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Antimicrobial Activity and the Chemical Composition of the Volatile Oil Blend from *Allium sativum* (Garlic Clove) and *Citrus reticulata* (Tangerine Fruit)

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ABSTRACT
The synergistic effect in the antimicrobial activity of the volatile oil blend from Garlic clove (*Allium sativum)* and tangerine fruits (*Citrus reticulata)* were investigated and compared to antimicrobial activity when the individual volatile oils were used alone. The volatile oils were extracted by steam distillation using Clevenger hydrodistillator apparatus and each oil was tested for antimicrobial activity, while equal volume of these oils were blended and tested for antimicrobial activity. The microorganisms used include, *Staphylococcus aureus* isolate, *Escherichia coli* isolate*, Pseudomonas aeruginosa*, *Salmonella typhi, Candida albicans isolate, Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 90028. The Minimum Inhibitory Concentrations (MICs) ranged from 9.31×10^{-13} – 7.88 mg/ml for garlic oil, 0.16 – 2.66 mg/ml for tangerine oil and 5.95×10^{-31} – 1.24 mg/ml for the essential oil blend. Minimum Inhibitory Concentration indicated that the Garlic oil and Tangerine oil blend was better at inhibiting the tested microorganisms than the individual oils except for *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. The Gas Chromatography-Mass Spectrometry revealed Trisulphide, di-2-propenyl (30.32%) as the major component in the garlic oil extract and 3-Cyclohexene-1-methanol, alpha 4-trimethyl (33.38%) in the tangerine oil. While the equal volume of the oil blend also revealed Trisulphide, di-2-propenyl (15.92%) and 3-Cyclohexene-1-methanol, alpha.4-trimethyl (12.02%) as the major constituents though in lower concentrations. Hence, the more potent antimicrobial properties demonstrated by the oil blend can be exploited further with a view to generate new effective antimicrobial compounds.

Keywords: *Allium sativum, Citrus reticulata,* Gas chromatography-Mass spectrometry, antimicrobial activity, Oil blend, Volatile oil.

INTRODUCTION

The antimicrobial activity of medicinal and aromatic plants have been known and described for several centuries. [1] Most of their properties are due to the secondary metabolites present in essential oils. [2-3] In recent years a large number of essential oils and their constituents have been investigated for their antimicrobial properties against bacteria and fungi. [4] Essential oils and extracts from several plant species are able to control microorganisms related to skin, dental caries and food spoilage, including gram-negative and gram-positive bacteria. [3]

The synergistic effect of medicinal plants occurs when the combined action of constituents is greater than would be expected from each individual plant. [5] There is a popular belief that any mixture of essential oils is synergistic, or that mixing oils with similar effects will result in synergy. These interactions, sometimes called matrix effects, may take place on one or more of three levels: Between essential oil constituents, between essential oils, between one or more essential oils and another type of substance. For example, citral has a zone of inhibition of 22 mm when tested against *Bacillus subtilis*, and myrcene has no effect at all, yet mixing myrcene and citral results in an inhibitory zone of 47 mm. [6] Garlic (*Allium sativum*) qualifies as a great vegetable because not only is it an indispensable cooking ingredient, it can also be delightfully eaten. The *Allium* genus belongs to the Liliaceae family comprising onions, leeks, shallots, asparagus etc. Garlic requires a sunny spot and rich soil. However the soil should not be too rich so that the tops will not overdevelop. Garlic is primarily used as an herb to enhance many food dishes in various cultures. It contains many substances which studies have shown to act together to

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prevent various disease such as hypertension, cancer and it has been shown to reduce plasma concentration of cholesterol and low-density lipoprotein in the blood and age-related conditions. Initial reports of the antimicrobial activity of garlic showed that Allicin (allyl-2-propene thiosulfinate); a notable flavonoid in garlic, is formed when garlic cloves are crushed. [7-8] Garlic also contains some sulphur-containing compounds such as alliin, ajoene, diallylsulphide, dithin, S-allylcysteine, enzymes as well as some non sulphur-containing compounds including vitamin B, proteins, minerals, saponins and flavonoids. Yukihiro *et al.,* [9] have reported a phytoalexin called allixin in garlic.

Tangerine oil is obtained from the fruit peel of *Citrus reticulata*, family Rutaceae. It is traditionally used as an antiseptic, antispasmodic, stomachic, and sedative, diuretic and to improve circulation. [10] Some chemical constituents of *C. reticulata* peel essential oil that have been reported include, *α*-pinene, β-pinene, Limonene, [11], Citronellal, Linalool, *β*-caryophyllene, *α*-farnesene, Dodecanal. [12] These chemical constituents have also been reported to have antimicrobial activities.

Since the discovery of antibiotics and their uses as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eradication of infectious diseases. However diseases and disease agents that were once thought to have been controlled by antibiotics are returning in new forms which are resistant to antibiotic therapies. Incidents of epidemics due to such drug resistant microorganisms are now a common global problem posing enormous public health concerns. [13]

In the present study, a more potent antimicrobial agent will be investigated from the synergistic effect that will be obtained when the essential oil blend from *Allium sativum* and *Citrus reticulata* is tested against against Gram +ve bacteria, Gram –ve bacteria and fungi and also to determine the chemical constituents of the individual oils (Garlic oil & Tangerine oil) and the equal volumes of the blended oil, that may be responsible for the antimicrobial activities, by using Gas Chromatography-Mass Spectrometry (GC MS).

MATERIALS AND METHODS

Collection and identification of plant materials

Tangerine fruits were purchased from Abeokuta, Ogun state, (in the South West region of Nigeria). Whilst the fresh Garlic cloves were collected from Sokoto, (in the Northern region of Nigeria) in February, 2011.

These fruits were identified at the Department of Botany, University of Lagos and given a voucher Herbarium number LUH107930 for Tangerine fruit and LUH 3899 for Garlic cloves.

Extraction of volatile oil by steam distillation

Garlic oil

The plant materials were peeled, washed under running tap water and grounded in a mortar in order to liberate the tissues. The plant tissues were mixed with water and then packed into the round bottom flask of a Clevenger-type hydrodistillator (Pyrex). The steam extraction of garlic oil produces two fractions: a hydro-soluble whitish fraction and a hydrophobic yellowish fraction. The essential oils (hydrophobic yellowish fraction) collected were then dehydrated using sodium sulphate anhydride before storage in the refrigerator at 4°C until tested. The yield of the oil (hydrophobic yellowish fraction) was recorded as volume: weight ratio. [11, 14]

Tangerine oil

Peels from 80 tangerine fruits were used in obtaining the tangerine oil by steam distillation [15] using the Clevenger hydrodistillator apparatus (Pyrex). The fresh tangerine peels were placed in the round bottom flask and filled with water to about three quarter full. The distillation apparatus was connected to the flask. The trap arm was filled with water to allow the oil to condense on the water layer. Heat was applied from the heating mantle and as the water in the flask boiled, steam carrying the volatile oil rose through the neck of the flask condensing on the surface of the condenser onto the water on the graduated trap arm. Distillation was continued until there was no more difference in successive readings of the oil volume. The oil was drained off and dried over anhydrous sodium sulphate before storage in the refrigerator at 4°C until tested. The yield of the oil was calculated and recorded as volume: weight ratio thus: [11, 14]

$$\text{Essential oil \% yield } (v/w) \text{ (dry weight} = \frac{\text{volume of essential oil (ml)}}{\text{Weight of raw materials (g)}} \times 100\%$$

Test organisms

The organisms used comprise of three gram-negative organisms- *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi,* one gram-positive organism- *Staphylococcus aureus* and a fungus *Candida albicans*. The test organisms were obtained from the research laboratory of Medical Microbiology and Parasitology of the College of Medicine, University of Lagos.

Control organisms

Control strains of *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *C. albicans* ATCC 90028 were used and tested along with the organisms.

Standardisation of inoculums

The test organisms were sub-cultured onto fresh plates of Mueller-Hinton agar (Oxoid, UK) for 24 h and Saboraud dextrose agar for 5-7 days at 37°C for bacteria and fungi, respectively. Colonies from these plates were suspended in Mueller-Hinton broth (Oxoid, UK) and Saboraud broth (Oxoid, UK) to a turbidity matching 0.5 McFarland standard (10^8cfu/ml). The media used for antimicrobial assays were Mueller-Hinton agar (Oxoid, UK) for bacteria and Saboraud dextrose agar (Oxoid, UK) for fungi. All were incubated appropriately as specified for each organism for a period of 18- 24 h. [16]

Antimicrobial assay (agar well diffusion)

Labelled media plates were uniformly seeded with the different test microorganisms, by means of a sterile swab rolled in the suspension and streaked on the plate's surfaces. Wells of 5 mm in diameter and 2 cm apart were punched on the culture media with a sterile cork borer. The various concentrations of the oils (100 µl) were dropped into each well to fullness. Ciprofloxacin antibiotic suspension (0.005%), the neat extracts for each oil and neat solvent (methanol) was dropped into each well to a volume of 100 µL. Dilutions were made using methanol as solvent. Each plate was kept in the refrigerator at 4°C for 1 h to allow for diffusion of extract, before incubating at 37°C for 24 h. [17]

Determination of minimum inhibitory concentration (MIC)

The diameter of the zone of inhibition around the well, measured in millimetre, is used as positive bioactivity. MIC is defined as the lowest concentrations able to inhibit any visible bacterial growth on the culture plates. This was determined graphically, by plotting zone diameter (in mm)

Table I: Zones of Inhibition (mm) of organisms to Garlic oil

Test organisms	Neat ext	D1	D2	D3	D4	D5	D6	D7	D8	D9	Met	Cip	MIC (mg/ml)
Concentrations (mg/ml)	**1051**	**525.5**	**262.75**	**131.36**	**65.69**	**32.84**	**16.42**	**8.21**	**4.11**	**2.05**		**0.05**	
Gram +ve Bacteria													
Staphylococcus aureus ATCC 25923	11	9	8	7	7	6	6	6	6	6	5	18	0.06
Staphylococcus aureus isolate	26	20	10	13	12	8	7	9	9	6	6	17	1.41
Gram –ve bacteria													
Salmonella typhi	11	8	7	6	8	6	6	7	6	6	5	27	0.06
Escherichia coli ATCC 25922	6	9	8	7	8	8	6	6	6	5	5	30	1.34
Escherichia coli isolate	6	10	12	8	8	9	6	6	7	5	6	30	1.75
Pseudomonas aeruginosa	6	30	30	30	25	20	15	6	6	5	6	15	2.83
Fungi													
Candida albicans ATCC 90028	30	30	30	30	30	30	30	28	26	26	5	5	9.31×10^{-13}
Candida albicans isolate	15	7	8	6	6	6	6	7	7	6	5	5	7.88

D1 – D9 = Extract Concentration; Met = Methanol; Cip = Ciprofloxacin, MIC= minimum inhibitory concentration

Table II: Zones of inhibition (mm) of organisms to Tangerine oil

Test organisms	Neat ext	D1	D2	D3	D4	D5	D6	D7	D8	D9	Met	Cip	MIC (mg/ml)
Concentrations (mg/ml)	**894**	**447**	**223.5**	**111.75**	**55.86**	**27.94**	**13.97**	**6.98**	**3.49**	**1.75**		**0.05**	
Gram +ve bacteria													
Staphylococcus aureus ATCC 25923	7	12	10	9	8	7	7	8	7	7	6	19	0.37
Staphylococcus aureus isolate	20	13	12	10	9	7	7	9	7	7	5	19	0.58
Gram –ve bacteria													
Salmonella typhi	9	15	12	10	7	7	6	7	6	6	5	27	2.69
Escherichia coli ATCC 25922	7	15	13	11	11	9	8	10	9	8	5	30	0.18
Escherichia coli isolate	7	13	11	10	11	6	6	9	6	6	5	30	1.32
Pseudomonas aeruginosa	7	8	6	8	8	8	6	7	7	7	6	17	2.63
Fungi													
Candida albicans ATCC 90028	15	30	25	20	16	14	15	15	15	15	5	5	0.16
Candida albicans isolate	15	30	30	30	30	30	30	10	9	8	5	5	0.55

D1 – D9 = Extract Concentration; Met = Methanol; Cip = Ciprofloxacin, MIC= minimum inhibitory concentration

Table III: Zones of inhibition (mm) of organisms to Garlic oil and Tangerine oil blend

Test organisms	Neat ext	D1	D2	D3	D4	D5	D6	D7	D8	D9	Met	Cip	MIC (mg/ml)
Concentrations (mg/ml)	**904.6**	**452.3**	**222.15**	**113.08**	**56.54**	**28.27**	**14.13**	**7.07**	**3.53**	**1.77**		**0.05**	
Gram +ve bacteria													
Staphylococcus aureus ATCC 25923	30	12	10	10	9	8	8	8	8	7	6	21	0.15
Staphylococcus aureus isolate	30	15	15	10	10	9	8	10	10	10	5	20	0.043
Gram –ve bacteria													
Salmonella typhi	6	10	10	9	8	7	8	7	7	7	5	28	0.16
Escherichia coli ATCC 25922	6	21	12	9	9	8	9	7	8	8	5	30	1.24
Escherichia coli isolate	30	24	18	12	10	9	8	10	10	10	7	30	0.82
Pseudomonas aeruginosa	30	30	30	30	15	13	12	10	12	11	7	18	1.21
Fungi													
Candida albicans ATCC 90028	30	30	30	30	30	30	30	30	28	28	6	5	5.95×10^{-31}
Candida albicans isolate	30	30	30	30	30	30	30	20	18	16	5	5	0.012

D1 – D9 = Extract Concentration; Met = Methanol; Cip = Ciprofloxacin, MIC= minimum inhibitory concentration

against the log concentration. The straight line obtained is extrapolated to a point equivalent to the diameter of the cup. The antilog of the corresponding concentration was taken as the MIC. [18]

Gas chromatography-mass spectrometry

The essential oils of *Citrus reticulata, Allium sativum* and the blend (*Citrus reticulata* and *Allium sativum)* were subjected to gas chromatography–mass spectra (GC/MS) analysis on an Agilent apparatus consisting of a model 7890A Network GC

Table IV: Compounds common to Garlic oil and/ or Tangerine oil found in oil blend

Compounds found in oil blend	Garlic oil		Tangerine oil		Oil blend	
	Retention time (Min)	% composition	Retention time (Min)	% composition	Retention time (Min)	% composition
Trisulfide, di-2-propenyl	5.907	30.32	-	-	5.788	15.92
3-Vinyl-1,2-dithiacyclohex-5-ene	4.381	5.68	-	-	4.525	3.26
3-Vinyl-1,2-dithiacyclohex-4-ene	4.057	3.91	-	-	4.147	1.99
Propanoic acid, 2-chloro-	6.627	3.72	-	-	6.600	4.14
Ar-tumerone	10.751	7.08	-	-	10.794	5.06
Cyclic octaatomic sulphur	15.015	1.48	-	-	15.150	1.73
Linoleic acid ethyl ester.	16.467	0.37	-	-	16.580	0.21
3-Cyclohexene-1-methanol, .alpha.,4-trimethyl-	-	-	4.382	33.38	4.306	12.02
2,6-Dimethyl-1,3,5,7-octatetraene, E,E	-	-	3.317	0.15	3.312	0.34
2-Methoxy-4-vinylphenol	-	-	8.868	1.37	5.987	0.33
Caryophyllene	-	-	7.168	1.78	7.207	0.72
alpha.-Farnesene	-	-	8.458	1.67	11.869	1.7
Santolina triene	-		11.916	7.84	11.676	0.90
n-Hexadecanoic acid	-	-	14.683	0.98	14.675	0.66

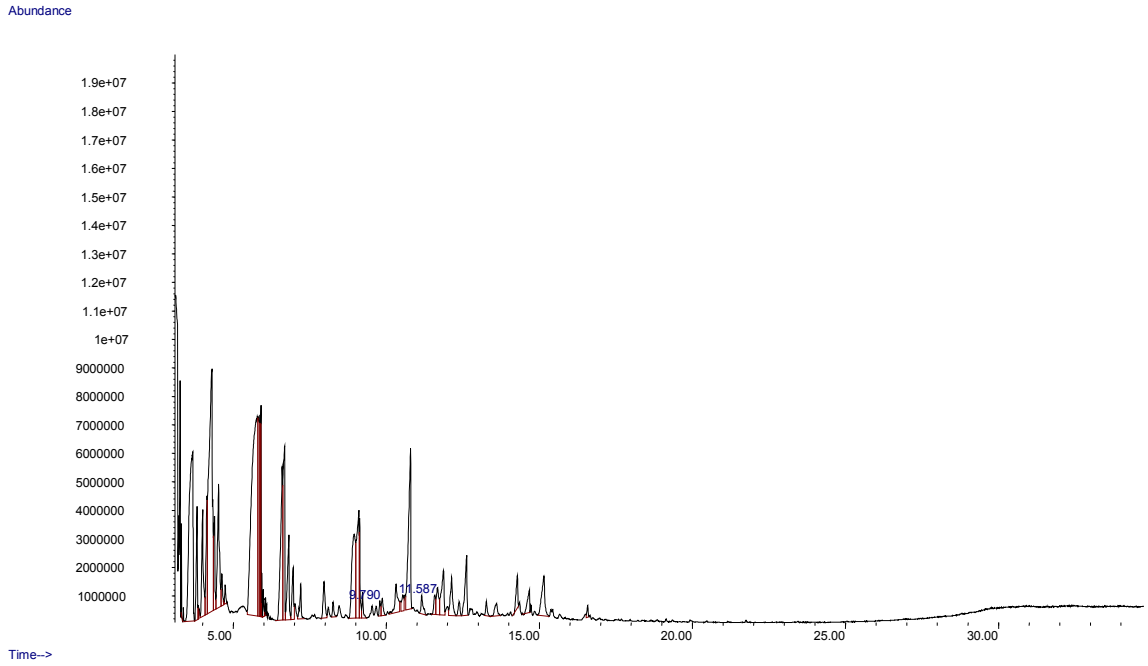

Fig. I: Gas chromatogram of blend oil extracts showing the various constituents

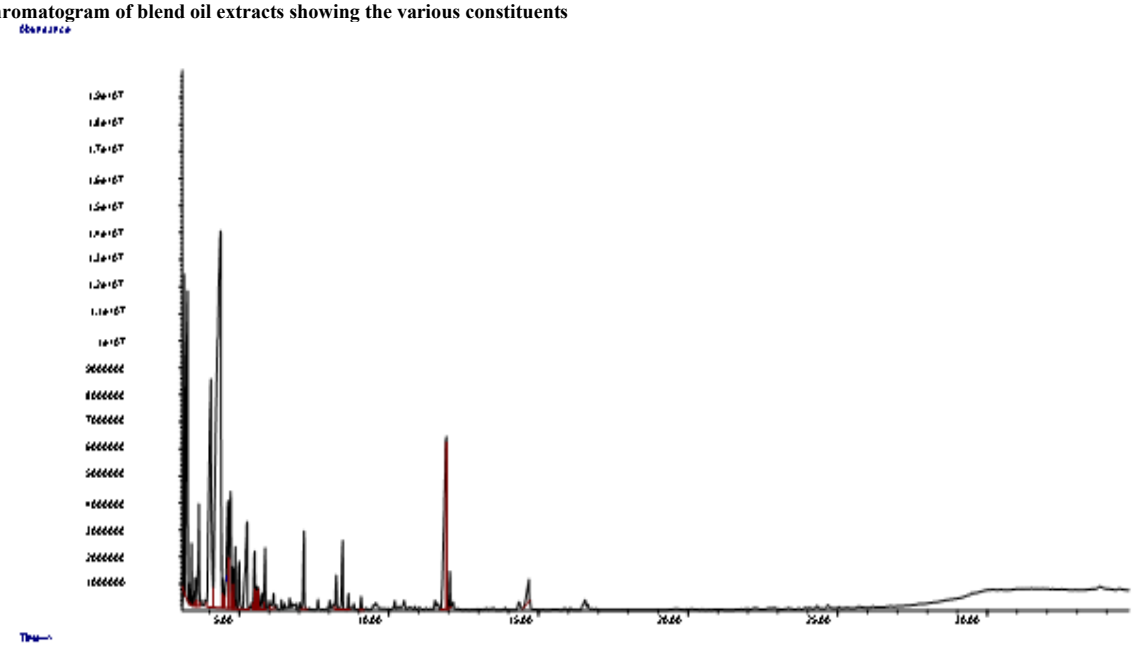

Fig. II: Gas chromatogram of tangerine oil extracts showing the various constituents

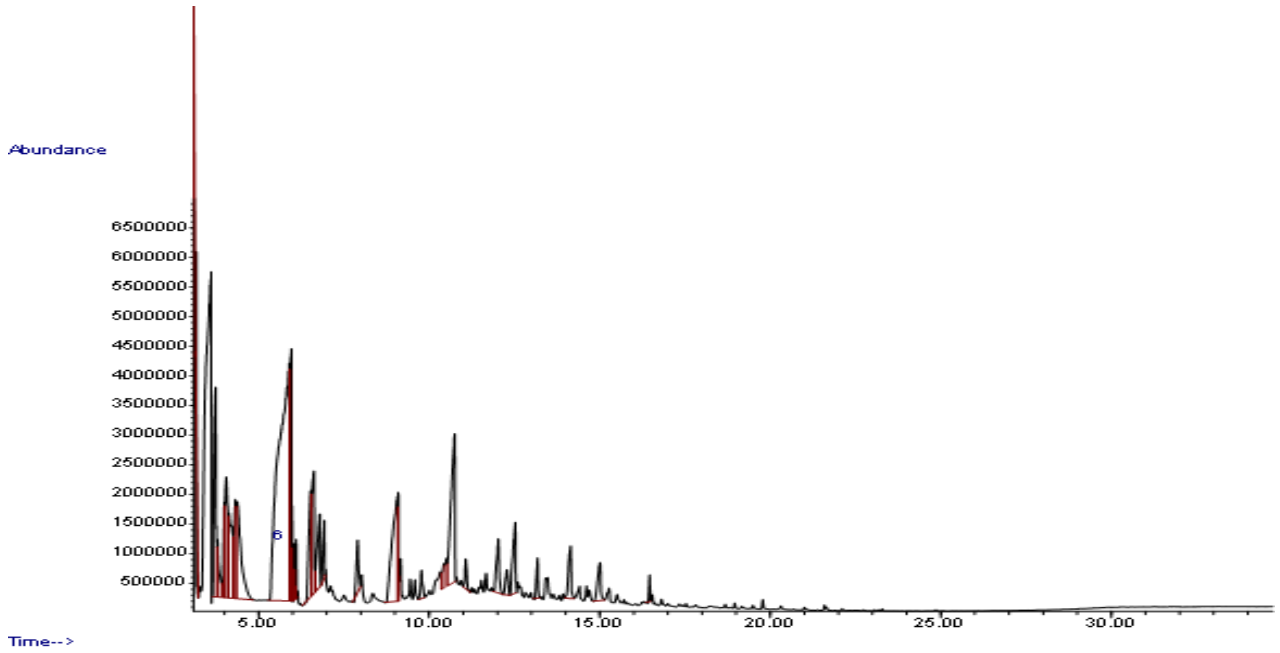

Fig. III: Gas chromatogram of garlic oil extracts showing the various constituents

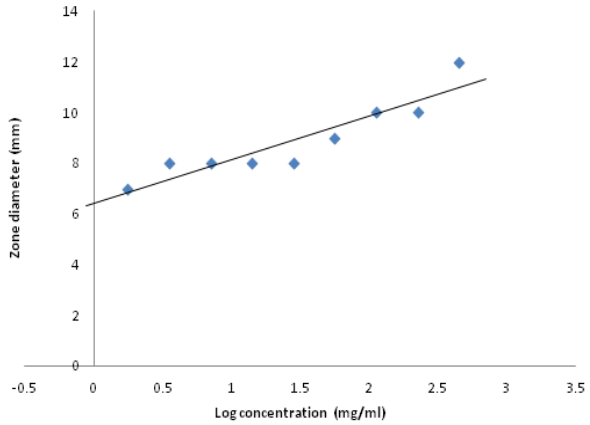

Fig. IV: Graph of zone diameter against log concentration for *staphylococcus aureus atcc 25923* for garlic oil and tangerine oil blend

system/ 5975C mass selective detector at 70 eV and 20°C. The capillary column HP-5MS was a 30 m long fused silica, with an interior diameter of 0.32 mm and a film thickness of 0.25 µm. The carrier gas was helium at a flow rate of 3.3 ml/min. The oven temperature was programmed from 80 to 300°C with an initial increase of 8°C/min. One microlitre of each essential oil sample prepared in acetone (1% concentration) was injected into the apparatus with final run time 34.75 min. The components of the test solution were identified by comparing the mass spectra with spectra of known compounds stored in NIST library 2005. The fragmented ions were separated by the analyzer, according to their various mass-to-charge (m/z) ratios.

RESULTS

The results of the study showed that the garlic clove contained 0.16% (v/w) essential oil (dried weight). The oil was a deep yellow liquid and possessed a distinct sharp garlic odour, with a density of 101.4 mg/ml. A light yellow liquid was obtained for tangerine oil with a yield of 0.5% (v/w) and density of 894 mg/ml. The mixture of an equal volume (1.5 ml each) of the essential oils of garlic and tangerine gave a yellow liquid, with a density of 904.6 mg/ml. The Minimum Inhibitory Concentration (MICs) of garlic oil ranges from 9.31×10^{-13} – 7.88 mg/ml, tangerine oil was 0.16 – 2.66 mg/ml and 5.95×10^{-31} – 1.24 mg/ml for the essential oil blend. The oils were soluble in methanol but insoluble in water.

The Gas Chromatography-Mass Spectrometry revealed Trisulphide, di-2-propenyl (30.32%) as the major component in the garlic oil extract with a total of twenty three constituents and 3-Cyclohexene-1-methanol, alpha 4-trimethyl (33.38%) in the tangerine oil with a total of thirty one constituents. While the equal volume of the oil blend also revealed Trisulphide, di-2-propenyl (15.92%) and 3-Cyclohexene-1-methanol, alpha.4-trimethyl (12.02%) as the major constituents though in lower concentrations and a total of forty three constituents.

DISCUSSION

The methanol solutions of the extracts were found to have a broad spectrum activity against all the micro-organisms tested. The undiluted extracts for tangerine oil and garlic oil, however, had potent activity against the gram positive bacteria and fungi but little activity against the gram negative bacteria as the zone diameter measured were similar to those measured for methanol (the diluting solvent) except for *Salmonella typhi* (Tables I and II). While the undiluted extract for the oil blend had potent activity against all the micro-organisms tested. For example S*taphylococcus aureus* ATCC 25923 had zone diameter of 11mm for garlic oil and 7mm for tangerine oil but 30mm for the oil blend. This is an evidence of interaction in the oil constituents that resulted in synergy in antimicrobial activity except for *Salmonella typhi* and *Escherichia coli* ATCC 25922 with zone diameter of 6 mm each (Table III). A minimum zone diameter of 6 mm for *Salmonella typhi and Ecsherichia coli* isolate and a maximum of 30 mm for *Candida albicans* isolate and *Candida albicans* ATCC 90028 on the Tangerine oil extract,

also for the Garlic oil extract, a minimum zone diameter of 5 mm for *Escherichia coli* ATCC 25922, *Ecsherichia coli* isolate and *Pseudomonas aeruginosa* and a miximum of 30 mm for *Candida albicans* ATCC 90028 was recorded. While for the oil blend, a minimum zone diameter of 7mm for *Staphylococcus aureus* ATCC 25923 and *Salmonella typhi* and a maximum of 30 mm for the Gram $_{+}$ve bacteria, Fungi, *Escherichia coli isolate* and *Pseudomonas aeruginosa* used.

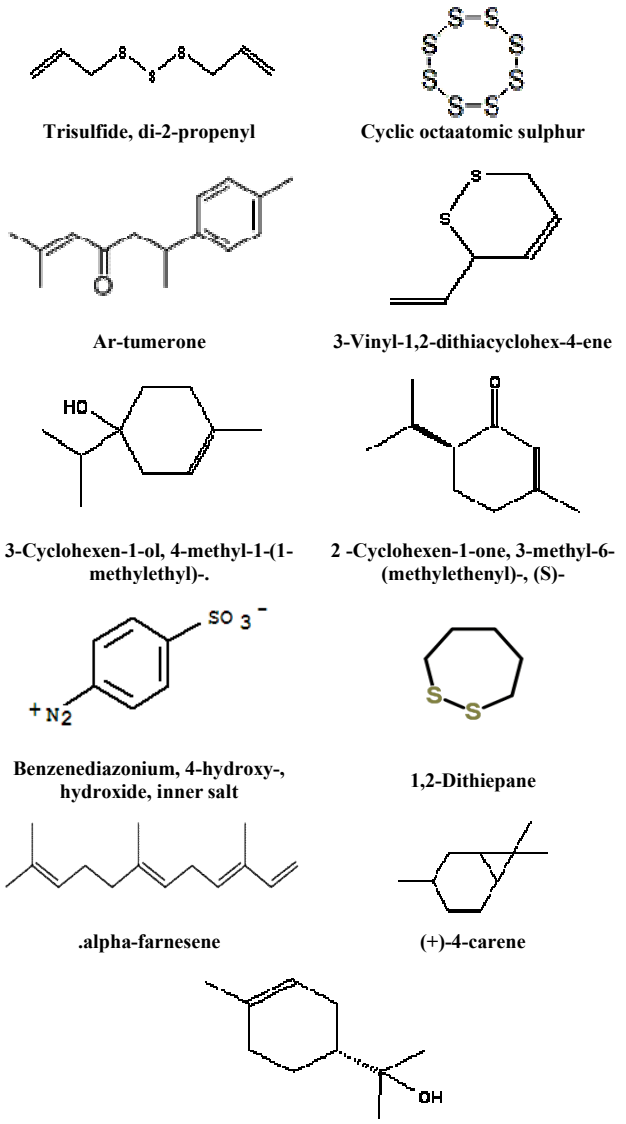

Fig. V: Structures of some identified compounds

The garlic oil was soluble in methanol but insoluble in water as expected. GC/MS analyses of the garlic oil resulted in the identification of twenty three components, accounting for the total composition of volatile oil of 81.77%. The major compounds were, Trisulphide, di-2-propenyl (30.32%), Ar-tumerone (7.08%), Tetrazolo[1,5-b]pyridazine, 6-chloro- (6.96%), 3-Vinyl-1,2-dithiacyclohex-5-ene (5.68%), 2-Hydroxyethyl ethyl disulfide (5. 64%), 3-Vinyl-1,2-dithiacyclohex-4-ene (3.91%), and Cyclic octa-atomic sulphur (1.48%). The dominant compound Trisulphide, di-2-propenyl has been reported as a common component in most garlic clove [19], which is responsible for the distinctive odour of alliums. [17, 20] The potent antimicrobial activity is likely to be related to the respective organosulphur derivatives and volatile sulphur compounds derived from the garlic oil. Garlic Oil is usually obtained by heating crushed garlic to boiling point and collecting the vapour as a distillate. It has been reported that during the heating process, allicin is converted to various sulphides or sulphur containing compounds [21-22], such as Trisulphide, di-2-propenyl, 3-Vinyl-1, 2-dithiacyclohex-5-ene, which are the major components of Garlic oil. Garlic oil and its constituent allyl sulphides inhibit microorganisms by reacting with sulphydryl groups of cellular protein. [23-24] 3 -Vinyl-1, 2-dithiacyclohex-5 -ene and 3-Vinyl-1, 2-dithiacyclohex-4-ene has been reported to be the characteristic flavour components in garlic essential oil. [25] Ar-tumerone may also be responsible for potent antimicrobial activity against the tested microorganisms. [26]

The total numbers of compounds identified in the essential oils of *Citrus reticulata* were thirty one, representing 98.61% of the total oil. Monoterpene hydrocarbon compounds were 85.55% in the oil, whereas sesquiterpene hydrocarbon compounds constituted 13.06%. The oil was rich in 3-Cyclohexene-1-methanol, alpha 4-trimethyl- (33.38%), 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (11.75%), 1,6-Octadien-3-ol, 3,7-dimethyl- (9.99%), (+)-4-Carene (4.93%), 2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis- (4.09%), 6-Octen-1-ol, 3,7-dimethyl-, formate (3.89%), 2-Cyclohexen-1-one, 3-methyl-6-(methylethenyl)-, (S)- (3.50%). The above named constituents are Limonene derivatives, which have been reported to be the major constituents of *C. reticulata.* [11] D-Limonene has been shown to have antimicrobial properties and so it is not surprising that the oil extract had potent antimicrobial properties against a wide range of organisms. [11, 27] The activity of the oil is expected to be related to the respective composition of plant volatile oils. Analysis of the MS data showed that these compounds were hydrocarbons. Fragmentation patterns showed stepwise cleavage of the alkyl groups.

The blend of equal volume of garlic oil and Tangerine oil is also soluble in methanol. GC/MS analyses of the oil blend, showed the presence of forty three components, accounting for the total composition of volatile oil of 87.03%. The GC-MS showed the presence of Trisulfide, di-2-propenyl (15.92%), 3-Cyclohexene-1-methanol,.alpha.,.alpha.,4-trimethyl-,(S)-(12.02%), 2-[2-[2-[2-[2-[2-[2-[2-(2-Acetyloxyethoxy)ethoxy]ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl acetate (8.99%), Ar-tumerone (5.06%), Pyridine, 3-(2-methylpropyl)- (4.31%), Propanoic acid, 2-chloro- (4.14), 3-Vinyl-1,2-dithiacyclohex-5-ene (3.27%), Cyclobutane, 1,1'-(1,1,2,2-tetrafluoro-1,2-ethanedily)bis [2,2,3,3-tetrafluoro- (3.71%), 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (2.37%), Benzenediazonium,4-hydroxy-,hydroxide, inner salt (2.03%), as some of the molecules in the oil blend.

It was evident that interactions occur between the garlic oil and tangerine oil blend from the percentage composition and retention time of the compounds that were found in both the individual oils and the oil blend as seen in (Table IV). Compounds that were not present in GC-MS analysis of Garlic oil and Tangerine oil but were present in the oil blend include Hydrazinecarbodithioic acid, 1-methyl-, methyl ester (1.71%), N-Methylrhodanine. (1.71%), 1-[1-Bromo-2-(2, 2, 3, 3-tetrafluorocyclobutyl)ethyl]-3-fluorobenzene (1.21%),

Hydrazine, (3-methoxyphenyl)- (1.19%), 1,3,5-Trithiane (1.74%), Pyridine, 3-(2-methylpropyl) (4.31%), Cyclobutane, 1,1'-(1,1,2,2-tetrafluoro-1,2-ethanedily)bis [2,2,3,3-tetrafluoro- (3.71%), 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (2.37%), Benzenediazonium,4-hydroxy-, hydroxide, inner salt (2.03%) and 1,3,5-Trithiane (1.74%). This can be seen as evidence that interaction occurred in the oil blend that resulted in synergism in the antimicrobial activity.

Therefore, the study revealed that a more potent antimicrobial activity was observed from the essential oil blend of *Allium sativum* and *Citrus reticulata* working synergistically against the micro-organisms tested and also the chemical constituents of the blended oil revealed Trisulphide, di-2-propenyl (15.92%) and 3-Cyclohexene-1-methanol, alpha 4-trimethyl (12.02%) as the major constituents though in lower concentrations when compared to the individual oils. This suggest that these two compounds can be further investigated for potential antimicrobial agents, however investigation of each constituents in the oil is important as they may also be a contributing factors to the overall activity of essential oil.

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