Original Article

Phytochemical Characterisation of *Argemone mexicana* Leaf Extracts: An Evidence for its Antiandrogenic and Antioxidant Activities

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ABSTRACT

Introduction: The naturally available herbs are used for medicinal purpose to promote health. The leaves of *Argemone mexicana* is one such herb used against different ailments. However, studies involving the characterization of different solvent extracts of the leaves and its antioxidant as well as antiandrogenic properties were limited. **Aim:** The present study aimed to characterize *A. mexicana* leaf extracts and determine its biological activities. **Materials and Methods:** The leaves were washed, shade dried, coarsely powdered and subjected to sequential extraction with solvents of increasing polarity using soxhlet apparatus. The extracts were dried and used to determine its phytochemical, and biological properties following standard protocols. **Results:** The results revealed that all the phytochemicals studied were present in ethanolic extract except protein compared to other extracts. Further, the quantitative estimation of phytochemicals showed that the ethanolic and cold water extracts had maximum amount of total polyphenols and flavonoids. The FTIR results of ethanolic extract showed a very broad peak for –OH groups accompanied by highest total polyphenol content, suggesting rational for its biological activities. Indeed, the *in vitro* antioxidant and antisteroidogenic activities were highest in the ethanolic extract than other extracts. **Conclusion:** This is the first comprehensive study reporting the characterization and antiandrogenic property of *A. mexicana* leaf. Hence, the ethanolic extract of *A. mexicana* leaf can be used as an antioxidant and antiandrogenic agent. Further, this extract can be considered for the treatment for pathological conditions with hyperandrogenic conditions.

Key words: Antioxidant, Polycystic ovary syndrome, Antiandrogenic, Phytochemistry, Polyphenols, Antisteroidogenic.

INTRODUCTION

Phytochemicals are the secondary metabolites/chemicals produced by plants to thrive or defend themselves against the threats or pathogens. Among several phytochemicals, polyphenols, flavonoids and alkaloids are usually recommended as supplements for their rich antioxidant properties.[1] The phytochemicals have beneficial properties in animals when administered and hence these properties can be exploited to enhance the human health status. Indeed, as per WHO[2], naturally available herbs are the best source of medicines. This may be due to the fact that the herbs have little or no side effects compared to synthetic drugs. In addition, India with rich vegetation/herbs and the Ayurvedic system of medicine provide additional benefit for the use of herbs to cure several diseased conditions. Further, recently pharmacological companies are relying on these phytocomponents to develop potent drugs. Hence, there is a dire need to study the phytochemicals present in the different parts of the plant to employ the same by the pharmaceutical companies. *Argemone mexicana*, commonly known as prickly poppy plant belongs to the family Papavaraceae found in several countries including India. Traditionally, the different parts of the plant and as a whole plant is used as a medicinal plant as they have various

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medicinal values. To cite a few, extracts of the plant *A. mexicana* has shown antibacterial,[3] and hepatoprotective[4] activities. Despite of several medicinal values, studies have reported that the seed oil of *Argemone* is known to be toxic.[5] The administration or adulteration with seed oil of argemone is toxic and shows the dropsy effect in humans.[6] Sood *et al.*[7] have reported that an alkaloid sanguinarine is responsible for the epidemic dropsy. However, the dose of the oil to be toxic is more than 0.001%, less than dose of 0.001 % has not shown any toxic effect[7] and used to treat ulcer, dysentery and intestinal infections.[8] In addition, the toxic effect of the other parts of this plant is not reported and are being used for medicinal purpose in traditional system of medicine.

As mentioned earlier, different parts of this plant are used for different medicinal purposes. However, the biological efficacies of leaves are less studied. Though, few studies have shown its beneficial properties, viz., to treat malarial fever, ulcers, cure diabetes, to maintain normal cholesterol level in humans,[9] and anti-inflammatory property,[10] further studies are necessary to explore the other health beneficial actions of the leaf extracts of *A. mexicana*. In addition, to understand the beneficial actions of the herb, it is important to know the phytocomponents present. Though there are studies reporting the phytochemical analysis of the leaf extracts of *A. mexicana,* the majority of the studies have used common crude method of extraction[11,12] but not improved methods, like sequential polarity based extraction using soxhlet which may ease the isolation of active components. The crude extracts consists of both polar and nonpolar constituents and separation of active components from these crude extracts is difficult. Indeed, thus far very few components have been isolated from the leaves of *A. mexicana* such as β-amyrin[13] isorhamnetin-3-O-β-Dglucopyanoside.[14] To avoid this difficulty, the present study aims to investigate the different phytoconstituents by subjecting the leaves of *A. mexicana* to sequential extraction with solvents based on increasing polarity so that polar and nonpolar components can be separated and biological activity of each can be understood.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The herb *A. mexicana* (Accession number - 169480) was collected from the Chandravana, Botanical garden maintained by the Mysore Medical College, Mysuru. The authentication of the herb was confirmed by an expert from the Department of Botany, University of Mysore, Mysuru-06. Plants were collected before flowering to avoid side effects as reported by earlier studies.

Extract Preparation

The leaves were separated from the herb, *A. mexicana* and washed in alcohol as well as distilled water. The leaves were then shade dried at room temperature and coarsely powdered. The coarsely powdered plant material was subjected to sequential extractions with solvents of increasing polarity, i.e., petroleum ether, benzene, chloroform, ethanol, cold water and hot water using soxhlet apparatus. The extract obtained is flash evaporated and the yield obtained after extraction was determined. The extracts obtained were stored in 4°C until further use.

Percentage Yield of the Extract

The percentage yield of the extract of different solvents was determined using the formula,

$$\% \text{ yield} = \frac{\text{Weight of the extract}}{\text{Weight of the powered sample used for extraction}} \times 100$$

Qualitative Phytochemical Analyses

The extracts obtained were individually analyzed for the presence of different phytocomponents/ phytoconstituents following the standard qualitative protocols.[15]

Quantitative Estimation of Chemical Constituency in the Leaves of *A. mexicana*

Based upon the preliminary qualitative phytochemical analysis, the quantitative estimation of the same was done following the standard protocols as briefed below.

Total phenolic content

The total phenolic content was estimated following the Folin-ciocalteu method.[16] Briefly, about 1 mg of plant extract was dissolved in 1 ml of distilled water. Different concentrations of extracts were made up to 1 ml, 5 ml of 1:10 diluted Folin-ciocalteu reagent and 4 ml of sodium carbonate was added. The total aliquot was mixed well and incubated at 45°C for 10 min and read at 765 nm. Different concentrations of gallic acid were used to prepare a standard calibration curve.

Total Flavonoids Content

The flavonoid content was estimated following the aluminium chloride colorimetry method.[17] Briefly, about 1 mg of plant extract was dissolved in 1 ml of

distilled water. The different concentrations of extracts were made up to 4 ml with distilled water. To this, 0.3 ml of 5% (w/v) sodium nitrite was added. After 5 min, 0.3 ml of 10% aluminium chloride solution was added. Further, 6 min post aluminium chloride addition, 2 ml of 1M sodium hydroxide solution was added to stop the reaction and the volume was made up to 10 ml with distilled water.

Total tannins content

The tannin content of different extracts of *A. mexicana* leaves were estimated following the method of Herald *et al.*[18] Briefly, the extracts were incubated with vanillin-HCl reagent in the dark for 20 min and read at 500 nm. The results were expressed as mg catechin equivalent/ 100 g leaf sample.

Total alkaloid content

The alkaloid content in different solvent extracts of *A. mexicana* leaves were determined by following the method of Fadhil *et al.*[19] Briefly, the known amount of extract was dissolved in 2N HCl, filtered and equal volume of phosphate buffer (pH 4.7) as well as bromocresol green solution were added. Further, the content was shaken with chloroform. The extracted content was collected in a volumetric flask and diluted with chloroform. The absorption was read at 470 nm against the blank. The results were expressed as mg atropine equivalent/ 100 g leaf sample.

Fourier-transform Infrared Spectroscopy (FTIR) Analysis of Different Solvent Extracts

The different solvent extracts of *A. mexicana* leaves were subjected to FTIR analysis to determine the functional groups present. Briefly, FTIR spectra was recorded at a resolution of 2 cm^{-1} (Tensor II, BrukerOptik GmbH, Germany) in the range of 400-4000 cm^{-1}.

Biological Activities

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

An antioxidant potential of different leaf extracts of the herb was determined by an *in vitro* DPPH assay following the standard protocol.[20]

In vitro ovarian steroidogenic enzyme activity

The anti-androgenic effect of different solvent extracts of *A. mexicana* leaves was determined by estimating the ovarian 3β- and 17β- hydroxysteroid dehydrogenase (HSDH) activities *in vitro*.[21] Briefly, 50 µg of different solvent extracts were incubated with the reaction aliquot for 1 hr at 37°C and read at 490 nm. The change in the activity against control was represented as nmol/mg/min.

Ethical Statement

The animal maintenance were according to the guidelines of CPCSEA and the protocols were approved by the institutional animal ethical committee (IAEC No. UOM/IAEC/03/2017).

Statistical Analysis

All the estimations were repeated in triplicates and the mean value of each parameter was computed. The mean values were compared using oneway ANOVA and judged significant if $P<0.05$.

RESULTS

Qualitative Phytochemical Analyses

The phytochemical profile of the different solvent extracts of the *A. mexicana* leaves were presented in the Table 1. The qualitative phytochemical analyses revealed that the ethanolic extract of the *A. mexicana* leaves showed the presence of majority of phytoconstituents followed by benzene and aqueous extracts whereas, petroleum ether and chloroform extracts showed the

Table 1: Qualitative estimation of phytochemicals from different solvent extracts of *A. mexicana* leaves.

Bioactives/ Solvent extracts	Total polyphenols	Flavonoids	Alkaloids	Tannins	Saponins	Carbohydrates	Proteins	Steroids
Petroleum ether	–	–	+	+	+	–	–	+
Benzene	+	–	+	+	–	+	–	+
Chloroform	+	+	+	+	–	–	–	+
Ethanol	+	+	+	+	+	+	–	+
Cold water	+	+	–	+	–	+	–	+
Hot water	+	+	–	+	–	+	–	+

Note : + present
– absent

least number of phytoconstituents. Tannins, total polyphenols and steroids were present in all the extracts except total polyphenols in petroleum ether extract. Alkaloids were absent in aqueous extracts, flavonoids were absent in petroleum ether and benzene extract, but saponins were present only in ethanol as well as petroleum ether extracts. Proteins were absent in all the extracts tested, however, carbohydrates were present in all the extracts except petroleum ether and chloroform extracts.

Percentage Yield of Solvent Extracts

The determination of percentage yield of different solvent extracts revealed that the cold water extract (8.39%) followed by petroleum ether (4.82%) and ethanolic (4.53%) extracts showed the highest yield compared to benzene, chloroform and hot water extracts which showed around 1 % yield (Table 2).

Quantitative Determination of the Chemical Constituents

Total polyphenolic content

The total polyphenolic content was significantly higher in the ethanolic extract (489.45 ± 25.80 mg/100g sample) followed by cold water extract (285.11 ± 126.62 mg/100g sample) (Table 2). The hot water (95.40 ± 15.64 mg/100g sample), benzene (91.72 ± 17.97 mg/100g sample) and chloroform (72.49 ± 13.03 mg/100g sample) extracts showed least phenolic content in the order mentioned and was absent in the petroleum ether extract of *A. mexicana* leaves (Table 2).

Total flavonoid content

A significant higher flavonoid content was found in ethanolic (3422.12 ± 676.86 mg/100g sample) and cold water (2164.41 ± 593.70 mg/100g sample) extracts compared to chloroform (1425.13 ± 369.15 mg/100g sample) and hot water (571.91 ± 71.29 mg/100g sample) extracts (Table 2). However, in the hot water extract it was found in very minimal concentration and was completely absent in the petroleum ether as well as benzene extracts (Table 2).

Total tannin content

The least amount of tannin was found in cold water extract (49.82 ± 14.85 mg/100g sample). The cold water extract was followed by ethanol (134.87 ± 12.92 mg/100g sample), benzene (139.13 ± 12.45 mg/100g sample), chloroform (179.43 ± 7.56 mg/100g sample), petroleum ether (248.04 ± 75.6 mg/100g sample) extracts respectively with the maximum amount in hot water extract (419.55 ± 23.46 mg/100g sample) compared to others (Table 2).

Total alkaloid content

The petroleum ether extract (1103.24 ± 156.83 mg/100g sample) of *A. mexicana* leaf showed higher concentrations of alkaloid followed by chloroform (494.56 ± 87.67 mg/100g sample), ethanolic (261.10 ± 12.95 mg/100g sample), and benzene (92.94 ± 15.83 mg/100g sample) extracts respectively. The alkaloid content was absent in cold and hot water extracts (Table 2).

Fourier-transform Infrared Spectroscopy (FTIR) Analysis of Different Solvent Extracts

The presence of functional groups in different solvent extracts of *A. mexicana* leaves were determined by FTIR analysis (Figure 1). The FTIR spectra of benzene and alcohol extracts showed a broad spectrum at 3300 cm^{-1} with a broad band at 3235 cm^{-1} in benzene extract and at 3319 cm^{-1} in alcohol extract indicating the presence of -OH group. In addition, the benzene extract showed

Table 2: Quantitative determination of phytochemicals and extract yield in different solvent extracts of *A. mexicana* leaves.

Solvent extracts	Percentage yield (%)	Total polyphenols (mg GAE / 100 g sample)	Flavonoids (mg CE/ 100 g sample)	Tannins (mg CE/ 100 g sample)	Alkaloid (mg AE/100g sample)
Petroleum ether	4.82	-	-	248.04 ± 75.6^{c}	1103.24 ± 156.83^{d}
Benzene	1.36	91.72 ± 17.97^{a}	-	$139.13 \pm 12.45^{a,b,c}$	$92.94 \pm 15.83^{a,b}$
Chloroform	1.56	72.49 ± 13.03^{a}	$1425.13 \pm 369.15^{b,c}$	$179.43 \pm 7.56^{b,c}$	494.56 ± 87.67^{c}
Ethanol	4.53	489.45 ± 25.80^{c}	3422.12 ± 676.86^{d}	$134.87 \pm 12.92^{a,b}$	261.10 ± 12.95^{b}
Cold water	8.39	285.11 ± 126.62^{b}	2164.41 ± 593.70^{c}	49.82 ± 14.85^{a}	-
Hot water	1.37	95.40 ± 15.64^{a}	$571.91 \pm 71.29^{a,b}$	419.55 ± 23.46^{d}	-
Significance	-	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001

Note: All the values are mean ± SEM

Mean values with same superscript letters in the given column are not significantly different, whereas those with different superscript letters are significantly (P<0.05) different as judged by Duncan's post-hoc test.

AE= Atropine equivalent; GAE= Gallic acid equivalent; CE= Catechin equivalent

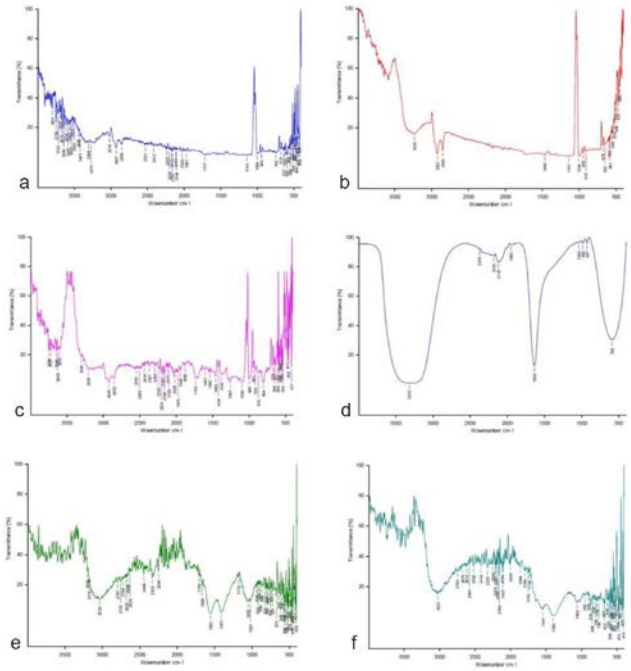

Figure 1: FTIR spectra of (a) petroleum ether extract (b) benzene extract (c) chloroform extract (d) ethanolic extract (e) cold water extract and (f) hot water of *A. mexicana* leaves showing bands of different functional groups.

Note the broad OH band at 3600-3000 cm^{-1} in (b) and (d) spectra suggesting the presence alcohol group.

medium, C-H stretching peak at 2922 cm^{-1} and 2851 cm^{-1} suggesting alkane group; medium NH stretching peak between 3304-3603 cm^{-1} indicating the aliphatic primary amine in chloroform extract; alcoholic extract showed medium C=C stretching peak at 1635 cm^{-1} and strong C-I stretching peak at 592 cm^{-1} suggesting the alkene group and halo compound respectively; cold water extract revealed weak broad OH stretching peak at 3038 cm^{-1} for intramolecular OH, weak (1551 cm^{-1}) and medium (1413 cm^{-1}) CH bending for aromatic compound and alkane methyl group as well as medium C-N stretching at 1021 cm^{-1} for the presence of amine group whereas, a strong broad OH stretching at 3023 cm^{-1} and medium OH bending at 1396 cm^{-1} suggests the presence of carboxylic acid and medium CN stretching at 1062 indicates the presence of amine group in hot water extract.

Biological Activities

DPPH radical scavenging activity

The results of DPPH scavenging assay revealed that the ethanol and petroleum ether extracts were more potent than other solvent extracts. This is because the IC_{50} value of ethanol and petroleum ether extracts was 29.33 ± 0.17 µg/mL and 31.29 ± 0.40 µg/mL respectively compared to that of standard ascorbic acid with IC_{50} value 131.05 ± 0.25µg/ mL (Table 3).

Table 3: DPPH scavenging activity of standard and different solvent extracts of *A. mexicana* leaves.

	IC_{50} value (µg/ mL)
Standard	131.05 ± 0.25
Petroleum ether	$31.29 \pm 0.40^{a,b}$
Benzene	35.19 ± 1.46^{c}
Chloroform	$33.74 \pm 0.79^{b,c}$
Ethanol	29.33 ± 0.17^{a}
Cold water	46.06 ± 1.35^{e}
Hot water	43.03 ± 1.18^{d}

Note: All the values are mean ± SEM

Mean values with same superscript letters in the given column are not significantly different, whereas those with different superscript letters are significantly (P<0.05) different as judged by Duncan's post-hoc test.

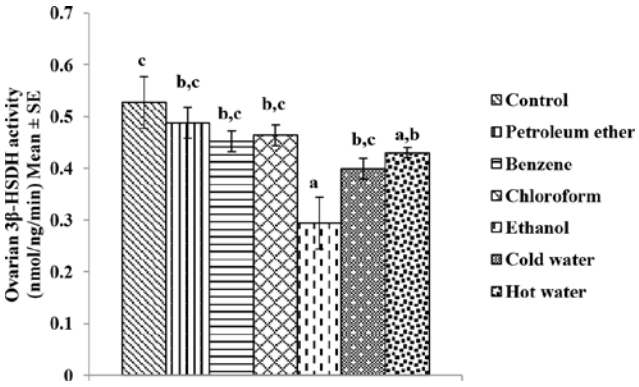

Figure 2: Vertical bars showing the effect of different solvent extracts of *A. mexicana* leaves on ovarian 3β-hydroxysteroid dehydrogenase activity.

Note: Bars with same superscript letters are not significantly different, whereas those with different superscript letters are significantly (*P*<0.05) different as judged by Duncan's multiple range test.

In vitro ovarian steroidogenic enzyme activity

The ovarian 3β- and 17β-HSDH activities were decreased by all the 6 solvent extracts studied, however, the ethanolic extract of *A. mexicana* leaves significantly decreased the activities of ovarian 3β-HSDH (Figure 2) and 17β-HSDH (Figure 3) compared to control.

DISCUSSION

The phytoconstituents are known to have biological values. In the present study, the preliminary qualitative analyses of leaves of the herb *A. mexicana* revealed the presence of total phenols, flavonoids, alkaloids, saponin, tannin and carbohydrates.The percentage yield of the extract was high in cold water extract followed by an ethanolic extract compared to other extracts studied. Further, the total phenolics and flavonoids were found to be highly concentrated in an ethanolic extract followed by the chloroform extract of the *A. mexicana* leaves. In addition, the alkaloid concentration was

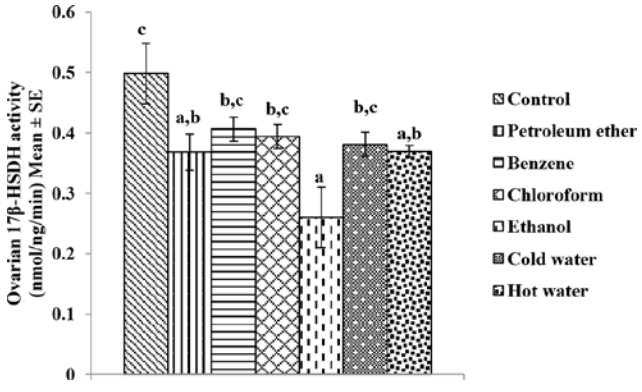

Figure 3: Vertical bars showing the effect of different solvent extracts of *A. mexicana* leaves on ovarian 17β-hydroxysteroid dehydrogenase activity.

Note: Bars with same superscript letters are not significantly different, whereas those with different superscript letters are significantly (P<0.05) different as judged by Duncan's multiple range test.

highest in petroleum ether extract followed by ethanolic extract and tannin content was observed to be least in cold water, alcohol and benzene extracts compared to other extracts. The differential content of secondary metabolites in different solvent extracts may be due to the polarity of the solvent, i.e., the solvent used for the extraction might have played a major role. The biological potency as an antioxidant agent was revealed with very low IC_{50} value for DPPH assay in the ethanolic extract compared to standard and other extracts. This is further supported by the presence of broad peak for OH groups in alcoholic extracts of the *A. mexicana* leaves as determined by FTIR analysis. In addition, the ethanolic extract of *A. mexicana* leaves could significantly reduce the steroidogenic enzyme activities. The results of the present study suggest that the ethanolic extract of *A. mexicana* leaves possess potent antioxidant property due to the presence of high concentration of total polyphenols and anti-steroidogenic property as well. Hence, the ethanolic extract can be used as a potent antioxidant and antisteroidogenic agent.

According to the plant physiology, to defend themselves against the threats, secondary metabolites are synthesized/produced by the plants.[22] These secondary metabolites can also show similar health beneficial effects in animal systems. This property is being exploited by the scientists/pharmacists since several decades. Regarding the *A. mexicana* leaves' pharmacological properties, studies have reported various medicinal values. For instance, antimalarial,[3,11,12,23] anti-inflammatory,[10] antioxidant,[11,12] and anticancer[24] activities has been reported.

The different biological/pharmaceutical properties of the *A. mexicana* leaf extracts may be due to the presence of secondary metabolites. The different secondary metabolites are known for various biological activities, to cite a few, polyphenols are good antioxidants,[25] alkaloids are widely used as antidiabetic agents,[26] etc. Thus far, the identification of presence of different secondary metabolities in *A. mexicana* leaves were reported in methanolic and ethanolic extracts,[23] methanolic extract,[12] n-hexane, ethyl acetate and methanolic extracts[11] and aqueous, acetone, ethanol, methanol, chloroform and petroleum ether extracts.[3] However, the quantification of these secondary metabolites was not determined except a single study by Apu *et al.,*[11] who reported only about the total polyphenols quantification but not other metabolites. The present study revealed both the qualitative and quantitative estimations of the secondary metabolites of leaf extracts of *A. mexicana.* The different solvent extracts of the *A. mexicana* leaves showed the presence of total phenolics, flavonoids, tannins, carbohydrates, alkaloids, saponins and steroids, but the proteins were found to be absent. These results are in agreement with the earlier studies.[12,23] In addition, the ethanolic extract showed the highest concentration of total polyphenols compared to other solvent extracts studied. The ethanolic extract is followed by the cold water and chloroform extracts. Further, flavonoids were found highly concentrated in the ethanolic and cold water extracts. The study showed highest extraction of polyphenols and flavonoids compared to earlier study by Srivastava *et al.*[27] This may be due the fact that, these polyphenols are highly soluble or extractable in higher amounts in polar solvents than the nonpolar solvents[28] and may also due to the sequential polarity of solvent based extraction using soxhlet apparatus. The tannins are high molecular weight polyphenols, are widely known as anti-nutritional component.[29] This is because, consumption of more tea, the rich source of tannin without milk lowers the absorption of calcium and may lead to iron deficiency.[30] However, recently reported that tannins are being isolated from various plant materials and it has ability to selectively inhibit HIV replication[31] along with its biological properties like antiviral, antibacterial and antitumor activities.[32] Hence, a good amount of tannin is also beneficial to the health and in our present study, the highest amount of tannin of about 0.4% and 0.2% was found in hot water and petroleum ether extracts, respectively, as well asthe lowest amount of about 0.05% and 0.1% were found in the cold water and ethanolic extracts respectively. Additionally, though alkaloids are also known for various health benefits, they are reported to have negative effects on female reproduction like antifertility, abortifacient, antiovulatory, etc.,[33] Therefore, in our

study petroleum ether and chloroform extracts are not preferable for pharmacological use since they have a higher amount of alkaloid.

The presence of abundant secondary metabolites in the leaf extracts of *A. mexicana* guarantees the rich biological properties. The few biological efficacies of the extracts, like antioxidant and antisteroidogenic properties were studied in the present study. Among various secondary metabolites of plant systems, the polyphenols are known to have potent antioxidant activity. The antioxidant system is the defense mechanism/system against oxidative stress caused by various internal and external factors. To determine the antioxidant potential of a plant extract, DPPH assay is widely used. DPPH is a stable free radical of purple color with absorption maxima at 517 nm. In the presence of an antioxidant, it accepts an electron/hydrogen radical and quench DPPH free radicals to become colorless 2,2-dipenyl-1-picryl hydrazine.[34] Generally, IC_{50} values will be determined for herbal extracts, i.e., minimum concentration required to quench the 50% of the DPPH, by comparing to the standard ascorbic acid. In the present study, the IC_{50} values are 31.29 mg, 35.19 mg, 33.74 mg, 29.33 mg, 46.06 mg and 43.03 mg in petroleum ether, benzene, chloroform, ethanol, cold water and hot water extracts of *A. mexicana* leaves respectively. This suggests that the ethanolic extract is with IC_{50} values less than other solvent extracts as well as that of standard ascorbic acid, i.e., 131.05 mg and hence ethanolic extract shows increased antioxidant potential than other extracts as well as standard. The leaf extracts of *A. mexicana* in the present study showed better DPPH scavenging activity than the earlier studies with different extraction methods (crude extraction) with different solvents/solutions.[11,27] This may be because of sequential extraction method we employed in the present study. The sequential extraction method has extracted higher concentration of polyphenols, inturn increased antioxidant potential as there is a direct association of polyphenol content and antioxidant activity can be related. Hence, the results suggest that ethanolic extract is potent antioxidant agents which can be exploited for the same.

In addition to the antioxidant property, the ethanolic extract of the herb *A. mexicana* showed significance in lowering the ovarian steroidogenic enzyme activities compared to other solvent extracts. It is well established that the reproductive process is highly regulated by hormones. Any impairment in the hormonal milieu alters the entire process. Further, the reproductive hormones are found to be elevated in several pathological conditions like cancer,[35] polycystic ovary syndrome,[36] etc. Since, the ethanolic extract of *A. mexicana* leaves lowers the activities of steroidogenic enzymes, it can be used in the treatment of above-said conditions to normalize the elevated hormonal levels. Furthermore, as the 17β-HSDH plays a key role in the synthesis of androgens in the ovary, ability of the ethanolic extract of *A. mexicana* leaves to decrease its activity can be exploited for the treatment of PCOS. Indeed, studies in this line are being carried out in our lab.

CONCLUSION

The sequential extraction method yield higher polyphenol content than the crude extraction by polar solvents from the plant source. In addition, ethanolic extract with higher concentrations of total phenols as well as flavonoids showed increased DPPH scavenging property compared to other extracts and standard. Further, this is the first report to show that ethanolic extract has ovarian steroidogenic enzyme activity lowering property. Furthermore, ethanolic extract showed lower amount of tannin and alkaloids which are reported to have negative effects upon higher concentration. Hence, ethanolic extract are potent antioxidant and anti-steroidogenic agent which can be further exploited for the pharmacological purpose.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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ABBREVIATIONS

ANOVA: Analysis of variance; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **HCL:** Hydrochloric acid; **HSDH:** Hydroxysteroid dehydrogenase; **FTIR:** Fourier transform infrared spectroscopy; **WHO:** World health organization.

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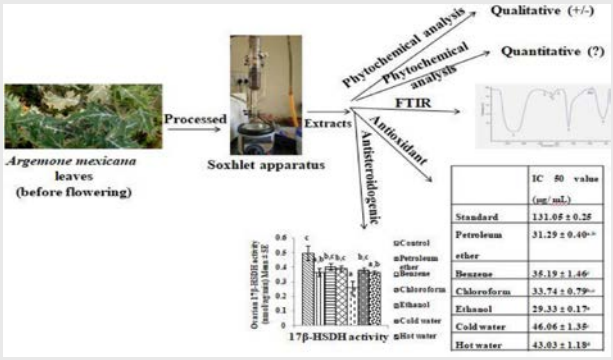
PICTORIAL ABSTRACT



	IC 50 value (µg/mL)
Standard	131.05 ± 0.25
Petroleum ether	31.29 ± 0.40^{a,b}
Benzene	36.19 ± 1.46^{c}
Chloroform	33.74 ± 0.79^{a,c}
Ethanol	29.33 ± 0.17^{b}
Cold water	46.06 ± 1.36^{d}
Hot water	43.03 ± 1.18^{d}

SUMMARY

Naturally herbs are being used recently for various ailments replacing English medicine because of the side effects of the latter. *Argemone mexicana* is known to have various health beneficiary properties. In the present study the edible part of the plant leaves were selected, processed and sequentially extracted to separate polar and non-polar components. The phytoconstituents were analysed by both qualitatively and quantitatively. The extract rich in polyphenols showed a potent antioxidant and antiandrogenic potential *in vitro*. This may be because of more –OH groups present in the extract as it was further supported by the broad peak for –OH in FTIR spectra.

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