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Studies on Phytoconstituents and Antioxidant Properties of *Argemone mexicana* Flower Extract

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ABSTRACT

In present study, the presence of bioactive compounds in *A. mexicana* flowers were analyzed and also evaluated the *in vitro* antioxidant properties of *A. mexicana* flowers. The flower extract showed the presence of alkaloids, flavonoids, saponins, tannins, phytosterol, triterpenoids, glycosides, anthraquinones and phenols. The total phenolic and flavonoid content were found to be 25.40 ± 1.20 µg gallic acid equivalents and 14.30 ± 0.20 µg quercetin equivalents, respectively. The carbohydrate and protein content was found to be 4.10 ± 0.24 mg/g and 2.10 ± 0.30 mg/g of the flower extract respectively. HPLC analysis of *A. mexicana* flower extract revealed the presence of biologically active components such as gallic acid, rutin, caffeic acid, quercetin and ferulic acid. *A. mexicana* flower extract exhibited 81.33% inhibition in DPPH assay, 85% in ABTS radical assay, 86% superoxide scavenging, 76% NO scavenging, 75% hydroxyl radical scavenging, 77% radicals indicating hydrogen peroxide radical scavenging potential of the flower extract. The antioxidant activity observed in the ethanolic extarct of *A. mexicana* flower may be related to the presence of significant amounts of total phenolics and flavonoids. Hence, *A. mexicana* flower extract could serve as natural sources of antioxidants and could be used in the treatment of free radical mediated diseases.

KEYWORDS

Argemone mexicana, Flavonoids, Phenols, Antioxidant potential, HPLC.

INTRODUCTION

Argemone mexicanab Linn. (Family: Papaveraceae) is a prickly, glabrous, branching annual herb with yellow juice and showy yellow flowers, naturalized throughout up to an altitude of 1500 m. It occurs as waste land weed in almost every part of India. *A. mexicana* is native of tropical America which has distributed in tropical and subtropical regions of the World. In India, it grows in the temperate region as a weed in waste lands, cultivating fields and road sides. The plant prefers light sandy well-drained soil and also grows in nutritionally poor acidic, neutral and basic (alkaline) soil [1].

The aerial part of the plant (without seeds) is used to treat malaria, dropsy and icterus, has analgesic and anti-parasitic activities and is considered as an antidote for snake bites *A. mexicana* Linn. also shows antihelmintic, anti-inflammatory, wound healing, antibacterial and antifungal activities [2,3].

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The plant contains several kinds of secondary metabolites such as polyphenols, tannins, saponins, flavonoids and alkaloids [4,5]. In present study, the presence of bioactive compounds of *A. mexicana* flowers were analyzed and also evaluated the *in vitro* antioxidant properties of *A. mexicana* flowers.

EXPERIMENTAL

Fresh and mature *Argemone mexicana* flowers were collected manually from the plant under natural conditions from the garden of A.V.V.M. Sri Pushpam College, Poondi, India. The flower was identified and authenticated by a taxonomist.

Preparation of *A. mexicana* flowers extract by delipidation and soxhalation: *A. mexicana* flowers were selectively removed and dried at room temperature and powdered in an electrical grinder, which was then stored in an airtight container at 5 ºC until further use. The powdered flowers were delipidated with petroleum ether (60-80 ºC) for overnight. It was then filtered and soxhalation was performed with 95% ethanol. Ethanol was evaporated in a rotary evaporator at 40-50 ºC under reduced pressure. Extractive value (%w/w) of ethanolic extract was 14.3 g. The ethanolic extract was stored at 5 ºC in a refrigerator.

Phytochemical screening: The ethanolic extract of *A. mexicana flowers* was subjected to phytochemical screening for the qualitative analysis of various plant constituents [6,7].

Determination of total phenolic content and total flavonoid content: Total polyphenol content in the ethanol extract of *A. mexicana* flower was determined according to the Folin-Ciocalteu colorimetric method [8,9]. Total flavonoid content in the ethanolic extract of *A. mexicana* flowers was determined according to the method of Quettier *et al.* [10] with minor modifications.

HPLC-DAD system for analysis of phenolic compounds: HPLC analysis was performed using Shimadzu HPLC system equipped with a diode array detector. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds and were detected using an external standard method.

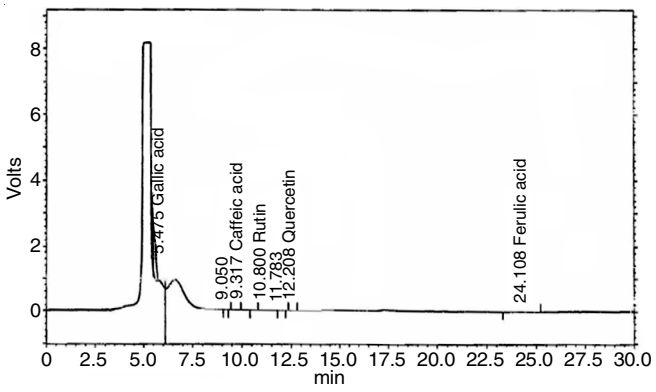
Free radical scavenging assays: The free radical scavenging capacity of the ethanolic extract was determined using DPPH [11]. ABTS radical scavenging activity of ethanolic extract of *A. mexicana* flower was determined according to the method of Re *et al.* [12]. Hydroxyl radical scavenging assay was performed according to the method of Smirnoff & Cumbes [13]. Hydrogen peroxide scavenging activity was performed according to the method of Ruch *et al.* [14]. Nitric oxide scavenging activity was estimated according to the method of Marcocci *et al.* [15]. The superoxide radical scavenging activity of *A. mexicana* flower was measured by the method of Fontana *et al.* [16].

RESULTS AND DISCUSSION

Argemone mexicana flower extract is found to contain alkaloids, flavonoids, saponins, tannins, phytosterol, triterpenoids, glycosides, anthraquinones and phenols.

The total phenolic and flavonoid content were found to be 25.40 ± 1.20 µg gallic acid equivalents and 14.30 ± 0.20 µg quercetin equivalents, respectively. The carbohydrate and protein content was found to be 4.10 ± 0.24 mg/g and 2.10 ± 0.30 mg/g of the flower extract, respectively

HPLC analysis of phytoconstituents of *A. mexicana* flower extract: The presence of phytoconstituents in *A. mexicana* flower extract by HPLC analysis is shown in Fig. 1. HPLC analysis of *A. mexicana* flower extract revealed the presence of biologically active components such as gallic acid, rutin, caffeic acid, quercetin and ferulic acid. Fig. 1a depicts the retention time, concentration and name of the phytoconstituents present in *A. mexicana* flower extract.



Retention time	Area	Height	Concentration (mg/kg)	Name of the compound
5.475	26487947	1516203	4668	Gallic acid
9.317	59504	472	0.034	Caffeic acid
10.800	4973	22	0.001	Rutin
12.208	4486	92	0.003	Quercetin
24.108	83165	2181	0.050	Ferulic acid

Fig. 1. HPLC analysis of phytoconstitutents of *A. mexicana* flower extract

DPPH and ABTS radicals scavenging activity of *A. mexicana* flower extract is depicted in Figs. 2 and 3 respectively.

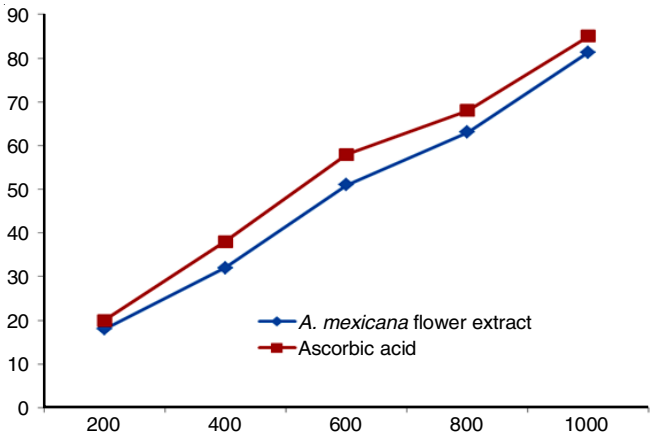


Fig. 2. DPPH scavenging potential of *A. mexicana* flower extract at a concentration of 1000 µg the extract scavenged 81.33% of DPPH

The *in vitro* superoxide scavenging activity of *A. mexicana* flower extract is graphically represented as Fig. 4. *A. mexicana* flower extract exhibited a maximum of 86% superoxide scavenging activity at a concentration of 1000 µg/mL. The extract exhibited a maximum of 76% NO scavenging potential (Fig. 5). Hydroxyl radical scavenging activity of *A. mexicana*

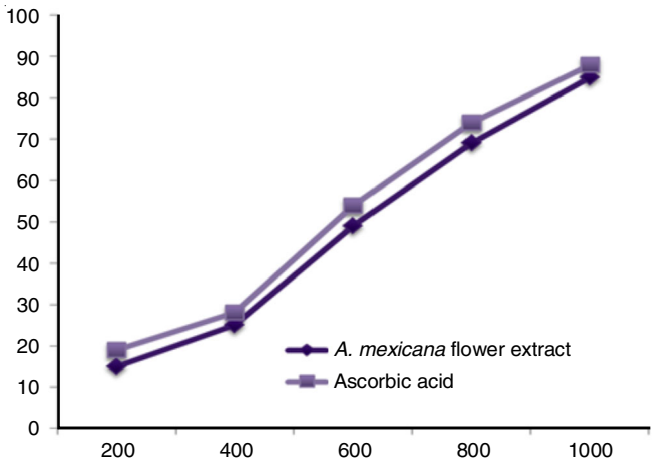


Fig. 3. ABTS scavenging potential of *A. mexicana* flower extract at a concentration of 1000 µg the extract scavenged 85% of ABTS

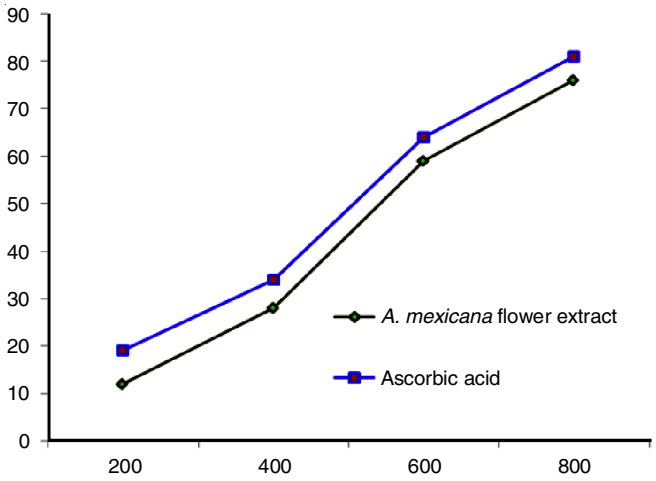


Fig. 4. Superoxide radical scavenging potential of *A. mexicana* flower extract at a concentration of 1000 µg the extract scavenged 86% of superoxide radicals

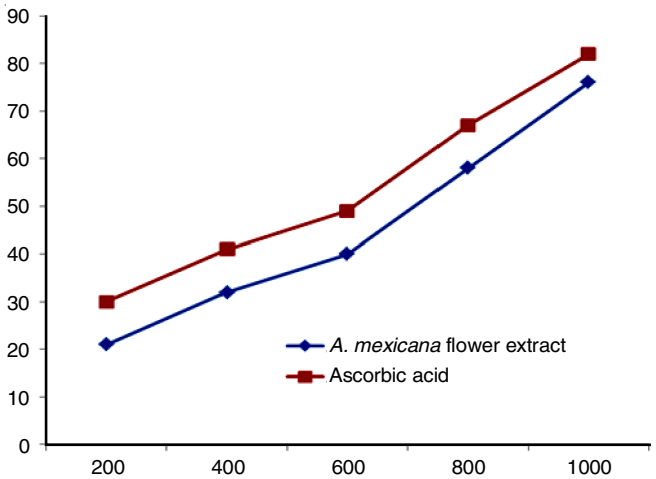


Fig. 5. Nitric oxide radical scavenging potential of *A. mexicana* flower extract at a concentration of 1000 µg the extract scavenged 76% of nitric oxide radicals

flower extract is shown in Fig. 6 (75% hydroxyl radical scavenging). Hydrogen peroxide radical scavenging activity (77% radical scavenging activity) of *A. mexicana* flower extract is represented as Fig. 7.

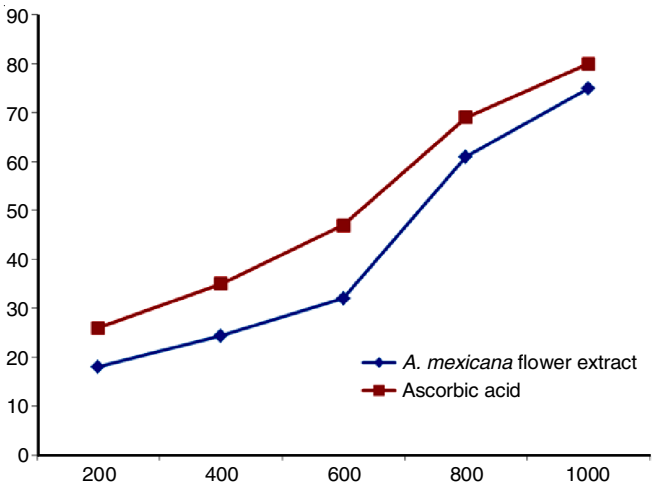


Fig. 6. Hydroxyl radical scavenging potential of *A. mexicana* flower extract at a concentration of 1000 µg the extract scavenged 75% of hydroxyl radicals

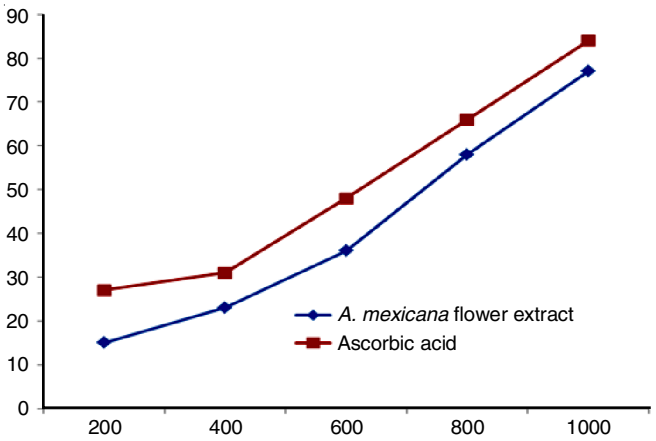


Fig. 7. Hydrogen peroxide radical scavenging potential of *A. mexicana* flower extract at a concentration of 1000 µg the extract scavenged 77% of hydrogen peroxide radicals

In present study, the flower extract contains appreciable amounts of flavonoids and phenols, which may be attributed to their strongest antioxidant potential. Plant metabolism is classified as primary or secondary. Compounds which are produced by primary metabolism (primary metabolites) include sugars, fatty acids, amino acids and nucleic acids. However, under *in vitro* antioxidant measurement assay conditions, the radical scavenging potential of alkaloids is reportedly moderate to non-existent. Most of the isolated compounds belong to the class of alkaloids; besides, terpenoids, flavonoids, phenolics, long-chain aliphatic compounds and few aromatic compounds are found to be other constituents of this plant. Present results are with accordance with the earlier findings of Joshi *et al.* [17].

HPLC analysis of *A. mexicana* flower extract revealed the presence of biologically active components such as gallic acid, rutin, caffeic acid, quercetin and ferulic acid.

***In vitro* antioxidant properties of *A. mexicana* flower extract:** DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was quantified in terms of percentage inhibition of a pre-formed free radical by antioxidants. Likewise, ABTS radical activity was quantified in terms of percentage inhibition of the ABTS radical cation by the antioxidant. *A.*

mexicana flower extract exhibited 81.33% inhibition at a concentration of 1000 µg in DPPH assay and 85% inhibition at a concentration of 1000 µg in ABTS radical assay which showed its significant radical scavenging capacity. *A. mexicana* flower extract showed the most antioxidant activity due to significant amounts of phenolics and flavonoids present in them. That indicated the direct correlation among antioxidant potential, total phenolics and flavonoids content [18].

Superoxide anions were generated *in vitro* enzymatically by hypoxanthine/xanthine oxidase system that reduces NBT and forms a blue coloured chromophore, formazone that can be measured at 560 nm. Superoxide radicals generated *in vitro* by the system was determined by NBT photoreduction method. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture [19]. *A. mexicana* flower extract exhibited a maximum of 86% superoxide scavenging activity at a concentration of 1000 µg/mL.

At a concentration of 1000 µg/mL, *A. mexicana* flower extract significantly scavenged 75% radicals indicating hydroxy radical scavenging potential of the flower extract. *A. mexicana* showed potent H_2O_2 scavenging activity which may be due to the presence of antioxidant compounds. As the antioxidant components present in the extracts are good electron donors, may accelerate the conversion of H_2O_2 to H_2O. At a concentration of 1000 µg/mL, *A. mexicana* flower extract significantly scavenged 77% radicals indicating hydrogen peroxide radical scavenging potential of the flower extract.

Conclusion

The antioxidant activity observed in the ethanolic extract of *Argemone mexicana* flower may be related to the presence of significant amounts of total phenolics and flavonoids. Hence, *A. mexicana* flower extract could serve as natural sources of antioxidants and could be used in the treatment of free radical mediated diseases.

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