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Research Article

INVESTIGATIONS OF ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF *ARGEMONE MEXICANA* MEDICINAL PLANT EXTRACTS AGAINST BACTERIA WITH GASTROINTESTINAL RELEVANCE

VENI T[1*], PUSHPANATHAN T[2]

[1]Department of Advanced Zoology and Biotechnology, Kamaraj College, Thoothukudi - 628 003, Tamilnadu, India. [2]Department of Zoology, St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamilnadu, India.Email: veni.xaviers@gmail.com

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ABSTRACT

Objective: The objective of the study was to evaluate antibacterial potential and phytochemical analysis of various extracts of *Argemone Mexicana.*

Methods: The antimicrobial activity of various extracts of *A. Mexicana* were analysed by using agar well disc diffusion method.

Results: The ethanolic and methanolic extracst of *A. mexicana* had showed significant antimicrobial activity. Similar result was not observed in other four extracts. The higher content of reducing sugar, flavanoids, tannin, sterol, terpene and alkaloid were found in all the extracts of *A. Mexicana* are comparable with Amoxicillin.

Conclusion: The results obtained from this study indicate that *A. mexicana* is a potential source of antimicrobial and thus could prevent many diseases.

Keywords: *A. mexicana,* Antimicrobial activity, Phytochemical analysis, Infectious diseases, Medicinal plants and Plant extract.

INTRODUCTION

Present time medicinal plants being the effective source of medicines, either it can be modern or traditional medicines, the advantage of medicines are they are useful for health. WHO had given the remark that traditional medicines are safe treatment for the infections originated from microbial and non microbial origin [1].Some antibiotics do not have capability to treat diseases because of drug resistance capacity of pathogens [2]. The uses of herbal treatment is one of the possible way to treat diseases caused by multi drug resistant bacteria.Though Many Pharmaceuticals industries have produced a number of antibiotics from several years but in many cases it was observed that the cultures were showing resistance against the medicines [3]. The use of plant extract with their antibacterial properties is a major work which was done from last few years and become major work in therapeutic treatment [4]. To prove efficiency the plant extract used as a drugs against different types of pathogens [5-10]. According to WHO the best source of medicines are medicinal plants, therefore such plant should be studied and evaluated properly to check there structural and functional properties as well as the particular activity of each parts of the plants [11].

Phytochemically the leaves contain flavonoids, steorls, tannins, alkaloids and glycosides [12]. Many reports have been carried out to investigate the antibacterial determines of *A. mexicana* extracts. [13] studied the antimicrobial activity of the essential oil of *A. mexicana***.** The earlier observations on *A. mexicana* leaf and seed extracts showed considerable antimicrobial activity [14, 15].

Argemone mexicana L. (Papaveraceae), commonly known as Prickly Poppy in English and Premathandu in Tamil found in Mexico and now has widely naturalised in the United States, India, Bangladesh and Ethiopia. It occurs as wasteland weed in almost every part of India [16, 17]. In Mexico, the seeds have been used as an antidote to snake poisoning [18]. In India, the smoke of the seeds is used to relieve toothache. The fresh yellow, milky seed extract contains protein-dissolving substances effective in the treatment of diuretic, anti-inflammatory, malarial fever, leprosy, scorpion sting, warts, cold sores, wound healing, skin diseases, itches, jaundice and an antidote to various poisons [19- 22]. The seeds are purgative and sedative (Ayurveda) [23], useful in skin diseases and leucoderma (Yunani) [24] and in Homeopathy, the tincture of the entire plant is reported to be used orally for bronchitis and whooping cough [25, 26]. The fresh juice of the leaves and the latex both are reported to be used externally as a disinfectant for open wounds and cuts [27, 28]. Various isoquinoline alkaloids viz. berberine, cryptopine, coptisine, muramine, scoulerine, stylopine, cheilkanthifoline, sanguinarine, sarguinarine, chelerytherine, sanguinarine, thalifoline and protopine have been reported from the plant [29].

The present study was undertaken to evaluate the antibacterial potentials and phytochemical analysis of *Argemone mexicana* root, stem and leaves extracts against some selected bacterial species with the possible use as a genuine antimicrobial agent in pharmacological industries.

MATERIALS AND METHODS

Plant Materials

The plant materials of *Argemone mexicana* Linn were collected from the surroundings of Tirunelveli District, Tamil Nadu, India. The plant species was authenticated by Dr.V. Chelladurai, Retired research officer a well known Botanist from Central council for Research in Ayurveda and siddha, Government of India.

Preparation of Plant Extracts

Root, stem and leaves of the plant samples were thoroughly washed with running tap water 2-3 times and then finally washed with distilled water followed by shade-dried for seven days and then dried in an oven below 50ºC. The dried plant materials were then powdered using mixer and grinder. 30g of plant powder were extracted with 100ml of aqueous, acetone, ethanol, methanol, chloroform and Petroleum ether for 72hrs by Soxhlet extractor. Then the extracts with different solvents were evaporated using rotary evaporator. Extracts were transferred into pre-weighed sample containers and were stored later was used for phytochemical screening, Antibacterial activity and [30, 31].

Growth and Maintenance of Test Microorganism for Antimicrobial Studies

Bacterial cultures of *Escherichia coli*, *Klebsiella pneumonia, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis,, Staphylococcus aureus* and *Proteus mirabilis* were obtained from the Vivek laboratories, Nagercoil, Kanyakumari district, Tamil nadu, India. The cells from lyophilized vials were transferred into the liquid nutrient broth medium, and then transferred into nutrient agar slants preserved at 4ºC in the refrigerator.

Screening for Antibacterial Activity

Agar well disc Diffusion method was used to test the antibacterial activity of the extracts against *Escherichia coli*, *Klebsiella pneumonia, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis,, Staphylococcus aureus* and *Proteus mirabilis* bacteria. The essential root, stem and leaves extracts were used for studying their antibacterial activity. A loopful of bacterial strains were inoculated into 5ml of nutrient broth and incubated for 24hrs at 37ºC to get active strain by using agar well disc diffusion method. Muller Hinton Agar plates were prepared by pouring 20ml of molten media into sterile Petri plates. After solidification of media, inoculum of strains was swabbed uniformly and the inoculum was allowed to dry for 5 minutes.

The extracts were dissolved in Dimethyl Sulfoxide (DMSO). The concentrations of extracts (500µg/disc) were loaded on 5mm sterile disc using micropipette. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5mins and the plates were kept for incubation at 37ºC for 24hrs. At the end of incubation, zones formed around the disc were measured with transparent ruler in millimetre. Based on the diameter of the zone of inhibition, antibacterial susceptibility was ranked [31].

Phytochemical screening

Extracts of root, stem and leaves of *A. mexicana* using aqueous, acetone, ethanol, methanol chloroform and petroleum ether were subjected to various chemical tests in order to determine the secondary plant constituents:

Test for reducing sugars

To an aliquot of 2 mL of any extract, an aliquot of 5 mL of a mixture (1:1) of Fehling's solution I and II was added and the mixture was boiled for 5min; a brick-red precipitate indicated the presence of free reducing sugars [32].

Test for the presence of anthraquinones

An aliquot of 0.5 mL of the extract was shaken with 10 mL of benzene, filtered and an aliquot 5 mL of 10% ammonia solution was added to the filtrate and the mixture was shaken, the presence of a pink, red or violet colour in the ammoniac (lower) phase indicated the presence of anthraquinones [33].

Test for saponins

An aliquot of 0.5 mL of an extract was dissolved in an aliquot of 10 mL of distilled water in a test-tube was shaken vigorously for 30 s and then allowed to stand for 45 min. The appearance of a frothing, which persists on warming indicated the presence of saponins [32].

Test for flavonoids

To a portion of the dissolved extract, a few drops of 10% ferric chloride solution were added. A green or blue colour indicated the presence of flavonoids [32].

Test for steroids/terpenes

A lot of 500 mg of the extract from the rotary evaporator was dissolved in an aliquot of 2 mL of acetic anhydride and cooled at 0 to 4 to which a few drops of 12 N sulphuric acid were carefully added. A colour change from violet to blue-green indicated the presence of a steroidal nucleus [34].

Test for tannins

A fraction of 0.5 g of the extract was dissolved in 5 mL of water followed by a few drops of 10% ferric chloride. A blueblack, green, or blue-green precipitate would indicate the presence of tannins [32].

Test for alkaloids

A lot of 0.5 g of ethanol extract (from rotary evaporator) was stirred with an aliquot of 5 mL of 1% HCl on a steam bath and filtrated; to an aliquot of 1 mL of the filtrate, a few drops of Mayer's reagent was added, and to another aliquot of 1 mL of the filtrate, a few drops of Dragendorff's reagent were added. Turbidity or precipitation in tubes due to either of these reagents indicated the presence of alkaloids in the extract [32].

Test for resins

To an aliquot of 10 mL of the extract an aliquot of 10 mL of cupper acetate solution 1% was added and shaken vigorously and, a separate green colour indicated the presence of resin [34].

Test for glycosides

An aliquot of 5 mL of each extract was mixed with an aliquot of 2 mL of glacial acetic acid (1.048-1.049 g/mL), one drop of ferric chloride solution (1%), and mixed thoroughly. To this mixture, an aliquot of 1 mL of 12 N H2SO4 was added. A brown ring at the interface indicated the presence of glycosides [32]

RESULTS AND DISCUSSIONS

In the present study, the in vitro antimicrobial activity of 18 extracts against seven microbial strains and their potential activity were assessed by the presence or absence of inhibition zones values. According to the results given in Table 1, the extracts of the investigated plant species showed in vitro antimicrobial activities against one or more bacterial strains tested. The antimicrobial activities of plant extracts are compared with standard antibiotics such as Ampicilin, which were used as positive control. Results of the antimicrobial activity obtained using the disc diffusion assay is summarised in Table 1.

The data indicated that the extracts displayed available degree of antimicrobial activity on different tested strains and the microorganisms tested exhibit variable sensitivity against 18 extracts of *A. mexican.* Gram-negative bacteria of *K. pneumoniae* were sensitive to 16 extracts out of 18 tested, while *S. thphi, E.coli, P. aeruginosa* and *P. mirabilis* were sensitive to 16 and 14 extracts of root, stem and leaves of *A. mexicana*, respectively. Among the two Gram-positive bacteria, *B. subtilis* was found to be more sensitive than *S. aureus* 15 and 13 extracts, respectively.

All the extracts showed wide spectrum of screening even though, the methanol and ethanol extracts of the *A. Mexicana* root, stem and leaves showed greater antibacterial activity than the corresponding other extracts. The range of inhibition of the bacterial growth summarized in Table 1, varied from 08.23-17.68mm, 07.11-20.68mm, 09.12-16.67mm for the root, stem and leaves extracts of *A. mexicana,* respectively. The aqueous, acetone, chloroform and petroleum ether extract of the root, stem and leaves was not active against all the tested bacteria, whereas a maximum inhibition was

observed against *S. typhi.* In most cases the methanol and ethanol extracts exhibited higher antibacterial effects than the corresponding other extracts. Impregnated paper discs containing only DMSO used as negative control did not show any inhibition zone.

Since the size of the zone of inhibition depends upon both the rate of diffusion of the active agent into the plate and of the rate of growth of the target microorganism, the sizes of the inhibition zone can only be interpreted as an indication of microbial susceptibility or resistance in a clinical setting with well characterised antibiotics [35].

Table 1: Antibacterial activities of hot extracts of *A. mexicana* using different solvents against monitored by the disc diffusion method, presented as zone of inhibition in mm.

Plant parts	Name of the solvents	Name of the Bacterial strains						
		Zone of Inhibition (mm)						
		E.coli	***K.pneumoniae***	***S.aureus***	***S.typhi***	***B.subtilis***	***P.aeuroginosa***	***P.mirabilis***
Root	Aqueous	10.35	10.52	14.04	-	8.23	-	11.45
	Acetone	12.56	10.45	15.05	-	13.45	-	-
	Ethanol	17.68	14.60	10.43	15.65	11.43	15.00	11.38
	Methanol	14.50	17.20	13.00	12.35	14.20	13.00	14.16
	Chloroform	-	09.58	-	12.32	13.37	13.21	14.40
	Petroleum ether	13.00	11.45	12.67	13.00	12.00	10.00	10.22
Stem	Aqueous	11.70	11.38	09.50	11.00	11.68	10.43	11.26
	Acetone	09.00	12.25	14.83	13.43	13.16	11.45	12.00
	Ethanol	13.20	20.68	18.30	14.43	13.56	13.00	14.40
	Methanol	15.56	10.22	11.76	14.33	15.45	14.76	16.00
	Chloroform	10.14	09.45	10.87	10.64	14.00	-	-
	Petroleum ether	-	07.11	08.24	-	-	13.67	-
Leaves	Aqueous	12.08	11.70	-	10.00	-	12.31	13.00
	Acetone	09.12	10.00	-	11.14	13.00	11.56	14.20
	Ethanol	10.28	12.00	12.16	09.06	12.87	14.04	16.23
	Methanol	15.00	15.43	10.65	16.00	15.00	16.67	11.45
	Chloroform	14.87	-	-	14.00	10.00	13.42	15.00
	Petroleum ether	-	-	-	11.00	-	12.87	-
Ampicilin	10ug/ml	21.00	28.00	26.00	24.00	23.00	30.00	22.00
NC	-	-	-	-	-	-	-	-

Data given are mean of triplicate, - indicates no zone of inhibition, NC- Negative control.

Among these bacteria, Gram-negative bacteria such as *S. thphi, E.coli, P. aeruginosa* and *P. mirabilis* can cause serious intestinal diseases. However, the tested extracts were found to be active against the above Gram- negative bacteria. This makes the selection of the tested plants (*A. mexicana*.) as antimicrobial agents advantageous for the further investigations. Thus, the antibacterial activity exhibited by the extracts against these organisms could justify their general use in treatment of infection diseases like diarrhoea, dysentery and other gastrointestinal diseases [36, 37]. *K. pneumoniae*, was the most resistant bacterium to the tested plant extracts regarding the inhibition zone diameters. This resistance could be explained by the fact that the outermost layer of *Klebsiella* consists of a large polysaccharide capsule. *A. mexicana* (ethanol and methanol extracts) showed to be most active against *K. pneumoniae* strain.

The Gram-positive bacterium *S. aureus* is the causative agent of gastrointestinal disease characterised by projectile vomiting, diarrhoea, fever, abdominal cramps, electrolyte imbalance and loss of fluids [38]. It is of great advantage that root, stem and leaves of *A. mexicana* can significantly inhibit the multiplication of *S. aureus*. *B. subtilis* is known to cause bacteraemia in immunocompromised patients as well as symptoms such as vomiting and diarrhoea [39]. The disease may result from the ingestion of the organisms or toxins produce by the organisms. It is of great interest that *A. mexicana* exhibited an appreciable activity against *B. Subtilis*.

This finding is interesting, because in the traditional method of treating a bacterial infection, decoction of the plant parts or boiling the plant in water is employed. Whereas, according to present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity, in accordance with the results obtained by [40]. These observations may be attributed to two reasons: firstly, the nature of biological active components whose activity can be enhanced in the presence of methanol; secondly, the stronger extraction capacity of methanol could have produced greater number of active constituents responsible for antibacterial activity.

Phytochemical analysis

During phytochemical analysis of methanol, chloroform, petroleum ether, acetone, ethanol and aqueous extracts of *A.mexicana* was found that tannins, sterols/terpenes and alkaloids were present in all the 6 types of extract. The result of other tests for reducing sugar, anthraquinone, flavonoids, saponins, resins and glycosides are summarised in Table 2.

Phytochemical Constituents in *A. Mexicana*

A mexicana is reported to possess alkaloids [41, 42] amino acids [43] phenolics [44] and fatty acids [45] as major phytochemical groups. A series of bioactive compounds have been reported and some of them are isolated from different parts of A. mexicana. The whole plant of *A. mexicana* was reported to possess isoquinoline alkaloids such as berberine, cheilanthifoline, coptisine, muramine, scoulerine, stylopine, cryptopine, thalifone, sanguinarine, protopine,

optisine, chelerytherine and benzylisoquinoline alkaloids [46-51]. Alkaloids such as berberine, tetrahydroberberine, protopine and Benzophenanthridines have been isolated from the plant [52]. Seed oil otherwise called as Argemone oil reported to contain sanguinarine and dihydrosanguinarine. It also contains palmitic, myristic, oleic and linoleic acids [53].

Previous reports on *A. mexicana* leaves and seeds extract showed considerable antibacterial activity [54-56]. In another research, stem and essential oil of A. mexicana was found to be good antimicrobial activity [57]. The phytoconstituents obtained from root, stem leaves, fruits, flowers and seeds of medicinal plants include phenolic compounds, essentials oils, proteins and antioxidants, together they performance as biocontrol agents [58]. The inhibition activity of plants extracts against the growth of microorganisms was attributed to the presence of antioxidants [59]. The results of the present work are found to be directly correlated with the observations of earlier researchers [60-65]. Other details are needed to isolate and characterize the bio therapeutic potentials to evolve current antimicrobial medicines.

Table 2: Preliminary phytochemical analyses of 6 extracts of *A.mexicana*

Properties	Methanol	Chloroform	Petroleum ether	Acetone	Ethanol	Aqueous
Reducing sugar	-	+	+	-	+	+
Anthraquinone	+	-	-	-	-	-
Flavonoids	+	+	-	+	+	+
Saponins	+	-	+	+	-	-
Tannins	+	+	+	+	+	+
Sterols/terpenes	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	-
Resins	-	-	-	-	-	-
Glycosides	+	-	-	-	-	+

+ Present - Absent

CONCLUSION

In conclusion, the results of this study suggest that *Argemone mexicana* organic extracts may act as an alternative to synthetic bactericides which might have significant applications in pharmaceutical or other industries for controlling pathogenic bacteria. However, if plant- based antimicrobials such as crude extracts are to be used for drug or food preservation, issues of safety and toxicity will always need to be addressed.

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