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A Natural Alternative to Conventional Antibiotics.

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ABSTRACT

Natural sources of treatment have long been sought by humans as a safe alternative to conventional, synthetic medications and antibiotics in order to avoid their complications. The repeated use of antibiotics, especially in minor illnesses results among other side effects and complications, in weakened immunity, antimicrobial resistance, and susceptibility to fungal infections as that caused by *Candida albicans*. The prolonged use of antibiotics also may lead to gastrointestinal tract disturbances, which usually results in discontinuation of the treatment. We have studied a natural therapy consisting of *Allium sativum*, *Zingiber officinale* and *Apis mellifera* for their potential use in infections. Each component was extracted and prepared according to standard methods and in-vitro tested as a single component and in combination on *Staph. aureus*, *Strep. pyogenes*, *E. coli* and *Candida albicans* and compared with the standard amoxicillin disk. The combination proved effective in-vitro and hence in-vivo testing was carried out on albino mice to test for antibacterial and analgesic activities as well as prophylactic potential. From both the in-vitro and in-vivo testing, it is concluded that these herbs are effective as antibacterial agents.

Keywords: *Allium sativum*, *Zingiber officinale* , *Apis mellifera*, antimicrobial activity.

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INTRODUCTION

Medicinal plants have been considered by many researchers as useful, safe and highly effective remedies for treating different types of infections as they are frequently used in popular medicine. They are preferred by several scientists as an alternative to the conventional antibiotics which have numerous side effects and complications namely toxicity, antimicrobial resistance and susceptibility to fungal infections. The prolonged use of antibiotics may also lead to gastrointestinal tract disturbances, which usually results in discontinuation of the treatment.

For these reasons, a triple antibacterial therapy consisting of *Zingiber officinale* (Ginger), *Allium sativum* (Garlic) and *Apis mellifera* (Honey) has been studied.

***Zingiber officinale* (Ginger)**

Familiar as a spice and flavouring, ginger is also one of the world's best medicines. Native to Asia, ginger is grown throughout the tropics. It is propagated by dividing the rootstock. Ginger has been well studied and its therapeutic properties are largely due to its volatile oil and oleoresin. It has been used traditionally to alleviate nausea and vomiting. It has antipyretic, analgesic properties and also it has antibacterial activity towards gram negative bacteria. [1, 2]

***Allium sativum* (Garlic)**

Garlic is renowned for its antifungal, anticancer and anti-microbial activities. These anti-microbial activities have been related to the presence of growth-inhibiting compounds, such as Allicin and related derivatives [3]. Previous studies proved that garlic is effective against gram negative bacteria. [2].

***Apis mellifera* (Honey)**

Honey is the product of the flower nectar produced by the beehive. It is an antibacterial agent, proven effective on external skin infections. [4, 5]. It has been recently regarded for its potential in treatment of burns and peptic ulcer, infected wounds, bacterial gastro-enteritis and eye infection. Honey increases the sensitivity of microorganisms to antibiotics and decreases the microbial resistance to antibiotics. [5]

The non-peroxide phytochemical components of Manuka Apinae honey from New Zealand (after removing hydrogen peroxide by treating with enzyme catalase) have been found to have substantial levels of antibacterial activity [6]

The following organisms were used in the study:

***Streptococcus pyogenes* (Strep. pyogenes)**

Strep throat is a common ailment that affects more than 700 million people and kills hundreds of thousands annually, around the world. [7]

Staphylococcus aureus (Staph. aureus)

Staph. aureus is a leading cause of many inflammatory diseases such as Otitis media, sinusitis, bronchitis, meningitis and many others. Each year, more than 500,000 patients contract a staphylococcal infection. [8]. More than 90% of *Staph. Aureus* strains are resistant to beta-lactam antibiotics and there are strains that are also resistant to erythromycin, gentamicin, ciprofloxacin and tetracyclines. [9]

Escherichia coli (E. coli)

Pathogenic variants cause intestinal and extra-intestinal infections, including gastroenteritis, urinary tract infection, meningitis, peritonitis and septicaemia. [10, 11]. Therapeutic options vary depending on the type of infection, with trimethoprim/sulphamethoxazole and fluoroquinolones being the common choice [12]. *E. coli* is sometimes used as a sentinel for monitoring antimicrobial drug resistance in faecal bacteria because it is found more frequently in a wide range of hosts and acquires resistance easily. [13]

Candida albicans (C. albicans)

It is a diploid fungus that is a casual agent of opportunistic infections in humans. Systemic fungal infections including those by *C. albicans* have emerged as important cause of morbidity and mortality in immunocompromised patients. It is usually treated using antifungals, topical, oral or vaginal depending on the site of infection. [14]

The repeated use of antibiotics, especially in minor cases, results in a weakened immunity and susceptibility to fungal infections such as that of *Candida albicans*. Antibiotics also lead to gastrointestinal tract disturbances, which usually results in discontinuation of the treatment. This results in resistance and sometimes, tolerance to the antibiotics. [15]

In order to avoid such complications, a natural therapy has been studied for its potential use in certain cases of infections.

MATERIALS AND METHODS

The natural products (garlic, ginger and honey) were obtained from the local market and identified by the botanist at the college.

Garlic was extracted by homogenisation and maceration in almond oil (ratio 1:1) for 48 hours. [16]

Ginger was crushed and its juice was filtered and refrigerated in a sterile jar. As a further step, ginger-infused honey was prepared by gentle warming of freshly chopped ginger in pure honey for one hour. It was then left to cool before refrigeration. [17]

Manuka honey from New Zealand was obtained from the market and stored at room temperature away from heat and light until it was needed for use.

Identification of the microorganisms was carried out before culture. After confirmation, the preparation of the microbiological media was done according to the standard methods. [18] After approval of the “Animal Ethical Committee of the college of Pharmacy and Health Sciences” and following the Principles of Laboratory Animal Care, a total of 60 adult, male albino mice were included in this study. The experiment period was 6 weeks. Mice were housed in their cages with an *ad libitum* access to the regular rat diet and water. They were maintained under 12:12 h light – dark cycle. After one week of habituation to housing conditions they were included in the study.

Experimental

Antimicrobial sensitivity testing was performed on Müller-Hinton agar, using each extract separately (garlic oil, ginger juice, ginger-honey and honey) and as a final combination, which contained 33.3% garlic extract and 33.3% ginger extract in a honey-almond oil base. Amoxicillin was chosen as the standard as it is one of the most commonly dispensed antibiotics. [19]

The culture was incubated for 24 hours, after which the inhibition zone was measured. Tests were conducted on the four organisms and repeated three times, with the closest two taken for average inhibition zone calculation.

For the antibacterial testing, the disc diffusion method was used [18]. The prepared extracts were used for the test. The test bacteria were cultured on Müller – Hinton agar as well as Sabouraud’s agar. Then, a well was made using the wide end of the standardized Pasteur pipette. In each well, one of the extracts was placed. The plate was then incubated aerobically for 24 hours and then the results were read and recorded. Each test was repeated three times with the average results used. The extracts were also tested on *strept. Pyogenes* using 5% blood added on Müller – Hinton. [18].

The formulated product was also tested *in-vivo* on albino mice infected with *Staph aureus*. Infection was carried out according to a modification of *Nakane et al.* method. [20]

Bacteria were cultured on blood agar for 24 hours at 37° C and were inoculated into nutrient broth for another 24 hours. The organisms were collected by centrifugation and were washed with 0.85% saline. A dilution of the bacterial solution was prepared in 0.01 M phosphate buffered saline. Mice were infected by injecting 0.2 ml of a solution containing standard amount of viable bacterial cells into the tail vein [21]. These mice were left for one day as an incubation period. [22]

For the *in-vivo* testing, the formulation was prepared and administered to each mouse as follows:

Ginger was given in a dose of 250 ugm /day for each mouse [23]. The dose of garlic extract is 1.5% of the mass of the mouse weighing 20 - 25 gm [24].

Calculation of Amoxicillin (Amoxil)^(R) dose

When reconstituted the suspension contained 125 mg of amoxicillin/5ml, the dose given to each mouse was 25mg/kg/day.

The 60 mice were divided into 3 sets

The first set, including 24 mice. Mice were infected by bacteria as before then divided into three groups each consisting of 8 mice for detection of recovery and antibacterial effect. Mice of the first group were given 5 ml of normal saline orally/day and considered as the control, Mice of the second group were given the combination of the three herbs orally as described before while the third group of mice was given Amoxicillin oral suspension orally in a dose of 0.025ml/ mouse/ day. Treatment continued for 7 days.

The second set, consisting of 16 mice were investigated for the prophylactic potential of the three herbs. Mice were infected as before, and then divided into two groups. The first group of 8 mice was considered as the control group and simultaneously given normal saline in a dose of 5 ml/ mouse, while the second group of 8 mice was simultaneously given the natural herbs in the previously described dose. These mice were monitored for development of signs of infection. Each mouse was monitored for two days and if it does not show any abnormal behavior (movement, feeding habits) or any sign of infection (described later), it was then returned back to the main cage and observed for another week for the normal activity and signs of infection.

The third set, consisting of 20 healthy mice were used to detect the analgesic activity using the Hot Plate analgesia meter [26] and were divided into 2 groups ; the first / control group of 10 mice were given normal saline as before, while the second group of 10 mice were given the combination of the 3 herbs orally as before. The reaction time before and after products administration was recorded. For the identification of analgesia, the reaction time was measured using the hot plate analgesia meter set at 50°C. Each mouse was first placed on the hot plate. Then the time taken for it to show signs of pain (elevation of tail, jumping and licking of paws) was recorded. After that, the mouse was given the formulation. After the intake of the formulation by one and a half hour, the measurement of the reaction time was repeated again.

RESULTS

In-vitro antimicrobial testing

After incubation of the culture media, the diameter of the inhibitory zones was measured using a ruler. Results were displayed in the following table (table 1) and figures (figures 1-6).

Table 1: Inhibitory zone (in mm) as measured on culture media following the use of the 3 herbs individually and in combination as compared to Amoxicillin.

Agent \ Microorganism	Garlic oil 1:1	Amoxicillin	Honey	Ginger	Ginger-Honey	Garlic, Ginger & Honey
<i>Strep. pyogenes</i>	30	23	9	0	11	35
<i>Staph. aureus</i>	17	8	17	0	18	25
<i>E.coli</i>	24	0	10	0	12	29
<i>C. albicans</i>	40	0	0	0	0	30

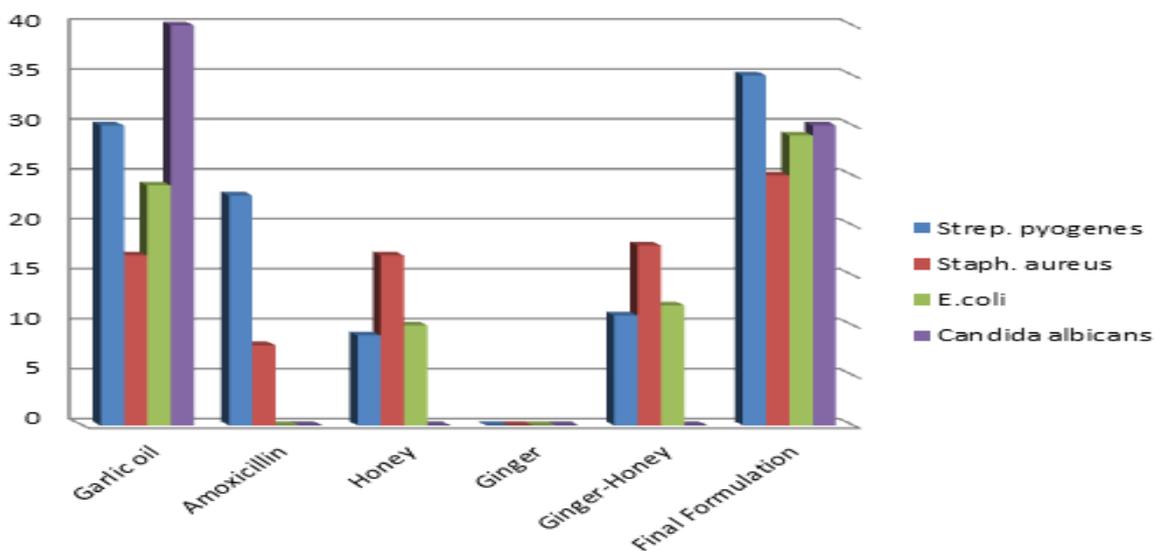


Figure 1: Inhibitory Zone (mm) Produced by Different Agents on the Microorganisms.

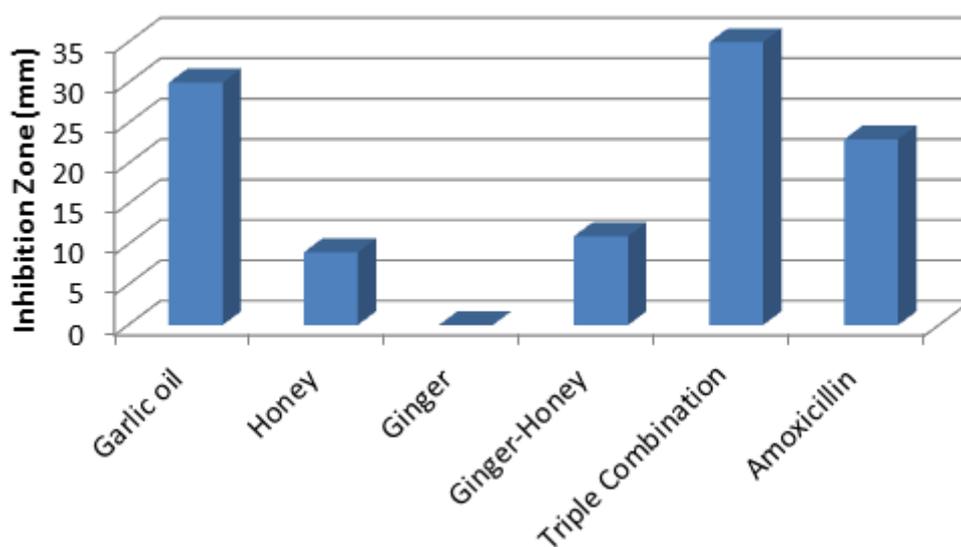


Figure 2: Antibacterial Effect of Different Agents on *Strept. Pyogenes*.

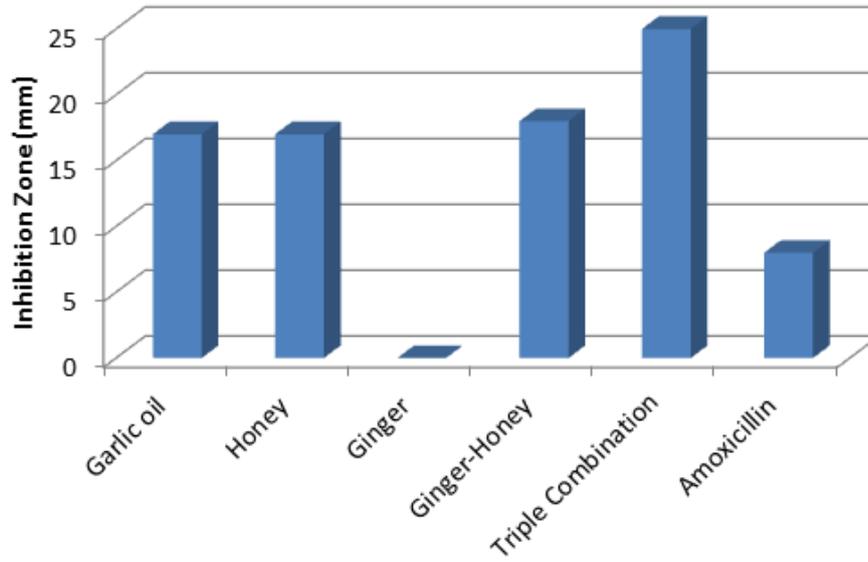


Figure 3: Antibacterial Effect of Different Agents on *Staph. Aureus*.

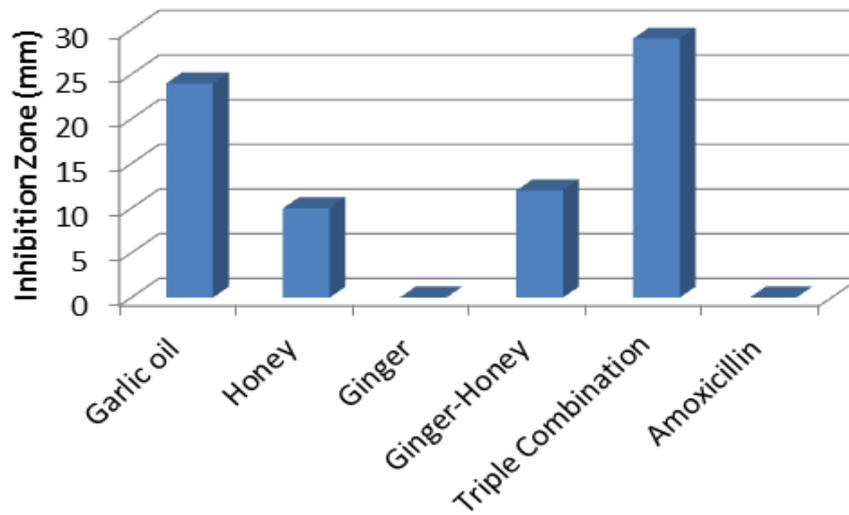


Figure 4: Antibacterial Effect of Different Agents on *E. coli*.

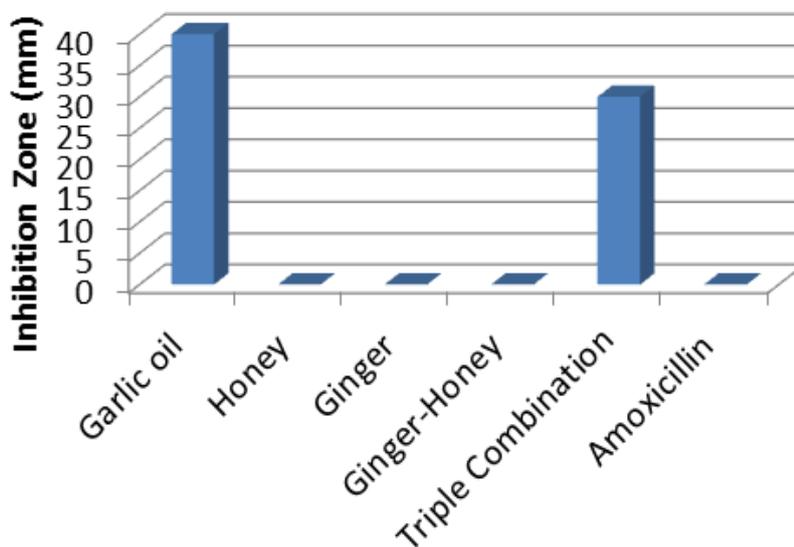


Figure 5: Antimicrobial Effect of Different Agents on *C. albicans*

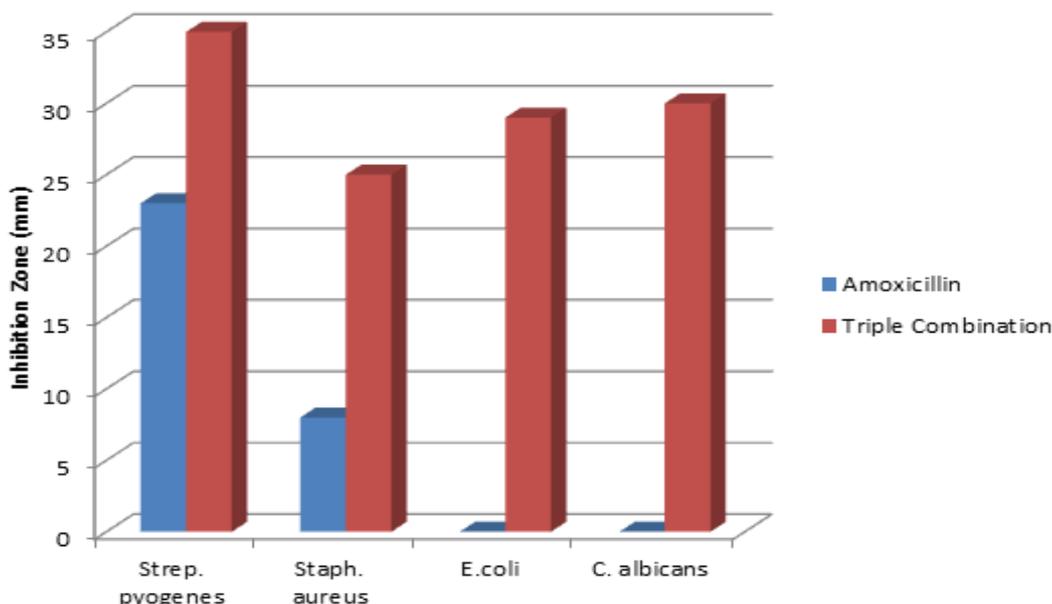


Figure 6: A comparison Between The Inhibitory Zone Produced by Standard Amoxicillin Disc and the Combination of The Three Herbs.

In-vivo antibacterial testing

Mice treated with the natural triple herbal combination recovered within seven days while mice treated with oral amoxicillin suspension recovered within six days. Mice given normal saline did not recover but they became inactive and lethargic. They showed signs of infection described by suffering from difficult labored breathing, discharge from the nose and eyes, sneezing, sniffing, lack of appetite, weight loss, dehydration, watery diarrhea, ruffled hair, hunched posture, rough hair coat, their condition deteriorated and eventually they died.

In-vivo prophylactic potential

Mice were monitored for a total of 9 days following the simultaneous bacterial inoculation and the administration of either normal saline or the natural herbal combination.

Mice administered the natural triple combination did not show the signs of infection described before and continued their normal activities such as exploratory movements, feeding behaviour and interaction with other mice, while mice of the control group (which were given normal saline) showed signs of infection explained before, their condition deteriorated and eventually they died.

In-vivo analgesic activity

After administration of natural herbal combination the average reaction time increased by 70% while after administration of normal saline it changed only by 14.3%. The average results for each group are displayed in table 2.

Table 2: Average Reaction Time before and After Administration of Saline (control group) and The 3 herbal combination on The Hot Plate Analgesia meter in seconds.

Mouse No	Average Reaction Time Before (seconds)	Average Reaction Time After (seconds)	Δ Time (Seconds)	% change in reaction time
Group1 Control (saline)	7	8	1	14.3%
Group2 (combination of 3 herbs)	10	17	7	70%

DISCUSSION

***In-vitro* antimicrobial testing**

Our study showed that *Allium sativum* (garlic) proved to be the most effective single component antibacterial and antifungal agent. The 3 herbal combination is next in efficacy against bacteria and fungus. In case of *E.coli* and *C.albicans* it is effective, where as amoxicillin showed no efficacy. Amoxicillin is the next agent in activity followed by honey. Ginger produced no bacterial or fungal inhibition, which was unexpected. However in a previous study, ginger powder water extract did not show bacterial growth inhibition on the test organisms as it has already been reported [27]. Another study proved that higher inhibition results were found for ginger ethanol or methanol extracts on the test organisms. The highest inhibition produced by this ginger powder extract using fresh ginger rhizome ethanol extract [28]. This difference could be explained by the loss of water soluble antioxidant volatile oils from the ginger powder up on dehydration [29].

***In-vivo* antibacterial testing**

The two groups of mice administered the 3 herbal combination and the amoxicillin oral suspension respectively recovered at almost the same time, with one day difference. This shows that the natural combination has a strong antibacterial potential.

This antimicrobial activity may be attributed to honey as the antimicrobial (antibacterial, antiviral, antifungal, and antiparasitic) activities of honeys were reported to be due to its high osmolarity, acidity, hydrogen peroxide, and phytochemicals. In vivo use of honey for human as therapeutic agent depends on the evaluation of the nonperoxide phytochemical components of honey as hydrogen peroxide can be destroyed by catalase in the body tissues and serum [30]. Similarly, the high osmolarity and acidity of honeys are destroyed in the digestion system or blood circulation of human. The nonperoxide phytochemical components of Manuka Apinae honey from New Zealand (after removing hydrogen peroxide by treating with enzyme catalase) have been found to have substantial levels of antibacterial activity [31]. The antimicrobial effects of different honeys might be also related to Phytochemicals such as Phenolic acids (benzoic and cinnamic acids) and flavonoids (flavanones, flavanols) which were reported for significant contribution of the antioxidant capacity of honey that varies greatly depending on the floral sources [32].

The medicinal properties of Ginger are due to variety of bioactive compounds such as tannins, flavonoid, glycosides, essential oils, furostanol, spirostanol, saponins, phytosterols, amides and alkaloids. These compounds have been isolated from the different parts of the plant and tested for their pharmacological actions. The plant was reported to have antimicrobial, anti-inflammatory, and immunomodulatory [33] and nephroprotective activities. Traditionally, ginger is reported to treat nausea, vomiting, asthma, cough, palpitation, inflammation, dyspepsia, loss of appetite, constipation, indigestion, common cold, stomachache, cough, fever, influenza and pain in different parts of the world. Mixtures of ginger rhizome powder and honeys are also used to treat different types of respiratory and gastrointestinal infections in traditional medicine. [34].

The antibacterial properties of *Allium Sativum* may be due to its potentially active chemical constituents as it contains at least 33 sulfur compounds, several enzymes and the minerals germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc; vitamins A, B1 and C, fiber and water. It also contains 17 amino acids namely: lysine, histidine, arginine, aspartic acid threonine, swine, glutamine, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tryptophan and phenylalanine. It has a higher concentration of sulfur compounds than any other *Allium* species which are responsible both for garlic's pungent odor and many of its medicinal effects. One of the most biologically active compounds in garlic is allicin (diallyl thiosulfinate or diallyldisulfide). The most abundant sulfur compound in garlic is alliin (S-allylcysteine sulfoxide), which is present at 10 and 30 mg/g in fresh and dry garlic, respectively. [35]

The combined antibacterial activity of honey-garlic (*Allium sativum*) [36] or honey and fresh ginger leaves or rhizome extract mixtures was reported superior over the use of these antimicrobial agents individually [37].

Oral administration of honey-ginger and garlic extract mixture, after clinical evaluation and pharmacological standardization, might be of therapeutic value for treating some drug resistant disease causing bacteria or fungal strains. The fact that these herbs are used in human nutrition and the effectiveness of their mixture as antimicrobial agent at very low concentration make them a novel source of effective drug for resistant bacteria strains. However, further clinical tests and pharmacological standardization is needed before using this mixture against drug resistant bacterial strains for therapeutic purposes.

***In-vivo* prophylaxis potential**

Our results proved that mice administered the *Zingiber officinale*, *Allium sativum* and *Apis mellifera* as a combination remained healthy even after bacterial inoculation, so it can be concluded that this combination holds some immune stimulant properties. It is a strong action since all three components have been scientifically proven to hold immune stimulant properties. [33]

***In-vivo* analgesic effect**

From our results, it is clear that the pain sensation was delayed after administration of the triple combination and was most significant as evidenced by prolongation of the

reaction time. This effect may be attributable to ginger. Previous studies indicated that ginger binds to human serotonin receptors and hence relieves pain. [26]

CONCLUSION AND RECOMMENDATIONS

From the previous it can be concluded that the combination of garlic, ginger and honey in non-aqueous vehicle is effective against gram positive bacteria and gram negative bacteria. This combination holds analgesic properties and can therefore be used in case of mild pain associated with infections. It has a potential to be used as an infection-preventive method.

Further studies should be done in the future using different varieties of the garlic and ginger. A study should also be done to investigate why ginger did not produce any bacterial inhibition. A study comparing different extraction methods should also be conducted. A larger number of mice should be used to further confirm safety and efficacy.

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