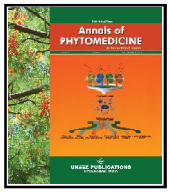
Annals of Phytomedicine 11(2): 494-499, 2022

DOI: http://dx.doi.org/10.54085/ap.2022.11.2.60

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Evaluation and comparison of the leaves and stem of *Argemone mexicana* L. in various solvents for total phenolics, total flavonoids and antioxidant activity

Neha Goel, Sachin Kumari◆, Sushila Singh, Vinod Sangwan*, Krishan Kumar Bhardwaj, Monika Moond, Monika Panghal and Indu Rani**

Department of Chemistry, College of Basic Sciences & Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar-125004, Haryana, India

**Department of Biochemistry, College of Basic Sciences & Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar-125004, Haryana, India*

***Department of Soil Science, College of Agriculture, Chaudhary Charan Singh Haryana Agricultural University, Hisar-125004, Haryana, India*

Article Info

Article history
Received 3 July 2022
Revised 18 August 2022
Accepted 19 August 2022
Published Online 30 December-2022

Keywords
Argemone mexicana L.
Extract
DPPH
Colorimetric
Soxhlet
Stem
Leaves
Antioxidants

Abstract

The use of plant leaves and stem is a common practice in traditional medicine. The naturally available herbs are used for medicinal purpose to promote health. The leaves and stem of *Argemone mexicana* L. is one such herb used against different ailments. Studies on the antioxidant, phenolic and flavonoid content of the leaves and stem using various solvent extracts were; however, scarce for the Haryana region. The aim of the research was to assess the antioxidant activity, total phenolics and total flavonoids of *A. mexicana* leaves and stem. Using the Soxhlet apparatus, extracts were prepared using a variety of solvents, including ethyl acetate, acetone, water and methanol, based on variable polarity. Among all solvents, methanol extract had the highest concentration of total phenolics as well as total flavonoids in leaves compared to stem. *A. mexicana* leaves and stem displayed a wide range of DPPH free radical scavenging activity, which was higher at higher concentrations (100 mg/ml). The leaves component showed the greatest DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity with IC_{50} values of 23.9 µg/ml in methanol extract and stem part with IC_{50} values of 26.2 µg/ml in the same extract. FTIR analysis showed that the presence of phenols and flavanoids. In terms of antioxidant potency, higher amounts of total phenolics as well as total flavonoids in methanol extract of leaves and stem was shown to be the most potent antioxidant. The leaves and stem of *A. mexicana* have a considerable amount of flavanoids, phenolics and antioxidant activity.

1. Introduction

Humans are given medicinal plants as a gift from nature, allowing them to enjoy a disease-free and healthy existence. Plants have been a valuable invention that has provided nearly everything required for human survival. Humans adopt diverse plants as medicines to treat a range of ailments (Sharma *et al*., 2021). 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 (Kumari *et al*., 2022). The study of therapeutic plants is currently a hot issue (Velavan, 2015). There are two types of metabolites found in medicinal plants: primary and secondary. Primary metabolites play a direct role in metabolic processes, whereas secondary metabolites do not. It has the ability to enhance all metabolic and catabolic responses (Joshi *et al*., 2013). Secondary metabolites phenols, tannins, alkaloids, flavonoids, polyphenols and saponins are in responsibility of *A. mexicana*'s therapeutic effectiveness (Ji *et al*., 2011). Medicinal plants possess biologically active secondary metabolites such iridoids, diterpenes, phytoecsteroids, flavonoids, sterol glycosides and phenylethonoid glycosides, according to Mamarasulov *et al*. (2020).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 (Haruna *et al*., 2019). Due to the presence of many active components, the plant's leaves, fruit, roots, stem, tubers and/or bark may be useful against certain infections (Ahmad, 2020). 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 (Rajalakshmi *et al*., 2016). *A. mexicana* 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 (Figure 1). It is a prickly annual herb that may reach 1.2 m in height and has spread throughout India up to 1,500 m in elevation (Rajvaidhya *et al*., 2012).The various

Corresponding author: Dr. Sachin Kumari
Department of Chemistry, College of Basic Sciences & Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar-125004, Haryana, India
E-mail: sachindalal@hau.ac.in
Tel.: +91-9812008680

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 (Alam and Khan, 2020). Exploration and utilisation of *A. mexicana* leaves and stem for total phenolic content, total flavonoid content and antioxidant activity for the Haryana region only. Using a range of solvents, including acetone, ethyl acetate, methanol and water with polarity increasing; the aim of this study was to measure the total phenolic content, total flavonoid content and antioxidant activity of *A. mexicana* leaves and stem sections.

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

2. Materials and Methods

2.1 Chemicals used

All of the chemicals and reagents utilized 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 and methanol (Mumbai, India). Prior to analysis, distilled water was utilised for sample preparation, dilution and rinsing apparatus.

2.2 Instruments

In the analysis, a FTIR (Thermo Fischer scientific), a UV-VIS spectrophotometer (Shimadzu Model UV 1900), a refrigerator, a remi-centrifuge and an electronic balance were employed.

2.3 Plant material and extract preparation

The leaves and stem of *A. mexicana* (Mexican poppy) (500 No's) were collected from the semi-arid region of Haryana and delivered to the lab within 2 h. Before being ground into powder, the samples were shade dried. An electric grinder was used to finely ground the dried leaves and stem components separately. After that, the powdered form was kept in airtight containers for assay of total phenols, flavonoids and antioxidant activity. The Soxhlet extraction was used to prepare the extracts (Luque *et al*., 1998). Then for sequential extraction acetone, ethyl acetate, methanol and water were utilized. 5 g of powdered leaves and stem 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 (acetone, ethyl acetate, methanol and water) were added up to one and a half syphons. The process was kept running for 5-6 h. using a syphon mechanism after completing 7-8 cycles with acetone, ethyl acetate and methanol as solvents. However, extracting 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. To quantitatively evaluate phenols, total flavonoids and antioxidant activity; all extracts were kept at 4°C in the refrigerator.

2.4 Estimation of total phenolic content

The total phenolic content of extracts was determined using the Folin-Ciocalteu method (Singleton *et al*., 1965). Each extract was mixed with the appropriate solvent before being treated with 1.0 ml of 1mol/lFolin-Ciocalteu reagent. Following that, 2.0 ml of Na_2CO_3 (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 min, the mixture was centrifuged at 6000 rpm for 10 min. 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 is often used as a standard (Figure 2). On a dry weight basis, the results were represented as mg GAE/g.

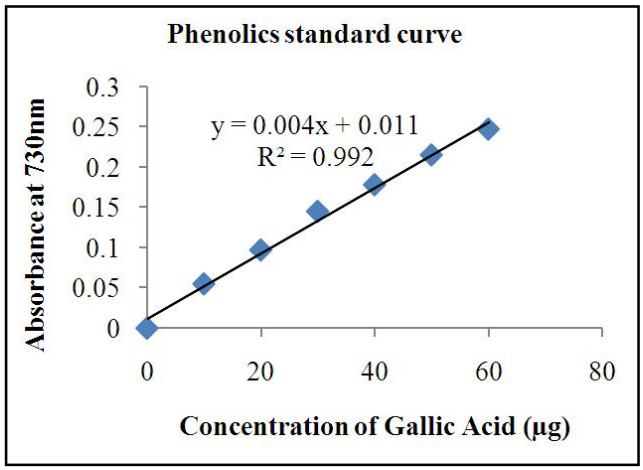


Figure 2: Standard curve of total phenols.

2.5 Estimation of total flavonoid content

Using the colorimetric approach, the total flavonoid content of extracts was examined (Marinova *et al*., 2005). In test tubes containing 1.0 ml of each extract, 4.0 ml of double distilled water and 0.3 ml of $NaNO_2$ (5 %w/v) were added. After 5 min, 0.3 ml (10% w/v) $AlCl_3$ was added. The entire volume was brought up to 10.0 ml with distilled water after immediately adding 2.0 ml 1M NaOH. A UV-VIS spectrophotometer (Model UV 1900, Shimadzu) was used to analyze the solution's absorbance at 510 nm in reference to a blank that was made in the same way but without extracts. A calibration curve was built using catechin served as a standard (Figure 3). The information was presented as dry weight-based mg CE/g.

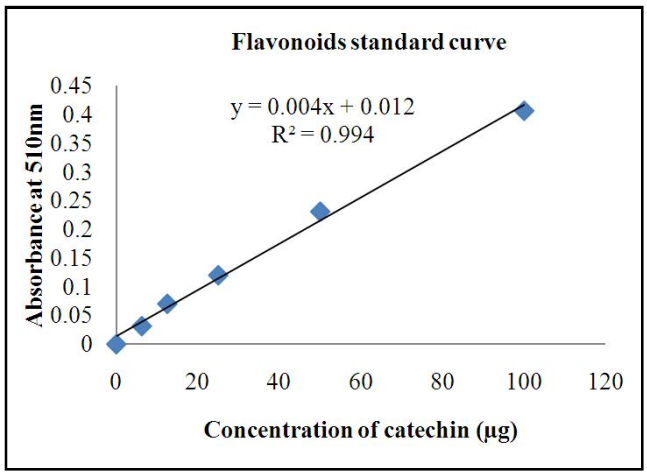


Figure 3: Standard curve of total flavonoids.

2.6 Antioxidant activity

The free radical scavenging 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) analysis was used to verify the extracts' antioxidant activity (Hatano *et al*., 1998). The weight of dry mass of *A. mexicana* leaves, stems were calculated using acetone, ethyl acetate, methanol and water extracts. To make the stock solution (5000 g/ml), the dry content of each extract was redissolved in an appropriate amount of methanol. The degree of discolouration indicates an antioxidant's scavenging potential in terms of hydrogen donating ability (Eberhardt *et al*., 2000). Using appropriate solvent dilutions, various concentrations (20, 40, 60, and 80,100 µg/ml) were obtained from stock solution (*i.e.* methanol for acetone, methanol, ethyl acetate 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 min to test antioxidant activity. A DPPH stock solution was produced in 50 % (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 min of incubation in the dark at room temperature. A quadratic regression equation ($y = ax^2 + bx + c$) was then 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.

2.7 FTIR analysis

Infrared spectra were recorded in the region of 4000-400 cm^{-1}. Five to ten mg of finely ground sample of leaves and stem was mixed homogenously with dry potassium bromide in the ratio of 1:20 by weight and pellets were made into discs by applying pressure.

3. Results

Many nations still heavily rely on traditional medical systems. Plant resources are being stressed for use as a source of medication for a wide variety of human ailments (Sethumathi *et al*., 2021). Medicinal plants have key roles in and contributions to knowledge and usage.The phytochemicals and antioxidant substances found in nature are a gift to us. The phytochemicals that have been investigated the most are phenolic compounds, flavanoids and carotenoids. The majority of plant species with phenolic content have high antioxidant potential (Kumari *et al*., 2022).This study appears to be the first for the Haryana region to validate the phenolic and flavonoid content, as well as the antioxidant activity of *A.mexicana* leaves and stem.

3.1 Total phenolic content

The total phenolic content of leaves and stem of *A. mexicana* was found to be highest in methanol that is 30.90 mg GAE/g and 24.69 mg GAE/g, respectively. When compared among solvent extracts, maximum found to be in methanol followed by water, acetone and ethyl acetate for leaves and stem as mentioned in Table 1.

Table 1: Total phenolics (mg GAE/g) content in extracts of *A. mexicana*

Sr.No.	Plant parts	Methanol	Water	Acetone	Ethyl acetate
1.	Stem	24.69 ± 0.14	23.19 ± 0.16	21.73 ± 0.21	20.50 ± 0.04
2.	Leaves	30.90 ± 1.29	29.84 ± 0.24	24.81 ±0.23	21.16 ± 0.22

Table 2: Total flavonoids (mg CE/g) content in extracts of *A. mexicana*

Sr.No.	Plant parts	Methanol	Water	Acetone	Ethyl acetate
1.	Stem	35.46 ± 0.47	21.88 ± 0.03	23.93 ± 0.33	21.67 ± 0.18
2.	Leaves	36.08 ± 0.43	24.72 ± 0.13	25.29 ± 0.85	31.22 ± 0.13

3.2 Total flavonoid content

The total flavonoid content of *A. mexicana* leaves and stem was indicated in Table 2. The maximum flavonoid concentration was observed in methanol extract of leaves (36.08 mg CE/g) and stems (35.46 mg CE/g). When comparing among solvent extracts of various polarity, it was discovered that methanol extract had the maximum flavonoid concentration followed by acetone, water and ethyl acetate.

3.3 DPPH free radical scavenging activity (%)

Different extracts of *A mexicana* leaves and stem displayed a wide range of DPPH free radical scavenging activity, which increased with increasing concentration levels. DPPH free radical scavenging activity ranged from 83.1% to 3.2%. The IC_{50} value of the *A.mexicana* leaves and stem extracts was found maximum in case of ethyl acetate followed by water, acetone and methanol. The DPPH free radical scavenging activity as well as the IC_{50} value of the stem and leaves of *A. mexicana* were depicted in Tables 3 and 4.

Table 3: DPPH free radical scavenging activity (%) of leaves of *A. mexicana*

Extracts	120 Conc. (mg/ml)	100 Conc. (mg/ml)	80 Conc. (mg/ml)	60 Conc. (mg/ml)	40 Conc. (mg/ml)	20 Conc. (mg/ml)	IC_{50} (µg/ml)
Methanol	83.1 ± 1.43	71.3 ± 1.40	46.1 ± 1.02	24.3 ± 1.50	19.8 ± 1.56	7.1 ± 0.85	23.9
Water	80.2 ± 1.01	64.2 ± 0.61	39.2 ± 0.38	25.0 ± 0.50	16.2 ± 0.53	4.1 ± 0.58	32.2
Acetone	81.0 ± 1.01	65.6 ± 1.05	40.1 ± 1.04	26.1 ± 1.01	17.5 ± 0.45	5.4 ± 0.53	30.5
Ethyl acetate	74.3 ± 1.26	61.0 ± 1.50	36.8 ± 0.80	24.1 ± 0.96	13.9 ± 1.13	4.0 ± 0.87	34.5

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

Extracts	120 Conc. (mg/ml)	100 Conc. (mg/ml)	80 Conc. (mg/ml)	60 Conc. (mg/ml)	40 Conc. (mg/ml)	20 Conc. (mg/ml)	IC_{50} (µg/ml)
Methanol	81.1 ± 1.33	67.3 ± 1.08	42.1 ± 0.90	27.3 ± 0.86	18.8 ± 1.97	6.1 ± 1.05	26.2
Water	80.2 ± 1.20	64.1 ± 0.41	38.2 ± 0.37	25.1 ± 0.70	14.2 ± 0.43	4.9 ± 0.48	33.3
Acetone	79.5 ± 1.28	63.6 ± 1.02	37.1 ± 1.09	24.1 ± 0.64	13.5 ± 0.72	3.4 ± 0.63	31.7
Ethyl acetate	73.3 ± 1.26	60.0 ± 1.05	35.8 ± 1.08	23.1 ± 0.66	11.9 ± 0.73	3.2 ± 0.67	35.0

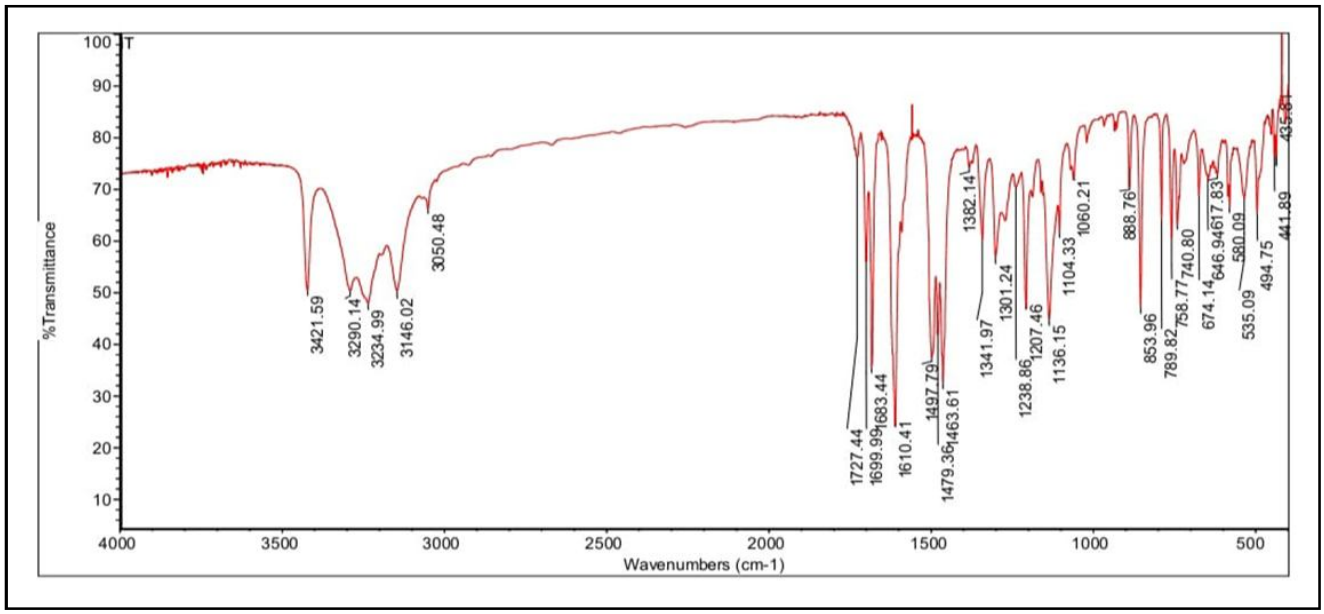


Figure 4: FTIR analysis related to *A. mexicana's* leaves and stem.

3.4 FTIR analysis

As shown in Figure 4, FTIR bands associated to *A. mexicana*'s stem and leaves 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 $-CH_3$ or $-CH_2$ groups.

4. Discussion

A. mexicana leaves and stem extracts were prepared in different solvents for assessment of the phenolic content, flavanoid content and antioxidant activity using standard procedures.The basic method for assessing secondary metabolites in plant extract is preliminary phytochemical screening. Phenols, flavonoids, proteins, tannins, sterols/terpenes and alkaloids are detected in the methanolic and ethanolic extracts of *A. mexicana* leaves (Ibrahim and Ibrahim, 2009). Extensive phytochemical screening of *A. mexicana* leaf, stem, and flower methanol extracts gave satisfactory outcomes for the most of medicinally important phytoconstituents related to curative,

antibacterial and antifungal action (Hussain *et al*., 2011). Gali *et al*. (2011) linked the anticancer effects of flavonoids to a methanol extract of *A. mexicana* leaves to support the plant's traditional usage in cancer prevention. Jaliwala *et al*. (2011), 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*. The higher concentration of phenolic or flavonoid chemicals in the plants that's what affords them their potential for antioxidant activity (Punit *et al*., 2019).The varying method of extract extraction, kind of solvent employed, amount of rainfall and meteorological conditions may have an impact on the findings of other researchers.

Our findings are consistent with Khan and Bhadauria (2019), who found 28.5 ± 1.15 mg GAE/g of plant extract in stems 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 H_2SO_4 and ether then with help of a separatory funnel the ether layers were separated and evaporated at room temperature after being filtered.

Our findings are in agreement with 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). Datkhile *et al*. (2020) estimated 32.5 mg QE/g flavonoid content in leaves, 6.25 mg QE/g in the stem, and 34.50 mg QE/g in flowers. Quercetin was used as the standard flavonoid by researchers.

Other researchers calculated the IC_{50} value of different portions of *A. mexicana* extracts. Datkhile *et al*. (2020), calculated the IC_{50} value of the whole plant to be 68.76 g/ml in aqueous extract, 50.66 g/ml in methanol extract and 39.39 g/ml in ethanol extract. *A. mexicana* L. Leaves ethanol extract demonstrated significant free radical scavenging action (Gawade and Farooqui, 2018). A DPPH-based antioxidant investigation found that methanol extract has remarkable free radical scavenging activity (Das *et al*., 2019).

5. Conclusion

The antioxidant activity of *A. mexicana* leaves and stem parts with higher phenolic and flavonoid content was assessed in this study, indicating that they could be a substantial source of natural antioxidants. The highest phenolic and flavonoid concentration was found in leaves and comparable to stem. As a result, leaves and stem of *A. mexicana*, 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.The results of the experiment showed that methanol was the best solvent and that it caused the greatest amount of molecular migration. It can be inferred that the compounds present in *A.mexicana* leaves and stem are likely polar in nature because methanol is a very polar organic solvent when compared to other extraction solvents like ethyl acetate and acetone. Prior reports have indicated that methanol is a superior extractant for the extraction of compounds (Kumari *et al*., 2022).

Acknowledgements

The authors are grateful to the Department of Chemistry, Biochemistry and Soil Science at CCSHAU, Hisar, for allowing them to conduct this study.

Conflict of interest

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

References

Ahmad, A. (2020). Herbs that heal: The philanthropic behavior of nature. Ann. Phytomed., **9**(1):7-17.http://dx.doi.org/10.21276/ap.2020.9.1.2

Alam, A. and Khan, Adnan A. (2020). *Argemone mexicana* L: A weed with versatile medicinal and pharmacological applications. Ann. Phytomed., **9**(1):218-223. http://dx.doi.org/10.21276/ap.2020.9.1.29.

Chang, C.; Yang, M.; Wen, H. and Chern, J. (2002). Estimation of total flavonoid content in *Propolis* by two complementary colorimetric methods. J. Food Drug Anal., **10**,178-182.

Das,K.; Saifulla, K. M.; Namratha, N.; Swetha, R. and Gezici, S. (2019). Comparative phytochemicalscreening, elemental content and chromatographic evaluation for detection and quantification of polyphenolic compounds for strong antioxidant activity of various extracts of *Abutilon indicum* (Link) Sweet leaves. Ann. Phytomed., **8**(1):36-44.

Datkhile, K .D.; Patil, S. R.; Patil, M. N.; Durgawale, P.; Jagdale, N. J. and Deshmukh,V. N.(2020). Studies on phytoconstituents, *In vitro* antioxidant, antibacterial, and cytotoxicity potential of *A. mexicana* Linn. (Family: Papaveraceae). Journal of Natural Science, Biology and Medicine, **11**(2):198-205.

Eberhardt, M. V.; Lee, C.Y. and Liu, R .H. (2000). Antioxidant activity of fresh apple. Nature, **405**:903-904.

Gawade, B. and Farooqui, M. (2018). Free radical scavenging potential of *A. mexicana* L. leaves. Chemical Science International Journal, **30**(8):39-46.

Goswami, M.; Yadav, A.; Bhardwaj, R.; Joshi,Y. C. and Sharma, R.A. (2014). Antioxidant properties of methanolic extracts of *Argemone mexicana*. Research Journal of Medicinal Plants, **8**:167-177.

Haruna, Y.; Magaji, M. B. and Muhammad, A. (2019). Characterization of antioxidant activity present in methanol extract *A. mexicana* leaf. Emergency Medicine and Critical Care, **3**:796-803.

Hatano, T.; Kagawa, H.;,Yasuhara, T. and Okuda, T. (1998). Two new flavonoids and other constituents in licorice root, their relative astringency and radical scavenging effects. Chemical and Pharmaceutical Bulletin, **36**:2090-2097.

Hussain, A.; Wahab, S.; Zarin, I. and Hussain, S. (2011). Antibacterial activity of the leaves of *Coccinia indica* (W.A.) of India. Advan. Biol. Res., **4**(5):241-248.

Ibrahim, H.A. and Ibrahim, H. (2009). Phytochemical screening and toxicity evaluation on the leaves of *Argemone mexicana* Linn. (Papaveraceae). Int. J. Pure App. Sci. Technol., **3**:39-43.

Jaliwala, Y.A.; Panda, P. K. ; Neha, C.; Kumar, B. N.; Amit, P. and Mohanty, P.K. (2011). *In vitro* anthelmintic activity of aerial parts of *Argemone mexicana* Linn. J. Pharm. Res., **4**(9):3173-3174.

Ji, G.; Shukla, S.K.; Dwived, P.; Sundaram, S. and Prakash, R. (2011). Inhibitive effect of *A. mexicana* L. plant extract on acid corrosion of mild steel. Ind. Eng. Chem. Res., **50**:11954-11959.

Joshi, N.; Bhatt, S.; Dhyani, S. and Nain, J. (2013). Phytochemical screening of secondary metabolites of *A. mexicana* L. flowers. Int. J. Curr. Pharm. Res, **5**(2):144-147.

Khan, A.M. and Bhadauria, S. (2019). Analysis of medicinally important phytocompounds from *A. mexicana*. Journal of King Saud University - Science, **31**(4):1020-1026.

Kumari, S.; Sindhu, M.; Goel, N.; Panghal, M.; Singh, S. and Rani, I. (2022). Effect of extraction techniques and evaluation of antimicrobial ativity of *Argemone mexicana* L. leaves and roots extracts in different solvents. Journal of Scientific Research and Reports, **8**(6):37-43.

Kumari, S.; Sindhu, M.; Singh, S.; Goel, N.; Rani, I. and Panghal, M. (2022). Determination of total phenolic, free radical scavenging activity and antimicrobial activity of root extracts of *Argemone mexicana* L. in methanol solvent. Ann. Phytomed., **11**(11):1-5. http://dx.doi.org/10.54085/ap.2022.11.1.0.

Luque, M. D.; Castro, D. and Garcýìa-Ayuso, L. E. (1998). Soxhlet extraction of solid materials: An outdated technique with a promising innovative future. Analytica Chimica. Acta., **369**(10):1-10.

Mamarasulov, B.; Davranov, K. and Jabborova, D. (2020). Phytochemical, pharmacological and biological properties of *Ajuga turkestanica* (Rgl.) Brig (Lamiaceae). Ann. Phytomed., **9**(1):44-57. http://dx.doi.org/10.21276/ap.2020.9.1.6.

Marinova, D.; Ribarova, F. and Atanassova, M. (2005). Total phenolics and total flavonoids in Bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy, **40**:255-260.

Punit, R.; Bhatt, U.; Patel, D.; Chirag, M.; Modi, K.; Pandya, B. and Patel, H. B. (2019). Thin-layerchromatography and *in vitro* free radical scavenging activity of few medicinal plants from the surroundings of Junagadh, Gujarat, India. Ann. Phytomed., **8**(1):45-55.

Rajalakshmi, P; Pugalenthi, M. and Vadivel, V. (2016). *In vitro* and inhibitory activity of pathogens on leaves of *A. mexicana* L. and *Premna tomentosa* L. International Journal of Herbal Medicine, **4**(5):84-90.

Rajvaidhy, S.; Nagori, B.P.; Singh, G. K.; Dubey, B. K. Desai, P. and Jain, S. (2012). A review on *A. mexicana* L.: An Indian medicinal plant. International Journal of pharmaceutical Sciences and Research, **3**(8):1995-2005.

Sethumathi, P.P.; Manjuparkavi, K.; Lalitha, V.; Sivakumar,T.; Menaka,M.; Jayanthi, A. and Kumar,B.A. (2021). Evaluation of *in vitro* antioxidant and antimicrobial activity of polyherbal formulation of *thirikadugu chooranam* and *parangipattai chooranam*. Ann. Phytomed., **10**(2):169-174. http://dx.doi.org/10.21276/ap.2021.10.2.23

Sharma, B.; Sharma, S. C. and Alam, A. (2021). Phytochemical screening and GC-MS analysis of *Tamarindus indica* L. (Angiosperms:Fabaceae). Ann. Phytomed., **10**(1):215-221. http://dx.doi.org/10.21276/ap.2021.10.1.23.

Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybidic- phosphotungstic acid reagents. American Journal of Enology and Viticulture, **16**:144-158.

Velavan, S. (2015). Phytochemical techniques: A review. World Journal of Science and Research, **1**(2):80-91.

Citation **Neha Goel, Sachin Kumari, Sushila Singh, Vinod Sangwan, Krishan Kumar Bhardwaj, Monika Moond, Monika Panghal and Indu Rani (2022). Evaluation and comparison of the leaves and stem of *Argemone mexicana* L. in various solvents for total phenolics, total flavonoids and antioxidant activity. Ann. Phytomed., 11(2):494-499. http://dx.doi.org/10.54085/ap.2022.11.2.60.**