Original Research Article

ANTIMICROBIAL EFFICACY OF SELECTED NATURAL PRODUCTS ON MICROORGANISMS ISOLATED FROM THROAT OF PATIENTS WITH THROAT INFECTION

ABSTRACT

Introduction: Natural products have been used in traditional medicines for treatment of infections due to the antimicrobial activity they exhibit. This study therefore evaluates the efficacy of honey, ginger (*Zingiber officinale*) and garlic (*Allium sativum*) extracts on microorganisms isolated from throat of patients with throat infection.

Methods: The antibacterial and antifungal efficacy of honey, ginger (*Zingiber officinale*) and garlic (*Allium sativum*) extracts was investigated against microorganisms isolated from throats of infected patients at the ENT Department of State Specialist Hospital, Akure, using agar disc diffusion and agar well diffusion technique respectively.

Results: Bacteria isolated from patients with throat infection were *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa,* and *Proteus mirabilis* while the fungal isolates were *Candida albicans* and *Candida tropicalis.* The antibacterial and antifungal assay results showed that all bacterial isolates were inhibited by honey, garlic and ginger extract. Honey, ginger and garlic showed highest inhibition against *P. mirabilis* (19.01±0.31 mm), *P. aeruginosa* (20.20±0.42 mm) and *S. aureus* (23.00±0.01 mm) respectively also, antifungal assay results showed that all the extracts had antifungal effect on the fungal isolates. The combination of equal concentrations of honey plus garlic showed the highest inhibitory effect on all the test bacteria followed by honey plus ginger then garlic plus ginger while the combination of honey plus garlic had the highest inhibitory effect on *Candida albicans* (21.63±0.02 mm) but garlic plus ginger combination showed the highest inhibitory effect on *Candida tropicalis* (21.68±0.04 mm).

Conclusion: The result of this study therefore showed that the bacteria and fungi isolated from throat of patients with throats infection demonstrated sensitivity towards the tested samples of honey, garlic and ginger and hence, can serve as effective therapeutic agents in the treatment of throat infections.

Keywords: Antibacterial activity, Antifungal activity, Natural products, Throat infection

INTRODUCTION

In recent years, a lot of attention has been focused on producing medicines and products that are natural. Several plants produce chemicals as primary and secondary metabolites which have beneficial long-term health effects and are used effectively to treat diseases [1]. Specifically, it is the secondary metabolites that exert therapeutic actions in humans. It has been stated that more than 30% of entire plant species, at one time or another, are used for medicinal purposes necessarily due to the amount and type of secondary metabolites they contain. These drugs of plant origin have saved lives of many residents of developing countries because of their good values in treating many infectious and non-infectious diseases [2]. Over the years, plants such as ginger, garlic and honey have been used in traditional medicines for treatment of infections due to the antimicrobial activity they exhibit [3, 4].

Ginger (*Zingiber officinale*) mostly used as spice and flavouring, is one of the world's best medicines. Although, native to Asia, ginger is grown throughout the tropics, its therapeutic potentials have been well studied and are reported to be largely due to its volatile oil and oleoresin. It has analgesic, antipyretic and also antibacterial properties [5, 6]. Garlic (*Allium sativum*) is well known for its antifungal, anticancer, antimicrobial activities. The antimicrobial activities of garlic have been related to the presence of growth-inhibiting compounds such as Allicin and related derivatives [3].

Honey is the product of flower nectar produced by beehive. It has been proven to have antibacterial activities. It is well-known for its treatment potential of burns and peptic ulcer, infected wounds, bacterial gastroenteritis and eye infection [4]. The high antimicrobial activity of honey has been attributed to its high osmotic effect, pH (3.2 – 4.5), hydrogen peroxide (H_2O_2), bee defensin, and its photochemical nature [5, 7]. High osmolarity has been considered a valuable tool in the treatment of infections, because it prevents the growth of bacteria [5]. Hence, Honey increases the sensitivity of microorganisms to antibiotics and decrease the microbial resistance to antibiotics [4, 8].

Throat infection can be because of various inflammatory and infective causes such as allergies, reflux disease, sinus drainage, and tonsillitis [6]. Throat infections can be of viral or infective etiology, bacteria and fungi has been a challenge for medical practitioners at the ENT department because the infection is difficult to treat with chemotherapy [4]. The difficulty in the treatment is due to the resistant of these microorganisms to antibiotics and the reoccurrence of throat infections after

few months or years of treatment with antibiotics has led to increase in the morbidity of the infection [9].

Due to the resistance of microorganisms to antibiotics, interest in finding alternative therapeutic measure for the treatment of throat infection has become necessary. In this regard, the present study aims at evaluating the antimicrobial activity of natural products namely honey, ginger and garlic on microorganisms causing throat infections.

MATERIALS AND METHODS

Study area and period

The study was conducted in the Ear, Nose and Throat (ENT) Department of the State Specialist Hospital, Akure and Federal University of Technology, Akure, Ondo state, Nigeria from March to June, 2017.

Specimen Collection

Swabs from throats and tonsils were collected from patients that attended the ENT clinic for a period of three weeks. Specimens were immediately transported in ice-packed containers to the Microbiology Laboratory of Federal University of Technology Akure, for microbiological analysis.

Ethical Approval

Approval was obtained from the Medical director of the State Specialist Hospital, Akure, Ondo state, Nigeria, the ethical approval number was FEB062017A.

Isolation and Identification of Microorganisms

Swabs from throats were screened and identification of microorganisms was done using standard bacteriological procedures as described by Cheesbrough [10]. Collected swabs were dipped into 1.0ml sterile physiological saline and allowed to stand for 10 minutes. It was homogenized and 0.1ml of the suspension was inoculated on MacConkey agar, Mannitol salt agar, Nutrient agar and incubated aerobically at 37^{o}C for 24 hours while Potato Dextrose agar was incubated at 28^{o}C for 48-72 hours. Grown isolates were identified by their colony morphology, Gram staining reaction and biochemical tests including catalase test, citrate utilization test, motility test, indole test, urease test, sugar fermentation test and coagulase test. The fungal isolates were identified based on morphology and microscopic characteristics.

Collection and Authentication of Plant Materials

The ginger, garlic and honey used were purchased at Oja-Oba market, Akure and authenticated at the Museum of the Department of Crop, Soil and Pest Management, FUTA, Ondo state, Nigeria.

Preparation of Plant Extracts

The crude ginger and garlic extracts were prepared according to the method described by Ogodo and Ekeleme [11]. The 500g of ginger and garlic were peeled and washed separately. They were then cut into smaller pieces, weighed and blended in a sterile blender. The blended ginger and garlic yielded 126ml and 173ml of juice respectively, the juice was filtered through a sterile muslin cloth after which the filtrates were purified by passing through Millipore membrane filter paper.

Sterility Check of the extract

Each of the extracts was tested for contaminants by inoculating them on nutrient agar followed by incubation at 37^{o}C for 24 hours after which the plates were observed for growth [12]. No growth in the extracts after incubation indicated that the extracts are sterile after which they were assessed for antimicrobial activity.

Antibacterial Susceptibility Testing

A suspension of 24 hours old pure culture of each bacterial isolate was prepared in nutrient broth (5ml) equivalent to McFarland turbidity standard. The suspensions were spread on to the surface of Mueller-Hinton agar (Oxoid, England) with sterile cotton swabs. The plates were briefly dried and then a circular paper disc which has been soaked overnight in concentrated honey, ginger, garlic, antimicrobial susceptibility assay for the combinations of the selected natural products were carried out by mixing 100ml of concentrated honey with 100ml of concentrated garlic and mixed thoroughly to give a mixture of honey mixed with garlic (1:1), this was repeated for; honey mixed with ginger (1:1), and garlic mixed with ginger (1:1) were added to each plates and incubated over night at 37^{o}C. The diameters of zones of inhibition were measured in millimeters, with a ruler [13].

For positive control, antibiotic susceptibility pattern of the bacterial isolates was tested with amoxicillin by disc diffusion method on Mueller-Hinton agar (Oxoid, England). The plates were incubated at 37^{o}C for 24 hours and observed for zone of inhibition after which the zones of inhibition were measured and interpreted according to Clinical and Laboratory Standard Institute [14].

Antifungal Susceptibility Testing

A suspension of the pure culture of each yeast isolate was prepared in yeast extract broth. The antifungal susceptibility of the isolates was performed by agar well diffusion method. Six equidistant wells of 5mm in diameter were drilled using a sterile cork borer at different sites on the plates. 100µL of each of the extract was aseptically introduced into each holes, and ketoconazole prepared

in solution was used as a positive control. The set up was allowed to stabilize for 3 hours before being incubated at 28^{o}C for 48-72 hours after which the zone of inhibition was measured in millimeters [15].

Statistical analysis

Results were expressed by means of ±SD. Statistical significance was established using one-way analysis of variance (ANOVA). Means were separated according to Duncan's New Multiple Range Test ($p< 0.05$) using software SPSS 20.0.

RESULTS

Isolation and Identification of Microorganisms

A total of 126 isolates were collected from throat swab of patients with throat infections over a 3 weeks' period. The bacterial isolates identified from the specimen collected include *Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Proteus mirabilis* while the fungal isolates include *Candida albicans* and *Candida tropicalis*.

The results revealed that the highest numbers of patients with throat infections were the male patients between the ages 10-20 and the highest microbial count was recorded among the male patients. Details of the demographic distribution of patients with throat infection and the total viable count of bacteria and fungi are presented in **Table 1** and **2** respectively.

Table 1: Demographic Distribution of Patients with Throat Infection

Age (years)	Male (%)	Female (%)	Total (%)
1 - 10	10 (20.41)	7 (14.29)	17 (34.69)
10 - 20	12 (24.49)	9 (18.37)	21 (42.86)
20 - 30	7 (14.29)	4 (8.16)	11 (22.45)
Total	29 (59.18)	20 (40.82)	49 (100)

Table 2: Total Viable Bacterial and Fungal Count of Patients with Throat Infection.

Gender	Bacterial counts (CFU/ml)	Yeast counts (CFU/ml)	Mould counts (SFU/ml)
Male	552.00±1.15^{b}	300.00±0.50^{b}	0.00±0.00^{a}
Female	450.00±0.54^{a}	230.00±1.54^{a}	0.00±0.00^{a}

Values are presented as mean ±SE. Values in the same column carrying different superscript are significantly different at ($p \leq 0.05$) using Duncan's New Multiple Range test.

Susceptibility Pattern of the Isolates to Honey, Ginger and Garlic

The antimicrobial activities of honey, garlic, ginger and their synergistic effects are presented for bacteria and fungi in **Figure 1 and 2** respectively. The highest inhibitory effect of honey was observed with *Proteus mirabilis*, garlic with *Staphylococcus aureus* while ginger showed the highest inhibitory activity against *Pseudomonas aeruginosa.* The synergistic effect of honey and garlic produced the highest inhibitory effect on the bacterial isolates compared to honey/ginger mixture and garlic/ginger mixture.

Candida albicans showed the highest sensitivity to garlic and ginger while the most sensitivity to honey was observed with *Candia tropicalis*. The synergistic effects of the natural products inhibited all the yeast isolates.

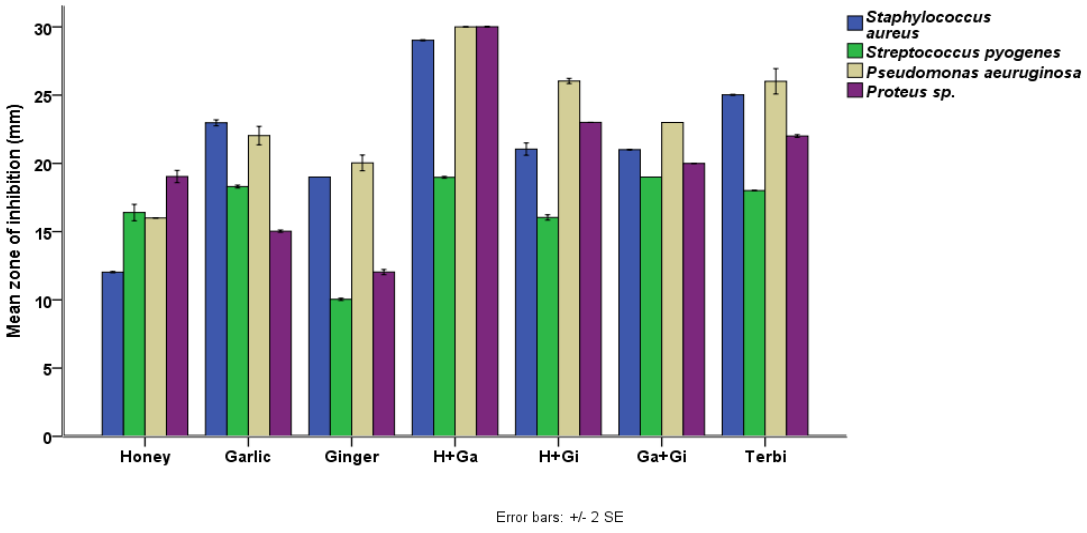

Fig. 1: Antibacterial susceptibility pattern of ginger, honey and garlic on bacterial isolated from throats of infected patients.

Key: H+Ga = Honey plus garlic, H+Gi = honey plus ginger, Ga+Gi = garlic plus ginger, Terbi = Antibiotic

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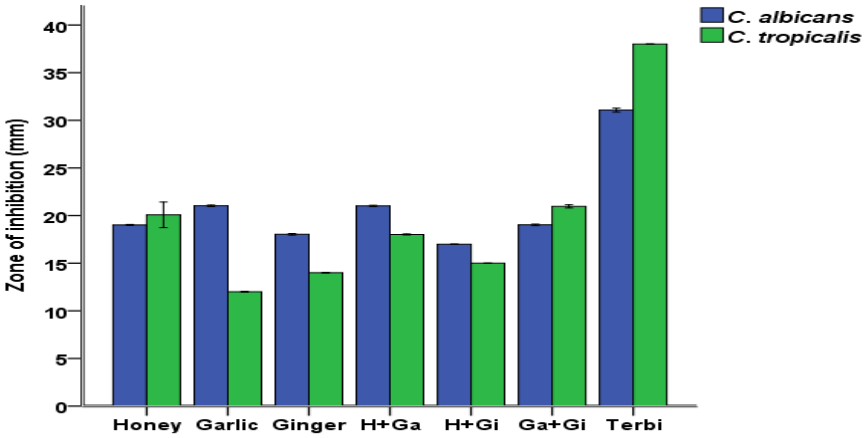

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Fig. 2: Antifungal susceptibility of fungal isolates from throat infection to honey, garlic and ginger

Key: H+Ga = Honey plus garlic, H+Gi = honey plus ginger, Ga+Gi = garlic plus ginger, Terbi = Antibiotic

DISCUSSION

This study has shown that throat infections are caused by bacteria and fungi. However, there were differences in the microbial load of male patients to that of the female patient at State Specialist Hospital Akure. The total viable bacterial and fungal counts observed in male patients was higher than what was observed in female patients. Variations in microbial load may be attributed to the differences in anatomy, lifestyle and socioeconomic differences [16]. The result of this work also revealed that different bacteria such as *Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeuruginosa,* and *Proteus mirabilis* and yeast such as *Candida tropicalis and Candida albicans* may be responsible for causing throat infections. This data collaborates with the previous work [17]. The presence of these bacteria in the throat could be as a result of contamination of the food and water that individuals eat or drink, environmental factors, or by the microflora of the throat [18].

All the tested bacterial and fungal isolates were completely susceptible to the tested samples of honey, ginger and garlic and their mixtures. This study further revealed that honey-garlic mixture produced the highest inhibitory effect on the test bacterial and fungal isolates compared to the single effects and the other combinations i.e. honey-ginger and ginger-garlic mixtures. This could be due to the synergistic effects of honey and garlic on the isolates as many compounds present in both the honey and garlic combined to inhibit the organisms. This result is in close proximity to the other results which stated that natural products have synergistic effect when used as a natural alternative to conventional antibiotics, antibacterial activity of garlic cloves and ginger rhizomes combination on food-borne pathogens were reported to be more effective [3, 11].

In previous study, local residents have been found to use honey for pharyngitis and respiratory ailmen [4]. The antimicrobial activity of honey is highly complex due to the involvement of multiple compounds and due to the large variation in the concentrations of these compounds among honeys. The use of honey where antibiotic treatments had failed to clear infection have been demonstrated in many studies [3, 4]. The control of infection by honey is said to be attributed to its high osmolarity while its hydrogen peroxide content, low pH, content of phenol (inhibin) and other unidentified properties are responsible for its antibacterial properties [19, 20, 21]. Acidity is also one of the factors that contributes to the antibacterial property of honey [20]. The medicinal properties of ginger

are due to variety of bioactive compounds such as tannins, flavonoid, glycosides, essential oils, saponins, phytosterols, amides and alkaloids [3, 11]. The antimicrobial properties of garlic may be due to its potentially active chemical constituents as it contains at least 33 sulphur compounds and several enzymes. One of the most biologically active compounds in garlic is allicin (diallyl thiosulfinate or diallyl disulfide) has been largely attributed to be responsible for the medicinal effects of garlic [3].

CONCLUSION

The single and combined samples of honey, ginger and garlic showed a high degree of antimicrobial activity on the tested bacterial and fungal isolates from throat infections, therefore, these natural products can serve as effective therapeutic agents and a natural alternative to conventional antibiotics in the treatment of throat infections. The combination of honey and garlic however show much promise in the development of phytomedicines in the treatment of throat infections.

Ethical Approval:
Approval was obtained from the Medical director of the State Specialist Hospital, Akure, Ondo state, Nigeria, the ethical approval number was FEB062017A.

Consent: NA

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