Anti-bacterial Activities of Crude extracts of Some East African Oleo gum resins *(Burceraceae*) and Their Respective Extraction Yield

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***Abstract*—**The present study is focus on anti-bacterial effects of methanol, acetone and hexane crude extracts of *Boswellia-frereana, Boswellia-carterii*, and *Commiphora-myrrha* in order to screen their inhibitory growth activities on *streptococcus mutans* and *lactobacillus spp.* Methanol extracts in all three oleo gum resins displayed anti-microbial activity against the tested bacterial strains while acetone and hexane extracts exhibited minimal activities respectively. *B.frereana*-methanol extract gave highest result with 19mm inhibition zone on tested bacteria. *B.frereana*-acetone and *C.myrrha*-acetone extracts gave 17mm and 15mm growth inhibition zone respectively. Hexane extracts generally gave low antimicrobial activity. Methanol extract gave highest yield of 68% for *B.carterii*, 30% for *C.myrrha*, and 65% for *B.frereana*. Hexane extraction gave 90% yield for *B.frereana* while 10% yield was recorded on *C.myrrha*. Methanol was selected as best solvent for yield and biological activity while *B.frereana* and *C.myrrha* showed highest efficiencies for biological activities respectively in both methanol and acetone solvents.

***Keywords*—**Anti-bacterial activity, *B.carterii, B.frereana, C.myrrha,* Crude extracts, Organic solvents.

I. Introduction

INTENSIVE research activities concerning efficacy of natural products in preventing oral diseases, especially plaque related diseases such as dental caries is increasing [1]. Aromatic plant species of family *Burceraceae* are important medicinal plants which are recommended for reducing occurrence of dental caries due to a range of therapeutic properties of their extracts and essential oils. Frankincense or Olibanum is an oleo-gum resin obtained from several species of the genus *Boswellia*, a member of the *Burseraceae* family of aromatic plants [2]. This complex material generally contain 5–9% essential oil, 65–85% alcohol-soluble resin, and the remaining water soluble gum (polysaccharide fraction)[3], the Myrrh which occurs in the same family *Burceraceae* is an oleo-gum resin exudates obtained from several species in the genus *Commiphora*, it contains 57–61% water-soluble gum, 7–17% volatile oils,25–40% alcohol-soluble resins and 3–4% impurities [4]. *C.myrrha* is an effective antimicrobial agent used in the treatment of mouth ulcers, gingivitis, sinusitis, glandular fever, brucellosis and as an anti-parasitic agent [5], [6]. Moreover, myrrh volatile oils and their crude extracts exhibited diverse biological activities such as cyto-toxic, anesthetic, anti-inflammatory and anti-microbial effects [7],[8]. Triterpenoids are major constituents isolated from Commiphora species resins, while flavonoids and lignans commonly occurred in the plant stems [9]. Therefore, the scope of the current study is to analyze the microbial activity and extracted yield for individual exudates, compare and select the best specie for farther studies.

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II. Material and Methods

A. Plant Materials

The oleo-gum resins of *C.myrrha* and *B.Frereana* were purchased from the incense collectors in Burco (Somaliland) while *B.Carterii* purchased from incense shops in Bosaso (Puntland) Somalia. All three different gum resins were kept at -80°C freezer overnight then crushed into fine powder (40mesh) using mortar and pestle, regular blender, and electric sieve system. After pulverization, the gum resins were kept in different containers and labeled accordingly then stored at-20°C freezer to maintain its physico-chemical structure until the further processing.

B. Preparation of Crude Extract

The pulverized form of all three oleo gum resin species, *B.carterii*, *B.frereana*, and *C.myrrha*(10g of each) were macerated separately in methanol, acetone, and hexane in

glass bottles. The bottles were labeled and put in an orbital shaker *(SatoriusCertomat IS, Germany).* Operating conditions were as follows; Time 5 hrs at temperature of 55°C and 250 rpm. The extracts were filtered with
What-man No.1 filter paper, then extract of each solvent were pooled and evaporated

Under rotary evaporator *(BUCHI, Rotavapor® R-215),* at 40°C to obtain crude extracts, and then weighed for subsequence analyzes..

Antimicrobial Activity

A. Microbial Strains

The bacteria strains used for anti-microbial activity evaluation were obtained from different sources, *Streptococcus mutans* were purchased from the Institute for medical research (IMR) Kuala Lumpur Malaysia, while *Lactobacillus spp.* was taken from isolated strains by bio-environmental research group in IIUM.

B. Culture medium and Inoculums

The stock cultures of microorganisms used in this study were maintained on test tube broth medium at 4◦C. Inoculums were prepared by suspending a loop full of bacterial cultures into 5 ml of BH broth and was incubated at 37◦C for 24h. About 100 µl of bacterial suspensions, adjusted to *(0.5 McFarland standard)* were taken and poured into Petri plates containing 4-5 ml sterilized Muller Hinton Agar. Bacterial suspensions were spread by glass rod to get a uniform lawn culture

C. Antimicrobial Activity Assay

The agar-well diffusion method was applied with some modification to detect antimicrobial activity [10]. Wells of 9 mm diameter were dug on the inoculated Mueller hinton agar medium and 100µl of different crude extracts dissolved in dimethylsulfoxide (DMSO) at concentration. (500mg/ml), were added in each well. The wells introduced with 100µl of DMSO were used as a negative control, whereas Ampicillin is used as a positive control. The plates were incubated at 37°C overnight and examined for the zone of inhibition. The diameter of the inhibition zone was measured in "mm". An extract was classified as active when the diameter of the inhibition was equal to or larger than 9 mm. All the assays were performed in triplicate and expressed as average values ± SD.

StatisticalAnalysis

All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD). Analysis of variance and significant differences among means were tested by one-way ANOVA

III. Results And Discussion

A. Antimicrobial Activity

The in vitro antimicrobial activity of *C.myrrha, B.frereana and B.carterii*, resin extracts of Methanol, Acetone and Hexane against *Streptococcusmutans* and *Lactobacillusspecies* were investigated. One-way ANOVA analysis showed significant differences ($P \leq 0.05$) in microorganisms' sensitivity among the studied extracts. The results presented in Table II showed that the Methanol extract at 500mg/ml concentration demonstrated highest in vitro antibacterial activity against the tested microorganisms, while hexane- extract and acetone at same concentration showed low or no antibacterial activity. There was no inhibition of bacterial growth observed with the negative control dimethyl sulfoxide (DMSO). The high potential of antibacterial activity of *B.frereana*-methanol extract might be attributed to the high polarity of methanol which is effective for more consistent extraction of different types of sesquiterpenoids particularly furanosesquiterpenoids, diterpenes, triterpenes and sterols [10]. It has been reported that crude extracts and essential oils from medicinal plants exercise antimicrobial activity by altering structural and functional damages to the microbial cell membrane [11]

B. Extraction Yield

As illustrated in Table IV crude extract yield varied with the variation of the solvent type and plant species. The highest yield (90% and 80%) was obtained when hexane was used on *B.frereana* and *B.carterii* gum resins respectively, whereas the lowest amount of crude extract (10%) was recorded with *C.myrrha*under same condition. However, no correlation was observed between the rate of yield and biological activities of the different extracts as could be seen in Tables II and IV

TABLE I
ANTIMICROBIAL ACTIVITY OF *C.MYRRHA*METHANOL, ACETONE, AND HEXANE EXTRACTS AT 500MG/ML CONCENTRATION BY AGAR WELL DIFFUSION METHOD.

Microorganism	Inhibition zone (mm)				
	Methanol	Acetone	Hexane	DMSO	Ampicillin
S. mutans	15±1	16±1.5	9±0.8	NI	16±0.7
L. bacillus spp.	14±0.8	15±1	11±1	NI	15±0.9

TABLE II
ANTIMICROBIAL ACTIVITY OF *B.FREREANA*METHANOL, ACETONE, AND HEXANE EXTRACTS AT 500MG/ML CONCENTRATION BY AGAR WELL DIFFUSION METHANOL.

Microorganism	Inhibition zone (mm)				
	Methanol	Acetone	Hexane	DMSO	Ampicillin
S. mutans	19±1.2	17±0.8	9±0.5	NI	16±0.7
L. bacillus spp.	19±1.4	15±1.1	11±0.9	NI	15±0.5

TABLE III
ANTIMICROBIAL ACTIVITY OF *B.CARTERII*METHANOL, ACETONE, AND HEXANE EXTRACTS AT 500MG/ML CONCENTRATION BY AGAR WELL DIFFUSION METHOD

Microorganism	Inhibition zone (mm)				
	Methanol	Acetone	Hexane	DMSO	Ampicillin
Streptococcus mutans	10±0.2	11±0.4	9±0.1	NI	16±0.2
Lacto bacillus spp.	10±0.3	10±0.3	11±0.3	NI	15±0.4

Values are mean inhibition zone (mm) ±SD of three replicates ,were Significantlydifferent($P \leq 0.05$). The diameter of the well(*9mm*) is included. NI: no inhibition zone

TABLE IV
YIELD % OBTAINED

Plant Species			
	Methanol	Acetone	Hexane
B.Frereana	65	78	90
B.Carterii	68	60	80
C.Myrrha	30	22	10

IV. CONCLUSION

The methanol and acetone solvent extracts of *Bfrereana* and *Cmyrrha* resins showed potential for use as antimicrobial activities. The methanol extract exhibited highest antimicrobial activity when compared to acetone and hexane extracts. All the tested microorganisms were sensitive to the methanol extract.Appendi.

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