BIOFARMASI J NAT PROD BIOCHEM
Volume 16, Number 1, February 2018
Pages: 36-50

E-ISSN: 2580-2550
DOI: 10.13057/biofar/f160105

Anti-bacterial properties and GC-MS analysis of extracts and essential oils of selected plant product

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Manuscript received: 4 September 2017. Revision accepted: 17 February 2018.

Abstract. *Nyaitondi OD, Wanjau R, Nyambaka H, Hassanali A. 2018. Anti-bacterial properties and GC-MS analysis of extracts and essential oils of selected plant product. Biofarmasi J Nat Prod Biochem 16: 36-50.* Plants are traditionally used to treat bacterial infections though not clinically regulated due to a lack of awareness and sufficient data to support the reported therapeutic claims. Some plants used as food and vegetables are hardly considered in such studies. This study aimed to investigate the antibacterial properties associated with garlic, ginger, turmeric, lemon, and onion in the form of juices, methanol extracts, and essential oils. These materials were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi*. Identification of suspected antibacterial compounds was made by comparing retention indices and the mass spectra with those in National Institute of Standards and Technology (NIST) libraries using GC-MS analyses. Garlic juice was bactericidal against all tested strains. Lemon/garlic juice exhibited significantly higher activity against *E. coli* and *S. typhi*. Turmeric/lemon/garlic methanol extracts blend was most active against *S. aureus*. Preliminary screening of the essential oils indicated significant antibacterial activity of lemon/garlic essential oil blend against *P. aeruginosa*. GC-MS analysis of the active samples confirmed the presence of compounds containing OOH,-OH,-N,-Cl,-F,-NH_2, and-S groups associated with bacterial inhibition in conventional antibiotics. The 10 major constituents obtained from samples suspected to contain antibacterial activity, include limonene; 3-vinyl-1,2-dithiacyclohex-4-ene; α-zingiberene; diallyl disulphide; 2-butanone,4-(-hydroxy-3-methoxyphenyl); 3-chlorothiophene; methanehydrazonic acid, N-[3-(methylthio)-1,-2,4-thiadiazol-5-yl]-,ethyl ester; n-hexadecanoic acid; γ-sitosterol and propanamide,2-amino-3-phenyl. Juices of garlic, lemon, and lemon/garlic blend were active against one or more bacteria tested, unlike methanol extracts and essential oils. They should be used in raw form as heating and drying are likely to render them inactive. Further studies on methanol extract and fresh juice of lemon/garlic blend need to be undertaken to elucidate the active principles in these extracts and may lead to the discovery of novel antimicrobial agents and models for the new generation of synthetic antibiotics.

Keywords: anti-bacterial, garlic, ginger, turmeric, lemon, onion, GC-MS analysis

INTRODUCTION

The continuous spread of multidrug-resistant pathogens has become a threat to public health and a significant concern for infection control practitioners worldwide (Borowitz and Naser 2011). Not only increasing the cost of drug regimens, but this scenario has also paved the way for the re-emergence of previously controlled diseases and has contributed substantially to the high frequency of opportunistic and chronic infection cases in developing countries (Collins et al. 1999). Among some pathogens, bacteria cause a wide range of infections, resulting in mild to life-threatening illnesses that require immediate interventions (Martin and Edzard 2003). Common bacterial infections include respiratory infections, gastrointestinal infections, ear infections, and skin disorders (Mandal et al. 2005; Arthur 2006).

In developing countries, outbreaks of bacterial infections occur most often in densely populated areas such as refugee camps and slums. Food vendors, slum dwellers, riparian communities, fishers, and school children are among the risk groups (Brooks et al. 2005; Change 2009). Studies conducted by the Center for Microbiology Research in KEMRI, Nairobi, Kenya, show that 41% of people contracted typhoid fever in 2008 (Kariuki 2008). The study further demonstrated that 52% of the cases affected children under 10 years old and 40% of people aged 15 and 45 years. More than half of these cases were from the informal settlements (slum areas) surrounding the capital city (Kariuki 2008). There have been studies of sporadic outbreaks of bacterial infections in many regions, including three districts in Central Kenya, Malindi, and Kwale in the Coast Province and some parts of Nyanza Province (Onyango 2005). However, not all outbreaks were confirmed, leading to a lack of reliable data on the prevalence of diseases caused by bacteria (WHO 2009).

Conventional antibiotics usually provide effective therapy for bacterial infections (Martin and Edzard 2003). Nevertheless, these bacteria have become resistant to one or more antibiotics, and the population of Multidrug-Resistant (MDR) bacteria is increasing (Stewart and Costerton 2001). Mechanisms that microorganisms have developed to resist antibiotics include inactivation of antibiotics by enzymes, alteration of drug target sites, blockage of drugs from entering into the cell membrane, and chromosomal and plasmid-mediated resistance.

Closely related to bacterial infections is malaria, caused by the *Plasmodium* parasite (Ndyomugyenyi et al., 2007;

Charles, 2010*)*. The symptoms include fever, shaking, chills, headache, muscle aches, tiredness, nausea, vomiting, and diarrhea (Ali et al. 2007; Mohanna et al. 200, similar to some bacterial infections (especially typhoid). Therefore, patients are focused on treating malaria instead of bacterial infections (Balentine 2009). The symptoms are more dramatic in children, and if untreated, they may kill fast (Onyango 2009).

Antibiotics such as ampicillin, chloramphenicol, Trimethoprim/Sulfamethoxazole (TMP-SMX), amoxicillin and ciprofloxacin have been commonly used to treat bacterial infections (Wain and Kidqell 2004). The bioactive parts against bacteria in these conventional antibiotics include structural moieties that include Cl,-F,-N,-NH_2,-S,-COOH, and-OH, which are also found in many herbs used traditionally against bacterial infections. Studies have shown that sulfur-containing compounds have strong inhibitory antibacterial activities (Julia and Ann 1947; Kyung and Fleming 1996; Yanyali et al. 2001). Nitrite exhibits toxic properties while nitrous acid is bactericidal; chlorine-releasing compounds such as chlorine dioxide (ClO_2) and acidic and alcoholic compounds act as antibacterial agents (Gerald and Russell 1999).

Some vegetable trials have been comparable to conventional treatments and provide therapy for bacterial infections (Martin and Edzard 2003). The compounds in drugs vary in different species. Even within a single species, the phytochemical composition may be affected by the plant‟s growing conditions, and various parts of a herb can have distinct chemical structures (Linda et al. 2008). Most herbs, foods, and spices contain antibacterial properties; for instance, allicin, a compound produced in garlic, was proven to be active against bacteria and fungi (Serge 2001; Onyeagba et al. 2007; Lian-fang et al. 2009; Pandey et al. 2011).

The objectives of this research were (i) To determine *in vitro* antibacterial activities of juices, methanol extracts, and essential oils of garlic (*A. sativum*), ginger *(Z. officinale*), onion (*A. cepa*), turmeric (*C. longa*), and lemon (*C. lemon*) individually and as blends. (ii) To determine the time-course antibacterial activities of garlic (*A. sativum*), ginger *(Z. officinale*), onion (*A. cepa*), turmeric (*C. longa*), and lemon (*C. lemon*) juices individually and as blends. (iii) To identify suspected antibacterial constituents of the active samples and blended essential oils by GC-MS.

MATERIALS AND METHODS

Experimental procedures

Over time, this study involved bioassay of garlic, ginger, turmeric onion, lemon juices, methanol extracts, and essential oils. Identification of active compounds was performed using GC-MS.

Sample collection and pretreatment

The vegetable materials (garlic, ginger, onion, and turmeric) and lemon were purchased from Githurai market in Nairobi. The ginger, turmeric, lemon fruit, and onion rhizomes were washed using tap water to remove dirt. The materials were dried up at room temperature for six hours, then stored in a dry cabin at room temperature awaiting extraction.

Instrumentation

The HP 5890 series II Gas Chromatograph was interfaced to a 5973 Mass Selective Detector (MSD) and controlled by HP Chemstation software (version b.02.05, 1989-1997). The chromatographic separation was achieved using an HP5-MS capillary column (30.0 m x 250 m x 0.25 m). The stationary column phase comprises a 5: 95% diphenyl: dimethylpolysiloxane blend. The operating GC condition was an initial oven temperature of 35 °C for 3 min, then programmed to 280^0 C at the rate of 10^0 C/min, and then kept constant at 280^0 C (23 min). The injector and detector temperatures were set at 270^0 C, and the carrier gas was nitrogen-flowing at a rate of 1.2 ml/min. The mass spectrometer was operated in the electron impact mode at 70 eV. Ion source and transfer line temperature were kept at 280^0 C. The mass spectra were obtained by centroid scan of the range from 40 to 800 amu. Retention index made identification of the constituents, library mass search database (NIST & WILEY, and compared with the mass spectral data.

Isolation and extractions

Methanol extractions

The vegetable materials and lemon were cut into small pieces and dried at room temperature for three weeks. The materials were ground into powder using a blender and soaked in methanol for 72 hours with occasional stirring. The extracts were filtered using Whatman's No. 1 filter paper (9 cm). The filtered extracts were then concentrated using a rotatory evaporator and dried to a paste in a hood. The crude extract was then used for bioassay.

Steam distillation

The essential oils were isolated from all the materials except onion since they did not produce a significant amount of hydrodistillate. The materials were chopped into small pieces. Using a round-bottomed flask, 1 kg of each material was mixed with 1 liter of water and then steamed distilled using Clevenger-type apparatus (Figure 3.2). A flask containing the homogenate was heated for three to four hours, and the oil was separated from water using a Pasteur pipette. The essential oils were put in amber-colored vials, labeled, and stored at −4° C before bioassay (Tassou et al. 1995)

The oil isolated from garlic was extracted using DCM. The mixture of oil and DCM was treated with anhydrous sodium sulfate to remove any dissolved water and evaporated using a rotatory evaporator. The oil was labeled, put in an amber-colored vial then stored at −4° C before bioassay.

Juice extractions

The bulbs of garlic and onion, rhizomes of ginger and turmeric, and lemon were cut into small pieces and crushed using a juice extractor. The juice was sieved, put in amber-colored vials, and concentrated by freeze-drying. The

extractions were done two hours before the commencement of the sensitivity test. The sensitivity test was done within five days of preparation.

Bioassays

Preparation of McFarland standard

McFarland equivalent turbidity standard (0.5 McFarland) was made by adding 0.6 ml of 1 % $BaCl_2$. $2H_2O$ to 99.4 ml of 1 % H_2SO_4 and mixed. About 5 ml of the turbid solution was transferred to a stopped test tube of the same type that was used to prepare the test and control inoculums, then stored in the darkroom at a temperature of 25^0 C. Exactly 0.5 McFarland gives an equivalent approximate density of bacteria $1x10^{-8}$ Colony Forming Units (CFU) (Baron and Yolken 1999).

Preparation of inoculums by direct colony suspension method

Microorganisms obtained from KEMRI included one gram-positive bacteria, *S. aureus* (ATCC 25923), and three gram-negative bacteria, *E. coli* (ATCC 25922), *S. typhi* (ATCC 20613), and *P. aeruginosa* (ATCC 27853). Before use, the test strains were tested biochemically for viability and purity (Elgayyer et al., 2000). Sterile water (small volume) was poured inside a test tube to which general colonies of the test organisms, and the suspension was adjusted to match the 0.5 McFarland's standard (10^8 CFU/ ml), which resembles the appearance of an overnight broth culture by adding distilled water (Azu et al. 2007).

Screening for antibacterial activity

Disc diffusion test. Antibacterial efficacy was tested using the filter paper disc diffusion method (Elgayyar et al., 2000). Each extract (3 g) was dissolved in DMSO and 10 µL (100 mg/mL) loaded onto 6 mm (Whatman's No. 3) filter paper discs and air-dried. The vegetable and lemon blends were made in a ratio of 1: 1. The nutrient agar (NA) was used in culturing the bacteria. The media (NA) was prepared using the manufacturer's instructions, while plates were prepared by adding Mueller-Hinton (MH) agar.

Each plate was inoculated with 0.1 ml of bacteria culture directly from the 24-hour broth culture and diluted to match the 0.5 McFarland standard. The discs loaded with the extracts were placed onto the seeded plates. The bacterial cultures were incubated at 37^0 C for 24 hours, after which zones of inhibition were measured and recorded in mm. Negative control plates had discs with DMSO and water; positive control had standard antibiotic discs of chloramphenicol, ciprofloxacin, and ampicillin. An inhibition zone of 9.0 mm was taken as the base, and any sample that recorded less value was treated as inactive against the test microorganism.

Minimum inhibitory concentration (MIC) Test. The active samples (with an inhibition zone of ≥9) from the antibacterial screening were tested for minimum inhibitory concentration (MIC). Different concentrations of essential oils, juices, and methanol extracts were prepared by dissolving 3.0 g of the crude samples in 2.0 ml of DMSO to determine the MIC. The blends were prepared by mixing the resultant mixtures in the ratio of 1: 1, and 100 µL of the samples were drawn into a 96-well microtiter plate. Concentrations of 750 mg/mL, 375 mg/mL, 188.5 mg/mL, 93.8 mg/mL, 46.9 mg/mL, 23.4 mg/mL, 11.7 mg/mL, 5.9 mg/mL, 2.9 mg/mL and 1.5 mg/mL were made using serial dilution method (Elgayyar et al. 2000; Kariba 2001).

The test strains adjusted to 0.5 McFarland standard were drawn into wells. Blends of active essential oils and methanol extracts were made at 1: 1. The MIC for bacteria was measured using a broth dilution method of the active extracts. Tubes containing only nutrient broth were seeded with the test organism, as described above, to serve as the control. The cultures were incubated at 37 °C for 24 hours and were examined for bacterial growth by observing turbidity. The MIC was the first tube showing no growth (the lowest concentration inhibited growth) (Kariba 2001; Michael et al. 2003).

Minimum bactericidal concentration (MBC). The minimum bactericidal concentration (MBC) of the active extracts was done by subculturing 0.1 ml (100µl) of all the tubes showing no growth on nutrient agar. After 24 hours of incubation at 37 °C, the first plate showing no growth was the MBC (Michael et al. 2003).

GC-MS analyses

Samples of 3.0 g garlic, ginger, lemon, and turmeric were crushed and dissolved separately in 5 ml of DCM. They were shaken and mixed using the ultrasound path for 3 min, then filtered using glass wool. The sample was drawn into small vials, and then 1 µL was injected into the GC-MS. China garlic was also prepared similarly and analyzed for comparison with garlic (used for bioassays). The active methanol extracts were blended in 1: 1, and 2 ml of pentane was added to each blend. The mixture was left overnight, filtered using glass wool, and 5 µL of the filtrate was dissolved in 1 ml of pentane. The sample (1 µL) was injected into the GC-MS for analysis. The active essential oil blends (in the ratio of 1: 1) were also drawn into small vials, and then 1 µL was analyzed.

Data analyses

The inhibition zone data obtained from juice and methanol extracts were subjected to analysis of variance (ANOVA). Individual essential oils recorded less than 9 mm activities, and their results were not subjected to ANOVA. The mean inhibition zones of their active juices and methanol extracts against *S. typhi*, *P. aeruginosa*, *S. aureus,* and *E. coli* were compared to their blends. Treatment means showing a significant difference ($p \leq 0.05$) were separated using Student-Newman-Keuls (SNK) at a 5% significance level. The GC-MS chromatograms acquired from each active sample were subjected to HP Chemstation software; each peak was analyzed for the most abundant compound that contains active constituents-OH,-COOH,-Cl,-S, N,-F, and-NH_2. The compounds were identified by directly comparing their mass spectra to the Wiley NBS and MIST database library of mass spectra.

RESULTS AND DISCUSSION

Antibacterial activities

Vegetable and lemon juices

The inhibition zones of juices on gram-positive and gram harmful bacteria were determined using the filter paper disc diffusion method (Elgayyar et al., 2000). The results are indicated in Table 1.

Garlic juice (Figure 1, sample 40) inhibited the growth of all bacteria tested to variable levels (10.0 mm for *P. aeruginosa*, Figure 1.A; 11.7 mm for *E. coli,* Figure 1.B; 14.7 mm for *S. aureus* Figure 1.C*;* and 17.7 mm for *S. typhi*, Figure 1.D.). Lemon juice inhibited only the growth of *S. typhi* with a zone of 11.0 mm. Turmeric, lemon, and ginger juices had no activity against *P. aeruginosa*, *E. coli,* and *S. aureus*; this can be attributed to the low concentration (10 µL) of samples used for bioassay. Earlier studies on their activity show that they had antifungal and antibacterial agents at concentrations of 50 µL and 100 µL (Gopalan et al. 2000; Jayaprakasha et al. 2002; Fisher and Phillips 2006).

The high antibacterial activity exhibited by garlic compared to lemon may be attributed to sulfur-based compounds such as alliin, which possess strong antibacterial activities (Larkcom 1976: Bocchini et al. 2001). These compounds are found in the intact bulbs, flavorants formed on cutting or crushing the bulbs, substances derived from further reactions of these flavorants, or metabolic degradation of these three types of compounds (John and Timothy 1997). The results agree with earlier reports where garlic was effective against a plethora of gram-positive and gram harmful bacteria such as *S. aureus*, *Proteus*, *Pseudomonas*, *E. coli*, *Salmonella,* and *Klebsiella* (O'Gara and Hill 2000). On the other hand, the activity of lemon can be attributed to the presence of-COOH and-OH group, which act against the bacteria (Angel 2006). However, *E. coli* was not susceptible to lemon juice, due to its unusual acid-resistant properties. The microorganism can survive and grow in acidified media (Greg and Ann 2007).

Research has found the aqueous extract of garlic to be more potent than organic extracts (Roy et al. 2006; Jaber and Al-Mossawi 2007). This could be a result of the fact that some phenolases and hydrolases are released when plant materials are ground in water . These enzymes might modulate the active compounds' activity in the extract (De and Ifeoma 2002). Since the herbalist usually uses water to prepare infusions and decoctions, and since most constituents of garlic are soluble in water, there is a likelihood that the herbalist can extract all the bioactive drug components in garlic, making it a proper home remedy against some infections.

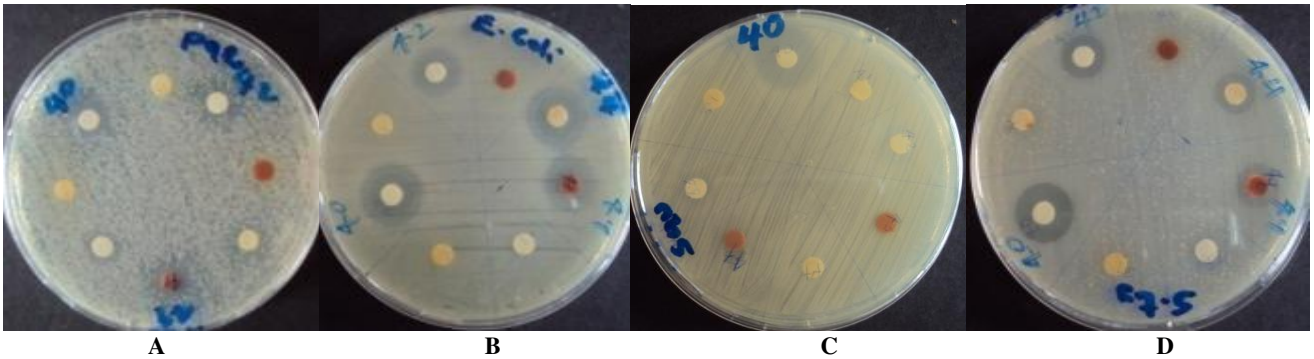
Positive controls had diverse activities depending on the type of sample used. The activity of ampicillin on *E. coli* and *S. typhi* was 11.7 mm and 18.7 mm, respectively (Table 1). Ciprofloxacin had an activity of 41.7 mm against *P. aeruginosa*, 30.7 mm against *E. coli*, and 17.3 mm against *S. aureus,* and 34.67 mm against *S. typhi*. Chloramphenicol had the highest activity against *E. coli* (35.0 mm) and *S. aureus* (34.0 mm) (Figure 2).

The activity of standards (+ve controls) is considerably high compared to the samples used. This can be accredited to the pure form of the standards and, therefore, no interferences from other compounds. The natural juices contain mixtures of compoun , including non-active constituents, which may dilute of the active constituents (Narayana et al. 2000).

Table 1. Antibacterial activity exhibited by various juices against *P. aeruginosa, E .coli, S. aureus,* and *S. typhi*

Juice/ antibiotic	Inhibition zone in mm[a]			
	P. aeruginosa	***E.coli***	***S.aureus***	***S.typhi***
Turmeric	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Lemon	6.0±0.0	6.0±0.0	6.0±0.0	11.0±1.0
Ginger	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Garlic	10.0±0.0	11.7±0.3	14.7±2.5	17.7±2.5
Onion	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
DMSO (-ve)	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Water (-ve)	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Ampicillin (+ve)	6.0±0.0	11.7±0.3	6.0±0.0	18.7±0.6
Ciprofloxacin (+ve)	41.7±0.6	30.7±0.3	17.3±2.1	34.7±0.6
Chloramphenicol (+ve)	20.0±0.0	35.0±0.0	34.0±0.0	30.7±0.3

Note: [a] includes the diameter (6 mm) of the disk used; +ve: positive control; -ve: negative control



A **B** **C** **D**

Figure 1. Plates showing inhibition zones of garlic against *P. Aeruginosa* (A), *E. coli* (B)*, S. aureus* (C), and *S. typhi* (D)

Figure 2. Plate showing inhibition zones of chloramphenicol and ciprofloxacin on *S. aureus.* Note: C-Chloramphenicol; CF-Ciprofloxacin

Juices of turmeric, ginger, and onion did not significantly inhibit the growth of any microorganism tested; their inhibition zones were 6.0±0.0 mm each. The sulfur-based compounds which are accredited to bacterial activities might have been destroyed during the cutting and crushing of onion, the bacteria may have developed resistance to the onion, ginger, and turmeric or the relative percentage of the active compounds in the samples was low (Griffiths et al. 2002). Onion has also proven ineffective against gram-negative bacteria such as *S. aureus*, *E. coli,* and *S. typhi* due to fewer amounts of allicin (Farbman et al. 1983).

Although ginger, turmeric, and onion juices tested individually showed no significant inhibition, some blends of these vegetables (1: 1, v/v) were active (Table 2). The highest activity was exhibited by lemon/garlic (15.0 mm) and lemon/garlic/turmeric (14.7 mm) against *E. coli.* The blends of lemon/garlic, ginger/garlic, lemon/garlic/ginger, turmeric/ginger/garlic, and lemon/garlic/turmeric had appreciable activities against *E. coli* and *S. typhi*. Ginger/ lemon, lemon/turmeric, ginger/turmeric, and lemon/ginger/ turmeric blends had no significant activity against all the four bacteria tested. *S. typhi* was susceptible to turmeric/ garlic and lemon/garlic/turmeric/ginger at a zone of 12.0 mm and 9.7 mm, respectively. The test bacteria, *P. aeruginosa* and *S. aureus,* did not record any activity when the juice blends were used.

Table 3 gives the results of the Student-Newman-Keuls (SNK) test on the mean inhibition zones of individual juices and their blends against *S. typhi*, *P. aeruginosa*, *S. aureus,* and *E. coli* bacteria. The mean inhibition zone of garlic juice against *S. typhi* was significantly different ($p < 0.05$) compared to other tested materials and not significantly different from ampicillin. Lemon/garlic/ turmeric blend gave inhibition zones against *E. coli* and *S. typhi* that are substantially different from pure garlic ($p < 0.05$).

From the mean inhibition zones, it can be noted that ginger and turmeric lower the activity of blends and the lemon/garlic blend has lower activity on *S. typhi* (12.0±0.0) compared to pure garlic (17.7±2.5). This may be due to the deactivating effect of citric acid on allinase, an enzyme that converts alliin to allicin (Bocchini et al. 2001). The transformation of alliin to allicin is exceptionally rapid, taking mere seconds. Even more intriguing is the instability of allicin (Blania and Spangenberg 1991). The allicin molecule's most crucial and reactive part is the sulfur-sulfur bond coupled to an oxygen atom (Mohammad et al., 2007). It remains active only for a short period before degrading when allicin degrades, 20 sulfur compounds are formed (Bocchini et al. 2001).

Blends that comprised garlic had antibacterial activity against one or more microorganisms tested. Studies on rats infected with Klebsiella pneumoniae using plant extracts (ginger and garlic) for seven days show that the garlic treated group recovered fully on day four. Still, all the animals in ginger managed group died. NHowever, no death wasrecorded in rats treated with the mixture of garlic and ginger (Olatunde et al. 2009). All tests performed against *P. aeruginosa* showed inactivity except for garlic. This might be a result of the bacteria developing resistance against individual juices and blends (Baliga 2005).

Methanol extracts

All individual methanol extracts except lemon showed no activity against the tested microorganism (Table 4). The lemon extract had an activity of 11.0±0.0 mm against *P. aeruginosa* and 10.0±0.0 mm against *S. aureus,* respectively.

The methanol blends, also made in the ratio of 1: 1 (v/v), had sensitivities against the bacteria, as shown in Table 5. The turmeric/lemon extract blend had the activity of 11.0±0.0mm against *S. aureus*. The increase in activity can be attributed to favorable interactions between the natural compounds present in the mixture leading to synergism (Bocchini et al. 2001). Addition of garlic to the mix of turmeric/lemon methanol extract increases the activity to 12.0±1.0 mm. The activity of turmeric/ginger/lemon extract on *S. aureus* is 10.0±0.0 mm, but on the addition of garlic, the activity reduces to 9.3±0.6 mm. The blend of turmeric/garlic/ginger/lemon/onion extracts had an activity of 9.0±0.0 mm against *S. aureus*. The methanol blends recorded an inhibition zone of less than 9.0±0.0 mm against *E. coli* and *S. typhi*, thus inactive. The factors associated with the reduced activities of the mixtures are not apparent and therefore require further studies to be undertaken on the blends.

Table 6 gives a summary of the overall mean inhibition zones of individual methanol extract and blends against *S. aureus*. The whole mean inhibition zone of turmeric/lemon/garlic methanol blend against *S. aureus* is significantly different ($p < 0.05$) to the other test materials. The activities of individual lemon and turmeric/ginger/ lemon, turmeric/garlic/ginger/lemon/onion, and turmeric/ garlic/ginger/lemon blends are not significantly different. The data acquired from the susceptibility tests on *P. aeruginosa, E. coli* and *S. typhi,* was not subjected to ANOVA as only lemon/ginger was active against *P. aeruginosa*.

The result of antibacterial susceptibility assay showed promising evidence for the antibacterial effects of lemon methanol extract against *S. aureus* (10.0±0.0 mm) and *P.*

aeruginosa (11.0±0.0 mm). This is in line with a study conducted by Pandey et al. (2011) which showed methanol extract of lemon to be effective against *P. aeruginosa* with an inhibition zone of 23 mm. The results of antibacterial testing revealed that methanol extract of lemon had inhibitory effect on *P. aeruginosa* (11.0 mm) and *S. aureus* (10.0 mm) due to better solubility in the organic solvent as compared to the juice (Malu 2009: Mohamma et al. 2009: Pandey et al. 2011).

Table 2. Antibacterial activity exhibited by various juice blends against *P. aeruginosa, E .coli, S. aureus* and *S. typhi*

Sample juice blends	Mean inhibition zone in mm [a]			
	P. aeruginosa	***E .coli***	***S. aureus***	***S. typhi***
Lemon/garlic	7.7±0.6	15.0±0.0	6.0±0.0	12.0±0.0
Ginger/garlic	6.0±0.0	12.0±0.0	6.0±0.0	11.0±0.0
Turmeric/garlic	6.0±0.0	6.0±0.0	6.0±0.0	12.0±0.0
Ginger/turmeric	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Ginger/lemon,	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Lemon/turmeric,	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Lemon/garlic/ginger	6.0±0.0	13.7±0.6	6.0±0.0	9.7±0.6
Turmeric/ginger/garlic	6.0±0.0	11.0±0.0	6.0±0.0	10.0±0.0
Lemon/garlic/turmeric	8.7±0.6	14.7±0.6	6.0±0.0	11.0±0.0
Lemon/ginger/turmeric	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Lemon/garlic/turmeric/ginger	6.0±0.0	6.0±0.0	6.0±0.0	9.7±0.6
DMSO (-ve)	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Water (-ve)	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Ampicillin (+ve)	6.0±0.0	11.7±0.3	6.0±0.0	18.7±0.6
Ciprofloxacin (+ve)	41.7±0.6	30.7±0.3	17.3±2.1	34.7±0.6
Chloramphenicol (+ve)	20.0±0.0	35.0±0.0	34.0±0.0	30.7±0.3

Note: [a] includes the diameter (6 mm) of the disk used

Table 3. The mean (±SD) inhibition zones exhibited by individual juices and their blends against *P. aeruginosa*, *E. coli, S. aureus* and *S. typhi*

Sample juice / antibiotic	Inhibition zone (mm) (±SD)			
	P. aeruginosa	***E. coli***	***S. aureus***	***S. typhi***
Lemon	N.A	N.A	N.A	11.0 ± 1.0^{a}
Garlic	10.0 ± 0.0^{a}	11.7 ± 0.6^{ab}	14.7 ± 2.5^{a}	17.7 ± 2.5^{b}
Lemon/garlic	N.A	15.0 ± 0.0^{d}	N.A	12.0 ± 0.0^{a}
Ginger/garlic	N.A	12.0 ± 0.0^{b}	N.A	11.0 ± 0.0^{a}
Turmeric/garlic	N.A	N.A	N.A	12.0 ± 0.0^{a}
Lemon/garlic/ginger	N.A	13.7 ± 0.6^{c}	N.A	9.7 ± 0.6^{a}
Turmeric/garlic/ginger	N.A	11.0 ± 0.0^{a}	N.A	10.0 ± 0.0^{a}
Lemon/garlic/turmeric	N.A	14.7 ± 0.6^{d}	N.A	11.0 ± 0.0^{a}
Lemon/garlic/turmeric/ginger	N.A	N.A	N.A	9.7 ± 0.6^{a}
Ampicillin	N.A	11.7 ± 0.3^{ab}	N.A	18.7 ± 0.6^{b}
Ciprofloxacin	41.7 ± 0.6^{c}	30.7 ± 0.6^{e}	17.3 ± 2.1^{a}	34.7 ± 0.6^{d}
Chloramphenicol	20.0 ± 0.0^{b}	35.0 ± 0.0^{f}	34.0 ± 0.0^{b}	30.7 ± 0.6^{c}

Note: Mean (±SD) followed by the same small letters within the same column are not significantly different at $\alpha = 0.05$ (Student-Newman-Keuls test). N.A-not active

Table 4. Antibacterial activity exhibited by individual methanol extracts against *P. aeruginosa*, *E. coli*, *S. aureus* and *S. typhi*

Sample methanol extract	Inhibition zone in mm[a]			
	P. aeruginosa	***E. coli***	***S. aureus***	***S. typhi***
Turmeric	6.0±0.0	6. 0±0.0	8.7±0.6	6. 0±0.0
Onion	7.7±0.6	7.3±0.6	6. 0±0.0	7.3±0.6
Lemon	11.0±0.0	7.3±0.6	10.0±0.0	6.3±0.6
Ginger	6. 0±0.0	6.0±0.0.	7.3±0.6	6. 0±0.0
Garlic	6. 0±0.0	6. 0±0.0	6. 0±0.0	6. 0±0.0
Ampicillin	6. 0±0.0	11.7±0.3	6. 0±0.0	18.7±0.6
Ciprofloxacin	41.7±0.6	30.7±0.6	17.3±2.1	34.7±0.6
Chloramphenicol	20.0±0.0	35.0±0.0	34.0±0.0	30.7±0.6

Note: [a] includes the diameter (6mm) of the disk used

Table 5. Antibacterial activity exhibited by various methanol extract blends against *P. aeruginosa E. coli*, *S. aureus* and *S. typhi*

Sample methanol extract	Mean Inhibition Zone in mm [a]			
	P. aeruginosa	***E. coli***	***S. aureus***	***S. typhi***
Turmeric/lemon	8. 0±0.0	6.3±0.6	11.0±0.0	8.3±0.6
Lemon /ginger	9.67±0.6	6.3±0.6	8.0±0.0	8.0±0.0
Ginger/garlic	6. 0±0.0	6.0±0.0	7.0±0.0	6.0±0.0
Turmeric/ginger/lemon	6· 0±0.0	6.0±0.0	10.0±0.0	6.0±0.0
Turmeric/lemon/garlic	6. 0±0.0	6.0±0.0	12.0±1.0	6.0±0.0
Turmeric/garlic/ginger/lemon	6. 0±0.0	7.0±1.0	9.3±0.6	8.0±0.0
Turmeric/garlic/ginger/lemon/onion	6· 0±0.0	6.7±0.6	9.0±0.0	8.0±0.0
Ampicillin	6. 0±0.0	11.7±0.3	6. 0±0.0	18.7±0.6
Ciprofloxacin	41.7±0.6	30.7±0.6	17.3±2.1	34.7±0.6
Chloramphenicol	20.0±0.0	35.0±0.0	34.0±0.0	30.7±0.6

Note: [a] includes the diameter (6mm) of the disk used

Table 6. The mean (±SD) inhibition zones exhibited by individual methanol extracts and blends against *S. aureus*

Sample/antibiotic	Mean inhibition zone (mm) of *S. aureus*(±SD)
Lemon	10.0±0.0[a]
Turmeric/lemon/garlic	12.0±1.0[b]
Turmeric/ginger/lemon	10.0±0.0[a]
Turmeric/garlic/ginger/lemon	9.3±0.6[a]
Turmeric/garlic/ginger/lemon/onion	9.0±0.0[a]

Note: Mean (±SD) followed by the same small letters within the same column are not significantly different at α = 0.05 (Student-Newman-Keuls test)

Table 7. Antibacterial activity exhibited by various essential oil blends against *P. aeruginosa E. coli*, *S. aureus* and *S. typhi*

Sample	Inhibition zone in mm [a]			
	P. aeruginosa	***E. coli***	***S. aureus***	***S. typhi***
Lemon	6.3±0.6	6 .0±0.0	6.3±0.6	6.3±0.6
Garlic	6.3±0.6	6 .0±0.0	7 .0±0.0	6 .7±0.6
Turmeric	6 .0±0.0	6 .0±0.0	6 .0±0.0	6 .0±0.0
Ginger	6 .0±0.0	6 .0±0.0	7 .0±0.0	6 .0±0.0
Lemon/garlic	10.0±0.0	6.3 ±2.1	6.7±0.6	6.3±0.6
Lemon/ginger	7.7±0.6	9.0±1.0	7.0±1.0	9.7±0.6
Lemon/garlic/turmeric	7.0±1.0	6 .0±0.0	9.3±0.6	6 .0±0.0
Ampicillin	6. 0±0.0	11.7±0.3	6. 0±0.0	18.7±0.6
Ciprofloxacin	41.7±0.6	30.7±0.6	17.3±2.1	34.7±0.6
Chloramphenicol	20.0±0.0	35.0±0.0	34.0±0.0	30.7±0.6

Note: [a] includes the diameter (6 mm) of the disk used

Essential oils

Essential oils of garlic, lemon, turmeric, and ginger were obtained through steam distillation using Clevenger-type apparatus. Onion did not yield sufficient oil with steam distillation using Clevenger-type apparatus. Bioassay of all the essential oils gave an inhibition zone of less than 9.0 mm and thus inactive against the test gram positive and gram harmful bacteria (Kariba et al. 2001). The inactivity of garlic may be attributed to the relative instability of the organosulphur compounds which might have been destroyed during hydrodistillation and drying (Ewa et al. 2002). Steam-distilled garlic does not contain significant amounts of alliin or allicin, but instead contains various products of allicin transformation; none appears to have as much physiological activity as fresh garlic (Mohammad et al. 2009: Salem et al. 2010).

Bioassay results obtained from blends of essential oils are summarized in Table 7. The lemon/garlic blend gave an inhibition zone of 10.0 mm with *P. aeruginosa*. Lemon/ginger essential oil blend was active against *E. coli* and *S. typhi* with an inhibition zone of 9.0 mm and 9.7 mm, respectively. Lemon/garlic/turmeric blend had an inhibition zone of 9.3 mm against *S. aureus.*

The results indicate that lemon/garlic essential oil blend showed an increase in the antibacterial activity against *P. aeruginosa* (10.0±0.0 mm) as compared to their essential oils. The increase may be due to synergistic interaction of essential oil constituents of lemon and garlic (Esimone et al. 2006). Lemon/ginger also showed an increase in the antibacterial activity against *E. coli* (9.0±1.0)and *S. typhi*(9.7±0.6). These results are consistent with the previous study which showed that some blends of plant essential oils could have higher *in vitro* activity against bacteria (Junior et al. 2005; Betoni et al. 2006; Horiuchi et al. 2007). Interestingly, although neither lemon nor garlic essential oil exhibited activity; the blend of the two was active, suggesting that the volatile constituents of lemon interact synergistically with the transformed products of garlic (Ewa et al. 2002).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The fresh juice of garlic inhibited the growth of *S. aureus*, *E. coli*, *S. typhi* and *P. aeruginosa* at a concentration of 375 mg/mL, 187.5 mg/mL, 93.8 mg/mL and 46.9 mg/mL respectively (Table 8).

Methanol extract of lemon and lemon/ginger inhibited the growth of *P. aeruginosa* at a concentration of 2.9 mg/mL and 5.9 mg/mL respectively. Lemon, turmeric/ lemon, turmeric/lemon/ginger, turmeric/lemon/ garlic, turmeric/ginger/garlic, turmeric/lemon/ginger/garlic, and turmeric/lemon/ginger/garlic/onion methanol extracts exhibited an MIC of 187.5 mg/mL, 23.4 mg/mL, 46.9 mg/mL, 93.8 mg/mL, 187.5 mg/mL, 187.5 mg/mL and 23.4 mg/mL against *S. aureus* respectively. All the methanol extracts had no activity against S. typhi. Essential oils of lemon/garlic inhibited growth of *P. aeruginosa* at a concentration of 187.5 mg/mL, and lemon/ginger inhibited growth of *E. coli* and *S. typhi* at 750 mg/mL and 187.5

mg/mL respectively. Lemon/garlic/turmeric essential oil had an MIC of 375.0 mg/mL against *S. aureus*.

The plates showing no growth on nutrient agar were sub-cultured and incubated for 24 hours at 37 °C. The MBC results obtained are displayed in Table 4.8. Garlic juice, methanol extracts, and essential oils were bactericidal on all the bacteria tested at concentrations similar to their MIC's except essential oil blend of lemon/ginger which was bacteriostatic against *E. coli* bacteria at 750 mg/mL.

Methanol extracts prevented the growth of bacteria at lower concentrations (2.9 mg/mL, 23.4 mg/mL, and 5.9 mg/mL) as compared to juices and essential oils. The bactericidal properties of the essential oils might have been evaporated, destroyed or transformed to other forms during hydrodistillation and drying while methanol extracted most of the components from the samples (Ewa et al. 2002). Interestingly, ginger and for relief during abdominal discomforts (Jayaprakasha et al. 2002; Apariman et al. 2006), but did not show unusual bactericidal activity on the tested microorganisms.

Time-course antibacterial efficacy

Juices which had recorded activity against any one or more bacteria of ≥9.0 mm were tested for effectiveness within five days and their results summarized in Table 9.

Garlic showed inhibitory activity against all the strains used for the five days (Figure 3). Turmeric and individual ginger juices recorded an inhibition zone of <9 and thus did not show activity against any of the tested bacteria.

Table 8. MIC and MBC results for active samples

		MIC's mg/mL				MBC's mg/mL			
	Sample	***P. aeruginosa***	***E. coli***	***S. aureus***	***S. typhi***	***P. aeruginosa***	***E. coli***	***S. aureus***	***S. typhi***
Fresh extracts	Garlic	46.9	187.5	375	93.8	46.9	187.5	375	93.8
Methanol	Lemon	2.9	ND	187.5	ND	2.9	ND	187.5	ND
extracts	turmeric/lemon	ND	ND	23.4	ND	ND	ND	23.4	ND
	lemon/ginger	5.9	ND	ND	ND	5.9	ND	ND	ND
	turmeric/lemon/ginger	ND	ND	46.9	ND	ND	ND	46.9	ND
	turmeric/lemon/garlic	ND	ND	93.8	ND	ND	ND	93.8	ND
	turmeric/ginger/garlic	ND	ND	187.5	ND	ND	ND	187.5	ND
	turmeric/lemon/ginger/garlic	ND	ND	187.5	ND	ND	ND	187.5	ND
	turmeric/lemon/ginger/garlic /onion	ND	ND	23.4	ND	ND	ND	23.4	ND
Essential oils	lemon/garlic	187.5	ND	ND	ND	187.5	ND	ND	ND
	lemon/ginger	ND	750	ND	187.5	ND	750(static)	ND	187.5
	lemon/garlic/turmeric	ND	ND	375	ND	ND	ND	375	ND

Note: ND: Test not done, static: bacteriostatic

Table 9. Antibacterial activity exhibited by juices and blends against *P. aeruginosa*, *E. coli*, *S. aureus* and *S. typhi* for a period of 5 days

Antibiotic/ Sample	**Bacteria**	**Mean (±SD) inhibition zone(mm) [a]**				
		Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Lemon	*S. aureus*	6.7±1.2^{b}	7.0±1.0 ^{b}	6.7±0.6 ^{b}	6.0±0.0 ^{b}	11.0±0.0 ^{a}
	S. typhi	11.3±0.6^{a}	7.0±1.0 ^{b}	6.7±1.2 ^{b}	6.3±0.6 ^{b}	6.3±0.6 ^{b}
Turmeric	*S. aureus*	6.0±0.0 ^{a}	6.0±0.0 ^{a}	6.0±0.0 ^{a}	6.0±0.0 ^{a}	6.0±0.0 ^{a}
Ginger	*S. typhi*	6.0±0.0 ^{a}	6.0±0.0 ^{a}	6.0±0.0 ^{a}	6.0±0.0 ^{a}	6.0±0.0 ^{a}
Garlic	*P. aeruginosa*	10.0±0.0^{b}	6.0±0.0^{c}	10.3±3.8 ^{b}	9.7±3.2 ^{b}	15.0±0.5 ^{a}
	E. coli	11.7±0.6 ^{ab}	19.7±3.8 ^{a}	17.0±3.6 ^{a}	10.3±3.8 ^{b}	6.0±0.0 ^{b}
	S. aureus	14.7±2.5^{b}	26.0±2.6 ^{a}	27.0±6.1 ^{a}	11.0±2.9 ^{b}	6.0±0.0 ^{b}
	S. typhi	17.7±2.5^{a}	13.7±1.2 ^{ab}	11.7±2.8 ^{b}	10.7±0.6 ^{b}	11.0±1.0 ^{b}
Lemon /garlic	*E. coli*	15.0±0.0^{a}	10.3±0.6^{c}	6.0±0.0^{d}	12.3±0.6 ^{b}	14.3±0.6 ^{a}
	S. typhi	12.3±0.6^{b}	11.3±0.6 ^{b}	7.0±1.0^{c}	11.7±2.1 ^{b}	18.7±1.2 ^{a}
Ginger/garlic	*E. coli*	12.3±0.6^{b}	9.3±0.6 ^{a}	7.0±1.0 ^{a}	7.3±1.5^{b}	7.7±1.5 ^{a}
	S. typhi	12.0±1.0^{a}	12.3±1.2 ^{a}	7.0±1.0^{b}	7.3±1.5 ^{b}	7.3±1.5 ^{b}
Turmeric/garlic	*S. typhi*	13.0±1.0^{a}	8.0±1.0 ^{b}	6.3±0.6^{b}	6.7±1.2 ^{b}	6.7±1.2 ^{b}
Lemon/garlic/ginger	*E. coli*	13.7±0.6 ^{a}	13.3±1.5 ^{a}	6.7±1.2^{b}	9.0±1.0 ^{b}	6.7±1.2 ^{b}
	*S. typh*i	10.3±0.6^{ab}	9.3±0.6 ^{b}	6.3±0.6^{c}	6.3±0.6^{c}	12.0±2.0 ^{a}
Turmeric/garlic/ginger	*E. coli*	12.3±1.5 ^{a}	7.7±1.5 ^{b}	6.3±0.6^{b}	7.0±1.0 ^{b}	7.0±1.7 ^{b}
	S. typhi	11.3±1.5 ^{a}	8.0±1.0 ^{b}	7.0±1.0^{b}	7.0±1.7 ^{b}	7.0±1.0 ^{b}
Lemon/garlic/turmeric	*E. coli*	14.7±0.6 ^{a}	13.3±1.5 ^{ab}	6.7±0.6^{c}	11.7±2.1 ^{b}	7.0±1.0^{c}
	S. typhi	11.7±1.2 ^{a}	10.3±0.6 ^{a}	6.7±1.2^{b}	7.3±1.5 ^{b}	6.7±1.2 ^{b}
Lemon/garlic/turmeric/ Ginger	*E. coli*	6.3±0.6 ^{b}	12.0±1.0 ^{a}	7.5±1.4^{b}	6.7±1.2 ^{b}	7.3±1.5^{b}
	S. typhi	10.0±1.0 ^{a}	6.7±0.6 ^{a}	7.7±1.5 ^{a}	7.0±1.7 ^{a}	7.3±1.5 ^{a}

Note: [a] includes the diameter (6mm) of the disk used

Lemon and garlic individual juices showed decreasing activities against *S. typhi* from day 1 to day 5. The individual juice of lemon and turmeric/garlic blend did not show any changes in activity against *E. coli*. Ginger/garlic, lemon/garlic/ginger, and turmeric/garlic/ginger blends show decreasing activities against *E. coli* with time. The activity of lemon/garlic against *E. coli* dropped from day 1 to 3 then increased again up to day 5 (Figure 4.4). The activity of lemon/garlic/turmeric against E. coli dropped from day 1 to 3 then raised back up to day 4 (Figure 4). Individual lemon juice and the other blended test materials did not record any activity against *P. aeruginosa* for the 5 days.

Lemon/garlic blend showed an interesting pattern against *S. typhi*: the activity dropped from 12.3±0.6 to 7.0±1.0 by day 3 but increased to 18.7±1.2 on day 5 (Figure 5). A similar pattern of activity was shown by lemon/garlic/ginger blend against the same bacterium (Figure 5). This pattern suggests that the intermediate products formed are inactive, but that their further transformation leads to products that are inhibitory to *S. typhi* (Farbman et al. 1983). Monitoring (by GC-MS or LC-MS) of the specific changes of the constituents that take place can shed light on these exciting findings.

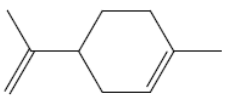
GC-MS analyses

Fresh juices

Juices of lemon, local garlic, ginger, and turmeric were analyzed by GC-MS, and each sample gave a chromatogram having several peaks. The suspected antibacterial compounds with their molecular formula and weight are listed in Table 10.

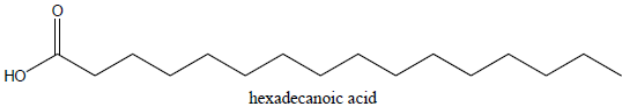
Garlic originating from China was also analyzed by GC-MS for comparison with garlic used (local garlic) in the bioassays. The candidate antibacterial compounds are listed with their molecular formula, percentage abundance and weight in Table 10.

The GC-MS analyses showed that lemon juice contained limonene (**14**) (85.08%), an antibacterial agent (Hiroyuk et al. 2006); 3-hexen-1-ol (0.16%); mentha-2,8-dien-1-ol (0.18%); hexadecanoic acid (**15**) (0.46%); 9,12-octadecadienoic acid (0.14%); 2-ethoxycarbonyl-3-methyl-7-nitro-4-azafluorenone,phenylimine (0.72%); phthalic acid, cyclohexylmethyl-3-phenylpropylester (0.40%) and α-terpineol (0.14), which may have confered bacterial inhibition property to this terpene (Angeh 2006; Fisher and Phillips 2006).



limonene

14



hexadecanoic acid

15

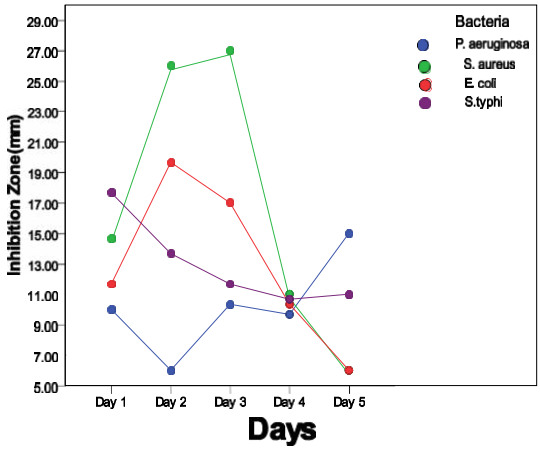


Figure 3. Comparison of mean (±SE) inhibition zones of garlic juice against *S. typhi, E. coli, P. aeruginosa* and *S. aureus*

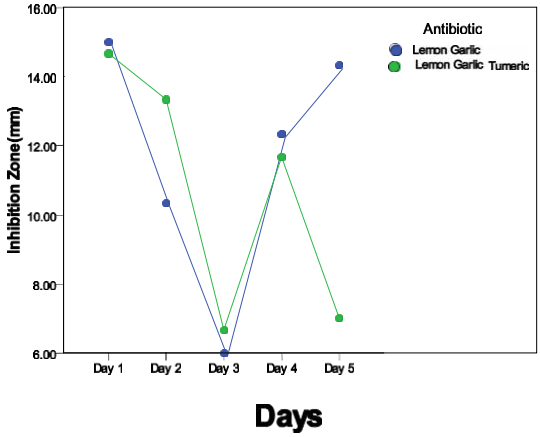


Figure 4. Comparison of mean (±SE) inhibition zones of two juice blends against *E. coli*

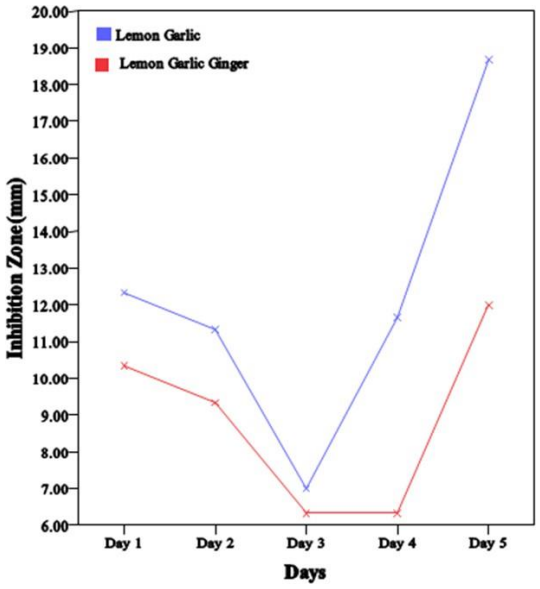
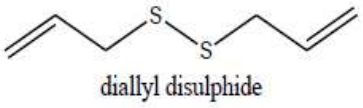


Figure 5. Comparison of mean (±SE) inhibition zones of two juice blends against *S. typhi*

Table 10. The GC-MS profile of compounds suspected to contain antibacterial properties identified in lemon, China garlic, local garlic, ginger and turmeric juices

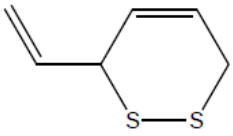
No	Compound	Molecular formula	M+ (g/mol)	Retention time (min)	Relative %				
					Lemon	China garlic	Local garlic	Ginger	Turmeric
1	α-Terpineol	$C_{10}H_{18}O$	154	14.562	0.41	-	-	0.61	-
2	Limonene	$C_{10}H_{16}$	136	11.906	85.08	-	-	-	-
3	3-Hexen-1-ol	$C_6H_{12}O$	100	8.154	0.16	-	-	-	-
4	Mentha-2,8-dien-1-ol	$C_{10}H_{16}O$	152	13.428	0.18	-	-	-	-
5	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	23.749	0.46	-	-	-	-
6	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	25.434	0.14	-	-	-	-
7	2-Ethoxycarbonyl-3-methyl-7-nitro-4-azafluorenone,phenylimine	$C_{22}H_{17}N_3O_4$	387	40.316	0.72	-	-	-	-
8	Pyrrolo[2,3-b] indole	$C_{14}H_{16}N_2O_4$	218	20.502	-	-	-	-	0.73
9	Methanehydrazonic acid, *N*-[3-(methylthio)-1,-2,4-thiadiazol-5-yl]-,ethylester	$C_6H_9N_4OS_2$	218	21.331	-	-	-	-	8.87
10	Selenourea, phenyl-	$C_7H_8N_2Se$	200	21.531	-	-	-	-	0.17
11	Imidazole, 4-methyl-5-[3,3,3-trifluoropropionylpropyl]-	$C_{10}H_{13}F_3N_2O$	234	22.248	-	-	-	-	0.47
12	1,6,10-Dodecatriene-3 ol,3,7,11-trimethyl-	$C_{15}H_{26}O$	222	22.358	-	-	-	-	0.48
13	2-Butenoic acid, 3-methyl-, methylester	$C_6H_{10}O_2$	114	22.43	-	-	-	-	0.60
14	2-Azabicyclo[3.2.1]octan-3-one	$C_7H_{11}NO$	125	22.58	-		-	-	0.12
15	2,4-Quinolnediol	$C_9H_7NO_2$	161	22.724	-	-	-	-	0.44
16	3-[4-Hydroxybenzoylhydrazono]-*N*-Mesitylbutyramide	$C_{20}H_{23}N_3O_3$	353	22.974	-	-	-	-	0.29
17	Phthalic acid, cyclohexylmethyl-3-phenylpropylester	$C_{24}H_{28}O$	380	23.14	0.40	-	-	-	0.53
18	Linalool	$C_{10}H_{18}O$	154	13.066	-	-	-	0.50	0.05
19	Terpinen-4-ol	$C_{10}H_{18}O_4$	154	14.563	-	-	-	-	0.05
20	Bicyclo[3.2.2]non-8-en-6-ol, (1R,5-cis,6-cis)-	$C_9H_{14}O$	138	16.105	-	-	-	-	0.03
21	Guaiacol<para-vinyl->	$C_9H_{10}O_2$	150	16.377	-	-	-	-	0.07
22	*N*-(2-Phenylethenyl)acetamide	$C_{10}H_{11}NO$	161	17.266	-	-	-	-	0.03
23	Ethanone,1-cyclopropyl-2-[3-pyridinyl]-	$C_{10}H_{11}NO$	161	19.5	-	-	-	-	0.73
24	1,5-Dimethyl-2-pyrrolecarbonitrile	$C_7H_8N_2$	120	20.104	-	-	-	-	0.61
25	6-Octen-1-yn-3-ol, 3,7-dimethyl-	$C_{10}H_{16}O$	152	20.207	-	-	-	-	1.11
26	Ethyl homovanillate	$C_{11}H_{14}O_4$	210	23.353	-	-	-	-	0.47
27	Ezlopitant , dehydro-	$C_{32}H_{24}N_2O$	452	32.758	-	-	-	-	0.14
28	Phenol, 4-pentyl-	$C_{11}H_{16}O$	164	33.341	-	-	-	-	0.76
29	[1,3,5]Triazine-2,4-diamine,6-	$C_9H_{13}N_7$	219	34.608	-	-	-	-	0.21
30	*O*-methoxy-α,-methylbenzyl alcohol	$C_9H_{12}O_2$	152	36.307	-	-	-	-	0.22
31	Methyl-4-deoxy-2-0-methyl.beta.1-threo-hex-4-enopyrid urinate	$C_8H_{12}O_4$	120	20.104	-	-	-	-	0.61
32	Benzenethiol	C_6H_6S	152	20.207	-	-	-	-	1.11
33	3,4-Dimethylthiophene	C_6H_8S	210	23.353	-	-	-	-	0.47
34	Ethylthiazole	C_5H_7NS	452	32.758	-	-	-	-	0.14
35	Thiophene, 3-methyl	C_5H_6S	164	33.341	-	-	-	-	0.76
36	Disulphide, methyl-2-propenyl	$C_4H_8S_2$	219	34.608	-	-	-	-	0.21
37	1-propene-3, 3-thiobis	$C_6H_{10}S$	152	36.307	-	-	-	-	0.22
38	Thiourea,*N*-*N*'-dimethyl	$C_3H_8N_2S$	204	5.878	-	3.77	-	-	-
39	Diallyl disulphide	$C_6H_{10}S_2$	110	8.952	-	1.18	-	-	-
40	3-Chlorothiophene	C_4H_3ClS	112	9.268	-	1.21	-	-	-
41	3-Vinyl-1,2-dithiacyclohex-4-ene	$C_6H_9S_2$	113	13.64	-	0.29	-		-
42	3-Vinyl-1,2-dithiacyclohex-5-ene	$C_6H_9S_2$	98	16.804	-	1.18	-	-	-
43	Cyclohexen-1-ol, 3-methyl	$C_7H_{12}O$	120	5.878	-	3.38	-	-	-
44	Ethyl trifluoromethyl trisulphide	$C_3H_5F_3S_3$	114	8.170	-	1.33	2.90	-	-
45	1,3-Dioxolane-2-[dichloromethyl]-	$C_4H_6Cl_2O_2$	104	9.869	-	0.48	0.84	-	-
46	Acetic acid, chloro-2-butoxyethyl ester	$C_6H_{15}ClO_3$	146	12.734	-	3.62	10.84	-	-
47	Acetamide,n-tetrahydrofurfuryl-2-methoxy	$C_8H_{15}NO_3$	173	14.250	-	1.44	1.35	-	-
48	Octadecanoic acid,3-hydroxy, methyl ester	$C_{19}H_{38}O_3$	314	20.178	-	-	0.66	-	-
49	1,2,3-Thiadiazole,5-methyl-	$C_3H_4N_2S$	100	14.727	-	2.59	-	-	-
50	1,4-benzenediol-2-chloro	$C_6H_5ClO_2$	144	16.656	-	1.61	-	-	-
51	Propanoic acid,2-chloro	$C_6H_5ClO_2$	108	17.163	-	1.86	-	-	-
52	3,4-Dimethylthiophene	C_6H_8S	112	9.268	-	1.46	-	-	-

53	Disulphide,methyl-2-propenyl	$C_4H_8S_2$	120	9.528		4.07	-	-	-
54	1,2-dithiolane	$C_3H_6S_2$	106	10.885	-	0.32	-	-	-
55	2-ethylthiacyclohexane	$C_7H_{14}S$	130	12.192	-	0.67	-	-	-
56	(methylthio)-acetonitrile	C_3H_5NS	87	13.738	-	0.88	-	-	-
57	3-Vinyl-1,3-dithiane	$C_6H_{10}S_2$	146	15.009	-	1.22	-	-	-
58	1,4-Diathiane	$C_4H_8S_2$	120	9.527	-	1.65-	3.176	-	-
59	Octadecanoic acid,3-hydroxy, methyl ester	$C_{19}H_{38}O_3$	314	20.179	-	1.24	-	-	-
60	N-Methoxy-N-methyl	$C_2H_6NF_2OP$	129	21.927	-	0.44	-	-	-
61	Amidinothiourea	$C_2H_6N_4S$	118	12.341	-	0.94	0.671	-	-
62	2-Heptanol	$C_7H_{16}O$	58	9.222	-	-	-	0.24	-
63	Borneol	$C_{10}H_{18}O$	154	14.49	-	-	-	0.81	-
64	Citronellol	$C_{10}H_{20}O$	156	15.067	-	-	-	0.50	-
65	Geraniol	$C_{10}H_{18}O$	154	15.458	-	-	-	1.05	-
66	Geranic acid	$C_{10}H_{16}O_2$	168	16.825	-	-	-	0.15	-
67	Elemol	$C_{10}H_{16}O_2$	222	19.447	-	-	-	0.73	-
68	E-Nerolidol	$C_6H_{26}O$	222	19.537	-	-	-	0.43	-
69	2-Butanone,4-(-hydroxy-3-methoxyphenyl	$C_{11}H_{14}O_3$	194	20.628	-	-	-	14.14	-
70	Ketone,1-cyclohexen-1-yl methyl,semicarbazone	$C_9H_{15}N_3O$	181	28.736	-	-	-	0.51	-
71	α-Zingiberene	$C_{15}H_{24}$	204	18.769	-	-	-	25.08	-



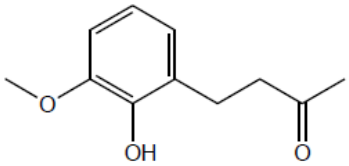
diallyl disulphide

16



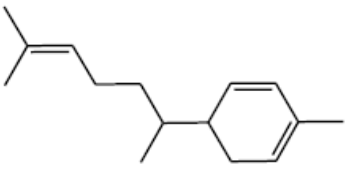
3-Vinyl-1,2-dithiacyclohex-4-ene

17



2-butanone,4-(-hydroxy-3-methoxyphenyl)-

18



19 (α-zingiberene)

GC-MS analysis of local garlic juice showed the presence of: diallyl disulphide (**16**) (10.84%); 3-chlorothiophene (6.49%); 3-vinyl-1,2-dithiacyclohex-4-ene (21.4%); 3-vinyl-1,2-dithiacyclohex-5-ene (**17**) (3.09%); acetic acid, chloro-2-butoxyethyl ester(2.73%); ethyl trifluoromethyl trisulphide (1.67%); acetamide, *n*-tetrahydrofurfuryl-2-methoxy (1.35%); 1-propene, 3,3‟thiobis (2.90%); 1,4-diathiane (3.18%); thiourea, N,N-dimethyl-(0,84%); octadecanoic acid,3-hydroxy, methyl ester (0.66%); Cyclohexen-1-ol, 3-methyl (0.62%); 1,3-Dioxolane-2-[dichloromethyl]-(0.36%) and amidinothiourea (0.67). All these compounds except acetamide n-tetrahydrofurfuryl-2-methoxy are sulphur-containing compounds, which might be responsible for antibacterial activity of garlic juice (Kathi 2000; O'Gara et al. 2000). China garlic gives additional sulphur compounds compared with local garlic. This may reflect some genetic or chemotypic differences between the two.

Ginger juice reaveled the presence of α-terpineol (0.61%); 2-heptanol (0.24%); linalool (0.50%); borneol (0.81%); citronellol (0.50%); geraniol (0.05%); geranic acid (0.15%); elemol (0.73%); *E*-nerolidol (0.43%); 2-butanone,4-(-hydroxy-3-methoxyphenyl)-(**18**) (14.14%); ketone,1-cyclohexen-1-ylmethyl,semicarbazone (0.51%) and α-zingiberene (**19**) (25.08%). These compounds are mainly terpenoids, some of which have shown strong inhibitory activity against pathogenic bacteria (Malu et al. 2009).

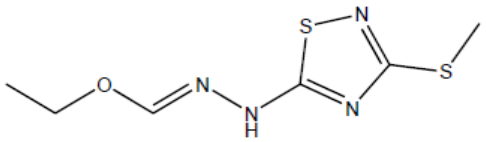
Turmeric had a wide range of suspected antibacterial components including; pyrrolo [2,3-b] indole (0.73%); Methanehydrazonic acid, *N*-[3-(methylthio)-1,-2,4-thiadiazol-5-yl]-,ethylester (**20**) (8.87%); Selenourea, phenyl-(0.17%); Imidazole, 4-methyl-5-[3,3,3-trifluoropropionyl-propyl]-(0.47%); 1,6,10-Dodecatriene-3 ol,3,7,11-trimethyl-(0.48%); 2-Butenoic acid, 3-methyl-, methylester (0.60%); 2-Azabicyclo[3.2.1]octan-3-one (0.12%); 2,4-Quinolnediol (0.44%); 3-[4-Hydroxybenzoylhydrazono]-*N*-mesityl-butyramide (0.29%); Phthalic acid, cyclohexylmethyl-3-phenylpropylester (0.53%); Linalool (0.05%); Terpinen-4-ol (0.05%); Bicyclo[3.2.2]non-8-en-6-ol, (1R,5-cis,6-cis)-(0.03%); Guaiacol<para-vinyl->(0.07%); *N*-(2-Phenylethenyl) acetamide (0.03%); Ethanone,1-cyclopropyl-2-[3-pyridinyl]-(0.73%); 1,5-Dimethyl-2-pyrrolecarbonitrile(0.61%); 6-

Octen-1-yn-3-ol, 3,7-dimethyl-(**21**) (1.11%); Ethyl homovanillate (0.47%); Ezlopitant , dehydro-(0.14%); Phenol, 4-pentyl-(0.76%); [1,3,5]Triazine-2,4-diamine,6-(0.21%) and *O*-methoxy-α,-methylbenzyl alcohol (0.22%) but they exhibited low or no anti-bacterial activity. This may be attributed to their low concentrations (Gopalan et al. 2000; Ghulam et al. 2009).

Methanol extracts

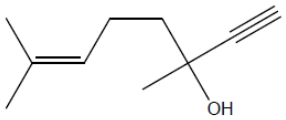
The suspected antibacterial compounds identified from active methanol extracts by GC-MS are listed in Table 11 with their relative percentage abundance, molecular formula, and weight.

The candidate antibacterial constituents obtained from methanol extracts include cyclohexanol,2-methylene-5-(1-methylene-5-[1-methylethenyl]-(4.41%); trans-carveol (1.49%); n-hexadecanoic acid (8.01%%); heptadecanoic acid (2.37%); γ-sitosterol (8.00%); borneol (0.59%); citronellol (0.64%); 2-butanone,4-[4-hydroxy-3-methoxy-phenyl]-**(22)** (5.15%); linoleic acid **(23)** (5.86%); ethyl hexadecanoate (1.28%); 2-[3-hydroxy-2-nitrocyclohexyl]-1-phenylethanone (2.53%); propanamide,2-amino-3-pheny (6.71%); 5,6,7,8-tetrahydroindolizine (1.03%); E-nerolidol (0.59%); 1,5-dimethyl-2-pyrrolecarbonitrile (1.10%); β-cadren-9-alpha-ol (0.69%); α-zingiberene (33.75%) and phenol,4-ethyl-2-methoxy-(0.94%). Methanol extracts contain aromatic hydrocarbons, ketones, phenols, organic acids and terpenes which have good inhibitory effect against gram positive and gram negative bacteria. Their varied occurrences in various blends may indicate that, their therapeutic effect(s) are not the direct effect of a single group or compound, but rather that the compounds possibly act in combination to bring about antibacterial effect (Abba et al. 2009).



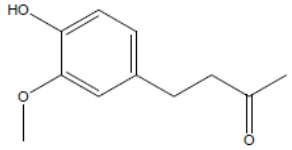
Methanehydrazonic acid, N-[3-(methylthio)-1,-2,4-thiadiazol-5-yl]-,ethylester

20



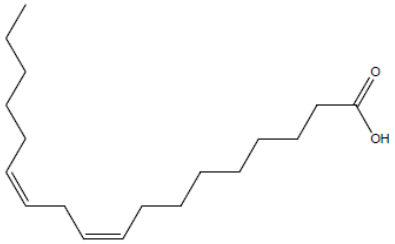
6-Octen-1-yn-3-ol, 3,7-dimethyl-

21



2-Butanone,4-[4-hydroxy-3-methoxyphenyl]-

22



Linoleic acid

23

Table 11. The GC-MS profile of compounds suspected to contain antibacterial properties identified in methanol crude extract and blends

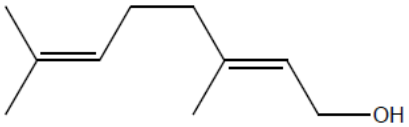
No.	Compound	Molecular formula	M⁺ (g/mol)	Retention time (min)	Relative %					
					Lemon	LG	GiLT	LGT	GGiT	LGTGi
1	Cyclohexanol,2-methylene-5-(1-methylene-5-[1-methylethenyl]-	$C_{10}H_{16}O$	152	14.517	4.41	-	-	-	-	-
2	Carveol	$C_{10}H_{16}O$	152	14.987	1.49	-	-	-	-	-
3	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	23.767	8.01	0.81		0.41	0.37	0.69
4	Linoleic acid	$C_{18}H_{32}O_2$	280	25.460	5.86	-	-	-	-	-
5	Heptadecanoic acid	$C_{17}H_{34}O_2$	312	25.933	2.37	-	-	-	-	-
6	γ-Sitosterol	$C_{29}H_{50}OH$	414	39.136	8.00	-	-	-	-	-
7	Borneol	$C_{10}H_{18}O$	154	14.197	-	0.59	-	-	-	-
8	Citronellol	$C_{10}H_{20}O$	156	15.076	-	0.64	-	-	-	-
9	2-Butanone,4-[4-hydroxy-3-methoxyphenyl]-	$C_{11}H_{14}O_3$	194	20.585	-	5.15	4.27	0.63	2.54	5.50
10	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	284	24.082	-	1.28	-	-	-	-
11	2-[3-Hydroxy-2-nitrocyclohexyl]-1-phenylethanone	$C_{14}H_{17}NO_4$	263	20.856	-	-	2.53	-	-	-
12	Propanamide,2-amino-3-phenyl	$C_9H_{12}N_2O$	164	21.216	-	-	6.71	-	-	-
13	5,6,7,8-Tetrahydroindolizine	$C_8H_{11}N$	121	21.089	-	-	-	1.03	-	-
14	E-Nerolidol	$C_6H_{26}O$	222	19.540	-	-	-	-	-	0.59
15	1,5-Dimethyl-2-pyrrolecarbonitrile	$C_7H_8N_2$	120	20.722	-	-	-	-	-	1.10
16	Beta-cadren-9-alpha-ol	$C_{15}H_{24}O$	220	22.407	-	-	-	-	-	0.69
17	Phenol, 4-ethyl-2-methoxy-	$C_9H_{12}O_2$	152	26.900	-	-	-	-	-	0.94
18	α-Zingiberene	$C_{15}H_{24}$	204	18.769		33.75				

Note: **LG:** Lemon/ginger, **GiLT:** Ginger/lemon/turmeric, **LGT:** Lemon/garlic/turmeric, **GGT:** Garlic/ginger/turmeric, **LGTG:** Lemon/garlic/ginger/turmeric

Table 12. The GC-MS constituents identified from three essential oil blends with antibacterial properties against *S. typhi*, *P. aeruginosa*, *E. coli* and *S. aureus*

No.	Compound	Molecular Formula	M^+ (g/mol)	Retention time (min)	Relative %		
					GL	LGi	GLT
1.	Diallyl disulphide	$C_6H_{10}S_2$	146	12.771	1.87	-	0.66
2.	Limonene	$C_{10}H_{16}$	136	11.921	84.27	49.78	36.16
3.	Linalool	$C_{10}H_{18}O$	154	14.612	0.91	1.13	-
4.	Terpinen-4-ol	$C_{10}H_{18}O$	154	13.113	4.46		1.72
5.	α-Terpineol	$C_{10}H_{18}O$	154	14.415	1.74	1.65	0.82
6.	[4-Aminophenyl]2-methylpiperidin-1-y1) methanone	$C_{13}H_{18}N_2O$	436	21.275	-	-	8.56
7.	Borneol	$C_{10}H_{18}O$	154	14.411	-	2.95	-
8.	Geraniol	$C_{10}H_{18}O$	154	15.510	-	0.09	-
9.	Elemol	$C_{10}H_{16}O_2$	222	19.490	-	0.67	-

Note: GL-Lemon/garlic, LGi-Lemon/ginger, GLT-Lemon/garlic/turmeric



geraniol

24

Essential oils

Three essential oil blends (lemon/garlic, lemon/ginger, and lemon/garlic/turmeric) that were active against *S. typhi*, *P. aeruginosa*, *E. coli,* and *S. aureus* were analyzed by GC-MS. The compounds suspected to have antibacterial properties with their molecular formula, mass and their relative proportions in the essential oils are given in Table 12 concerning the sample of origin.

The compounds suspected to have antibacterial properties are fewer in the essential oils as compared to juices and methanol extracts. The compounds which were present include: diallyl disulfide; [4-Aminophenyl]2-methylpiperidin-1-yl) methanone; limonene; terpinen-4-ol; α-terpineol; borneol; geraniol (**24**) and elemol. Limonene and α-terpineol are present in all the analyzed essential oils. Lemon/garlic essential oil does not show any sulphur derived compound in the GC-MS analysis due to the fact that during cutting and heating of garlic to obtain the oil, the compounds might have escaped (Lawson 1991; Yongabi et al. 2009; (Ahmet et al. 2006; Hérent et al. 2007; Ahmed et al. 2009; Mohamed et al. 2010).

Citrus essential oils contain significant amounts of terpenes, oxygenated derivatives and aromatic hydrocarbons (Ahmet et al. 2006; Hérent et al. 2007; Ahmed et al. 2009; Mohamed et al. 2010). Among the components (limonene and linalool) limonene was more abundant than linalool. Limonene shows the lowest effect against microorganisms. (Hérent et al. 2007; Tao et al. 2009; Palakawong et al. 2010). The inhibitory effect against microorganisms resulted from linalool rather than limonene (Fisher and Phillips 2006). Results of the previous report showed that greater antimicrobial potential could be ascribed to the oxygenated terpenes, including phenols (Maruti et al. 2011).

Conclusion

The most potent sample among the juices was garlic juice which inhibited the growth of all bacteria tested. The mean inhibition zones of Lemon/garlic juice against *E. coli* and *S. Typhi* were significantly higher among the juice blends. Among methanol extract samples, lemon had the highest activity against *P. aeruginosa* and *E. coli*. The results of antibacterial testing revealed that the juices of garlic and lemon had higher inhibitory effects as compared to methanol extracts and essential oils. The results of this study support the traditional usage of the studied vegetables and lemon and suggest that some of the extracts possess compounds suspected to have antimicrobial properties that can be used as agents in new drugs for therapy of infectious diseases caused by pathogens.

GC-MS analyses revealed that the compounds which were present in all the samples contain one or more of the following functional groups:-COOH,-OH,-N,-Cl,-F,-NH_2 and-S groups which may be associated with bacterial inhibition and found in conventional antibiotics. Individual juices and methanol extracts contained more compounds that were suspected to have antibacterial properties as compared to the blends. For example, lemon and garlic individual juices had a total of 22 compounds that were suspected to have antibacterial properties, lemon methanol extract contained 6, while lemon/garlic blend contained only 6 compounds.

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