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ANTIFUNGAL POTENTIAL OF ARGEMONE MEXICANA L

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ABSTRACT

Medicinal plants are widely used to treat infectious diseases. Infectious diseases produced by different types of micro-organism are still the leading cause of morbidity and mortality in the world today. A huge number of synthetic antimicrobial agents are available in the market. These antimicrobial agents are very effective for the treatment of related disorders. However, due to the capacity of many microbes to acquire resistance to various antimicrobial therapies, their effectiveness is limited. The majority of these antimicrobials have side effects also. As a result, infectious diseases produced by the microbes pose a severe threat to worldwide public health. So there is a great need to develop natural anti-microbial agents. With reference to many of Ayurvedic literature keen interest has been developed to work on this plant. The introduction of natural antifungal agents to treat the fungal disease will be a great mile stone for the most significant public health accomplishment. The aim of this study was to evaluate the antifungal potential of methanol and aqueous extracts of the leaf of *Argemone mexicana*. In this study aqueous and methanolic extracts were prepared from A. mexicana leaves. These extracts were tested for their antifungal activities by using the agar well diffusion method. It was observed that both aqueous and methanolic extracts of A. mexicana leaves inhibited the growth of *Trichophyton rubrum, Epidermophyton floccusum,* and *Microsporum canis*. Antifungal activity of the extract was comparable to that of ketoconezole. In this study it was observed that the leaf of A. mexicana exhibit good antifungal potential.

KEYWORDS: *Argemone mexicana*; Antifungal potential; Aqueous and Methanolic extract.

1. INTRODUCTION

Ayurveda is a system of holistic health care. Ayurveda is India's traditional health science,

which was widely practiced even during the Vedic period, about 5,000 years ago. Ayurvedic formulations are usually prepared from the different parts of medicinal plants. Various infectious diseases caused by bacteria, fungi, virus and worms are effectively treated with herbal/Ayurvedic formulations.[1]

The increasing resistance of pathogenic micro-organism against synthetic antimicrobial agents highlights the need of searching new antimicrobial agents from alternative sources. Many plants derived products have been reported showing strong antimicrobial properties. It has been reported that the *A. mexicana L*. (Papaveraceae), has antimicrobial potential against several different types of micro-organism. Even also against some worm its potential has been investigated.

Argemone mexicana belongs to family of Papaveraceae is commonly known as Mexican poppy or Prickly poppy. In India it is known as "*Satyanasi* or *Bhatkatiya*". It grows in the tropical and subtropical region as a weed in waste lands, cultivating fields and road sides (Bhalke & Gosavi, 2009).

It is an herb has showy yellow flowers of six petals and yellow juice, brings us to its Sanskrit name, *Svarnakshiri*. [2] When the plant is injured the yellow colour juice exudes bitter in taste, has long been used in India as traditional medicine for dropsy, jaundice, ophthalmia, scabies and cutaneous affections[3] Traditionally, its leaves are also used in cough, wounds, ulcer, warts, cold sores, skin diseases, itches etc.

In another study Argmone mexicana leaves has tested against four different fungal strains viz. *Aspergillus niger, Aspergillus terreus, Aspergillus flavus and Rhizopus stolonifera*[4] A good antifungal activity was observed at temperature 20°C - 25°C. Among the various extracts, methanol extracts have been reported to show maximum potency.

Ringworm is a fungal infection of the skin in humans. Ringworm is caused by several different fungus organisms that all belong to a group called "Dermatophytes". The species of the genera of Microsporum, Trichophyton and Epidermophyton areknown as dermatophytes.

2. MATERIAL AND METHOD

Materials

Fungal culture maintenance

Fungal strains were provided by MTCC, IMTECH, Chandigarh, India at DSRRAU, Jodhpur.

Following Three species of dermaophytes are selected for this study- *Trichophyton rubrum* (MTCC NO.296), *Epidermophyton floccosum* (MTCC NO.7880), and *Microsporum cannis* (MTCC NO.2820).

The fungal stains procured from MTCC, IMTECH Chandigarh were in freeze dried condition (in dormant form). So, the revival of the stains was done. After revival all fungal cultures were allowed to grow in incubator at 200 rpm and 25°C to 30°C temperature for 7to 10 days by using potato dextrose broth. After incubation the turbidity in each flask confirms the growth of culture and placed them at 4°C till further use. Thereafter the fungal cultures were used for antimicrobial studies.

Parts of *A. mexicana* plant

Argemone maxicana leaves were collected from a village of Alwar district, Rajasthan. It was collected in the last week of April month. The leaves were thoroughly washed with running tap water 2-3 times and finally washed with sterile distilled water followed by shade-drying on paper towel at room temperature for 15 days. Leaves were exposed to direct sunlight also for a few days. After drying, the plant materials were ground in a grinding machine.

Methods

Methanolic extracts

In a tightly sealed container at room temperature, fifty grams of grounded plant material was extracted with 150 ml methanol. The extract was protected from light and kept overnight on a rotary shaker at Seminal Applied Sciences Pvt. Ltd. Jaipur, Rajasthan in India. The extract was filtered with a five layered sterile muslin cloth. The procedure was repeated three times to obtain clear and colorless filtrate. The methanol from the filtrate was removed by rotary evaporation. Extracts were stored at 16 °C overnight and were subsequently freeze-dried at 60 °C in a 20 mL vacuum for 24 h. Theextract was then sterilized with UV and stored in an airtight container at 4 °C for further use.

Aqueous extracts

Fifty grams of grounded plant material was extracted with 150 ml sterile double distilled water for 24 h as in the case of methanol. The mixture was filtered with sterile five-layered muslin cloth and centrifuged at 5000 rpm. The supernatant obtained was concentrated to N/5 volume with rotary evaporator. The concentrated extract was then UV sterilized and stored at 4 °C for further use.

Antifungal potentiality test

Agar well diffusion technique (Adeniyi *et al)*[5] was adopted for this study with some modification. Potato Dextrose Agar media (Hi-Media M096) poured into pre-sterilized petri-plates. Fungal suspensions (1 ml of each) were spread over the solidified potato dextrose agar plates and allowed to dry for few minutes. Stains were incubated at 25°C or 30°C for 30 days according to MTCC protocol to enhance sporulation.

Thereafter five wells were punched with a sterile cork borer. The extracts were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations of 20 mg/ml. Four wells were filled with 20 µl, 40 µl, 60 µl and 80 µl of diluted extract. In fifth well ketoconazole (40 µl) was filled as the standard for comparison of antifungal activity. Plates were then incubated at 37 °C for 72 h. Following an incubation period of 72 hrs, plates were removed from the incubator and antifungal activity was evaluated by measuring zones of inhibition of fungal growth.

OBSERVATION AND RESULTS

The methanolic and aqueous extracts prepared from the leaves of *A. mexicana* along with ketoconazole were inoculated into wells punched in pre-seeded agar plates. After incubation at 37 °C for 72 h, the clear growth inhibition zone around the well was measured and recorded as a measure of antifungal activity. The results represented in Figure 1 show the antifungal potential of methanolic extract along with ketoconazole against *Trichophyton rubrum and Epidermophyton floccosum.* and Figure B show that aqueous of *A. mexicana* leaves extract along with ketoconezole exhibit significantantifungal activity.

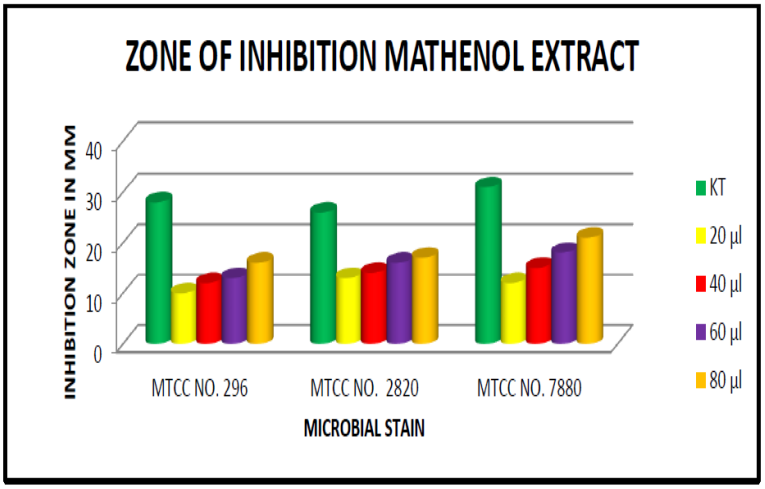

Figure 1: Antifungal potential of *A. mexicana* methanolic extracts: (1) *MTCC NO. 296-*

***Trichophyton rubrum* (2) *MTCC NO.2820- Epidermophyton floccosum* and (3) *MTCC NO. 7880 – Microsporum canis*. Antifungal activity seen within *A. mexicana* leaf extract with 20 µl (yellow bar), 40 µl (red bar), 60 µl (purple bar), 80 µl (orange bar) while (greenbars) indicate antifungal activity of ketoconazole.**

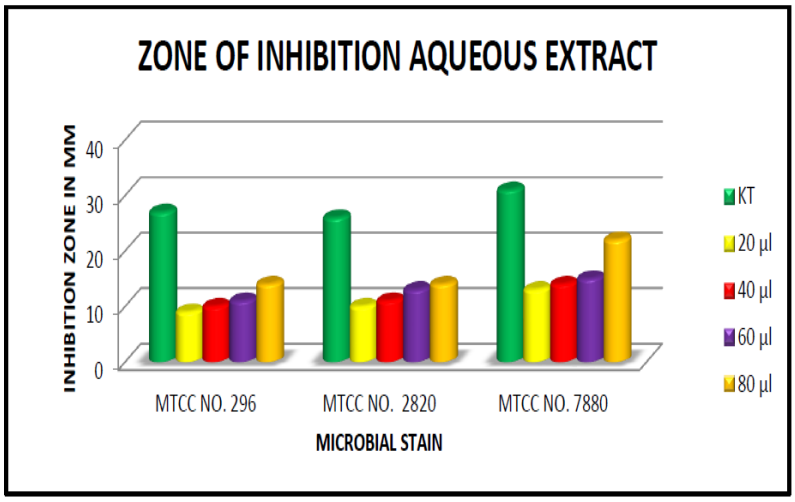

Figure 2: Antifungal potential of *A. mexicana* aqueous extracts: (1) *MTCC NO. 296- Trichophyton rubrum* (2) *MTCC NO.2820- Epidermophyton floccosum* and (3) *MTCC NO. 7880 – Microsporum canis*. Antifungal activity seen within *A. mexicana* leaf extract with 20 µl (yellow bar), 40 µl (red bar), 60 µl (purple bar), 80 µl (orange bar) while (greenbars) indicate antifungal activity of ketoconazole.

Table: Zone of inhibition and MIC of both extracts of leaf on selected fungal stains.

Name of extract / drug	Concentration of the exracts / drug (in µl)	Inhibition zone for different fungal stains		
		Trichophyton rubrum	Epidermophyton floccosum	Microsporum cannis
		MTCC NO. 296	MTCC NO. 2820	MTCC NO. 7880
Ketoconazole	40 µl	27	26	31
Aqueous Extract	20 µl	9	10	13
	40 µl	10	11	14
	60 µl	11	13	15
	80 µl	14	14	22
Ketoconazole	40 µl	28	26	31
Mathanol extract	20 µl	10	13	12
	40 µl	12	14	15
	60 µl	13	16	18
	80 µl	16	17	21

DISCUSSION

Sensitivity of drug sample can be explained on the basis the following scale which was

developed by Arora D. S. et al (1997).

Table: Relation between zone of inhibition and drug sensitivity.

S. No.	Zone of inhibition (in mm)	Drug sensitivity
1	Below 6	Insensitive
2	6 to <9	Less sensitivity
3	9 to <12	Moderate sensitivity
4	≥12	High sensitivity

It is clear that the antifungal component within the extract could successfully inhibit fungal growth. On the basis of above scale, we can explain-

- Trochophytom rubrum was found moderate sensitive to aqueous extract with the dose of 20 µl. when the doses were increased sensitivity was also increased and it was found also moderately sensitive to 40 and 60 µl doses. High sensitivity was showed with 80 µl dose.
- Trichophyton rubrum showed moderate sensitivity to 20 µl of methanol extract and high sensitivity to the rest of three doses.
- Epidermophyton floccosum was found moderately sensitivity to 20 µl and 40 µl of aqueous extract but highly sensitive with the rest of all doses of both extracts.
- Microsporum cannis was showed high sensitivity to all selected doses.
- Methanol extract is more sensitive in comparison of same dose of water extract against all three organisms.

Both extracts showed moderate and high sensitivity against all organisms. Methanol extract showed slightly higher potential than water extract in the same volume. There was also slight augmentation in sensitivity with increasing concentration of both extracts. The extracts have more potential against *Microsporum canis* besides rest of two organisms.

Many other researchers have also confirmed great antimicrobial activity of *A. mexicana* seeds and leaves against gram-positive as well as gram-negative bacteria, fungi and other pathogenic micro-organisms.[6]

Phytochemical compounds are known to play important role in bioactivity of medicinal plants and these help to produce definite physiological action on the human body.[7]

Recent scientific studies suggest that antifungal potential of *Argemone maxicana* is also due the presence of some phytochemicals. According to Singh et al., 2009 the alkaloids,

dehydrocorydalmine and oxyberberine isolated from *A. mexicana*, were found to exhibit antifungal activities against some fungal strains.[8] In the view of one another study its alkaloids are mostly beneficial when it is used superficially; it shows active inhibitor against bacteria and fungus. It inhibits the bacterial cell wall permeability and leak out its cytoplasm from the cell and bacterial cell unable to survive.[9]

CONCLUSIONS

In conclusion, experiments described in this article demonstrate that the *A. mexicana* extracts exhibit the antifungal potential. More experiments are required to elucidate molecules that have antifungal potential from *A. mexicana* leaves.

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Conflicts of Interest

The authors declare no conflict of interest.

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