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Anti-cariogenic activities of some East African oleo gum resin crude extracts and essential oils

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Abstract

Oleo gum resins are plant exudates commonly used in folk medicine for treating several disease conditions. Anti-cariogenic properties of essential oil (E. oil) and crude extracts obtained from *Boswellia frereana (B. frereana), Boswellia carterii (B. carterii)* and *Commiphora myrrha (C. myrrha)* were investigated. Methanol and acetone extracts of the three plants inhibited *Streptococcus mutans (S. mutans)* and *Lactobacillus* spp. growth. Hexane extracts showed low anti-microbial activity. The average microbial inhibition was 14.6 mm for *S. mutans* and 13.8 mm for *Lactobacillus* spp. regardless of solvent type. *B. frereana* produced 8% E. oil while *B. carterii* and *C. myrrha* gave 5% and 6%. *B. frereana* E. oil inhibited *S. mutans* and *Lactobacillus* spp. more than *B. carterii* and *C. myrrha* E. oils.

Introduction

Frankincense plant exudates contain about 5–9% essential oil (E oil), 65–85% alcohol-soluble resin and water-soluble polysaccharide fraction (Mathe *et al*., 2004). The chemistry and pharmacological applications of frankincense resin are well documented (Shah *et al*., 2009; Al-Harrasi *et al*., 2014). However, The *C. myrrha* species belonging to the family Burseraceae, contains 57–61% water-soluble gum, 25 – 40% alcohol-soluble resins and 7–17% volatile oil (Massoud *et al*., 2007). The gum contains polysaccharides and proteins, while the volatile oil composed of steroids, sterols and terpenes. Triterpenoids are major constituents isolated from the gum resins of *Commiphora* species, while flavonoids and lignans commonly occurred in the plant stems (Albayrak *et al*., 2010); therefore, the characteristic fragrance of myrrh is derived from Furano-sesqui-terpenes (Abdul-Ghani *et al*., 2009).

In folk medicine, *C. myrrha* has been used as anti-microbial agent in the treatment of mouth ulcers, gingivitis, sinusitis, glandular fever and brucellosis. *C. myrrha* extracts are reported to contain anti-parasitic agents (Abdel-Hay *et al*., 2002; Abdul-Ghani *et al*., 2009). Other studies reported the anti-bacterial effects of terpenes obtained from the oleo-resin of *C. molmol* (another name for *C. myrrha*) against several strains of *Staphylococcus aureus* (Rahman *et al*., 2008; Abdallah *et al*., 2009). In the present study, crude extracts and E. oils of *B. frereana, B. carterii* and *C. myrrha* were investigated for their *in-vitro* anti-cariogenic activities using the two main causative agents of the disease –*S. mutans* and *Lactobacillus* spp.

Material and Methods

Plant materials

C. myrrha and *B. frereana* were purchased from the incense collectors in Burco (Somaliland) while *B. carterii* was procured from the incense shops in Bosaso (Puntland) Somalia. All three different oleo gum resins were kept at -80°C overnight then crushed into fine powder using mortar and pestle, regular blender. The fine powder was sieved with 40 mesh size screens (0.4 mm). The gum resins were stored in airtight container at -20°C to maintain their physico-chemical properties.

Preparation of crude extracts

About 10 g of each pulverized oleo gum resin species, were macerated separately in methanol, acetone, and hexane in glass Erlenmeyer flasks

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(solid solvent ratio of 1:10). The flasks were fixed on orbital shaker (Satorius Certomat IS, Germany) at 55°C, 250 rpm and extraction was performed for 5h. The extracts were filtered with Whatman No.1 filter paper, then extract of each solvent were pooled together and evaporated under reduced pressure in a rotary evaporator (BUCHI, Rotavapor® R-215), at 40°C for 20 min.

Antimicrobial activity assay

The agar-well diffusion method was applied with some modifications to detect antimicrobial activity (Shen *et al*., 2012). Wells of 9 mm diameter were dug in the sterilized Mueller Hinton agar medium and then 100 µl of each extract with dimethylsulfoxide (DMSO) 500 µl/ml concentration were added to each well. Ampicillin and DMSO were used as positive and negative controls respectively. The plates were incubated at 37°C overnight and examined for inhibition zone; the diameter of inhibition zone was measured ((diameter of the well (9 mm) is included)). All the assays were performed in triplicates and expressed as an average values ± standard deviations (SD) (Mirghani *et al*., 2012).

Preparation of essential oils

100 g of each pulverized samples were placed in hydro-distillation flasks and mixed with de-ionized water at ratios of 1:10. Hot plate incorporated with magnetic stirrer (EMS-HP-7000) was used as a source of heat and agitation. Distillation was carried out between 3 to 5 h.

Microbial strains

The bacteria strains used for anti-microbial activity evaluation were obtained from reliable sources; *S. mutans* (IMRS246) were purchased from the Institute for Medical Research (IMR) Kuala Lumpur Malaysia, while *Lactobacillus* spp. was taken from isolated strains kept at the Bioenvironmental research centre, Department of Biotechnology Engineering, International Islamic University Malaysia (IIUM).

Culture medium and inoculums

The stock cultures of microorganisms used in this study were maintained on test tube slant of appropriate agar medium at 4°C. Inoculums were prepared by suspending a loop full of bacterial cultures into 5 ml of Muller Hinton broth (MHB) and were incubated at 37°C for 24 h. 100 µl of bacterial suspensions, adjusted to 0.5 McFarland standard were taken and poured into Petri plates containing 20 ml sterilized Muller Hinton Agar (Mirghani *et al.*, 2012). Bacterial suspensions were spread by glass rod to get a uniform layer culture.

Statistical analysis

All tests were conducted in triplicates and the standard deviations (SD) between the samples were measured. Analysis of variance (ANOVA) and significant differences among means were tested by one-way ANOVA.

Results and Discussions

Effects of solvents on yield of crude extract

The extraction yield of the oleoresin extracts obtained varied with the different solvent and plant species used (Figure 1). The highest extraction yield was recorded when n-hexane was used on *B. frereana* and *B. Carterii* while the lowest extraction yield was recorded on *C. myrrha* under the same solvent. Similar study on *C. holtziana* reported a yield of crude extract that is significantly lower than current result when hexane was used for extraction (Chiteva *et al*., 2013). *C. myrrha* methanol crude extract yield was higher than other investigated plant species and it is slightly more than the value obtained in previous study where 29.65% yield was recorded via ethanol extraction (Ashry *et al.*, 2010). *B. carterii* acetone extract yield of 60% in this study is higher than the 40% obtained in previous reports using 70% of aqueous acetone at ambient temperature (Fan *et al*., 2005). Moreover, 50.2% of crude extract yield was obtained from *Boswellia sacra* using methanol at ambient temperature (Al-Harrasi *et al*., 2014). The current result study surpassed 67% and the reason could be the moderate extraction temperature (55°C) and inclusion of agitation.

Antimicrobial activities of oleo gum resin crude extracts

The *in-vitro* antimicrobial activity of *C. myrrha, B. frereana* and *B. carterii* oleo gum resin extracts from methanol, acetone and hexane against *S. mutans* and *Lactobacillus* spp. were investigated. The results showed that methanol and acetone extracts at 500 µl/ml concentration showed highest *in-vitro* antibacterial activity against the tested bacteria, while hexane extracts at the same concentration (500 µl/ml) exhibited low or no antibacterial activity (Figures 2, 3, and 4). One-way analysis of variance (ANOVA) showed significant difference at $P \leq 0.05$ among the bacteria sensitivity to the extracts. No anti-bacteria activity was recorded for Dimethylsulfoxide (DMSO) while profound activity existed in the positive control as also stated by Adewale *et al*., (2012).

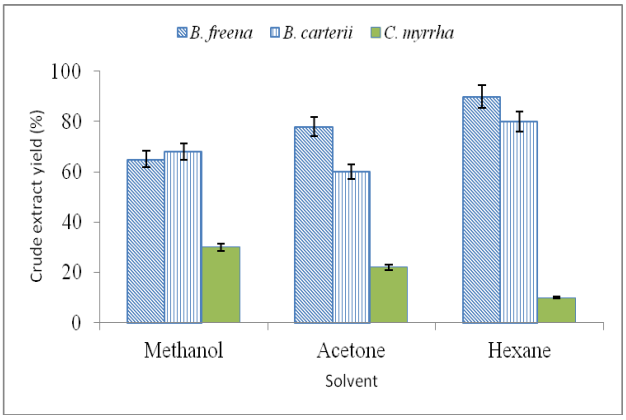

Figure 1. Yield of solvent crude extract of oleo gum resins

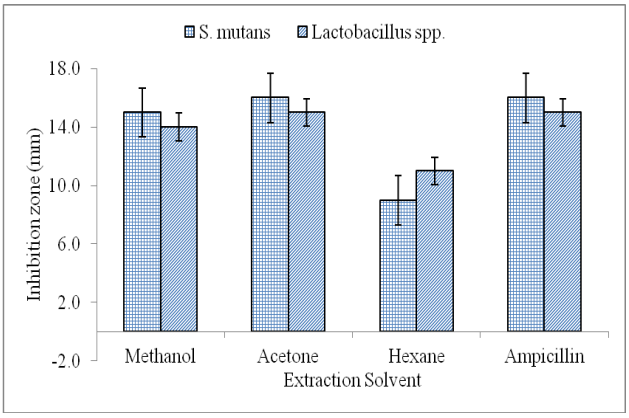

Figure 2. Anti-microbial activity of *C. myrrha* Methanol, Acetone and Hexane at 500µl/ml Concentration

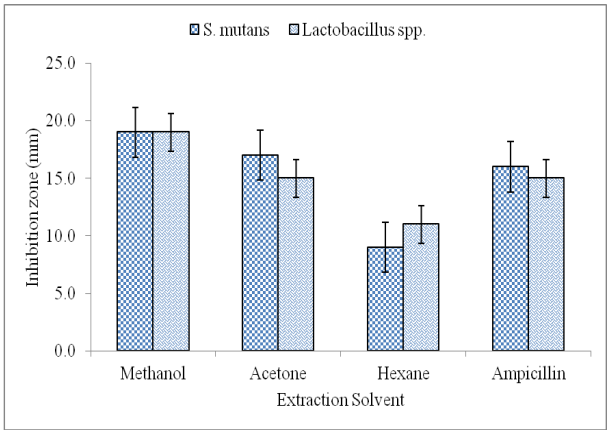

Figure 3. Antimicrobial activity of *B. frereana* methanol, acetone and hexane extracts at 500µl/ml concentration

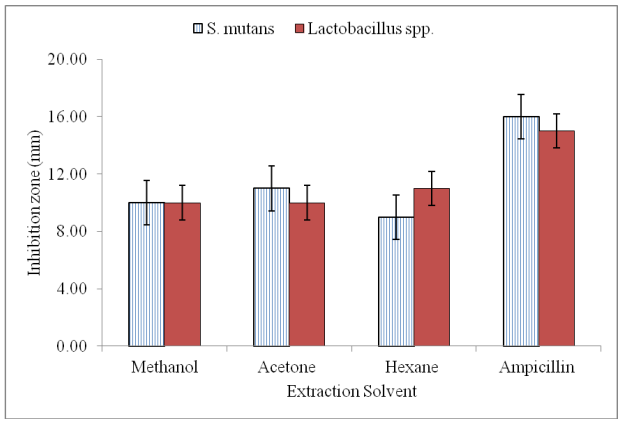

Figure 4. Anti bacteria activity of *B. carterii* methanol, acetone, hexane extracts at 500µl/ml

The high potential of antibacterial activity of *B. frereana* methanol extract might be attributed to the polarity of methanol which is effective for more consistent extraction of different types of sesqui-terpenoids particularly furano-sesqui-terpenoids, diterpenes, triterpenes and sterols (Shen *et al*., 2012). 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 (Mohamed *et al.*, 2014). The *C. myrrha* extract (Figure 2) showed its highest inhibition of *S. mutans* when extracted with acetone, followed by methanol extract. However, the n-hexane extracts demonstrated very low activities against Lactobacillus spp and *S. mutans*, respectively. DMSO doesn't produce considerable inhibition zone, while the positive control (Ampicillin at 1µl/ml) displayed activities equal to that of *C. myrrha* acetone extract. Present investigation is consistent with previous observation where anti-microbial and antioxidant activities of *C. myrrha* methanol crude extracts and E oils were found to exhibit high antioxidant and antimicrobial activity when compared to ethyl acetate extract and E oil at the same concentration Mohamed *et al*., 2014. *C. myrrha* methanol extracts exhibited the profound antibacterial activity against *Staphylococcus aureus* (Abdallah *et al*., 2009).

B. frereana methanol extract was selected as the best of the three species, considering its high anti-bacterial activity on both bacteria species compared with other crude extracts (Figure 3). Acetone extract demonstrated effective anti-bacteria activity against *S. mutans* and *Lactobacillus* spp, while n-hexane extracts exhibited low activity. However, this study is not in agreement with methanolic extracts of *B. sacra* from the Suqotra (Yemen) and Dhofar (Oman) regions as they showed higher antibacterial activity than *B. frereana* collected from Somalia (Hasson *et al.*, 2011).

All *B. carterii* Crude Extracts displayed less activity against *S. mutans* and *Lactobacillus* spp, regardless the type of solvent is used (Figure 4). In a report, four boswellic acid molecules from *Boswellia serrata* were tested with some cariogenic bacteria with conclusion that acetyle-11-Keto-β-boswellic Acid (AKBA) was the most active of the four boswellic acids against all bacterial pathogens (Raja *et al.*, 2011)

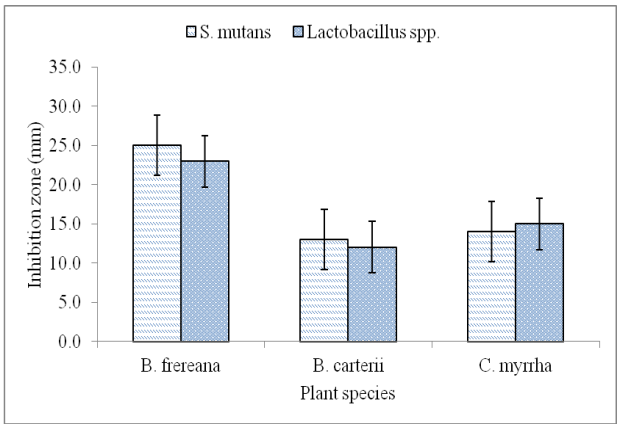

Figure 5. Anti-microbial activity of *B. frereana*, *B. carterii* and *C. myrrha* essential oils

Effects of hydro-distillation on essential oil yield

Yield of E. oils obtained from the different oleo gum resins using hydro-distillation process were varied greatly among the different oleo gum resin species. Hydro-distillation of *B. frereana* oleo gum resin produced the highest yield of E. oil when compared to the other two oleo gum resin species. *B. frereana* gave 8% E. oil while *B. carterii* and *C. myrrha* produced 5% and 6% respectively. The result is consistent with another hydro-distillation investigation of *C. myrrha* where paltry 2.97% E oil was produced (Mohamed *et al*., 2014). This difference in E. oil production could be due to postharvest conditions, environmental and climate differences or both. Also, E. oil production could be specie dependent as evident in high oil production (9%) by *C. holtziana* (Tuker, 1986; Chiteva *et al*., 2013).

Antimicrobial effects of essential oils

All three E. oils investigated for their Anti-microbial activity demonstrated promising inhibitory effects against *S. mutans* and *Lactobacillus* spp. *B. frereana* E. oil gave highest antibacterial effect among the three oleo gum resins while *B. carterii* E oil exhibited the lowest inhibition against tested bacteria strains (Figure 5).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of E. oils

The MIC values of B. frereana E. oil and *C. myrrha* E. oil showed that slightly low concentration of the E. oil from the two species of oleo gum resin is required to greatly inhibit growth of cariogenic bacteria. Previous studies conducted by Salvat *et al.*, (2004) reported that plant extracts with MIC's less than or around 0.5 mg/ml indicate a good antibacterial activity, whereas, VanVuuren (2008) stated that, E. oils having MIC values of 2 mg/ml or lower are considered to be noteworthy. Moreover, (Shen *et al.*, 2012) reported that chemical compound (Mansum binoic acid) isolated from *Commiphara* spp possessed potent antibacterial activity against a multidrug-resistant strain *Staphylococcus aureus* with a MIC value of 4 µg/ml. Therefore, the oleo gum resin E. oils in the present study exhibited profound antimicrobial activity against the tested microorganisms.

Conclusion

Three oleo gum resin species of *Burceraceae* class were screened for their potential anti cariogenic activity. Organic solvent extraction of oleo resins yielded profound crude extracts with promising antibacterial activities. *B. frereana* and *B. carterii* crude extract obtained by organic solvents was higher than *C. myrrha*. Hydro distillation of the samples produced comparable E oils yield. However, *B. frereana* methanol extract and *C. myrrha* extracted by acetone exhibited good anti-bacterial activities while hexane extracts despite high yield had low anti-bacterial activity. Low concentration of E oil was needed to greatly inhibit the growth of tested cariogenic bacteria.

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