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IN VITRO ANTIMICROBIAL ACTIVITY OF THE LEAF EXTRACTS OF *ARGEMONE MEXICANA* AGAINST SELECTED PATHOGENIC MICROORGANISMS

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ABSTRACT

This article describes the antimicrobial properties of *Argemone mexicana* a plant that grows in tropical and subtropical regions. The antimicrobial efficacy of different solvent extracts (Ethyl acetate, methanol and aqueous) of *Argemone mexicana* leaves against different pathogenic microorganisms was investigated using the disc diffusion method. The aqueous and methanolic extracts exhibited potent antibacterial activity against two Gram positive (*Enterococcus fecalis, Staphylococcus aureus)* and two Gram negative (*Klebsiella pneumoniae, Proteus mirabilis)* bacteria and moderate antifungal activity against *Aspergillus fumigatus* and*, Cryptococcus neoformans*. The zone of inhibition was found to be in the range of 10.0 - 25.0 mm when tested at a concentration range of 75 - 500 µg/disc. Differences in the antimicrobial activity can be attributed to the presence of different phytochemicals present in these extracts. Phytochemical analysis of different fractions that have shown efficacy may contribute to the development of new antimicrobial agents.

KEYWORDS : ***Argemone mexicana,*** **Antimicrobials, Disc diffusion method, Zone of inhibition, Phytochemicals, Pathogenic bacteria.**

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INTRODUCTION

Argemone mexicana (A.mexicana) commonly known as prickly poppy, is used as a medicinal plant. In Mexico, the seeds are considered as an antidote to snake venom. In India, the smoke from the seeds is used to relieve toothache. The fresh yellow, milky seed extract contains protein-dissolving substances, effective in the treatment of warts, cold sores, cutaneous infections, skin diseases, itches, dropsy and jaundice [4]. Traditional healers in Mali use *A. mexicana* to treat malaria [15]. The plant extracts have been shown to cure skin diseases, leprosy and inflammation. The fresh juice of the leaves and the latex are reported to be used externally as a disinfectant for open wounds and cuts [15]. The therapeutic efficacies could be due to the presence of several phytochemicals present in *A. mexicana* which include phenolic compounds, alkaloids and isoquinoline alkaloids (Table 1 and 2). Aqueous extract of leaves and alkaloid fractions of the roots have been reported to possess anti-inflammatory activity and strong uterine stimulant effect [2].

Table 1
Phytochemicals present in different extracts of A. mexicana leaves

S.N^{o}	Chemical test	Methanolic extract	Aqueous extract
1	Alkaloids	++	-
2	Glycosides	+	++
3	Saponins	++	-
4	Flavonoids	++	+
5	Tannins and phenolic compounds	+	+
6	Gums and mucilages	-	+
7	Carbohydrates	+	-
8	Reducing sugars	++	+
9	Proteins	+	-

++ = Moderately present, + = Present - = Absent

Table 2
Phytochemicals present in A. mexicana leaves

Isoquinoline alkaloids	Alkaloids	Aliphatic compounds	Phenolic compounds
Cheilanthifoline	Berberine	Mexicanol	Eriodictyol
Coptisine	Protopine	Mexicanic acid	Argemexitin 5,7-dihydroxychromone-7-neohesperidoside
Cryptopine	Sarguanerine		
Muramine	Muramine		
Scoulerine	Chelerytherine		
Stylopine			
Thalifoline			
Dihydropalmitine hydroxide			
Oxyhydrastinine			

Indiscriminate use of chemotherapeutic agents often leads to drug resistance, treatment failure, suppression of immunity against the diseases and cross reactivity due to their residues in the tissues. Phytochemical constituents in plants have high potential to develop safe and potent antimicrobial agents. Such plant derived agents could be inexpensive alternatives to synthetic drugs. The seeds and leaves of *A. mexicana* are known to exhibit antibacterial activity [3, 14]. The ethanolic extract of the whole plant has been reported to possess antiviral, hypotensive and smooth muscle stimulant activity [11] and found to be active against pathogenic bacteria including *Proteus vulgaris, Sarcina lutea, Candida albicans, Escherichia coli, Pseudomonas aeruginosa, Salmonella newport, Shigella flexneri, Staphylococcus albus* and *Serratia marcescens* [10]. There are no studies on the antimicrobial activity of *A. mexicana.* The purpose of this study is to investigate whether the leaf extracts (ethyl acetate, methanol and aqueous) exhibit any antimicrobial activity against the most common human pathogens i.e. *Klebsiella pneumoniae, Proteus mirabilis, Enterococcus fecalis, Staphylococcus aureus, Cryptococcus neoformans* and *Aspergllus fumigatus.*

MATERIALS AND METHODS

Collection of plant material

The plant material used in this study consisted of leaves of *A. mexicana,* collected from Tirupati, Andhra Pradesh, India, during summer (April-May, 2012) identified by the botanist, Department of Botany, Sri Venkateswara University (Tirupati) and a voucher specimen of the plant (No.367/Bot/SVU/2012) has been deposited in the herbarium for further reference.

Preparation of leaf extracts

The fresh leaves were harvested, washed with tap water and rinsed with sterile distilled water. The plant material was then shade dried and pulverized in a mechanical grinder followed by sieving to obtain coarse powder. The coarse powder was stored in air tight sterile containers protected from sunlight to prevent the loss of active components. Ethyl acetate (1000ml) was mixed with 250 grams of the powdered leaf. The mixture was kept for 48 h in tightly sealed vessels at room temperature, protected from sunlight and mixed several times periodically. After 48 hrs this mixture was filtered through Whatman no. 1 filter paper and the residue was collected for methanol extraction. The ethyl acetate fraction was subjected to soxhlet extraction to remove the ethyl acetate [1] and this is used as the ethyl acetate fraction. By following the above procedure the methanolic extract was prepared and the residue was used for making aqueous extract. All the three solvent extracts were stored in air tight containers at 4^{o}C for further use.

Test organisms

Microorganisms used for the study were obtained from the IMTECH, Chandigarh. The four bacterial strains, *Enterococcus fecalis, Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumoniae* and the two fungal strains *Aspergillus fumigatus, Cryptococcus neoformans* were used in this study. The bacteria were grown in nutrient broth (Himedia, M002) at 37±1^{o}C and fungi were grown in Sabouraud dextrose broth at 25±1^{o}C (Himedia, M033) and maintained on nutrient agar and Sabouraud dextrose agar slants at 4^{o}C.

Determination of Zone of Inhibition (ZOI)

The zone inhibition assay was performed by disc diffusion method as suggested by CLSI (Clinical Laboratory Standards Institute). The plant extracts were dissolved in di methyl sulfoxide (DMSO) and dilutions were made as needed. The discs were impregnated with 20 µl (micro liters) of the desired extracts at different concentrations (75, 125, 250 and 500 µg/disc) and placed on the inoculated agar plate. Negative controls were prepared using the same solvent employed to dissolve the extracts. Ciprofloxacin (5µg) for bacteria and fluconazole (25 µg) for fungi served as positive controls to determine the sensitivity (Himedia Laboratories). The inoculated plates were incubated at 37±1^{o}C for 24 hrs for

bacterial strains and 25±1^{o}C for 48 hrs for fungal strains. Antimicrobial activity was determined by measuring the zone of inhibition (mm) against the tested microorganisms. Each experiment was repeated three times and the average diameter of zone of inhibition is given in table.3.

RESULTS

Results of the different extracts against tested microorganisms are shown in Table.3. Both Gram positive and Gram negative bacteria were sensitive to the standard drug i.e. ciprofloxacin. The *Cryptococcus neoformans* and *Aspergillus fumigatus* were sensitive to the antifungal agent, fluconazole. Negative controls employing the solvent (DMSO) did not show any activity against the tested microorganisms. The aqueous extracts exhibited moderate antibacterial effects against two Gram positive bacteria (*Enterococcus fecalis, Staphylococcus aureus)* with inhibition zone of 20 and 10 mm respectively at 250 µg/disc concentration. With the Gram negative bacteria (*Proteus mirabilis, Klebsiella pneumoniae*) the inhibition zone was 16 mm at 250 µg/disc concentration. Higher antibacterial activity was observed (25mm) at 500 µg of the extract. However, the aqueous extract exhibited antifungal activity at 500 µg/disc concentration only against *Cryptococcus neoformans* and did not show any antifungal activity on *Aspergillus fumigatus*. The methanolic extract of *A. mexicana* exhibited significant antimicrobial activity at 125, 250 and 500µg /disc (zone of inhibition, 10-20mm). The methanolic extract was more effective against fungi compared to the aqueous extract. On the other hand, the ethyl acetate extract exhibited mild antibacterial activity on Gram negative bacteria and antifungal activity on *Cryptococcus neoformans* at higher concentrations (500 µg/disc, zone of inhibition, 15mm). The ethyl acetate extract did not show any activity against Gram positive bacteria and *Aspergillus fumigatus.*

Table 3
Antimicrobial activity of different solvent extracts of A. mexicana leaves

Name of the Extract/Drug	Conc. (µg / disc)	Diameter of Zone inhibition (mm)					
		Proteus mirabilis	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Enterococcus faecalis*	*Aspergillus fumigatus*	*Cryptococcus neoformans*
Ethyl acetate	75	-	-	-	-	-	-
	125	-	-	-	-	-	-
	250	13	10	-	-	-	12
	500	17	14	-	-	-	15
Methanol	75	-	-	-	10	-	-
	125	10	11	10	13	10	9
	250	13	14	12	15	15	14
	500	15	20	16	18	20	18
Aqueous	75	-	-	-	-	-	-
	125	12	-	-	12	-	-
	250	16	16	10	20	-	-
	500	24	22	12	25	-	15
Ciprofloxacin	5	28	25	22	25	-	-
Fluconazole	30	-	-	-	-	20	22
NC	-	-	-	-	-	-	-

mm- millimeter, - indicates no zone of inhibition, NC- Negative control.

DISCUSSION

In the present work, *in vitro* antimicrobial study was performed with the leaf extracts of *A. mexicana* on different bacterial and fungal species. In recent years, several researchers have reported that the alkaloids, phenolics, triterpenoids, glycosides and tannins, have high potential that could be developed as antimicrobial compounds against pathogenic microorganisms [8, 9]. Phytochemicals identified in the leaves of *A. mexicana* are given in Table.2. [12]. Presence of berberine and potassium nitrate salts in the yellow juice of the plant [5] indicate that these compounds might be responsible for the observed antimicrobial activity. Mexicanol, mexicanic acid, argemonic acid, alkoloids, phenolics present in the seeds of *A. mexicana* could be responsible for antimicrobial activity [6]. Oxyhydrastinine and cryptopine isoquinoline alkaloids from *A. mexicana* have been shown to inhibit the pathogenic microorganisms [7]. The results presented in the current report suggest that the methanolic and aqueous extracts of *A. mexicana* inhibit the growth of human pathogenic Gram positive and Gram negative bacteria and fungi. The results indicate that the leaf extract contains phytochemicals that exhibit broad spectrum antimicrobial effects. Preliminary phytochemical screening of *A. mexicana* extracts primarily revealed the presence of alkaloids, tannins and flavonoids which are reported to be effective against a wide range of microorganisms [13]. Among the tested extracts, aqueous extract was found to be more effective than the other extracts (methanol and ethyl acetate). The efficacy could be due to some of the phytochemicals that have been shown to exhibit antimicrobial activity [5, 6, 7]. In conclusion, results of the present study suggest that *A. mexicana* extracts may act as an alternative to synthetic antimicrobial compounds. This study is the first finding about the antimicrobial activity of *A. mexicana* against human pathogenic microorganisms. Further studies are needed to determine the future application of this plant extracts for industrial and therapeutic purposes. Identifying the phytochemicals that inhibit the growth of the microorganisms and testing the safety and toxicity of such agents is necessary before they could be used for therapeutic purposes.

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