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Research Article

DPPH FREE RADICAL SCAVENGING ACTIVITY OF PHENOLIC COMPOUNDS IN *ARGEMONE MEXICANA* LINN.

R A SHARMA, ANKITA YADAV* AND RICHA BHARDWAJ

Plant Physiology and Biochemistry Laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India.
Email: yadanki22@gmail.com

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ABSTRACT

The present study was aimed to evaluate the total phenolic contents (TPC), total flavonoid contents (TFC) and the antioxidant efficacy of methanolic extracts of different plant parts (leaves, stems, roots, flowers and fruits) of *Argemone mexicana*. The results showed significant variation in the total phenolic and flavonoid content and antioxidant potential among the different plant parts. The highest total phenolic (23.5mg GAE/gdw) and flavonoidal content (34.5mg QE/gdw) was recorded in flowers. Antioxidant evaluation of *A.mexicana* was carried out using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) free radical scavenging assay and exhibited considerable antioxidant potential and showed good correlation with the total phenolic and total flavonoidal content. The highest radical scavenging effect was observed in flowers of *A. mexicana* with IC_{50} = 23.75µg/ml. The results of the study showed that *A.mexicana* possess significant antioxidant activity. Owning to these results, the plant has the potential to be used as a medicine against the diseases caused by free radicals.

Keywords: *Argemone mexicana L., TPC, TFC, DPPH free radical scavenging assay.*

INTRODUCTION

Medicinal plants are the main source of natural antioxidants. *Argemone mexicana* Linn belongs to family Papaveracae and also known as mexican prickly poppy [1]. It grows wildly in tropical and subtropical countries like Mexico, United States, India and Ethopia [2]. The seed are used as emetic, demulcent, laxative [3] and antitode in snake poisoning. It has also been investigated for anticatalepsy activity, antihistamic activity [4], hepatoprotective activity [5], antimicrobial activity [6], antiallergic activity, antistress, larvicidal activity, antidiabetic activity [7], antioxidant, anti-inflammatory activity [8, 9] and wound healing activity [10]. It is also used in the treatment of jaundice, leprosy, piles, dysentery and warts [11].

Antioxidants are compounds that protect cells against the damaging effects of ROS (reactive oxygen species) such as superoxide, hydroxyl radicals, singlet oxygen, peroxy radicals etc. These active oxygen species and free radicals can attack molecules in biological membranes and tissues and thus inducing oxidative stress that further has been associated with cancer, ageing, inflammation, neurodegenerative diseases, hypertension, artherosclerosis [12,13,14,15]. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. The main characteristic of an antioxidant is its ability to trap free radicals [16]. It has been mentioned that antioxidant activity of plants might be due to their antioxidant compounds like vitamin C, vitamin E, carotenes, phenolic acids, phytoestrogen, phytate, tocophenols, benzoic acids, folic acid etc [17].

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized according to chemical structure. Phenolics are characterized by at least one aromatic rings (C_6) bearing one or more hydroxyl groups [18]. Recently flavonoids and phenols have aroused considerable interest because of their beneficial effects on human health mainly antioxidant activity that depends on their molecular structure.

There are very few studies of reactive oxygen species in relation to methanol extracts of *Argemone mexicana* against free radicals like DPPH and their relation to total phenolic and flavonoidal contents. Therefore, in this study an attempt has been made to investigate the TPC and TFC and to evaluate the antioxidant activities of methanolic extracts of leaves, stems, flowers, fruits and roots of *A.mexicana* Linn. by using widely accepted free radical scavenging model system i.e DPPH.

MATERIALS AND METHODS

Plant material

The different plant parts (roots, stems, leaves, fruits and flowers) of *A.mexicana* were collected in the month of November-December from the Jaipur-Delhi highway. It was washed with tap water, dried at room temperature and ground to fine powder. The species specimen was submitted in herbarium, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India and got the voucher specimen No. RUBL20874.

Chemicals

All the chemicals used were of analytical grade and purchased from Hi Media from Hi-media Laboratory Pvt. Ltd. Mumbai.

Total Phenolic and Flavanoidal Contents

Plant Extraction

2gm each of the dry material (leaves, stems, roots, flowers and fruits) was extracted with 25ml of methanol at room temperature for 48 hours, filtered through Whatman paper no 1 filter paper, stored and used for quantification.

Total Phenolic Content

Total phenolic compound contents were determined by the Folin-Ciocalteau method [19, 20, 21, 22].The extract samples (0.5 ml; 1; 10 diluted) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric method at 765 nm. The standard curve was prepared using the standard solution of Gallic acid in methanol in the range 20-200µg/ml (R^2=0.987). Total phenol values are expressed in terms of Gallic acid equivalent (mg/ g of dry mass), which is a common reference compound. Total phenolic contents can be calculated from the formula:

$$T = \frac{C.V}{M}$$

Where,

T=Total Phenolic concentration

C= Concentration of gallic acid from caliberation curve (µg/ml)

V= Volume of extract (ml)

M= Wt. of methanol plant extract

Total Flavonoidal Content

Total flavonoid content was determined by using aluminium chloride colorimetric method ($AlCl_3$) according to the known method [23, 24] with slight modifications using quercetin as standard. 1ml of test material was added to 10ml volumetric flask containing 4ml of water. To above mixture, 0.3ml of 5% $NaNO_2$ was added. After 5mins, 0.3ml of 10% $AlCl_3$ was added. After 6min, 2ml of 1M NaOH was added and the total volume was made up to 10ml with distilled water. Then the solutions were mixed well and absorbance was measured against blank at 510nm. The standard curve was prepared using the standard solution of quercetin in methanol in the range 0.5- 5.0mg/ml (R^2=0.991). Total flavonoidal content of the extracts was expressed in milligram of quercetin equivalents/gdw. Total flavonoidal content can be calculated from the formula:

$$T = \frac{C.V}{M}$$

Where,

T=Total flavonoidal concentration

C= Concentration of quercetin from caliberation curve (mg/ml)

V= Volume of extract (ml)

M= Wt of methanol plant extract

DPPH Radical Scavenging Activity

Plant extraction

10gm each of the plant material was soxhlet extracted with methanol for 24hours. The extract was filtered with Whatman filter paper no 1 and the crude extract was concentrated to dryness in a rotary flash evaporator under reduced pressure and controlled temperature (40–50°C). The extract was preserved in vacuum desiccators for subsequent use in antioxidant assay.

Assay

The antioxidant activities were determined using 1, 1, diphenyl-2-picryhydrazyl (DPPH) as a free radical. Experiments were initiated by preparing a 0.25mM solution of DPPH and 1mg/ml solution of different plant parts extracts (stock) in methanol. To the methanolic solutions of DPPH an equal volume of the extract dissolved in methanol was added at various concentrations. An equal amount of alcohol was added to the control. The setup was left at dark in room temperature and the absorption was monitored after 20 minutes. Ascorbic acid was used as a control. Experiment was performed in triplicate [25, 26]. A control reaction was carried out without the test sample. Absorbance values were corrected for radicals decay using blank solution. The inhibitory effect of DPPH was calculated according to the following formula:

$$\% \text{ Inhibition} = [1 - (Abs_{SAMPLE} / Abs_{CONTROL})] \times 100$$

Linear graph of concentration Vs percentage inhibition was prepared and IC_{50} values were calculated. The antioxidant activity of each sample was expressed in terms of IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve [27, 28, 29].

Statistical analysis

Experimental results are expressed as means ± standard deviation (SD). All measurements were replicated three times. IC_{50} values were also calculated by linear regression analysis. Experiments results were further analyzed for Pearson correlation coefficient(r) between total phenolic, flavonoid and DPPH radical scavenging assay using the Microsoft Excel 2007 software and two way analysis of variance (ANOVA) was applied to investigate the differences among means by using the Microsoft Excel 2007. The values were considered to be significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

The total phenolics and flavonoid contents of the different plant parts of *A.mexicana* were exhibited in Table 1. Total phenols and flavonoids were expressed in terms of gallic acid equivalent (GAE) and Quercetin equivalent (QE) per gram of the dry weight basis. The results revealed that total phenolics and flavonoid content varies among different plant parts. The flowers methanolic extracts showed highest phenolic content (23.5 GAE/gdw) and decreases in the order stem (13.5 GAE/gdw), fruits(10.5 GAE/gdw), leaves(4.5 GAE/gdw) and roots(4.0 GAE/gdw). Whereas the total flavonoid content was found to be highest in the flowers extracts (34.5 QE/gdw) when compared to that of the leaves (32.5 QE/gdw), fruits (9.3 QE/gdw), stems (6.35 QE/gdw) and roots (3.5 QE/gdw).

Table 1: Total Phenolic and Flavonoidal content in different plant parts of *A. mexicana Linn*

Plant Part	Total Phenolic content(mg GAE/gdw)	Total flavonoidal content(mg QE/gdw)
Leaves	4.5 ± 0.0357	32.5 ± 3.284
Stems	13.5 ± 2.312	6.25 ± 0.335
Flowers	23.5 ± 1.01	34.5 ± 0.221
Fruits	10.5 ± 0.73	9.375 ± 0.36
Roots	4.0 ± 0.426	3.5 ± 0.35

Each value is expressed as mean ± S.E(Standa Error) (n=3)

Antioxidants through their scavenging power are useful for the management of various diseases. The antioxidant activities of the methanol extracts of *A.mexicana* were measured by using widely used antioxidant assay, i.e. DPPH free radical scavenging assay. Table 2 shows the IC_{50} (µg/ml) values of methanolic extracts of various plant parts for free radical scavenging activity by DPPH. It was observed that the extract of flowers exhibited the highest radical-scavenging activity (lowest IC_{50} value= 23.75 µg/ml). The elevated DPPH radical scavenging ability of the flowers extracts might be due to the presence of high contents of phenolics and flavonoids. Previously also plant has been investigated for antioxidant activity [30, 31]. It has been reported that the antioxidant activity of many botanicals were proportional to their phenolic content, suggesting a causative relationship between them [32].

Table 2: The IC_{50} values of different plant parts of *A.mexicana* Linn of DPPH radical scavenging assay (µg/ml)

Plant Part	IC_{50} values(µg/ml)
Leaves	254 ± 1.51
Stems	114 ± 3.072
Flowers	23.75 ± 0.795
Fruits	633.66 ± 19.014
Roots	327.51±8.382

Each value is expressed as mean ± S.E.(Standard Error) (n=3)

To find the relationship between the antioxidant activity, phenolics and flavonoid contents, we performed linear regression and correlation analyses of the values of total antioxidant capacity by DPPH method with the TPC and TFC and the correlation coefficient values are depicted in Table 3.

Table 3: Correlations between the IC_{50} values of DPPH assay, phenolic and flavonoids content of *A. mexicana*

Plant Part	Correlations(r)	
	TPC and DPPH	TFC and DPPH
Leaves	0.624	0.728
Stems	0.705	-0.527
Flowers	0.926	0.899
Fruits	-0.025	-0.749
Roots	0.908	0.896

The correlations of TPC and TFC against the antioxidant activity based on the DPPH assay in all the plant parts were significant. In flowers, TPC and TFC showed a very strong correlation with DPPH, but the coefficient of correlation was negative in case of fruits. Further the negative correlation between TPC, TFC and antioxidant activity suggested that it could be related to other antioxidant compounds contained in the plants [33]. Leaves show good correlation with the flavonoids compared to phenols that

shows that flavonoids act through scavenging or chelating process. The nature of phenolics contributing to their electron transfer/hydrogen donating ability is also known to be related to the DPPH radical scavenging activity [29, 34]. Stems show negative correlation with the flavonoids. The high free radical scavenging capacity of the wild plants might be attributed not only to the phenolic composition, but also to the presence of other bioactive compounds, such as vitamins (tocopherols) and pigments (anthocyanins) as well as the structural interaction among these compounds [35].

Table 4: ANNOVA analysis for the antioxidant activity, Total phenolic content and Total flavonoidal content

Source of variation	SS	Df	MS	F	P value	F crit
Rows	67186.97031	4	16796.74	0.868305	0.522621	3.837853
Column	219178.5716	2	109589.3	5.665198	0.029336	4.45897
Error	219178.5716	2	109589.3	5.665198	0.029336	4.45897
Total	441119.9245	14				

As drawn from table 4, there is statistically no significant difference between the rows i.e the content of phenols, flavonoids and DPPH radical scavenging activity(F value < F_{crit}), but when columns are taken into account , there is a significant difference in the content of phenols, flavonoids and DPPH radical scavenging activity in all plant parts (F value > F_{crit}).

CONCLUSION

The total phenolic and flavonoidal content and antioxidant activity of different plant parts was measured in the methanol extract of *A. mexicana.* All these vary significantly in different plant parts; however, maximum activity was recorded in flowers. Significant correlations was found between the antioxidant activities and total phenolic and flavonoid contents indicating that these phytocompounds are the major contributors of antioxidant capacities of this plant. The results of the present study suggests that *A. mexicana* contained potential antioxidant bioactive compounds, which if properly and extensively studied could provide source of biologically active drug candidates and it also shows its great importance as therapeutic agent(especially the flowers) in preventing or curing the diseases caused due to oxidative stress.

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