

## *In vitro* evaluation of antioxidant activity and estimation of total flavonoids in seeds of *Psoralea corylifolia* plant extracts

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### Abstract

The present study aims at *in-vitro* evaluation of anti oxidant activity of n-hexane and ethyl acetate extracts of seeds of *Psoralea corylifolia*. For evaluating this study 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method and reducing power method was used. For DPPH method Butylated Hydroxy Anisole (BHA) was used as a positive control and for Reducing power method ascorbic acid was used as a standard reducing agent. All the analysis was made with the use of UV-Visible Spectrophotometer. The content of total flavonoids was measured spectrophotometrically by using the aluminium chloride colorimetric assay where catechin was used as a standard. The extracts showed good DPPH radical scavenging activity and reducing power activity which was found to increase with the increasing concentration of the extracts. The total flavonoid content was found to be 73.09 mg CE/100g. Ethyl acetate extract of *Psoralea corylifolia* showed more prominent activity when compared to n-hexane extract which could be attributed to the presence of flavonoid content. The study could be further extended to isolate and characterize phytoconstituents from these extracts.

**Keywords:** *Psoralea corylifolia*, DPPH Method, Reducing power method, n-hexane extract, ethyl acetate extract, total flavonoid content

### Introduction

Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias [1]. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties [2].

Plants are potent biochemical factories and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plants based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, etc[3] i.e. any part of the plant may contain active components. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct [4]. Antioxidant-based drugs/formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke,

diabetes, Alzheimer's disease, and cancer have appeared during the last 3 decades [5].

This has attracted a great deal of research interest in natural antioxidants. Subsequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables has increased. Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger, and several Chinese medicinal plants extracts[6]. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, beta-carotene, and tocopherol are known to possess antioxidant potential [7].

Dry seeds of leguminous plant *Psoralea corylifolia* Linn. (syn: *Cullen corylifolium* Linn.) is one of the most popular Traditional Chinese Medicine and officially listed in Chinese Pharmacopoeia.[8] *P. corylifolia* is an annual herb growing throughout the plains of India.

The plant classification details are [9]

Kingdom: Plantae  
Division: Angiospermae  
Class: Dicotyledoneae

Order: Rosales  
 Family: Leguminosae  
 Subfamily: Papilionaceae  
 Genus: *Psoralea*  
 Species: *corylifolia* Linn.

Seeds contain phyto constituents like stigmasterol, triaconate, and  $\beta$ -sitosterol-D-glucoside [10]. The seeds also contain flavonoids, such as corylifolean, corylifolin, corylifolinin, [11] bakuchicin, psoralidin, isopsoralidin, bavachin, isobavachin, bavachinin, bavachalcone, isobavachalcone [12]. The plant is of immense biological importance, and it has been widely exploited since ages for its magical effect against several skin diseases, such as psoriasis, leukoderma, and leprosy [13]. In the present study the n-hexane and ethyl acetate extracts of *Psoralea corylifolia* seeds were screened for in-vitro anti oxidant activity using DPPH and reducing power method. The total flavonoid content of the plant was carried out using aluminum chloride colorimetric assay.

## Materials and Methods

### Chemicals

All the solvents used were of analytical grade. N-hexane, ethyl acetate, methanol, aluminium chloride was obtained from Merck. Butylated hydroxyl anisole (BHA), potassium ferricyanide, trichloro acetic acid, ferric chloride, ascorbic acid, were purchased from R&M chemicals. 1,1-Diphenyl picryl hydrazyl (DPPH) and catechin were purchased from Sigma-Aldrich chemical.

### Plant Material

Seeds of selected plant materials were collected from local areas of Hyderabad and further authenticated by botany department of Osmania University. Voucher specimens were deposited at the same department. Seeds were dried under shade for five days. Dried seeds were ground into a uniform powder using a pulveriser and stored in polythene bags at room temperature.

### Preparation of extracts

The shade dried plant materials were extracted successively with n-hexane, chloroform, ethyl acetate and ethanol using a soxhlet extractor. The extracts obtained were concentrated using rota evaporator and stored in a refrigerator.

### Phytochemical investigation

The preliminary phytochemical investigation of the extracts showed the presence of steroids and flavonoids in n-hexane and ethyl acetate extract respectively as major constituents. The details of the chemical tests are as follows [14].

### Test for sterols

Salkowski test : When few drops of concentrated sulphuric acid is added to the test solution, shaken and allowed to stand, lower layer turns red indicating the presence of sterols.

Liebermann Burchardt test: The test solution treated with few drops of acetic anhydride and mixed well. When concentrated sulphuric acid is added from the sides of the test tube, it shows a brown ring at the junction of the two layers and the upper layer turns green.

Sulphur test: Sulphur when added into the test solution, it sinks in it.

### Test for flavonoids

Ferric chloride test: Test solution with few drops of ferric chloride solution shows intense green colour.

Shinoda test: Test solution with few fragments of magnesium ribbon and concentrated hydrochloric acid, shows pink to magenta red colour.

Alkaline reagent test: Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.

Lead acetate solution test: Test solution with few drops of lead acetate solution (10%) gives yellow precipitate.

### Antioxidant assay

The antioxidant activity of plant extracts were determined by different in-vitro methods such as, the DPPH free radical scavenging assay and reducing power methods. The different extracts were dissolved in methanol at the concentration of 2mg/ml. All the assays were carried out in triplicate and average value was considered.

### DPPH Radical scavenging activity

According to the method [15],[16], a 0.2 ml of methanolic solution of plant extract samples at different concentration (50-250 $\mu$ g/ml) was mixed with 0.8 ml of Tris HCl buffer (100Mm, pH 7.4). One ml DPPH (500 M in methanol) solution was added to above mixture. The mixture was shaken vigorously and incubated for 30min in room temperature. Absorbance of the resulting solution was measured at 517nm UV-Visible Spectrophotometer (Systronics UV-Visible Spectrophotometer 117, INDIA). All the assays were carried out in triplicates with BHA (Butylated Hydroxy Anisole) as a positive control. Blank was prepared without the addition of DPPH and for control 0.2 ml of methanol (without plant extract) was added. Percentage of DPPH scavenging activity determined as follows.

% scavenging DPPH free radical =  $100 (1-AE/AD)$

Where AE is absorbance of the sample solution and AD is the absorbance of the DPPH solution with nothing added (blank, without extract).

Purified sample 2mg/ml of *Psoralea corylifolia* extracts were taken for antioxidant activity with a standard BHA (Butylated Hydroxy Anisole) antioxidant. Decreased absorbance of the reaction mixture



indicates stronger DPPH radical-scavenging activity. In this study-hexane and ethyl acetate extracts of *Psoralea corylifolia* seeds were used.

(b) Reducing power This was carried out as described previously [17,18]. A 1 ml of plant extract solution (final concentration 100-500 mg/l) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [ $K_3Fe(CN_6)$ ] (10g/l), then mixture was incubated at 50 degree C for 20 minutes. A 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, a 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml FeCl<sub>3</sub> (1g/l) and absorbance measured at 700nm in UV-Visible Spectrophotometer (Systronics UV-Visible Spectrophotometer 117, INDIA). As a control, ascorbic acid was used (final concentration 15 mg/ml).

Increased absorbance of the reaction mixture indicates stronger reducing power.

### Determination of total flavonoids

The total flavonoid content was measured by the aluminium chloride colorimetric assay[19].

### Method

#### Sample preparation

A ground, freeze dried sample of 0.5 g was weighted and flavonoid compounds were extracted with 50 ml 80% aqueous methanol on an ultrasonic bath for 20 min. An aliquot (2 ml) of the extracts was ultracentrifuged for 5 min at 14000 rpm.

#### Total flavonoid assay

An aliquot of extracts or standard solution of catechin(20,40,60,80 and 100 mg/l) was added to 10 ml volumetric flask containing 4 ml of dd H<sub>2</sub>O. To the flask was added 0.3ml 5% NaNO<sub>2</sub>. After 5 min, 0.3 ml 10% AlCl<sub>3</sub> was added. At 6th min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with dd H<sub>2</sub>O. The solution was mixed well and the 250 µg/ml of extract absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content of the plant was expressed as mg catechin equivalents (CE)/100g fresh mass. Samples were analysed in duplicates.

## Result and Discussion

### Antioxidant Assay

#### DPPH scavenging activity

The percentage of DPPH radical scavenging activity of n-hexane extract of *Psoralea corylifolia* presented in Table 1(a). The DPPH radical scavenging activity of the extract increases with increasing concentration, only 16.75% DPPH radical scavenging was present for 250µg/ml of extract. Nevertheless; it was 75.32% in the presence of 100 mg/l BHA. Although this plant extract shows lower scavenging activity in comparison to BHA. Plant extract exhibited

antioxidative potential and increased concentration of plant extract has shown increased antioxidative potential.

**Table 1a:** DPPH scavenging activity of n-hexane extract of *Psoralea corylifolia* seeds

Sample( µg/ml)	Absorbance at 517 nm			% of activity	
	OD	BHA( mg/l)	OD	Sample	Standard
50	1.078	2	0.873	2.88	21.35
100	1.041	4	0.692	6.21	38.83
150	1.012	6	0.482	8.82	56.58
200	0.963	8	0.358	13.24	67.66
250	0.924	10	0.274	16.75	75.32

OD: Optical density

Control OD AT 517 nm-----1.110

The percentage of DPPH radical scavenging activity of ethyl acetate extract of *Psoralea corylifolia* presented in Table 1(b).

**Table 1b:** DPPH scavenging activity of ethyl acetate extract of *psoralea corylifolia* seeds

Sample (µg/ml)	Absorbance at 517 nm			% of activity	
	OD	BHA (mg/l)	OD	Sample	Standard
50	1.038	2	0.894	6.4	19.4
100	0.962	4	0.693	13.33	37.5
150	0.914	6	0.488	17.65	56.0
200	0.813	8	0.369	26.75	66.7
250	0.715	10	0.273	35.58	75.4

OD: Optical density

Control OD AT 517 nm-----1.110

The DPPH radical scavenging activity of the extract increases with increasing concentration. Only 35.58% DPPH scavenging activity was exhibited by 250µg/ml of extract. Nevertheless, it was 75.40% in the presence of 100 mg/l BHA. These results suggest that ethyl acetate extracts exhibited better activity than n-hexane extract.

#### Reducing power

Different extracts of seeds of *psoralea corylifolia* exhibited good reducing power. The reducing power of n-hexane extract of *Psoralea corylifolia* (100-500mg/ml) concentrations is presented in Table 2(a). High absorbance indicates high reducing power. However, this reducing power is lower than that of ascorbic acid which was used as control. Therefore, the absorbance of ascorbic acid at concentration 10 mg/l was 0.76 while at the 500mg/l n-hexane extract concentration it was 0.44.



**Table 2a:** Reducing power of n-hexane extract of *Psoralea corylifolia* seeds

Sample	Concentration(mg/ml)	Absorbance (700nm)
*Control	0	0.07±0.06
Psoralea corylifolia	100	0.11±0.013
	200	0.26±0.047
	500	0.44±0.07
Ascorbic acid	5	0.39±0.006
	10	0.76±0.006
	15	1.10±0.006

\*The control was test sample without plant extract.High absorbance indicates high reducing power

The reducing power of ethyl acetate extract of *Psoralea corylifolia* is presented in (Table 2b) .The absorbance of ascorbic acid in a sample (10mg/l) was 0.86 while at the 500mg/l ethyl acetate extract concentration it was 0.73.The reducing power of ethyl acetate extract of *Psoralea corylifolia* seed extract has shown good reducing power than n-hexane extract. As the amount of extract increases, the reducing power also increases. In both cases of n-hexane and ethyl acetate extracts of *Psoralea corylifolia* there is a remarkable concentration dependent reducing power was exhibited. This variation in reducing activity may be due to crude nature of plant extracts and availability of different phytochemicals in these plants.

**Table 2b:** Reducing power of ethyl acetate extract of *Psoralea corylifolia* seeds

Sample	Concentration(mg/ml)	Absorbance(700nm)
*Control	0	0.08±0.06
Psoralea corylifolia	100	0.28±0.013
	200	0.58±0.047
	500	0.73±0.07
Ascorbic acid	5	0.489±0.006
	10	0.86±0.006
	15	1.30±0.006

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\*The control was test sample without plant extract.High absorbance indicates high reducing power

## Total flavonoid content

The total flavonoid content of the plant material was found to be 73.09 mg CE/100g.

## Conclusions

Against the backdrop of many known medicinal properties of these plants, results from the present work suggest that relatively low values of antioxidant and reducing power may not imply a low medicinal value. Emerging trends in antioxidant research point to the fact that low levels of phenolics (and other phytochemicals) and low value of antioxidant indices in plants do not translate to poor medicinal properties. The present investigation indicates that though *Psoralea corylifolia* seed extracts has been described as plants of low economic values, these are not worthless. As ethyl acetate extract showed very prominent activity, it was attributed to the flavonoids present in the extract. The total flavonoid content was found to be 73.09mgCE/100g. There is prospectus for the commercial utilization especially in the view abundant and widespread nature. Attempts would be made to isolate and characterize phytoconstituents in n-hexane and ethyl acetate extract.

## Author's contribution

Aruna Pai has performed the experiments under the guidance of J Madhu Rajendra and the results were interpreted by JVLN Seshagiri Rao.

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