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Phytochemical screening and evaluation of antioxidant activity of *Parkinsonia aculeata* L. (Family-Leguminoseae) leaves extract

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Abstract: The present study states the phytochemical investigation and the therapeutic importance of *Parkinsonia aculeata* Linn. (Family-Leguminoseae). It can be proved that plant possess potent medicinal value. Worldwide trend towards the utilization of natural plant remedies has created an enormous need for the use of medicinal plants. This study involves the preliminary phytochemical screening of the methanolic crude extract of *P. aculeata* leaves. Phytochemical analysis of the methanolic extract prepared from *P. aculeata* leaves revealed the presence of alkaloids, flavonoids, C-glycosides, terpenoids and saponins. The results showed that the phytochemical properties of the leaves can be used for curing various diseases. Out of seven fractions fraction-2, 4, 5, 6 and 7 were screened for DPPH antioxidant activity again. The crude $CHCl_3$ fraction showed 71.7% inhibition and fr-4 showed 85. 4 % inhibition which is more potent than standard gallic acid which was measured 83.5%. IC_{50} of 0.41, 0.29, 0.38 and 0.33 mg/ml were recorded for $CHCl_3$, EtOH, Aqueous and EtOAc extract. For $CHCl_3$ fractions IC_{50} reported were 0.32, 0.36, 0.37 and 0.37 mg/ml for fraction F2, F4, F5 and F7. The *P. aculeata* leaves is a potential source of various types of bioactive compounds with diverse chemical structures as well as pharmacological activities.

Keywords: Antioxidant, phytochemical, terpenoids, flavonoids, saponins, C-glycosides, alkaloids.

Introduction

There has been an increasing interest worldwide on therapeutic values of natural products. The nature provides the mankind vast therapeutic flora with a wide variety of medicinal potential. The revival of interest in plant derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs many of which have adverse side effects. The need of the hour is to screen a number of medicinal plants for promising biological activity (1). In recent years, phytochemicals in vegetables have received a great deal of attention mainly on their role in preventing diseases caused as a result of oxidative stress which releases reactive oxygen species such as singlet oxygen and various radicals as a damaging side effect of aerobic metabolism. These radicals are possibly involved in a number of disorders including cardiovascular malfunctions, tissue injury, DNA damage and tumor promotion. Several studies suggest that antioxidants could prevent accumulation of these reactive oxygen species and be beneficial for treatment of these pathologies. Diets rich in fruits and vegetables have been reported to associate consistently with reduced risk of a variety of tumors, especially epithelial cancers of respiratory and gastrointestinal tract.

The term antioxidant refers to the activity of numerous vitamins, minerals and other phytochemicals to protect against the damage caused by reactive oxygen species (2). Free radicals are implicated in several degenerative diseases such as atherosclerosis, diabetes, arthritis and cancer (3). Synthetic antioxidants can be carcinogenic so natural antioxidants can prove a promising alternative. Medicinal plants possess a variety of compounds of known therapeutic property (4, 5, 6).

Materials and methods

Collection of plant material

The plants of *Parkinsonia aculeata* were collected from areas around Hoshangabad road, Bhopal, M.P. in India. Identification of plant was carried out and authenticated by Dr. Jagriti Tripathi, HOD, Department of Botany Unique College, Bhopal (M.P.), India and a voucher specimen was procured in herbarium record maintained at the Laboratory, in the Department of Zoology, S.S.L. Jain P.G., College, Vidisha (M.P.). The plant material was thoroughly washed with water and was kept for drying in shade at room temperature at for 20 days. The thoroughly air dried plant material was grinded to powder to about 40-60 mesh size weighted and stored in large plastic bottles for future extraction and chemical testing.

Solvent Extraction:

In this method the 40-60-mesh size powdered plant material was extracted with soxhlet apparatus using 80 and 50 % methanol for 38 hrs. after defatting with petroleum ether for 24 hrs.. The extraction was done for 48 hours duration or till 5 cycles are completed. The extract is concentrated on a rotatory evaporator below 40°C. Almost all the chlorophyll and lipid is deposited on the side of the flask and with skill it was removed.

The concentrated crude was pipette off skillfully, almost completely free of lipid impurities. The concentrated crude extract was then extracted with $CHCl_3$, EtOAc and EtOH according to Harborne,1995 with some modifications.

Alternatively it was also extracted by hydrolyzing plant powder with 2M HCl for 30 minutes at 100°C. The cooled solution is filtered by muslin cloth and then filtrate is extracted thrice with ethyl acetate ant the combined extract was taken to dryness and collected in glass vials for further processing.

The $CHCl_3$ crude extract was subjected to purification process by silica gel G – Emerk (120 mesh) column chromatography.

Phytochemical screening

Phytochemical screening were performed to assess the qualitative chemical composition of different crude extracts using commonly employed precipitation and coloration reactions to identify the major secondary metabolites like alkaloids, terpenes, flavonoids, saponins, steroids, phenolic compounds, tannins and aminoacids. The phytochemical analyses were carried out using standard procedures (7,8).

Test for flavonoids (Shinoda test)

To the extract, add 5 ml 95% ethanol, few drops of conc. HCl and 0.5g magnesium turnings. Pink coloration indicates the presence of flavonoids.

Test for alkaloids (Wagner's test)

Evaporate the aqueous, alcoholic, $CHCl_3$ or ethyl acetate extracts. To residue add dil. HCl. Shake well and filter. 2-3 ml filtrate add few drops of Wagner's reagent. Reddish brown ppt. indicates the presence of alkaloids.

Test for C-glycosides (modified Borntrager's test)
To 5 ml of extract add 5ml of 5% $FeCl_3$ and 5ml dil. HCl. Heat for 5 min. in boiling waterbath. Cool and add benzene or any organic solvent. Shake well. Separate the organic layer and add equal volume of dil. Ammonia. Ammonical layer shows pinkish red color.

Test for terpenoids (Salkowski test)
To 0.5g of the extract 2ml of $CHCl_3$ was added. 3ml of conc. H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for saponin glycaosides (Foam test)
To 0.5g of extract 5ml of distilled water was added. The solution was shaken vigorously. Persistent foam indicates the presence of Saponins.

Test for phenolic compounds
To 2-3ml of aq. Or alc. Extract few drops of 10% aq ferric chloride solution was added. Formation of blue or green color indicates the presence of phenolic compounds.

Test for gums and mucilages
10 ml of extract was slowly added to 25 ml of absolute alcohol under constant stirring. Precipitation indicates the presence of gums and mucilages.

Test for reducing sugars (Fehling's test)
The aqueous ethanol extract (0.5g in 5ml of water) was added to boiling fehling's solution(A+B). brick red ppt. at the bottom of the test tube indicates the presence of reducing sugars.

Test for tannins
5ml of extract was added with few drops of 1% lead acetate. Formation of yellow or white ppt. indicates the presence of tannins.

Determination of antioxidant activity
For the determination of scavenging activity of DPPH free radical in the extract solution a solution of 0.135mM DPPH in methanol was prepared and 1.0ml of this solution was mixed with 1.0ml of extract prepared in methanol containing 0.025-0.5mg and standard drugs separately (Gallic acid). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30min. the absorbance of the mixture was measured spectrophotometrically at 517nm(9).
The ability of plant extract to scavenge DPPH radical was calculated by the equation-

$$\text{DPPH radical scavenging activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Statistical Analysis
Tests were carried out in triplicate experiments and mean values were calculated with EXCEL program from MS office package. IC_{50} were graphically estimated using a linear regression algorithm.

Table 1: Phytochemistry of *Parkinsonia aculeata* extracts

S.No.	Compound	Aqueous extract	Ethanol extract	Chloroform extract	Ethyl acetate extract
1	C-Glycosydes	+	-	+	+
2	Terpenes	+	-	-	-
3	Flavonoids	-	+	+	+
4	Alkaloids	+	+	+	-
5	Saponin		-	+	-
6	Tannins and Phenolic Compounds	+	-	_	-
7	Gums and Mucilages	+	-	_	-
8	Reducing Sugars	+	-	+	-

Table 2: % Inhibition of different extracts of *Parkinsonia aculeate* leaves

S.no.	Extract	% I	IC_{50} mg/ml
1	$CHCl_3$ Extract	71.7	0.41
2	EtOH Extract	86.7	0.29
3	Aqueous Extract	70.81	0.38
4	EtOAc Extract	75.29	0.33
5	Gallic acid	83.5	

Table 3: % Inhibition of different fractions of $CHCl_3$ extract of *Parkinsonia aculeata* leaves

S.no.	Fraction	% Inhibition at different concentrations					IC_{50}
		0.025 mg/ml	0.05 mg/ml	0.1 mg/ml	0.2 mg/ml	0.5 mg/ml	
1	F_2	5.7	24.5	50.2	72.9	81	0.32
2	F_4	5.9	6.4	29	57.8	85.4	0.36
3	F_5	-	0.7	19.76	29.81	59.27	0.37
4	F_6	60.5	59.5	58.9	-	-	-
5	F_7	13.6	26.1	60.7	65.25	70.3	0.37
6	Gallic acid	79.8	82.1	82.5	82.9	83.5	

Results

The phytochemical screening of *Parkinsonia aculeata* Linn. (Family- Leguminoseae) showed the presence of flavonoids, terpenoids, alkaloids, saponins, tannins, phenolic compounds, C-glycosides and reducing sugars (table-1).

IC_{50} of 0.41, 0.29, 0.38 and 0.33 mg/ml were recorded for $CHCl_3$, EtOH, Aqueous and EtOAc extract (table-2). For $CHCl_3$ fractions IC_{50} reported were 0.32, 0.36, 0.37 and 0.37 mg/ml for fraction F2, F4, F5 and F7 (table-3).

The phytochemical screening of the *P.aculeata* Linn. Leaves extract showed the presence of flavonoids, terpenes, alkaloids, saponins, tannins, C-glycosides, phenolic compounds and reducing sugars.

Fig:1 DPPH free radical scavenging activity of different crude leaf extracts of *P. aculeata*

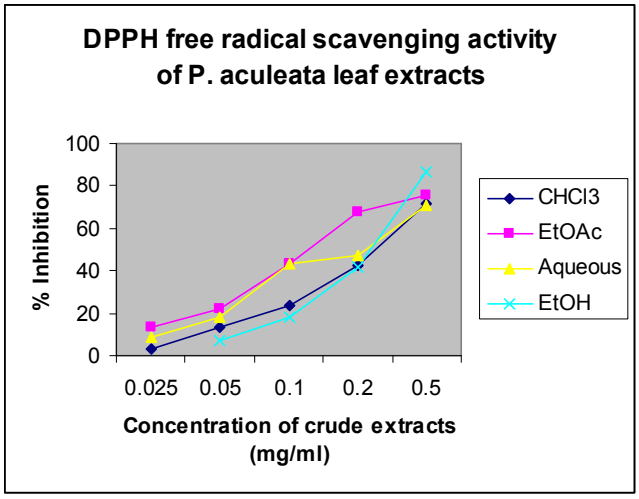
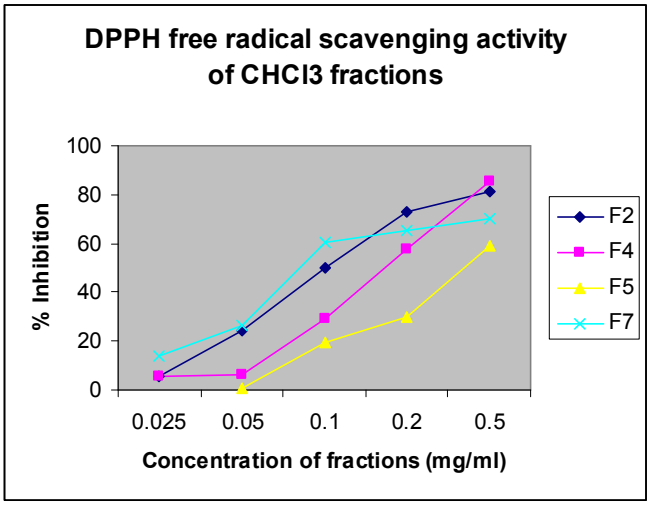


Fig: 2 DPPH free radical scavenging activity of $CHCl_3$ fractions of *P.aculeata* leaves



Discussion

Available literature indicated that medicinal plants are the backbone of traditional medicine and the antibacterial activity of plant extract is due to different chemical agent in the extract which were classified as active antimicrobial compounds (10). Alkaloids are formed as metabolic byproducts and have been reported to be responsible for the antibacterial activity (11). Glycosides serve as defence mechanisms against predation by many microorganisms, insects and herbivores (12). The demonstration of antimicrobial activity against both gram positive and gram negative bacteria by the plant may be indicative of the presence of broad spectrum of antibiotic compounds (13). The optimal effectiveness of a medicinal

plant may not be due to the one main active constituent, but may be due to the combined action of different compounds originally in the plant (14). The phenolic compounds such as flavonoids, phenolic acids and tannins are considered to be major contributors to the antioxidant capacity of plants. The diverse biological activities may be related to their antioxidant activity (15). Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti- inflammatory effects (16,17). Tannins play a major role as antihaemorrhagic agent and has been shown to have immense significance as antihyper cholesterol, hypotensive and cardiac depressant properties (18). Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities (19). Steroids, saponins and triterpenoids showed the analgesic properties (20, 21). It has been reported that saponins possess hypocholesterolemic and antidiabetic properties (22). Flavonoid compounds especially quercetin and genistein have antitumor activity. These compounds are cytotoxic to cancer cells but have no or insignificant activity in normal cells. (23). It has been reported that flavonoid, apigenin holds great promise as a chemopreventive agent for a variety of cancers and exhibits significant activity against UV induced DNA damage and thus protect against skin cancer (24). It also inhibits the growth of a variety of human cancer cells including leukemia, brest, colon, skin, thyroid and prostate cancers (25). Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants of free radical scavengers (26).

The DPPH antioxidant assay provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517nm in visible region. The degree of reduction in absorbance measurement is indicative of the radical scavenging potential of the extract. The ethanol and ethyl acetate extract appeared to be as potent as Gallic acid with a maximum inhibition of 86.7% and 75.29% at 0.5mg/ml which is more potent as comparable to 83% for Gallic acid at similar concentration.

It has been concluded that different extract of *P. aculeata* leaves exhibits significant antioxidant activity which participate in various pathophysiological conditions of different diseases. The present study shows that *P. aculeata* leaf extract is a rich source of natural antioxidants that can be important in disease prevention. 7 fractions were isolated from $CHCl_3$ extract obtained after acidification of direct 80% and 50% methanolic extract obtained from soxhlet by 2M HCl and after addition of $CHCl_3$ and separation of $CHCl_3$ fraction by separating funnel. Fraction 2,3,4,5,6 and 7 showed positive test for flavonoids Out of seven fractions fraction-2,4,5,6 and 7 were screened for DPPH antioxidant activity again the crude $CHCl_3$ fraction showed 71.7% inhibition and fr-4 showed 85.4 % inhibition which is more potent than standard Gallic acid (table-2). Further screening is needed to identify the bioactive component responsible for antioxidant activities and its use in treatment of various diseases.

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