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Evaluation of antifungal activity and phytochemical analysis of selected plant extracts against *Malassezia Furfur*

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Abstract

The fungus *Malassezia furfur* is lipophilic dimorphic yeast, widely accepted as the causative agent of superficial skin infections like pityriosis versicolor, seborrhoeic dermatitis, dandruff and atopic dermatitis. The currently available synthetic drugs possess limitations, due to this drawback of conventional drugs; attention is shifted towards herbal remedies with medicinal plants as alternative drugs. The study was carried out to evaluate the antifungal activity of nine selected plant extracts of *Sida cardifolia*, *Senna alexandrina, Mentha citrata, Trigonella foenumgraecum, Pelargonium geaveolens, Nelumbo nucifera, Datura stramonium, Tagetes erecta* and *Lantana camara* with ketoconazole as the positive reference standard. The anti-malassezial efficacy was tested using different solvent extracts and the preliminary screening of plant extracts was determined by the Kirby Bauer disc diffusion method and Minimum Inhibitory concentration was determined by the micro broth dilution method wherein the MIC was showcased to be 0.78mg/ml, 0.156 mg/ml and 1.25mg/ml respectively. The preliminary phytochemical analysis of secondary metabolites by standard chemical methods revealed the presence of different secondary metabolites. Such as phenolics, alkaloids, terpenoids, saponins, flavonoids, tannins.

Keywords: phytochemicals, *M. furfur*, mic, medicinal plants, secondary metabolites

Introduction

Malassezia spp belong to basidiomycetous fungi and naturally found on the skin surfaces of many animals, including humans [1]. *Malassezia furfur* is a lipophilic yeast resident on the skin, it is known to produce certain chemicals which reduce the pigment on the skin and leave white patches [2] and also known to cause superficial skin infections such as pityriosis versicolor, pityriasis folliculitis, seborrhea dermatitis and dandruff, atopic eczema also known to cause systemic infections in immune compromised patients [3, 4].

Due to toxic side effects of synthetic or conventional agents, there is need for affordable, effective and nontoxic alternatives, which has led to the search for compounds from natural sources such as plants [5]. The use of natural products has been one of the most successful strategies for the discovery of new drugs; medicinal plants are being used from 1000s of years as folk medicine and are believed as a novel source of antimicrobial agents [6].

From the past ancient period of time, plant species are known to be the store houses of bioactive compounds possess therapeutic properties [7]. In current years large extent of work is being carried out on antimicrobial properties of plant extracts. Several works demonstrated in the laboratory studies have shown that various plant parts like roots, stem, bark, leaves and seeds possess inhibitory properties against bacteria, fungi and insects. The studies in the last decade have emphasized for their properties [8].

Since plant and plant products are extremely effective without negative side effects and due to compatibility with the human body and known to act on multiple targets, their demand is increasing day by day, emphasizing the more research for novel plant constituents and natural products have afforded a rich source of compounds having found many applications in the fields of medicine pharmacy.[9]

Presently bioactive secondary metabolites from the plants are more widely used for medicinal purposes both in the native and modified forms; beginning from the ancient period, plant and plant extracts were used as medicine for healing purposes ethno medicine around the world [10].

From the beginning of history plants are being used as medicines, in fact studies of animals demonstrated that they feed on plants to cure certain diseases. Especially in the continent Asia, been use of herbal medicine is more and also documented. Most of the medicinal plants have globally recognised as a medicine are originated from China and India [11].

The knowledge related to medicinal properties of plants and plant products to treat infectious diseases and to maintain a healthy life was in practice in mankind. Nature is a constant source of hope and also it is well known that the plants are reservoirs of secondary metabolites which include flavonoids, phenolics, alkaloids, quinones, terpenoids and saponins which are known to be the therapeutic agents [12, 13]

The aim of this study was to evaluate antifungal activity of selected plant extracts on *Malassezia furfur*. The inhibitory activity and also the minimum concentration that inhibited the growth of the organism were determined.

Materials and Methods

Collection and Maintenance of the Test Organism

The organism used in this study was *Malassezia furfur* (strain MTCC1374), procured from Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh, India. The culture was maintained by using Leeming-Notmann (LN) agar medium.

Preparation of Plant Extraction

The plants used for this study were selected based on mainly focused on their medicinal properties according to ayurvedic system of medicine. Plant materials were collected from Gandi Krishi Vignyan Kendra (Bangalore).The collected plant materials were washed to remove the dust particles and blot dried, powdered. The powdered plant material was subjected to extraction

Aqueous Extraction

The dried powder was macerated with distilled water in the ratio (1:3% W/V) in a blender for 10-15 mins. The obtained macerate was filtered and centrifuged at 8000 rpm for 10 mins and the supernatant was filtered through Whatman no.1 filter paper and concentrated in preheated water bath 30 mins and stored at 4℃ for further use [14].

Solvent Extraction

The solvent extraction was carried out by using soxhelt apparatus, where the powdered material was placed in the thimble and extracted with 250ml of solvents the obtained extracts were evaporated by using rotary vacuum evaporator and the dried extracts were stored in refrigerator at 4℃.

Screening of Plants Extracts for Antifungal Activity

Antimicrobial Screening of Plant Extracts by Disc Diffusion Method

To the Lemming Notmann agar medium 50µl of microbial inoculum was added and evenly spread with glass rod. Four sterile discs were placed equidistantly and dispensed with the 10µl of (20 mg/ml and 10 mg/ml) concentration of plant extract the positive control Ketoconazole (50µg/disc) and 5% DMSO as negative control and incubated at 32±2℃. The plates were checked for zone of inhibition as diameter. The experiment was carried in triplicates [15].

Determination of Minimum Inhibition Concentration (Mic) by Microbroth Dilution Technique.

MIC was carried out according to the National Committee for Clinical Laboratory Standards (NCCLS) method for broth dilution M27-A of antifungal susceptibility testing of yeast. Different concentrations of the plant extracts were prepared from 10 to 0.019 mg/ml by double dilution method. The experiment was performed in 96-well plate. 100 µl of LN media was pipette to all the wells aseptically and 100 µl of plant extract was added and serially diluted and to all the wells, 20µl of (0.5 McFarland) inoculums added and incubated for 48hrs at 32± 2℃ [16].

Further, Minimum Yeast-cidal concentration (MYC) was evaluated by inoculating the contents from 96-well plates on to LN agar medium by streak method and results were observed after incubation at 32±2℃ for 48hrs. The lowest concentration of the test samples which restricted the growth of the yeast was considered as MYC.

Phytochemical Screening of Plants

Phytochemical analysis for the identification of the chemical groups of those plants which showed bioactivity was carried out using standard procedures as described in standard books and research articles [17, 18]. The aqueous and methanol extracts were evaluated for the presence of alkaloids, flavonoids, phenols, tannins, saponins, glycosides and terpenoids.

Statistical Analysis

Data are expressed as mean±SD, with students “t” test and 95% confidence level was used for analysis of the results.

Results and Discussion

The present study was carried out to test the antimicrobial efficacy of nine plants which belong to different families against *M. furfur*. The plants were selected based on their traditional use, and antimicrobial activity.

Table 1: List of the plants selected for antifungal activity

Plant species	Family	Common name	Part used
Sida cardifolia	Malvaceae	Country mallow	Leaves
Senna alexandrina	Fabaceae	Cassia senna	Leaves
Mentha citrata	Lamiaceae	Peppermint	Leaves
Trigonella foenum graecum	Fabaceae	Fenugreek	Seeds
Nelumbo nucifera	Nelumbonzceae	Indian lotus	Leaves
Datura stramonium	solanaceae	Jimson weed, thorn apple	Leaves
Lantana camara	verbenaceae	Kadu jola	Leaves
Pelargonium graveolens	Geraniaceae	Geranium	Leaves
Tagetes erecta	Asteraceae	Mexican marigold	Flower

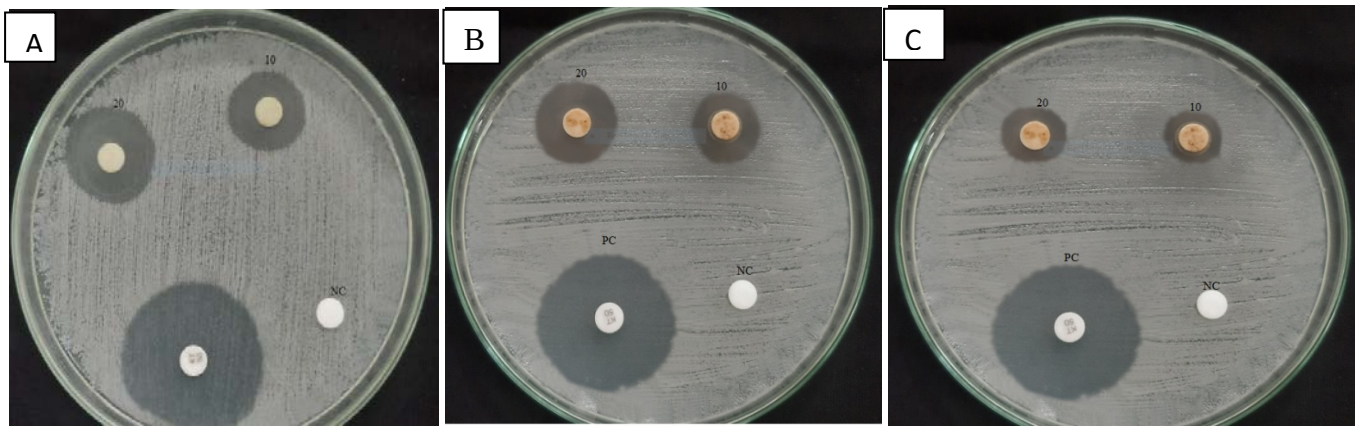

Fig 1: A. *P. graveolens;* B. *M. citrata* C. *S. cardifolia* [1. 1-20mg/ml, 2- 10mg/ml, PC- Positive control; (Ketoconazole) NC- Negative control (5% DMSO)].

Antimicrobial Screening of Plant Extracts by Disc Diffusion Method

The plant extracts were taken at two concentrations 20mg/ml and 10mg/ml. Among the nine plant extracts aqueous extract of *P. graveolens* exhibited the highest zone of inhibition with 19.99±0.42 mm followed by methanol extract of *M. citrata* with zone of inhibition 18.9 ±0.42mm and hexane extract of *S. cardifolia* exhibited a zone of 12.7±0.29 mm diameter which are illustrated in (Fig.1) and the results are tabulated in table.2. The activity is compared with standard drug ketoconazole 50µg/disc. Our results are comparable with the reports where Balakrishnan *et al.*, 2010 reported the antifungal activity of various plant extracts such as *Terminalia bellarica, Embilica officinalis, Terminalia chebula* and *Lantana camara*. Among these, the extract of *Lantana camara* exhibited highest zone of inhibition of 22±0.2mm. Mamatha pingali (2016) reported that extracts of Amla, Fenugreek and reetha showed potent activity and effectively inhibited the growth of *M. furfur*. Arumugan, Akdhailappan 2012 have screened the antifungal inhibitory activity of *N. nucifera* seed extract against the *M. furfur*, *Trichophyton rubrum* and reported the potent activity at the concentration of 25mg/ml. Alirezanaeini 2017 found that *Pelargonium* oil showed effective activity against Malassezia species with the zone of inhibition 50 mm exhibiting higher antifungal activity compared to that of positive control Ketoconazole.

Table 2: Antifungal activity of phytoextracts on *M. furfur*

Sl. No.	Plants	Extraction Solvent	Zone of Inhibition (in mm)	
			20 mg/ml	10mg/ml
1	*Sida cardifolia*	Water	9.8 ± 0.29	NA
		Ethanol	10 ± 0.30	8.2 ± 0.28
		Methanol	8.6±0.4	NA
		n-Hexane	12.7±0.29	9.23±0.29
2.	*Senna alexandrina*	Water	9.8± 1.2	NA
		Ethanol	8.8± 0.9	NA
		Methanol	7.3±2	NA
		n-Hexane	10.4±0.4	NA
3.	*Mentha citrata*	Water	12.0±0.11	9.6± 0.43
		Ethanol	13.2± 0.32	10.2± 0.12
		Methanol	18.9± 0.34	15±0.21
		n-Hexane	12±0.21	10.5±0.14
4.	*Trigonella foenum graceum*	Water	10.2± 0.11	8 ± 0.31
		Ethanol	10 ± 0.21	9 ± 0.42
		Methanol	9.4 ± 0.31	NA
		n-Hexane	NA	NA
5.	*Lantana camara*	Water	NA	NA
		Ethanol	11± 0.21	9± 0.41
		Methanol	9 ± 0.66	NA
		n-Hexane	8.2± 0.11	NA
6.	*Nelumbo nucifera*	Water	11± 0.21	9.2± 0.11
		Ethanol	11.8± 0.42	8.5 ± 0.32
		Methanol	12± 0.4	9.3 ± 0.21
		n-Hexane	NA	NA
7.	*Datura stramonium*	Water	12.1± 0.32	11± 0.43
		Ethanol	9.3 ± 0.12	NA
		Methanol	8 ± 0.32	NA
		n-Hexane	NA	NA
8.	*Pelargonium graveolens*	Water	19.99±0.2	17.2±0.21
		Ethanol	15±0.7	12.4±0.42
		Methanol	13±0.5	10.8±0.3
		n-Hexane	11.6±0.42	8.99±0.12
9.	*Tagetes erecta*	Water	11.2±0.21	7.9±0.2
		Ethanol	NA	NA
		Methanol	8.6±0.34	NA
		n-Hexane	7.5±0.66	NA
	Positive control: Ketoconazole 50µg/disc		29.0±0.2	
	Negative control: (DMSO)		-	

± Standard deviation; NA- no activity

Minimum Inhibitory Concentration of Selected Plant Extracts.

The minimum inhibitory concentration values of plant extracts using microdilution assay showed that the aqueous extract of *P. graveolense* exhibited MIC value of 0.78mg/ml, methanol extract of *M. citrata* exhibited 0.156mg/ml and the hexane extract of *S. cardifolia* exhibited 1.25mg/ml.

Qualitative Phytochemical Analysis

The different qualitative chemical tests were performed for establishing profile of potent plant extracts for their

phytoconstituents responsible for their activity. The methanolic extract of *M. citrata* and showed presence for phenols, terpinoids, flavonoids, glycosides and alkaloids and aqueous extract of *P. graveolense* showed the positive results for phenols, terpinoids, flavonoids, glycosides, saponins and alkaloids whereas hexane extract of *S. cardifolia* showed the result for alkaloids, phenols, tannins, glycosides, flavonoids. Minakshi and Jharnard 2016 reported that the ethanolic extracts of *Mentha citrata* were rich in various phytochemicals such as saponins, tannins, flavonoids, terpenoids and glycosides indicating the influence in the inhibition in the growth of various pathogenic bacteria and fungi. The phytoconstituents present plants were summarized in the table 3.

Table 3: Phytochemical analysis of aqueous extract of *Pelargonium graveolens*, Methanol extract of *Mentha citrata* and Hexane extract of *Sida cardifolia*

Phytochemical constituents	*M. citrata* (Methanol extract)	*P. graveolens* (aqueous extract)	*S. cardifolia* (Hexane extract)
Phenols	+	+	+
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	-	-	+
Saponins	_	+	+
Terpenoids	+	+	_
Glycosides	+	+	+

Conclusion

The results of antimicrobial testing of potent plant extracts of *M. citrata, P. graveolens* and *S. cardifolia* in drug development as a candidate source of antifungal agent against *Malassezia furfur*. More over the current study strongly supports the ethnomedicinal use of plant extracts in treatment of skin disorders caused by *M. furfur* and can be concluded that *Mentha citrata, Pelargonium graveolens* and *Sida cardifolia* could be potential source of antifungal agents. So further studies has to be carried out to identify the active constituents responsible for the activity**.**

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