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Phytochemical Screening and Antioxidant Activity of Ethanol Extract of *Psoralea corylifolia* seeds

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Abstract

Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom. Flavonoids of different classes exhibited various pharmacological and biological activities. The present study was undertaken to investigate the total flavonoids and antioxidant effect of *Psoralea corylifolia* seeds. The ethanol extract of *Psoralea corylifolia* seeds were prepared and performed its phytochemical screening. The total flavonoids and polyphenol were investigated to quantify the presence of polyphenol compounds. The 2, 2-diphenyl-1-picryl-hydrazil stable radical (DPPH) and Superoxide scavenging radical were used to determine extract antioxidant activity. The concentrations of flavonoids and polyphenol in ethanol extract of *Psoralea corylifolia* seeds were 60.63 QE. mg/gm and 74.35 GAE mg/gm respectively. The extract exhibited the strongest antioxidant activity with the lowest IC50 - value for DPPH and Superoxide scavenging. The IC50 value for DPPH and Superoxide scavenging were 166.61 µg/ml and 177.69 µg/ml respectively. The strongest antioxidant activity of ethanol extract could be due to the presence of flavonoids and phenols.

1 Introduction

Psoralea corylifolia L. commonly known as 'Babachi'. Belongs to family Fabaceae (Leguminosae). Its other common names are Malay Tea, Cot Chu, Ku Tzu Malaysia, Scurfpea, Malaysian Scurfpea, Po Ku Chih, Pha Cot Chi. It is an erect annual herb, 30-180 cm. high, found almost throughout India. *Psoralea corylifolia* grows as winter season weed. Leaves are broadly elliptic, arranged in racemes. incisodentate, flower yellow or bluish purple and seeds are smooth, adhering to the pericarp, dark brown and elongated[1] in dense axillary, long peduneled heads, pods are small 3.5-4.5 mm x 2.0-3.0 mm, ovoid- oblong, somewhat compressed, mucronate, dark chocolate to almost black. The plant is harvested for the drug industry when it sets into flowering in Nov-March. Seed setting commences around April -May and seed geminate immediately after shading. The major active constituents of *Psoralea corylifolia* are corylifols a-c (prenylfoavanoids) that are present in the seeds[2]. Other active compound such as psoralen, isopsoralen and neobavaislflavones are found in the dried ripe fruits[3] Daidzein (4:7 dihydroxuisoflavon) and genistein (4'5'7 trihydroxyisoflavon) are present in natural plants of *Psoralea corylifolia* as well as *in-vitro* cultures[4]. Other active constituents have since been identified, including neoba-vaislfloavone, borachin, Bavaislfavooz, bavachalcone, bavachromene psoralidin, corylifolinin, barachini psoralenoside, isopsoralenoside and coumarins[5, 6] , have been isolated from this plant.. A number of chemical constituents, including flavonoids and coumarins, have been isolated from this plant. Since a number of prenylflavonoids and related compounds were isolated from *Psoralea corylifolia*[2, 7]. The plant species is expected to be a resource of lead compounds for new anti-MRSA drugs. Previous studies reported the presence of several new and known compounds like, furanocoumarins[3], prenyl flavonoids[2], aromatic terpenoids and chromenes[9]. Several chemical compounds were identified and documented from the *Psoralea corylifolia* including flavonoids (bavachalcone, bavachinin, bavachin, corylin, and 6-prenylnaringenin etc), coumarins (psoralidin, psoralen, isopsoralen and angelicin) and meroterpenes (bakuchiol and 3-hydroxybakuchiol)[10].

Almost the plant is used by the Indian traditional system of medicine for the treatment of various skin diseases in human being. In particularly, the seed of *Psoralea corylifolia* has significant medicinal properties. The seeds are used in indigenous medicine as laxative, aphrodisiac, anthelminitic, diuretic and diaphoretic in febrile conditions. The seeds have been specially recommended in the treatment for leucoderma, leprosy, psoriasis and inflammatory diseases of the skin[11]. The seed extracts inhibits the growth of *Staphylococus citreus, S. aurues* and *S. albus* including strains resistant to penicillin's. The seeds posses anthlemintic activity against earth worms, psoralen being the active principle. The essential oil shows a selective activity against the skin *Streptococci* and used in the treatment of skin affections. The seeds are used locally in the preparation of certain types of medicated oils and incense preparations. The root is useful in the caries of teeth[11]. The one-seeded fruits are highly regarded as an aphrodisiac and tonic to the genital organs. The seed is anthelmintic, antibacterial, aphrodisiac, astringent, cardiac, cytotoxic, deobstruent, diaphoretic, diuretic, stimulant, stomachic and tonic. It is used in the treatment of febrile diseases, premature ejaculation, impotence, lower back pains, frequent urination, incontinence, bed wetting etc. The seed extracts have been reported to have antiplatelet[12], antitumor, immunomodulatory properties[13] *in vitro* antimicrobial[14] and antioxidant activities[15] have also been reported. The isoflavones daidzein and genistein have been reported to reduce the chances of breast and colon cancer[16]. The seeds are used locally in the preparation of certain types of medicated oils and incense. The plant yields a useful medicinal oleoresin, coumarins, flavonoids, and merotepenes such as psoralen, isopsoralen, neobavaislfoavone, bovachin, bavaislfoavone, bavachromene, psoralidin, corylifolinin, bavachinin, bavachalcone[17]. Therefore, the study was planned phytochemical screening and antioxidant activity of ethanol extract of *Psoralea corylifolia* seeds.

2 Materials and Methods

2.1 Plant collection

The mature seeds of *Psoralea corylifolia* were collected from the MPCST Bhopal (M.P.) India during month of January-February2016. Collected material were shade dried in an open air and grinded into powder for further use.

2.2 Preparation of the crude extracts

The powder of the *Psoralea corylifolia* seeds**,** were packed in the Soxhlet apparatus and extracted with ethanol, until the completion of extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely, and later dried in a desiccator. After that ethanol extract of seeds was kept in air tight container for further study.

2.3 Preliminary Phytochemical analysis

The extracts were analyzed by the following procedures. To test for the presence of the alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, volatile oils and reducing sugars.

2.3.1 Test for alkaloids

(a) Dragendorff's test: To 1 ml of the extract, add 1 ml of Dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

(b) Mayer's test: To 1 ml of the extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

(c) Hager's test: To 1 ml of the extract, add 3ml of Hager's reagent (Saturated aqueous solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids.

(d) Wagner's test: To 1 ml of the extract, add 2 ml of Wagner's reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

2.3.2 Test for saponins

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

2.3.3 Test for Glycosides

(a) Legal test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

(b) Baljet test: To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

(c) Keller-Killiani test: 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with5ml of chloroform. The chloroform layer is separated in a porcelein dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

(d) Borntrager's test: Add a few ml of dilute Sulphuric acid to1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinones glycosides.

2.3.4 Test for carbohydrates and sugars

(a) Molisch's test: To 2 ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

(b) Fehling's test: To 1 ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

(c) Benedict's test: To 5 ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

2.3.5 Test for tannins and phenolic compounds

(a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

(b) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.

(c) The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

2.3.6 Test for flavonoids

(a) The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light, formation of fluorescence indicates the presence of flavonoids.

(b) Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution formed, disappears on addition of an acid indicates the presence of flavonoids.

(c) Shinoda's test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

(d) The extract is treated with sodium hydroxide, formation of yellow colour indicates the presence of flavones.

(e) The extract is treated with concentrated H_2SO_4, formation of yellow or orange colour indicates flavones.

2.3.7 Test for steroids

(a) Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

(b) Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H_2SO_4. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

2.3.8 Test for triterpenoids

Noller's test: Dissolve two or three granules or tin metal in 2ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink colour indicates the presence of triterpenoids[18-21].

2.4 In vitro antioxidant activity

2.4.1 Total flavonol content

Flavones and flavonols contents were analyzed by the colorimetric method. 9.8 ml of the prepared extract was mixed with a 10% solution of aluminum chloride (200 µl). After 30 min, absorption was measured at a 425 nm wavelength. The amount was calculated using quercetin calibration curve. The results were expressed as the quercetin equivalent (QE) mg per 100 ml of the sample.

2.4.2 Total polyphenol content

Total polyphenol content was determined using colorimetric method. 2.0 ml of the prepared extract was oxidized using Folin - Ciocalteu reagent (400 µl), and sodium carbonate solution (75 g/l) was then added to the reaction mixture to reach a 10.0 ml volume. After 2 h, the suspension was centrifuged for 10 min at 5000 rpm, and absorption was measured at a 760 nm wavelength. The amount was calculated using the gallic acid calibration curve. The results were expressed as gallic acid equivalent (GAE) mg per 100 ml of the sample.

2.4.3 Hydrogen-donating activity

This assay was used in many studies for testing antioxidant activity. 2,2-diphenyl-1-picryl-hydrazil stable radical (DPPH) evidently offers a convenient and accurate method for titrating the oxidizable groups of natural and synthetic antioxidants. This assay was based on the reduction of a methanolic solution of the colored free radical DPPH by free radical scavenger. The degradation of DPPH was evaluated by comparison with a control sample without hydrogen-donating compounds. The decrease in absorbance of DPPH at its absorbance maximum of 517 nm was proportional to the concentration of free radical scavenger added to DPPH reagent solution. Lower absorbance of reaction mixture indicated higher antioxidant activity. In this study, methanolic solution of DPPH (100 mM, 2.95 ml), 0.05 ml of extracts dissolved in methanol was added at different concentrations (50-250 µg/ml). Reaction mixture was shaken and after 30 min at room temperature, the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity (% AA). Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract, was calculated by the following equation.

% AA = 100 – {[(Abs sample – Abs blank) / AbsDPPH] x 100}.

2.4.4 Superoxide scavenging activity

Superoxide scavenging (SOD) was carried out by using alkaline Dimethyl sulfoxide (DMSO). Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use. Filtrate (200 ml) was added to 2.8ml of an aqueous solution containing nitrobluetetrazolium (56 mM), EDTA (10 mM) and potassium phosphate buffer (10 mM, pH 7.4). Sample extract (1 ml) at various concentrations (50-250 µg/ml) in water was added and the absorbance was recorded at 560 nm against a control in which pure DMSO has been added instead of alkaline DMSO[22-24].

3 Results

3.1 Phytochemical screening of extract

The phytochemical screening of *Psoralea corylifolia* seeds**,** powder of ethanol extracts demonstrated the presence of alkaloids, polyphenol, tannins, flavonoids and glycoside (Table1). The presence of these phytochemical components may be responsible for the various pharmacological activity of the plant leaves extract.

Table 1: Phytochemical analysis of ethanol extracts of *Psoralea corylifolia* seeds

Plant constituents	Ethanol extract
Alkaloids	+
Polyphenols	+
Carbohydrates	-
Saponins	-
Flavonoids	+
Tannins	-
Glycosides	-
Triterpenes	+
Steroids	+

-ive: absence of plant constituents, +ive: presence of plant constituents

3.2 In vitro antioxidant activity

3.2.1 Total flavonol content of extract

The concentration of flavonoids in ethanol extract of *Psoralea corylifolia* seeds was determined spectrophotometrically using aluminum chloride. The content of flavonoids was expressed in terms of quercetin equivalents. The content of flavonoids identified in the tested extracts is shown in table 2. The concentrations of flavonoids in ethanol extract of *Psoralea corylifolia* seeds, was 60.63 QE mg/gm.

3.2.2 Total polyphenol content of extract

The ethanol extract of *Psoralea corylifolia* was evaluated for investigation of the total phenolic content concentrations in extracts. The total phenolic content of ethanol extract of *Psoralea corylifolia* was found to be 74.35 GAE mg/gm (Table 2).

Table 2: Determination of total flavonol and polyphenol content of *Psoralea corylifolia* seeds

Extract	Total flavonol content (QE mg/gm)	Total polyphenol content (GAE mg/gm)
Ethanol extract	60.63 ± 0.66	74.35 ± 0.70

Values are mean ± SEM of triplicate determinations

3.2.3 Hydrogen-donating activity of extract

2,2-Diphenyl-1-picrylhydrazyl radical is a stable organic free radical with an absorption band at 517 nm. It loses this absorption when it accepts an electron or a free radical species, resulting in a visually noticeable discoloration from purple to yellow. Ethanol extract of *Psoralea corylifolia* seeds, strongly scavenged DPPH radical with the IC_{50} being 166.61 µg/ml (Table 3 & Fig 1). The scavenging was found to dose dependent.

Table 3: Free radical scavenging capacity of ethanol extracts of *Psoralea corylifolia* seeds

Concentration (µg/ml)	DPPH Scavenging %	
	EthanolExtract	Ascorbic Acid
50	20.11± 0.12	80.13±0.12
100	32.55±0.94	-
150	43.51±0.57	-
200	59.33±0.41	-
250	72.63±0.62	-
IC50	166.61	-

Values are mean ± SEM of six determinations

3.2.4. Superoxide scavenging activity of extract

As it is a reactive oxygen species, superoxide has some damaging properties that can be imposed to the cells and DNA and subsequently invites various diseases. Thus, a proposal has been established to gauge the comparative interceptive ability of the antioxidant extracts to scavenge the superoxide radical. Table 3 demonstrated the changes in the activity of SOD upon treatment with the extracts. The ethanol extract of *Spilanthes acmella* scavenged superoxide radical with the IC_{50} values of 177.69 µg/ml (Table 4 & Fig 2).

Table 4: Superoxide scavenging capacity of ethanol extracts of *Psoralea corylifolia* seeds

Concentration (µg/ml)	Superoxide scavenging%	
	Ethanol Extract	Ascorbic Acid
50	22.5±0.58	74.11±0.51
100	32.65±0.94	-
150	43.41±0.57	-
200	59.33±0.41	-
250	62.53±0.62	-
IC50	177.69	-

Values are mean ± SEM of six determinations

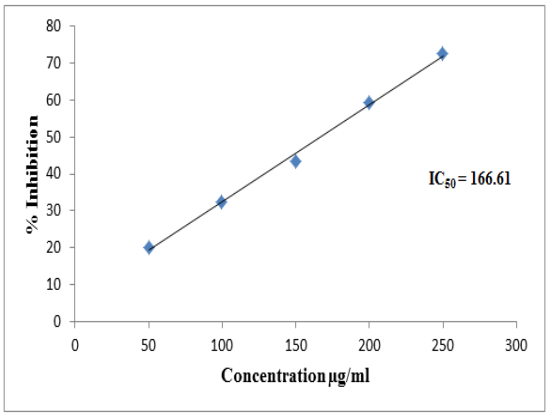

Fig 1: IC_{50} values of ethanol extract of *Psoralea corylifolia* seeds by DPPH scavenging capacity

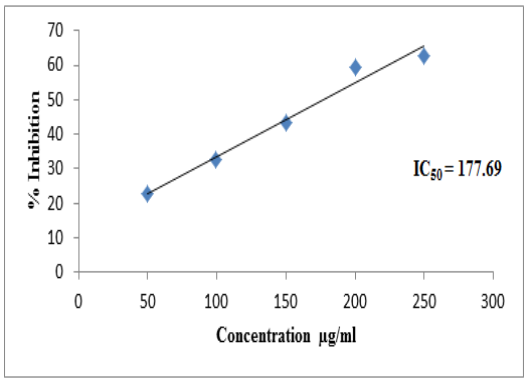

Fig 2: IC_{50} values of ethanol extract of *Psoralea corylifolia* seeds by superoxide scavenging capacity

4 Discussions

Preliminary phytochemical screening showed the presence of alkaloids, polyphenol, flavonoids Triterpenes and Steroids in ethanol extract of *Psoralea corylifolia* seeds**.** It was supported by earlier reports for the presences of phenols and flavonoids in the *Psoralea corylifolia* seeds. Flavonoids and phenols exhibit a wide range of biological activities, one of which is they have the properties of antioxidant activity. Being plant secondary metabolites, the phenolics or polyphenols are very important judging from the virtue of their antioxidant activities by chelating redox-active metal ions, inactivating lipid free radical chains, and avoiding the hydro peroxide conversions into reactive oxyradicals. The ethanol extract of *Psoralea corylifolia* seeds, illustrated the highest total flavonol and polyphenol content. The rich-flavonoid plants could manifest themselves as good sources of antioxidants that would assist in the enhancement of the overall antioxidant capacity of an organism and protection against lipid peroxidation. The total flavonoid content results were entirely synchronous with those of the total phenolic. It was successfully shown that samples with high level of phenolic content also contain flavonoids in great amount. The rich-flavonoid plants could be a good antioxidant source that would help increase the overall antioxidant capacity of an organism and guard it against lipid peroxidation[25]. The plant extract was able to reduce the stable free radical of DPPH to the yellow coloured diphenylpicrylhydrazine. This evidences that the *Psoralea corylifolia* seed, extract contains some active constituents that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. DPPH radical scavenging method has been proven to be good because its results are not affected by substrate polarity. Scavenging ability of the *Psoralea corylifolia* seed, extract shows the potential decrease in the concentration of DPPH. Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals and also very harmful to cellular components. It has been reported that flavonoids are found to be most effective antioxidants mainly because they can easily scavenge superoxide anions[26]. The results suggest that radical scavenging effect of extract was significant.

5 Conclusion

The findings of study indicates that *Psoralea corylifolia* seed extract contains large amounts of phenolic and flavonoid compounds and exhibits high antioxidant and free radical scavenging activities. The *in vitro* assays of antioxidant exhibit *Psoralea corylifolia* seed extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Further studies are in progress for the isolation of active constituents responsible for antioxidant activity.

6 Conflicts of Interests

We have not declared any conflict of interest.

7 Author's contributions

NGN carried out the complete experimental work. The entire work was carried out under the supervision of MS.

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