

The Effect of Using Frankincense (*Boswellia sacra*) Chewing Gum on the Microbial Contents of Buccal/Oral Cavity, Taif, KSA

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Abstract: Frankincense (*Boswellia sacra* / Oleo-gum-resin), the main effective contents were alcohol-soluble resins is 78%, water-soluble gum 23% and volatile essential oil 7% respectively. The contents have anti-microbial effects, on moth flora. The target was to detect the anti-microbial effects on microbial contents of buccal/oral cavity which are the sources of infection. Selection was of 5 female persons aged (25-35yrs.), for the experiment under study as the usage of Frankincense chewing gum for 5hrs. continually. As well as they were tested for microbial contents of buccal/oral cavity through microbial identification of saliva. The main CFUs/ml saliva were 13.000, 8.200, 6.100, 3.900, 2.200, and 740 at Zero, 1, 2, 3, 4, and 5hrs. The differences of CFUs/ml saliva were 4.800, 2.100, 2.200, 1.700, and 1.460 between (Zero hr.-1hr.), (1hr.-2hr.), (2hr.-3hr.), (3hr.-4hr.), and (4hr.-5hr.) respectively. CFUs/ml saliva were 1.3×10^4 , 8.2×10^3 , 6.1×10^3 , 3.9×10^3 , 2.2×10^3 , and 7.4×10^2 at Zero, 1, 2, 3, 4, 5hr. respectively. Frankincense chewing gum is a safe and low-cost herbal product, applied to improve buccal/oral cavity hygiene by anti-microbial effects which decrease the sources of microbial infection in buccal/oral cavity and support mouth hygiene for all ages. It has been indicated to have wide pharmacological and industrial potentials as well.

Key words: Frankincense, *Boswellia sacra*, Oleo-gum-resin, Buccal, Oral, Microbial, CFUs/ml

I. Introduction:

Frankincense (*Boswellia sacra*) is a tree indigenous to Dhofar region and is one of the most famous plant of Sultanate of Oman. It is also known as Luban, Bakhor or Kendar in Arabic region^[1]. Its composed of about 5–9% essential oil, 65–85% alcohol-soluble resins, and the remaining water soluble gums 30–40%^[2]. Oil portion contains 62.1% ester, 15.4% alcohol, 9.9% mono-terpene hydrocarbons and 7.1% di-terpenes, contain α -pinene, α -thujene, β -pinene, camphene, myrcene, o-methylanisole, α -terpinene, methoxytoluene, hexyl acetate, limonene, 1, 8-cineole, n-octanol, linalool, octyl acetate, bornyl acetate, cembrene A, incensole, incensole acetate, sabinene, o-cymene, p-cymene, 1,8-cineole, cis- β -ocimene, trans- β -ocimene, γ -terpinene, 1-octanol, terpinolene, linalool, 1-decanol, terpinen-4-ol, α -terpineol, 1-octyl acetate, citronellyl acetate, neryl acetate, geranyl acetate, hexyl hexanoate, 1-decyl acetate, hexyl octanoate, α -campholenic aldehyde, caproic acid, oenanthic acid, caprylic acid, pelargonic acid, lauric acid, and verbenone^[3]. It's the most important multipurpose tree species in Central and Eastern Africa. possess multiple economic and ecological benefits in Africa. It is found in Ethiopia, Nigeria, Cameroon, Central African Republic, Chad, Sudan, Uganda and Eritrea^[4]. It produced by several species of *Boswellia*. "Frankincense or Pure- incense" was known to the most of the ancient civilizations who used it in rituals and prayers to the gods. It is used in the traditional medicine in Oman, India and African countries for the treatment of variety of diseases. The oldest written evidence which mentions Frankincense as a drug is papyrus Ebers^[5]. Essential oil was dominated by α -thujene 10% and p-cymene 4-3%^[6]. Boswellic acids have an anti-inflammatory action^[7], active constituents for many of its medicinal uses^[8]. Non-steroidal anti-inflammatory drugs (NSAIDS) used for inflammatory conditions, among the constituents of boswellic acid, β -boswellic acids (the major constituents of boswellic acids) was shown to have anti-inflammatory and anti-arthritic pain activity, which was found to be due to their ability to inhibit 5-lipoxygenase activity^[9]. Frankincense consists of essential oils, gum, and terpenoids. The boswellic acids in the terpenoid portion are the active constituents in *Boswellia*^[10]. The fresh gum obtained from the tree is hot dry with a pleasant flavor and slightly bitter in taste. When burned produces a brilliant flame and produces a pleasant aroma. It was widely used by ancient Egyptians, Greeks and Romans as prized incense, fumigant as well as a multipurpose aromatic. In the past it was used for trading across Asia and Europe. It was the most powerful fuel for the Arab economy as it was once more valuable than gold. The essential oil contains E- β -ocimene and limonene as the major mono-terpenes 97.3% however, E- caryophyllene is the main constituent of 2.7% sesquiterpenes, resin contains a mixture of four penta-cyclic triterpene acids: β -boswellic acid (the most abundant), 3-O-acetyl β -boswellic acid (ABA), 11-keto- β -boswellic acid, and 3-O-acetyl-11-keto- β -boswellic acid (AKBA), collectively called boswellic acids^[11]. Boswellic acids are further known to contain β -boswellic acid, acetyl- α -boswellic acid, and acetyl- β -boswellic acid, and associated 3-acetyl-11-hydroxy- β -boswellic acid and 11-keto- β -boswellic acid acetate chemicals^[12]. AKBA components of the boswellic acids are also identified to be among the biologically active and most potent constituents^[13]. Boswellic acid and their derivatives were investigated

for anti-inflammatory. Essential oil showed anti-bacterial, anti-fungal and immuno-stimulating activity^[14]. Mono-terpenoids seem to be the dominant class of compounds found in the oils. The main compounds essential oil were α -pinene 45.7 %, α -terpinene 11.5% and trans-sabinene hydrate^[15]. Resin oils showed similar chemical profiles, with iso-incensole and iso-incensole acetate as the main di-terpenic components. Both n-octanol and n-octyl acetate, along with the diterpenic components incensole and incensole acetate, were the characteristic compounds of *B. papyrifera* oleogum resin oil. The anti-microbial activities of the essential oils were individually evaluated against fungi, Gram-positive and Gram-negative bacteria strains. Resins destroy microbial cell wall and stop protein synthesis in *Strept. salivaris*, *Staph. aureus*, and *Bacillus megaterium*^[16]. Pharmacological activities of Frankincense, as crude extracts, the distilled essential oil and isolated compounds reported to exhibit in-vitro anti-bacterial, anti-fungal and immuno-modulatory activity. Volatile oils make sensitization of microbial cell contents and have anti-inflammatory effect on *Staph. aureus*, *Staph. epidermidis*, *Staph. hominis*, *Bacillus cereus*, *E. coli*, *Proteus vulgaris* and *Candida albicans*^[17]. Frankincense application can lead to remarkable decrease in inflammatory indices in comparison to drug therapy. It's a safe and low cost herbal medicine, may be feasibly applied to improve inflammation based disease of gingival as an adjunct to the conventional mechanical therapy^[18]. The daily and ritual use of *Boswellia* is characteristic cultural of the horn of Africa which transcends the ethical and religious memberships. The essential oil and methanol extract of *Boswellia* showed activity against bacterial spp. than against yeast. Water soluble gum has sticky character which stop the reactions between substances^[19]. In general, most of the respondents were aware of beneficial therapeutic uses of luban, but 3/4 of the participants hardly had an idea that incense is considered as potential chemo preventive agent in modern medicine. Similarly, (38-46%) of the participants correctly believed that luban is having anti-inflammatory actions, a fact substantiated by scientific evidences and its commercial availability in the pharmaceutical market. More than 1/2 of the study population suggested that luban could be used in cosmetic industry because of its aroma and fragrance. Frankincense was once and still considered to be a national treasure^[20]. The human mouth is an ideal environment of the existence and growth of micro-organisms. It provides a source of water and nutrients, as well as a moderate temperature. Resident bacteria of the mouth adhere to the teeth and gums to resist mechanical flushing from the mouth to stomach where they are destroyed by hydrochloric acid^[21]. Anaerobic bacteria in the oral cavity include: *Actinomyces*, *Bacteriodes*, *Bifidobacterium*, *Eubacterium*, *Fusobacterium*, *Lactobacillus*^[22]. The role of oral micro-organisms in the two major dental diseases: dental caries and periodontal disease. The mouth harbors a diverse, abundant and complex microbial community. This highly diverse micro flora inhabits the various surfaces of the normal mouth. Bacteria accumulate on both the hard and soft oral tissues in biofilms. Bacterial adhesion is particularly important for oral bacteria. Oral bacteria have evolved mechanisms to sense their environment and evade or modify the host. Bacteria occupy the ecological niche provided by both the tooth surface and gingival epithelium. However, a highly efficient innate host defense system constantly monitors the bacterial colonization and prevents bacterial invasion of local tissues. A dynamic equilibrium exists between dental plaque bacteria and the innate host defense system. Oral bacteria include *Strept.*, *lactobacilli*, *staph.*, *Corynebacterium*, and various anaerobes in particular *Bacteriodes*. The oral cavity of the new-born baby does not contain bacteria but rapidly becomes colonized with bacteria such as *Strept. salivaris*. With the appearance of the teeth during the first year colonization by *Strept. mutans* and *Strept. sanguinis* occurs as these organisms colonize the dental surface and gingiva. Other strains of *Strept.* adhere strongly to the gums and cheeks but not to the teeth. The gingival crevice area (supporting structures of the teeth) provides a habitat for a variety of anaerobic species. *Bacteriodes* and *Spirochetes* colonize the mouth around puberty^[23]. *Spirochetes* and *Fusiform bacilli* live as normal flora in the mouth, but in the case of bleeding in the oral cavity, the bacteria can cause infection and diseases to oral cavity. Acute necrotizing ulcerative gingivitis (ANUG), Vincent angina with a membrane covering the throat area. Gram-negative anaerobic Cocci. It is thought that this species thrives in the acidic environment of caries and is thought to slow the development of dental caries. It converts the acidic products of other spp. to less acidic products. *Aggregatibacter Actinomycete mcomitans* is considered an oral pathogen due to its virulence factors, its association with localized aggressive periodontitis in young adolescents^[24]. Dental plaque is the material that adheres to the teeth and consists of bacterial cells (mainly *Strept. mutans* and *Strept. sanguinis*), salivary polymers and bacterial extracellular products. Plaque is a biofilm on the surfaces of the teeth. This accumulation of micro-organisms subject the teeth and gingival tissues to high concentrations of bacterial metabolites which results in dental disease. If not taken care of, via brushing or flossing, the plaque can turn into tartar (its hardened form) and lead to gingivitis or periodontal disease. Most of the bacterial species found in the mouth belong to microbial communities, called biofilms, a feature of which is inter-bacterial communication. Cell-cell contact is mediated by specific protein adhesins and often, as in the case of inter-species aggregation, by complementary polysaccharide receptors. Another method of communication involves cell-cell signaling molecules, which are of two classes: those used for intra-spp. and those used for inter-spp. signaling. An example of intra-spp. communication is quorum sensing. Oral bacteria have been shown to produce small peptides, such as competence stimulating peptides, which can help promote

single-spp. biofilm formation. A common form of inter-spp. signaling is mediated by 4, 5-dihydroxy-2, 3-pentanedione (DPD) or Autoinducer-2 (AI-2)^[25]. Some Lactobacillus spp., have been associated with dental caries although these bacteria are normally symbiotic in humans and are found in the gut flora^[26].

The aim of the experiment: The usage of raw Frankincense (Boswellia sacra) at Taif, KSA, with the stress of anti-microbial activities for simple and chip herbal materials which using by all ladies in Arabian region. Taking into our consideration anti-microbial effects, low-cost, availability of Frankincense in the herbal markets, relatively high frequency of use in all ages and making good hygienic condition for oral cavity.

II. Materials and Methods:

Frankincense chewing gum preparation: It was chosen and purchased from famous herbal market as a pure type at Taif Souk. It was weighted as separately specimens (1±0.2gm) for each understudy persons with extra samples for any dismissing in the experiment.

Understudy persons preparation: Experiment advertise was making at Taif region, it was stay for 2days only. Ten female were accepted as experimental understudy persons, 5female were selected from them as final chosen for experiment, (healthy, diseased free and don't take any medication). The experiment was explained and consent forms were gotten from them for the experimental work.

Experiment steps: It was started at Zero hr. time considered as control baseline. Specimens were collected from all understudy persons at Zero hr. time for control microbial scan. Then understudy persons started the usage of gum and adjusted the collection of others specimens for everyone hr. till 5hrs., as well specimens were sent to Microbiology Laboratory for microbial scan.

Microbial scan: Specimens were collected at the time recorded for the experiment. Whole saliva collected by spitting (at least 1ml) from each understudy person obtained in sterile container and labeled. Each specimen was seeded onto bacterial and fungal culture media at that moment, or placed immediately into an Eppendorff tube and kept deep-frozen carbon dioxide at (-70°C) until sent for analysis in Microbial Laboratory by standard methods. Specimens were collected transported to Microbiology Laboratory within 1hr. of collection and processed for microbial scan immediately. Microbial determination for CFUs/ml was performed for understudy persons, CFUs/ml were calculated by: $y \times 10^{-d} \times 1/v$ (where d=dilution v=volume plated and y=colony count on the plates, between 30 and 300)^[27-28].

Data Analysis: The data were recorded and entered into Microsoft excel sheet, then summarized and analyzed^[29].

III. Results and discussion:

Table and figure 1: The main effective contents of Frankincense chewing gum

Contents	Main %
Volatile essential oil	7%
Water-soluble gum	23%
Alcohol-soluble resins	78%

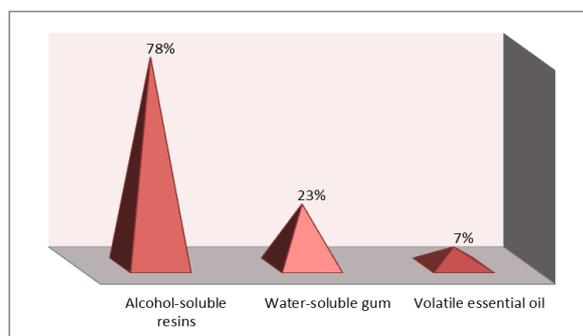


Table and figure 1 show the main effective contents of Frankincense chewing gum were alcohol-soluble resins is 78%, water-soluble gum 23% and volatile essential oil 7% respectively. Frankincense (Boswellia sacra) is a tree indigenous to Dhofar region and is one of the most famous plant of Sultanate of Oman. It is also known as Luban, Bakhor or Kendar in Arabic region^[1]. Its composed of about 5–9% essential oil, 65–85% alcohol-soluble resins, and the remaining water soluble gums 30–40%^[2]. Frankincense consists of essential oils, gum, and terpenoids. The boswellic acids in the terpenoid portion are the active constituents in Boswellia^[10]. Boswellic acids are further known to contain β-boswellic acid, acetyl-α-boswellic acid, and acetyl-β-boswellic acid, and associated 3-acetyl-11-hydroxy-β-boswellic acid and 11-keto-β-boswellic acid

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Table 2 and figure (2 & 3): Microbial contents of the buccal/oral cavity for each person using rankincense chewing gum

Hr.	CFUs/ml					The main	Differences
	Person1	Person2	Person3	Person4	Person5		
Zero hr.	13000	13200	12900	12800	13100	(65000/5) 13000	Zero hr.- 1hr. 4800
1hr.	8100	8300	8200	8400	8000	(41000/5) 8200	1hr.-2hr. 2100
2hr.	6300	6100	6200	5900	6000	(30500/5) 6100	2hr.-3hr. 2200
3hr.	3900	3700	4000	4100	3800	(19500/5) 3900	3hr.-4hr. 1700
4hr.	2100	2000	2400	2300	2200	(11000/5) 2200	4hr.-5hr. 1460
5hr.	730	720	750	760	740	(3700/5) 740	

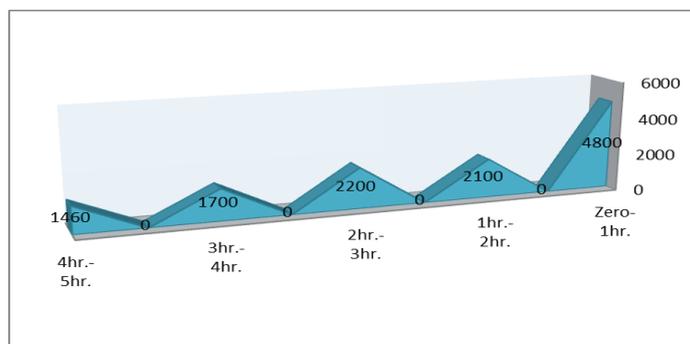
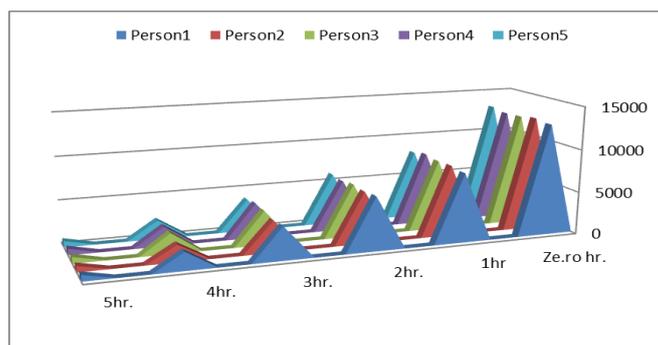


Table 2 and figure (2 & 3) show microbial contents of the buccal/oral cavity for each person using Frankincense chewing gum. The main CFUs/ml of saliva were 13.000, 8.200, 6.100, 3.900, 2.200, and 740 at Zero, 1, 2, 3, 4, and 5hrs. The differences of CFUs/ml were 4.800, 2.100, 2.200, 1.700, and 1.460 between (Zero hr.-1hr.), (1hr.-2hr.), (2hr.-3hr.), (3hr.-4hr.), and (4hr.-5hr.) respectively.

Table 3 and figure 4: Incidence of the main microbial contents of the buccal/oral cavity with using Frankincense chewing gum

Hrs.	The main CFUs/ml	The main No./ml
Zero hr.	1.3×10^4	13.000
1hr.	8.2×10^3	8.200
2hr.	6.1×10^3	6.100
3hr.	3.9×10^3	3.900
4hr.	2.2×10^3	2.200
5hr.	7.4×10^2	740

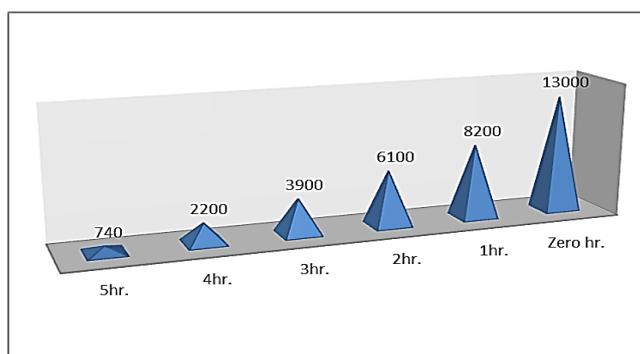


Table 3 and figure 4 show incidence of the main microbial contents of the buccal/oral cavity with using Frankincense chewing gum. CFUs/ml were 1.3×10^4 , 8.2×10^3 , 6.1×10^3 , 3.9×10^3 , 2.2×10^3 , and 7.4×10^2 at Zero, 1, 2, 3, 4, 5hr. respectively. Non-steroidal anti-inflammatory drugs (NSAIDS) used for inflammatory conditions, among the constituents of boswellic acid, β -boswellic acids (the major constituents of boswellic acids) was shown to have anti-inflammatory and anti-arthritis pain activity, which was found to be due to their ability to inhibit 5-lipoxygenase activity^[9], also identified to be among the biologically active and most potent constituents^[13]. Boswellic acid and their derivatives were investigated for anti-inflammatory. Essential oil showed anti-bacterial, anti-fungal and immuno-stimulating activity^[14]. The anti-microbial activities of the essential oils were individually evaluated against fungi, Gram-positive and Gram-negative bacteria strains. Resins destroy microbial cell wall and stop protein synthesis in *Strept. salivaris*, *Staph. aureus*, and *Bacillus megaterium*^[16]. Pharmacological activities of Frankincense, as crude extracts, the distilled essential oil and isolated compounds reported to exhibit in-vitro anti-bacterial, anti-fungal and immuno-modulatory activity. Volatile oils make sensitization of microbial cell contents and have anti-inflammatory effect on *Staph. aureus*, *Staph. epidermidis*, *Staph. hominis*, *Bacillus cereus*, *E. coli*, *Proteus vulgaris* and *Candida albicans*^[17]. Frankincense application can lead to remarkable decrease in inflammatory indices in comparison to drug therapy. It's a safe and low cost herbal medicine, may be feasibly applied to improve inflammation based disease of gingival as an adjunct to the conventional mechanical therapy^[18]. The human mouth is an ideal environment of the existence and growth of micro-organisms. It provides a source of water and nutrients, as well as a moderate temperature. Resident bacteria of the mouth adhere to the teeth and gums to resist mechanical flushing from the mouth to stomach where they are destroyed by hydrochloric acid^[21]. Anaerobic bacteria in the oral cavity include: *Actinomyces*, *Bacteriodes*, *Bifidobacterium*, *Eubacterium*, *Fusobacterium*, *Lactobacillus*^[22]. The role of oral micro-organisms in the two major dental diseases: dental caries and periodontal disease^[23].

IV. Conclusion

Frankincense has been indicated to have wide pharmacological and industrial potentials. Result of this experiment displayed anti-microbial properties on microbial contents of buccal/oral cavity as a source of infection. This herbal product may be feasibly and safely applied to improve mouth hygiene for all ages.

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