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# Study of *Commiphora myrrha* (Nees) Engl. var. molmol Extract and Its Antibiogram against Some Microbial Pathogens

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**Abstract**

The aim of this study was to determinate the effects of *Commiphora myrrha* (*C. myrrha*) resin extract on the cellular morphology of Gram-positive bacteria (***Bacillus subtilis*)**, Gram-negative bacteria (*Escherichia coli*), fungi (*Aspergillus niger*) and yeast (*Candida albicans*). Oleo-gum resins of *C. myrrha* were collected from Wadi Noeman at Makkah. Ethanolic extract (85%) of *C. myrrha* resins obviously inhibited the growth of the tested pathogens. The minimum inhibitory concentrations (MIC) and the minimum bactericidal/fungicidal concentrations (MBC/MFC) were tested. The phytochemical screening of myrrha resin extract was performed to find the presence of active chemical constituents. Cellular morphology of the tested microorganisms was examined using Scanning electron microscope (SEM). Finally, the obtained data were statistically analyzed. *C. myrrha* extract was found to have antimicrobial activities causing a zone of inhibition against *Escherichia coli*; 29mm, followed by *Staphylococcus epidermidis*, *Candida albicans*; 27mm and *Aspergillus brasiliensis;* 16mm. Phytochemical analyses of *Commiphora myrrha* extract showed high concentrations of tannins, isoprenoids and sterols. The FTIR spectrum of *C. myrrha* resin showed the presence of hydroxyl radical which may has a role in its antimicrobial activity. The effects of *C. myrrha* resin extract on the cellular morphology of the tested pathogens were observed using the SEM which revealed that the ethanolic extract of *C. myrrha* resin showed a strong antimicrobial activity. The *C. myrrha* resin extract showed a considerable antibacterial activity against the tested pathogens which give the hope to use it in the future in medical as well as pharmaceutical applications.

***Keywords****: Commiphora myrrha,* molmol, antibiogram assay, pathogenic bacteria.

## 1. Introduction

The *Commiphora* genus belongs to the family *Burseraceae* including over 200 species and native to the seasonally dry tropic of Africa, Saudi Arabia, and India **[1]**.

Saudi Arabia has a wide range of native plants and herbs, known for their great effect in inhibiting and/or killing many bacterial strains that cause many diseases. Jeddah area is too close to the Holy Mosque in Mecca, which is characterized by periodic visitors of large numbers of all nationalities in the seasons of Hajj and Umrah resulting in the spread of many diseases and epidemics which can be dangerous in some cases. Hence this study aimed to evaluate the oleo-gum extract (*Commiphora myrrha*) which is widely spread in the local markets in Saudi Arabia as a natural antimicrobial agent. Higher plants produce broad spectrum of secondary metabolites including polyphenols, tannins, quinones, alkaloids, essential oils, sterols, and saponins etc. **[2]**. *Commiphora myrrha* is one of the most common herbal products that was used in Saudi Arabia for a long period of time. It was massively used in ancient traditional medicine to relief pain, as a disinfectant and antimicrobial agent **[3]**. *Commiphora myrrha*, a yellow fragrant oleo-gum resin, collected from the damaged bark of *Commiphora* genus **[4]**. It is known locally (in Saudi Arabia) as Morr Hijazi or myrrha and commercially as molmol, Arabian myrrha or Karam (True myrrha). In the Kingdom of Saudi Arabia, the plant widely grows at Jizan on the Red Sea coast, a distinct so bare and dry that it is called "Tehama" meaning very hot "hell" **[5]**. Myrrha contains volatile oils, resins and gums which could be hydrolyzed to form arabinose, galactose, xylose, and 4-*O*-methylglucuronic acid **[6, 7]**. It is widely used in medicine as an antiseptic, anti-inflammatory, antipyretic, expectorant, carminative, emmenagogue, antidiabetic **[8]** and antimicrobial **[9,**

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**10].** It is also used as a wash for spongy gums, ulcerated throat and aphthous stomatitis and the tincture is applied to indolent ulcers **[11]**. Myrrha contains myrrin, volatile oil, gum, terpenes, sesquiterpenes which possess mutagenic activity **[12]**, it also contains aldehydes and eugenols that are oxygen radical scavengers and have anti mutagenic and antilipemic potentials in addition to resin acid and protein **[13]**. Three new tetraterpenyl esters which may be used as chromatographic markers for quality control of the drugs and two aliphatic esters have been identified. An extract of myrrha had minimum inhibitory concentrations of 0.18– 2.8µg/ml when used against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* as well as antifungal effect against *Candida albicans* **[14]**. The novelty of the present study was to find alternative antimicrobial agents from plant origin that may be considered as safe, cheap and efficient alternatives to antibiotics to be used in the treatment of infectious diseases.

## 2. Materials and methods

### Collection of plant resins

Oleo-gum resins of *C. myrrha* (used as aromatic plant and in traditional medicine in Saudi Arabia) were collected (August 2019) from wild tree growing in Wadi Noeman at Makkah, Saudi Arabia (21°21'55.98"N 40°11'27.03"E). Prof. Fadl M. (Professor of plant taxonomy at Taief University) kindly identified the tree. The collected samples were deposited in the Herbarium of the Biology Department at Taif University and the ID number for the voucher specimen is: Wadi Noeman, 2019, 10512 (TUH) Roushdy M.M. The criteria for choosing the best and ideal form of Oleo-gum resin of *C. myrrha* included some important characters such as its transparency, color, odor and time of storage. The gum should not be stored more than 3 month and should be transparent with golden to brown yellowish color.

### Preparation of the Extracts

The dried powdered resin (100g) of *C. myrrha* was washed with distilled water and left to dry at 60°C overnight. It was then directly subjected to extraction with ethanol (85%). The dried powdered resin was left in ethanol (500ml) at room temperature for 48 h. After filtrations through a Whatman No. 1 paper, the filtrates were concentrated using rotary evaporator under reduced pressure and controlled temperature, followed by drying at room temperature. A stock solution was prepared by dissolving the dried extract in dimethyl sulfoxide (DMSO 2%, v/v) (Merck, Germany) for phytochemical and antimicrobial analyses.

### Microorganisms

Extract of myrrha resin was evaluated against various pathogenic bacterial strains. The strains used for the antimicrobial assays were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). Gram-Negative strains were *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 27853), *Pseudomonas fluorescens* (ATCC 13525) and *Klebsiella pneumoniae* (ATCC 10031), while the Gram-Positive bacteria comprised *Bacillus subtilis* (ATCC 11774), *Streptococcus pyogenes* (ATCC 12344) and *Staphylococcus epidermidis* (ATCC 12228). The fungal strains used in this study were *Aspergillus niger* (ATCC 16888), *Aspergillus brasiliensis* (ATCC 16404) and *Candida albicans* (ATCC 10231). Bacteria as well as filamentous fungi were cultivated on Mueller-Hinton agar medium and Sabouraud's dextrose agar (SDA) medium, respectively at pH 7.4. The agar plates were incubated at 37°C for 24 h in the case of bacteria and at 28°C for 4 days in the case of fungi. The yeast, *C. albicans* was cultivated on Yeast Peptone Dextrose (YPD) agar at 30ºC.

### Antibacterial assay using agar disc diffusion method

The dried extract of myrrha resin was dissolved in dimethyl sulfoxide (DMSO; 2% v/v). The antibacterial activity of myrrha resin was carried out using agar disc diffusion method **[15]**. Each bacterial strain was firstly cultivated in nutrient broth at 37ºC for 24h. Each bacterial suspension was diluted with nutrient broth to obtain inocula of ~$1x10^6$ CFU/ml **[16]**. One mL of the standardized inoculum of each test bacterium was spread with the help of sterile spreader onto a sterile nutrient agar plate. The plates were allowed to dry. A Whatman No. 1 sterile filter paper disc (6mm diameter) was impregnated with 100µl of (10mg/ml) of the stock solution (myrrha resin dissolved in DMSO; 2% v/v). Preparation of negative controls were carried out using the same solvent evolved to dissolve the samples. Subsequently, the plates were refrigerated for at least 1h for diffusion to take place and then incubated at 37ºC for 24h. Evaluation of antibacterial activity was determined through the measuring of the resulted inhibition zones diameter against the tested bacteria. Three replicates of the experiment were carried out, and the zone of inhibition was measured in mm **[17]**. One hundred microlitres of ciprofloxacin (positive control) and 2% DMSO (Negative or solvent control) loaded onto filter papers were used as positive and negative control, respectively.

### Antifungal assay using agar disc diffusion method

For determining the antifungal activity of the extract of *C. myrrha* resin, the fungal strains were subcultured on Sabouraud's Dextrose Agar (SDA) at 28ºC for 3-4 days. Sterilized SDA plates were taken, and the same steps were carried out, to prepare filter paper disks impregnated with the extract of the same

concentration and allowed to diffuse in refrigerator for one hour before incubation at 28ºC for 3 days. Each fungal suspension was diluted with SD broth to obtain inocula of ~1x10$^3$ CFU/ml **[18]**. Evaluation of antifungal activity was evaluated through the measuring of resulted inhibition zones diameter against the tested fungi. The presence of inhibition zones was recorded as a positive antimicrobial action of the tested extract. *C. albicans* strain was cultivated in Yeast Peptone Dextrose (YPD) agar at 30ºC to determine the activity of the tested extract. The suspension was diluted with YPD broth to obtain inocula of ~1x10$^3$ CFU/ml **[18]**. Three replicates of the experiment were carried out. Fluconazole was used as positive control at 100 µl. On the other hand, 2% DMSO loaded onto filter paper discs was used as negative control.

**Purification and identification of the extracted substance**

*C. myrrha* crude extract was purified using thin layer chromatography (TLC) technique. The extract sample was loaded and eluted with DMSO; 2% v/v. The substance was collected, dried, and the residue was scratched and collected in a clean sterile Stoppard glass container as an amorphous brown powder to be used in MIC, MBC and MFC experiments. Bioautography was used again to confirm the purity of the bioactive substance for spectral IR analysis, which was carried out in National Research Center, Dokki, Giza, Egypt.

**Determination of Minimum Inhibitory Concentration (MIC)**

The previous test cultures were used as test organisms. The minimal inhibitory concentration (MIC) of the active substance was determined by the broth macro-dilution method as recommended by **NCCLS [19]**. Growth inhibition was examined after 24 hours of incubation at 37°C on nutrient broth for bacteria. The same procedure was used for fungi, except that SD and YPD broth were used for filamentous and yeast strains, respectively. All the cultured tubes were incubated at 28°C. The highest dilution of the active agent preventing growth of the test organism was recorded.

**Determination of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)**:

MBC/MFC (minimal bactericidal/fungicidal concentration) was determined by transferring 100 µl of broth from the MIC tubes (the previous test cultures were used as test organisms) with no visible growth to new agar plates (nutrient agar, SDA and YPD for bacterial, fungal and yeast strains respectively) of the respective media. MBC/MFC were tested by sub-culturing the test dilutions onto a fresh solid medium and incubated for 18-48 h. The highest dilution that yielded no bacterial/fungal growth on solid medium was recorded as MBC/MFC **[20]**.

**Phytochemical Screening**

The phytochemical screening of myrrha resin extract was performed to find the presence of active chemical constituents such as sterols, terpenoids (isoprenoids), steroids and tannins. Phytochemical analyses were carried out for myrrha TLC residues using the method described by **Crombie [21]**.

**Cell morphology analyses using Scanning Electron Microscope (SEM)**

The purified myrrha extract (at ½ MIC) was added to YPD broth inoculated with *C. albicans* strain, nutrient broth inoculated with *Bacillus subtilis* and *E. coli* respectively, while the active substance was added to a broth of SD inoculated with the fungal strain *A. niger*. After incubation, the mode of action of myrrha extract on the tested strains (treated) was examined against the tested organisms without addition of myrrha extract (untreated). The scanning electron microscopy (JEOL-JSM-5500 LV) was used, where the samples were coated by gold sputter coat (SPI-Module) and examined by using high vacuum mode. This procedure was carried out at the Regional Center of Mycology and Biotechnology, Cairo, Egypt.

**Statistical analysis**

The obtained data were presented as mean ± standard deviation (SD) of three replicates and then analyzed using the analysis of variance (one way ANOVA) Minitab version 19.

## 3. Results

The ethanolic extract of *C. myrrha* oleo-gum resins showed a promising growth inhibition of the tested pathogens as shown in **Table 1**. The maximum inhibition zones were found against *Escherichia coli* (ATCC 10536) (29mm) followed by *Staphylococcus epidermidis* (ATCC 12228) (28mm), *Bacillus subtilis* (ATCC 11774) (27mm), *Candida albicans* (ATCC 10231) (27mm), *Streptococcus pyogenes* (ATCC 12344) (26mm), *Klebsiella pneumoniae* (ATCC 10031) (25mm), *Pseudomonas aeruginosa* (ATCC 27853) (22mm), *Pseudomonas fluorescens* (ATCC 13525) (21mm), *Salmonella typhimurium* (ATCC 13311) (18mm), *Aspergillus brasiliensis* (ATCC 16404) (16mm) and *Aspergillus niger* (ATCC 16888) (13mm) respectively. The oleo resin extract significantly showed a high activity against all the tested strains ($p$ value <0.05) when compared to the referenced antibiotics (ciprofloxacin for bacteria, and fluconazole for fungal strains)..

**Table 1. *In vitro* antimicrobial activity of *C. myrrha* oleo-gum extract against tested bacteria and fungi**

| Test Species | Zone of inhibition (mm)* | | |
|---|---|---|---|
| | Resin Extract | Ciprofloxacin | Fluconazole |
| *Salmonella typhimurium* (ATCC 13311) | 18.0±0.056$^{f}$ | 16±0.029$^{f}$ | ND$^{d}$ |
| *Escherichia coli* (ATCC 10536) | 29.0±0.044$^{a}$ | 20±0.076$^{c}$ | ND$^{d}$ |
| *Pseudomonas aeruginosa* (ATCC 27853) | 22.0±0.012$^{e}$ | 18±0.088$^{d}$ | ND$^{d}$ |
| *Pseudomonas fluorescens* (ATCC 13525) | 21.0±0.015$^{e}$ | 14±0.009$^{g}$ | ND$^{d}$ |
| *Klebsiella pneumoniae* (ATCC 10031) | 25.0±0.035$^{d}$ | 17±0.053$^{e}$ | ND$^{d}$ |
| *Bacillus subtilis* (ATCC 11774) | 27.0±0.048b$^{c}$ | 20±0.039$^{b}$ | ND$^{d}$ |
| *Streptococcus pyogenes* (ATCC 12344) | 26.0±0.103$^{c}$ | 21±0.011$^{b}$ | ND$^{d}$ |
| *Staphylococcus epidermidis* (ATCC 12228) | 28.0±0.099$^{b}$ | 23±0.047$^{a}$ | ND$^{d}$ |
| *Aspergillus niger* (ATCC 16888) | 13.0±0.056$^{h}$ | ND*$^{h}$ | 12.**0**±0.030$^{c}$ |
| *Aspergillus brasiliensis* (ATCC 16404) | 16.0±0.098$^{g}$ | ND$^{h}$ | 14.**0**±0.077$^{b}$ |
| *Candida albicans* (ATCC 10231) | 27.0±0.022**c** | ND$^{h}$ | 22.**0**±0.021$^{a}$ |

* Concentration of extracts 10 mg/ml (100µg/disc), inhibition zones were the mean of three repl58icates. *ND= Not Detected

The MIC and MBC/MFC for the ethanolic *C. myrrha* extract against the previous test strains were recorded **(Table 2)**. The highest dilution of the active substance (MIC) of *C. myrrha* extract that prevented growth of *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 27853), *Pseudomonas fluorescens* (ATCC 13525), *Klebsiella pneumoniae* (ATCC 10031), *Bacillus subtilis* (ATCC 11774), *Streptococcus pyogenes* (ATCC 12344), *Staphylococcus epidermidis* (ATCC 12228) and *Candida albicans* (ATCC 10231) was 15.6µg/ml, while the rest of the test strains recorded 31.25, for *Salmonella typhimurium* (ATCC 13311) and *Aspergillus brasiliensis* (ATCC 16404) and 62.5 for *Aspergillus niger* (ATCC 16888). The lowest dilution of the active substance (MBC/MFC) that killed bacteria and fungi (no growth in the subculture) was 31.25µg/ml recorded for *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 27853), *Pseudomonas fluorescens* (ATCC 13525), *Klebsiella pneumoniae* (ATCC 10031), *Bacillus subtilis* (ATCC 11774), *Streptococcus pyogenes* (ATCC 12344), *Staphylococcus epidermidis* (ATCC 12228), *Candida albicans* (ATCC 10231) *and Aspergillus brasiliensis* (ATCC 16404), while the rest of the test strains recorded 62.50 and 500µg/ml for *Salmonella typhimurium* (ATCC 13311) and *Aspergillus niger* (ATCC 16888) respectively.

Subsequently, resins of *Commiphora myrrha* were phytochemically analyzed **(Table 3)**. The obtained results revealed the presence of high concentrations of tannins, sterols, and Isoprenoids (Terpenoids) while steroids were found in moderate concentrations.

The FTIR spectrum of *C. myrrha* resin (**Fig. 1**) showed the presence of broad bands located at 3450, 1630, and 1550cm$^{-1}$ which resemble the stretching of OH, COOH, and C=C as well as the shifting of C=O vibration of COOH groups. An intense peak at 1630cm$^{-1}$ represents the molecular vibration of myrrha resin, the bands at 2930cm$^{-1}$ seem to be analogous to the asymmetric stretching of the C–H bonds; the broad band at 3440cm$^{-1}$ could be attributed to the stretching vibrations of various groups in alcohols (OH).

The effects of *C. myrrha* resin extract on the cellular morphology of Gram-positive bacteria (***Bacillus subtilis*, ATCC 11774)**, Gram-negative bacteria (*Escherichia coli*, ATCC 10536), fungi (*Aspergillus niger*, ATCC 16888) and yeast (*Candida albicans*,

ATCC 10231) during their logarithmic growth phase as observed under the SEM were shown in **Figures 2 to 5**. SEM micrographs of untreated *Bacillus subtilis* cells **(Fig. 2A)** were bacilli in shape with smooth surface. The exposure of the bacterial cells to the *C. myrrha* resin extract at ½ MIC showed obvious change **(Fig. 2B)**, where bleb-like structures were observed on the surface of some cells.

**Table 2. MIC and MBC/MFC values for ethanolic *C. myrrha* extract against susceptible strains**

| Test Species | *C. myrrha* extract | | Ciprofloxacin | | Fluconazole | |
|---|---|---|---|---|---|---|
| | MIC (µg/ml) | MBC/MFC (µg/ml) | MIC (µg/ml) | MBC (µg/ml) | MIC (µg/ml) | MFC (µg/ml) |
| *Salmonella typhimurium* (ATCC 13311) | 31.25±0.00 | 62.50±0.09 | 15.60±0.032 | 31.25±0.055 | ND* | ND |
| *Escherichia coli* (ATCC 10536) | 15.60±0.01 | 31.25±0.00 | 15.60±0.033 | 31.25±0.086 | ND | ND |
| *Pseudomonas aeruginosa* (ATCC 27853) | 15.60±0.015 | 31.25±0.002 | 15.60±0.046 | 31.25±0.093 | ND | ND |
| *Pseudomonas fluorescens* (ATCC 13525) | 15.60±0.014 | 31.25±0.003 | 15.60±0.087 | 62.50±0.018 | ND | ND |
| *Klebsiella pneumoniae* (ATCC 10031) | 15.60±0.017 | 31.25±0.002 | 15.60±0.10 | 62.50±0.090 | ND | ND |
| *Bacillus subtilis* (ATCC 11774) | 15.60±0.011 | 31.25±0.001 | 15.60±0.099 | 31.25±0.022 | ND | ND |
| *Streptococcus pyogenes* (ATCC 12344) | 15.60±0.011 | 31.25±0.005 | 15.60±0.085 | 31.25±0.045 | ND | ND |
| *Staphylococcus epidermidis* (ATCC 12228) | 15.60±0.018 | 31.25±0.004 | 15.60±0.076 | 31.25±0.002 | ND | ND |
| *Aspergillus niger* (ATCC 16888) | 62.50±0.098 | 500.0±0.043 | ND | ND | 62.50±0.098 | 500.0±0.040 |
| *Aspergillus brasiliensis* (ATCC 16404) | 31.25±0.001 | 31.25±0.004 | ND | ND | 62.50±0.076 | 500.0±0.074 |
| *Candida albicans* (ATCC 10231) | 15.60±0.011 | 31.25±0.002 | ND | ND | 31.25±0.028 | 500.0±0.088 |

*ND: Not detected.

**Table 3. Phytochemical screening of *Commiphora myrrha* oleo-gum resins specimens**

| Constituents | Level* |
|---|---|
| Terpenoids | +++ |
| Sterols | +++ |
| Steroids | ++ |
| Tannins | +++ |

*++: Medium concentration, +++: High concentration,

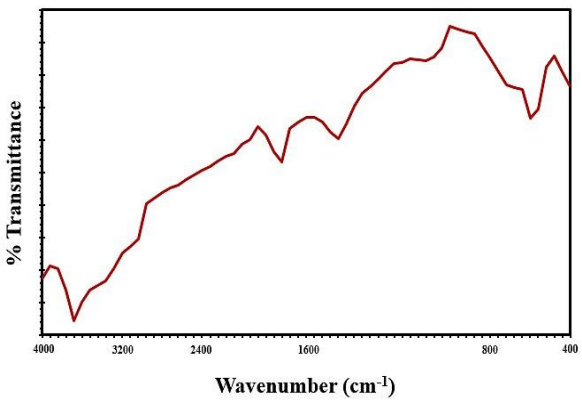


**Figure 1. FT-IR spectrum of the myrrha extract**

On the other hand, exposure of Gram-negative (*Escherichia coli*, ATCC 10536) bacterial cells to the *C. myrrha* resin extract at ½ MIC had led to some morphological changes **(Fig. 3).** As shown in **Figure 3B** (treated cells with myrrha extract), there was obvious cellular deformation with the appearance of holes (pores) on the surfaces of some cells, unlike the normal structures depicted in **Figure 3A** of the bacterial cells where the cells appeared as short rods with smooth surfaces.

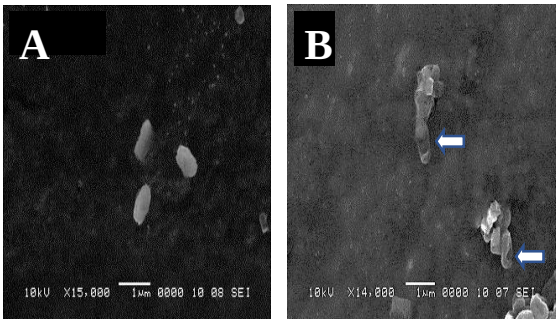


**Figure 2: Scanning electron images of *Bacillus subtilis* (ATCC 11774) treated with the *C. myrrha* resin extract at ½ MIC. A= Untreated bacterial cells were typically Bacilli in shape (magnification 15.000 times); B=The cells treated showing lumpy surface (magnification 14.000 times) (see arrows).**

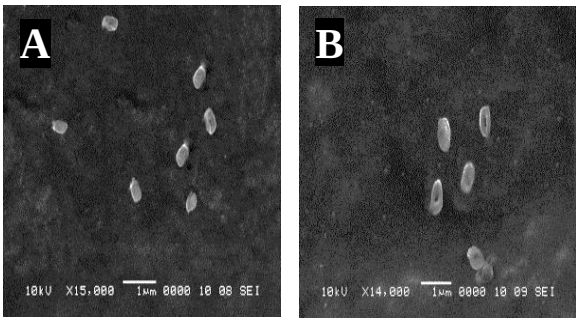


**Figure 3: Scanning electron images of *Escherichia coli*, ATCC 10536 treated with the *C. myrrha* resin extract at ½ MIC. A= Untreated bacterial cells were typically short rods in shape (magnification 15.000 times); B=Some cells treated with the *C. myrrha* resin extract showing pores in their surfaces (magnification 14.000 times) (see arrows).**

The effect of myrrha extract on the morphology of *A. niger* (ATCC 16888) was carried out by inoculating the fungus on SD amended with ½ MIC. All tubes were incubated at 28ºC for 48 hours and the growth was checked then examined by SEM (**Fig. 4**). Scanning Electron micrograph (**Fig.4B**) showed obvious deformations in the fine structure of the *A. niger* spores when compared to the normal fungal spores (**Fig.4A**). The *C. myrrha* resin extract affected the external layer of spore and as a result may have affected the inner layer. To explain this result, it is thought that these spores lost their viability, where the micrograph shown in Fig.(4B) exhibited damage of the fungal spores by spore disintegration or disruption. This result supports the idea that *C. myrrha* resin extract degraded the outer layers of fungal spores which exposed the spore core to the action of active substances of the plant extract.

Obviously, the cells were drastically destroyed when treated with *C. myrrha* resin extract and observable changes in the membrane of *C. albicans* clearly appeared after treatment. The treated cells showed aggregation of the outer membrane and their shape became irregular (**Fig. 5B**) compared to the untreated cells (**Fig. 5A**).

Subsequently, visible deformation, protrusion, or clumping was noted after treatment. The mechanism by which the ***C. myrrha* resin extract** caused an obvious change in the shape of *C. albicans* cells may be related to the ability of tannins to weaken the membrane of the treated cells. All these results are promising to use the pure ***C. myrrha*** resin extract as antimicrobial agent or even as food preservative against several microbial and food pathogens**.** Information of ultrastructural changes inside the cells is also useful to give a better understanding of correlation between ultrastructural changes and the mechanisms of antibacterial, antifungal and anticandidal activities that causes biochemical alternations within the cells.

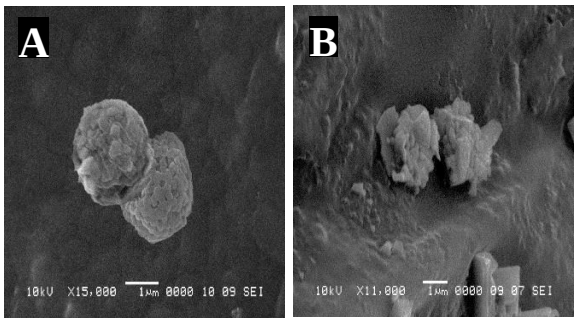


**Figure 4: Scanning electron micrograph of *A. niger* (ATCC 16888) treated with the *C. myrrha* resin extract at ½ MIC. A= Untreated, the fungal spores are normal and intact (magnification 15.000 times); B= Treated with the *C. myrrha* resin extract, the fungal spores are damaged by spore disruption or disintegration leading to leakage of the spore contents (magnification 11.000 times).**

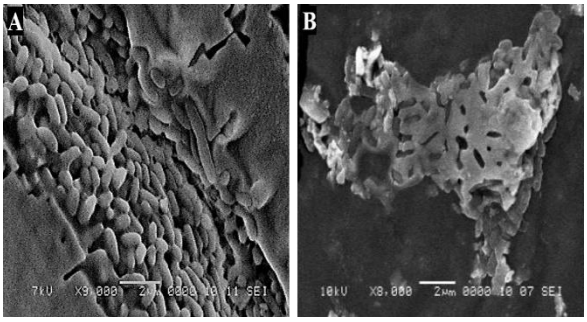


**Figure 5: Scanning electron micrograph of *C. albicans* (ATCC 10231) treated with the *C. myrrha* resin extract at ½ MIC. A= Untreated, the cells had well defined, intact shapes with smooth surfaces (magnification 9.000 times); B= Treated with the *C. myrrha* resin extract, the cells were starting to show collapse of the outer membrane and their shape was becoming irregular (magnification 8.000 times).**

## 4. Discussion

The present study aimed to determinate the effects of *C. myrrha* resin extract on the cellular morphology of Gram-positive bacteria (***Bacillus subtilis*)**, Gram-negative bacteria (*Escherichia coli*), fungi (*Aspergillus niger*) and yeast (*Candida albicans*). Generally, as mentioned by **Grove and Randall [22]** and **Saadabi [23]**, the plant extracts are considered as antibacterial and antifungal when the inhibition zone is greater than 6 mm. The ethanolic ectract of *C. myrrha* oleo-gum resins showed a promising inhibition of the growth of the tested pathogens. Solubility of *C. myrrha* oleo-gum resins in ethyl alcohol is in accordance to **Hanus [6]** who stated that myrrha has a solubility in ethanol. The antibiogram of *C. myrrha* resin extract towards the bacterial and fungal strains under investigation is in agreement with the results obtained by **Omer [9]** and in **Alhussaini**

**[24]**. MIC, MBC and MFC were caried out for all of the tested microorganisms. The results revealed that the ethanolic extract of *C. myrrha* resin showed considerable activities against the tested pathogens even at lower concentrations. Microbiological techniques that are commonly used to evaluate the bactericidal or fungicidal activity of antimicrobial agents include the MBC or MFC **[25, 26]**. Subsequently, resins of *C. myrrha* were phytochemically analyzed. The obtained results represented the presence of high concentrations of tannins, sterols, and Isoprenoids (Terpenoids) while steroids were found in moderate concentration. Accordingly, **Hanus [6]** mentioned that 41 compounds were identified in myrrha which were mainly sesquiterpenoids**.** Myrrh has many effective medicinal uses and has been used to cure tumors, fever, disorders of gall bladder, dysmenorrhea, and skin infections **[27, 28]**. Many previous investigations have demonstrated the presence of many phytochemicals possess different biological constituents as flavonoids, terpenoids, carbohydrates, lignans, steroids, and others **[29]**. **Ahmad [30]** also studied the phytochemical composition of myrrha resin. He stated that myrrha contains sesquiterpenoids which possess antibacterial, antifungal, and anesthetic activities. High total tannin content in the *C. myrrha* extract suggests that it may be responsible for the antimicrobial properties against several microorganisms **[31, 32]**. The FTIR spectrum of *C. myrrha* resin showed the presence of broad bands located at 3450, 1630, and 1550cm$^{-1}$ which resemble the stretching of OH, COOH, and C=C as well as the shifting of C=O vibration of COOH groups. An intense peak at 1630cm$^{-1}$ represents the molecular vibration of myrrha resin **[33]**, the bands at 2930cm$^{-1}$ seem to be analogous to the asymmetric stretching of the C–H bonds **[34]**; the broad band at 3440cm$^{-1}$ could be attributed to the stretching vibrations of various groups in alcohols (OH) **[33]**. **Chen [35]** stated that, the spectral bands of a volatile compound will decrease synchronously if this compound evaporates when the sample is heated. Previous studies have shown that Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are susceptible towards both methanolic and aqueous *Quercus infectoria* (QI) gall extracts **[36, 37, 38]**. The mechanism of action of *C. myrrha* resin extract on *E. coli* cells may be explained as previously mentioned by **Abd [39]** who studied the effect of *Quercus infectoria* (QI) gall methanolic extract on the morphology of *Proteus vulgaris* as Gram negative bacteria where he found some morphological abnormalities such as cell lumpiness and loss of appendages which were clearly observed on the treated *P. vulgaris* cells and subsequently affected their activity which led to defective cell wall and finally cell death.

Accordingly, **Xiong [40]** proved that *B. subtilis* cells treated with 1-octen3-ol (a natural product was first isolated from mushroom and thereafter from other fungi and plants) at MIC caused a severe destruction on the cell morphology, showed an irregular coarse, wrinkled outer surface of the tested cells.

This may confirm the idea that 1-octen-3-ol treatment may result in damage to the bacterial cell wall and cytoplasmic membrane.

In accordance to similar studies, **Khayyat and Al-Kattan [41]** revealed that the *Microsporum gypseum* hyphae and spores were damaged and the morphological shape was changed when the fungus was treated by essential oil extract of Indian Costus and Sea-Qust roots. Other study carried out by **Elsayed and Shabana [42]** revealed that the treatment of *A. niger* by camphor oil (40%, v/v) caused some malformation and eruption of the fungal spores of compared to the oil-free control fungus. This result may be contributing to the suggestion of **Ahmad [30]** who studied the phytochemical composition of myrrha resin and he revealed that myrrha contains sesquiterpenoids which possess antifungal activities. SEM images of *C. albicans* (ATCC 10231) cells untreated and treated with ***C. myrrha*** **resin extract** revealed ultrastructural changes in the *C. albicans* due to the action of the plant extract.

Untreated cells showed well defined, intact shapes with smooth surfaces. On the other hand the treated cells showed considerable morphological changes including shrinkage and deformation. Obviously, the cells were drastically destroyed when treated with ***C. myrrha*** **resin extract** and observable changes in the membrane of *C. albicans* clearly appeared after treatment**. The treated** cells showed aggregation of the outer membrane and their shape became irregular compared to the untreated cells. Subsequently, visible deformation, protrusion, or clumping was noted after treatment. This result is in agreement with **Abd [39]** who studied the effect of *Quercus infectoria* (QI) gall methanolic extract on the morphology of *C. albicans.* He stated that the effect of QI appeared destructive as observed under the SEM with the presence of the un-dividing cells, and biconcave form on the cell surface. The mechanism by which the *C. myrrha* resin extract caused an obvious change in the shape of *C. albicans* cells may be related to the ability of tannins to weaken the membrane of the treated cells as mentioned before by **Suwalak and Voravuthikunchai [43]**. All these results are promising to use the pure ***C. myrrha*** resin extract as antimicrobial agent or even as food preservative against several microbial and food pathogens. However, further studies are required to improve the findings by comparing the effects of ***C. myrrha*** **resin** extract on the morphology of Gram-positive bacteria, Gram-negative bacteria, fungi and yeast using Transmission electron microscope (TEM).

## 5. Conclusions

The antimicrobial activities of *C. myrrha* resin extract were evaluated in this study against Gram-positive bacteria, Gram-negative bacteria, fungi and yeast. It showed a considerable antibacterial activity against the tested bacteria, and it can also inhibit fungal growth as well as spore germination. SEM observations suggested that *C. myrrha* resin extract exerted its antibacterial effect by affecting the permeability of the cell membrane which leads to leakage of some cellular components as well as subsequent cellular deformations. Comparative analyses found that the hydroxyl radical in the plant extract may play an important role in its antimicrobial activity. In conclusion, *C. myrrha* resin extract has a promising action to be used as antimicrobial agent. Further studies are required to fully understand the antimicrobial efficacy of *C. myrrha* resin extract, such as its effect against other pathogenic bacteria and fungi, and its antifungal mechanism, in order to promote its application in the future.

## 6. Conflict of interest

The authors have no conflict of interest

## 7. Acknowledgments

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