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Potential scavenging, antimicrobial activities and total phenolic content achieved by resins for five species of *Commiphora*

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Abstract

In this study, we will study the biochemical characters of the resins for the different species of *Commiphora* genus through investigation of the *in vitro* anti-oxidant activity of the crude extracts of resins for five species of *Commiphora*. The free radical scavenging activity will be measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Also the total phenolic content was evaluated according to the Folin-Ciocalteu procedure. On the other hand the determination of the inhibitory effects of each of the extracts on the growth of selected bacterial and fungal was investigated on the pathogenic selected species. The results of this study are aiming to help in the development of biochemical analysis of resins which contributed to distinguish between plant species, to employ the plant extracts in the medical field and the possibility of their use in the treatment of many microbial diseases.

Keywords: Commiphora, resins, DPPH, antimicrobial and total phenolic content

Introduction

Genus *Commiphora* is one of the "Burseraceae" species that includes 190 species, widespread in tropical regions of the world, in Africa, Madagascar, the Arabian Peninsula, India, and America. It is pointed out that the existence of new varieties of species *Commiphora* was not at hand for study and comparison (Collenette, 1985).

The genus *Commiphora* "Burseraceae" forms a major part or the dominant vegetation in the western coastal plains and regions under the mountains of Saudi Arabia (Chaudhary, 2001). The family "Burseraceae" includes two important species- *Boswellia sacra* and *Commiphora myrrha*, the source of frankincense or the luban and myrrh, respectively. It can be said that these two plants are at the heart of the different layers and shaped the past course of civilization in the Middle East and Europe at least. *Boswellia sacra* the source of the true frankincense is found in the southern parts of the Arabian Peninsula, specifically Dhofar and does not occur in Saudi Arabia. The genus *Commiphora* however, is represented by 6 or 7 species and is a very prominent component of the Saudi Arabian flora in the western parts, particularly the southwest (Chaudhary and Al-Juwaid, 1999).

Incense ornamental trees, are used as gums and incense, pasture for camels and sheep, feed on them and increase milk. Wood is taken from their huge trunks, and miswak (toothpick) from their tender trunks which purifies the mouth, and make the smell beautiful. Mugs, spoons, pots are made from its woods, and used its leaves to dye and perfume hair.

Resin is used as incense and flavor for foods to get nice smell and delicious taste. The aqueous resin is used as a quick impact vaccine for deadly snakes' poisons, and effective treatment for stomach ulcers, and mixed with oil for the treatment of joints pain, diseases of the chest and throat, and diseases of the abdomen, and is used as an incense for women in puerperium period accelerating the cure. All resin cleans wounds, heals them, and mixed with honey to treatgangrene, so the patient is cured with God willing (Gushash, 2006).

Major components found in plants,with antimicrobial activity are phenolic compounds, terpenes, aliphatic alcohols, aldehydes, ketones, acids, and isoflavonoids. As a rule, it has been reported that the antimicrobial activity of essential oils depends on the chemical structure of their components and on their concentration. Essential oils of a large number of plants possess useful biologic and therapeutic activities, and the oils are extensively utilized in the preparation of pharmacologic drugs. They are commercially recovered from plant materials primarily by steam distillation, and their use in the food industry is influenced by the nature of their constituents (Alzamora *et al.* 2000).

Plants provide a rich source of natural antioxidants. These include vitamin C, carotenoids, and phenolic compounds. Thus, plants use a myriad of antioxidant compounds to deal with these in order to survive. Many of these compounds have basic molecular similarities in that all have at least one aromatic ring and a hydroxyl group. These include phenolic acids, flavonoids and isoflavones, gallate esters (hydrolysable tannins), lignans, flavonones, and oligomeric proanthrocyanidins (Shahidi, 1997).

Materials and Methods

Plant materials

Several field trips have been made to the study area (Al-Baha region) in different seasons (December 2010, November 2011, March 2012) to collect *Commiphora* species growing naturally in this area, take into consideration the period of flowering and fruiting plants and to follow the spread and growth of *Commiphora* plants widely and diversified in the region (Figure: 1)

C. habessinica ***C. kataf*** ***C. gileadensis*** ***C. myrrha*** ***C. quadricincta***

Figure 1: Resin in *Commiphora* species

By referring to the references that dealt with the "Flora of the kingdom of Saudi Arabia" and its wild plants including *Commiphora* genus: (Collenette, 1985, Migahid, 1988, Chaudhary and Al-Juwaid, 1999, Chaudhary, 2001), we can identify five species of *Commiphora* growing naturally in the study area merge between trees and shrubs.

Preparation of resin extracts

5 g of each resin species underwent extraction in a conical flask.Absolute ethanol (99.5 %), methanol (HPLC) grade & petroleum ether 60-80 °C PRS are used as a solvent. Approximately 20 ml of each solvent is added to the flask and covered with par film. The flask is left over night at ambient temperature (25°C) for 24 hours, followed by filtration by Whatman filter paper (No.1) into test tubes. The extraction process is repeated to ensure maximal extraction.

DPPH free radical and scavenging activity

The hydrogen atom or electron donation ability of the corresponding extracts is measured from the bleaching of purple colored methanol solution of DPPH (Cuendet *et al*. 1997 & Burits and Bucar, 2000).

The DPPH radical is a stable free radical and the DPPH radical-scavenging activity was determined by the decrease in absorbance at 517 nm, due to reduction by the antioxidant (AH) or reaction with a radical species, as shown in Eqs. (1) and (2):

$$DPPH^{\cdot} + AH \longrightarrow DPPH — H + A^{\cdot} \quad \text{Eq (1)}$$
$$DPPH^{\cdot} + R^{\cdot} \longrightarrow DPPH — R \quad \text{Eq (2)}$$

50 µl of plant extract, are added to 5ml of 0.004% methanolic solution of DPPH.
After for 50 min of incubation at dark, the absorbance is read against a blank at 517 nm.
Inhibition free radical DPPH in percent (*I* %) was calculated as in Eq (3):

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100 \quad \text{Eq (3)}$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test sample.

Determination of total phenolics content

The total phenolic content in the plant extracts is determined by using Folin-Ciocalteu reagent as described by Gulluce *et al*. (2005).One ml plant extract is added to 10 ml deionized water and 2.0 ml Folin-Ciocalteu reagent. The mixture is then allowed to stand at room temperature for 5 min, and 1.5 ml sodium carbonate (2%, w/v) is added. Reaction mixture is further left at room temperature for 2 h with intermittent shaking, then the absorbance is measured at 765 nm using a spectrophotometer (UV-model PD-303 UV Spectrum).

The concentration of total phenolic compounds in all plant extracts is expressed as milligram of gallic acid equivalents per gram wet weight of plant.

The linear Eq (4) in derived from Eq (5) is determined from known concentration of gallic acid standard similarity. Data are reported as a mean ± standard deviation for three replicates.

Absorbance (at 765 nm) constant × (gallic acid concentration) Eq (4)

Gallic acid equivalents = Absorbance × (at 765 nm)/0.0508 Eq (5)

Antimicrobial activity

Plant extracts are individually tested against a panel of gram positive *(Bacillus subtilis* and *Staphylococcus aureus*) -negative bacterial *(E. Coli* and *Pseudomonas*) and fungal pathogens (*Candida albicans* and *Aspergillus niger*) Antimicrobial tests are then carried out by the agar well diffusion method (Perez *et al*. 1990) using 100µl of suspension containing 10^8CFU/ml of pathological tested bacteria spread on nutrient agar.

Results

DPPH free radical scavenging activity

In this study it was found that crude extracts using three types of solvents (Ethanol, Methanol, Petroleum Ether) for the resins of five species of *Commiphora*genus showed considerable free radical scavenging activities (Figure 2), the data indicated that methanol solvent is the strongest one.

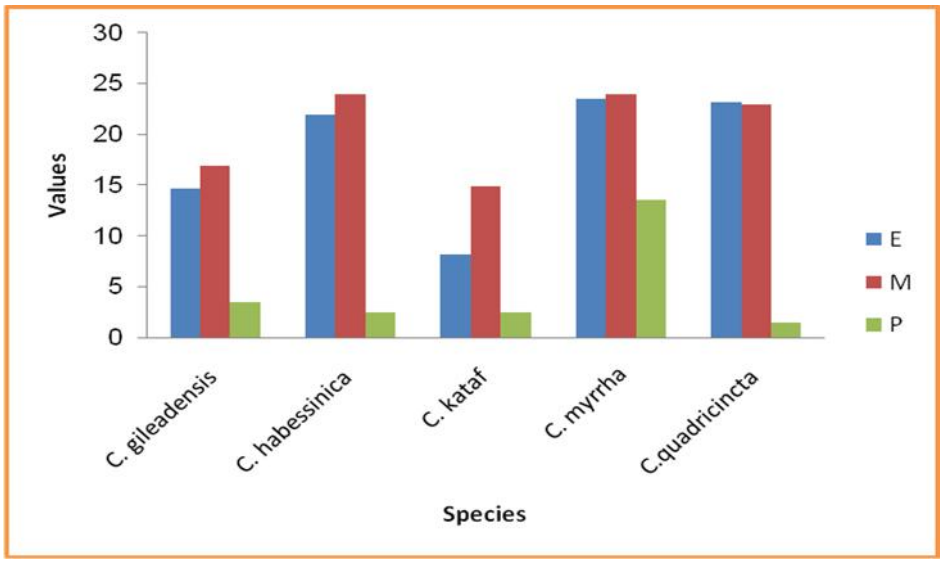

Figure 2: Radical scavenging capacityof each of the *Commiphora* species (resin) extracts.
*** E** = (Ethanol), **M** = (Methanol), **P** = (Petroleum Ether).

In general, the methanol extracts of *Commiphora*, displayed antioxidant activity in the DPPH assay with values ranging between 5.64-23.92 %. It was found the resin of showed the highest antioxidant activity of *C. habessinica*, *C. myrrha* 23,92% respectively and *C. quadricincta* 22.98%. While the medium values were recorded of the species *C. gileadensis* and *C. kataf* 16.93%, 14.91% respectively (Figure2).

Determination of total phenolics content

The total phenolic contents in extracts using three types of solvents (Ethanol,Methanol,Petroleum Ether)of the resins of five species of *Commiphora* genus in units of mg gallic acid equivalent of phenolic compounds as shown in (Figure 3), the data indicated that methanol solvent is the strongest one. And also, it will be our dependence on the resulting data from methanol extracts of the resins of five investigated species in *Commiphora* genus. The methanol extracts of *Commiphora*, displayed total phenolicscontent with values ranging between 14.71-98.17 %.

The total phenolics content of resin of the species *C. kataf* 50.07%, *C. gileadensis* 48.88%, *C. quadricincta* 45.32%, and *C. habessinica*, *C. myrrha* 44.77% (Figure 3).

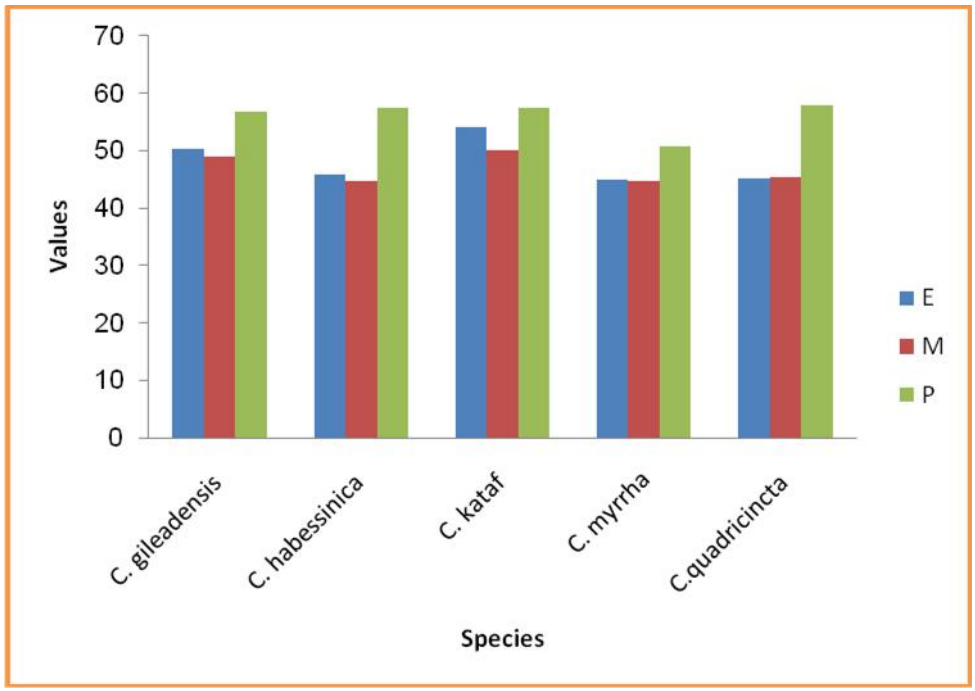

Figure 3: Total phenolic content of each of the *Commiphora* species (resin) extracts.
*** E** = (Ethanol), **M** = (Methanol), **P** = (Petroleum Ether).

Antimicrobial activity

The antimicrobial activities in extracts of the resins of five species of *Commiphora* genus against target pathogens were examined qualitatively and quantitatively by the presence or absence of inhibition zones and zone diameter.The results obtained for the pathogensbacteria kinetics endangered to *Commiphora* genus extracts are shown, the antibacterial activity was observed to begin after approximately 30 min of the exposure of bacterial to the different concentrations of resins extract. This observation was made through the reduction in colony forming units over time. All concentrations exhibited antibacterial activity, with a complete bactericidal effect being achieved by all test concentrations by the 24th hour. It was noted that by the 48th hour by fungal, re-growth had begun in the two lowest test concentrations.Results showed that most *Commiphora* genus extracts have antimicrobial activity against the tested pathogenic bacteria (gram positive-negative) and fungal (Figure 4).

Antimicrobial activity against Gram-negative bacteria was shown to be less, and the least sensitivity towards the extracts effective than the activity against Gram-positive bacteria).

The strongest activity against *Candida albicans* were from *C. kataf*, *C. myrrha*, and *C. quadricincta*. Greater activity was observed against *Bacillus subtilis*, when compared to activity against *E. Coli*, with activities ranging between 16-32 mm (Figure 5). The sensitivity with respect to *Staphylococcus aureus* and *Pseudomonas* among the resin species, indicating poor antimicrobial activity, with the exception*C. Myrrha* against *Pseudomonas* (Figure 6)

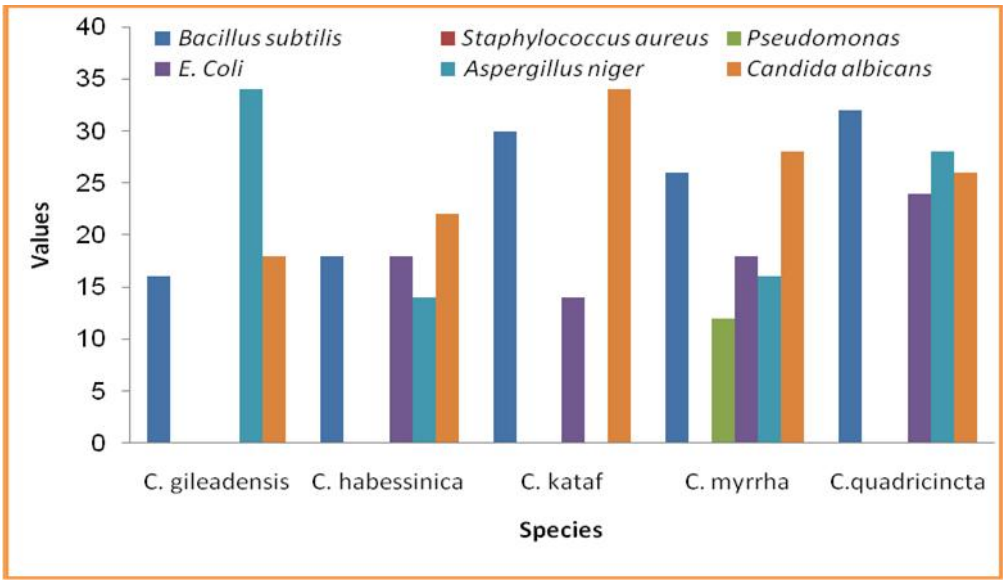

Figure 4: Antimicrobial activity of each of the *Commiphora* species (resin) extracts

C.m

C.q
C.k

C.gCh

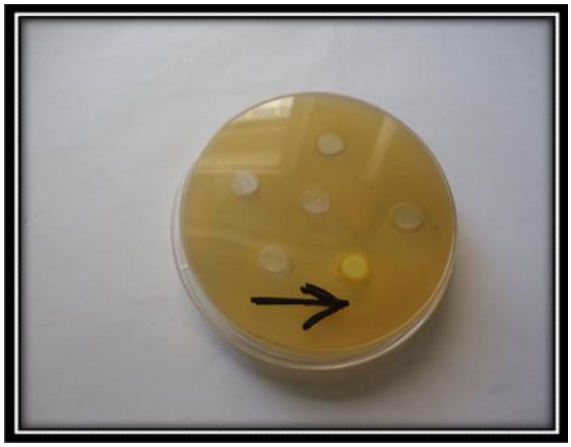

Figure 5: Antimicrobial activity of the five species *Commiphora* (resin) extracts against on *Candida albicans.*

C.qC.k

C.gCh

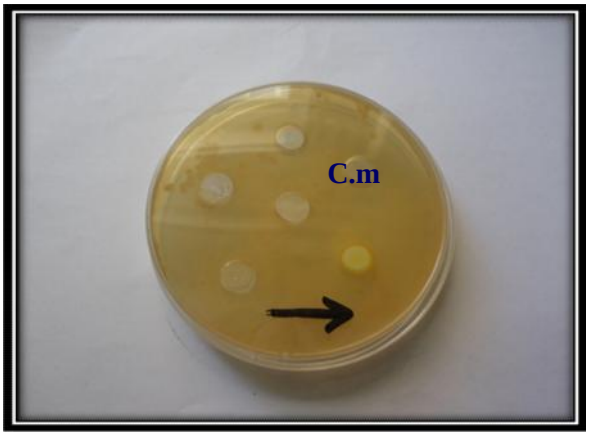

Figure 6: Antimicrobial activity of the five species *Commiphora*(resin) extracts against on gram-negative bacteria (*E. Coli).*

Discussion

In this research we have studied five plant species belonging to *Commiphora* "Burseraceae" that grow in Al-Baha region Kingdome of Saudi Arabia using the biochemical characters serves as a preliminary screening. The process of the DPPH free radical scavenging activity, determination of total phenolics content and antimicrobial activity of the extracts *Commiphora* genus may not directly correspond of all the results obtained in the independent assays, as there is a definite concentration difference between the extracts analysed by biochemical assays and dependence on the resulting data from methanol extracts employed in the assays, because it's strongest one.

In this study, the DPPH method was selected to evaluate the antioxidant activity of the five investigated species in *Commiphora* genus because it is one of the most effective methods for evaluating the concentration of radical-scavenging materials active by a chain-breaking mechanism.

The DPPH radical has a deep purple color and absorbs strongly at a wavelength of 517 nm. The radical scavenging potential of *Commiphora* species.

Upon comparison of the anti-oxidant activity, as determined by the DPPH assay, it was observed that resin extracts, with the exception of *C. gileadensis*, *C. habessinica* and *C. Quadricincta* (stem), *C. habessinica*, *C. myrrha* and *C. quadricincta* (resin).

The radical scavenging potential against the DPPH organic radical directly depends on the number of hydroxyl groups, with an increase in the number of hydroxyl groups resulting in an increase in radical scavenging activity (Rusak *et al*. 2005). The mechanism by which DPPH is scavenged, aids in elucidating the structure-activity relationship (SAR) of the antioxidant, and, in so doing, may be beneficial in the rational design of novel anti-oxidants with improved pharmacological profiles (Wangand Zhang, 2003).

Factors contributing to this activity variation may include the quantity of molecules available to react or the presence of molecules acting antagonistically to those molecules that are available with a greater scavenging potential.

Phenolic compounds exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydro-peroxides into free radicals.

The Folin-Ciocalteu method is a rapid and widely-used assay, to investigate the total phenolic content but it is known that different phenolic compounds have different responses in the Folin-Ciocalteu method.

The determination of total phenolics content include substances such as tannins, flavonoids, tocopherols, and catecheses. Tannins are, at least in part, responsible for the strong free radical scavenging activities working synergistically with other anti-oxidant substances. Organic acids and protein additionally act as anti-oxidants (Dapkevicius *et al*.1998).

The phenomenon that the resin extracts demonstrated substantial total phenolic content capacity is not completely surprising, in light of the expectancy of the high radical scavenging. Limited (TPC) was displayed by *C.gileadensis* (leaf)and *C.kataf* (stem), with 14.71, 98.17 values of mg/g dw % respectively, the phenolic content of *C.kataf* (stem) was very high, compared to that obtained from *C. gileadensis*, *C.kataf* (leaf) and *C.myrrha* (stem), 14.71, 17.40,17.64values of mg/gdw % respectively.

This research was conducted specifically to determine the in *vitro* antimicrobial activity of indigenous *Commiphora* species, and to determine whether the therapeutic properties of some of the species used in traditional medicine correlates with laboratory-generated findings.

Resin extracts with the strongest antimicrobial activity of the plant extracts among the resin species exhibited poor and unvaried activity against *staphylococcus aureus* and *pseudomonas*, indicating the resistance of this bacterium to the plant resin extracts.

The lipophilic or hydrophilic nature of compounds also plays a role in the activity, or lack thereof, against the micro-organisms. Compounds considered to be more effective against Gram-negative bacteria are considerably less lipophilic (Denyer &Maillard. 2002). This is as a result of the structure of the Gram-negative cell wall (Figure7), which also has higher lipid content (Linfield *et al*. 1982). Interactions of lipophilic compounds with hydrophilic parts of the membrane will bring about a more toxic effect against the micro-organism (Sikkema *et al*. 1995).

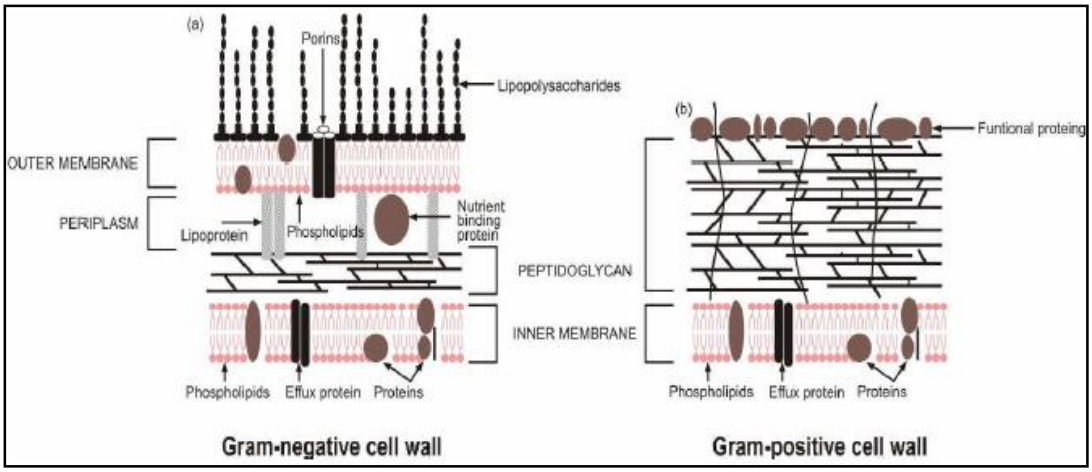

Figure 7: The comparative structural complexity of the outer membranes and cell walls of Gram-negative and Gram-positive bacteria.

The fact that many harmful agents, are including antibiotics are either hydrophobic or relatively large hydrophilic compounds, and thus hardly able to penetrate the outer membrane. It has also been suggested that the polysaccharide constituents of the outer membrane aid the bacterial cell in evasion of phagocytosis and protect the deeper parts of the outer membrane from complement and antibody binding (Vaara, 1992). In comparison, Gram-positive bacteria possess a much thicker peptidoglycan layer, which does not act as an effective barrier to permeation, and inhibitors are thus able to pass through more easily (Scherrer and Gerhardt, 1971). The current results are consistent with the pattern of in vitro activity emerging from other studies.

The results obtained in this study demonstrates the importance of investigating natural products for antimicrobial activity against fungal such as *Candida albicans* and *Aspergillus niger*. The screening of *Commiphora* species, both in the present results and in those conducted on non-indigenous species (Fatope *et al*. 2003, Claeson *et al*. 2003&Tipton *et al*.2003) has yielded fairly strong antimicrobial activity against both *Candida albicans*, and stated that *Aspergillus niger* produces butyrolactone derivatives which have a strong antimicrobial activity.

On the other hand, *Candida albicans* is an opportunistic pathogen able to cause both systemic and local fungal infections, especially in patients with compromised immune systems and those undergoing antibiotic therapy over extended periods of time(Duarte *et al*.2005).There thus exists an ever-increasing need for the development of novel and improved drugs for the management of fungal infections.

Antimicrobial activity may involve complex mechanisms, like the inhibition of the synthesis of cell walls and cell membranes, nucleic acids and proteins, as well as the inhibition of the metabolism of nuclide acids (Oyaizu *et al*. 2003). Taking into consideration the properties of the organic solvent used for the extraction, the extract seams to contain diverse substances, ranging from non-polar to polar compounds.

Conclusions

The current study included five resin species belonging to the genus *Commiphora* in Al-Baha region of Saudi Arabia. We can surmise some of the recommendations that we have reached through the morphological and biochemical study:

Plant *Commiphora* is one of the old Albkhurih trees and which has been used millions of years ago for the Pharaohs and the Greeks in worship and religious rituals, which draws attention to take advantage of this plant such as economic sales and aromatic extracts in the age of harvested high side of the national economy.

We recommend doing medical research on this genus until utilizing in extracting and pharmaceutical industry for its contribution in the treatment of many skin diseases, eczema, gangrene, the branches of the genus *Commiphora* are used as mouth antiseptic killing bacteria, and particles emanating from the incense leading to kill germs and clean the skin, and powder stems of *Commiphora* trees was placed on the hair as an accessory because of its impact for the protection and abundant hair.

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