WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 12, 1-13 Research Article ISSN 2278 – 4357

EVALUATION OF THE ANTIMICROBIAL EFFECTS OF *SYZYGIUM AROMTICUM* (CLOVE) AND *GARCINIA KOLA* (BITTER KOLA) EXTRACTS SINGLY AND IN COMBINATION, ON SOME BACTERIA

Ugwu C. C.*[1], Ezeonu I. M.[2], Mbah-Omeje K.[3], Agu C. G.[4] and [5]Onuorah S. C.

[1,3]Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology.

[2]Departmentof Microbiology, University of Nigeria, Nsukka.

[4]Bacterial Vaccine Production Division, National Veterinary Research Institute Vom, Plateau State, Nigeria.

[5]Nnamdi Azikiwe University.

Article Received on
21 Sept. 2017,
Revised on 11 October 2017,
Accepted on 01 Nov. 2017
DOI: 10.20959/wjpps201712-10481

***Corresponding Author**
Ugwu C. C.
Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology.

ABSTRACT

Aqueous and organic(or ethanolic and methanolic) extracts of *Syzygium aromaticum* (clove) and *Garcinia kola* (bitter kola) were evaluated for their antimicrobial effects against *Staphylococcus aureus*, *Bacillus cereus (*NRRL-B-14727) and *Pseudomonas aeruginosa* using agar well and disc diffusion methods. The ethanolic extract had more inhibitory effect than the aqueous and methanolic extract. The inhibition zone diameters (IZD) for the ethanolic extract of clove ranged between 10 and 35 mm while that of bitter kola ranged between 10 and 20mm. For the aqueous extract, the IZD ranged between 10 and 18 mm for clove and 8 and 18 mm for bitter kola. The IZD for the methanolic extract ranged between 9 and 16mm and 8 and 18mm for *Garcinia kola*. The combination of these plant extracts produced greater zones of inhibition than the aqueous and methanol extracts used separately. The MIC varied between 3.125 and 6.25 mg/ml as well as 3.125 and 50.0mg/ml for aqueous, ethanolic and methanolic extracts respectively. *S. aureus* was found to show the greatest sensitivity, while *P. aeruginosa* showed the least sensitivity of all the isolates. Phytochemical analyses of the extracts revealed the presence of alkaloids, tannins, saponins, flavonoids and essential oils. This study shows that extracts of these plants possess antimicrobial properties which could be used as alternative to conventional antibiotics.

KEYWORDS: *Syzygium aromaticum*, *Garcinia kola*, Synergy, Antimicrobial activity, MIC.

INTRODUCTION

The use of plant extracts in the treatment of diseases have become an important interest over the years. This is as a result of the fact that microorganisms are developing resistance to many drugs and as such created situation where some of the common and less expensive antimicrobial agents are losing effectiveness (Montefore *et al*., 1989). In view of this, there is an urgent need to find the alternative to chemotherapeutic drugs in disease treatment particularly those of plants origin which are easily available and have considerably less side effects (khulbe and Sati, 2009). In the past, man has used plant to treat common infectious diseases and even long before mankind discovered the existence of microbes; the idea that certain plant had healing potential was well accepted (Rios and Recio, 2005). A medicinal plant is any plant which, in one or more of its organs, contains assistances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Anyanwu and Nwosu, 2014). A number of plants have been used in traditional medicine for many years due to their antimicrobial properties (Sofowora, 1993). Specifically, the medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human or animal body (Edeoga *et al*., 2005). The most important of these bioactive constituents which are mainly secondary metabolities are alkaloids, flavonoids, tannins and phenolic compounds (Anyanwu and Nwosu, 2014). These phytochemicals are toxic to microbial cells. Medicinal plants generally contain a number of compounds which may be potential natural antibacterial for the treatment of common bacterial infections (Ratnasooriya *et al*., 2005). Plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefit and more affordable treatment (kareem *et al*., 2010). Cloves, botanically known as *Syzygium aromaticum* are one of the highly prized spices, widely recognize all over the world for their medicinal and culinary quantities. Cloves are the aromatic flower buds of a tree in the family Myrtaceae. They are nature to the Maluku Islands in Indonesia and are commonly used as a spice. The most important constituent of Clove is the phenylpropene eugenol. The use of Clove as a food ingredient spice is common in oriental foods (Arora and kaur, 1999). It has been used in folklore treatment of toothaches, insect bites, gastroenteritis and intestinal parasites. It has been used as a sedative and asa cement material in dentistry (Markowitz *et al.*, 1992).Furthermore, anti oxidant (Decker, 1997; Fugisawa *et al.*, 2002; Atsumi *et al.*, 2005) and anti-herpes simplex virus (Tragoolpua and Jatisatienr, 2007).*Garcinia kola*, commonly called bitter cola belongs to the family

Guttiferae (Adesuyi *et al*., 2012). The plant contains the following active ingredients: flavonoids, saponins, apigenin, kolaviron, bifiavonoid-ametoflavone, taninins and resin (Gill, 1992; Okunji *et al*., 2002). The stem bark and seeds are used for acute fever, cough, liver disorders and as an anti-vomiting agent (Odugbemi, 2008; Gill, 1992). It is also used as a remedy for inflammation of respiratory tract, bronchitis, throat, stomach ache and gastritis (Adegboye *et al*., 2008 Ajebesine and Aina, 2004).The seed extract) is very efficacious for hepatitis, antiseptic and is active against Gram-positive and negative bacteria (Gill, 1992). The decoction of the root is used as aphrodisiac, evacuant, anti cancer and also recommended for dysentery, headache, malignant tumours and respiratory ailments (Odugbemi, 2006). The root is chewed for clearing teeth and toothache (Gill, 1992). Several studies have confirmed that cloves have antimicrobial and antifungal properties (Saeed *et al*., 2013; Kim *et al*., 1994; Chaieb *et al*., 2007b). In the same vein, some studies have confirmed that *Garcinia kola* has both antimicrobial and antifungal properties (Amalu *et al*., 2014, Akinnibosun and Itedjere, 2013). However, not much work has been carried out to investigate the synergistic effect of Cloves and *Garcinia kola* on some organisms. It is believed that herbal medicines are more effective when taking in combination.

The aim of this study was to assess the antimicrobial activities of *Syzygium aromaticum* (Cloves) and *Garcinia kola* (Bitter kola) and compared the antimicrobial efficiency of the synergy of Cloves and *Garcinia kola* on some bacteria.

MATERIALS AND METHODS

Collection of plant material

The seeds of *Garcinia kola* and cloves were purchased from Ogbete market, Enugu, Enugu state. The plants were identified and authenticated at the herbarium of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology Enugu.

Test Organisms

The test organisms used in this research consist of both Gram-negative and Gram-positive bacteria namely, *Bacillus cereus* (NRRL-B-14727), *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These organisms were obtained from the clinical laboratory, University of Nigeria Nsukka, Enugu State.

Preparation of aqueous extract

Fifty (50) grams of each dried grinded powder of plants were dissolved in 200ml of sterile distilled water for 24h. The mixtures were filtered using whatman's filter paper no. 1 to obtain solution free of solids. The filtrate was concentrated by drying at 37^0c and stored at 4^0c.

Preparation of ethanolic extract

Fifty (50) grams of each dried grinded powder of plants were dissolved in 200ml of 95% ethanol for 24h. The mixtures were filtered using whatman's filter paper no. 1 to obtain solution free of solids. The filtrate was placed into evaporator to drive-off the solvent and stored at 4^0c.

Preparation of methanolic extract

Fifty (50) grams of each dried grinded powder of plants were dissolved in 200ml of methanol for 24h. The mixtures were filtered using whatman's filter paper no. 1 to obtain solution free of solids. The filtrate was placed into evaporator to drive-off- the solvent and stored at 4^0c.The stored extracts were reconstituted using dimethyl sulfoxide (DMSO) to obtain extracts of several concentrations 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/ml and stored at 4^0c prior to determination of the minimum inhibitory concentration.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration was determined by agar well diffusion method as described by Ogata *et al*. (2000). The plates containing Mueller Hinton agar medium were spread with 0.1ml of the bacterial inoculum. Wells (6mm in diameter) were cut from agar plates using a sterilized stainless steel borer and wells were filled with 0.1ml of each extract. The plates were incubated at 37^0c for 24h and the diameter of resultant zone of inhibition was measured. The least concentration that inhibited the growth of microorganism was termed the MIC.

Disc diffusion assay

A disc diffusion assay using the Kirby-Bauer method (Prescott *et al*., 2005) was applied in testing pure cultures of the isolates for their antimicrobial activities. The sensitivity discused for the test were punched from whatman no. 1 filter paper. Fifty (50) pieces of the sterilized discs were placed in a petri dishes and were impregnated with 2ml of each of the raw extracts separately (Onyeagba *et al*., 2004). Muellar Hinton agar plates were prepared for each test

organisms per plant extract. 0.1ml of the overnight culture of the test organisms were inoculated and spread evenly, with the aid of a forceps the disc were harvested and placed onto the surface of the medium. This was then incubated for 24 h at 37^0c and the plates were observed for zones of inhibition.

Antibiotics Sensitivity Test

A known antibiotic disc was used to test bacteria isolates susceptibility to antibiotics. A broad spectrum antibiotics were employed to compare and contrast the antimicrobial activities of the plant extract.

Synergy test

To test the combined extracts, equal volumes (0.1:0.1) of each concentrations of each extract was tested on the organisms. 0.1ml of each mixture was added into the wells which has been inoculated with the test organisms. This was incubated for 24h at 37^0c. The zones of inhibition were noted.

Phytochemical analysis

The phytochemical analysis of the ground power of cloves and *Garcinia kola* were performed following the methods described by Trease and Evans (1989) and Harbone (1998). The phytochemicals analysed for were plant secondary metabolities which included flavonoids, tannins, spaonins, alkaloids and glycosides.

Statistical Analysis

All data collected were analysed using one-way ANOVA.

RESULTS

Antimicrobial activity of the extracts

The results of the antimicrobial activity of the extracts against the test organisms, namely, *S.aureus, Bacillus cereus* and *Pseudomonas aeruginosa* are shown in Table 1, 2 and 3. The extracts showed varying degrees of growth inhibition against the isolates. The mean zones of inhibition of growth of the isolates are a function of relative antimicrobial activity of the extracts. The ethanol extract showed higher growth inhibition (10-40mm) than the aqueous (2-11mm) and methanol extracts (2-12mm) against all the isolates.Ethanol used as positive control showed activity against the test organisms (6-10 mm) while distilled water used as negative control showed no activity against the test organisms.

Table 1: Antimicrobial activity of the aqueous extracts of *Syzygium aromaticum* (clove) on *Bacillus cereus, Pseudo monas aeruginosa* and *Staphylococcus aureus*.

			Mean zones of inhibition (mm)						
Plant	Test organisms	200	100	50	25	12.5	6.25	3.125	1.56
Syzygium	*Bacillus cereus*	16.0	15.0	14.0	14.0	13.0	13.0	10.0	0
***aromaticum* (clove)**	*S. aureus*	17.0	16.0	16.0	15.0	14.0	12.0	10.0	0
	P.aeruginosa	13.0	12.0	11.0	10.0	10.0	9.0	0.0	0.0
Garcinia kola	*B. cereus*	18.0	17.0	17.0	16.0	15.0	12.0	0.0	0.0
(bitter cola)	*S. aureus*	15.0	14.0	14.0	13.0	12.0	11.0	10.0	0.0
	P. aeruginosa	11.0	10.0	10.0	9.0	9.0	8.0	8.0	0.0

200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56 mg/ml, different concentrations.

***+ve control: with ethanol; 12mm**

***-ve control: with water; no inhibition 0.00mm**

Table 2: Antimicrobial activity of the ethanol extracts of *Syzygium aromaticum* (clove) and *Garcinia kola* (bitter cola) mg/ml against the test organisms.

Mean zones of inhibition (mm)									
Plant	Test organisms	200	100	50	25	12.5	6.25	3.125	1.56
Syzygium	Bacillus cereus	35.0	30.0	20.0	18.0	15.0	10.0	0	0.0
***aromaticum* (clove)**	*S. aureus*	35.0	30.0	28.0	20.0	13.0	10.0	10.0	0.0
	P.aenginosa	40.0	37.0	27.0	20.0	15.0	14.0	12.0	0.0
Garcinia kola	*B. cereus*	18.0	15.0	15.0	12.0	11.0	10.0	10.0	0.0
(bitter cola)	*S. aureus*	20.0	18.0	15.0	14.0	13.0	10.0	0	0.0
	P. aeruginosa	20.0	15.0	14.0	14.0	13.0	12.0	10.0	0.0

200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56 mg/ml, different concentrations.

***+ve control: with ethanol; 12mm**

***-ve control: with water; no inhibition 0.00mm**

Table 3: Antimicrobial activity of the methanol extracts of *Syzygium aromaticum* (clove) and *Garcinia kola* (bitter kola) mg/ml against the test organisms.

Mean zones of inhibition (mm)									
Plant	**Test organisms**	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.56**
Syzygium	*B. cereus*	16.0	14.0	13.0	12.0	10.0	10.0	9.0	0
***aromaticum* (clove)**	*S. aureus*	20.0	19.0	18.0	17.0	15.0	10.0	9.0	0
	P. aeruginosa	10.0	10.0	10.0	9.5	8.0	8.0	0	0
Garcinia kola	*B. cereus*	18.0	17.0	16.0	16.0	15.0	15.0	0	0
(bitter cola)	*S. aureus*	17.0	16.0	15.0	15.0	12.0	0	0	0.
	P. aeruginosa	13.0	13.0	12.0	0	0	0	0	0

***+ve control: with ethanol; 12mm**

***-ve control: with water; no inhibition 0.00mm**

Minimum Inhibitory Concentration (MIC) of the extracts

The results of the MIC of the extracts against the tested isolates are shown in Table 4. The MIC of the ethanol and aqueous extracts for the different organism ranged between 3.125 mg/ml and 6.25 mg/ml respectively while that of the methanol extract ranged between 3.125 mg/ml and 50.0 mg/ml. Lower MIC values were obtained for both ethanol and aqueous extracts. Higher concentrations of the methanolic extract of Garcinia kola (bitter kola) were needed to inhibit P aeruginosa when compared with the other extracts on other organism.

Table 4: Minimum Inhibitory Concentration of the extracts.

Plant	Test Organism	aqueous extract (mg/ml)	ethanol extract(mg/l)	methanol extract(mg/mi)
Syzygium	*B. cereus*	3.125	6.25	3.125
aromaticum	*S. aureus*	3.125	3.125	3.125
(clove)	*P. aeruginosa*	6.25	3.125	6.25
Garcinia	*B. cereus*	6.25	3.125	6.25
kola(bitter	*S. aureus*	3.125	6.25	12.5
kola)	*P. aeruginosa*	3.125	3.125	50.0

***+ve control: with ethanol; 12mm**

***-ve control: with water; no inhibition 0.00mm**

Disc Diffusion Assay

The results of the disc diffusion assay of the ethanol and methanol extracts on the test organisms were shown in Table 5. The disc of the ethanol extracts of *Garcinia kola* had more activity on *S. aureus* and *B. cereus* than *P. aeruginosa* while the ethanol extracts of *Syzygium aromaticum* (clove) had more activity on*S. aureus* and *B. cereus* than P. aeruginosa. From the results, it was observed that the agar well diffusion had more activity than the disc diffusion assay.

Table 5: The disc diffusion assay of the ethanol and methanol extracts of *Garcinia kola* (bitter kola) and *Syzygium aromaticum* (clove) on test organisms.

Plant	Test Organism	Ethanol extract (mg/ml)	Methanol extract(mg/l)
Syzygium	*B. cereus*	18.0	13.0
aromaticum	*S. aureus*	14.0	13.0
(clove)	*P. aeruginosa*	12.0	13.0
Garcinia	*B. cereus*	18.0	16.0
***kola*(bitter**	*S. aureus*	18.0	16.0
kola)	*P. aeruginosa*	13.0	12.0

***+ve control: with ethanol; 12mm**
***-ve control: with water; no inhibition 0.00mm**

Antibiotic Sensitivity Test

The results of the antibiotic sensitivity test were shown in Table 6.

From the result, it was observed that most antibiotics were effective on the *S. aureus* while *P. aeruginosa* was resistant to most of the Gram-negative antibiotics tested.

Table 6: Antibiogram – zone of inhibition (mm) of antibiotic discs on tested organisms.

Test organism				**Zone of inhibition (mm)**						
Gram positive bacteria	E	LEV	CN	APX	RD	AMX	S	NB	CH	CPX
s. aureus	15.0	20.0	18.0	0.0	15.0	0.0	15.0	0.0	15.0	18.0
Gram negative bacteria	PEF	NA	OFX	S	CPX	CN	AU	SEXT	PN	CEP
p. aeruginosa	20.0	18.0	20.0	15.0	10.0	10.0	0.0	0.0	0.0	0.0

Key: Gram Positive. E- erythromycin, CPX- ciproflox, CN- gentamycin, AMX- amoxicillin, RD- raflampicin, CH- chloramphericol,. APX- ampiclox, LEV- levofloxacin, NB- norfloxacin, S-streptomycin, Gram negative, PEF- reflacin, CPX – ciprofiox, S – streptomycin, SXT- septtrin, AU-augumentin, OFX-tarivid, CEP – ceporex, CN-gentamycin, NA – nalidixic acid, PN – ampicillin.

Synergy test

The synergistic effects of ethanol extracts of*Syzygium aromaticum* (clove) and *Garcinia kola* (bitter kola) were shown in Table 7. From the result, it was observed that the combination of these two extracts had more effect than the use of single extract of the test on the test organisms especially on *Pseudomonas aeruginosa* which hadless inhibition zones to the ethanolic extracts of *Garcinia kola* and *Syzygium aromaticum*.

Table 7: The zone of inhibition (mm) on the synergistic effects of ethanolic extracts of *Syzygiumaromaticum* (clove) and *Garcinia kola* (bitter kola) on test organisms.

Mean zones of inhibition (mm)									
Plant	**Test organism**	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.56**
***Syzygium aromaticum* (clove) and *Garcinia kola* (biter kola)**	*B.cereus*	20.0	19.0	18.0	17.0	16.0	15.0	12.0	11.0
	S.aureus	22.0	20.0	19.0	19.0	18.0	17.0	17.0	15.0
	P.aeruginosa	15.0	14.0	13.0	12.0	11.0	10.0	10.0	9.0

Phytochemical analysis

The phytochemcial analysis of *Syzygium aromaticum* (clove) and *Garcinia kola* (bitter kola) revealed the presence of flavonoids, tannins, saponins, alkaloids and essential oils.

DISCUSSION

The results of this study showed that the extracts of Syzygium aromaticum (clove) and Garcinia kola (bitter kola) inhibited the growth of all the bacterial isolates tested (Tables 1, 2 & 3) indicating that the plant extracts had broad antibacterial spectrums (Bankole, 1992). The data obtained showed that the inhibitory effects of extract on the various tested organisms were dose-dependent.

This is in agreement with the study of Puangpronpitag *et al*. (2009) who found out that clove had an inhibitory effect on *S.aureus* (ATCC 25923) with zone diameter of 23.7mm. This study also matched the results obtained by (Prabuseenivasan *et al*., 2006; Al-Barrak and Mohammed, 2011; Betoni *et al*., 2006). This study also is in agreement with the study of Amit Pandey and Parulsingh, (2011) who found out that clove had antimicrobial effect on *S.aureus* and *P. aeruginosa* with zone diameter of 16 mm and 20mm for the ethanolic extracts respectively and 24mm and 19mm for methanolic extract respectively. Clove oil is known for its antibacterial activity which is due to several constituents and may be tested as an alternative to conventional antibiotics therapy (Ali *et al*., 2009). Cloves are strongly pungent due to their high content of eugenol, which is known to inhibit growth of Gram negative, Gram positive and Acid fast bacteria (Abd Rahim and Khan, 2006). The antimicrobial activity of *Garcinia kola* (bitter kola) on the test organisms in this study is similar to the work of Adegboye *et al*. (2008), who showed that the crude extract of *G. kola* exhibited antimicrobial activities in vitro against both Gram-positive and Gram-negative organisms. This study is in agreement with that of Akinnibosun and Itedjere, 2013 and Amalu *et al*., 2014 who found that *G. kola* had antibacterial activity on some microorganism.*G. kola* has been medicinally used as an antimicrobial (Akinnibosun and Itedjene, 2013). The seeds are used in the treatment of bronchitis and throat infections (Mboto *et al*., 2009). The antimicrobial properties of this plant are attributed to the benzophenone and flavonones. Studies have shown that it have very good antibacterial and antiviral properties (Adesuyi *et al*., 2012, Terashima *et al*., 2002). *G. kola* seed is believed to contain a wide spectrum of organic compounds such as flavonoids which confer on it some antibacterial and antifungal actions against Gram negative and Gram positive microorganisms (Adesuyi *et al*., 2011).

The synergistic effects of *Syzygium aromaticum* (clove) and *Garcinia kola* (bitter kola) extracts produced greater zone of inhibition than the aqueous and methanol extracts of these plants when used separately as shown in Tables 1, 3 and 7. The mixture showed better

antimicrobial activity against the test organisms when compared with standard antibiotics as shown in Table 6.Statistical analysis showed that *S.aureus*, *P.areruginosa* and *Bcereus* showed significant differences ($p< 0.05$) in their sensitivities to the synergistic mixture and separate use of *Syzygium aromaticum* and *G. kola* extracts. This study also showed that the disc diffusion assay of the ethanol and methanol extracts of these plants had appreciable inhibitory effect on the test organism as shown in Table 5. This work is similar to that ofAmalu *et al*. (2014) who reported that aqueous suspensions of Garcina kola impregnated into discs had inhibitory activity on *S.auerus*. The MIC of the plant extracts against these test organisms were also determined. The MIC varied between 3.125 and 6.25 mg/ml as well as 3.125 and 50.0mg/ml for aqueous, ethanol and methanol extracts of *Syzygium aromaticum* and *Garcinia kola* respectively. The results of MIC showed that the synergistic uses of *Syzygium aromaticum* and *Garcinia kola* extracts are more active against the test organisms even at low concentrations as shown in Tables 4 and 7.

From this study, it was observed that ethanol extracts exhibited higher inhibitory activity on the test organism than the aqueous and methanol extract. This can be as a result of the ability of ethanol to extract more of the essential oils and secondary plant metabolites which are believed to extert antibacterial activity on the test organism (Nwinyi *et al*., 2009). This study however, justify the scientific use of these plants in traditional medicine in the treatment of infections caused by the test organisms.

REFERENCES

1. Abd Rahim, Z.H and Khan, H.B. Comparative studies on the effect of crude aqueous and solvent extract of clove oil on the cariogenic properties of *S. mutanus*. *J. Sci*., 2006; 48(3): 117-123.
2. Adegboye, M.F., Akinpelu, D. A. and Okoh, A. The bioactive and phytochemical properties of *Garcinia kola* (Heckel) seed extract on some pathogens. *Afr. J. Biotechnol*., 2008; 7(21): 3934-3938.
3. Adesuyi, A. O., Elumm, K., Adaramola, F.B. and Nwokocha, A. G. M. Nutritional and phytochemcial screening of *Garcinia kola*. *Adv. J. Food Science Technol*., 2012; 4(1): 9-14.
4. Ajebesone, P. E. and Aina, J.O. Potential African substances for hops in tropical beer brewing. *J. food Technol. Afr*., 2004; 9(1): 13-16.

5. Akinnibosun, F.I. and Itedjere, F. Evaluation of the antibacterial properties and synergistic effect of *Garcinia kola* Heckel (family: Guttiferae) seed extract and honey on some bacteria. *Afr. J. Microb. Res*., 2013; 7(3): 174-180.
6. Al- Barrak, A.Y. and Mohammed, Z. A. Effect of clove oil on adhesion of *Staphylococcus aureus* to buccal cavity epithelial cells *in vitro*. *Iraqi J. Comm. Med*., 2011; 24(4): 295-301.
7. Ali, H.S., Kamal, M. and Mohamed, S.B. In vitro clove oil activity against periodontal bacteria. *J. Sc. Tech*., 2009; 10: 1
8. Amalu, P. C., Chukwuezi, F.O. and Ugwu, O.P.C. Antimicrobial effects of bitter kola (*Garcinia kola*) nut on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. *IOSR J. Dental Med. Sci*., 2014; 13: 29-32.
9. Amit Pandey and Parul Singh Antibacterial activity of *Syzygium aromaticum* (clove) with metal ion effect against food borne pathogens. *Asian J. Plant Sci. Res*., 2011; 1(2): 69-80.
10. Anyanwu, C. and Nwosu, G. C. Assessment of the antimicrobial activity of aqueous and ethanolic extracts of *Piper guineense* leaves. *J. Med. Plant Research*, 2011; 8(10): 436-440.
11. Arora, D.S. and Kaur, I. Antimicrobial activity of spices. Int. *J. Antimicrob. Agents*, 1999; 12: 257-262.
12. Atsumi, T. Fujisawa, S. and Tonosaki, K. A comparative study of the antioxidant/ prooxidant activities of eugenol and isoeugenol with various concentrations and oxidation condition. Toxicol. *in vitro*, 2005l; 19: 1025-1033.
13. Bankole, A.A. The antibacterial activity of the crude seed extracts of three plants used in Nigerian ethnomedicine. University of Benin, Benin City, 1992; 45.
14. Betoni, J.F, Mantovani, R.P., Barbosa, I.N. and Junior, F.A. Synergism between plant extract of antimicrobial drug used on Staphylococcal disease. *Mem. Int. Oswaldo, Cruz*, 2006; 101(4): 387-390.
15. Chaieb, K. Zmantar, T., Ksoun, R., Hajlaoui, H., Mahdouani, K., Abdelly, C. and Bakhrouf, A. Antioxidant properties of the essential oil of eugenia caryo phyliata and its antifungal activity against a large number of clinical *Candida species*. *Mycoses*, 2007b; 50: 403-406.
16. Decker, E. A. Phenolics: proxidiant or antioxidants? *Nutr. Rev*., 1997; 55: 396-398.
17. Edeoga, H. O; Okwu, D. E, Mbaeble, B. O. Phytochemical constituents of some Nigerian medicinal plants. *African J. Biotechnol*., 2005; 4: 685-688.

18. Fugisawa, S. Atsumi, T., Kaduma, Y. and Sakagarnid, H. Antioxidant and prooxidant action of eugenol-related compound and their cytotoxicity: *Toxicology*, 2002; 177: 39-54.
19. Gil, L.S. Ethnomedicinal uses of plants in Nigeria. University of Benin press. Benin City, Edo State, Nigeria, 1992; 276.
20. Kareem, K.T., Kareem, S. O. Adeyemo, O.J. and Egberongbe, R.K. *In vitro* antimicrobial properties of *Bridelia ferruginea* and some clinical isolates. *Agric. Biol. J. North. Am*., 2010; 1(3): 416-420.
21. Khuibe, K. and Sati, S.C. Antibacterial activity of *Boenninghavsenia albiflora* Reichb (Rutaceae). *African J. Biotechnol*, 2009; 8(22): 6346-6348.
22. Kim, S.Y., Kim, J. H., Kim, S.K., Oh, M. J. and Jung, M.U. Antioxidant activities of selected oriental herb extracts. *J. Am. Oil Chem. Society*, 1994; 71: 633-640.
23. Markowitz, K., Moynihan, M., Liu, M. and Kim, S. Biological properties of eugenol and zinc oxide-eugenol. *Oral Surg. Oral. Med. Oral. Pathol*, 1992; 73: 729-737.
24. Montefore, D., Rotimi, Y.O. and Adeyemi-Doro, F.A The problem of antibacterial resistance to antibiotics among strains from hospital patients in Lagos and Ibadan. Nigeria. *J. Antimicrob. Chemother*, 1989; 23: 604.
25. Nwinyi, O.C., Chinedu, N.S., Ajani, O.O., Ikpo, C. O. and Ogunniran, K .O. Antibacterial effects of extracts of *Ocimum gratissimum* and Piper guineense on *Escherichia coli* and *Staphylococcus aureus*, *Afri. J. Food Sci*., 2009; 3(3): 77.81.
26. Odugbemi, T. and Akinasuline, D. Medicinal plants by species names. In: T. Odugbemi (ed). Outlines and pictures of medicinal plants from Nigeria. University of Lagos press, Lagos State, Nigeria, 2006.
27. Ogata, M., Hoshi, M., Urano, S. and Endo, T. Antidudant activity of eugenol, and related monomeric and dimeric compounds. *Chem. Pharm. Bull*, 2000; 48: 1467-1489.
28. Okunji, C.O. Tantalia, A. W. Hicks, R. P. Iwu, M.M. and Skanchy, D.J. Capillary electrophoresis determination of bifavonomes from *Garcinia kola* in three traditional Africana medicinal formulations. *Plant Med*., 2012; 68: 440-444.
29. Onyeagba, R.A., Ugbogu, O.C., Okeke, C. U. and Iroakasi, O. Studies on the antimicrobial effects of garlic. (*Allium sativum linn*), ginger (*Zingiber officinale roscoe*) and lime (*Citrus aurantifolia linn*). *Afri. J. Biotech*., 2004; 3(10): 552-554.
30. Prabuseenivasan, S., Jayakumar, M. and Ignacimuthu, S. *In vitro* antibacterial activity of some plant essential oils, 2006; 6: 39.
31. Prescott, L. M., Harley, J.P. and Klein, D.A. Microbiology. 6th ed. McGraw-Hill, Boston. 2005; 992.

32. Puangpronpitag, D., Niamsa, N. and Sittiwet, C. Anti-microbial properties of clove (*Eugenia caryophyllum* Bullock and Harrison) Agueous extract against food- borne pathogenic bacteria. *Inter. J. Pharm*., 2009; 5: 281-284
33. Ratnasooriya, W.D., Jayakody, J.R., Premakumara, G.A. and Edriweera, E.R. Antioxidant activity of water extract of *Scoparia dutcis*. *Fitoterapia*, 2005; 76(2): 220-222.
34. Rios, J. l. and Recio, M. C. Medicinal plants and antimicrobial activity. *J. Enthopharmacol*., 2005; 100: 80-84.
35. Saeed, M., Nadeem, M., Khan, M.F., Shabbir, M.A., Shehzad, A. and Amir, R.M. Antimicrobial activity of *Syzgium aromaticum* extracts against food spoilage bacteria, 2013.
36. Sofowora, A. Medicinal plants and traditional medicine in Africa. Spectrum books limited, Ibadan, Nigeria, 1993; 346.
37. Terashima, K., Takaya, Y. and Niwa, M. Powerful antioxidative agents based on garcinoic acid from *Garcinia kola*. *Bioorganic Med. Chem*., 2002; 10(5): 1619-1625.
38. Tragooipua, Y. and Jatisatienr, A. Anti-herpes simplex virus activity of eugenia caryophyllus (spreng) Bullock and S.G. Harrison and essential oil, eugenol. *Eugenol Phytother. Res*., 2007; 21: 1153-1156.