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Phytochemical screening and antimicrobial activities of *Costus speciosus* and Sea Qust.

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Abstract

The present work reveals the preliminary phytochemical screening and the study of antimicrobial activities of *Costus speciosus* (Koen) and Sea Qust. These are medicinal plants of considerable importance. Carbohydrates, Tannins, Steroids, Anthocyanates and Proteins were detected in both plants. Alkaloids, saponines, Anthraquinones were present in *Costus speciosus* but where absent in Sea Qust. The antibacterial and antifungal activity of essential oils and plant extracts of *Costus Speciosus* and Sea Qust was investigated against a few pathogens viz. *M. gypseum*, *M. canis*, *C. albicans* and *C. tropicalis*, *P. aeruginosa* and S. aureus, *P. aeruginosa* and *St. aureus*. The data obtained validated their wide application for therapeutic purposes in alternative therapy.

Keywords: *Costus speciosus* (Koen.), Phytochemical, Extracted compounds, Antimicrobial activities.

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Introduction

There has been a virtual explosion of interest in alternative therapy. The use of the herbal remedies has become more and more popular because of most people believe that herbal products are superior to manufactured products, less expensive than synthetic drugs and they are not satisfied with the result they get with manufactured medicines [1]. In addition to this most of herbal products are categorized under GRAS (Generally Recognized as Safe) for human consumption, and efficient and rarely have side effects [2].

The medicinal properties and the biological evaluation of plants have been valued by every culture and civilization in the world. It is focused on the botany, phytochemical composition [3]. Medicinal plants contain some important organic compounds such as tannins, carbohydrates, terpenes, alkaloids, steroids, falvonoids and coumarines. Under *in vitro* conditions, these organic constituents inhibit the growth of all types of microorganisms [4]. For those reasons, the medicinal plants are currently used as alternative therapy to antibiotics for many pathogenic microorganisms. Studied indicated that crude ethanol extracts obtained from plant species had antifungal activities against *C. albicans* and *Cladosporium cucumerinum*. Further, the ethanol extracts also had bacteriostatic and even bactericidal effects on *S. aureus* and *E. faecalis*. These are pathogenic microorganism for the human [5]. *Trichophyton mentagrophytes* and *Trichophyton rubrum* are a species of dermatophytes, and they recorded a high sensitivity when the treatment by methanol extracts of *Eupatorium buniifolium* and *Terminalia triflora*.

The MICs ranging was of 100 to 250 microg/ml [6]. *Costus afer* is one of 218 plants which were registered for antibacterial activity against *Staphylococcus aureus*, *Mycobacterium fortuitum*, *Bacillus cereus* and *Candida albican*s [7]. *Costus specious* and Sea-Qust are belong to Costaceae (Zingiberaceae) family which constitutes a vital group of rhizomatous medicinal and aromatic plants. Besides their therapeutic significance, they are also important source for the extraction of export-quality essential oils and oleoresins [3]. The dried root and aquatic extracts of Indian Costus and Sea-Qust were a high inhibitory on *Aspergillus niger*, *A. flavus* and *C. albicans* growth [8]. Hence the aim of this study was to obtain the important organic compounds of Sea-Qust roots and examine their effectiveness against pathogenic microbes. It has confirmed the fatal influence of dried root and aquatic extracts of Indian Costus and Sea-Qust on *Escherichia coli*, *P. aeruginosa*, S. aureus and *K. pneumonia* [9].

Material and Methods

Chemistry

IR spectra were performed on a Perkin-Elmer 16 FPC FT-IR spectrophotometer. ^{1}HNMR and ^{13}CNMR spectra were performed on a Brucker AVANCE D.P.X. 600 MHz apparatus. GC-MS were determined by Joel JMS 600H, GC Hewlett Packerd, HP 6890 Series. Thin layer chromatography (TLC)

and preparative layer chromatography (PLC) was done by Polygram SIL G/W 254, Mecherey-Nagel. A rotatory evaporator (at 200°C/15 torr) was used to remove the solvents.

Plant material

(Indian Costus and Sea Qust) rhizomes were washed with water and dried and then ground well. All the rhizomes collected from several herbalists at (Jeddah, Saudi Arabia). The powder of rhizomes has been used in the chemical experiments [8-10].

Extraction of essential oil

(500 g) of dried roots of Indian Costus or Sea Qust were using for steam distillation for four hours. About 45 ml of the distillates were collected and extracted with chloroform (3 × 100 mL) and dried over anhydrous sodium sulphate (Merck, Germany), and the solvent was removed by evaporation. The yields were (0.60-0.65%) stored in a refrigerator (+ 6°C).

Preparation of plant extracts

200 g of the dried and powdered form roots of Indian Costus or Sea Qust were extracted successively using cold percolation system [11] ethanol, methanol, distilled water or chloform (400 ml. for each) for 4 days, using a stirring apparatus. Then collected solutions were filtered through Whatman filter paper. The extracts were concentrated by using a Rotary evaporator at 600°C. The respective extracts were stored under freeze condition at -180°C until used for further analysis.

Preparation of alcoholic extracts for screening

200 gm of dried powder of Indian Costus or Sea Qust was extracted with about 800 ml of 70% v/v methanol for 4 days at room temperature using a stirring apparatus. The extract was filtered and the solvent was distilled off in a rotatory evaporator at 400°C. The extract was concentrated to dry residue in a desiccator over anhydrous Sodium Sulphate. The resulting extracts were filled into sample container.

Phytochemical screening

Standard procedures were adopted for the phytochemical screening.

Test for tannins: To about two grams of the ethanol extract of the sample, a few drops of 5% ferric chloride solution were added. A dark green or bluish-black coloration show the presence of tannins [12].

Test for flavonoids (Shinoda Test): To two grams of the ethanol extract, a few fragments of magnesium ribbon were introduced. To this, 6 drops of concentrated hydrochloric acid were added. If a pink or red colour is obtained, the presence of flavonoids is indicated [13].

Test for saponins: To about five grams of ethanol extract, 5 ml de-ionized distilled water was added. On vigorous shaking, the formation of a persistent froth that lasted for 15 minutes indicated the presence of saponins [14].

Test for terpenoids (Salkowwski Test): In a test tube 0.5 gm of the extract was taken and about 2 ml of chloroform was added to it. To this, 3 ml of conc. H_2SO_4 was carefully introduced to form a layer. If a reddish brown colour is obtained, presence of terpenoids is indicated [15].

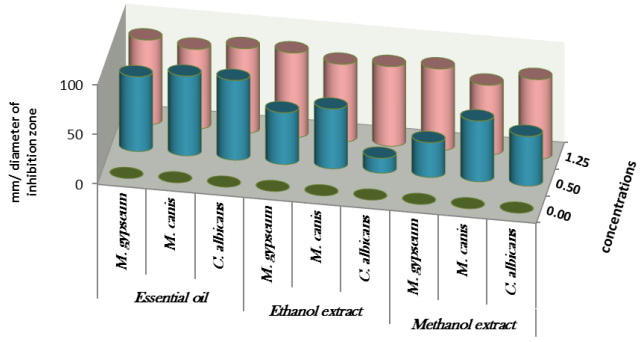

Figure 1. *Effect of various concentrations of Sea Qust extracts against Microsporum gypseum, Microsporum canis and Cadida albicans grown on sabaround dexterous media (mm/diameter of inhibition zone).*

Test for carbohydrates (Molisch's Test): One gram of the ethanol extract was dissolved in a few drops of water. Then 1 ml of conc. Sulphuric acid was added along the walls of the test tube. On this addition, if a red or violet zone is visible at the interphase of the oil-water layers, carbohydrates and /or glycosides are indicated in the sample [16].

Test for anthraquinone (Bontrager's Test): To one gram of the ethanol extract, 5 ml of benzene was added. Then it was shaken and filtered. Five ml of 10% NH_4OH was added to the filtrate, followed by shaking of the contents. The formation of a red, pink or violet colour in the lower ammoniacal phase confirmed that free anthraquinones are present in the sample [16].

Test for cardiac glycosides (Keller-Kilani Test): To the Ethanol extract, 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$ was added. This mixture was then introduced into another test tube that contained 2 ml of concentrated H_2SO_4. The appearance of brown ring at the interphase indicated that cardiac glycosides are present in the sample [13].

Test for coumarins: One gram of ethanol extract of the sample was taken in a test tube. The test tube was then covered with a filter paper that is moistened with dil. NaOH. The sample was then heated on water bath for a few minutes. The filter paper was then examined under UV (365 nm). If a yellow fluorescence is obtained, the presence of coumarins is indicated [17].

Test for steroids (Liebermann-Burchard Test): One gram of ethanol extract was taken. This was followed by the addition of 2 ml of acetic acid. The solution was cooled in an ice bath. After the cooling, conc. Sulphuric acid was added carefully.

The development of colour from violet to blue or bluish-green is a positive test for the presence of a steroidal ring [16].

Test for alkaloids: To one gram of ethanol extract, 2 ml of 1% HCl was added and the contents were heated gently. This was followed by adding 2-3 drops of Mayer's reagent. The appearance of white or cream precipitate confirm the presence of alkaloids [13,14]. Table 1 shows the presence of phytochemical screening of ethanolic extract of rhizomes of *Costus specious* and Sea-Qust.

Antibacterial activity

Test organisms fungi and yeast pathogenic: *M. gypseum*, *M. canis*, *C. albicans* and *C. tropicalis* isolates were obtained from King Faisal Specialist Hospital & Research Centre-Jeddah, Saudi Arabia. Culture medium was the sabaroud dexterous ager (Oxoid CM 41), it used to the growth of fungi and yeast.

Bacterial pathogenic: *P. aeruginosa* and *S. aureus* were obtained from King Faisal Specialist Hospital & Research Centre-Jeddah, Saudi Arabia. The blood ager (Oxoid) was culture medium for the bacterial pathogenic.

Antimicrobial activities of essential oils and plant extracts of Indian Costus

About 0.5 and 1.25 ml of essential oil, ethanolic and methanolic extracts of Sea Qust have been added into sabaroud dexterous ager media and blood ager media using agar disc diffusion method. All the media were inoculated by 1 ml from suspension of *M. gypseum*, *M. canis*, *C. albicans* and *C. tropicalis* (sabaroud dexterous ager) and *P. aeruginosa* and *St. aureus* (blood ager). The incubation of fungi and yeast using agar disc diffusion method. Days and 48 hours respectively at 25°C. Whereas, The bacterial incubation was 24 hours at 37°C, then the diameter of inhibition zones was measured by millimeters [18-20].

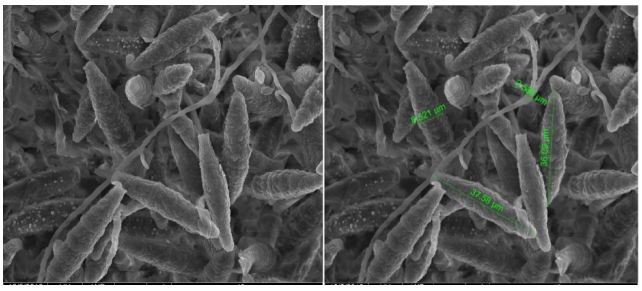

Figure 2. *The control sample of Microsporum gypseum show a normal growth of spores (L=37.58 µm & W=8.52 µm) and hyphae (W=2.58µm).*

Results and Discussion

The results of the qualitative phytochemical analysis and screening of *Costus Speciosus* and Sea Qust are presented in Table 1. These results indicate the differences in the presence of medically active components in these two plants. It could be inferred from Table 1 that carbohydrates, tannins, steroids and anthocyanates were present in both the *Costus speciosus* and the Sea Qust. Others like cardiac glycosides, coumarines, terpenoids were absent in both. Alkaloids, saponines, Anthraquinones were present in *Costus speciosus* but were absent in Sea Qust. Sea Qust extracts demonstrated a high antimicrobial activities on the tested fungi, yeast and bacteria.

Table 1. *Phytochemical constituents of rhizomes of Indian costus and Sea-qust.*

Phytoconstituents	Indian Costus	Sea-Qust
Carbohydrates	+	+
Cardiac glycosides	-	-
Alkaloids	+	-
Tannins	+	+
Saponins	+	-
Steroids	+	+
Flavonoids	+	-
Coumarines	-	-
Anthraquinones	+	-
Terpenoids	-	-
Anthocyanates	+	+
Protein	+	+

At 0.5 and 1.25 m of essential oil the inhibition zone was increased. The measures were (75, 85, 80 and 85 mm) for *M. gypseum*, *M. canis* and *C. albicans*. Ethanol and methanol extracts showed same efficiency at 1.25 m, but the effect decreased at 0.5 m relatively when compared to essential oil results (Figure 1). *M. gypseum* hyphae and spores damaged and the morphological shape changed when *M. gypseum* treated by essential oil extract, that effect showed in SEM images (Figures 2 and 3) . The results of Sea Qust extracts showed same effects on pathogenic bacteria, whereas their growth has been affected when the treatment by 1.25 m of extracts (Figure 4).

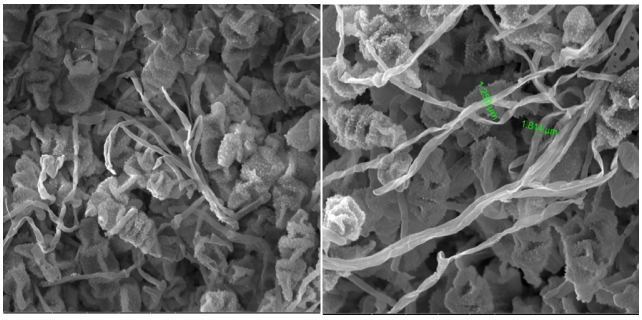

Figure 3. *SEM images of Microsporum gypseum fungus after treatment by 1.25% essential oil and ethanol extract of Sea Qust showing the change in spore forms and disruption hyphae.*

Essential oil and methanol of Sea Qust were highest effect then ethanol extract on *P. aeruginosa*, the measures of inhibition zone were 75, 80 and 85 mm at 0.5 and 1.25 m of it. The

growth of *S. aureus* was most sensitivity and the measures were 75&80 mm when the treatment by Sea Qust ethanolic. These results indicated that efficacy of chemical extracts on the microbial tested.

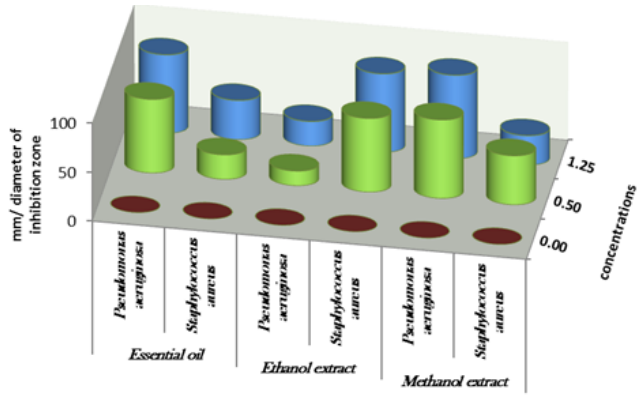

Figure 4. *Effect of various concentration of Sea Quest extracts against Peudomonas aeruginosa and Staphylococcus aureus grown on blood media (mm/diameter of inhibition zone).*

The recorded results in this study demonstrated efficiency of chemical extracts of Sea Qust. Our results are compared with Habsah et al. [21] who found a very high antibacterial and antifungal activities in dichloromethane and methanol extracts of Alpinia, *Costus* and *Zingiber* species. The essential oil, methanol and ethanol extracts of *Costus arabicus* show antimicrobial properties against wide spectrum of bacteria (both gram-positive and three gram-negative species), one fungal stain and three resistant *Staphylococcus* strains. This plant is used as traditional medicine for human cancer cell [22]. The medicinal plant *Costus speciosus* has antioxidant activity as evident from the treatment of diabetic male treatment by costunolide and eremanthin. These constituents were isolated from this plant and ananlyzed by gas chromatography-mass spectrometry (GC-MS) analysis [23,24]. Dried roots and aquatic extracts (hot and cold) of Indian Costus and Sea Qust have exhibited antifungal activities against *Aspergillus niger*, *A. flavus*, *A. fumigatus* and *C. albicans* which cause respiratory diseases [8,25]. Presently, AL-Kattan observed fatal effects of Indian Costus and Sea- Qust roots on *S. areus* and *K. pneumonia* [9]. The percentage inhibition was 100% at 15 & 20%. This effect continued when the bacterial growth was taken after 48h and repeated it on the same concentration three times. Furthermore, it is showed that effect on *C. albicans* and A. niger 7 whereas, the growth and budding of yeast decreased at 25% of Indian Costus (hot and cold) aquatic extracts [8]. Also *A. niger* spores didn't grow and hyphae disruption at same concentration of Sea Qust. Consequently those results have been comported with our SEM figures in this study. Thus, chemical extracts of Sea Qust have antimicrobial activities on dermatophytes which can be used as natural alternatives to treat these diseases.

Conclusion

In the present study, the qualitative phytochemical analysis and screening of *Costus speciosus* and Sea Qust were done. *Costus speciosus* and Sea Qust are shown to exhibit antifungal activities against *Aspergillus niger*, *A. flavus*, *A. fumigatus* and *C. albicans*. This study showed that *Costus speciosus*, Sea Qust and its extracts have good antimicrobial activity.

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