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Short Communication

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In Vitro Antimicrobial Evaluation of Lozenges Containing Extract of Garlic and Ginger

Abstract

Purpose: The present work is an antimicrobial evaluation of lozenge dosage forms containing garlic and ginger extract.

Methods: Lozenges containing pulverized garlic and ginger were produced by the moulding method and was evaluated against oropharyngeal microbial isolates after time-release in a normal saline-saliva sink solution. The solution was withdrawn at different intervals and screened for antimicrobial activities using the agar diffusion method against *Escherichia coli, Staphylococcus aureus,* and *Candida albicans.* Nystatin tablets were used as standard.

Results: There was inhibition of growth by Nystatin tablet but garlic-ginger combination only inhibited growth of laboratory strains of *C. albicans*.

Conclusion: The result of this study showed that the garlic and ginger can be formulated into lozenges and used in non-resistant oral thrush.

Keywords: Antimicrobial evaluation, Lozenges, Garlic extract, Ginger extract, Oral thrush.

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Introduction

Herbal medicine, a form of complimentary medicine, holds a great promise as source of easily available effective therapy for diseases to the people, particularly in developing countries, including Africa. Up to 80% of the world's population depends directly on the traditional medicine for their primarily health care [1-2]. Traditional medicinal practice has employed medicinal plants as remedies for many diseases due to underlying microbial infections [3]. WHO has described traditional medicine as one of the surest means to achieve total health care coverage of the world's population. In pursuance of its goal of providing accessible and culturally acceptable health care for the global population, WHO has encouraged the rational use of traditional plant-based medicines by member States and has continued to monitor the usage of such herbal medicines [4-5]. While there is a need for the continued search for new medicinal preparations to combat the array of diseases facing humanity, there appears an apparently more looming need for assessment and scientific validation of a host of herbal medicinal plants parts that have been employed both in folkloric and present day practice.

Garlic (*Allium sativum*), a hardy perennial of Asiatic origin, is probably one of the earliest known medicinal plants [6-8]. It has traditional dietary and medicinal applications as an anti-infective agent [9-10]. The medicinal parts are principally the bulbs (cloves) and the oil [11]. *A. sativum* has remained popular as a cure-all among some traditional healing traditions [12-14], and also useful as a health supplement [15]. Chronic cancer and *Helicobacter pylori* infections have been shown to be reduced following consumption of garlic [16-17]. The antimicrobial effects of aqueous garlic extracts are also well established [18]. The *in vitro* evidence of the antimicrobial activity of fresh and freeze-dried garlic extracts indicated that it can be used effectively against the treatment of a variety of fungi, bacterial and viral infections [19-26]. On the other hand, ginger (Zingiber officinale) which is a member of the Zingiberaceae (ginger) family, occurs in horizontal, laterally flattened irregularly branching piece; 3-16cm long, 2-4cm wide, up to 3cm thick, sometimes split longitudinally, pale yellowish buff or light brown externally striated, some what fibrous, branches known as fingers arise obliquely form the rhizome, are flattish, obviate, short, about 1-3cm long, fracture, short and starchy with projecting fibers [27-28]. It has a characteristic aromatic taste and colour, internally pale yellow to brown [27, 29]. The plant is probably native to South-East Asia and is cultivated in the tropical regions in both the eastern and Western hemispheres. It is commercially grown in Africa, China, India, Jamaica; India is the world largest producer [27, 29,30-32]. It is noted for its actions to safely relieve nausea from many causes including morning sickness, labyrinthis, and motion sickness [33]. It helps improve digestion [34], lower cholesterol [35], and prevent seizures [36]. It also prevents cancer [37], the formation of blood clots which may cause heart attacks or strokes [38-39], protects the liver from chemical injury [40-41] and alleviates pain from arthritis [42-43]. Above all, it is noted for its antioxidant [44] and antimicrobial activities [45].

In view of the fact that crude garlic and ginger are characterized by a debilitating disagreeable taste and odour, and the observation that quite a lot of people chew garlic and ginger in their crude form with the aim of combating an underlying oropharyngeal infection, the need to formulate garlic and ginger into lozenges became apparent. Lozenges are large tablets prepared by moulding, cutting or by compression, that are intended to dissolve or erode slowly while in the mouth medicating the throat for a relatively prolonged time. They are expected to stay in the mouth for between 10 and 15 minutes [46]. Lozenges are termed "medicated lozenges" when they contain an antimicrobial agent or an anaesthetic which produces local effect on the mouth or the throat [47]. This present work is therefore aimed at formulating crude garlic and ginger extracts into lozenges to mask their taste, and evaluating their *in vitro* antimicrobial activities against common oral pathogens. The outcome of this study is expected to form necessary baseline towards an effective formulation of ginger and garlic in useable and reliable oral dosage form for some

cases of oral thrush, and oropharyngeal infections.

Materials and Methods

Plant material

The bulbs of *Allium sativum* (garlic) and rhizome of *Zingiber officinale* (ginger) were purchased from Nsukka main market and authenticated by a plant taxonomist in the Department of Botany, University of Nigeria, Nsukka.

Test microorganisms

Five (5) clinical strains of *Candida albicans* were isolated from primary school pupils of ages 6-14 years. Laboratory strains of *Staphylococcus arueus*, *Escherichia coli* and *Candida albicans* obtained from the Pharmaceutical Microbiology Laboratory, University of Nigeria, Nsukka were also used in the studies. The bacteria and fungal isolates were identified and authenticated using routine laboratory techniques and maintained in agar slant at 37 ^{o}C and 24 ^{o}C, respectively.

Drugs and reagents

The following materials were employed in this study: sodium chloride (Merck, Germany), sucrose (Merck, Germany), polyvinyl pyrrolidone (Merck, Germany), sodium carboxymethylcellulose (Merck, Germany). Nutrient agar (Fluka, Spain) was used as medium for the bacterial growth while Sabouraud dextrose agar (LAB M^{TM}) was used as medium for fungi growth.

Preparation and Physical evaluation of garlic-ginger lozenges

The moulding method was employed is this study. The formula used to produce 10 g of the total weight is

Minced garlic ---------- 15%
Minced ginger ---------- 15%
Sodium carboxymethylcellulose - 7%
Polyvinyl pyrrolodone (RP) ------ 3%
Sucrose---------- 60%

Lozenges each containing 15%, respectively, of garlic and ginger extracts were then prepared by mixing together appropriate amounts of minced garlic, minced ginger, sodium carboxymethyl-cellulose, polyvinyl pyrrolidone and sucrose to form a uniform paste which was poured into a mould to produce the lozenges of uniform weights. Weight variation of the lozenges was determined using analytical balance.

Preparation of saliva-normal saline mixture

Sodium chloride (0.9 g) was dissolved in 95 ml distilled water and the volume was made up to 100 ml with human saliva. This preparation makes provision for isotonicity of actual human saliva, as well as necessary presence of resident salivary enzymes which may impact on lozenge activity in normal clinical use condition. The mixture was sterilized by autoclaving at 121 °C for 15 min.

Susceptibility testing of garlic-ginger lozenge on test organisms

Two 25 ml of sterile normal saline plus saliva were transferred each to a sterile 50 ml round bottom flask. A lozenge was dropped into each flask containing the 25 ml normal saline-saliva mixture and fastened to a flask shaker. A 1 ml volume of sample was withdrawn at 5, 10, 20, 30 and 60 min. The samples were stored in sterile Bijou bottles and the same procedure was repeated for the nystatin tablet.

A 0.1 ml of 10^7 cfu/ml of the microorganisms (*E. coli*, *S. aureus* and *C. albicans*) were pipetted into agar plates. Nutrient agar (20 ml each; sabouraud dextrose agar was used for *C. albicans*) was poured into the plates and the contents swirled gently to produce uniform mixtures. The agar plates were allowed to cool and solidify and sterile cork borers of diameter 4 mm were used to form wells at equidistance in the agar plates. Two drops of the lozenge release solution collected at different time intervals (5, 10, 20, 30 and 60 min respectively) were introduced into the respective wells using sterile pipettes. Plain saliva-normal saline mixture was used as control in all cases and replicates were made. The plates were incubated at 37 °C for 24 hr (*E. coli* and *S.*

aureus) and 25 °C for 48 hr (*C. albicans*) and the diameters of the zones of inhibition were then measured.

Susceptibility testing of garlic-ginger lozenge and nystatin tablet on clinical isolates of *C. albicans*

Five clinical isolates of *C. albicans* (0.1 ml of 10^7 cfu/ml each) were pipetted into agar plates. Sterile sabouraud dextrose agar (20 ml) was poured into each of the plates and the contents swirled gently to produce uniform mixture. The plates were allowed to set on a horizontal plane to produce agar layers with uniform thickness. Sterile cork borer was used to form six wells at equidistance in the plates. Two drops of each lozenge release solution were introduced into the respective cups using sterile pipette. Nystatin was employed as standard against the *C. albicans* isolates. The plates where incubated at 25 °C for 48 hr and the diameters of the zones of inhibition were measured. Replicates determinations were carried out.

Results and Discussion

Taking 5% deviation (from the 250 mg weight of each tablet) as limit for passing the pharmacopoeia weight uniformity, only those lozenges that met the weight uniformity requirements were employed for the *in vitro* antimicrobial evaluation. The garlic-ginger lozenge demonstrated pronounced antifungal activity recording inhibition zone diameters of 17, 19, 20, 23, and 25 mm at 5, 10, 20, 30, and 60 min release times, respectively, against the laboratory isolate of *Candida albicans* but not against the clinical isolates of the same organism while Nystatin gave inhibition zone diameter values of 21 to 41 mm (Table 1). *E. coli* and *S. aureus* strains were resistant.

Previous reports on the antimicrobial properties of garlic by other workers has shown it to be active against microbial pathogens such as *Candida albicans s*pecies, *E. coli* and *S. aureus* [20, 22, 23, 25,26] which are implicated in oropharyngeal infections [48]. Lozenges are large tablets that are intended to dissolve slowly in the saliva, thus releasing the active ingredient over a relatively prolonged period thereby producing a local effect on the mouth or the throat. It is therefore expected that for any lozenges formulation containing garlic-ginger extract to qualify as a suitable base for the garlic-ginger extract, it should allow and promote the release of the antimicrobial extract from the dosage form. The progressive increase in the size of the inhibition zone diameters (17- 25 mm) with increasing times (5- 60 min) is indicative of a good correlation between extent of release of the garlic-ginger extract and antimicrobial activity. It is however relatively unclear as to why the clinical isolates of *C. albicans* used in this study did not show susceptibility to the garlic-ginger release sample used, given that the antifungal effect of garlic has been previously well established [19,21,24-26]; especially when this is compared to the results obtained for the standard nystatin tablet where the *C. albicans* strains showed susceptibility.

Table 1: Inhibition zone diameters of nystatin against five clinical isolates of *C. albicans* (mm)

Isolates	Time (min)				
	5	10	20	30	60
1	21.00	24.00	35.00	32.00	30.00
2	30.00	32.00	32.00	34.00	30.00
3	22.50	35.00	40.00	41.00	42.00
4	-	-	-	-	-
5	23.00	24.00	25.00	27.00	28.00

It is worthy to note that in most cases of infection, a combination of antimicrobial activity and one or more other biological effects, such as immunomodulation, could be responsible for overall effect of a natural product [49]. Garlic and ginger have been known to possess immunological and cytoprotective effects in the biological host [16, 24, 37, 40-41]. Immune activation or immunomodulation within the host play a role in defense and elimination of infection, and to minimize any damage that seem to arise as a result of that infection [48]. It is therefore likely that a combination of these biological effects of garlic-ginger and the

demonstrated antimicrobial effect from this study may explain its usefulness in the management of oropharyngeal infections, especially those of fungal origin in folklore medicine. The resistance of the bacteria employed to the antimicrobial activity of the lozenges may be related to factors accruing due to the combined effect of garlic and ginger in the lozenge or inherent resistance of the micro organism.

Conclusion

Garlic-ginger extract has been successfully formulated as a lozenge for the purpose of taste-masking, crude drug release, and consequent antimicrobial activity. The formulated product showed inhibitory activity against non-resistant *C. albicans* infections thus proving a very good release matrix for the garlic-ginger combined extract.

Further studies are required to fully standardize this garlic-ginger combination for maximum antimicrobial activity without compromising the other desirable properties of either garlic or ginger, and screen various community strains of fungi and bacteria.

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