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Antifungal Study of Essential Oils of *Avicennia marina* and *Suaeda monoica* against some Pathogenic Fungi

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Abstract

Essential oils are one of the most active components which can be found in medicinal plants. These compounds have high antimicrobial activity against various microorganisms since old time. Here, the aimed of this paper evaluate the antidermatotic and biochemical activity of the essential oil of two species of marine plant leaves (*A. marina* and *S. monoica*). Antidermatophytic activity of essential oils of *A. marina* and *S. monoica* leaves were evaluated against selected fugus using well-cut diffusion method. *M. gallina* was the most sensitive for *A. marina* oil while *M. gallinae*, *M. gypseum* and *M. canis* were the most sensitive for *S. monoica* oil. Essential oils were further subjected to the determination the anti-scavenging activity and TPC. Essential oil of *S. monoica* displayed higher activity (81 %), while higher total phenolic content was found with oils of *A. marina* (1.170 mg/gdw). In addition, we found the best percentage of cytotoxic effect with *S. monoica* oil (61,3 %).

These results indicated that essential oils of *A. marina* and *S. monoica* good antidermatophytic activity with high biochemical properties.

Key word: Antidermatophitic , Avicennia marina , Essential Oils and Suaeda monoica

Introduction:

Earth has been a reported as a characteristic area for parasites which cover individual realm with development [1]. Among all growth, dermatophyte is one of the cutaneous parasites. Dermatophytosis can be brought on by them invading keratinized tissue in both animals and humans. Dermatophytosis has a few unmistakable cutaneous signs. Dermatophytosis has a few unmistakable cutaneous signs. The seriousness of the illness relies upon different variables including strain or types of contaminating dermatophyte, the responsiveness of the host and the site of disease [2].

Dermatophyte has a place with three gathering named as Trichophyton, Epidermophyton and Microsporum. Further they are isolated into anthopophilic, zoophilic and geophilic as per their regular territory [3]. Dermatophytosis sore is called annular injury. It takes single or different ring shape injuries with incendiary edges, Tingling, redness, and scaling edges with rankle is likewise notifiable [4]. One more term utilized as fungus contamination as indicated by their physical area like fungus capitis, mouth fungus, fungus corporis, fungus cruris, fungus manuum, athlete's foot and fungus unguium [5]. Both solid and resistant compromised patients are impacted with this contamination [2].

Their geological dispersion is broadly factor. Environment, way of life, inclusion of open air exercises, previous co-morbidities (diabetes mellitus, hypothyroidism, ailing health and so on) are liable for the heterogeneous commonness [6-7]. Pervasiveness of dermatophytosis shifts between 13% to 49% relying upon the geological circulation of the nation's [8]. Even though dermatophytes are not perilous growth, it transforms into significant general medical issue because of high dismalness as well as surface level harm [9]. Dermatophytic diseases have a few regular highlights. It is occasionally confused with other skin conditions [10].

It is notable that plant parts in general or their concentrates in various solvents are applied for different wellbeing infirmities since days of yore. Since normal natural cures of illnesses are the result of many long periods of cautious assessment of their restorative viability and dangers, aftereffects, and properties of these natural based medicines are well known. Further, the current shoppers' advantage is for such normal food varieties that ought to be powerful antimicrobial specialists with no aftereffects [11].

Marine plants are a gathering of trees and bushes that live in the beach front intertidal zone. Marine trees can develop under unfavorable natural circumstances like low-oxygen soil, high temperature, high saltiness, and so forth. The dispersion of marine woods is for the most part at the tropical and subtropical scopes [12]. *Avicennia marina*, the Red Sea's most common species, is thought to be one of the best sources of bioactive chemicals [13]. It is ordered in the family Acanthaceae and the phytochemical constituents of its leaves are found to have a place with terpenoids, unsaturated fats and their esters [14]. *S. monoica* is a very much concentrated on waterfront halophyte that fills in marine conditions [15]. It was accounted for to have different actives like antiviral, cell reinforcement, wound recuperating [16], phytoremediation [17], and antimicrobial exercises [18]. A past report on *S. monoica* in Saudi Arabia announced that this plant is utilized to treat different illnesses like stiffness, loss of motion, asthma, and snakebites [19].

MATERIALS AND METHODS

Plant material

The new leaves of (*Avicennia marina* and *Suaeda monoica)* plants were gathered by hand from marine coast in Yanbu area. The leaves cleaned from epiphytes, sand and rock by utilizing new water after brought to research center in plastic sacks, then, at that point, they were air dried in the shade at room temperature. Air dried leaves were grounded in an

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electric plant and put away in stoppered bottles at room temperature. The medicinal plants chosen to test their antidermatopytic properties came from the Yanbu region's marine coast. The area of study is situated between Scope (24o 2.742 N), Longitude (38o 6.840 E) and it is portrayed by a tropical to subtropical environment.

Fungal isolates

Tried dermatophytes species incorporated the accompanying: *M. gallinae, M. gypseum, T. verrucosum T. mentagrophytes, M. canis,* and *E. floccosum* and the yeasts *C. albicans* and *C. tropicalis* were acquired from Ruler Fahed Emergency clinic in Jeddah. They cause diseases in human.

Extraction of essential oil

Around (500 g) of dried leaves were utilizing for steam refining for four hours. A rotatory evaporator was used to remove the solvent from about 45-50 mL of the distillates after they were collected, extracted three times with chloroform (3100 mL), and dried over anhydrous sodium sulfate (Merck, Germany). The yields were (0.60-0.65%) put away in a cooler (+ 6°C) [20].

Antidermatophyitc assay

The well-cut diffusion method

Antidermatophytic activity was performed by well-cut diffusion technique. Wells were cutted from the plate using a sterile (10 mm) cork borer. Different concentrations of oil (0.25 – 0.5 ml) was placed in each well in inoculated plates, then were incubated at (4 ˚C) for (2 h) to slow fungal growth and gives suitable time for the antifungal agent to diffuse. The plates were later incubated at (28 ˚C) for (10 days) for dermatophytes and (48 h) for yeasts [21]. The inhibition zones measured by using (Scan 500) equipment as (mm).

Biochemical assay

A- Determination of total antioxidant activity

The hydrogen atom or electron donation ability of the corresponding oil was measured using the method of Burits and Bucar [22]. Inhibition free radical DPPH in percent (I %) was calculated as in Eq (1):

$I\,\% = (A \text{ blank} - A \text{ sample} / A \text{ blank}) \times 100$ (1)

where *A* blank is the absorbance of the blank (containing all reagents except the tested compound) and *A* sample is the absorbance of the test sample.

B- Determination of total phenolic contents (TPC)

The total phenolic content in the oil was determined by using Folin–Ciocalteu reagent [23]. The concentration of total phenolic compounds in oil was expressed as (mg) of gallic acid equivalents per gram dry weights of samples, using the linear Eq (3) derived from Eq (2), which was determined from known concentration of gallic acid standard similarity. Absorbance (at 765 nm) constant × gallic acid concentration (2)

Gallic acid equivalents =(Absorbance at 765 nm)/0.0508 (3)

Cytotoxic effect on human normal epithelial cell line (BJ1)

Cell viability was assessed on normal skin fibroblast by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [24] under similar circumstances [25-26]. The percentage of change in viability was calculated according to the formula: ((Reading of extract / Reading of negative control) -1) x 100

Statistical analysis

Result are presented as the mean of three or four replicates ± standard error (SE). The statistical analyses were carried out using SPSS program (version 22). Data obtained were analyzed statistically to determine the degree of significance using one way (ANOVA) at probability level $p \leq 0.05$ levels of significant.

RESULTS and DISCUSSION

The well- cut diffusion method

M. gallinae, *E. flocossum* and *M. gypesum* were the most sensitive to essential oil of *A. marina* (0.5 ml) and they exhibited inhibition zone (61.5, 59.6 and 55.9 mm) respectively. The moderate level of inhibition zones observed against *T. vercosum*, *T. mentagrophytes* and *M. canis* and they exhibited inhibition zone (45.3, 44.4 and 43.9 mm) respectively.

Table (1): Effect of different concentrations of *A. marina* and *S. monoica* oils on the tested pathogenic fungi and yeasts

Types of oils		*Aviccenia marina*				*Suaeda monoica*			
Concentrations of oil		Control	0.25	0.5	P-value	Control	0.25	0.5	P-value
Fungi	*E. flocossum*	10.0 ± 0.0	42.6 ± 1.9	59.6 ± 0.7	0.000	10.0 ± 0.0	34.4 ± 0.7	38.3 ± 2.0	0.001
	M. canis	10.0 ± 0.0	29.2 ± 2.9	43.9 ± 1.3	0.002	10.0 ± 0.0	39.4 ± 0.9	80.0 ± 0.0	0.000
	M. gallina	10.0 ± 0.0	39.2 ± 0.7	61.5 ± 2.6	0.000	10.0 ± 0.0	55.7 ± 7.2	80.0 ± 0.0	0.003
	M. gypesum	10.0 ± 0.0	42.6 ± 2.2	55.9 ± 1.1	0.000	10.0 ± 0.0	46.8 ± 10.5	80.0 ± 0.0	0.009
	T. vercosum	10.0 ± 0.0	26.7 ± 1.1	45.3 ± 5.3	0.010	10.0 ± 0.0	32.6 ± 0.4	62.0 ± 4.2	0.002
	T. mentagrophytes	10.0 ± 0.0	42.4 ± 1.3	44.4 ± 2.3	0.001	10.0 ± 0.0	33.7 ± 13.3	59.6 ± 6.6	0.039
	C. albicans	10.0 ± 0.0	16.7 ± 1.5	23.5 ± 0.5	0.004	10.0 ± 0.0	17.0 ± 0.3	22.4 ± 0.5	0.000
	C. tropicals	10.0 ± 0.0	14.6 ± 0.5	22.6 ± 0.7	0.001	10.0 ± 0.0	16.9 ± 1.3	26.8 ± 4.2	0.042

Each value is the mean of 3 replicates ± SE

* =There is a significant effect of concentrations on tested pathogenic fungi by using One Way ANOVA at<0.05

C. albicans and *C. tropicals* were the lowest sensitive to essential oil of *A. marina* and they exhibited inhibition zone (23.5 and 22.6 mm) respectively. Essential oil of *A. marina* was more active against *M. gallina*, which inhibited by (61.5 mm). The susceptibility of other fungi to the extract was decreased respectively according to the recorded inhibition zone ranged from (59.6 - 23.5 mm). Whereas, *C. tropicalis*

was the least susceptible to the extract as shown by (22.6 mm).

This results agreement with Machado [27] who discovered the growth of *C. albicans*, *S. aureus*, *P. aeruginosa*, and *E. coli* inhibited by using essential oil from the leaves of *A. schaueriana*. *P. aeruginosa* was the most sensitive organism for both oils, presenting the highest growth inhibition. Whereas, essential oil of *S. monoica* (0.5 ml) showed the highest inhibition activity against *M.* canis M. gallina and M. gypesum were observed that effect by maximum inhibition zone (80.0.mm). Followed by T. vercosum and T. mentagrophytes exhibited inhibition zone (62.0 and 59.6 mm) respectively. C. tropicals and C. albicans showed the smallest halo (26.8 and 22.4 mm) respectively.

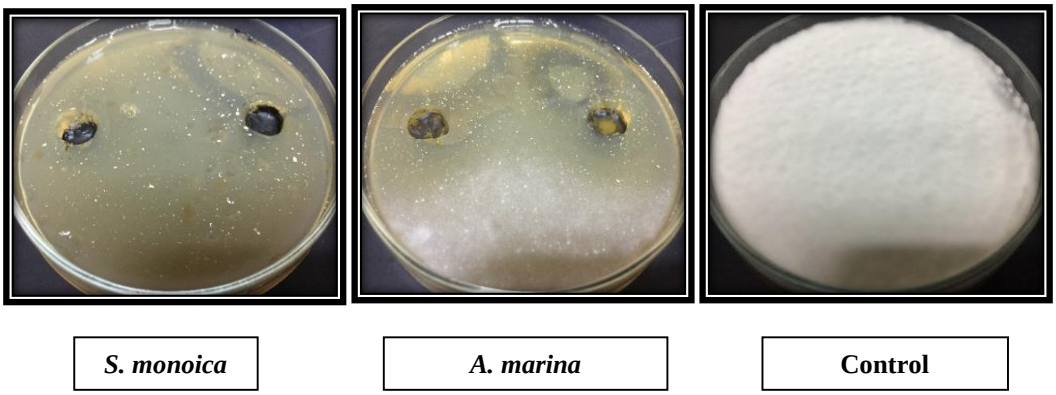

Fig (1): Effect of 0.5 ml of *A. marina* and *S. monoica* oils on *M. gallin*

Effect of mangrove extract on microorganisms was reported in the previous references such as Das [28] who revealed that ethanol leaves and barks extracts of A. officinalis were able to inhibit the growth of bacterial and fungal strains to different extent (B.subtilis, E. coli, P. aeruginosa, S. aureus, C. albicans and C. krusei).

Also, Okla [11] showed that ethanolic roots extract of A. marina have significant antibacterial effect on the growth of (P. aeruginosa, B. subtilis, S. aureus and E. coli), whereas ethyl acetate A. marina leaf extract able to inhibit the growth of (S. aureus and E. coli), while ethanolic extract of the root, fruits and seeds of A. marina exhibit antifungal effect against A. fumigatus by using agar diffusion method. This result accordance with Belmimoun [29] showed a varying degree of antimicrobial activity of essential oils of *M. communis* and *Z. album* against bacteria (*S. aureus, clostridium sp., E. fecalis, S. typhi, E. coli, shigella sp.*, and *B. subtilis)*. *Suaeda monoica* as been using make an antiseptic ointment for skin eruptions [30]. Further, Dinesh [31] found that Methanol and Petroleum ether extracts of *S. maritima* and *S. monoica* showed antibacterial activity against clinical pathogens bacteria (*B. subtilis, B. cereus, S. aureus, E. coli and P. aeureuginosa*) also they inhibited the growth of fungal strains (*A. flavus*, *Mucor* sp. and *C. albicans*). As well, Paper disc method and well diffusion method showed that *F. oxysporum*, *M. phaseolina* and *R. solani* were inhibited by leaves and stem extract of *S. imbricata* at 100 % w/v [32].

Our result same with Inouye [33] screening assay of 72 EO against *T. mentagrophytes*. The most active oils were *Origanum vulgare, Thymus serpyllum, Eugenia caryophyllata, Cymbopogon nardus, Pelargonium roseum, Lindera umbellata, Aniba rosaeodora, Thymus vulgaris, Lavandula latifolia, L. angustifolia* and *Melaleuca alternifolia*.

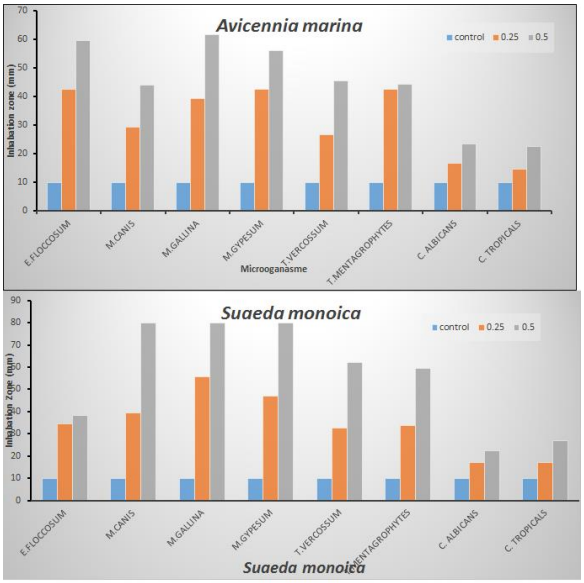

Fig (2): Effect of different concentrations of *A. marina* and *S. monoica* oils on the tested pathogenic fungi

On the other hand, essential oil of *S. monoica* was more effective against *M. canis*, *M. gallina* and *M. gypesum*were were observed that effect by maximum inhibition zone (80 mm) followed by *T. vercosum*, *T. mentagrophytes*, *E. flocossum* and *C.tropical* respectively. The less effective of oil was against *C. albicans* as it recorded minimum inhibition zone (22.4 mm).

Cytotoxic effect on human normal epithelial cell line (BJ1)

Essential oil of *S.* was tested against the normal *monoica* human epithelial cell line (normal skin fibroblast) (BJ1) *in vitro* to evaluate its cytotoxic activity by using MTT assay.

The cytotoxicity activity of oil on (BJ1) normal skin fibroblast cell line with the best percentage (61.3 %).

Total antioxidant activity and total phenolic contents

Essential oil of *S. monoica* displayed higher activity (81 %), while higher total phenolic content was found with oils of *A. marina* (1.170 mg/gdw). Phenolics are not the only components in the extracts that could possess antioxidant activity also by other components such as Gallic acid, Catechin, Coffeic acid, Syringic acid, Rutin, Coumaric Acid, Vanillin, Querectin and Cinnamic acid that purified from plants extracts by HPLC method with other unknown substances in fungal filtrates [36].

Table (2): Total antioxidant activity and total phenolic contents of *A. marina* and *S. monoica* oils

Oils	Inhibition (%)	TPC (mg/gdw)
S. monoica	81 %	0.176
A. marina	67.1 %	1.170

Mangroves are related with marine living space and subsequently a portion of the variation exercises are found in this plant, which incite them to orchestrate various particles for protecting themselves from various burdens established by the climate. These particles are subsequently detailed as the most encouraging atoms for medical advantage. Alkaloids, saponins, phenols, and tannins, all of which have distinct antimicrobial properties, are the chemical constituents that they produce as they grow to overcome the adverse environmental stress conditions. The traditional knowledge, now these mangrove plants are of great interest in the scientific world for the development of potential bioactive compounds and herbal drugs [37].

Conclusions

The results of this study showed that essential oils of *A. marina* and *S. monoica* as marine plant have high biological activity against different species of dermatophytes and yeasts with more biochemical activity. That suggested, these plants need more studies for the ability of using these oils for treatment skin fungal diseases.

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