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Evaluation and Comparision of Total Phenolics, Total Flavonoids and Antioxidant Activity of A. Mexicana Aerial Parts in Different Solvents

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Abstract

The study's goal was to determine the total phenolics, total flavonoids, and antioxidant activity of *A. mexicana* aerial parts. Extracts were prepared using different solvents namely n-hexane, ethyl acetate, acetone, methanol, and water on basis of increasing polarity using soxhlet appratus. Aqueous extract from the flower contained the highest level of total phenolics, followed by that of the stem and leaves. The flower methanol extract was found to contain the most total flavonoids. With IC_{50} values of 24.98 g/ml in acetone extract, the stem component demonstrated the most DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity, followed by leaves with IC_{50} values of 36.29 g/ml in hexane extract and flowers with IC_{50} values of 38.33 g/ml in acetone extract. FTIR analysis showed that the presence of phenols and flavanoids. In different solvents, the DPPH free radical scavenging activity of aerial portions of *A. mexicana* varied greatly, and it increased with increasing concentration levels. In terms of antioxidant potency, higher amounts of total phenolics in aqueous extract, total flavonoids in methanol extract, and acetone extract were shown to be the most potent antioxidant. The aerial parts of A. mexicana have a considerable **amount of flavanoids, phenolics, and antioxidant activity.**

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Introduction

Humans are given medicinal plants as a gift from nature, allowing them to enjoy a disease-free and healthy existence. India is one of the world's most medically and culturally diverse countries, and the medicinal plant industry has a lengthy history that is still respected today [16]. The study of therapeutic plants is currently a hot issue [22]. In medicinal plants, there are two categories of metabolites: main and secondary. Secondary metabolites are not directly involved in metabolic processes, but primary metabolites are. It has the ability to enhance all metabolic and catabolic responses [14]. Polyphenols, flavonoids, alkaloids, phenols, tannins, and saponins are secondary metabolites responsible for A. mexicana's therapeutic efficacy [13]. The phenolic/antioxidant compounds are commonly found in extracts of natural products from plants and fungus, and they can have a variety of biological effects, including antioxidant activity. The antioxidative effect is mostly due to phenolic components such as flavonoids, phenolic diterpenes, and phenolic acid. The activities of such compounds are determined by their redox properties [8]. Antioxidants are necessary nutrients that protect the body from oxidative stress

caused by free radicals. Exogenous and endogenous antioxidants, whether synthetic or natural, can aid to prevent free radical formation by scavenging or encouraging their decomposition, as well as prevent diseases caused by them [19]. A. Mexican Linn is a plant native to northern India that belongs to the Argemone genus of the Papaveraceae family. A. mexicana is a common weed that grows along roadsides and in fields in India. It's a prickly annual herb that may reach 1.2 metres in height and has spread throughout India up to 1,500 meters in elevation [20]. The various portions of this weed have strong emetic and sedative qualities, and have been used to cure syphilis and numerous skin ailments for centuries. The entire plant is used to cure asthma in ethnobotany [1]. For the Haryana region exclusively, exploration and exploitation of aerial portions of A. mexicana for total phenolic content, total flavonoid content, and antioxidant activity. The goal of this study was to determine the total phenolic content, total flavonoids content, and antioxidant activity of A. mexicana aerial parts using a variety of solvents, including methanol, acetone, water, ethyl acetate, and hexane, with polarities ranging from nonpolar to most polar.

Materials and methods

Chemicals used

All of the chemicals and reagents utilised were analytical reagent grade . Hi-media Pvt. Ltd., India, provided DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, gallic acid, aluminium chloride, catechin, sodium carbonate, and the Folin–Ciocalteu reagent. Sigma-Aldrich provided the solvents acetone, ethyl acetate, methanol, and hexane (Mumbai, India). Prior to analysis, distilled water was utilised for sample preparation, dilution, and rinsing apparatus.

Instruments

In the analysis, a FTIR, a UV-VIS spectrophotometer (Shimadzu Model UV 1900), a refrigerator, a remi centrifuge, a scalpel, a grinder, a measuring cylinder, Whatman filter paper (No. 42) micropipettes, an electronic balance, a separatory funnel, and aluminium foil were employed.

Plant material and extract preparation

The stem, leaves, and flowers of A. mexicana (Mexican poppy) were picked in the semi-arid region of Haryana and delivered to the lab within 2 hours. Before being ground into powder, the samples were shade dried. An electric grinder was used to finely ground the dried stem, leaves, and flower components. After that, the powdered form was kept in airtight containers for assay of total phenols, flavonoids, and antioxidant activity. The Soxhlet extraction device was used to prepare the extracts [17]. Then, for sequential extraction, hexane, ethyl acetate, acetone, methanol, and distilled water were utilised in that order (from non-polar to polar). 5g of powdered stem, leaf, and flower samples were placed in Whatman No. 1 thimble filter paper in a 250 mL round bottom flask of a standard soxhlet setup to prepare these extracts. 150 mL of the solvents (distilled water, methanol, acetone, ethyl acetate, and hexane) were added up to one and a half syphons. The process was kept running for 5-6 hours using a syphon mechanism after completing 7-8 cycles with methanol, acetone, ethyl acetate, and hexane as solvents. However, extracting distilled water by the syphon mechanism takes longer, taking more time to complete the 7-8 cycles because each cycle takes longer. The volume of each filtered solvent was measured after extraction. All extracts were kept at 4°C in the refrigerator for further used to estimate total phenols, total flavonoids and antioxidant activity.

Estimation of total phenolics content

The total phenolic content of extracts was determined using the Folin-Ciocalteu method [21]. After

diluting each extract with the appropriate solvent, 1.0 mL of 1mol/L Folin-Ciocalteu reagent was applied. Following that, 2.0 mL of Na2CO3 (20% w/v) was added. The solution was agitated on a stirrer, and water was added to bring the total volume to 10.0 mL. After 8 minutes, the mixture was centrifuged at 6000 rpm for 10 minutes. A UV-VIS spectrophotometer (Model UV 1900, Shimadzu) was used to compare the absorbance of the supernatant solution to a blank made in the same fashion but without the extracts at 730 nm. A calibration curve was built using gallic acid as a standard. The results were expressed as mg GAE/g on a dry weight basis.

Estimation of total flavonoids content

The total flavonoids content of extracts was determined using the colorimetric assay [18]. In test tubes containing 1.0 mL of each extract, 4.0 mL of double distilled water and 0.3 mL of NaNO2 (5 percent, w/v) were added. After 5 minutes, 0.3 mL (10% w/v) AlCl3 was added. The entire volume was brought up to 10.0 mL with distilled water after immediately adding 2.0 mL 1M NaOH. The solution was thoroughly mixed, and the absorbance was measured using a UV-VIS spectrophotometer (Model UV 1900, Shimadzu) at 510 nm against a blank prepared similarly but without extracts. A calibration curve was built using catechin as a standard. The data were expressed as mg CE/g on a dry weight basis.

Antioxidant activity

The antioxidant activity of the extracts was assessed using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay [9]. The weight of dry mass of A. mexicana leaves, stems, and flowers was calculated using acetone, methanol, aqueous, ethyl acetate, and hexane extracts. To make the stock solution (5000 g/mL), the dry mass of all the different extracts was redissolved in a suitable volume of methanol. The degree of discolouration indicates an antioxidant's scavenging potential in terms of hydrogen donating ability [5]. Using appropriate solvent dilutions, various concentrations were obtained from stock solution (i.e. methanol for acetone, methanol, ethyl acetate & hexane extracts, and with methanol: water for water 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 extracts (different concentrations) and rapidly agitated on a stirrer for 5 minutes to test antioxidant activity. A DPPH stock solution was produced in 50 percent (v/v) methanol: water for antioxidant activity in water extracts (different concentrations), and the rest of the technique was the same. Instead of extract, 0.2 mL of each solvent was used as a control. The absorbance of the sample and control was measured at 517 nm using a UV-VIS spectrophotometer (Model UV 1900, Shimadzu) against a blank containing the matching solvent after 30 minutes of incubation in the dark at room temperature. The percent DPPH free radical scavenging activity (y-axis) was plotted versus extract concentration (x-axis) to generate a graph. A quadratic regression equation ($y = ax2+bx+c$) was then generated using Microsoft Excel. On inserting $y = 50\%$ to the equation $y = ax2+bx+c$, it was changed to the form $ax2+bx+c = 0$. The following formula was used to derive the IC50 from the equation $ax2+bx+c = 0$:

Where, x = IC50 (µg/mL)

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

Where,

A control is the absorbance of the control,

A sample is the absorbance of the sample.

Results and Discussion

Medicinal plants play significant roles in and contributions to knowledge and utilisation, and this work appears to be the first for Haryana region to validate the phenolic and flavonoid content, as well as the antioxidant activity of A.mexicana aerial parts. Because of the variable method of extract extraction, kind of solvent employed, rainfall, and meteorological conditions, the results of other researchers varies. The easiest method for detecting secondary metabolites in plant extract is preliminary phytochemical screening. Phenols, flavonoids, proteins, tannins, sterols/terpenes, and alkaloids are found in the methanolic and ethanolic extracts of A. mexicana leaves (Ibrahim and Ibrahim, 2009). A. mexicana leaf, stem, and flower methanol extracts revealed positive results for most medicinally relevant phytochemical elements related to curative, antibacterial, and antifungal action in early phytochemical screening [10]. [13] linked the anticancer effects of flavonoids to a methanol extract of A. mexicana L. leaves to support the plant's traditional usage in cancer prevention. [12], also reported the preliminary discovery of similar types of phytocompounds. The results of this investigation correspond with those of Goswami et al. (2014), who found that phenolic compounds were highest in methanol extracts of A. mexicana and that total phenolic content and total flavanoid content were found in flowers (23.5 mg GAE/g DW and 34.5 mg QE/g DW, respectively).

Total phenolic content

The total phenolic content of aerial parts of A. mexicana varied greatly, as indicated in Table-1. Flowers had the highest phenolic content, with 35.75 mg GAE/g, compared to 26.59 mg GAE/g in the stem and 21.44 mg GAE/g in the leaves. When compared to solvent extracts [aqueous (32.16 mg GAE/g), methanol (31.08 mg GAE/g), acetone (26.46 mg GAE/g), ethyl acetate (26.02 mg GAE/g), and hexane (23.89 mg GAE/g)] stem, leaves, and flowers of A. mexicana revealed the greatest phenolic content, i.e. 32.16 mg GAE/g]. Our findings are consistent with Khan et al. (2019), who found 28.5± 1.15 mg GAE/ g of plant extract in stems, 22.23 ±0.61 mg GAE/g in flower, and 20.89 ±0.89 mg GAE/g in leaves of A. Mexicana. Chang et al. (2002), found 106.65 mg GAE/g phenolic content in an ethyl acetate extract of A. mexicana leaves and 70.19 mg GAE/g in a methanol extract.The results vary due to the numerous methods of extract preparation, which include utilising a Soxhlet extraction device and then adding 7 percent H2SO4 and ether then with help of a separatory funnel the ether layers were separated and evaporated at room temperature after being filtered.

Total flavonoid content

The total flavonoids content of various portions of A. mexicana varied greatly, as indicated in Table-2. Flowers (31.20 mg CE/g) had the maximum flavonoid concentration followed by leaves (29.58 mg CE/ g) and stems (28.40 mg CE/g). When comparing among solvent extracts of various polarity, it was discovered that methanol extract (36.98 mg CE/g) had the maximum flavonoid concentration followed by aqueous (34.44 mg CE/g), ethyl acetate (31.76 mg CE/g), hexane (31.20 mg CE/g) and acetone (24.19 mg CE/g). Goswami et al., 2014 reported that flowers had the highest flavonoid concentration in A. mexicana methanolic extract (41.76 0.74 mg QE/g), followed by leaf (30.59 1.27 mg QE/g), and stem (22.83 0.83 mg QE/g). Variation in the result due to modified method used by researcher and quercetin was used as the standard flavonoid. Datkhile et al., 2020 estimated 32.5mg QE/g flavonoid content in leaves, 6.25mg QE/g in the stem, and 34.50mg QE/g in flowers.

DPPH free radical scavenging activity (%)

Other researchers calculated the IC50 value of different portions of A. mexicana extracts. Datkhile et al.

Table 1. Total phenolics (mg GAE/g) among different solvent (aqueous, methanol, acetone, ethyl acetate, and hexane) extracts of A. mexicana stem, leaves, and flowers

Sr. No.	Plant parts	Total phenolics(mg GAE/g)					
		Extracts					
		Methanol	Acetone	Water	Ethyl acetate	Hexane	Mean
1.	Stem	23.19±0.16	20.51±0.03	24.69±0.14	20.50±0.04	18.32±0.03	21.44
2.	Leaves	29.84±0.24	24.81±0.23	30.90±1.29	24.16±0.05	23.24±0.02	26.59
3.	Flow-	40.22±0.57	34.08±0.36	40.90±1.29	33.42±0.22	30.13±0.01	35.75
	Mean	31.08	26.46	32.16	26.02	23.89	

Table 2. Total flavonoids (mg CE/g) among different solvent (aqueous, methanol, acetone, ethyl acetate, and hexane) extracts of A. mexicana stem, leaves, and flowers

Sr. No.	Plant parts	Total phenolics (mg GAE/g)	Total phenolics (mg GAE/g)	Total phenolics (mg GAE/g)	Total phe-nolics(mg GAE/g)	Total phe-nolics(mg GAE/g)	Total phe-nolics(mg GAE/g)
		Extracts					
		Methanol	Acetone	Water	Ethyl ace-tate	Hexane	Mean
1.	Stem	36.08±0.43	21.88±0.03	31.93±0.33	31.67±0.18	20.48±0.04	28.40
2.	Leaves	35.46±0.47	24.72±0.13	35.29±0.85	31.22±0.13	21.25±0.37	29.58
3.	Flowers	39.31±0.13	25.99±0.09	36.12±0.37	32.41±0.12	22.19±0.04	31.20
	Mean	36.98	24.19	34.44	31.76	31.20	

Table 3. DPPH free radical scavenging activity (%) of methanol, acetone, aqueous, ethyl acetate, and hexane extracts of leaves of A. mexicana

Sr. No.	Extracts	DPPH Free Radical Scavenging Activity (%)						
		Conc. (mg/ml)						
		120	100	80	60	40	20	IC_{50} (µg/ml)
1.	Methanol	88.7	80.3	70.3	58.9	46.4	29.4	45.95
2.	Acetone	86.2	70.4	60.9	46.9	29.9	11.1	62.29
3.	Water	80.3	75.6	73.9	66.8	55.5	33	37.28
4.	Ethyl Acetate	75.6	64.9	57.2	42.5	26.9	19.8	68.91
5.	Hexane	99.5	94.4	82.5	72.9	54.6	33.6	36.29

Table 4. DPPH free radical scavenging activity (%) of methanol, acetone, aqueous, ethyl acetate, and hexane extracts of stem of A. mexicana

Sr. No.	Extracts	DPPH Free Radical Scavenging Activity (%)						
		Conc. (µg/mL)						
		120	100	80	60	40	20	IC_{50}
1.	Methanol	87.9	80.6	74.8	58.5	40.2	21.5	48.70
2.	Acetone	99.3	97.6	94.6	88.3	74.9	46.7	24.98
3.	Water	71.6	67.5	63.1	52.4	31.3	23.7	58.88
4.	Ethyl Acetate	78.4	69.2	64.5	58.8	41.7	26.6	48.02
5.	Hexane	97.7	92.9	83.7	64.6	43.5	27.1	41.81

Table 5. DPPH free radical scavenging activity (%) of methanol, acetone, aqueous, ethyl acetate, and hexane extracts of flowers of A. mexicana

Sr. No.	Extracts	DPPH Free Radical Scavenging Activity (%)						
		Conc. (mg/mL)						
		120	100	80	60	40	20	IC_{50}
1.	Methanol	82.5	76.5	64.1	51.6	34.3	21.5	55.18
2.	Acetone	99.2	87.09	77.6	67.6	54.9	36.4	38.33
3.	Water	78	76.5	75.8	63.2	47.2	21.2	41.92
4.	Ethyl Acetate	92.4	83.6	72.5	61.3	51.9	28.3	42.37
5.	Hexane	79.8	71.3	66.7	60.3	46.3	28.7	45.74

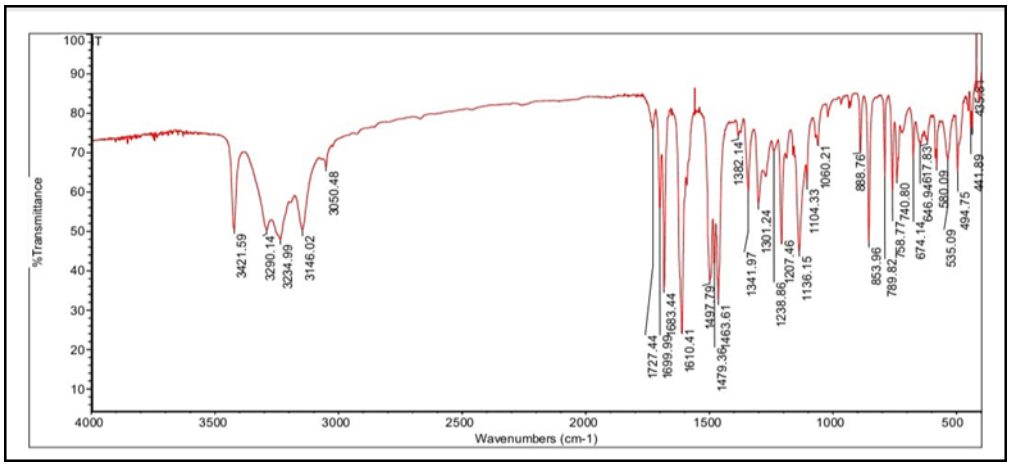

Figure 1. FTIR bands related to A. mexicana's aerial parts for phenols and flavanoids

(2020), calculated the IC50 value of the whole plant to be 68.76g/ml in aqueous extract, 50.66g/ml in methanol extract, and 39.39g/ml in ethanol extract. A. mexicana Linn. leaf ethanol extract demonstrated significant free radical scavenging action [6].

DPPH free radical scavenging activity (%) of methanol, acetone, aqueous, ethyl acetate, and hexane extracts of leaves of A. mexicana

Different leaf extracts displayed a wide range of DPPH free radical scavenging activity, which increased with increasing concentration levels. Methanol extract scavenging activity against DPPH free radicals ranged from 88.7% to 29.4%. It ranged from 86.2 percent to 11.1 percent in the case of acetone extract. It ranged from 80.3 percent to 33 percent in the case of aqueous. Similarly, DPPH activity ranged from 75.6 percent to 19.8 percent in the case of ethyl acetate extract, and from 99.5 percent to 33.6 percent in the case of hexane extract. The IC50 value of the ethyl acetate extract was 68.91g/ml, followed by 62.29g/ml for the acetone extract, 45.95g/ml for the methanol extract, 37.28g/ml for the aqueous extract, and 36.29g/ml for the hexane extract as in Table 3.

DPPH free radical scavenging activity (%) of methanol, acetone, aqueous, ethyl acetate, and hexane extracts of stem of A.mexicana

Different stem extracts displayed a wide range of DPPH free radical scavenging activity, which increased with increasing concentration levels. Methanol extract's DPPH free radical scavenging activity ranged from 87.9% to 21.5 percent. It ranged from 99.3 percent to 46.7 percent in the case of acetone extract and from 71.6 percent to 23.7 percent in the case of aqueous extract. Similarly, it ranged from 78.4 percent to 26.6 percent in the case of ethyl acetate, and from 97.7 percent to 27.1 percent in the case of hexane extract. The IC50 value of the aqueous extract was 58.88 g/ml, followed by 48.70 g/ml for methanol, 48.02 g/ml for ethyl acetate, 41.81 g/ml for hexane, and 24.98 g/ml for acetone, showing that the aqueous extract has the highest activity, as shown in Table 4.

DPPH free radical scavenging activity (%) of methanol, acetone, aqueous, ethyl acetate, and hexane extracts of flowers of A. mexicana

Different flower extracts displayed a wide range of DPPH free radical scavenging activity, which increased with increasing concentration levels. Methanol extract's DPPH free radical scavenging activity ranged from 82.5 to 21.5 percent. It ranged from 99.2 percent to 36.4 percent in the case of acetone extract and from 78 percent to 21.2 percent in the case of aqueous extract. Similarly, ethyl acetate extract ranged from 92.4 percent to 28.3 percent, and hexane extract ranged from 79.8% to 28.7 percent. The methanol extract had the highest activity, with an IC50 value of 55.18g/ml, followed by 45.74g/ml for the hexane extract, 42.37g/ml for the ethyl acetate extract, 41.92g/ml for the aqueous extract, and 38.33g/ml for the acetone extract, as shown in Table 5.

FTIR Analysis

As shown in Figure 1, FTIR bands associated to A. mexicana's aerial parts revealed the presence of phenols and flavanoids. FTIR analysis showed bands at 3421 cm-1 ,3290 cm-1, 3234 cm-1,3146 cm-1, probably related to -NH and bonded -OH groups of carboxylic acids. The band at 1727cm-1, 1699 cm-1 could be related to C=C stretching vibration of aromatic rings and to the vibration of N-H of amines, C=O of amides and carboxylic groups , in addition, this band could be related to flavonoids and amino acids. The band at 1341 cm-1 could be related to C-O stretching of acid groups or to bending vibrations of -CH3 or -CH2 groups.

Conclusion

The antioxidant activity of A.mexicana aerial parts with higher phenolic and flavonoid content was assessed in this study, indicating that they could be a substantial source of natural antioxidants. The presence of total phenolics, flavonoids, and antioxidant activity in methanol, acetone, aqueous, ethyl acetate, and hexane extracts of A. mexicana stem, leaves, and flowers was discovered in this study. The highest phenolic and flavonoid concentration was found in flowers. As a result, among the stems, leaves, and flowers of A. mexicana, flowers are largely employed for therapeutic purposes. To better comprehend their potential to regulate diseases that have a substantial impact on quality of life, more research into the separation and identification of responsible antioxidant components, as well as their mechanism of action, is required as well as biological activity.

Conflict of interests

There are no conflicts of interest among the authors. All authors contributed equally to the study work and agreed to have it published.

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