

## *In Vitro* Antidermatophitic and Biochemical Studies for Solvent Extracts of Marine Plants

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### ABSTRACT

Human fungal diseases demonstrate a dangerous medical problem. For thousands of years, plant metabolites have performed a highly significant part in health preservation and protection from diseases. The current study detects the potentiality of solvent extracts of A. marina and S. monoicaas an antifungal agent. Solvent extracts of A. marina and S. monoica were prepared in (ethanol, methanol and acetone). Antidermatophytic activity of them was evaluated against T. mentagrophytes, T. verrucosum M. gallinae, M. gypseum, M. canis, E. floccosum, C. albicans and C. tropicalis using dry weight method. The results show that M. gypseum was the most sensitive for ethanol and methanol extracts of S. monoica while M. gallinae was the most sensitive for acetone extract of A. marina. The acetone extracts of A. marina and S. monoica were moreover undergo to the determination of the minimal inhibitory concentrations using different concentrations which the MIC value of different extracts was found to be different but in the range of (0.075- 0.5 mg/ml). The anti-oxidant activity and total phenolic content for all solvent extracts and defined, acetone extract of S. monoicahave the highest anti-oxidant activity (77 %) whereas methanol extract of A. marina have the highest amount of phenolic content (47.04 mg/gdw). In addition, some bioactive compounds from solvent extracts separated and estimated by using high performance liquid chromatography.

Key Words: A.Marina,S. Monoica,Dermatophytes. Yeast, Anti-oxidant, TPC, HPLC, MIC, Dry weight.

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### **INTRODUCTION**

Cutaneous mycoses are among the most widely recognized parasitic contaminations and are for the most part brought about by filamentous keratinophilic growths called dermatophytes that utilization keratin as a supplement during skin, hair and nail disease [1]. They additionally debase paws, quills, hooves, horns, and fleeces in creatures [2]. The disease is empowered by warm, moist conditions and pitiable cleanliness conditions everywhere throughout the tropical and mild areas of the earth [3].

Be that as it may, Pityriasis Versicolor, Saccharomyces cerevisiae and Candida sp. pioneering pathogenic growths are fit for causing mycotic contaminations in humans [4]. As of late, there has been a reestablished enthusiasm for regular item examination because of the disappointment of elective medication revelation techniques to convey many

lead mixes in key helpful regions, for example, immunosuppression, hostile to infectives and metabolic ailments. Characteristic items examine keeps on investigating an assortment of lead structures, which might be utilized as layouts for the improvement of new medications by the pharmaceutical business. There is no uncertainty that characteristic items have been and will be significant wellsprings of new pharmaceutical mixes [5]. Although the concoction segments of most mangrove plants still have not been contemplated widely, examinations have driven so far to the revelation of a few novel mixes with an imminent restorative incentive for the disclosure of new chemotherapeutic operators. The mangrove natural surroundings get nourishment and a wide assortment of conventional items and antiques from mangroves [6]. Also, these plants are a rich wellspring of steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and sugars [7].

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Concentrates from various mangrove plants are accounted for to have assorted restorative properties, for example, antibacterial and antihelminthics [6]. Likewise, utilized in society drug against fever, asthma, angina, dying, looseness of the bowels, diarrhea, and tuberculosis [8] just recuperating, as astringent, tonic, hemostatic, antimicrobial, antitumor and antiulcerogenic properties [9]. Leaf and natural products remove in relieving skin infections, toothache, disease, premature births and has antifungal properties [6]. Mangrove removes demonstrated antimicrobial action against certain microorganisms, including Shigella sp., Staphylococcus sp. what's more, Pseudomonas sp. [10].

A. marina is regularly known as dark or white mangrove, are types of mangrove tree ordered in the plant family Acanthaceae [11]. Notwithstanding essential metabolites including higher polysaccharides speaking to up to (50 %) plant weight, for example, cellulose, a significant item for paper, polymer, nourishmentand biofuel industry [12]. These are found in bark, leaves, roots, stems, and seeds [6]. They are normally utilized for the treatment of ulcers [13], ailment, little pox and different afflictions [6].

S. monoica is an Annual herb adjusted to saline soil and lives in salt bogs or parched saline soil. Amaranthaceae family incorporates around (1300) species overall range from yearly herbs to trees [14]. The leaf of S. monoica is referred to use as a drug for hepatitis and experimentally it is accounted for to be utilized as a salve for wounds and have antiviral action [15], antidiabetic and toothache [16]. The current study was conducted for evaluation of the antidermatophytic activity of (ethanol, methanoland acetone) extracts of some marine medicinal plants against some dermatophytes and yeasts.

#### **MATERIALS AND METHODS**

#### **Marine plants**

The fresh leaves of plants (*A. marina*. *S. monoica*) were collected by hand from the marine coast in the Yanbu region. The area of study is located between Latitude (24° 2.742 N), Longitude (38° 6.840 E) and it is characterized by a tropical to subtropical climate. The leaves were prepared according to [17].

#### **Fungal Isolates**

Tested dermatophytes species included the following: *T. mentagrophytes, T. verrucosum M. gallinae, M. gypseum, M. canis,* and *E. floccosum* and the yeasts *C. albicans* and *C. tropicalis* were obtained from King Fahed Hospital in Jeddah. They cause infections in humans.

#### **Extract Preparation**

The extraction was carried out according to [17]. The extracts were filtered and stored at refrigerator

temperature (4 °C) in an airtight bottle.

#### **Antidermatophyitc Assay**

#### Dry weight of Dermatophytes and Yeasts

To determine the effect of plant extracts on the fungal biomass, various concentrations of it were added to sterilized Sabouraud dextrose broth(SDB) and completed to (100 ml) in sterile conical flasks (250 ml) capacity to get the required concentrations. Notwithstanding the control test, the conelike flagons were immunized by circle (10 mm) of the terminal development of settlements of (10 days) old, brooded at (28 °C) for (seven days) for M. gallinae, M. gypseum, and M. canis, (2 weeks) for T. mentagrophytes and T. verrucosum and (3 weeks) for E. floccosum. Toward the finish of the hatching time frame, dermatophytes were filtrated by utilizing realized weight channel papers, dried medium-term in an electric stove at (80 °C), at that point, steady weight was gotten. In the yeast, the cone-shaped carafes were vaccinated by (1 ml) C. albicans or C. tropicalis suspension. After brooding at (28 °C) for (48 h), (1 ml) of the yeast development was moved to rotator containers of known loads, centrifuged at (3500 rpm) (Ilettich-MIKRO 22 R) for (15 min). The supernatant was disposed of and the pellet was stove dried medium-term at (80 °C), at that point consistent weight was acquired. The dry weight was resolved as (mg) [18, 19].

## **Determination of Minimal Inhibitory Concentrations** (MICs)

Serial dilutions of the most potent plant extract (100, 50, 40, 30, 20, 10, 5, 3, 2, 1, 0.5, 0.3, 0.2 and 0.1 mg/ml) were added to sterilized plates containing freshly (SDA) prepared with standard number of cells for tested fungi to determine the minimal inhibitory concentration [20].

#### **Biochemical Assay**

## Determination of Total Antioxidant Activity by Using DPPH Free Radical and Scavenging Activity

The hydrogen atom or electron donation ability of the corresponding extracts was measured from the bleaching of a purple-colored methanol solution of Diphenyl picrylhydrazyl (DPPH) [21].

#### **Determination of Total Phenolic contents (TPC)**

The total phenolic content in the extracts was determined by using Folin–Ciocalteu reagent [22]. The concentration of total phenolic compounds in all extracts, filtrates and new products was expressed as (mg) of gallic acid equivalents per gram dry weights of samples.

#### High-performance Liquid Chromatography (HPLC)

HPLC analysis was carried out using an Agilent (1260) series. The separation was carried out using (C18) column

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(4.6 mm x 250 mm i.d., 5  $\mu$ m). The mobile phase consisted of (2 %) acetic acid (A) and acetonitrile (B) at a flow rate (0.8 ml/min). The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (85 % A); 0–15 min (50 % A); 15-17 min (20 % A); 17-19 min (85 % A) and 19-25 min (85 % A) [23].

#### Statistical analysis

The result is displayed as the mean of three or four repeats  $\pm$  standard blunder (SE). The factual examinations were done utilizing the SPSS program (variant 22). Information acquired was dissected measurably to decide the level of importance utilizing one way (ANOVA) at likelihood level P  $\leq$  0.05 degrees of significance.

#### RESULTS

# Effect of the solvent marine plant extracts on the dry weight of the dermatophytes and yeasts

## **Ethanol extracts**

*T. verrucosum, M. gypseum*, and *M. gallinae* were the most sensitive to extract of *A. marina* (6 ml) and they are inhibited by (94.5, 94.4 and 93.1 %) respectively. The moderate level of inhibition percentage was observed against *C.albicans* (63.5 %). The same concentration of *S. Monoica* extractshowed the highest inhibition activity against *M. Gypseum*(97.2%) whereas the inhibition percentage of *T. mentagrophytes, E. floccsum*, and *C. albicans* were (96.2, 95.7 and 70.5 %) respectively compared with control untreated.

#### **Methanol extracts**

At (6 ml) of the extractof *A. marina* showed the most significant effect on *M. gypseum*, *E. floccsum* and *M. gallinae*, which they inhibited by (97.0, 96.1 and 95.3 %) respectively, while *C. albicans* was inhibited by (68.7 %). On the other hand, the data showed the extract of *S. monoica* (6 ml) was the most effective in reducing the weight of *M. gypseum*, *M. gallinae* and *M. canis* with inhibition percentages (98.0, 96.5 and 90.9 %) respectively then, *C. albicans* (64.1%) after incubation time.

#### **Acetone extracts**

The highest level of inhibition percentage of *A. marina* extract (6 ml)observed against *M. gallinae* (95.7 %) followed by *M. canis* and *M. gypseum* (94.0 and 88.1 %) respectively, in the finally *C. albicans* (73.6 %). While the same solvent extract of *S. Monoica*(6 ml) recorded the strongest inhibition against *M. canis* and *M. gypseum* with percentage (93.0 and 90.1 %) respectively, followed by *T. verrucosum* and *C. albicans* (85.1 and 75.0 %) respectively contract with the untreated sample.

## Determination of minimal inhibitory concentrations (MICs) the most potent solvent extracts of marine plant

Acetone extract of marine plants were the most effective solvent extracts against tested fungi and yeast. Whereas, MIC values of extract were in the range (0.5 - 0.075 mg/ml). *A. marina* extract inhibit *T.verrucosum* by (0.5 mg/ml) then, *M. gallinae* inhibited by (0.3 mg/ml) followed by *M. gypseum* and *E. floccosum* inhibited by (0.2 mg/ml) whereas *M. canis, T. mentagrophytes* and *C.tropicalis* inhibited by (0.05 mg/ml), in the finally *C. albicans* inhibited by (0.075 mg/ml) then *M. gallinae* and *T. mentagrophytes* inhibited by (0.1 mg/ml) followed by *M. gypseum* hibited by (0.2 mg/ml), in the finally *C. albicans* inhibited by (0.1 mg/ml) then *M. gallinae* and *T. mentagrophytes* inhibited by (0.1 mg/ml) whereas *M. canis, E. floccosum* and *C. albicans* inhibited by (0.075 mg/ml), in the finally *C. albicans* inhibited by (0.1 mg/ml) whereas *M. canis, E. floccosum* and *C. albicans* inhibited by (0.075 mg/ml).

## Determination of total antioxidant by using DPPH free radical scavenging activity

Acetone extract of *S. monoica* displayed the highest activity (77 %). In the last, methanol extract of *S. monoica* (49.5 %).

#### **Determination of total phenolic contents (TPC)**

The lowest total phenolic content was found with ethanol extract of *A. marina* (18.04 mg/gdw). The methanol extract of *A. marina* recorded the highest value of the total phenolic content (47.04 mg/gdw).

## High-performance liquid chromatography (HPLC) of plant extracts and fungal filtrates

*S. monoica* (Ethanol) extract has a high content of Gallic Acid (10.5 mg/100gdw), Whereas, *S. monoica* (Methanol) extract had a high content of Catechin and Syringic Acid (23.1 and 2.3 mg/100gdw) respectively. Also, *S. monoica* (Acetone) has high content of Vanillin (9 mg/100gdw).

*A. marina* (Methanol) extract had a high content of Caffeic Acid, Rutin and Coumaric Acid (4, 85.7 and 13.9 mg/100gdw) respectively. Whereas, *A. marina* (Acetone) has high content of Quercetin and Cinnamic Acid (234.6 and 5 mg/100gdw).

### DISCUSSION

Countless plants in the various area around the globe have been removed, semi-purged to explore exclusively their antimicrobial movement. Restorative plants are endowments of nature to fix various sicknesses among individuals. Their concentrates have picked up significance as potential antibacterial operators. Optional metabolites of plants, including the tannins, flavonoidsand alkaloids have been found to have antimicrobial properties in vitro. Nonetheless, next to no data is accessible on such action of restorative plants and out of the (400,000) plant species on earth, just a limited quantity has been

deliberately researched for their antimicrobial exercises [24, 25].

| IS                        |            | Dermatophytes |      |           |            |          |      |                      |           |               |          |             |           |  |
|---------------------------|------------|---------------|------|-----------|------------|----------|------|----------------------|-----------|---------------|----------|-------------|-----------|--|
| utration                  | Plant      | M. gallinae   |      | M. gypse  | M. gypseum |          | vis  | T.<br>mentagrophytes |           | T. verrucosum |          | E. floccsum |           |  |
| Concel                    | extracts   | А             | В    | А         | В          | А        | В    | А                    | В         | А             | В        | А           | В         |  |
| 0.0                       | A. marina  | 870±1.        | 22   | 1085±0    | .70        | 900±0.   | 81   | 1075±0               | 1075±0.70 |               | 920±0.40 |             | 1185±1.08 |  |
|                           | S. monoica |               |      |           |            |          |      |                      |           |               |          |             |           |  |
| 0.5                       | A. marina  | 135±0.70      | 84.4 | 145±0.70  | 86.6       | 370±1.08 | 58.8 | 400±0.70             | 62.7      | 340±1.22      | 63.0     | 570±0.40    | 51.8      |  |
|                           | S. monoica | 500±0.40      | 42.5 | 1020±1.22 | 5.9        | 660±0.70 | 26.6 | 830±1.08             | 22.7      | 650±0.70      | 29.3     | 485±0.40    | 59.0      |  |
| 1.0                       | A. marina  | 105±0.70      | 87.9 | 120±1.22  | 88.9       | 220±0.70 | 75.5 | 280±0.70             | 73.9      | 320±1.22      | 65.2     | 540±0.70    | 54.4      |  |
|                           | S. monoica | 470±0.70      | 45.9 | 980±1.22  | 9.6        | 500±0.70 | 44.4 | 340±0.40             | 68.3      | 270±1.08      | 70.6     | 460±0.40    | 61.1      |  |
| 2.0                       | A. marina  | 80±1.22       | 90.8 | 90±1.22   | 91.7       | 95±0.70  | 89.4 | 130±0.40             | 87.9      | 60±0.70       | 93.4     | 515±0.81    | 56.5      |  |
|                           | S. monoica | 415±0.81      | 52.2 | 800±0.70  | 26.2       | 145±0.40 | 83.8 | 70±0.70              | 93.4      | 240±1.08      | 73.9     | 180±1.22    | 84.8      |  |
| 4.0                       | A. marina  | 60±0.81       | 93.1 | 75±0.81   | 93.0       | 80±0.81  | 91.1 | 125±1.22             | 88.3      | 55±1.08       | 94.0     | 495±1.08    | 58.2      |  |
|                           | S. monoica | 400±1.08      | 54.0 | 260±1.08  | 76.0       | 130±1.22 | 85.5 | 60±0.81              | 94.4      | 220±0.81      | 76.0     | 70±0.81     | 94.0      |  |
| 6.0                       | A. marina  | 60±0.70       | 93.1 | 60±0.70   | 94.4       | 70±0.81  | 92.2 | 120±0.00             | 88.8      | 50±0.81       | 94.5     | 480±1.22    | 59.4      |  |
|                           | S. monoica | 180±1.22      | 79.3 | 30±0.81   | 97.2       | 110±0.81 | 87.7 | 40±0.81              | 96.2      | 60±0.40       | 93.4     | 50±0.70     | 95.7      |  |
| P-value ( A. marina<br>)  |            | 0.0001        | *    | 0.0006*   |            | 0.0008*  |      | 0.0005*              |           | 0.0007*       |          | 0.0004*     |           |  |
| P-value ( S.<br>monoica ) |            | 0.0003        | *    | 0.0002*   |            | 0.0001*  |      | 0.0008*              |           | 0.0005*       |          | 0.0006*     |           |  |

| Table 1: Effect of different concentrations of ethanol extracts of A. marina and S. monoica (ml) on dry weight |
|--|
| (mg) and inhibition percentage (%) of the dermatophytes  |

 Table 2: Effect of different concentrations of methanol extracts of A. marina and S. monoica (ml) on dry weight (mg) and inhibition percentage (%) of the dermatophytes

| io                      |                    | Dermatophytes |            |            |      |          |         |             |         |               |         |             |         |  |
|-------------------------|--------------------|---------------|------------|------------|------|----------|---------|-------------|---------|---------------|---------|-------------|---------|--|
| Concentrat<br>ns        | Plant              | M. galli      | nae        | M. gypseum |      | M. can   | eis     | T. mentagro | phytes  | T. verrucosum |         | E. floccsum |         |  |
|                         | extracts           | А             | В          | А          | В    | А        | В       | А           | В       | А             | В       | А           | В       |  |
| 0.0                     | A. marina          | 860±0.        | 70         | 1013±0     | .81  | 773±1.22 |         | 1025±1.     | .08     | 920±0.40      |         | 1035±0.70   |         |  |
|                         | S. monoica         |               |            |            |      |          |         |             |         |               |         |             |         |  |
| 0.5                     | A. marina          | 110±0.70      | 87.2       | 405±0.40   | 60.0 | 280±0.81 | 63.7    | 160±0.81    | 84.3    | 195±0.81      | 78.8    | 800±1.22    | 22.7    |  |
|                         | S. monoica         | 160±1.22      | 81.3       | 730±0.81   | 27.9 | 750±0.81 | 2.9     | 740±0.81    | 27.3    | 795±0.40      | 13.5    | 540±0.70    | 47.8    |  |
| 1.0                     | A. marina          | 80±0.81       | 90.6       | 380±0.81   | 62.4 | 180±0.81 | 76.7    | 130±1.22    | 87.3    | 170±1.08      | 81.5    | 280±1.08    | 72.9    |  |
|                         | S. monoica         | 140±1.08      | 83.7       | 600±1.08   | 40.7 | 710±1.22 | 8.1     | 710±0.81    | 30.7    | 770±0.81      | 16.3    | 510±0.81    | 50.7    |  |
| 2.0                     | A. marina          | 50±1.22       | 94.1       | 350±1.08   | 65.4 | 75±0.70  | 90.2    | 115±0.40    | 88.7    | 90±0.70       | 90.2    | 175±0.81    | 83.0    |  |
|                         | S. monoica         | 110±0.81      | 87.2       | 380±0.70   | 62.4 | 200±0.40 | 74.1    | 630±0.70    | 38.5    | 660±1.08      | 28.2    | 485±1.22    | 53.1    |  |
| 4.0                     | A. marina          | 45±0.40       | 94.7       | 30±1.08    | 97.0 | 60±0.40  | 92.2    | 100±0.70    | 90.2    | 70±1.22       | 92.3    | 160±0.70    | 84.5    |  |
|                         | S. monoica         | 90±0.70       | 89.5       | 170±1.22   | 83.2 | 120±0.70 | 84.4    | 220±0.40    | 78.5    | 640±1.08      | 30.4    | 465±0.40    | 55.0    |  |
| 6.0                     | A. marina          | 40±0.00       | 95.3       | 30±0.70    | 97.0 | 40±1.08  | 94.8    | 100±0.70    | 90.2    | 60±1.22       | 93.4    | 40±0.00     | 96.1    |  |
|                         | S. monoica         | 30±0.70       | 96.5       | 20±1.22    | 98.0 | 70±0.70  | 90.9    | 170±1.08    | 83.4    | 150±0.70      | 83.6    | 450±0.40    | 56.5    |  |
| ( <u>A</u> .            | P-value<br>marina) | 0.0009*       |            | 0.0007*    |      | 0.0003*  |         | 0.0001*     |         | 0.0002*       |         | 0.0006*     |         |  |
| P-value<br>(S. monoica) |                    | 0.0006        | <u>5</u> * | 0.0001*    |      | 0.0005   | 0.0005* |             | 0.0004* |               | 0.0006* |             | 0.0002* |  |

| s                       | _                          |          | Dermatophytes |           |      |           |         |                 |         |            |         |           |         |  |
|-------------------------|----------------------------|----------|---------------|-----------|------|-----------|---------|-----------------|---------|------------|---------|-----------|---------|--|
| Concentration           | Plant ey                   | M. galli | M. gallinae   |           | eum  | M. car    | nis     | T.<br>mentagrop | ohytes  | T. verruce | osum    | E. flocc  | sum     |  |
|                         | tracts                     | А        | В             | А         | В    | А         | В       | А               | В       | А          | В       | А         | В       |  |
| 0.0                     | A. marina<br>S.<br>monoica | 940±0.40 |               | 1015±1.08 |      | 1010±0.81 |         | 1090±1.08       |         | 945±1.22   |         | 1310±0.81 |         |  |
| 0.5                     | A. marina                  | 220±0.81 | 76.5          | 370±0.81  | 63.5 | 305±0.81  | 69.8    | 760±1.22        | 30.2    | 475±1.08   | 49.7    | 595±1.08  | 54.5    |  |
|                         | S.<br>monoica              | 295±1.08 | 68.6          | 330±1.08  | 67.4 | 550±1.22  | 45.5    | 280±0.81        | 74.3    | 400±0.81   | 57.6    | 290±0.81  | 77.8    |  |
| 1.0                     | A. marina                  | 190±0.70 | 79.7          | 340±0.40  | 66.5 | 290±0.81  | 71.2    | 760±0.81        | 30.2    | 450±0.81   | 52.3    | 570±1.22  | 56.4    |  |
|                         | S.<br>monoica              | 270±1.22 | 71.2          | 190±0.81  | 81.2 | 520±0.81  | 48.5    | 255±0.81        | 76.6    | 315±0.40   | 66.6    | 260±0.70  | 80.1    |  |
|                         | A. marina                  | 165±0.40 | 82.4          | 335±1.08  | 66.9 | 260±0.40  | 74.2    | 735±0.70        | 32.5    | 420±1.22   | 55.5    | 540±0.70  | 58.7    |  |
| 2.0                     | S.<br>monoica              | 240±0.70 | 74.4          | 150±1.22  | 85.2 | 290±0.70  | 71.2    | 225±0.40        | 79.3    | 290±1.08   | 69.3    | 235±0.40  | 82.0    |  |
| 4.0                     | A. marina                  | 150±1.22 | 84.0          | 320±1.08  | 68.4 | 240±0.70  | 76.2    | 720±0.40        | 33.9    | 400±0.70   | 57.6    | 410±0.81  | 68.7    |  |
|                         | S.<br>monoica              | 220±0.81 | 76.5          | 125±0.70  | 87.6 | 160±0.40  | 84.1    | 210±0.70        | 80.7    | 190±1.08   | 79.8    | 215±1.22  | 83.5    |  |
| 6.0                     | A. marina                  | 40±0.40  | 95.7          | 120±0.70  | 88.1 | 60±1.08   | 94.0    | 250±0.70        | 77.0    | 130±1.22   | 86.2    | 280±0.40  | 78.6    |  |
|                         | S.<br>monoica              | 150±0.40 | 84.0          | 100±1.22  | 90.1 | 70±0.70   | 93.0    | 190±1.08        | 82.5    | 140±0.70   | 85.1    | 200±0.40  | 84.7    |  |
| (A.                     | P-value<br>marina)         | 0.0001   | *             | 0.0008*   |      | 0.0005*   |         | 0.0003*         |         | 0.0002*    |         | 0.0006*   |         |  |
| P-value<br>(S. monoica) |                            | 0.0002   | )*            | 0.0006*   |      | 0.0009    | 0.0009* |                 | 0.0004* |            | 0.0005* |           | 0.0007* |  |

## Table 3: Effect of different concentrations of acetone extracts of A. marina and S. monoica (ml) on dry weight (mg) and inhibition percentage (%) of the dermatophytes

 Table 4: Effect of different concentrations of ethanol, methanol and acetone extracts of A. marina and S.

 monoica (ml) on dry weight (mg) and inhibition percentage (%) of the yeasts

|               | Cc      | Plant extracts |            |            |      |                |      |                |      |                |      |              |      |
|---------------|---------|----------------|------------|------------|------|----------------|------|----------------|------|----------------|------|--------------|------|
| Ye            | once    | Ethanol        |            |            |      |                | Meth | nanol          |      |                | Ace  | cetone       |      |
| asts          | ntrati  | A. mari        | ina        | S. monoica |      | A. marina      |      | S. monoica     |      | A. marina      |      | S. monoica   |      |
|               | on      | А              | В          | А          | В    | А              | В    | А              | В    | А              | В    | А            | В    |
| C. albicans   | 0.0     | 170±0.40       |            |            |      | 195±0.81       |      |                |      | 220±1.08       |      |              |      |
|               | 0.5     | 135±1.08       | 20.5       | 117±0.81   | 31.1 | 150±1.22       | 23.0 | $152 \pm 0.70$ | 22.0 | $141 \pm 1.08$ | 35.9 | 147±1.22     | 33.1 |
|               | 1.0     | 116±1.22       | 31.7       | 98±0.70    | 42.3 | 131±1.22       | 32.8 | 133±0.40       | 31.7 | 130±1.08       | 40.9 | 120±0.70     | 45.4 |
|               | 2.0     | 95±0.70        | 44.1       | 77±0.40    | 54.7 | $105 \pm 1.08$ | 46.1 | $107 \pm 0.40$ | 45.1 | $100 \pm 0.81$ | 54.5 | 94±0.81      | 57.2 |
|               | 4.0     | 79±0.40        | 53.5       | 57±1.08    | 66.4 | 86±0.81        | 55.8 | 87±1.22        | 55.3 | 71±0.70        | 67.7 | 69±0.81      | 68.6 |
|               | 6.0     | 62±0.70        | 63.5       | 50±1.22    | 70.5 | 61±0.40        | 68.7 | 70±0.81        | 64.1 | 58±0.40        | 73.6 | 55±0.40      | 75.0 |
|               | P-value | 0.0004         | <b>!</b> * | 0.000      | 6*   | 0.0003         | *    | 0.0007         | *    | 0.0001         | *    | 0.0002       | 2*   |
| C. tropicalis | 0.0     |                | 190±       | 0.81       |      | 215±1.22       |      |                |      | 240±0.70       |      |              |      |
|               | 0.5     | $150\pm0.70$   | 21.0       | 144±1.22   | 24.2 | 160±0.81       | 25.5 | $170\pm0.40$   | 20.9 | $164 \pm 0.40$ | 31.5 | 166±1.08     | 30.8 |
|               | 1.0     | 126±1.08       | 33.6       | 128±0.40   | 32.6 | 136±0.70       | 36.7 | $140 \pm 0.70$ | 34.8 | $140 \pm 0.81$ | 41.6 | 139±0.81     | 42.0 |
|               | 2.0     | $109 \pm 1.22$ | 42.6       | 107±0.81   | 43.6 | 120±0.40       | 44.1 | $118 \pm 0.81$ | 45.1 | 120±0.81       | 50.0 | $115\pm0.40$ | 52.0 |
|               | 4.0     | 92±0.40        | 51.5       | 85±0.70    | 55.2 | 95±1.08        | 55.8 | 100±1.22       | 53.4 | 90±1.08        | 62.5 | 85±0.70      | 64.5 |
|               | 6.0     | 75±0.81        | 60.5       | 64±0.70    | 66.3 | 74±1.08        | 65.5 | 85±0.70        | 60.4 | 70±1.22        | 70.8 | 65±1.08      | 72.9 |
|               | P-value | 0.0005         | 5*         | 0.0002*    |      | 0.0009*        |      | 0.0006*        |      | 0.0004*        |      | 0.0003*      |      |

| monoreu   |   |  |  |  |  |  |  |
|-----------|---|--|--|--|--|--|--|
| A. marina | S. monoica  |  |  |  |  |  |  |
| 0.3       | 0.3   |  |  |  |  |  |  |
| 0.2       | 0.1   |  |  |  |  |  |  |
| 0.05      | 0.05  |  |  |  |  |  |  |
| 0.05      | 0.3   |  |  |  |  |  |  |
| 0.5       | 0.5   |  |  |  |  |  |  |
| 0.2       | 0.05  |  |  |  |  |  |  |
| 0.075     | 0.05  |  |  |  |  |  |  |
| 0.05      | 0.075   |  |  |  |  |  |  |
|           | A. marina           0.3           0.2           0.05           0.05           0.5           0.2           0.05           0.5           0.2           0.05 |  |  |  |  |  |  |

 Table 5: Minimal inhibitory concentrations (MICs)
 (mg/ml) of acetone extracts of A. marina and S.

 monoica
 monoica

| Table 6: Anti-oxidant | t activity | and to | otal phenol        | ic |
|-----------------------|------------|--------|--------------------|----|
| contents of S.monoi   | caand A    | marin  | <i>ia</i> extracts |    |

| Diant autroata       | Inhibition | TPC      |
|----------------------|------------|----------|
| Plant extracts       | (%)        | (mg/gdw) |
| S.monoica (Methanol) | 49.5 %     | 31.1     |
| S. monoica (Ethanol) | 59 %       | 34.02    |
| S. monoica (Acetone) | 77 %       | 31.92    |
| A. marina (Methanol) | 73 %       | 47.04    |
| A. marina (Ethanol)  | 64.5 %     | 18.04    |
| A. marina (Acetone)  | 70.5 %     | 30.28    |

Ethanol extract of A. marina significantly inhibited the growth of T. verrucosum with inhibition percentage (94.5 %). The susceptibility of other fungi to the extract was decreased respectively according to the recorded inhibition percentage ranged from (94.4 - 60.5 %). This data contract with. [26] and [27] who discovered that the mangrove (A. marina) leaves ethanolic extract had inhibition effect on (A. citri, P. digitatum, A. Flavus, and P. italicum)in 20, 40, 60 and 80 mg/ml.Crude extricates demonstrated better hindrance against every single tried parasite strains, showing that dynamic fixings in plant materials could be separated into ethanol. [28] detailed the majority of the antimicrobial dynamic mixes were dissolvable in polar dissolvable, for example, ethanolic rather than water and [29] recommended that ethanol extricate uncovered a higher antimutagenic movement than the water separate.

Moreover, [30] reported that ethanol extract of *A. marina* and *R. mucronata* leaves reduced the growth of(*P. purpurogenum*, *A. niger*, *P. chrysogenum*, *P. notatum*, *A. Alternate*, and *A. flavus*). The ethanol extracts of both species have high antioxidant activities and rich in polyphenols and tannins [31].

Also, [32] found that hexane, ethyl acetate, acetone and methanol extracts of *A. marina* leaves and stem have antifungal activity on (*C. albicans* and *C. neoformans*) also

the same solvents extracts have antibacterial activity against Gram-positive bacteria (B. cereus, B. subtilis, C. rubrum, S. aureus) and Gram-negative bacteria(E. coli and S. Typhimurium). Among the solvent extracts, acetone extract of the stem had maximum TPC. On the other hand, leaf solvent extracts had the almost same amount of TPC. The methanol extract of A. marina showed more effect on M. gypseum, which inhibited by (97.0 %). The growth inhibition by the extract against other pathogenic fungi ranged from (96.1 - 65.5 %). This result agreement with [33] reported that methanol leaves extracts of Avicennia sp., Rhizophora sp., C. decandra showed high antimicrobial effect against (S. aureus and P. aeruginosa) and the methanol extract of Thillai sp. showed high antimicrobial effect against (Pseudomonas sp.). [10] investigated the antibacterial effects of A. marina and reported that the extracted by the solvent methanol and ethanol had the highest antibacterial activity.

Likewise, [34] evaluated the antifungal activity of methanol plant extracts of *L. racemosa* and *R. mangle* leaves and bark. They found the extracts have antifungal activity on all tested dermatophytes (*M. gypseum*, *T. Mentagrophytes* and *T. rubrum*) and have inhibition effect on (*C. glabrata*, *T. pullulans*, *T. beigelii* and *C. parakrusei*). This is because tannins were the representative group in the plants followed by flavonoids.

Acetone extract of *A. marina* decreased the growth of *M. gallinae* with inhibition percentage (95.7 %). Whereas, the extract inhibited other fungi ranged from (94.0 - 70.8 %). The same result appeared with [35] demonstrated that methanol, acetone and ethanol extracts of *P. acidula* and *C. Tagal* leaves and bark have antimicrobial activity against pathogenic bacteria (*P. aeruginosa, K. pneumonia, V. parahaemolyticus, S. aureus*, and *V. cholera*). In almost all tests, crude methanolic extracts showed better inhibition against all tested bacterial strains, indicating that active ingredients in plant materials could be extracted into methanol.

Furthermore, ethyl acetate, ethyl ether and ethanol extracts of *A. marina* and *R. stylosa* leave inhibited growth of the tested fungi (*P. digitatum*, *F. oxysporum*, and *C. albicans*)also have inhibition activity against pathogenic bacteria(*E. coli*, *S. aureus* and *B. subtilis*) with different degrees of inhibition. Concentrates by solvents of the two plants stifled the development of the tried strains to differing degrees, showing nearness of wide range inhibitory standards likewise both A. marina and R. stylosa leaves contain antibacterial just as antifungal mixes [36, 37]. Ethyl acetate extracts showed the highest inhibition activity more than other extracts, probably due to the extraction of more effective bioactive principles of *A.marina* and *R. stylosa* leaves [38].

Also, [39] demonstrated that ethyl acetate, ethyl alcohol, chloroform, and ethyl methyl ketone extracts of *A. marina* 

leaves showed wide inhibition against the tested fungi(*R. solani*, *C. gleosporioides*, *Curvularia lunata*, *F. oxysporum*, and *C. albicans*)also have antibacterial activity against (*P. aeruginosa* and *B. subtilis*). Ethanolic leaf extract of *A. marina* had a good inhibitory activity for both fungi and bacteria.

Ethanol extract of *S. monoica* was high inhibition activity against *M. gypseum*, which inhibited by(97.2 %). The susceptibility of other fungi to the extract was decreased respectively according to the recorded inhibition percentage ranged from (96.2-66.3 %). This data similar to [40] demonstrated that ethanol extract of *S. alba, R. mucronata*, and *E. agallocha* inhibited the tested bacteria (*S. aureus, Streptococcus* sp., *P. mirabilis, S. Typhi* and *P. Vulgaris*). Ethanol removes demonstrated the nearness of a few phytochemicals making it increasingly dynamic against bacterial strains in contrast with the watery concentrate.

As well, [41] reported the different solvent extracts (hexane, benzene, chloroform, ethylacetate, acetone, and methanol) of Suaedanudiflora showed varied antibacterial activity against tested bacteria (Micrococcus luteus, Arthrobacter protophormiae, Rhodococcus rhodochrous, B. subtilis, S. aureus, B. megaterium, E. faecalis, Streptococcus mutans, L. acidophilus, Alcaligens faecalis, P. Vulgaris, P. mirabilis, P. aeruginosa, and E. aerogenes). Ethyl acetate and acetone fractions of different concentrations exhibited higher free radical scavenging activity than the control when compared to all other extracts. The methanol extract showed the next higher scavenging activity, whereas hexane, benzene, and chloroform extracts revealed low free radical scavenging activity. [42] reported the antimicrobial activity of petroleum ether, ethyl acetate and methanol extracts of S. monoica against both bacteria and fungi.

The methanol extract of S.monoica was the most effective against M. gypseum, which inhibited by (98.0 %). The growth inhibition by the extract against other pathogenic fungi ranged from (96.5-60.4%). The same result appeared with [43] demonstrated that methanol and petroleum ether of S. monoica and S. maritime leaves extracts have antifungal activity againstclinical fungal pathogens(A. flavus, Mucor sp. and C. albicans) also they able to inhibit the growth of clinical pathogenic bacteria(E. coli, P. aeruginosa, S. aureus, B. subtilis, and S. aerginosa). The phytochemical analysis of S. Maritima indicates the presence of tannins and flavonoids [44]. Flavonoids are known to possess a wide range of biological activities such as antioxidant, antimicrobial, anti-inflammatory and anticancer activities [45] also the potential of free radical scavengers of the phenolic compound have been reported by [46]. The antibacterial activity against both Gramnegative bacteria and Gram-positive bacteria by acetone,

ethanol, methanol and aqueous extracts of *S. Maritima* was also reported earlier [47].

Moreover, [48] reported the antimicrobial compounds from marine halophytes (*Salicornia brachiata, S. Maritima* and *Sesuvium portulacastrum*) revealed that antimicrobial activity was due to the presence of bioactive components such as sulfated polysaccharides. [49] studied biology and antimicrobial activities of salt marsh and coastal plants. He examined the ethanolic extracts of *S. monoica* and *S. Maritima* salt marsh plant showed effective antimicrobial activities towards dreadful pathogens.

Acetone extract of *S. monoica* was high inhibition activity against *M. canis*, which inhibited by(93.0 %). Whereas, the extract inhibited other fungi ranged from(90.1- 72.9 %). This result similar with [7] reported that hexane, chloroformand methanolextracts of *S. monoica* leaves and shoots exhibited the different degrees of growth inhibition against tested fungal strains (*C. albicans*, *M. recemosus*, *R. solani*, *R. stolonifer*, and *S. cerevisiae*) also exhibited the different degrees of growth inhibition against tested bacteria (*B. subtilis*, *B. megaterium*, *L. acidophilus*, *E. coli*, *E. aerogenes*, *E. cloacae*, and *K. pneumonia*).

From our results, the methanol extract of *A. marina* and acetone extract of *S. monoica* more active than other solvents extracts against the tested dermatophytes and yeasts, they displayed the highest antioxidant activity (73 and 77 %) and total phenolic contents(31.92 and 47.04 mg/gdw) respectively. Phenolics are by all account not the only parts in the concentrates that could have cancer prevention agent action [32] likewise by different segments, for example, Gallic corrosive, Catechin, Coffeic corrosive, Syringic corrosive, Rutin, Coumaric Acid, Vanillin, Quercetin and Cinnamic corrosive, that purified from plants extracts by HPLC method with other unknown substances in plant extracts.

MIC values of acetone extracts in the range of (0.5 - 0.075)mg/ml). T. verrucosum the most resistant to the A. marina and S. monoica extract was inhibited by (0.5 mg/ml) whereas C. albicans the most sensitive to the A. marina extract was inhibited by (0.075 mg/ml) and C. tropicalis the most sensitive to the S. monoica extract was inhibited by (0.075 mg/ml). This results in agreement with [50] suggested the most minimal MIC esteem is seen as that of A. marina root-chloroform separate (0.25 mg/ml) against (B. subtilis) and (0.98 mg/ml) against (S. cerevisiae). The A. marina leaf-ethanol and Avicennia alba bark-methanol removes are found to have the most noteworthy MIC esteem (7.81 mg/ml) against (B. subtilis). A. marina barkhexane, A. alba leaf-chloroform, A. alba wood-ethanol, Clerodendrum inerme leaf-hexane and C. inermebarkhexane extricates have shown high MIC values (>31.1 mg/ml) against (S. cerevisiae). The concentrates of the test mangrove plants have critical antimicrobial exercises. When all is said in done, methanol, chloroform, and hexane

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extricate demonstrated noteworthy antibacterial and antifungal exercises.

Also, [51] demonstrated that the values of MIC was found to be in the range of (1.25 - 5.0 mg/100µl) for leaf and stem (hexane, benzene, ethyl acetate, acetone, methanol, and ethanol) extracts of *A. marina* against all the bacteria tested (*E. coli, E. aerogenes, K. pneumoniae, P. aeruginosa, B. subtilis, Lactobacillus delbrueckii, S. aureus* and *S. pyogens*). The *A. marina* can also be strongly recommended for consideration as a valuable source for identification, isolation and characterization of potential bioactive compounds with antibacterial property.

Furthermore, The ethyl acetate extract of *S. nudiflora* total plant MIC values range of (25-75 mg/ml) whereas the MIC values of acetone extract range of (50-75 mg/ml) against (*M. luteus, A. protophormiae, R. rhodochrous, B. megaterium, B. subtilis, E. faecalis, S. mutans, S. aureus, L. acidophilus, A. faecalis, P. mirabilis, P. Vulgaris, <i>E. aerogenes* and *P. aeruginosa*). All the extracts of *S. nudiflora* exhibited free radical scavenging activity and the presence of different phytochemicals like tannins, steroids, flavonoids, alkaloids, and terpenoids [41].

Also, [52] demonstrated that the chloroform extract of *S. melongena* was found to be the most active extract with lower MIC values as compared to the other tested plant *J. gendarussa.* MIC values for *S. melongena* ranged from (3.12- 6.25mg/ml) with chloroform extract, ranged from (6.25-12.5mg/ml) with methanol extract whereas MIC values for *J. gendarussa* greater than (12.5mg/ml) with all extracts against the tested dermatophyte samples (*T. mentagrophytes, T. rubrum, M. gypseum,* and *M. fulvum*) that show the nearness of antifungal operators in the tried plants which were discovered full of feeling in restraining the development of both (*Trichophyton and Microsporum*) species.

Whereas, [53] concluded that the MIC values for the different methanolic plant extracts (*Calendula officinalis*, *Acacia arabica*, *Ginkgo biloba*, *Juglans regia*, *Osimum basilicum*, *Solanum nigrum*, *Hypericum perforatum*, and *Anagalis arvensis*) were ranged from (0.2 - 12.5 mg/ml) against (*M. canis*, *M. gypseum*, *T. mentagrophytes*, *T. rubrum*, *T. schoenleinii* and *E. floccosum*).In this way, demonstrating the remedial possibilities of concentrates. It indicated the nearness of bioactive mixes just as the antifungal properties of methanolic extract. The more saponins are present the higher the rate [54].

### CONCLUSION

This paper is a successful trial of phytochemical properties and antidermatophytic efficiency of *A. marina* and *S. monoica* screening as mangrove plants can be used as a biological effect. Furthermore, attentiveness has to pay to purification and formulation may be needed to understand the mechanisms through which this effect is exerted.

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