wjpmr, 2017,3(4), 01-10

SJIF Impact Factor: 4.103

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

SYNERGISTIC ACTIVITY OF GARLIC, GINGER AND MORINGA ON EAR, NOSE AND THROAT ASSOCIATED FUNGI AND VANCOMYCIN RESISTANT COCCI AT AMINU KANO TEACHING HOSPITAL, KANO, NIGERIA

Mohammed Y[1], Dr. Mukhtar M. D*[2] and Orah S.A[2]

[1]Department of Medical Microbiology and Parasitology, Bayero University, Kano, Nigeria
[2]Department of Microbiology, Bayero University, Kano, Nigeria.

***Corresponding Author: Dr. Mukhtar, M. D**

Department of Microbiology, Bayero University, Kano, Nigeria.

Article Received on 26/02/2017 **Article Revised on 19/03/2017** **Article Accepted on 08/04/2017**

ABSTRACT

This study was aimed at evaluating the synergistic activity of Garlic, Ginger and Moringa extracts on ear, nose and throat associated fungi and vancomycin resistant cocci. The plant materials were extracted with methanol and petroleum ether. The extracts were screened for phytochemical contents as well. The microbial isolates were obtained from 33 patients (45.5% females and 54.5% males, 78.8% children and 21.2% adults) attending ear, nose and throat clinic at Aminu Kano Teaching Hospital. Coccal bacteria and fungi were isolated accordingly. The cocci were screened for vancomycin resistance. The antimicrobial assay was carried out by combining the plant extracts at concentrations 100 and 50mg/mL into different ratios (1:1:1, 1:2:1, 2:1:2, 1:2:2 and 2:2:1 w/v). Brine shrimp toxicity test was also conducted. *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Candida albicans* and *Aspergillus fumigatus* were isolated. Twenty one point four percent (21.4%) *S. aureus* were vancomycin resistant, 20% of *S. pneumoniae* isolated were vancomycin resistant and 16.7% *S. pyogenes* were vancomycin resistant. The most susceptible organism to the extracts was *C. albicans* and the least susceptible was *S. pyogenes*. Though the most active combined ratio for both the methanol and petroleum ether extract is the 1:1:1 w/v ratio which gave zones of inhibition ranging from 08mm-30mm but the best ratio was 2:2:1 w/v ratio. The brine shrimp lethality assay showed LC_{50} value ranging from 0.006-3.691µg/mL for all the extracts except Moringa methanol extract which was mildly toxic with LC_{50} value 0f 93.48µg/mL. In conclusion, the ratios showed appreciable activity against the fungal isolates and vancomycin resistant cocci associated with Ear, Nose and Throat symptoms. The search for novel cytotoxic fractions in Moringa and a better combined ratio of use of these candidate extracts should be encouraged.

KEYWORDS: Antimicrobial, Garlic, Ginger Moringa, Ear, Nose, Throat, Fungi,Vancomycin.

INTRODUCTION

Medicinal plants have been used from ancient time for their medicinal values as well as to impact flavor to food. Nowadays, the crude extracts and dry powder samples from medicinal and aromatic plants species are used for the development and preparation of alternative medicine and food additives (Baydar *et al*., 2004). Garlic, Ginger and Moringa are among these plants with medicinal and culinary purposes. Garlic (*Allium sativum*) belongs to the family Alliaceae. Garlic is a plant high in a sulphur compound called Allicin, which is believed to bring most of its health benefits. Its close relatives include the onion, shallot and leek (Block, 2010).

Ginger (*Zingiber officinale*) is a member of the family Zingiberaceae, a small family with more than 45 genera, and 800 species (Foster, 2011). Ginger is truly a world domestic remedy. It is used in India and other places like the ancient Chinese where the fresh and dried roots were considered distinct medicinal products also in Nigeria it is included in soups and drinks which gives a rich, hot and spicy flavor to the soup and drinks. Moringa, native to parts of Africa and Asia, is the sole genus in the flowering plant family Moringaceae. Important medicinal properties of the plant include antipyretic, antiepileptic, antiinflammatory, antiulcerative (Pal *et al*., 1995), antihypertensive (Dahot, 1988) cholesterol lowering (Mehta *et al*., 2003), antioxidant (Nickson *et al*., 2003), anti diabetic, hepatoprotective (Ruckmani *et al*., 1998), antibacterial and antifungal activities (Nickson *et al*., 2003).

Vancomycin is an antibiotic used to treat a number of bacterial infections (American society of health system pharmacist, 2015). It is recommended intravenously as a first-line treatment for complicated skin infections, bloodstream infections, endocarditis, bone and joint

infections, and meningitis caused by methicillin-resistant *Staphylococcus aureus* (Liu *et al*., 2011). In addition to natural circumstances, misuse of vancomycin has led to vancomycin resistance. The reasons for clinical failure of vancomycin are many and have been hypothesized to include poor penetration of the drug to certain tissues (Leclercq *et al.*, 1988; Frieden *et al.*, 1993).

Wide varieties of Ear, Nose and Throat diseases are usually presented to the Otorhinologist (head and neck surgeons) (Ibekwe *et al*., 2005). The pattern of these diseases may vary from community to community or hospital to hospital based on the availability of specialist personnel or facilities for the management of such diseases which are either congenital or acquired in origin. Ear, nose and throat diseases are serious public health problems with universal distribution affecting all age groups (Kishve *et al*., 2010).

One of the research problems facing chemotherapy today is that microorganisms are now gaining resistance to vancomycin, which has been considered to be the reference standard for the treatment of bacterial infection. In Nigeria today, ear, nose and throat- related conditions constitute a major burden of infections. Yet the majority of the citizens are ignorant of ENT treatment options. Disease of the ear, nose and throat (ENT) affect the functioning of adults as well as children, often with significant impairment of the daily life of affected patients (Witsell *et al*., 2001). Due to the emergence of vancomycin resistance which is the last resort antibiotic where other antibiotic has failed and ignorance of the severity of ear, nose and throat infections, there is need for an easy, effective and affordable means to cure infections of the ear, nose and throat (ENT). Garlic, Ginger and Moringa are known for their numerous medicinal properties one of which is their antimicrobial activity (Ankri and Mirelman, 1999; Kamrul *et al*., 2010; Renitta *et al*., 2009). It is very common worldwide to find people consuming these plants in combination or separately as remedies against symptoms believed to be associated with the selected microorganisms targeted in this work. There is a need to find out if these plants have potent antimicrobial activity against ENT fungi and vancomycin resistant cocci. These plants are easy to afford. They can be included in our foods and drinks e.g. tea and soups (Hellsing *et al*., 2013).

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Garlic and Ginger samples were obtained from Yankura market by buying as any other purchaser in Kano State while the Moringa leaves were collected from Naibawa in Kano state. They were all identified and compared to voucher specimens with voucher numbers (Garlic BUKHAN 0297, Ginger BUKHAN 0296, Moringa BUKHAN 0011) at the department of Plant Biology Bayero University, Kano Herbarium with the assistance of Baha'uddeen Said Adam and with reference to Demetrio *et al.* (2015).

Processing and Extraction of Plant Materials

The garlic cloves, ginger rhizomes and moringa leaves were thoroughly washed with distilled water and air dried in a shady environment for two weeks and made into powdered form using a clean pestle and mortar, and then they were sieved through a mesh to obtain fine powder of approximately 20µm particle size. These were stored at room temperature in sealed containers until required for use as demonstrated by (Bashir *et al*., 2013). Accordingly, one hundred grams (100g) of each of the powdered plant material were extracted separately with methanol and petroleum ether using soxhlet apparatus as demonstrated by (James *et al*., 2014).

Confirmation of the Bioactive Components of the Plants

Phytochemical screening was carried out to confirm the bioactive components of the plants as follows:

Test for Alkaloids

This was carried out qualitatively as demonstrated by Cuilie (1994). Using a pipette, 1.0 ml of the extracts was placed in two separate test tubes. Using a dropper, three drops of Meyer's reagent was added separately. A white precipitate with Meyer's reagent indicated the presence of alkaloids.

Test for Saponins

This was carried out as demonstrated by the method reported by Brain and Turner (1975). 0.5g each of the extracts was placed in a test-tube, 5.0ml of sterile distilled water was added to the extract in the test-tubes and shaken vigorously. A froth that persisted for 15 minutes was an indication of the presence of saponins.

Test for Steroids

This was carried out as demonstrated by Cuilie (1994). 2g of each the extracts was placed in a test tube and evaporated to dryness. The extract was then dissolved in acetic anhydride followed by the addition of chloroform and then concentrated sulphuric acid was added by the side of the test tube. Appearance of a brown ring at the interface of the two liquids and the appearance of violet colour in the supernatant layer indicated the presence of steroids in the extract.

Test for Reducing Sugar

This was carried out as demonstrated by the method of Brain and Turner (1975). Here, 1g each of the extracts was weighed and introduced into separate test tubes. The extracts were diluted with 2.0ml each of dimethyl sulphoxide (DMSO) and sterile distilled water respectively. Fehling's solution was added to the solution obtained, and then the mixture was warmed. A brick-red precipitate at the bottom of the test tubes indicated the presence of reducing sugar.

Test for Tannins
2ml of each of the plant was diluted with distilled water in separate test tubes and 2-3 drops of 5% ferric chloride ($fecl_3$) were added. A green-black or blue-black colouration indicated the presence of tannin as demonstrated by (Cuilie, 1994).

Test for Flavonoids
This was carried out as demonstrated by Garba *et al.*, (2011). A 4mg weight of the extracts and a piece of magnesium ribbon were added together followed by concentrated HCL drop-wise. A colour change from crimson to magenta indicated the presence of flavonoids in the extracts.

Test for Terpenoids
0.5ml of the extracts was added to 2ml of chloroform, 3ml of concentrated H_2S0_4 was added to form a layer. A reddish brown colouration at the interface indicates the presence of terpenoids as demonstrated by (Garba *et al.*, 2011).

Test for Anthraquinone
0.5ml of the extract was taken into a dry test-tube and 5ml of chloroform was added and shaken for 5mins. The extract was filtered and drops of ammonia solution were added. A pink violet or red colour in the ammonical layer (lower layer) indicates positive results. This is as demonstrated by (Garba *et al.*, 2011).

Tests for Phenol
Few drops (0.5%) of dilute ferric chloride solution was added 0.5 ml of each of the extracts, the formation of a dark green colour shows the presence of phenol according to the method of Ghumare *et al.*, (2012).

Collection and Identification of Test Isolates
The isolation was carried out in Aminu Kano Teaching Hospital after ethical clearance has been approved. The isolation was carried out under the supervision of a medical laboratory technician. Thirty three specimens were collected from patients attending ENT clinic in any age group. The organisms were isolated from the ear, nose and throat swabs. The specimen was cultured on Sabouraud Dextrose Agar (Manufacturing date, 2016; Expiring date, 2018) for the isolation of fungi. After 3-7days of incubation the fungi isolates were identified macroscopically and microscopically with the help of scheme (Laila, 2014).

The specimens were cultured on Chocolate agar for the isolation of cocci bacteria. The cocci were identified macroscopically in the culture plates after 24 hours of incubation, after which gram staining was carried. This was followed by catalase and coagulase test to confirm the species of Staphylococci. Optochin, bacitracin disc and bile solubility test were used to confirm the species of streptococci. The identified cocci were subjected to vancomycin sensitivity disc (30µg) and the cocci that were found to be resistant to the vancomycin were used for this study as demonstrated by (Demetrio *et al*., 2015).

Bioassay
Preparation of Extracts Concentrations
This was carried out according to the method described by (Cheesbrough, 2006). Stock solution of the garlic, ginger and moringa, methanol and petroleum ether crude extracts were prepared by dissolving 0.6g of each of the plant extracts in 6mL of dimetyhylsulphoxide (DMSO) in glass vial bottles. Therefore, each stock solution would have a concentration of 100000µg/mL (100mg/mL). the stock solution was double-diluted to get three varied extracts concentrations in addition to it to make them four different concentrations of 100mg/mL, 50mg/mL, 25mg/mL and 12.5mg/mL (Kalpana *et al.*, 2013; Garba *et al.*, 2011; Hiba *et al*., 2011).

Standardization of Inoculum
The isolates were adjusted to 0.5 McFarland standard (1.5 X 10^8 CFU/mL) turbidity for bacteria isolates and 1 x 10^6spores/mL for the fungi isolates by adding sterile normal saline. McFarland standards were used as a reference to adjust the turbidity of microbial suspension so that the number of microorganisms will be within a given range. For the preparation of the 0.5 McFarland standard, 0.05mL of barium chloride ($BaCl_2$) (1.17% w/v $BaCl_2.2H_2O$) was added to 9.95ml of 0.18M H_2SO_4 (1.0% w/v) with constant stirring. To aid comparison the standard was compared against a white background with a contrasting black line (Andrews, 2002).

Preparation of Antibiotic Dilution
The antibiotic ciprofloxacin and fluconazole were purchased from Lamco pharmacy Kano State, Nigeria and was reconstituted by dissolving 3g of the ciprofloxacin and fluconazole powder in 100ml of distilled water so as to get a concentration of 30mg/mL. The prepared dilution of the antibiotics was used for subsequent antimicrobial test as positive control (Garba *et al.*, 2011).

Antimicrobial Assay
The bioassay was carried out using the agar well diffusion method described by cheesbrough (2006). 0.1mL of the standardized inoculums (1.5 x 10^8 CFU/ml) of *Staphylococcus aureus* was inoculated onto sterile prepared Mueller Hinton Agar and was spread with a sterile swab while *Streptococcus pneumoniae* on chocolate agar and *Streptococcus pyogenes* was inoculated on sterile blood agar plates. *Aspergillus fumigatus* and *Candida albicans* were inoculated on sterile Sabouraud dextrose agar plates. Six wells were made with a 6mm sterile cork borer into the agar plates containing the bacterial and fungal inoculums and 0.lmL of the four different concentrations from the stock solution of the extracts at concentrations (100, 50, 25, and 12.5mg/mL) were introduced into their respective wells. 0.1mL of DMSO was introduced into the fifth well

to serve as negative control while 0.1ml of 30mg/mL of ciprofloxacin was introduced into the sixth well to serve as a positive control for the bacterial isolates and fluconazole was used for the fungal isolates. The inoculated plates were left to stand for about 30 minutes to allow diffusion of extract before incubating at 37◦C for 24 hours for the bacterial isolates and the fungal isolates were incubated for 37^{o}C for 3 days. The zones of clearance produced around the wells after incubation were observed and measured using a vernier caliper and recorded (in mm). Each of the experiment was conducted thrice and mean results were taken for the test organisms (Kaniz *et al*., 2012; Garba *et al*., 2011; Hiba *et al*., 2011).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)/ Minimum Fungicidal Concentration (MFC)

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that inhibited visible growth of microorganisms after overnight incubation (Andrews, 2002; Thongson *et al*., 2004). The doubling macrodilution broth method was used to determine the MIC. Two (2) mL of the reconstituted crude extract at a concentration of 100000µg/ml was added to 2mL sterile Mueller Hinton broth for the bacterial isolates, 2mL of the reconstituted crude extract was added to 2mL of Sabouraud dextrose broth for the fungal isolates. Two (2) mL of this extract concentration was transferred to another test-tube and this dilution continued until the 12th test-tube was reached, giving extract concentrations ranging from 800-0.39mg/mL in different test tubes. 0.1mL of an 18h culture of bacteria and 3 days culture of fungi previously adjusted to 0.5 MacFarland standard was inoculated into each of the test tubes and the contents were thoroughly mixed. A test tube containing the broth and extract was used as positive control while a test tube containing the broth and bacteria/fungal inoculum was used as negative control. The inoculated culture tubes were incubated at 37^{o}C and were observed for growth after 24 hours for the bacterial isolates and 3days for the fungal isolates. The lowest concentration of extract showing no visible growth when compared with the control was considered as the MIC as demonstrated by (Andrews, 2002).

The minimum bactericidal/fungicidal concentration is the lowest concentration of antimicrobial agent that prevented the growth of an organism. 0.1mL aliquot from the tubes that showed no visible bacterial/fungal growth from the determination of minimum inhibitory concentration was inoculated on a sterile Mueller Hinton Agar for 24 hours at 37◦C for the bacterial isolate while the fungal isolates were inoculated on sterile Sabouraud dextrose agar at 37^{o}C. The lowest concentration in which no growth occurred was taken as the minimum bactericidal concentration (MBC/MFC) as demonstrated by (Andrews, 2002).

Determination of the Synergistic Effect of Garlic, Ginger and Moringa

Concentrations 100 and 50 mg/mL of garlic, ginger and moringa extracts were mixed at ratios 1:1:1, 1:2:1, 2:1:2, 1:2:2 and 2:2:1 and placed in wells on the agar plate where the standardized inoculum has been streaked already. Ciprofloxacin/fluconazole was used as positive control and DMSO served as negative control. The plates were incubated at 37^{o}C for 24 hours for the bacterial isolates and 3 days for fungi isolates. MIC and MBC/MFC were also conducted as demonstrated by (Palaksha *et al*., 2010).

Assay for Lc50 of the Plant Extracts by Brine Shrimp Lethality Test

The eggs were hatched in a hatching chamber containing ocean sea salt water (75ml). Natural light was allowed to penetrate into the hatching chamber for 48 hours so that the eggs will hatch into the shrimp larvae. Twenty milligram (20mg) of the extracts were separately dissolved in 2ml of methanol and equally a positive control of which 20mg of the extracts was dissolved in 2ml of distilled water. 500, 50 and 5ml of the solution equivalent to 1000, 100 and 10 µg/mL respectively was transferred into vials. A negative control which is simply the solvent (methanol) without the test extracts was also prepared. Each concentration were tested in triplicate, therefore each extracts had 9 test tubes. The methanol in the test tubes containing the extracts were allowed to evaporate to dryness for about 48 hours at room temperature and were subjected to test for their activity against Brine shrimp larvae (*Artemia salina*). To each test sample vial, sea water was added and a drop of DMSO solvent was added in order to facilitate the solubility of each test samples in the sea water. Ten (10) shrimps were transferred using a Pasteur pipette and natural sea water was added to make a total volume of 5ml. After 24 hours, the number of surviving shrimps at each concentration was counted and recorded. LC_{50} values were determined at 95% confidence intervals by analyzing the data on a computer loaded with a "Finney Programme." The LC_{50} values of the brine shrimps obtained for extracts of the plants studied were recorded. **(**Adoum *et al*., 2005).

Statistical Analysis

The differences in the antimicrobial activity of Garlic, Ginger and Moringa methanol and petroleum ether extracts were analyzed with Graphpad instat3 and SPSS software for windows 2006. The mean average was used to analyze the best active ratio in the synergism of garlic ginger and moringa.

RESULTS AND DISCUSSION

Phytochemical contents of garlic, ginger and moringa plant extracts

Some Phytochemical components of garlic, ginger and moringa plant extracts is presented in Table 1. The data showed that, Phenol was present in all the extracts. Alkaloid was present in all the extract except moringa

petroleum extract. Saponins was absent in garlic methanol extract, garlic petroleum extract and moringa petroleum extract but was present in all other extracts. Steroids, reducing sugar, flavonoid and terpenoids were present in all the extracts. Tannins were present in all the extracts except garlic methanol extract. Anthraquinone was present in all the extracts except Ginger methanol extract.

Inhibitory Activity of Garlic, Ginger and Moringa Extracts on ENT Associated Fungi and Vancomycin Resistant Cocci

The inhibitory activity of Garlic, Ginger and Moringa methanol and petroleum ether extracts is presented in Table 2. Ginger methanol exhibited the highest inhibition on *Staphylococcus aureus* with a 17mm zone of inhibition at a concentration of 100mg/mL. Moringa methanol exhibited the highest zone of inhibition on *Streptococcus pyogenes* with a 14mm zone of inhibition at 100mg/mL. Garlic petroleum ether extracts showed the highest zone of inhibition (15mm) on *Streptococcus pneumoniae* at 100mg/mL. Ginger methanol extract showed the highest zone of inhibition on *Aspergillus fumigatus* with a 20mm zone of inhibition at 100mg/mL. Ginger petroleum ether extract exhibited the highest zone of inhibition (30mm) on *Candida albicans* at a 100mg/mL. The inhibitory activity of the Synergy of Garlic, Ginger and Moringa methanol and petroleum ether extracts on the test organisms is presented in Table 3. Although all the ratios have similar activity the 1:1:1 combination of Garlic, Ginger and Moringa methanol and petroleum ether extracts was the most active ratio with zones of inhibition ranging from 08mm-30mm. Ratio 1:2:1 was the next active ratio with zones of inhibition ranging from 09mm-23mm. Ratio 2:2:1 have zones of inhibition ranging from 08mm-21mm. Ratio 2:1:2 have zones of inhibition ranging from 10mm-20mm. Ratio 1:2:2 was the least active ratio with zones of inhibition ranging from 09mm-19mm.

MIC and MBC/MFC of the Plant Extracts on the Test Organisms

The MIC and MBC/MFC of the methanol extracts on the test Organisms is presented in Table 4. From the data presented, the MIC for the test organism ranged from 0.39 to 12.5mg/mL while the MBC/MFC ranged from 3.125 to 200mg/mL. Table 5, presents the result of the petroleum ether extracts on the test organisms, from the table MIC ranged from 0.78 to 400 while the MBC/MFC ranged from 6.25 to 800mg/mL.

Assay for the LC_{50} of the plant extracts by Brine Shrimp Lethality Test

Brine shrimp lethality toxicity assay of the plant extracts is presented in table 6. The brine shrimp results in this study are interpreted as follows: LC_{50} <1.0 µg/mL – highly toxic; LC_{50} 1.0-10.0 µg/mL – toxic; LC_{50} 10.0-30.0 µg/mL – moderately toxic; LC_{50} >30 <100µg/mL – mildly toxic, and > 100µg/ml as non-toxic (Moshi *et al*., 2010). From the data presented in table 3, the LC_{50} for GaME is 2.14µg/mL, GaPE is 2.95µg/mL, GiME is 0.30µg/mL, GiPE is 0.45µg/mL, MME is 93.48µg/mL, MPE is 3.691µg/mL, CME is 0.060µg/mL, CPE is 0.006µg/mL. From this result, GiME, GiPE, CME and CPE are highly toxic, GaME, GaPE and MPE are toxic while MME is mildly toxic.

Table 1: Phytochemical Components of Garlic, Ginger and Moringa Identified.

Plant Extracts						
Test	**GarME**	**GarPE**	**GinME**	**GinPE**	**MME**	**MPE**
Phenols	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	-
Saponins	-	-	+	+	+	-
Steroids	+	+	+	+	+	+
Reducing Sugar	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins	-	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Anthraquinone	+	+	-	+	+	+

KEY: GarME=Garlic Methanolic Extract, GarPE= Garlic Petroleum Ether, GinME= Ginger Methanolic Extract, GinPE= Ginger Petroleum Ether Extract, MME= Moringa Methanolic Extract, MPE= Moringa Petroleum Ether Extract + = Presence of Secondary Metabolite, - = Absence of Secondary Metabolite

Table 2: Inhibitory Activity of Garlic, Ginger and Moringa Methanol and Petroleum ether Extracts.

Organisms	GaME	GaPE	GiME	GiPE	MME	MPE
S. aureus	14	10	17	13	10	00
S. pyogenes	12	08	12	10	14	00
S. pneumoniae	13	15	12	09	10	00
A. fumigatus	16	12	20	22	19	00
C. albicans	22	15	24	30	20	09

KEY: GarME=Garlic Methanolic Extract, GarPE= Garlic Petroleum Ether, GinME= Ginger Methanolic Extract, GinPE= Ginger Petroleum Ether Extract, MME= Moringa Methanolic Extract, MPE= Moringa Petroleum Ether Extract

Table 3: Synergistic Action of Garlic, Ginger and Moringa Methanol Extracts on ENT Fungi and Vancomycin Resistant Cocci Zones of inhibition in mm.

	CME					CPE						
Organisms	1:1:1	1:2:1	2:1:2	1:2:2	2:2:1	1:1:1	1:2:1	2:1:2	1:2:2	2:2:1	DMSO	Cip/Flu
S. aureus	12	13	13	12	13	08	09	10	09	08	00	22
S. pyogenes	16	11	11	10	11	12	09	11	09	08	00	28
S. pneumoniae	14	11	10	10	11	12	12	10	12	10	00	25
A. fumigatus	17	16	14	14	15	23	14	16	13	12	00	00
C. albicans	24	21	20	19	21	30	23	20	19	13	00	09

KEY: CPE= Combination of garlic, ginger and moringa petroleum ether extract, Cip= Ciprofloxacin, Flu= Fluconazole

Table 4: MIC and MBC/MFC of the Petroleum ether Extracts on ENT Fungi and Vancomycin Resistant Cocci.

Organisms	GaPE		GiPE		MPE		CPE	
	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC
S. aureus	50	200	25	100	200	800	25	100
S. pyogenes	100	400	50	200	100	200	50	200
S. pneumoniae	6.25	100	6.25	50	100	400	6.25	100
A. fumigatus	50	100	6.25	25	100	200	3.125	25
C. albicans	25	100	1.56	6.25	50	200	0.78	25

KEY: MIC= Minimum Inhibitory Concentration, MBC/MFC = Minimum Bactericidal Concentration/Minimum Fungicidal Concentration

Table 5: MIC and MBC/MFC of the Methanol Extracts on ENT Fungi and Vancomycin Resistant Cocci.

Organisms	GaME		GiME		MME		CME	
	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC
S. aureus	50	200	12.5	50	50	100	0.78	25
S. pyogenes	25	100	25	200	50	200	12.5	100
S. pneumoniae	6.25	50	0.78	12.5	6.25	50	0.78	12.5
A. fumigatus	12.5	50	6.25	25	6.25	25	6.25	25
C. albicans	1.56	12.5	0.39	6.25	0.78	6.25	0.39	6.25

KEY: MIC= Minimum Inhibitory Concentration, MBC/MFC = Minimum Bactericidal Concentration/Minimum Fungicidal Concentration.

Table 6: Brine Shrimp Lethality Toxicity Test.

Extracts	Concentrations (µg/mL)	Total Survival	% Mortality	LC_{50}(µg/ml)
GaMe	1000	5	83.3	2.14
	100	20	33.3	
	10	26	13.3	
GaPe	1000	8	73.3	2.95
	100	22	26.7	
	10	22	96.7	
GinMe	1000	1	73.3	0.30
	100	8	70	
	10	9	96.7	
GinPe	1000	1	93.3	0.45
	100	2	63.3	
	10	11	63.3	
MME	1000	11	26.7	93.48
	100	22	6.7	
	10	28	53.3	
MPE	1000	14	46.7	3.691
	100	16	26.7	
	10	22	26.7	
CME	1000	0	100	0.060
	100	20	33.3	
	10	20	33.3	
CPE	100	4	86.7	0.006
	100	7	76.7	
	10	10	53.3	

KEY: GarME=Garlic Methanolic Extract, GarPE= Garlic Petroleum Ether, GinME= Ginger Methanolic Extract, GinPE= Ginger Petroleum Ether Extract, MME= Moringa Methanolic Extract, MPE= Moringa Petroleum Ether Extract, CME= Combination methanol extract, CPE= Combination petroleum ether extract.

Findings from this study indicated that garlic, ginger and moringa contain some secondary metabolites. These metabolites are responsible for their bioactive properties. These secondary metabolites also serve to protect the plants themselves against bacterial, fungal and viral infections (El-Mahmood and Amey, 2007). These bioactive compounds are known to work synergistically to produce various effects on the human and animal subjects (Amagase, 2006).

In this research, saponins was absent in both garlic methanol and petroleum ether extract, while tannins was absent in only garlic methanol. All other phytochemicals screened for were present. This agrees with the work of Garba *et al*., 2011 and Ameh *et al*., 2012. However, anthraquinone was absent in the study carried out by Garba *et al* for the methanol extract, also Ameh *et al*., claimed that tannins and terpenoid were absent in their research. Antimicrobial evaluation of methanol and petroleum ether extracts of garlic revealed a significant antimicrobial potency against the test organisms this concurs with the study of Ameh *et al*., 2012, who also reported that garlic has potent antimicrobial effect. The phytoconstituents of garlic have long been known and its antimicrobial properties have been widely reported (Roy *et al*., 2006). The zones of inhibition produced by the garlic extracts against the test organisms indicated the potency of the active principle in them. Drugs present in plants are known as active principle and these active principles are divided chemically into a number of chemical classes including glycosides, alkaloids, volatile oils, steroids flavonoids, resins and sterols. Most of these active principles have measurable antimicrobial activities against microorganisms.

In this study ginger methanol and petroleum ether extract also revealed a lot of phytochemicals such as phenols, alkaloids, saponins, steroids, reducing sugar, flavonoids, tannins, terpenoids. Anthraquinone was only present in ginger petroleum ether extract and absent in ginger methanol extract This findings is similar to the findings of Shipra *et al*, 2012 but steroids was absent in their findings. The findings of Matthew, 2007, also concurs with this study. In this studies ginger methanol and petroleum ether extract were shown to exhibit antimicrobial effect on the organisms there were tested on. The antimicrobial activity is due to its numerous phytochemical constituents and also the active components found in ginger as reported by (Park *et al*., 2008; Habib *et al*., 2011).

In this study moringa methanol was found to be more effective than moringa petroleum ether extract. Moringa methanol inhibited the growth of all the organisms it was tested on. Moringa petroleum ether extract however inhibited only the growth of *Candida albicans* at a concentration of 100mg/mL which gave a 09 mm zone of inhibition. However when the concentration was

increased from 200-800mg/mL it was found to inhibit the growth of the organisms and it also had bactericidal and fungicidal effect. A similar study was conducted and moringa leaf petroleum ether extract was found to be active at this same concentration as reported by Kalpana *et al*., (2013). It was also revealed in this study that moringa methanol extract possessed all the phytochemicals it was screened for. Alkaloids and saponins were absent in moringa petroleum ether extracts, this could be the reason for its poor activity. However, a similar research carried out by Nweke, (2012), reported the presence of Alkaloid and Saponin but in low amount. Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs (Rhoades and David, 1979). Saponins cause hemolysis of red blood cells (Winter *et a*l., 1993). They were no much significant difference in the antimicrobial activity of garlic, ginger and moringa combined and the antimicrobial activity of the individual plant. Findings from this study also showed that the fungi isolates were more susceptible than the bacteria isolates this could be because of the cell wall components of bacteria and fungi. Bacteria cell wall is made up of peptidoglycan which is made up of peptide bonds while that of fungi is made up of chitin which is made up of hydrogen bond. The peptide bond is stronger than the hydrogen bond, this could be the reason why the fungi isolates were more susceptible.

This investigation has shown that there is no significant difference ($P>0.05$) in the antimicrobial activity of Garlic, Ginger and Moringa in both methanol and petroleum ether extract in the treatment of ear, nose and throat associated fungi and vancomycin resistant cocci.

Findings from this study indicated that GiME, GiPE, CME and CPE are highly toxic, GaME, GaPE and MPE are toxic while MME is mildly toxic. The toxicity of Garlic agrees with that of Abu-garbia *et al*., (2014) who reported that garlic ethanol extract is toxic at Lc50 value of 10. They was no article to compare the toxicity of *Zingiber officinale*, however a study by Augusto *et al*., (2013) revealed the toxicity of *Zingiber. zerumbet* extracts a close relative of *Zingiber officinale* in the Zingiberaceae family, showed significant toxicity against *Artemia salina* for up with an LC_{50} of 30.9µg/mL for the Dichloromethane extract, and an LC_{50} of 64.0µg/mL for the Methanol extract. The toxicity of Moringa was confirmed by a study carried out by Kaniz *et al*., 2010. Their study reported that all the fractions (chloroform, ethyl acetate and petroleum ether) of moringa leave were found to have potential cytotoxic activity having LC_{50} values ranging from 0.43-1.18 µg/ml. However, in this study, Moringa methanol extract was moderately toxic, probably that was what imparts the low antibacterial effect of the petroleum ether extract excepting on *Candida albicans*, fungal isolate.

Findings in this study showed that the synergy of Garlic, Ginger and Moringa methanol and petroleum ether extracts were highly toxic. This could be due to the toxic effect of the individual plant, due to this fact if this plants must be combined it is preferable to reduce the concentration of garlic and ginger. In this work, the best combined ratio adviced to be used is the 2:2:1 w/v ratio.

CONCLUSION

The present study deduced no significant difference in the antimicrobial activity of Garlic, Ginger and Moringa extracted by methanol and petroleum ether although moringa petroleum ether extract was experimentally more active on *C. albicans* than on vancomycin resistant coccal bacteria. However, suitable combinations (1:1:1, 1:2:1, 2:1:2, 1:2:2 and 2:2:1) w/v of these three plant extracts have demonstrated appreciable activity but the best ratio to be used is the 2:2:1 w/v ratio due to its low concentration of garlic and ginger since they are toxic and moringa is mildly toxic.

Aspergillus fumigatus was the most predominant fungus while *Staphylococcus aureus* was the most predominant cocci associated with ENT at the time of this study. Ginger methanol extract was most active on *S. aureus*, the combination of garlic, ginger and moringa methanol extract was most active on *S. pyogenes*, Garlic petroleum extract was most active on *S. pneumoniae*. However, combination of garlic, ginger and moringa petroleum ether extract was most active on *A. fumigatus*. Ginger petroleum ether extract and the combination of garlic, ginger and moringa petroleum ether extracts were the most active plant extracts on *C. albicans*.

The synergy of garlic, ginger and moringa methanol and petroleum ether extracts were not significantly different from the activity of the individual plant extracts. Although the most susceptible isolates to the synergy were *S. pneumoniae* and *C.albicans*, it is less likely to advice for the combination as no positive outcome could be obtained economically.

RECOMMENDATIONS

The study demonstrated that Moringa extracts was active, therefore, the search for the novel cytotoxic components in Moringa should be encouraged.

1) Individual compounds of the plants should be isolated, purified, characterized and tested.
2) Other extraction methods and solvents should be used for the extraction of the plant materials to see if their activity will be better.
3) The plant extracts should be evaluated *in vitro* to ascertain their activity on ear, nose and throat fungi and vancomycin resistant cocci and also to check their effect on vital organs in the body.
4) Combination of Moringa with plants that have lower toxicity should be encouraged.

REFERENCES

1. Abu-garbia, M., Elmaghraby, O.M., Sotan, E.M., El-raheem, M., Shalaby, E.A. Study of the Antimicrobial Efficacy of some Plant Extracts Against Oral Pathogens and Comparative and Analysis of their Efficiency Against Commercialy Available Tooth Paste and Mouth Rinse. A PhD thesis of the Department of Botany, Faculty of Science Sohag Egypt, 2014.
2. Adoum, O. A. A PhD thesis, Determination of Toxicity Levels of Some Savannah Plants Using Brine Shrimp Test (Bst). Department of Pure and Industrial Chemistry, Bayero University, P.M.B. 3011, Kano – Nigeria, 2005.
3. American Society of Health – System Pharmacists. Retrieved, Sep 4, 2015.
4. Amagase, H. "Clarifying the real bioactive constituent of garlic".*Journal of nutrition*, 2006; 136: 716-725.
5. Ameh, G.I., Eze, S.C. and Omeje, F.U., A PhD thesis, Phytochemical screening and antimicrobial studies on the methanolic bulb extract of *Allium sativum* L. Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu, 2012.
6. Andrews, J.M. Determination of minimum inhibitory concentration. *J. Antimicrob. Chemother.* 48: 5-16, 2002.
7. Ankri, S. and Mirelman, D. Antimicrobial properties of allicin from garlic. *Microbes infect*, 1999; 2: 125-129.
8. Bashir, S.F., Kawo, A.H., Bala, J.A. and Dabai, Y.U. A PhD Thesis, Invitro antibacterial activities and preliminary phytochemical screening of the aqueous and ethanolic extracts of *Zingiber officinale*., Department of Medical Laboratory Science, Faculty of Medicine, Bayero University, Kano, 2013.
9. Baydar, H., Sağdiç, O., Özkan, G., Karadoğan, T. Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey. *Food Control*, 2004; 15:169–172.
10. Block, E. Garlic and Other Alliums: The Lore and the Science. *Royal Society of Chemistry*, 2010; ISBN 0-85404-190-7.
11. Brain, K.R. and Turner, T.D. Practical Evaluation of Phyto-pharmaceuticals. Wright Scientechica, Bristol, United Kingdom, 1975; 57-58.
12. Cheesbrough, M. District Laboratory practice in tropical countries. Cambridge, United Kingdom: Press Syndicate of the University of Cambridge, 2006; 194-201.
13. Cuilei, I. Methodology for the Analysis of Vegetables and Drugs. Chemical Industry Division, NNIDO Romania, 1994; 24-67.
14. Dahot, M.U. Vitamin contents of flowers and seeds of M. oleifera. Pak. J. *Biochem*, 1988; 21: 1-24.
15. Demetrio, L., Valle, J.R., Jeannie, I., Andrade, Windell, L.R. Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. *Asian Journal of Tropical Biomedicine*, 2015.
16. El-Mahmood, A.M. and Amey, J.M. "Invitro antibacterial activity of Parkia biglobosa (Jacq) root bark extract against some microorganisms associated with urinary infections". *Afr. J. biotechnol*, 2007; 6(11): 1272-1275.
17. Foster, S. (2011). Ginger *Zingiber officinale*-Your food is your medicine. [Online] Available from: http://www.stevenfoster.com/education/monograph/ginger.html.
18. Friden, T.R., Munsiff, S.S., Low, D.E., Willey, B.M., William, G., Faur, Y.E., Warren, S., Kreiswirth, B. Emergence of vancomycin-resistant enterococcin. New York City. *Lance*t, 1993; 342: 76-79.
19. Garba, I., Umar, A.I., Abdulrahman, A.B., Tijjani, M.B., Aliyu, M.S., Zango, U.U. and Muhammad, A. MSc Dissertation, Phytochemical and Antibacterial Properties of Garlic Extracts. Department of Medical Microbiology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University Sokoto, Nigeria, 2011.
20. Ghumare, P., Jirekar, D.B., Farooqui, M. and Naikwade, S.D. Phytochemical analysis of petroleum ether extract of some selected medicinal plants leaves. Anandrao Dhonde Alias Babaji Mahavidyalaya Kada. Dist. Beed. (India) Dr. Rafiq Zakeria College for Women, Aurangabad, India, 2012.
21. Hellsing, M.S., Kwaambwa, H.M., Nermark, F.M., Nkoane, B.B.M., Jackson, A.J., Wasbrough, M.J., Berts, I., Porcar, L., Rennie, A.R. "Structure of flocs of latex particles formed by addition of protein from Moringa seeds". Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2013; 460: 460. doi:10.1016/j.colsurfa.2013.11.038.
22. Hiba, A.H., Ayad, R.R., Basama, M.A., Bassam, A.H. Chemical Composition and Antimicrobial Activity of the Crude Extracts Isolated from Zingiber Officinale by Different Solvents.Department of Clinical Laboratory Science, College of Pharmacy, Al-Mustansiriya University, Baghdad, Iraq, 2011.
23. Ibekwe, T.S., Nwaorgu, O.G.B., Onakoya, P.A., Ibekwe, P.U. Spectrum of Otorhinolaryngological emergencies in elderly in Ibadan, Nigeria. *Nig J Med*, 2005; 14(4): 411-414.
24. James, R., Malcolm, K., Dariel, B. and Joanna, V. *J Microbiol Biol Educ*, 2014; 15(1): 45-46.
25. Kalpana, S., Moorth, S. and Sushila, K. Antimicrobial activity of different extracts of leaf of *Moringa oleifera* (Lam) against gram positive and gram negative bacteria. Department of Biochemistry, Asan memorial college of Arts and Science, Chennai, India. *Int.J.Curr.Microbiol.App.Sci*, 2013; 2(12): 514-518.
26. Kamrul, I., Asma, A., Rowsni, M.D., Murad, K. and Shahidul, K. Antimicrobial Activity of Ginger

(*Zingiber Officinale*) Extracts Against Food-Borne Pathogenic Bacteria. Department of Microbiology, Stamford University, Bangladesh. *Journal of Microbiology and Antimicrobials*, 2011; 3(1): 18-22.
27. Kaniz, F.U., Nurul, H.M., Abu, H., Zulfiker, M.D., Kamal, H., Kaiser, H. Comparative Anti-microbial activity and brine shrimp lethality bioassay of different parts of the plant *Moringa oleifera* lam. Department of Pharmacy Jahangirnagar University, Dhaka, Bangladesh, 2012.
28. Kishve, S.P., Kumar, N., Kishve, P.S., Aarif, A.M.M., Kalakoti, P. Ear, Nose and Throat disorders in paediatric patients at a rural hospital in India. *Australasian Medical Journal*, 2010; 3(12): 786-790.
29. Laila, A.N. (2014). Molecular identification of isolated fungi, microbial and heavy metal contamination of canned meat products sold in Riyadh, Saudi Arabia. *Saudi J Biol Sci*, 2015 Sep; 22(5): 513-520.
30. Leclercq, R., Derlot, E., Duval, J., Courvalin, P. Plasmid- mediated resistance to vancomycin and teichoplanin in Enterococcus faecium. *N. England J Med*, 1988; 319: 157-61.
31. Liu, C., Bayer, A., Cosgrove, S.E., Daum, R.S., Fridkin, S.K., Gorwitz, R.J., Kaplan, S.L., Karchmer, A.W., Levine, D.P., Murray, B.E., Rybak, J., Talan, D.A., Chambers, H.F. "Clinical Practice guidelines by the infections diseases Society of America for the treatment of methicilin – resistant *Staphylococcus aureus* infections in adults and children executive summary; Clinical infectious diseases: an official publication of the infectious diseases society of America, 2011; 52(3): 285-92.
32. Matthew, E.E. A PhD Thssis, Comparative Efficacy of Garlic and Antibiotics. Department of Microbiology, University of Calabar, 2007.
33. Mehta, L.K., Balaraman, R., Amin, A.H., Baffa, P.A. and Gulati, O.D. Effects of fruits of *M. oleifera* on the lipid profile of normal and hypercholesterolaemic rabbits. *J. Ethnopharmacol*, 2003; 86: 191-195.
34. Moshi, M.J., Innocent, E., Magadula, J.J., Otieno, D.F., Weisheit, A., Mbabazi, P.K. and Nondo, R.S.O. Brine shrimp toxicity of some plants used as traditional medicines in Kagera Region, north western Tanzania. Department of Biological and Preclinical Studies, Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, 2010.
35. Nickon, F., Saud, Z.A., Rehman, M.H. and Haque, M.E. In vitro antimicrobial activity of the compound isolated from chlor;form extract of M. oleifera Lam. Pak. *J. Biol. Sci*, 2003; 22: 1888-1890.
36. Nweke, F. U. A PhD Thesis, Antifungal Activity of Petroluem Ether Extracts of Moringa oleifera Leaves and Stem Bark against Some Plant Pathogenic Fungi Faculty of Agriculture, Delta State University, Asaba Campus, P.M.B. 95074, Asaba, Nigeria, 2012.
37. Palaksha, M.N., Ahmed, M., Das, S. Antibacterial activity of garlic extract on streptomycin-resistant *Staphylococcus aureus* and *Escherichia coli* solely and in synergism with streptomycin. *J Nat Sci Biol Med*, 2010; 1: 12-5.
38. Pal, S.K., Mukherjee, P.K. and Saha, B.P. Studies on the antiulcer activity of M. oleifera leaf extract on gastric ulcer models in rats. Phytother. Res, 1995; 9: 463-465.
39. Park, M. Antibacterial activity of [10]-gingerol and [12]-gingerol Isolated from ginger rhizome against periodontal bacteria, 2008.
40. Renitta, R.E, J. Anitha, and Napolean, P. Isolation, analysis and identification of phytochemicals of antimicrobial activity of *Moringa oleifera* Lam. *Current. Biotica*, 2009; 3(1): 33-37.
41. Rhoades, and David, F. Evolution of Plant Chemical Defense against Herbivores. In Rosenthal, Gerald A., and Janzen, Daniel H. Herbivores: Their Interaction with Secondary Plant Metabolites. New York; Academic Press, 1979; 41.
42. Roy, J., Shakaya, D.M., Callery, P.S. and Thomas, J.G. "Chemical constituents and antimicrobial activity of a traditional herb medicine containing garlic and black cumen". *Afr. J. Trad.* CAM, 2006; 3(20): 1-7.
43. Ruckmani, K.S., Davimani, B., Jayakar and Anandan, R. Anti-ulcer activity of the alkali preparation of the root and fresh leaf juice of Moringa oleifera Lam. *Ancient Science of life*, 1998; 17(3): 220-223, DIG.
44. Shipra, B., Kshipra, D., Amla, B., Asha, S., Bharti, M. *Zingiber Officinale:* Chemical and phytochemical screening and evaluation of its antimicrobial activities. Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India, 2012.
45. Winter, W.P., Mason, K.T. and Ford, T.D. Mechanism of saponininduced red cell hemolysis: reexamination; *Blood, 82: Suppl*, 1993; 1: 461.
46. Witsell, D.L., Dolor, R.J., Bolte, J.M., Stinnet, S.S. Exploring health-related quality of life in patients with diseases of the ear, nose and throat; A multicenter observation study. Otolaryngology-Head and neck surgery, 2001; 125.