

Original Article

ANTIBACTERIAL ACTIVITY OF THE AQUATIC EXTRACT OF FRESH, DRY POWDER GINGER, APPLE VINEGAR EXTRACT OF FRESH GINGER AND CRUDE OIL OF GINGER (ZINGIBER OFFICINALE) AGAINST DIFFERENT TYPES OF BACTERIA IN HILLA CITY, IRAQ

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ABSTRACT

Objective: An evaluation for antibacterial activity of extracts of *Zingiber officinale* (ginger) against gram positive bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Streptococcus faecalis*, *Streptococcus mutans*, *Streptococcus faecalis* and gram negative bacteria *Escherichia coli*, *Salmonella typhi*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter spp.*, *Acinetobacter*, *Serratia spp.*

Methods: Four ginger products were used to determine the antibacterial activity of aquatic extracts of fresh ginger, aquatic extract of powder ginger, crude oil of ginger and apple vinegar extract of fresh ginger. Agar well diffusion method was used in this study.

Results: Apple vinegar extract of fresh ginger exhibited excellent and best antibacterial activity against both gram positive and gram negative bacteria and show inhibition zone better than other ginger product, while aquatic extract of fresh, dry powder ginger showed antibacterial activity against gram positive and gram negative bacteria and it show zone of inhibition better than crude oil of ginger.

Conclusion: Most of ginger's extracts show high antibacterial activity against both gram positive and gram negative bacteria, therefore ginger can provide protection to a certain extent against our natural enemies like bacterial pathogens.

Keywords: Ginger, Antibacterial, *Zingiber officinale* antibacterial activity.

INTRODUCTION

Medicinal plants are finding use as pharmaceuticals, nutraceuticals, cosmetics and food supplements. Plant derived products have been used for medicinal purposes for centuries. In traditional Indian medicine or Ayurveda, *Zingiber officinale* and many other herbs have been used as medicine [1]. Zingiberaceae is among the plant families that are widely distributed throughout the tropics, particularly in Southeast Asia. It is an important natural resource that provides man with many useful products for food, spices, medicines, dyes, perfume and aesthetics [2]. Ginger root's immune-system benefits may help protect brain function. A study found that ginger may help inhibit some forms of cancer. Ginger provides non-toxic benefits that activate the immune system to prevent cancer from developing, kill existing cancer cells and prevent tumors from spreading. Ginger inhibits colon cancer, lung cancer, breast cancer, prostate cancer [3-6].

The tissue culture study found that fresh ginger prevented human respiratory syncytial virus, or HRSV, from attaching to and infecting upper respiratory tract cells. Doses of 300 micrograms per milliliter of fresh ginger stimulated the respiratory cells to secrete an antiviral protein called interferon-beta [7]. Further, ginger was also well-regarded for its ability to fight inflammation, to clean colon, to reduce spasms and cramps, and to stimulate circulation. So, it was well justified for the India's Ayurvedic and the ancient Chinese herbalists that had used ginger for 5,000 years as a medical panacea for curing various illnesses [8-10].

The characteristic odor and flavor of ginger root come from a volatile oil composed of shogaol and gingerols. Gingerols have been investigated for analgesic, sedative, antipyretic, antibacterial, and gastrointestinal tract motility effects. They have been found to inhibit Gram-positive and Gram-negative bacteria [11]. In humans, ginger is thought to act directly on the gastrointestinal system to reduce nausea. Traditionally, ginger has been used to treat intestinal infections, especially related with digestive problems. Equally, its antibacterial 'power' is effective against preventing numerous intestinal problems that take place as a result of the alteration of the

intestinal flora. This is ideal to avoid the formation of ulcers by eliminating the *Helicobacter pylori*, a bacterium whose secretions of ammonia are responsible for many ulcers, especially those of the duodena, and for other stomach problems like gastritis, since the plant is able to neutralize the excess of gastric acid that is another of the causes that favors the formation of ulcers [12]. The objectives of this study were to compare the antimicrobial activity of the essential oils and aquatic extract of fresh ginger, aquatic extract of dry powder ginger and apple vinegar extract of fresh ginger against pathogenic bacteria, including Gram positive bacteria and gram negative bacteria.

MATERIALS AND METHODS

Plant collection

Preparation of aquatic extracts of fresh ginger (Aqueous extract was soaked 50 gram of ginger powder by 100 ml distilled water, and allowed to stand for 72 hr, and sterilized by filtration (using Millipore 0.45 filter paper). Aqueous ginger extract preparation fresh ginger were collected from a retail food store (Al-Hilla) 2014. Then, the ginger were cleaned, peeled, sun dried, and cut into small pieces; it was then ground using an electric blender and placed in clean container. Aqueous extract was soaked 50 gram of ginger powder by 100 ml distilled water, and allowed to stand for 72 hr, and sterilized by filtration (using Millipore 0.45 filter paper). This extract was considered as the 50% concentration of the extract. Preparation of apple vinegar, and apple vinegar- ginger extract was dissolved 50 gram of ginger fresh in 100 ml of apple vinegar (weakly acidity), and sterilized by filtration. Oil of ginger were collected from a retail food store (Al-Hilla) 2014 [13].

Bacterial Isolates

Different fifteen clinical microbial isolates (Gram positive, Gram negative (listed at table-1) were isolated and identified by using conventional biochemical tests and Api system (Biomeraux, France) [14] and cultivated in pure culture, at microbiological laboratory/college of Medicine / Babylon University.

Table 1: Bacterial isolates used in this study

| Gram positive bacteria | Gram negative bacteria |
|-----------------------------------|-------------------------------|
| <i>Staphylococcus aureus</i> | <i>Moraxallia catarrhalis</i> |
| <i>Staphylococcus epidermidis</i> | <i>Pseudomonas aeruginosa</i> |
| <i>Streptococcus pneumonia</i> | <i>Proteus mirabilis</i> |
| <i>Streptococcus pyogenes</i> | <i>Klebsiella pneumonia</i> |
| <i>Streptococcus mutanus</i> | <i>Enterobacter spp.</i> |
| <i>Streptococcus faecalis</i> | <i>Acinetobacter</i> |
| <i>Streptococcus galactia</i> | <i>E. coli</i> |
| | <i>Serratia spp</i> |
| | <i>Salmonella typhi</i> |

In vitro Antibacterial activity testing using Agar well diffusion assay NCCLS [15]

Loop full growths from bacterial isolates were inoculated into nutrient broth incubated at 37 °C for 18 hours. The bacterial suspensions were diluted with normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to yield a uniform suspension containing 1.5×10⁸ CFU / ml. Cotton swab was dipped and streak into adjustment suspension the entire Mueller-Hinton agar (for all tested bacteria) surface of plates and the plates were left for one 5 -15 minutes at room temperature to dry. Media were cut into four wells (5mm diameter) by cork borer and add 20µ of the ginger extracts solutions or apple vinegar solution or ginger extracts-vinegar, oil of ginger (The plates were performed in triplicates). All plate of the tested organisms was then allowed to incubate at 37°C for overnight. After 24 h of incubation, each extract was noted for zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm).

Statistical analysis

Bonferroni test recommended by Danial [16] was used for statistical analysis (P ≤ 0.05) to show if there is any significant differences between results of ginger extract, apple vinegar, and ginger extract & apple vinegar combination.

RESULTS

The screening of antimicrobial activity of aquatic extracts of fresh ginger, aquatic extract of powder ginger, Crude oil of Ginger and Apple vinegar extract of fresh Ginger was carried out using the well-agar diffusion test and the results were shown in (Figure-1, 2, 3 and 4).

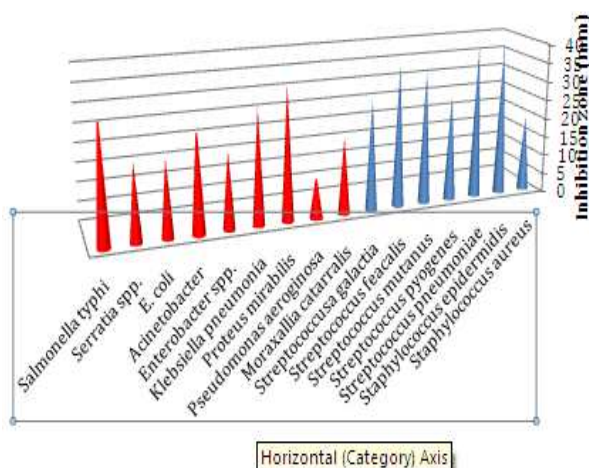


Fig. 1: Antibacterial activity of Apple vinegar extract of fresh Ginger against Gram positive and gram negative bacteria.

These results showed that the Apple vinegar extract of fresh Ginger produced the highest inhibition activity whether against all G+, G- with inhibition zone range 40-10 mm. This high activity followed by the effect of aquatic extract of powder ginger and aquatic extracts of fresh ginger as it inhibited the growth of all the studied isolates with

inhibition zone 7-25mm. On the other hand, the lowest effect obtained from the Crude oil of Ginger as it inhibit the growth of only seven from sixteen isolates (4 G+ and 3 G-) with lowest inhibition zone range from 12-7mm (figure 4) . Statistical analysis showed no significant differences between effect of aquatic extract of fresh Ginger and Ginger-apple vinegar extract combination on bacterial isolates, there were no significant differences between aquatic extract of powder ginger and Ginger -vinegar extract combination on bacterial isolates, there were no significant differences between aquatic extract of fresh Ginger and aquatic extract of powder Ginger extract on bacterial isolates, there were significant differences between aquatic extract of fresh Ginger and Crude oil of Ginger extract on bacterial isolates, there were significant differences between aquatic extract of powder Ginger and Crude oil of Ginger extract on bacterial isolates, and there were significant differences between Ginger -vinegar extract and Crude oil of Ginger extract on bacterial isolates, at level (P≤ 0.05).

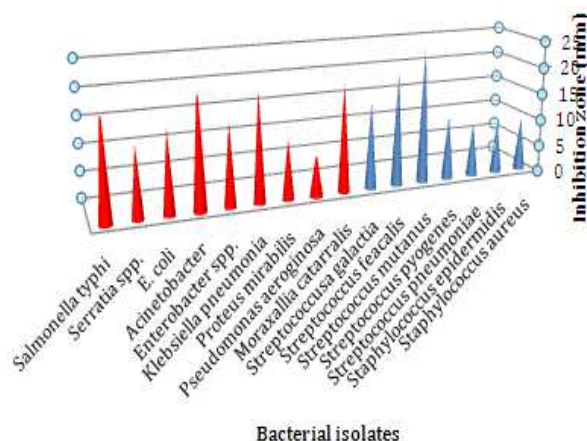


Fig. 2: Antibacterial activity of aquatic extract of powder ginger against Gram positive and gram negative bacteria.

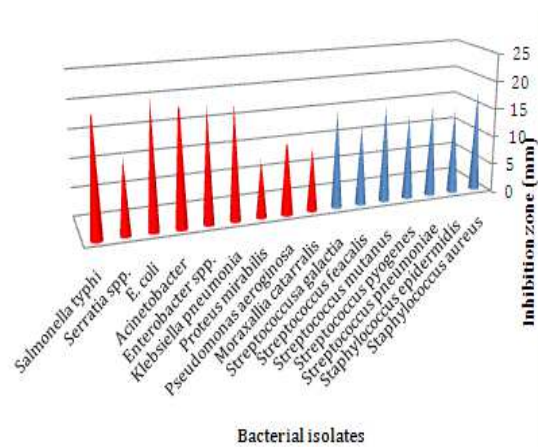


Fig. 3: Antibacterial activity of aquatic extract of fresh ginger against Gram positive and gram negative bacteria.

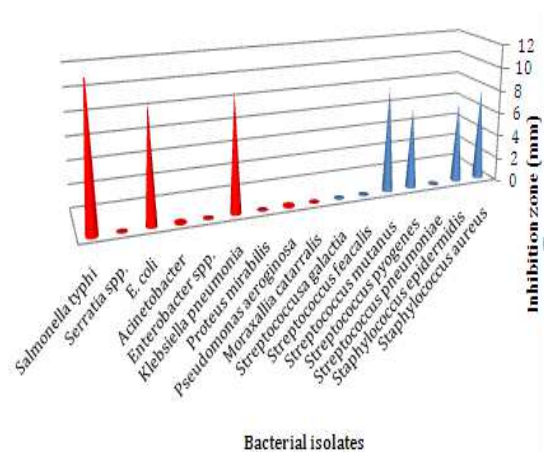


Fig. 4: Antibacterial activity of Crude oil of Ginger against Gram negative and gram negative bacteria.

DISCUSSION

Our present study design to obtain preliminary information on the in vitro antimicrobial activity of *Zingiber officinale* on 16th pathogenic gram positive and gram negative bacteria, the agar-well diffusion method was preferred to be used in this study.

The results show that the extracts of ginger have an antimicrobial activity against both gram negative and gram positive bacteria. This may be caused as a result of the presence of gingerol and shogaol as active ingredient within ginger.

The result of this work indicates that the various soluble extracts of ginger have antibacterial properties. When the extracts were tested on both gram negative and gram positive bacteria, the widest zones of inhibition was obtained with *Proteus mirabilis*, *Klebsiella pneumoniae*, *Salmonella typhi* followed by *Acinetobacter*, *Escherichia coli*, *Moraxallia catarrhalis*, *Serratia spp.*. Then *Pseudomonas aeruginosa* using Apple vinegar extract of fresh Ginger.

The present work shows that the combination of ginger with apple vinegar show the highest antibacterial effect against most of gram positive and gram negative bacteria. Onyeagba *et al* have been reported that mixture of lime and ginger have the highest inhibition zone against *Staphylococcus aureus*; *Bacillus spp.*, *Escherichia coli* and *Salmonella spp* than using ginger alone [12].

Also the results for all extracts were more effective against the Gram-positive bacteria compared to the results for the Gram-negative ones. The higher resistance of the Gram-negative bacteria could be due to the complexity of the cell wall of this group of microorganisms. Indeed, the external membrane of Gram-negative bacteria renders highly hydrophilic surfaces whereas the negative charge of the surface of the Gram-positive wall may reduce their resistance to antibacterial compounds [17].

Other in vitro studies have shown that active constituents of ginger inhibit multiplication of colon bacteria, these bacteria ferment undigested carbohydrates causing flatulence, this can be counteracted with ginger [17]. It inhibits the growth of *Escherichia coli*, *Proteus sp*, *Staphylococci*, *Streptococci* and *Salmonella* [18,19].

Study of Suhad *et al* showed that aqueous extract of ginger gave highest activity against *Klebsiella pneumoniae*, *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus*. Antimicrobial activity of ginger extract was compared with a number of antibiotics that known for their ability include nalidixic acid, trimethoprim, chloromphenicol, gentamicin and erythromycin by using antibiogram test. She found that antimicrobial activity of ginger was better than that to Chloromphenicol, Gentamicin against *Klebsiella pneumoniae*, *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus* [20].

Results shows there is differences in the zones of inhibition may be directly related to the susceptibility of each test organisms to the ginger extracts. The factors responsible for this high susceptibility of *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Escherichia coli* to the extracts may be attributed to the presence of secondary plant metabolites [20].

The result showed that the crude oil extract of ginger have less range of bacterial inhibition than other type of extract, it can inhibit just *Klebsiella pneumoniae*, *E. coli* and *Salmonella* but the zones of inhibition were significantly lower than the zone of inhibition using another extracts type.

Aquatic extract of dry powder ginger and aquatic extract of fresh ginger can inhibit gram negative bacteria but their ability of inhibition were lower than the ability of Apple vinegar extract of fresh Ginger. Similar results were obtained using the four types of Ginger products with gram positive bacteria.

Ginger can inhibit an antibiotic resistant strain of *E. coli*, a bacterium that causes intestinal and food-borne illness. In recent years, several reports have been published concerning the composition and/or the biological properties (antimicrobial, antioxidant, anticancer and a stimulated effect on the immune system) of Zingiberaceae extracts oils extracted from different species or varieties. These variations are likely to influence the antimicrobial activity of the oil and are generally a function of three factors: genetically determined properties, the age of the plant and the environment [2].

Results shows that all Ginger products can inhibit *E. coli*, researchers approved that ginger can inhibited an antibiotic resistant strain of *E. coli*.

other study tested both Garlic and Ginger extracts against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Shigella sonnei*, *Staphylococcus epidermidis* and *Salmonella typhi* while the inhibitory effect with Ginger alone was less [17,18].

Study of Masniari, 2011 found that the red ginger the traditional remedy was effective in controlling *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus agalactiae* the three bacteria were responsible for mastitis [19].

Nwaopara *et al.* (2009) reported that: ginger had strong antibacterial and to some extent antifungal properties. *In vitro* studies had shown that active constituents of ginger inhibited multiplication of colon bacteria. Ginger inhibited the growth of *Escherichia coli*, *Proteus sp.*, *Staphylococci*, *Streptococci* and *Salmonella*. Hence, ginger should have impact on the growth of *Bacillus cereus*, which mainly caused diarrhoea and nausea [21].

Ginger compounds are active against specific type of diarrhea which is leading to cause death in infant in developing countries. Moreover, it has been found that ginger is effective in treating nausea caused by sea sickness, morning sickness and chemotherapy, though it was found superior over a place for post-operative nausea [3]. In addition, it has been reported that the main ingredients of ginger like volatile oil, gingerol, shogaol and diarylheptanoids work as antioxidant, anti-inflammatory, anti-lipid, anti-diabetic, analgesic, antipyretic and anti-tumor [22].

The gingerols have analgesic, sedative, antipyretic, antibacterial and gastrointestinal tract motility effects. Ginger has the capacity to eliminate harmful bacteria, such as *Escherichia coli*, responsible for most of the diarrhoea, especially in children. Ginger eases both diarrhea and constipation; hence it should have impact on the growth of *Bacillus cereus*, which mainly causes diarrhoea and nausea. It has been shown to reduce the stickiness of blood platelets, hence may help reduce risk of atherosclerosis [23].

It has been reported that ginger extract and its pungent compounds demonstrated greater antibacterial activity against a variety of bacterial species including *Helicobacter pylori*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, although mixed result is attributed to different ginger preparations and varying strength [11].

Today, most pathogenic organisms are becoming resistant to antibiotics. To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Most of the spices extracted either in water or in organic solvents have biologically active compounds, which can be used in the synthesis of potent drugs. Thus spices, which are normal ingredients of our routine food preparations, can provide protection to a certain extent against our natural enemies like bacterial pathogens[20].

CONCLUSION

The present study showed that apple vinegar extract of fresh ginger exhibited strong antibacterial activities followed by the activity of aquatic extracts of fresh ginger and aquatic extract of powder ginger while crude oil of ginger exhibited the lowest antibacterial activities.

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