-

PE: petroleum ether, CHL: chloroform, EA: ethyl acetate, ME: methanol extracts

(+)-Present, (-) Absent

Fig. 1 *Psoralea corylifolia plant and seeds*

The plants are the rich source of biomolecules. The use of botanicals and antimicrobial agents of plant origin is time honored practice for plant and human diseases. The necessity to develop a non-toxic, safe and biodegradable agent alternative to synthetic bactericides and antibiotics in recent years has led to a concentrated effort for developing new sources from plant parts.

Thus, the present research was focused on the above considerations and comprises the antibacterial activity of *Psoralea corylifolia* against human pathogenic bacteria where the soxhlet extract of ethyl acetate and methanol seed extract of *Psoralea corylifolia* showed the good activity against all the tested bacteria. Antibacterial activity of seed extract of *Psoralea corylifolia* was also reported by Bhawya and Anilkumar [9], Chanda et al [10] and Moon and Moon [11], against the tested bacteria. There are very less reports of antibacterial activity of ethyl acetate extract of *Psoralea corylifolia* but in the present study ethyl acetate extract has shown activity against all the tested bacteria with the inhibition zone ranging 16-22 mm.

DPPH, hydroxyl radical and nitric oxide scavenging methods are selected for the determination of antioxidant activity in the current study. The scavenging ability of the samples is the measure of antioxidant activity. In DPPH method, a stable radical is used as a substrate to evaluate the antioxidant activity. All the extracts of *P. corylifolea* have good scavenging activity at concentrations lower than that of standard.

Hydroxyl radicals are the most reactive reduced form of dioxygen that are known to initiate cell damage. The scavenging effect of the extracts on hydroxyl radicals generated by Fenton´s system is quantified spectrophotometrically at 532 nm. The chloroform (22 µg/ml) and ethyl acetate (40 µg/ml) extracts have showed good scavenging activity compared to standard ascorbic acid (39 µg/ml). While petroleum ether, ethyl acetate and methanol extracts have showed good scavenging activity with IC_{50} values ranging from 20 – 37 µg/ml.

Nitrite oxide radical scavenging activity was determined by the ability of the extract to inhibit the formation of nitrite ions that can be produced by the interaction of nitric oxide generated by sodium nitroprusside in aqueous solution at physiological pH [12]. The reduced nitrite ions are quantified spectrophotometrically at 546 nm. Only methanol extract of *P. coryifolea* showed good scavenging activity with least IC_{50} values

The seeds of *P.corylifolia* have good scavenging capacity and it can be used for potential application in food systems as an antioxidant and in biological system as a neutraceutical [13]. The results indicate that *Psoraleacorylifolia* has better scavenging activity in all the performed methods

In the present study, phytochemical screening of methanol seed extract of *Psoraleacorylifolia* showed the presence of alkaloids, flavonoids, terpenoids, tannins, steroids and proteins. The ethyl acetate and petroleum ether extract showed the presence of alkaloids, flavonoids and proteins. Chloroform extract showed the presence of alkaloids only.

CONCLUSION

Currently there is growing interest of plant-based medicine all over the world making drug therapy for various pharmacological activities by natural products being the focus of interest in the recent years. In conclusion, the present study of a potent antibacterial activity against a band of pathogenic bacteria is exhibited by *Psoralea corylifolia* extracts especially methanol extract and good scavenging ability of all the extracts leads to scientific validation of the plant. A natural substance which is a part of daily diet nutritional supplement with antimicrobial and antioxidant property constitutes a new source of herbal drug.

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Conflict of Interest: Nil

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