

## Study Effect of Indian Costus and Sea-Qust Oil Extract on Some Opportunistic Bacteria and Yeast.

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**Abstract:** *Klebsiella pneumoniae* (*K. pneumonia*), *Staphylococcus aureus*, *Candida albicans* and *Candida tropicalis* are opportunistic microbes associated with certain diseases such as diabetes, deep wounds and immunodeficiency. Those microbes have evolved their resistance to antibiotics in recent years. The aim of this study is to prepare oil extracts from the dried roots of Indian Costus and sea-Qust and test that oil's effectiveness on some bacteria and yeast. The results have recorded significantly high antimicrobial activity against *K. pneumonia* and *C. tropicalis* during treatment with Indian Costus oil, the sea-Qust oil was also very effective on *C. albicans* and *S. aureus*. Because the cell wall (SEM) of *C. albicans* was rough and wrinkled. Therefore, the budding of yeast became less with a treatment of Indian Costus oil. Thus, this result demonstrates that oil extract of Costus-type plants is effective that their oil extract could be considered as a natural alternative to antibiotics.

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### 1. Introduction:

In recent years, the number of opportunistic pathogens have been steadily increasing for several reasons, as seen in the prevalence of pathogen bacteria such as, *Pseudomonas aeruginosa*, *K. pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* which is isolated from foot infections in diabetic patients (Viswanathan *et al.*, 2002, Sharma *et al.*, 2006 and Raja, 2007). Both *P. aeruginosa* and *C. albicans* are opportunistic pathogens and persistent in the hospital environment, where they are responsible for the ventilator-associated pneumonia, *C. albicans* usually colonises the tracheo-bronchial tract of patients going through mechanical ventilation in intensive care units, and it's resistance to antibiotics has increased (Pierce, 2005, Ader *et al.*, 2008 and Sausseureau & Debarbieux, 2012). Furthermore, *C. albicans*, *C. tropicalis*, and *C. parapsilosis* are the most common cutaneous candidiasis (Odds, 1979 and Arendorf & Walker, 1980) and are causative agents in human fungal infections, they have been especially noted to cause infection when the skin is inoculated in vivo by low and high concentration of yeasts and followed for 1 - 5 days, and then electron microscopy observations revealed that all three tested yeasts adhered to the skin but *C. albicans* covered the skin to a higher extent than *C. tropicalis* or *C. parapsilosis* (Raz-Pasteur *et al.*, 2011).

Moreover, there is a worldwide concern that antibiotics are being overused. This is one factor that contributes to the growing number of bacterial and fungal infections that are becoming resistant to

antibiotic medications. Although most antibiotics are safe considering their volume of use, some of them have the potential for life-threatening side effects (Cunha, 2001 and Butler *et al.*, 2006). Antimicrobial side effects present as adverse drug reactions involving one or more organ systems. For the stated reasons, it is important to discover natural alternatives for treatment and many medicinal plants have been used in traditional healing since ancient times. Indian Costus roots are species of the Zingiberaceae family and native Indian plants, which characterized by the presence of volatile oils and oleoresins of export value also, the rhizomes of Costus constitute a vital group and aromatic plants (Singh *et al.*, 1972, Anaga *et al.*, 2004 and Nahak & Kanta sahu 2011). Habsah *et al.*, (2000) showed the antimicrobial and antioxidant activities of dichloromethane and methanol extracts the *Alpinia*, *Costus* and *Zingiber*, and the methanol extract of *Costus discolor* exhibited antifungal activity against *Aspergillus ochraceous* (MID, 15.6 µg per disc). Additionally, Pandey *et al.* (2007) indicated the important medicinal applications of *Saussurea lappa* (costus) in treatments for asthma, inflammatory diseases, ulcers and stomach problems. The *Saussurea* (costus) has remarkable biological activity for that it's necessary to prepare it as a medicine. This study will test the preparation of Costus oil from low-cost and natural trading materials, with the main active substance derived from dried roots of Indian Costus and Sea-Qust and will prove its effectiveness as an antibiotic on the yeast and bacteria tested.

## 2. Materials and methods:

### 1-Plant material:

The Indian Costus and sea-Qust roots were collected from numerous herb stores in Saudi Arabia (Jeddah, Makkah), washed, left to dry and then, ground into a powder (Ody,1999).

### 2- Oil Extracts Preparation:

About 250 g. powder of both dried Costus roots were added to 500 ml. of oil, and then the mixture was heated for three hours. Thereafter, the oil extracts were left in the dark for two weeks and were used having been filtered (Ody, 1999) in the laboratory experiments on the tested microorganisms.

### 3- Test organisms:

#### 3-1. Bacterial pathogen:

*Staphylococcus aureus* and *Klebsiella pneumonia* were obtained from King Faisal Specialist Hospital & Research Centre – Jeddah, Saudi Arabia. Blood agar was the media (Oxoid) used for the cultivation of pathogenic bacteria at 37 °C (Madigan & Martinko, 2005).

#### 3-2. Yeast pathogen:

*Candida albicans* and *C.tropicalis* were obtained from King Faisal Specialist Hospital & Research Centre – Jeddah, Saudi Arabia. They were cultured on Sabaroud dexteros agar media (Oxoid CM 41) at 25°C.

#### 4- Antibacterial activities of the Costus oil extracts:

Added 0.5 and 1.0 ml of Costus oils to Sabaroud dexteros agar inoculated with 1 ml. from a suspension of *S. aureus* and *K. pneumonia* (106 CFU/μl) at 37 °C for 24 hours. Then the antibacterial activities was determined by method of agar disc diffusion (Baker & Breach, 1980 and Hasenekoglu, 1990).

#### 5-Dry weight estimation of the bacteria and yeast tested:

Approximately 0.5 and 1.0 ml of Costus oils were mixed in Sabaroud dexteros agar and then inoculated with the pathogenic microbes. After the plates

incubated at 37 °C for 48 hours the estimation of the dry weight for yeast and bacteria were determined by method of Al-kattan, 2006.

#### 6- Scanning electron microscopy:

All picture scanning have been provided by electron microscopy at King Fahd Center for Medical Research - King Abdulaziz University - Jeddah, Saudi Arabia.

### 3. Results:

The Costus oil extracts have multicomponent of medical groups, whereas these components were highly active on the bacteria and yeast tested in this study. The inhibition zone results in (Table 1) indicated that antibacterial efficiency appeared around the diffusion disc. Whereas, the Indian Costus oil inhibited *S. aureus* effectively, Sea-Qust oil provided measures of the inhibition zone at 7.10 mm and 1.55mm for *K. pneumonia*. In addition, data present in (Table 2) showed antibacterial activity against *S. aureus* and *K. pneumonia* also, the dry weight of bacteria was decreased with increasing concentration especially at 1.0%. Their growth rates were 0.036, 0.157, 0.232 and 0.090 percent with of treatment by the oil extracts. Furthermore, the dry weight of *C.albicans* and *C.tropicalis* was sensitised by the treatment of Costus oils. Indian Costus oil affected on *C. albicans* growth at 1.0 %, and that result was reduced slightly at the same concentration on *C.tropicalis*. When the yeast treated with Sea-Qust oil, the growth rate of *C.tropicalis* was reduced more than occurred with *C.albicans* at 0.5% and 1.0% (Table 3). The SEM showed that cell wall sensitivity of *C.albicans* at 25% of Indian Costus oil, it was rough and wrinkled fig. (1, 2) and, the budding of yeast became less fig. (1, 3). All the results were compared to control tests. Consequently, the results of treatment with Indian Costus oil produced demonstrable effect against *C. albicans* and *S. aureus*, but the Sea- Qust oil was a very efficient against *C.tropicalis* and *K.pneumonia* tested.

Table (1):Antibacterial effects of 1.0 concentration of Indian Costus and sea-Qust oil (100 μg/cm).

Treatment	Inhibition zone (mm)	
	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumonia</i>
Indian Costus oil	7.10	0.33
sea-Qust oil	1.10	1.55

Table (2):Effect of Various Concentrations of Indian Costus and sea-Qust oil on the dry weight of *S. aureus* and *K. pneumoniae* after 24 h (Mean of Replicates ± SE)

Treatment	Concentration	Pathogenic bacteria	
		<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>
Control	0.0	0.269 + 0.00	0.215 + 0.00
Indian Costus oil	0.5	0.117 + 0.01 **	0.118 + 0.00 *
	1.0	0.036 + 0.02 **	0.157 + 0.02 *
Sea-Qust oil	0.5	0.205 + 0.00 *	0.052 + 0.01
	1.0	0.232 + 0.02	0.090 + 0.00 *

\*Significant at 5%

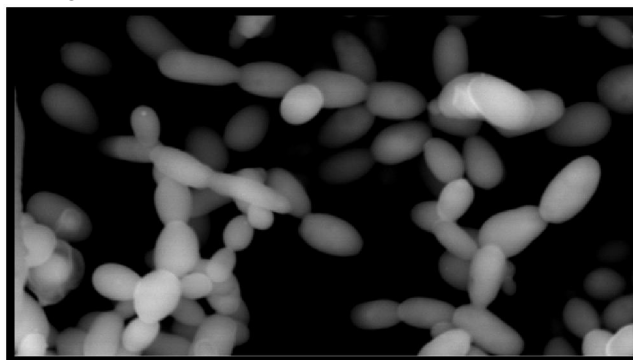
\*\* Significant at 1%

**Table (3):**Effect of Various Concentrations of Indian Costus and sea-Qust oil on the dry weight of *Candida albicans* and *C. tropicalis* after 24 h (Mean of Replicates  $\pm$  SE)

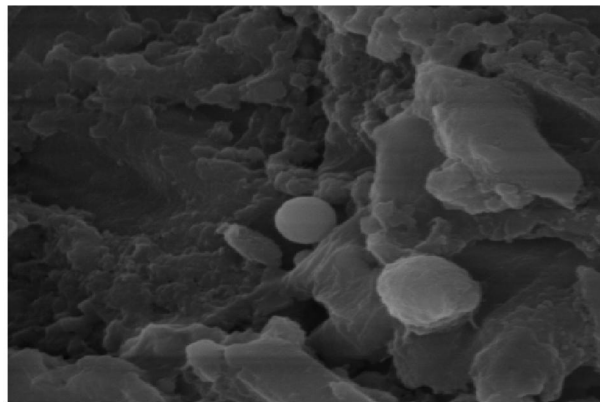
Treatment	Yeast		
	Concentration	<i>Candida albicans</i>	<i>Candida tropicalis</i>
Control	0.0	184.52 + 14.50	137.17 + 7.13
Indian Costus oil	0.5	162.48 + 9.96	160.00 + 1.30 **
	1.0	129.05 + 1.97 *	169.24 + 0.81 **
Sea-Qust oil	0.5	191.29 + 12.09	120.00 + 9.92 **
	1.0	240.09 + 57.96 **	99.01 + 1.00 **

\*Significant at 5%

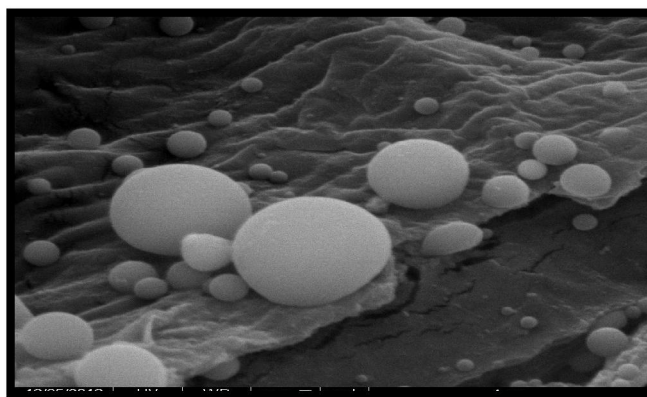
\*\* Significant at 1%



**Figure (1):** Scanning Electron Microscopy of *C. albicans*: Normal shape of *C. albicans* (control).



**Figure (2):** The cell wall appeared rough due to wrinkles and changes in cell shape which became round as a result of 25% Indian Costus oil treatment.



**Figure (3):** The wall separated and decreased budding of *C. albicans*.

#### 4. Discussion:

This study has added new natural alternatives to antibiotics for treatment of opportunistic pathogens it found an alternative that reduced the presence of *S. aureus*, *K.pneumonia*, *C.albicans* and *C.tropicalis* which cause diabetic foot, skin, lung, burn, oral, gastrointestinal and vaginal infections. It tested, the *Costus* species, a member of the Zingiberaceae family, used worldwide as medicinal plants, pharmacology cures, health tonics and antiseptics for wounds (Singh *et al.*, 1972; Viel *et al.*, 1999; Kala *et al.*, 2006; Pandey *et al.*, 2007 and Ibn Qayim, 2008). The rhizomes and roots of *Costus speciosus* Koen are bitter taste and employed in India as traditional medicine because, they create many pharmacology responses as anthelmintics, antioxidants and stimulants (Vijayalakshmi and Sarada, 2008; Nadkarni, 2009; Gupta, 2010 and Nahak and Kanta sahu, 2011). Mothana *et al.* (2009) confirmed the chemical extracts of *Costus arabicus* roots have antimicrobial activity whereas, the essential oil, methanol, ethanol and aqueous extracts efficiently inhibited against gram-positive, three gram-negative bacterial and three resistant *Staphylococcus* strains. Al-Kattan (2013) observed the fatal influence of Indian *Costus* and Sea-*Qust* roots on *S. aureus* and *K. pneumonia* when, their growth is repeated at same concentration by inoculation, especially at 15 and 20% of the dried roots.

The SEM images taken in this study demonstrate that *Costus* oil is effective in reducing the budding and damaged cell walls of *C. albicans*, these results combined with (Mostert *et al.*, 2000 and Al-Kattan & Al-Sheikh 2011) same inhibitory effect on *Aspergillus niger* and *C. albicans* growth when treated by the aquatic extracts of Indian *Costus* and Sea-*Qust* and a high effectiveness with the dried roots against *Aspergillusniger*, *A. flavus*, *A.fumigatus* and *C. albicans*. Thus, the oil extract preparation of Indian *Costus* and Sea-*Qust* are a new antimicrobial treatment against opportunistic pathogens and can be employed for diabetic patients' foot infections to reduce the side effects caused by the antibiotics currently in use.

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