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Research Article
Comparative Phytochemical Characterization of the *Argemone mexicana* and *Thevetia peruviana* Leaves Extracts

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Abstract

Background and Objective: Pharmaceutical property of any plant extract is due to the presence of various phytochemicals and also due to the antioxidant potential of the extract. The two plant species namely *Argemone mexicana* and *Thevetia peruviana* have great importance due to their medicinal values. The present examination illustrated the phytochemical analysis, total antioxidative potential and DPPH free radical scavenging activities of the leaves extracts of these plants. **Materials and Methods:** Powdered leaves of plants were extracted by using a Soxhlet apparatus in hexane. Their total antioxidant and DPPH free radical scavenging activities and the qualitative test for flavonoids, quinones, saponins, phenols, steroids, tannins, terpenoids, cardiac glycoside, alkaloid and carbohydrates. Phenols, steroids, tannins, terpenoids, cardiac glycoside, alkaloid and carbohydrates have been performed by using standard protocols. **Results:** The qualitative phytochemical studies have clearly demonstrated that the plant, *Argemone mexicana* and *Thevetia peruviana* contained flavonoids, quinones, phenols, steroids, tannins, terpenoids, cardiac glycoside, alkaloid and carbohydrates. Further, *A. mexicana* and *T. Peruviana* displayed 41 and 33 μM g^{-1} Fe^{++} FRAP values and 200 μg and 175 μg IC_{50} values, respectively against DPPH. **Conclusion:** The results obtained from this study suggested that the leaves extracts from the *A. mexicana* and *T. peruviana* rich in phytochemicals and also have antioxidant potential.

Key words: *Argemone mexicana*, *Thevetia peruviana*, phytochemicals, antioxidant potential

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Argemone mexicana L. (*A. mexicana*) and *Thevetia peruviana* L. (*T. peruviana*) belong to Papaveraceae and Apocynaceae family, respectively. Both of these plant species contain various phytochemicals with medicinal properties and their therapeutic applications include anti-fungal, anti-microbial, anti-inflammatory and anti-termite properties of both the plants[1]. A number of plant secondary metabolites like alkaloids and flavonoids have been reported as anti-viral, anti-bacterial, anti-amoebal and anti-cancer agents[2,3]. In addition, *A. mexicana* and *T. peruviana* possess some toxic constituents which adversely impact the health of both the humans and animals. *A. mexicana* is an exotic weed comprising approximately 30-32 species. Among these species, *A. mexicana* which is commonly known as Mexican Poppy is used as medicinal plant in rural areas of Mexico. This is a very common herbaceous plant with latex[4]. The morphological features of *A. mexicana* included its prickly, glabrous, branching herb with yellow juice and showy yellow flowers[5]. Saranappa and Vidyasagar have been illustrated the botanical description of *A. mexicana*. The height of the plant is about 1 m with usually 5-11 cm long leaves. The stem is prominently sinuate-lobed and spiny. The flowers of this plant are 4-5 cm in diameter and are yellow, terminal and scentless and the length of the capsule is about 3 cm (spiny, obovate or elliptic-oblong). Moreover the shapes of seeds are spherical, black and pitted[6]. *Argemone mexicana* is considered as an important medicinal plant and its leaves and seeds are also reported to find application in maintaining normal blood circulation and cholesterol level in the human body and these plant parts possess anti-venom property as well. The leaves of *A. mexicana* are also traditionally used as anti-asthmatic. The wide variety of the traditional uses of the plant may be due to the presence of various phytochemicals like alkaloids, amino acids, phenolics and fatty acids[7] (Fig. 1a).

Thevetia peruviana commonly known as yellow oleander/yellow bell, cultivated as an ornamental plant in tropical and subtropical regions of the world, including India, Australia and China. These medicinal plants as a group comprise approximately 8000 species[8]. The plant is frequently grown throughout the tropical and subtropical regions of the world, including India[9]. Morphologically, it is a small evergreen tree with about 3-4 m in height. Its fruits are small in size of 3-4 cm in diameter with deep green color and contain 2-4 flat gray seeds. Its flowers are generally yellow, but there are varieties with red or white color. Flowers are funnel shaped with five petals. Leaves are green in color with a length of 10-15 cm and lance shaped. Leaves are covered with a waxy coating to reduce water loss[10]. The tips of leaves are pointed with a dark green color. Its stem is green. *Thevetia peruviana* produces a large number of secondary metabolites which have found various therapeutic uses from time immemorial. The early history of modern medicine contains descriptions of plant-derived phytochemicals, many of which are still in use. *Thevetia peruviana* is potentially lethal plant after ingestion (Fig. 1b). These are toxic to most vertebrates as they contain cardiac glycosides. The toxins are cardenolides called the vetin A and B, others include peruvoside, neriifolin, the vetoxin and riverside. These are not destroyed by drying or heating and produced gastric and cardio effects. The plant or its individual parts can be used for the treatment of various disorders in human beings such as diabetes, liver toxicity, fungal infection, microbial infection, inflammation, pyrexia and to relieve pain[11].

Fig. 1(a-b): Representative morphology of (a) *A. mexicana* and (b) *T. peruviana* plants

The plant-based therapeutics are known to play an important role in the public health care system and in traditional system of medicine. A number of different classes of secondary metabolites have been reported in *A. mexicana* and *T. peruviana* including alkaloids, flavonoids, steroids, cardiac glycosides, terpenoids, tannins and saponins etc. The present investigation has been carried out to characterize the phytochemicals of hexane extracts of the leaves of *A. mexicana* and *T. peruviana* collected from Allahabad, India and also to assess their antioxidative potential.

MATERIALS AND METHODS

The present research work was carried out in Allahabad region and performed in 4 weeks during April, 2019.

Chemicals: Ferric chloride, Hydrochloric acid, Sulfuric acid were purchased from SRL. Pvt. Ltd. (India). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) were purchased from Alfa Aesar and hexane was purchased from spectrochem. All other chemicals used were of analytical and molecular grade.

Collection of plant leaves and preparation of extract: *Argemone mexicana* and *T. peruviana* leaves were collected from Allahabad, washed thoroughly under running water and dried in shade on paper. These dried leaves were used for the preparation of powder. The extract was prepared in hexane using Soxhlet. The ratio of the plant material and the solvent was 1:10 (w/v). The liquid was removed on rotatory evaporator at the temperature equivalent to the boiling point (68°C) of hexane. The extraction of plant leaves was followed by using Laddaa and Magdumb, with some required modifications[12].

Analysis of phytochemicals in plant extract: For the analysis of phytochemicals present in the extract was carried out using the procedure with slight modification as described elsewhere[13,14]. The procedure in brief for the analysis of different phytochemicals is described as follows.

Phenols: About 1 mL of extract was mixed with 2 mL of distilled water, followed by few drops of 10% ferric chloride. It resulted into appearance of the blue or green color.

Flavonoids: About 1 mL of extract was mixed with few drops of dilute sodium hydroxide, which gave an intense yellow color. This color disappears after the addition of few drops of diluted HCl.

Quinones: About 1 mL of extract was added to 1 mL of concentrated sulfuric acid, which led to the formation of red colored complex.

Tannins: About 1 mL of 5% ferric chloride added to solvent free extract 1 mL turned the extract to produce bluish black or greenish black precipitate.

Saponins: About 1 mL extract was diluted with 20 mL distilled water and was agitated in a graduated cylinder for 15 min, resulting in the formation of 1 cm layer foam.

Cardiac glycosides: About 5 mL of plant extract was mixed with 2 mL of glacial acetic acid containing a drop of ferric chloride solution (5% w/v) followed by the addition of 1 mL of concentrated sulfuric acid resulting into development of a brown ring of a deoxy sugar at the interface.

Steroids: About 1 mL of extract was mixed with 10 mL chloroform and 10 mL of sulfuric acid. The upper layer turned red and sulfuric acid layer showed yellow with green fluorescence.

Terpenoids: About 5 mL of extract was added with 2 mL of chloroform followed by the addition of 3 mL concentrated sulfuric acid. It resulted in the appearance of reddish-brown precipitate at the interface.

Alkaloids: About 1 mL of the extract was in DDW and followed by the addition of 5 drops of 1% HCl and passed through steam again followed by 6 drops of Wagner's reagent. Brown-red precipitate was observed.

Determination of total antioxidant activity: The method described by Benzie and Strain with some modifications was employed for the estimation of antioxidant activity by FRAP assay[15]. The stock solution included 300 mM acetate buffer, pH 3.6, 10 mM of 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl at 50°C in the water bath for 30-40 min till it completely dissolves. The fresh working solution was prepared by mixing 10:1:1 of acetate buffer, TPTZ and $FeCl_3$. $6H_2O$, respectively. The temperature of the working solution was maintained to 37°C before starting the reaction by adding the plant extract (100 µL) to 2 mL of the FRAP solution. The reaction was incubated for 30 min in the dark condition. The optical density (OD) of the colored complex (ferrous tripyridyltriazine complex) was recorded at 593 nm. The standard curve was linear between 20 and 100 µL $FeSO_4.7\ H_2O$. The results were expressed as Fe^{++} µM g^{-1} dry mass.

DPPH free radical scavenging activity: The DPPH radical scavenging activity assay was carried out as described by Siatka and Kašparová[16]. The solution of DPPH (0.1 mM) in methanol was prepared and 1.0 mL of this solution was added to different concentrations (10, 100 and 1000 µg mL^{-1}) of plant extract. After 30 min incubation, the absorbance was measured at 517 nm. A blank was prepared without adding the extract. Ascorbic acid (1% w/v) at various concentrations was used as a standard.

Statistical analysis: The experiments have been conducted 3 times and the average value of the data has presented.

RESULTS

Analysis of phytochemicals in plant extract: In order to evaluate the presence of some phytochemicals in *A. mexicana* and *T. peruviana* leaves extracts, the following tests were performed. The performed tests revealed that the flavonoids, quinones, phenols, steroids, tannins, terpenoids, cardiac glycoside, alkaloids and carbohydrates are present in both *A. mexicana* and *T. peruviana* extracts, while saponins is absent in both the extracts. Plus (+) and minus (-) signs denotes the presence and absence of that phytochemical. The increase in plus sign indicated the increase in intensity. The extracts were rich in phytochemical activity, as shown in Table 1.

Determination of total antioxidant activity: When *A. mexicana* and *T. peruviana* extracts were subjected to FRAP assay, the extracts were found to contain antioxidant potential. The *A. mexicana* and *T. peruviana* were found to contain FRAP values to be 41 and 33 µM g^{-1} Fe^{++}, respectively (Table 2).

DPPH free radical scavenging activity: The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging method is an antioxidant assay. It is based on electron-transfer that produces a violet solution in methanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless methanol solution. The results of the present study indicated the IC_{50} values to be 200 and 175 µg for *A. mexicana* and *T. peruviana*, respectively (Table 3).

Table 1: Phytochemicals detected in the extracts of *A. mexicana* and *T. peruviana* leaves

Phytochemicals	*A. mexicana*	*T. peruviana*
Flavonoids	+++	+++
Quinones	+	++
Saponins	-	-
Phenols	+	+
Steroids	++	+++
Tannins	++	+
Terpenoids	++	++
Cardiac glycoside	++	+++
Alkaloid	++	++
Carbohydrates	++++	++

+: Presence, -: Absence, Increase in the number of (+) sign indicates increase intensity

Table 2: Total antioxidant activities in the extracts of *A. mexicana* and *T. peruviana* leaves

Leaves extracts	FRAP values (µM g^{-1} Fe^{++})
A. mexicana	41
T. peruviana	33

Table 3: DPPH free radical scavenging activities of extracts of *A. mexicana* and *T. peruviana* leaves

Leaves extracts	DPPH free radical scavenging activities (µg)
A. mexicana	200
T. peruviana	175

DISCUSSION

In the present investigation, we have performed the qualitative assessment of phytochemicals from the leaves extracts of *A. mexicana* and *T. peruviana* prepared in hexane and also determined the total antioxidant and DPPH free radical scavenging activities of the extracts. The obtained results have suggested the presence of flavonoids, quinones, saponins, phenols, steroids, tannins, terpenoids, cardiac glycoside, alkaloid and carbohydrates in the tested samples.

Generally, the curative properties of medicinal plants can be attributed to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, sterols and coumarins etc. Using similar assays, Apu *et al.*[4] performed phytochemical screening of leave extracts of *A. mexicana* and displayed the presence of various bioactive compounds such as alkaloids, flavonoids, saponins and steroids. The qualitative study carried out by Desai *et al.*[17] indicated the presence of alkaloids, flavonoids, glycoside, saponin, tannin, phenol, lignin, steroid and terpenes in methanolic extracts of *A. mexicana* leaves. The researchers have also reported the presence of these phytochemicals in the leaves and fruits of *T. peruviana*, extracted in different solvents of varying polarities[11]. The presence of alkaloids, flavonoids, phenols, tannins and saponins in the aqueous

extract of the *A. mexicana* has been investigated by some workers[18]. Bhattacharjee *et al.*[19] has also been confirmed the presence of steroids and terpenoids in the seed of *A. mexicana* using chloroform column chromatographic fractions.

Basically, the antioxidant potential of any plant extract depends on its chemical composition and also on the assessment system. In FRAP assay, the measurement of antioxidant capacity of any plant extract is done by the help of an oxidant i.e., Fe^{3+}. The reductants i.e. antioxidants present in test sample reduce the Fe(III)/tripyridyl triazine complex into the blue ferrous (Fe II) form. The estimation of FRAP values has been done in hexanolic extracts of *A. mexicana* and *T. peruviana*. The FRAP values of the both extracts suggested that *A. mexicana* has little more antioxidant potential than the *T. peruviana* extract. It was previously reported that the FRAP values for aqueous extracts of *A. mexicana* and *T. peruviana* to be 33.6±0.14 and 15.8±0.07 μM Fe^{++} g^{-1}, respectively[14]. Further, it was previously evaluated the total antioxidant (FRAP) activity of leaves of *A. mexicana* extracted in methanol, ethyl acetate and hexane with FRAP values of 249.19, 51.80 and 22.33 μM Fe^{++} g^{-1}, respectively[20].

The DPPH free radical scavenging assay is used to quantitate free radical scavenging activities of varied compounds. Scavenging of free radicals is one of the major anti-oxidation mechanisms to inhibit chain reaction of oxidation. The present study involved the use of extracts of *A. mexicana* and *T. peruviana* to find out their radicals scavenging activity. The results of the present investigation clearly demonstrated that the *T. peruviana* have more antioxidant potential in comparison to *A. mexicana*. Another study, had reported the IC_{50} value of *A. mexicana* to be 151.02±0.24 μg mL^{-1} using methanolic extracts[21]. The DPPH scavenging assay has also been evaluated by Al-Madhagi *et al.*[22] and they have demonstrated that methanolic extract of *A. mexicana* leaves exhibited IC_{50} value 21.83 μg mL^{-1}.

Thus, the information obtained from the present investigation indicated the presence of probable bioactive compounds such as flavonoids, quinones, phenols, steroids, tannins, terpenoids, cardiac glycoside, alkaloids and carbohydrates. Based on the presence of these molecules, these extracts may be utilized as nutraceuticals and/or pharmaceuticals in the therapy of many ailments.

CONCLUSION

The results obtained from this study suggested that the leaves from the *A. mexicana* and *T. peruviana* were quite rich in containing various phytochemicals with significantly high antioxidant potential, which could be exploited in the development of therapeutics and nutritional supplements.

SIGNIFICANCE STATEMENT

This article presents an updated account of the presence of different phytochemicals from the leaves of the plants, *A. mexicana* and *T. peruviana* and their varied chemical, biochemical and pharmacological properties. The information imbibed in this article indicate that the application of these extracts should be carefully done as in addition to the presence of plenty of anti-oxidants, some phyto-constituents from these plants may exhibit toxic properties.

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