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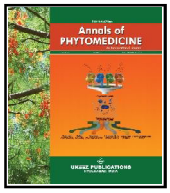

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Mineral content, bioactive ingredient identification and antioxidant activity of *Argemone mexicana* L. flowers extracts

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Abstract

The goal of this study was to look into the mineral content, bioactive ingredient identification and antioxidant activity of *Argemone mexicana* L. flowers extracts. Methanol and aqueous extracts were prepared using speed extractor. Using a flame atomic absorption spectrometer, the mineral content of *A. mexicana* was determined. The obtained flowers extracts were tested for their antioxidant activity using DPPH and assessed for the DPPH radical scavenging activity. Results indicated that the extracts contained mineral element named chromium, copper, zinc, vanadium, iron and nickel. Gas chromatography-mass spectrometry (GC/MS) analysis revealed the presence of ten different compounds in the methanol extract as well as in aqueous extract. In the DPPH scavenging assay, it was shown that methanol extract had a greater level of radical scavenging activity with an IC_{50} value of 38.33 g/ml than aqueous extract with a value of 41.92 g/ml.

1. Introduction

The Papaveraceae family includes the flowering plant, *Argemone mexicana* L., Satyanashi and Mexican Poppy are two popular names for the Papaveraceae plant, *A. mexicana*. The plant can reach heights of 0.3 to 1.2 metres (Schwarzbach and Kadereit, 1999). It is a small herbaceous plant with latex (Figure 1). The plant is common in India, it can be found in agricultural and wasteland weed regions (Mukherjee and Namahata, 1990). Throughout subtropical India, including Haryana, Madhya Pradesh, Uttar Pradesh, the Punjab plains and the North-Western states of Gujarat and Rajasthan; this plant can be found growing on the edges of roadways or on vacant lots (Kumari *et al*., 2022). Around the world, *A. mexicana* is used to treat a variety of illnesses, including tumours, warts, skin conditions, inflammations, rheumatism, jaundice, leprosy, microbiological infections and malaria. Flowers have been used to cure coughs because they are known to be expectorants (Brahmachari *et al*., 2010). Natural elements that can be extracted from the plant's leaves, flowers, roots, fruits, seeds and other parts as well. The quantities of trace elements in plants have received much interest as they are regarded to be vital for their effective operation. These substances are found in enzymes and activate them, having a crucial impact on the biochemical activity. Plants are a significant source of bioactive secondary metabolites, which are used in drug development (Jose and Thomas, 2014). Based on their functions in plant metabolism, bioactive components are separated into two groups: primary constituents and secondary constituents. Proteins, carbohydrates, amino acids and chlorophyll comprise up the primary bioactive components, whereas alkaloids, terpenoids, phenolic compounds, flavonoids and tannins make up the secondary bioactive components (Chugh *et al*., 2012). Extracting plant components is required for separating biologically active molecules, studying their role in disease prevention and therapy and determining their risk (Srivastava *et al*., 2012). Secondary bioactive metabolites protect the plant from disease-causing organisms, fungus and viruses by performing a number of general offensive actions (*e.g*., antioxidant, free radical scavenging, UV light absorbing and antiproliferative agents (Kenedy and Wightman, 2011). Antioxidant properties have been credited to medicinal plants because plants have to counteract themselves from stress caused by oxygen and it is very much necessary to screen medicinal plants for their antioxidant properties (Mahapatra *et al*., 2013). According to the literature that is presently available, relatively little research has been done on the mineral content, bioactive ingredient identification and antioxidant potential of *A. mexicana* in Haryana. The current study was designed to yield relevant information about diverse mineral content, bioactive ingredient identification and antioxidant activity.

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2. Materials and methods

2.1 Plant materials

The *A. mexicana* flowers (500 no's) were collected from roadside and vacant plots in the Haryana districts of Bhiwani and Hisar in late March 2021. After being cleaned with running tap water for two to three times, flowers were washed with double distilled water. It then had undergone a 30-day period of shade drying. Flowers were ground into a powder after being dried. Samples were stored in sealed containers.

2.2 Chemicals and reagents

All of the chemicals and reagents utilised were analytical reagent grade. Hi-media Pvt. Ltd., India, provided DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium hydroxide, lead acetate, nitric acid, sulfuric acid, perchloric acid and hydrochloric acid. Sigma-Aldrich provided the solvents methanol and butylated hydroxyl anisole (Mumbai, India). Prior to analysis, distilled water was utilised for sample preparation, dilution and rinsing apparatus.

2.3 Preparation of extracts

Using the speed extraction technique, the sample was extracted at 40°C, 100 bar pressure and 1 ml/min solvent flow rate (Samar *et al*., 2018). Aqueous and methanol were the two extraction solvents that were employed. Using a rota-evaporator (Heidolph,Germany), the extracts were concentrated to dryness under vacuum and the solution was regenerated in acetone for GC-MS analysis and antioxidant activity.

2.4 Preliminary phytochemical screening

The aqueous and methanol extracts were subjected to preliminary phytochemical screening for alkaloids (Iodine and Wagners tests), flavonoids (NaOH test), glycosides (Keller-Killani, Conc. H_2SO_4, and Molisch tests), lignin (Labat and Lignin tests), phenols (Phenol test), saponins (Foam test) and tannins (Lead acetate test) as per Bhatt and Dhyani (2011) .

2.5 Mineral analysis

The thoroughly dried and pulverized samples were deposited in a vitreosil crucible overnight in an electric muffle furnace at 400°C as element loss occurs at temperatures above 400°C. Ashing will completely eliminate the samples' entire organic components. The crucible containing pure ash was then taken out of the muffle furnace and kept in desiccators (Kar *et al*., 1999). The triple acid mixture of nitric acid, sulfuric acid and perchloric acid (11: 6: 3) was used to digest the ash, in hydrochloric acid; the material was dissolved to give a clear solution. Using a flame atomic absorption spectrometer (GBC- SensAA, Australia), the trace element concentration of *A.mexicana* flowers were determined. The FAAS's operational parameters were given in Table 1.

Table 1: Operating conditions of FAAS

Flame type	Wave length (nm)	Slit width (nm)	Gas flow (l/min)	Lamp current (mA)	Detection limits (ppm)
N_2O /Air Acetylene	245-360	0.2-0.7	1.0-2.0	5-25	0.01-0.05

2.6 GC-MS analysis of extracts

By using a GC-MS (Shimadzu) technique, bioactive chemical identification of *A. mexicana* flowers were performed. The GC separations were carried out in SH-Rxi-5Sil (30 m × 0.25 mm × 0.25 mm film thickness of 5% diphenyl and 95% di-methyl polysiloxane). The oven was operated at 80°C for two min, then ramped up to 180°C, then increased by 5°C/min to 300°C for three min. Temperatures of the injector port were 250°C, the ion source was 200°C and the interface is 250°C. Using a column split ratio of 1:10, argon (99.9999 %) was used as the carrier gas at an initial flow rate of 1.46 ml/min.

2.7 Antioxidant activity

The free radical scavenging 2,2'-diphenyl-1-picrylhydrazyl (DPPH) test was used to measure the extracts' antioxidant activity (Hatano *et al*., 1998). The stock solution (5000 g/ml) was made by redissolving the dry mass of flower extracts in the appropriate volume of methanol. The level of discoloration reveals an antioxidant's capacity to scavenge by donating hydrogen (Eberhardt *et al*., 2000). Using appropriate solvent dilutions, various concentrations were obtained from stock solution (*i.e.*, methanol for methanol and with methanol: water for water extracts). To assess the antioxidant activity of the extracts, 3,0 ml of 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH; 0.1 mM in 100% methanol) was added to 0.2 ml of the extracts at various concentrations. As a control, 0.2 ml of each solvent was used. The absorbance of the sample and control was measured at 517 nm using a UV-VIS spectrophotometer (Model UV 1900, Shimadzu) against a blank after 30 min of incubation in the dark at room temperature. A quadratic regression equation ($y = ax^2 + bx + c$) was generated using microsoft excel. On inserting y = 50% to the equation $y = ax^2+bx+c$, it was changed to the form $ax^2 + bx + c = 0$. The following formula was used to derive the IC_{50} from the equation $ax^2 + bx + c = 0$:

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

where, $x = IC_{50}$ (µg/ml)

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.

2.8 Statistical analysis

Triplicates of each sample were used for statistical analysis and the results were reported as mean, standard error. One-way and two-way analysis of variances (ANOVA) was employed in online statistical analysis (OPSTAT) to examine, if there were any significant differences between the sample means.

3. Results

3.1 Preliminary phytochemical screening

The two extracts (aqueous and methanol) of *A. mexicana* were subjected to a preliminary phytochemical screening, which revealed the presence of alkaloids, sterols, glycosides, lipids, phenolic compounds, tannins, gum and mucilage, flavonoids, and saponins.

3.2 Mineral analysis

Table 2 showed that the six mineral element named chromium, copper, zinc, vanadium, iron and nickel were present in *A. mexicana* flowers. The concentrations of minerals were found to be highest for iron followed by zinc, copper, chromium, nickel and vanadium.

Table 2: Minerals content (ppm) in *A.mexicana* flowers

Minerals	Conc. in ppm	SE(m)	CD @5%
Chromium	5.70 ± 0.12	0.07	0.24
Copper	6.90 ± 0.06	0.03	0.10
Iron	92.37 ± 4.09	3.80	12.56
Nickel	4.07 ± 0.24	0.14	0.48
Vanadium	3.40 ± 0.06	3.40	0.06
Zinc	46.44 ± 6.30	0.07	0.23

Figure 1: *Argemone mexicana* L., Papaveraceae.

Table 3: Bioactive compounds identified in *A. mexicana* flower extract in two solvents

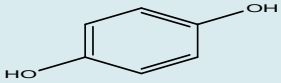
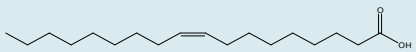
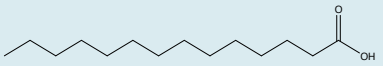
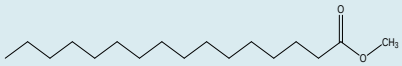
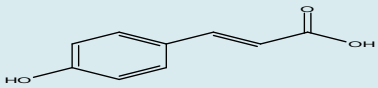
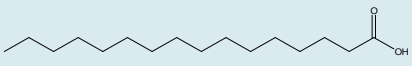
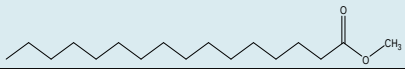
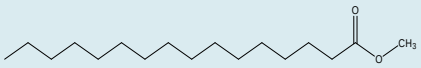
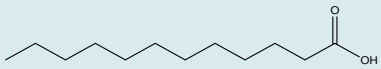
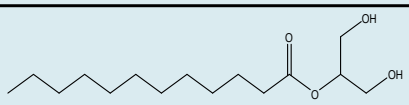
Retention time	Structure of the compound	Name of the compound	Category
5.825		1,4-benzenediol	Phenol
8.215		Dodecanoic acid	Fatty acid
11.051		n-Tetradecanoic acid	Carboxylic acid
12.149		Palmitic acid, methyl ester	Fatty acid ester
16.343		3-(4-hydroxyphenyl)-2-Propenoic acid	Phenyl acids
17.408		n-Hexadecanoic acid	Fatty acid
19.175		Linoleic acid, methyl ester	Fatty acid ester
19.325		Oleic acid	Fatty acid
25.876		9,12-Octadecadienoic acid(z,z)-	Fatty acid ester
26.204		2-hydroxy-1-(hydroxymethyl) ethyl ester	Fatty acid ester

Table 4: DPPH free radical scavenging activity (%) of *A. mexicana* flower extracts

Sr. No.	Conc. (mg/ml) → / Extracts ↓	DPPH free radical scavenging activity (%)								
		120	100	80	60	40	20	IC_{50} (g/ml)	Regression equation	Correlation coefficient R^2
2.	Methanol	99.2	87.09	77.6	67.6	54.9	36.4	38.33	y = 0.2407 × + 2.4611	0.9703
3.	Aqueous	78	76.5	75.8	63.2	47.2	21.2	41.92	y = 0.2028 × + 3.0964	0.9832

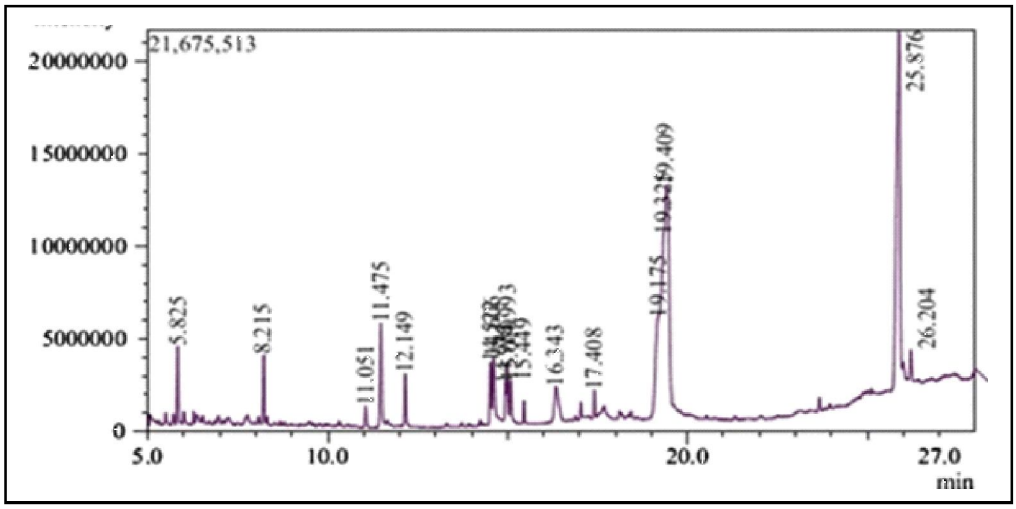


Figure 2: GC-MS analysis of *A. mexicana* flower extract.

3.3 GC-MS analysis of extracts

Gas chromatography and mass spectroscopy are used to identify the bioactive components in the methanol and water extracts of *A. mexicana* flowers. Table 3 lists the active principles along with their retention time, structure, chemical name and class. Figure 2 depicts the existence of bioactive phytochemical components in *A. mexicana* flower extract.

3.4 Antioxidant activity

DPPH method has been widely used to evaluate the antioxidant activity as researched by Wang *et al.* (2002). The extracts' ability to scavenge DPPH was assessed as a test of their antioxidant activities. Antioxidant activity and free radical scavenging is not quite the same thing, yet they are interconnected. Because of this, the test's activity suggests the existence of possible antioxidants. 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 benchmark was BHA (butylated hydroxyl anisole). Values for the IC_{50} (half maximum inhibitory concentration) were determined using regression equations from microsoft excel. As shown in Table 4, the DPPH free radical scavenging activity of flower extracts of *A. mexicana* increased with increasing concentration levels. The IC_{50} value of methanol root extract was minimum (38.33 g/ml) and that clearly indicated the highest free radical scavenging activity as in table

4. Discussion

Plant material analysis and extraction are crucial for development, modernization and quality assurance. Studying medicinal plants also makes it easier to understand plant toxicity and aids in shielding humans and animals from environmental toxins. Determining the mineral content, bioactive compounds and antioxidant activity of *A. mexicana* flowers was the purpose of the current study. According to the study's findings, the flower extract contains both bioactive chemical components and mineral elements. Bioactive chemical components that can donate hydrogen to free radicals in order to scavenge potential damage.

4.1 Mineral analysis

Variable parts of the plants contain different amounts of mineral elements, especially the flowers, roots, seeds and leaves (Van Soest *et al.*, 1967). It is important to identify mineral elements in plants. Some plants contain significant amounts of minerals; their presence and abundance are influenced by the plant's genealogy, history and phytochemical characteristics (Lokhande *et al.*, 2010). The plant's medicinal strength is influenced by a range of phytochemical and elemental compositions. The flowers of *A. mexicana* included six mineral elements: chromium, copper, zinc, vanadium, iron and nickel. Iron was discovered to have the highest mineral concentrations, followed by zinc, copper, chromium, nickel and vanadium.

4.2 GC-MS analysis of extracts

Gas chromatography-mass spectrometry analysis revealed the presence of ten compounds in the methanol extract as well as in aqueous extract. Dodecanoic acid, n-tetradecanoic acid and 3-(4-hydroxyphenyl)-2-propenoic acid are three of the reported phytocompounds that have antioxidant properties (Abirami and

Rajendran, 2011; Kala *et al*., 2011). According to earlier researchers, octadecadienoic acid (Z, Z) exhibits anti-inflammatory, hypocholesterolemic and antiarthritic properties (Ponnamma and Manjunath, 2012). 1,4-benzenediol is a phenolic molecule and it is generally accepted that these molecules have physiological functions as a chemical defence against some pathogens that cause diseases in both humans and animals. The chemical 1,4-benzenediol has antimicrobial and colon cancer-preventive properties (Rao *et al*., 1998). In addition to its antibacterial capabilities, 1,4-benzenediol has also been linked to antitumor, antipesticide, antioxidant, chemopreventive, gastropreventive and hepatoprotective actions (Katerere *et al*., 2003). Esters are commonly utilised in pharmaceuticals, cosmetics, detergents, perfumes and personal care products. Esters (ethyl oleates) can also be employed as hydraulic fluids, plasticizers, lubricants and biological additives (Hazarika *et al*., 2002). The flower section of *A. mexicana* may be employed in a variety of pharmacological and industrial applications due to the presence of the bioactive compounds (Alam and Khan, 2020).

4.3 Antioxidant activity

Using solution of 2, 22 -diphenyl-1-picrylhydrazyl radical (DPPH), researchers can assess that how natural compounds donate electrons (Nunes *et al*., 2012). The technique relies on the DPPH being scavenged by the addition of an antioxidant or radical species that makes the DPPH solution less colourful. The concentration and potency of the antioxidants are inversely correlated with the degree of colour change (Krishnaiah *et al*., 2011). Methanol extracts demonstrated a much higher percentage of inhibition than aqueous in the current investigation among the flower part of *A. mexicana*. In contrast to those extracted in water, plant extracts in methanol solvent offered more consistent antioxidant activity, according to our research. These findings can be explained by the polarity of the molecules that each solvent is extracting.

5. Conclusion

The *A. mexicana* flower extracts were quantitatively analyzed and it was observed that they included a variety of bioactive and mineral constituents. The preliminary phytochemical screening showed the presence of alkaloids, sterols, glycosides, lipids, phenolic compounds, tannins, gum and mucilage, flavonoids and saponins. The antioxidant activity of flower extracts was demonstrated; as a result, they are a better source of antioxidants. As a result, *A. mexicana* flower extracts are considered to be potent antioxidants with a variety of bioactive and mineral constituents. The extract of *A. mexicana* flowers may have therapeutic and antioxidant effects due to the presence of bioactive components. Therefore, knowledge of this plant's bioactive components, mineral elements and antioxidant activity may be useful to researchers planning to conduct additional research on it. Studies on *A. mexicana's* bioactive components, mineral components and antioxidant activity are found to be very promising, which begs for more systematic research on this medicinal plant.

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Conflict of interest

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

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