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IN-VITRO ANTIFUNGAL ACTIVITY OF *ARGEMONE MEXICANA* AGAINST KERATINOPHILIC FUNGI

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Keratinophilic fungi are ecologically important fungi which degrade keratinous waste materials into simpler forms. Some of keratinophilic fungi are pathogenic for humans and animals and cause skin diseases. The aim of present study is to study the antifungal activity of leaves stem and flower extracts of *Argemone mexicana* against some isolated keratinophilic fungi viz. *Chaetomium seminis-citrulli, Penicillium guttulosum, Aphanoascus terreus, Aphanoascus durus* and *Chrysosporium zonatum*. The plant extracts were prepared with Soxhlet extraction in 80 percent methanol and further with diethyl ether using separating funnel. The antifungal activity of plant extracts was analysed by agar disc diffusion method on Sabouraud's Dextrose Agar (SDA) medium. Ketoconazole was used as the reference antifungal disc. The results were analysed in diameter of zone of inhibition. The result of present study indicated that *Argemone mexicana* have significant antifungal activity against all tested fungi. The bound flavonoid extract of *Argemone mexicana* recorded highest antifungal activity against keratinophilic fungi. *Aphanoascus durus* found to be the most susceptible fungal isolate among the tested fungi. The herbal antifungal discs in this study, were found more effective antifungal agents than reference antifungal drug.

Keywords: Antifungal activity, *Argemone mexicana,* Inhibition, Keratinophilic fungi

Introduction

Keratinophilic fungi are ecologically important and recently have great concern throughout the world as they have important role in degradation of kerationous waste [1-3]. Many keratinophilic fungal species frequently cause dermatophytosis in humans and animals on keratinous tissue, such as skin, nails and hair in man and animals. The dermatophytes and related keratinophilic fungi cause superficial mycoses in humans and domestic animals[4]. These infections are might be transmitted from soil to humans.

For treatment of these superficial mycoses, a number of topical as well as systemic antifungal agents have been developed[5]. The dermatophytes and keratinophilic fungi show different susceptibility to different antifungal agents. A wide range of new antifungal agents are currently utilized including azole group[6]. The antifungal agents are used as standard for different antibiotic sensitivity tests. The indiscriminate use of these antifungal agents can led to the development of antibiotic resistance in the fungal species. Therefore, plant based antifungal drugs are used mostly in recent years.

Medicinal plants are useful in the treatment of many infectious diseases[7]. Recently, medicinal plants are being utilized as antimicrobial agents against bacteria and

fungi[8]. In this study, antifungal activity of *Argemone mexicana* was analyzed against isolated keratinophilic fungi. The antifungal activity was observed in free and bound flavonoid extracts of leaves, stem and flower of *Argemone mexicana*. The antifungal susceptibility of dermatophytes and filamentous fungi are mostly performed by agar disc diffusion method[9]. The agar disc diffusion method was earlier used for determining the MIC values of itraconazole, terbinafine and ketoconazole against different dermatophyte species. Several methods have been used for susceptibility testing e.g. disc diffusion method, broth macro and microdilution method, colorimetric micro-dilution method, E-test etc. [10-12]

Material and Methods

Collection and processing of plant materials:

Freshly harvested leaves, stem and flower were collected from Amer area, Jaipur between December to February month. The plants were placed in laboratory of Department of microbiology, JECRC University for further processing.

The collected plant parts were washed thoroughly with running tap water to remove the soil and dirt. Then leaves, stem and flowers were separated from plants and shade dried for 10 days. The dried plant parts were grinded separately to prepare powder extract and stored in air tight vessels separately.

Solvent extraction:

Plant extraction was carried out with Soxhlet extraction and extraction with separating funnel. For Soxhlet extraction, 200 grams of plant part powder was filled in the thimble and extracted 80 percent methanol with regular heating for 24 hours on heating mantle[13]. After extraction, extracts were filtered separately through Whatman No.1 filter paper.

The filtrate of each extract was further extracted subsequently with petroleum ether, ethyl ether and ethyl acetate in separating funnel. Petroleum ether fraction was discarded; ether fraction was used for isolation of free flavonoids whereas ethyl acetate fraction was used for isolation of bound flavonoids. Ethyl acetate fraction was further hydrolyzed with 7% H_2SO_4 for three hours and was re-extracted in ethyl acetate using separating funnel. This fraction was washed with distilled water for neutrality and further dried for final plant product[14, 15]. Ethyl ether and ethyl ether factions were further used for antifungal activity against keratinophilic fungi.

Fungal species used:

Isolates of *Chaetomium seminis-citrulli, Penicillium guttulosum, Aphanoascus terreus, Aphanoascus durus* and *Chrysosporium zonatum* were used .The fungi were isolated from soil using hair baiting technique and cultures were maintained on Sabouraud's Dextrose Agar (SDA) medium.

Drug used for antifungal activity:

Ketoconazole was used as reference standard for antifungal activity. Three replicates were kept for each treatment and incubated at 28±2˚C. The radial growth of mycelium to ascertain the effect of the plants were measured every day for five to six days and compared with the results of control.

Antifungal activity of plant extracts against selected keratinophilic fungi:

The antifungal efficacy of the free flavonoid and bound flavonoids of *Adhatoda vasica* and *Argemone maxicana* was evaluated against selected fungi. The therapeutic value of the *Adhatoda vasica* and *Argemone mexicana* was assessed against keratinophilic fungi and compared to commercially available antifungal discs of Ketoconazole. Whatman filter paper no**.** 1 was used for the preparation of antifungal disc. The discs and Petri dishes were autoclaved for sterilization**.** The discs were dipped in each plant extract separately for 24

hours for complete absorption of plant extraction**.**

Screening of anti-keratinophilic activity:

The antifungal activity of free flavonoid and bound flavonoid extracts of *Adhatoda vasica* and *Argemone maxicana* was evaluated using modified agar disc diffusion method as described by Gould and Bowie (1952)[16] and Bauer and Kirby (1966)[17]. Sabouraud's Dextrose Agar (SDA) media was poured in sterile Petri dishes and allowed to solidify. The pouring is done in such a way that thickness of SDA media is equal in each Petri-plate. The test fungi selected for antifungal sensitivity include: *Chaetomium seminis-citrulli, Penicillium guttulosum, Aphanoascus terreus, Aphanoascus durus* and *Chrysosporium zonatum.*

Test cultures were scraped using sterile cotton swabs (Hi-media) and streaked onto Sabouraud's Dextrose Agar plates. Each plate was homogenized to ensure uniform distribution of the inoculum. Plant extract discs, prepared from respective plant extracts, were then kept on the SDA plate using sterile forceps. Similarly, the standard antifungal discs were also placed on media surface for comparative analysis. The plates were incubated at 30°C for 72 hours. The antimicrobial activity was noted by measuring the diameter of zone of inhibition for each plant extract. All the experiments and measurements were done in three replicates. The Activity Index of each extract of both plants was calculated against all test fungi using following formula:

$$\text{Activity Index} = \frac{\text{Inhibition zone of sample (mm)}}{\text{Inhibition zone of standard (mm)}}$$

Results and Discussion

Antifungal activity of plant extracts against selected keratinophilic fungi:

In the present study, antikeratinophilic activity of free and bound flavonoid extract of *Adhatoda vasica* and *Argemone mexicana* was assayed. The antifungal activity these two plants was tested against *Chaetomium seminis-citrulli, Penicillium guttulosum, Aphanoascus terreus, Aphanoascus durus* and *Chrysosporim zonatum*. Both of the plants exhibited very good inhibition activity against all the test fungi**.** The antimicrobial activity was evaluated by measuring the zone of inhibition. The Activity Index of all plant extract was also calculated by comparing zone of inhibition of plant extract with zone of inhibition of standard antifungal disc for each test fungi (Table No. 1 and 2). Ketoconazole was the most effective antifungal drugs with zone of inhibition measuring for *Chaetomium seminis-citrulli* (21 mm)*, Penicillium guttulosum* (12 mm)*, Aphanoascus terreus* (10 mm)*, Aphanoascus durus* (13 mm) and *Chrysosporium zonatum* (26 mm).

Antifungal activity of free flavonoid extract from *Argemone mexicana* :

Argemone mexicana free flavonoids extracts of leaf, stem and flower were screened for antifungal efficacy against selected test fungi. Free flavonoids extracts were found effective against the selected test fungi. The maximum inhibition was expressed by flower extract against *Aphanoascus durus* (53 mm) followed by *Chrysosporium zonatum* (46 mm) and *Chaetomium seminis-citrulli* (42 mm). The stem extract did not show antifungal activity against *Chrysosporium zonatum* and *Aphanoascus terreus.* The leaf extract was also found to be effective against all tested fungi. The maximum Activity Index was shown by flower extract (Table 1).

Antifungal activity of bound flavonoid extracts from *Argemone mexicana:*

Argemone mexicana bound flavonoids extracts of leaf, stem and flower were screened for antifungal efficacy against selected test fungi. *Argemone mexicana* bound flavonoids extracts expressed significant inhibition to all tested fungi. The maximum inhibition of *Aphanoascus durus* was observed in flower extract (65 mm)

followed by stem extract (56 mm). The maximum inhibition of *Chaetomium seminis-citrulli* was observed in stem (60 mm) extract and flower extract (41 mm). The maximum zone of inhibition of *Chrysosporium zonatum* was observed in stem extract (49 mm) and flower extract (41 mm). The antifungal activity of *Penicillium guttulosum* was also observed in leaf, flower and stem extract bound flavonoid extracts of *Argemone mexicana*.

Table 1: Activity Index of free flavonoid extracts of *Argemone mexicana* against isolated fungi

Test fungi	Leaf		Stem		Flower	
	Inhibition Zone (mm)	Activity Index	Inhibition Zone (mm)	Activity Index	Inhibition Zone (mm)	Activity Index
Chaetomium seminis-citrulli	11 ± 0.57	0.52	14 ± 0.66	0.66	42 ± 1.57	2.0
Penicillium guttulosum	10 ± 0.65	0.83	11 ± 0.77	0.92	17 ± 1.05	1.41
Aphanoascus terreus	15 ± 0.89	1.5	-ve	0	15 ± 0.58	1.5
Aphanoascus durus	22 ± 0.97	1.69	32 ± 1.17	2.46	53 ± 1.73	4.07
Chrysosporium zonatum	18 ±0.78	0.69	-ve	0	46 ± 2.19	1.75

*Zone of inhibition of Ketoconazole against *Chaetomium seminis-citrulli* = 21mm, *Penicillium guttulosum* =12 mm, *Aphanoascus terreus* = 10 mm, *Aphanoascus durus*= 13 mm and *Chrysosporium zonatum* = 26 mm

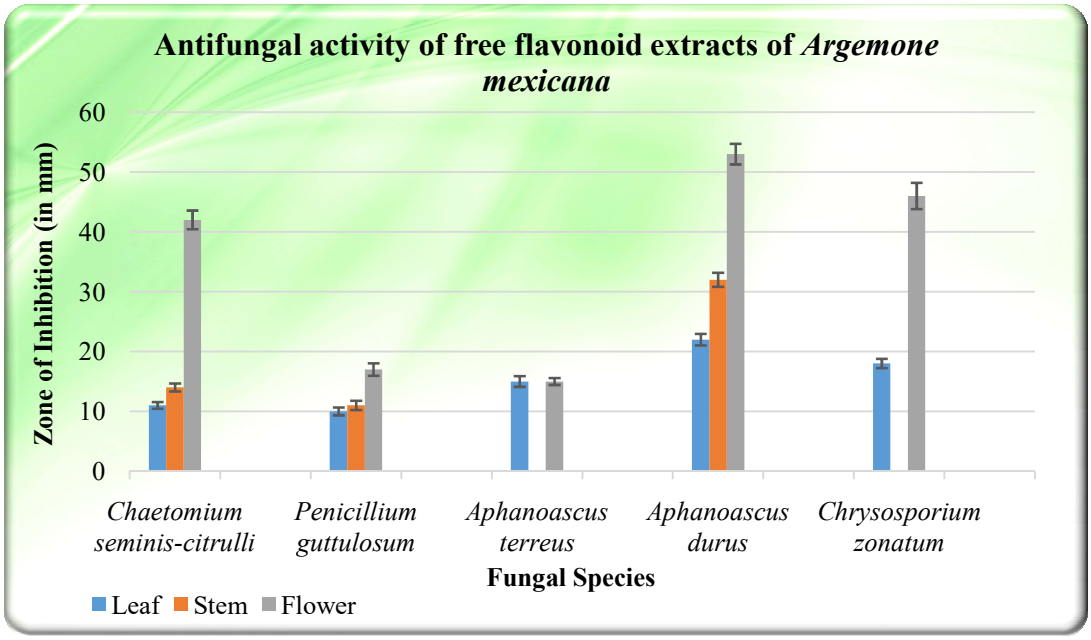

Figure 1: Antifungal activity of free flavonoid extract of *Argemone mexicana*

Table 2: Activity Index of bound flavonoid extracts of *Argemone mexicana* against isolated fungi

Test fungi	Leaf		Stem		Flower	
	Inhibition Zone (mm)	Activity Index	Inhibition Zone (mm)	Activity Index	Inhibition Zone (mm)	Activity Index
Chaetomium seminis-citrulli	16 ± 0.46	0.76	60 ± 3.23	2.85	41 ± 1.56	1.95
Penicillium guttulosum	11 ± 0.39	0.92	33 ± 1.56	2.75	26 ± 0.68	2.16
Aphanoascus terreus	14 ± 0.66	1.4	32 ± 1.29	3.2	22 ± 0.39	2.2
Aphanoascus durus	30 ± 0.83	2.30	56 ± 2.43	4.30	65 ± 2.65	5
Chrysosporium zonatum	21 ± 0.79	0.80	49 ± 1.68	1.88	41 ± 2.38	1.57

***Zone of inhibition of Ketoconazole against** *Chaetomium seminis-citrulli* = 21mm, *Penicillium guttulosum* =12 mm, *Aphanoascus terreus* = 10 mm, *Aphanoascus durus*= 13 mm and *Chrysosporium zonatum* = 26 mm

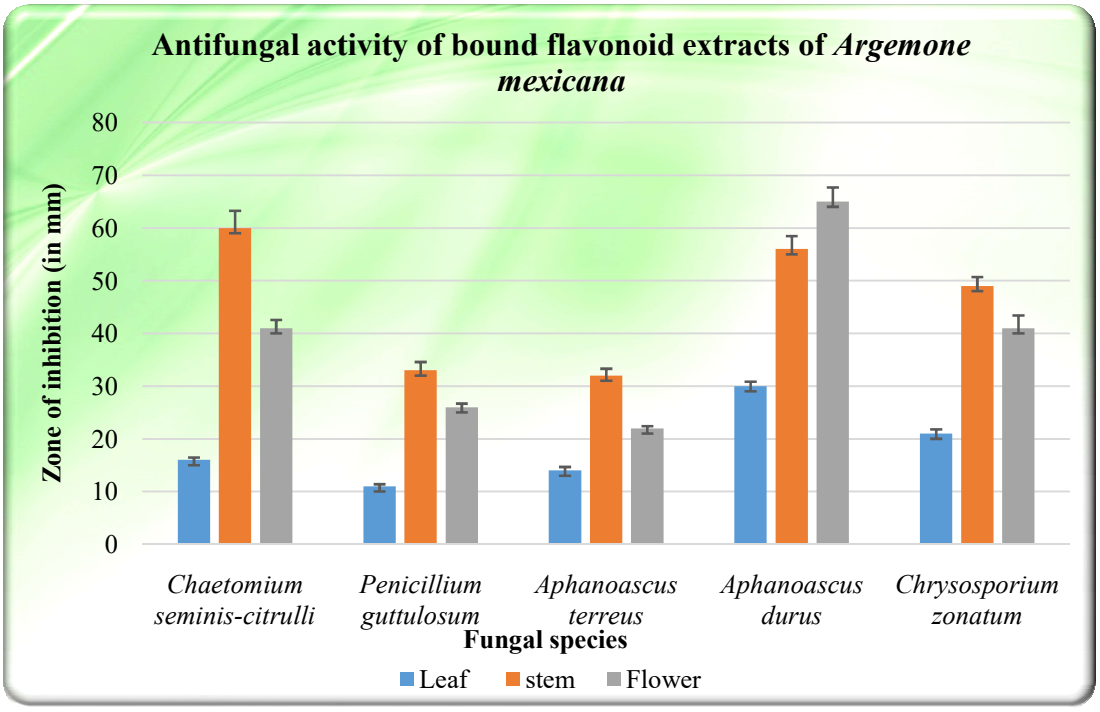

Figure 2: Antifungal activity of bound flavonoid extract of *Argemone mexicana*

The maximum inhibition of fungal isolates was found in *Aphanoscus durus* followed by *Chaetomium seminis-citrulli, Chrysosporium zonatum*, *Penicillium guttulosum* and *Aphanoscus terreus* (Figure 1 and 2). *Argemone maxicana* stem bound flavonoid and *Argemone maxicana* flower also showed inhibition of fungal strains. The bound flavonoid extract of *Argemone mexicana* recorded highest antifungal activity against keratinophilic fungi. The herbal antifungal discs in this study, were found more effective antifungal agents than reference antifungal drug.

Several authors have reported that herbal extracts contain antimicrobial potential [18,19,20]. These herbal extracts contain some biological important compound responsible for antimicrobial activity by inhibition of microbial metabolism system or inhibition of cell-wall formation[21].

In the present study, the antifungal effect of the extracts of *Argemone maxicana* was examined on some keratinophilic fungi namely *Chaetomium seminis-citrulli, Penicillium guttulosum, Aphanoascus terreus, Aphanoascus durus* and *Chrysosporium zonatum*. *Argemone maxicana* stem and flower bound flavonoid extracts showed very good antifungal efficacy against the fungal growth. *Aphanoscus durus* was found the most susceptible fungi against all parts of plant followed by *Chaetomium seminis-citrulli, Chrysosporium zonatum*, *Penicillium guttulosum* and *Aphanoscus terreus*. Ketoconazole was used as standard antibiotic against isolated fungi. The present study proves that plant extracts showed better efficacy as compared to standard antibiotic discs. The *Argemone mexicana* leaves, stems and flower extracts showed good results and proved to be good antifungal agents [22,23].

Siva *et al.,* (2008) reported that acetone and ethanol extract of *Adhatoda vasica* leaf showed 100% inhibition of *F. oxysporuim*. *Argemone mexicana* plant extracts are also used in antimicrobial activities[24]. Singh *et al.,* (2010) screened the antifungal activity of *Argemone mexicana* against *Aspergillus flavus* by food poisoning method on potato dextrose agar (PDA) medium using silver nanoparticles and observed a zone of inhibition of 10 ± 0.2 mm in diameter[25]. Osho and Adetunji, (2010) studied sensitivity of *Argemone mexicana* aerial and root part essential oil against *Candida albicans* and *Candida stellatoidea.* The fungal species were found susceptible to essential oil from the aerial parts, but the root part was found to be effective only against *C. Stellatoidea*[26]. Prasad and Dhanapal, (2010) studied antifungal activity of leaf extracts of *A. mexicana* at the concentrations 40µl, 70µl and 100µl. The plant inhibited the growth of *Aspergillus niger, Fusarium moniliforme, Candida albicans* and *Mucor plumbeus*[27]. The antifungal activity of *A. mexicana* plant extracts was described later by Apu *et al.* (2012)[28].

Plant extracts of *Argemone mexicana*, used in this study, have not been analysed by any researcher in previous studies. Thus the findings of present study fulfill the requirement of new, natural antifungal drugs against pathogenic dermatophytes and keratinophilic fungi which is safe and more effective than commercial available drugs[29].

Conclusion

The *Argemone mexicana* bound flavonoid extract was more effective against keratinophilic fungi. *Argemone mexicana* free flavonoid extract also exhibited strong inhibition of the test fungi.

The present study provides a scientific validation of *Argemone mexicana* as important plant for antifungal activity. The positive antifungal activity of *Argemone mexicana* against some pathogenic fungi, justifies the ethno-medical use of this plant for treatment of mycotic infections. The plant extracts used in this study exhibited good results than commercially available drugs with safe treatment indicating that naturally prepared antifungal drugs are more effective against pathogenic fungi with no side effects.

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