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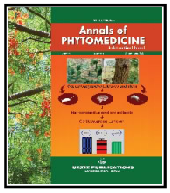

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Determination of total phenolic, free radical scavenging activity and antimicrobial activity of root extracts of *Argemone mexicana* L. in methanol solvent

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Abstract

Argemone mexicana L. (Papaveraceae), tropical yearly wild plant, called as Mexican prickly poppy is a species of poppy, found in Mexico and now widely naturalized in many parts of the world with broad range of activities. The main aim of this study was to evaluate total phenolic content, antioxidant potential and antimicrobial activity in root part of *A. mexicana*. Root parts of *A. mexicana* were extracted with methanol using Soxhlet apparatus and further concentrated on rotatory vacuum evapoprator. The total phenolic content of methanol root extracts was quantified using the Folin-Ciocalteu method, antioxidant activity was assessed using the DPPH method, and antibacterial activity was tested using the agar well diffusion method. Total phenolics in root extracts were found to be 8.91 mg GAE/g. Methanolic root extract of *A. mexicana* showed that DPPH free radical scavenging activity varied widely and it increased with increase in concentration levels. The IC_{50} value of the methanolic extract was lowest 24.98 µg/ml and indicating the highest DPPH free radical scavenging activity. The higher antifungal activity is exhibited by methanol root extracts as comparable to standard. Methanol root extract of *A. mexicana* was said to be valuable for antioxidant potential as well as for fine antifungal activity.

1. Introduction

Plants have long been documented as prospective sources of different classes of chemical compounds known as phytochemicals, having diverse biological and therapeutic activities, which are effective in controlling or treating various diseases. There is a very important role of medicines based on plant system that continues to play in the healthcare system. An about 80% of the world inhabitants relying mainly on plant based medicines for their primary healthcare. There is an increasing faith in herbal medicine as a result of growing recognition of medicinal plants. The medicinal plants are continuing appreciation due to growing trust in herbal medicine (Dutta *et al*., 2014). The plant parts of the medicinal plants such as stem, bark, leaves, fruits, roots and seeds have been a part of phytomedicine and produces specific physiological action on human body. The medicinal plants contained natural bioactive constituents such as alkaloids, tannins, flavonoids and phenolic compounds (Chaudhuri *et al*., 2012). Medicinal plants also contain massive amounts of antioxidants, such as polyphenols, vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein and other carotenoids, which play important roles in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Bind *et al*., 2014). In various places, *A. mexicana*, also known as prickly poppy or satyanashi, is utilised as a medicinal plant. This plant can be found growing on the borders of roads or on vacant lots throughout subtropical India, including Haryana, Madhya Pradesh, Uttar Pradesh, Punjab plains and the North-Western states of Gujarat and Rajasthan. *A. mexicana* possess a wide-range of biological activities such as antibacterial as reported in earlier studies (Bhattacharjee *et al*., 2006; Rahman *et al*., 2011; Rahman *et al*., 2006 ; Sahu *et al*., 2012) as well as antifungal activities (Kushtwar *et al*., 2017; Singh *et al*., 2009; More *et al*., 2016; Andleeb *et al*., 2020). Several researchers and academic groups have studied that *A. mexicana*'s strong medicinal potential to identify its key secondary metabolites, which include phenolics, flavonoids, tannins, terpenoids (such as glycosides), N-containing chemicals (such as alkaloids), saponins, and steroids (Ibrahim and Ibrahim, 2009). In the work herein, a comprehensive evaluation of the total phenolic, free radical scavenging activity and antimicrobial activity of methanol root extracts of *A. mexicana* is provided. Methanol extracts of roots were tested against gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*), Gram-ve bacteria (*Xanthomonas campesteris*)and fungal species *Fusarium oxysporum, Macrophomina phaseolina* and *Candida albicans* that had not previously been examined against *A. mexicana* in the literature for Haryana region. Most of the work cited in literature for the *A. mexicana* aerial parts as there is a little work on root part.

2. Materials and Methods

2.1 Plant materials

A. mexicana fresh roots were collected. The plant roots were washed under tap water to remove dust and then with double distilled water to remove other particulate matter. After washing to dryness, roots were cut into small pieces, shade dried for 30 days and then

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kept in a hot air oven at 100°C till dryness for two days and further it was crush manually. Then grind in a grinder to fine powdered form and sieved. It was stored in an air tight container at room temperature for further use.

2.2 Chemicals and reagents used

For experimental design, HPLC grade chemicals were used. The other solvents and chemicals used were NaOH (sodium hydroxide), HCl (hydrochloric acid), H_3BO_3 (boric acid), 1,1-diphenyl-picryl-hydrazyl (DPPH), gallic acid, Folin-Ciocalteu phenol reagent (FCP), $FeCl_2$ (ferric-chloride), potassium-ferricyanide, ascorbic acid, $FeCl_3$ (ferrous chloride), Na_2CO_3 (sodium-carbonate), catechin, H_2O_2 (hydrogen-peroxide) purchased from Hi media and Sigma Chemicals Limited. Freshly prepared solutions were used and utilized on the same day of the assay used for evaluation of total phenolic content, free radical scavenging and antimicrobial activity.

2.3 Extraction

Extracts were produced using the Soxhlet equipment for estimating total phenolics and evaluating antioxidant and antibacterial activity in methanol. For extracts preparation, 4 g of powdered samples of *A. mexicana* roots were placed in a Whatman No.1 filter paper and form a thimble that fitted in a classical Soxhlet apparatus with a 250 ml round bottom flask. The analytical grade methanol solvent was added in such a way first up to one and secondly a half siphons that is just about 150 ml. Extraction step for these samples completed in three cycles; the first extraction step completed in 5 h, after that extraction is repeated, that completed in 2 h and then further in 1 h. Suitable amount of methanol solvent was added at each step for maintaining a final volume 150 ml in the siphon. Filtrates of methanol solvent from three extraction steps were taken and their volumes were noted. These extracts were filtered. Methanol root extracts were concentrated further on rotary vacuum evaporator (Buchi R-300) and used for estimation of total phenolics contents, evaluation of antioxidant activity and antimicrobial activity.

2.4 Estimation of total phenolics content

Folin-Ciocalteu method (Singleton *et al.*, 1965) was used for determination of total phenolics content of root extracts quantitatively. In brief, various amounts of materials mixed with double distilled water to achieve a final volume of 3 ml. FCP (0.5 ml) reagent was also combined and incubated at room temperature for 10 min. Then, 7 per cent Na_2CO_3 (2 ml) solution was added and the mixture was heated for one to two minutes on a hot plate. Absorbance was measured at 650 nm after cooling of the solution (as there is no separate absorbance maximum wavelength for polyphenols, so we were used 650 nm wavelength). As a standard, gallic acid was used. The total phenolic content was measured in milligram gallic acid equivalent per gram of extract (mg GAE/g).

2.5 Evaluation of DPPH free radical scavenging

The antioxidant activity in methanolic root extracts of *A. mexicana* were checked on the basis of 1,1-diphenyl-picryl-hydrazyl method. The antioxidant present in the root extracts reduced DPPH* to DPPH-H, then there is a decrease in absorbance occurs. An antioxidant's scavenging power in terms of hydrogen donating ability is determined by its degree of discolouration (Eberhardt *et al.*, 2000). The DPPH solution (500 g/ml, 0.5 milli-molar in methanol) was combined with various amounts of sample and the final volume was 3.5 ml with methanol. The mixture was incubated in the dark for 45 min at room temperature. Absorbance was measured at 515 nm using a spectrophotometer (Shimadzu UV-2600. I). Three ml of methanol and 0.5 ml of DPPH solution was mixed. That solution was used as a positive control. For blank sample, we were used methanol without DPPH solution for elimination of the absorbance of sample extracts. For the blank and standard, methanol and BHA (butylated hydroxyl anisole) were used respectively. For each sample, three replications were carried out. By plotting per cent DPPH free radical scavenging activity (y-axis) against extract concentration (x-axis) graph was drawn. From microsoft excel, quadratic regression equation ($y = ax^2 + bx + c$) was obtained. IC_{50} was calculated from the equation $ax^2 + bx + c = 0$ using following formulae:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

where, $x = IC_{50}$ (µg/ml)

Calculation

The percentage of DPPH scavenged (% DPPH*sc) was estimated using the following formula:

$$\%DPPH^*sc = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where,

$A_{control}$ is the absorbance of the control,

A_{sample} is the absorbance of the sample.

2.6 Evaluation of antimicrobial activity

Agar well diffusion method (Bayer *et al.*, 1966) was used for the evaluation of antimicrobial activity of the methanolic root extracts. To prepare the bacterial suspension with the turbidity of 0.5 McFarland {equal to 1.5×10^8 colony-forming units (CFU)/ml)} eighteen to twenty-four hours single colonies on agar plates were used. Turbidity of the bacterial suspension was measured at 600 nm (Thermoscientific UV-Visible Spectrophotometer). Agar plates were inoculated with 10 µl of the test microorganisms, dispersed equally using a spreader, and left to dry for 5 min. Under aseptic circumstances, mueller hinton agar plates and potato dextrose agar were inoculated with bacterial and fungus strains, respectively, and 30 µl of the test samples were poured into wells (diameter = 6 mm) and incubated at 37°C for 24 h for bacteria and 72 h for fungi. After the incubation time, the diameter of the growth inhibition zones was assessed. After 24 h for bacteria and 72 h for fungi, zone of inhibition around each well was measured. To reduce error, we performed all the experiments in triplicate. For fungi cycloheximide was used as standard and for bacteria, chloramphenicol was used. The antimicrobial activity of methanolic root extracts obtained was tested against gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus)* and gram-ve bacteria (*Xanthomonas campesteris*) and fungal species (*Fusarium oxysporum*,

Macrophomina phaseolina and *Candida albicans*) and their zones of inhibition in mm are measured.

3. Results

The natural world offering us gift in the form of phytochemicals and antioxidant compounds.The phenolic compounds and carotenoids are most studied phytochemicals. Most of the plant species that contained phenolics, expressed as antioxidant potential (Silva *et al*., 2012). Antioxidants are chemicals that protect the human body from free radicals by reducing the oxidation of oxidizable compounds, hence slowing the progression of many chronic diseases (Speisky *et al*., 2012). Antioxidants may be called as enzymatic or non-enzymatic.

3.1 Extract yield

Extract yield of *A. mexicana* roots was found to be 9.95 g100g in root extracts.

3.2 Total phenolics content

Plant phenolics are an important class of chemicals that act as major antioxidants or free radical terminators. As a result, the total amount in the sample chosen for extraction must be estimated. Quantitative estimation of methanoilic root extracts of *A.mexicana* showed that the presence of phenolic compounds and total phenolics in methanol root extract were found to be 8.91 mg GAE/g.

Table1: DPPH free radical scavenging activity (%) of root extracts of *A. mexicana*

Extract / Conc. (mg/ml) →	DPPH free radical scavenging activity (%)					
	120	100	80	60	40	20
Methanol	99.3	97.6	94.6	88.3	74.9	46.7

Table 2: Quadratic regression equation and IC_{50} value for the root extracts of *A. mexicana*

Parameters	Methanol extract
Quadraticregression equation	$y = -0.010x^2 + 2.053x + 4.954 R^2 = 0.982$
IC_{50} (µg/ml)	24.98

Table 3: Antimicrobial activity of root extracts of *A. mexicana*

	Antimicrobial activity; Zone of inhibition (mm)					
	Antibacterial activity (mm)			Antifungal activity (mm)		
	Bacillus sp.	*Xanthomonas campestris*	*Staphylococcus aureus*	*Candida albicans*	*Fusarium oxysporum*	*Macrophomina phaseolina*
Methanol	13	-	14	34	30	32
Chloramphenicol	21	16	18	-	-	-
Cycloheximide	--	--	--	13	15	10
% inhibition	-	-	-	28%	15%	22%

3.3 Evaluation of DPPH free radical scavenging activity

For scavenging free radicals of DPPH, the antioxidant activity of methanol root extracts was calculated. DPPH method has been widely used to evaluate the antioxidant activity as researched by Wang *et al*. (2002). While free radical scavenging and antioxidant activity are not synonymous, they are related. The DPPH radical is scavenged by most powerful natural antioxidants, such as tocopherol, carnosol, and ascorbic acid. As a result, the presence of activity in the test indicates the presence of potential antioxidants. The standard used was BHA (butylated hydroxyl anisole). Using regression equations from microsoft excel, IC_{50} (half maximal inhibitory concentration) values were calculated. As shown in Table 1, the DPPH free radical scavenging activity of methanol root extract of *A. mexicana* increased with increasing concentration levels. The IC_{50} value of methanol root extract was minimum (24.98 µg/ml) and that clearly indicated the highest free radical scavenging activity as in Tables 2, 3.

3.4 Evaluation of antimicrobial activity

Fungal species (*Fusarium oxysporum* and *Macrophomina phaseolina*) used in this study causes soil borne infection in crops, vegetables (soybean, sorghum, groundnut) and alternatively decreases the yield. Methanol extract of *A. mexicana* showed good activity against pathogenic bacterial culture, *Bacillus* sp. and *Staphylococcus aureus* as well as antifungal activity against *Candida albicans*, *F. oxysporum* and *M. phaseolina* as shown in Table 3. The antibacterial activity in terms of inhibition zone against *Bacillus* sp. (13 mm), *Xanthomonas campestris* (4 mm) and *Staphylococcus aureus* (14 mm) was observed. The antifungal activity in terms of inhibition zone against *C. albicans* (34 mm), *M. phaseolina* (32 mm) and *F. oxysporum* (30 mm) was observed. Antifungal activity shown by methanol extract were better as compared to positive control (cycloheximide).

4. Discussion

A. mexicana root extract was prepared in methanol for assessment of the phenolic content, free radical scavenging activity and antimicrobial activity using standard procedures. The root extract of *A. mexicana* contains phenolic content and was found to be 8.91 mg GAE/g. Ibrahim and Ibrahim (2009), agreed with our findings as well as Jain *et al.* (2011), obtained positive results for most of the medicinally important phytochemicals and components identified for curative, antibacterial, and antifungal activity in *A. mexicana* aerial parts (leaf, stem, and flower) extracts in methanol.The preliminary finding of phytocompounds were also reported by Jaliwala *et al.* (2011). The findings of this investigation revealed that root extracts contain a significant level of total phenolic content. According to Apu *et al.* (2012), methanol is the top solvent (most polar) with the highest phenolic concentration in *A. mexicana*. The DPPH free radical scavenging activity of *A. mexicana* methanolic root extract was altered, and it increased as concentration levels increased.The IC_{50}(minimum inhibitory concentration) value of methanol root extract was found to be minimum (24.98 µg/ml). With antibacterial and antioxidant characteristics, *A. mexicana* has been used to treat a wide range of illnesses. Other researchers were also in agreement with our finding that efficient free radical scavenging potential with IC_{50} value 32.50 µg/ml in *A. mexicana* roots was reported by Gawade *et al.* (2018). Ethanol extract of *A. mexicana* roots possesses antioxidant activity at a dose of 100 µg/ml concentration, the extract showed high scavenging activity against DPPH (85.17%) assessed by Perumal *et al.* (2010). *A. mexicana* has an endless potential as a drug discovery candidate due to its profusion of secondary metabolites. The antibacterial activity of *A. mexicana* methanol root extracts was modest, with the largest impact against the fungal species tested and less activity against gram-positive bacteria.The methanol extracts of the roots of the *A.mexicana* showed greater antifungal activity than the corresponding standard. Antibacterial activity was found to be moderate. The results of ultra-high-resolution liquid chromato-graphy coupled with mass spectrometry and subsequent nuclear magnetic resonance analysis of the root and leaf methanol fractions by Orozco-Nunnelly *et al*. (2021) revealed that two main antibacterial compounds, chelerythrine and berberine, were effective for antimicrobial activity. Decoction of plant parts or boiling of plant parts in water are used in traditional treatments for treating fungal and bacterial infections. Our findings are extremely astounding, according to the current study. It was discovered that extracting with an organic solvent improved antifungal and antibacterial activities.The results obtained by Bhatacharjee *et al*. (2006), in methanol extract that showed maximum inhibition against the tested microorganisms that is two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*). According to Nair *et al*. (2005), antimicrobial activity can be attributed to two factors: first, the nature of biological active components whose activity can be enhanced in the presence of methanol; and second, methanol's stronger extraction capacity may have produced a greater number of active constituents responsible for antibacterial activity. Plant parts from *A. mexicana* (roots, leaves, stem, flowers, and seed) could be used to identify bioactive natural compounds that could lead to the creation of new medications. Such selection of various natural organic compounds and identification of active agents must be measured as a productive approach in the look for novel herbal drugs based on medicinal plants. Moreover, root extracts were found to be more valuable as contained good phenolics, good free radical scavengers and good antimicrobial. This gathered information about the plant could help future researchers for the work related to use and development of pharmacological drugs that could be pocket friendly and easily available with good amount of efficiency, this could be anticipated in future.

5. Conclusion

The quantitative analysis of the *A. mexicana* methanol root extracts revealed that they contained a significant amount of total phenolics. Root extracts were exhibited antioxidant activity, hence, are better source of antioxidants. The antimicrobial activity of root extracts was found effective in case of fungal species tested as comparable with standard. Thus, methanolic root extracts of *A.mexicana* can be regarded as good antioxidants with moderate value of phenolic contents and good antimicrobial. The presence of phytochemicals in the methanol root extract of *A.mexicana* might be responsible for its therapeutic and antioxidant effect. This is the first report of the effectiveness of methanol root extract of *A. mexicana* in Haryana.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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