Microbiology Research Journal International

22(3): 1-9, 2017; Article no.MRJI.37904
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Antibacterial Activities of Three Spices on Some Human Bacterial Pathogens

D. J. Ajose[1*], B. A. Adeniyi[2] and T. A. Bamidele[3]

[1]Department of Biological Sciences, College of Natural and Applied Sciences, Bells University of Technology, Ota, Ogun State, Nigeria.
[2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Oyo State, Nigeria.
[3]Department of Molecular Biology and Biotechnology, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author DJA wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Author BAA designed the study. Authors BAA and TAB managed the analyses of the study. Author TAB managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2017/37904
Editor(s):
(1) Essam Hussein Abdel-Shakour, Professor, Department of Botany and Microbiology, Faculty of Sciences, Al-Azhar University, Cairo, Egypt.
Reviewers:
(1) Gorkem Dulger, Duzce University, Turkey.
(2) Muhammad Ali, Kano University of Science and Technology, Nigeria.
(3) Ejikeugwu E. P. C., Ebonyi State University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/22160

Original Research Article

Received 1st November 2017
Accepted 20th November 2017
Published 5th December 2017

ABSTRACT

Aim: The aim of this study was to determine the susceptibility pattern of bacterial pathogens against spice extracts.
Place of Study: Microbiology laboratory of Bells University of Technology; between August, 2015 to July, 2016.
Methodology: The three spices; *Capsicum annuum* (cayenne), *Curcuma longa* (turmeric) and *Piper guineense* (black pepper) were analyzed for the presence and absence of metabolites using standard methods and also tested for their activity against some clinical bacterial pathogens namely: *Bacillus subtilis, Staphylococcus* species, *Escherichia coli, Enterobacter agglomerans, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Acinetobacter baumannii.* Antibacterial

**Corresponding author: E-mail: ajosedj@yahoo.com;*

testing was done using agar diffusion method on Mueller-Hinton agar plates following standard methods.
Results: This study demonstrated that the three spices contained alkaloids, tannins, saponins, flavonoids, steroids, and terpenoids. *Piper guineense* extract was most active against all tested isolates with MIC of 2.5% ^{v}/v against *Staphylococcus* species and *Escherichia coli* and 5% ^{v}/v against *Klebsiella pneumoniae.*
Conclusion: All the tested extracts showed varying spectra of inhibitions of the indicator organisms with *Piper guineense* the most active.

Keywords: Spice; human pathogen; antibacterial; agar diffusion.

1. INTRODUCTION

The "Spice" is a culinary term not a botanical category, it does not refer to a specific kind of plant or plant part [1]. Spices are flavoring agents made from various parts of plants. Each spice has a unique aroma and flavour derived from compounds known as phytochemicals or secondary compounds. These chemicals evolved in plants to protect them against herbivorous insects, vertebrates, fungi, pathogens, and parasites [2]. Spices are used as substances that increase the taste and variation of food [3]. Naturally occurring compounds in spices such as sulphur compounds, terpenes and terpene derivatives, phenols, esters, aldehydes, alcohols and glycosides have shown antimicrobial functions [4]. For thousands of years, aromatic plant materials have been used in food preparation and preservation. Some spices are reported to have bactericidal or bacteriostatic activities. The inhibitory effects of spices are mostly due to the volatile oils present in their composition [5].

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficacy [6,7]. The antimicrobial properties of plant volatile oils and their constituents from a wide variety of plants have been reviewed and accessed [8]. It is clear from these studies that these plant secondary metabolites can have applications in the cosmetic, food and pharmaceutical industries [9]. Medicinal plants typical of spices (thyme, black pepper and chilli) have been used for centuries as food preservatives, flavonoids, home remedy, drug, perfume and insecticide [10]. Essential oils and their phyto-constituents have shown promising antifungal activity *in vitro* and *in vivo*, where they have been extensively studied against *Candida* spp., *Trichophyton* spp. and *Aspergillus* spp. [11,12,13,14]. The antimicrobial properties of medicinal plants are now being reported from all over the world [15,16,17] and these plants are used in the treatment of many diseases such as malaria [18], AIDS and sexually transmitted diseases [19,20], ulcer [21,22,23,24] and tuberculosis [25,26].

Currently, man is facing a major medical crisis: antibiotics are becoming less effective as bacteria are developing resistance to them. Drug-resistant pathogens are a growing menace to all people, regardless of age, gender, or socioeconomic background. Over the last few decades the great advances in our understanding of the causes, transmission, treatment and prevention of infectious diseases have fostered complacency about infections in a society that has access to vaccines, antibiotics and other drugs as a result haven long applied poultices and infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory by people on all continents.

Infectious diseases remain a major cause of death worldwide and these are attributed to increases in respiratory tract infections and antibiotic resistance in both nosocomial and community acquired infections [27]. In view of this, there is need for renewed strategies on treatment and prevention. Plants containing alkaloids and other secondary metabolites used in traditional African system of medicine have been found to be active against a wide variety of microorganisms [28]. The present study was therefore designed to evaluate the activities of three commonly used local spices, *Capsicum annuum* (cayenne), *Curcuma longa* (turmeric) and *Piper guineense* (black pepper) against selected human bacterial pathogens.

2. MATERIALS AND METHODS

Mueller-Hinton Agar (Oxoid, UK CM0337) was used to cultivate the bacteria. Gram-negative and Gram-positive (Oxoid, UK) multi discs and Nystatin (Ns 100µg) (Oxoid, UK) single disc were

procured. Ethanol was distilled at the boiling point of 78°C. Sterile Petri dishes were used.

2.1 Plant Identification, Collection and Processing

The plants (turmeric, black pepper and cayenne) were bought from an herb vendor at Ketu market, Agboyi-Ketu Local Council Development Area, Lagos State, Nigeria for authentication by Mr. Tola at the herbarium section of the Department of Botany, Faculty of Science, University of Lagos and specimen samples deposited.

Curcuma longa LUH 6278 (Turmeric)

Piper guineense LUH 6279 (Black pepper)

Capsicum annuum LUH 6283 (Cayenne)

The plants were commercially obtained afterwards from Agege market, Agege Local Government, Lagos, Nigeria. These were air-dried for several days until completely dried and then reduced to powder using a local grinding mill.

2.2 Solvent Processing

The solvent (ethanol) was distilled at the boiling point of 78 °C in order to remove all forms of impurities and the resultant solvent (100% ethanol) was used for extraction. The distillation was as described by [29].

2.3 Plant Extraction

The extraction was done by standard method [30]. Briefly, powdered Cayenne sample weighted 300 g was loaded into the thimble inside the Soxhlet apparatus and plugged with cotton wool. The apparatus was connected to a condenser above and to a round-bottom flask below containing the solvent; 2 liters of absolute ethanol. The solvent was heated using an isomantle (water bath) at 70 – 80°C. Continuous extraction was done until the condensate turned colourless which signified that the powdered-spice had been exhaustively extracted.

After exhaustive extraction, the solvent (absolute ethanol) was recovered using a rotary evaporator. The extract was then concentrated *in vacuo*. The extraction process was repeated for 350 g of powdered turmeric sample and 700 g of powdered black pepper sample using 5 liters of absolute ethanol as solvent for each sample.

2.4 Phytochemical Screening of Plant Extracts

2.4.1 Alkaloids test

This was done according to the method described by [31]. Briefly, 100 mg of each powdered sample was dissolved in 5 ml of methanol and filtered. Then 2 ml of filtrate was mixed with 5 ml of 1% aqueous HCl. One ml of mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent were added in one tube and occurrence of orange-red precipitate was taken as positive for the presence of alkaloids. To the second tube Mayer's reagent was added and appearance of buff-colored precipitate was taken as positive test for the presence of alkaloids.

2.4.2 Flavonoids test

This was done according to standard methods [32]. Five hundred milligram of each powdered sample was dissolved in 5 ml of ethanol, slightly warmed and then filtered. Few pieces of magnesium chips were added to the filtrate followed by addition of few drops of conc. HCl. A pink, orange, or red to purple coloration was taken as a confirmation for the presence of flavonoids.

2.4.3 Saponins test

Test for saponins was done according to standard methods previously described [31]. One gram of each powdered sample was boiled in 10 ml of distilled water and then filtered. Three milliliter of distilled water was added to filtrate and shaken vigorously for about 5 min. Formation of foam after shaking was taken as a confirmation for the presence of saponins.

2.4.4 Steroids test (liebermann–burchard)

This was done according to standard technique [31]. Two hundred milligram of each powdered sample was dissolved in 2 ml of acetic acid separately; solutions were cooled followed by the addition of few drops of conc. H_2SO_4. Color development from violet to blue or bluish-green was taken as positive test steroidal ring.

2.4.5 Tannins test

This was done according to standard methods [32]. Five hundred milligram of each powdered sample was mixed with 10 ml of distilled water

and then filtered followed by the addition of few drops of 1% ferric chloride solution. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

2.4.6 Terpenoids test (salkowki's test)

This was done according to standard technique [31]. One milliliter of chloroform was added to 2 ml of the extract followed by a few drops of concentrated sulfuric acid (H_2SO_4). A reddish brown precipitate produced immediately indicated the presence of terpenoids.

2.4.7 Antibiotic susceptibility test

This was done according to standard technique [33]. The indicator organisms; *Bacillus subtilis, Staphylococcus* species*, Escherichia coli, Enterobacter agglomerans, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Acinetobacter baumannii* were collected at culture bank of the Nigerian Institute of Medical Research. Antibiotic Sensitivity multi ring disc manufactured by Abtek Biologicals Limited was used. A sterile swab stick was used to take inoculums from a solution of the test bacterial isolate (0.5 McFarland standards) and the excess was drained off by pressing the swab stick against the inside of the test tube above the level of the suspension. The entire surface of the Muller Hinton agar (MHA) plates was uniformly swabbed with the inoculums of test organisms and allowed to dry by allowing them to stand on the sterile work bench for 3 - 5 min. Carefully, a sterile forceps was used to pick the appropriate antibiotic discs and placed on the surface of the inoculated agar media and labeled accordingly. The plates were incubated overnight at 37°C in an inverted position and the zones of inhibition measured and interpreted using the Clinical and Laboratory Standard Institute [33] guidelines.

2.5 Antimicrobial Screening of Crude Extracts: Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MIC) were determined according to standard methods [34,35]. The extracts of the 3 spices were reconstituted in ethanol at a concentration of 50 % (^{v}/v). The crude extracts were screened for antibacterial activity using Mueller Hinton agar dilution method.

One mL of each dilution of the extract was mixed with 19 ml of sterile Mueller-Hinton agar (1 in 20 dilution) to give final concentrations of 5, 2.5, 1.25 and 0.625 (% ^{v}/v), poured into Petri dishes and allowed to set. The surface of the set agar plates were allowed to dry before streaking with an overnight broth culture of the selected isolates and then incubated appropriately. The plates were then examined for the presence/absence of growth. The lowest concentration of the extract inhibiting the visible growth of each organism on the agar plate was regarded as the minimum inhibitory concentration. All experiments were conducted in triplicates.

3. RESULTS AND DISCUSSION

Phytochemicals Present in Plant Extracts and Antimicrobial susceptibility of tested isolates.

Different phytochemicals were present in the spice extracts which include tannins, alkaloids, flavonoids, saponins, terpenoids and sterols. The susceptibility patterns of the tested pathogens/isolates to antibiotics (Table 1) showed that all tested Gram negative bacteria isolates were totally resistant to amoxicillin, cotrimoxazole and augmentin. It was shown that all isolates were resistant to at least two classes of antibiotics [36,37]. In this study, *K. pneumoniae* was observed to be the predominant resistant pathogen. Highest level of susceptibility was observed for ofloxacin for all tested isolates. The Gram positive bacteria isolates, *B. subtilis* was susceptible to all antibiotics, while *Staphylococcus* species was resistant to cloxacillin, erythromycin, streptomycin and chloramphenicol. The resistance pattern shown by the Gram negative bacteria isolates and *Staphylococcus* species indicates that they are multi-drug resistant organisms.

The ethno-pharmacological claims of the plants were also investigated which revealed the efficacy and potency of the plant extracts against certain microbes: zones of inhibition were determined and thus represented in Table 2. All tested isolates showed varying patterns to the reconstituted spice extracts. Susceptibility testing of the ethanolic extract from this study revealed that the highest inhibitory activity was exhibited by extracts of *P. guineense* with zone of inhibition at 14 ± 0.8 mm, the most active being extracts of *P. guineense* on *Bacillus subtilis. C. longa* and *C. annuum* had a lower diameter zone of inhibition ranging between 9 ± 0.3 mm and 16 ± 0.3 mm on *A. baumannii* and *B. subtilis*.

Table 1. Antimicrobial susceptibility pattern of the isolates

Organism	Antibiotics (± SD) Gram negative bacteria							
	Tet (30 µg)	**Amx (25 µg)**	**Cot (25 µg)**	**Nit (300 µg)**	**Gen (10 µg)**	**Nal (30 µg)**	**Ofl (30 µg)**	**Aug (30 µg)**
A.baumannii	7±1.5	-	-	9±1.3	20±1.5	21±1.6	36±1.8	-
P. aeruginosa	6±0.5	-	-	-	16±1.2	6±0.4	30±0.7	6±0.4
E. agglomerans	9±1.0	-	-	7±0.3	19±2.0	20±2.0	34±2.0	-
E. coli	8±0.8	-	-	22±1.9	16±0.8	16±0.4	22±0.3	-
K. pneumoniae	-	-	-	9±0.7	7±0.2	-	9±0.6	-
	Gram positive bacteria							
	Cot (25 µg)	**Cxc (5 µg)**	**Ery (5 µg)**	**Gen (10 µg)**	**Aug (30 µg)**	**Str (10 µg)**	**Tet (30 µg)**	**Chl (10 µg)**
B. subtilis	22±1.7	8±0.8	8±0.3	10±1.2	10±0.5	16±1.0	18±0.9	14±1.2
Staphylococcus sp.	19±0.7	-	-	9±0.4	9±0.3	-	9±1.0	-

NOTE: Zones of inhibition (mm) are average of triplicate experiment and standard deviation.

Tet (30 µg) - tetracycline, Amx (25 µg) - Amoxycillin, Cot (25 µg) - Cotrimoxazole, Nit (300 µg) - Nitrofurantoin, Gen (10 µg) - Gentamycin, Nal (30 µg) – Nalidixic acid, Ofl (30µg) - Ofloxacin, Aug (30 µg) – Augmentin, Cxc (5µg) – Cloxacillin, Ery (5 µg) – Erythromycin, Str (10 µg) – Streptomycin, Chl (10 µg) – Chloramphenicol

- = resistant

The minimum inhibitory concentration of the extracts against the selected tested isolates is represented in Table 3. The MIC of turmeric against all the selected tested isolates and cayenne against *E. coli* and *K. pneumoniae* was seen to be slightly high at greater than 5 % (^{v}/v), while it was 5 % (^{v}/v) for *Staphylococcus* sp. whereas, for black pepper MIC values ranged between 2.5 % (^{v}/v) *E. coli* and *Staphylococcus* sp. and 5 % (^{v}/v) against *K. pneumoniae.*

Majority of the treatment of upper respiratory and urinary tract infections begin or are done totally empirically. Hence to avoid the emergence of bacterial resistance knowledge about common upper respiratory tract pathogens and uropathogens and their regional susceptibility pattern is crucial to optimize the therapeutic strategy. Previous work by other researchers [38,39] also revealed that these spices contained various phytochemicals such as tannins, alkaloids, flavonoids, saponins, terpenoids and sterols. Again, [28] showed that plants have been known to contain myriads of antimicrobial compounds such as polyphenols and flavonoids.

All isolates were resistant to one or two of antibiotics which is mostly pronounced in *Klebsiella pneumoniae* resistance to fluoroquinolone (ciprofloxacin). This is in accordance with the study of [36,37]. The resistance pattern observed indicates that the isolates are multidrug resistant organisms [40]. Resistance of bacteria means that these bacteria have antibiotic resistance genes and the latter may be on chromosome or on plasmids. It is well known that plasmids are major vectors for the dissemination of both antibiotic resistance and virulence determinants among bacterial populations [41]. The exchanging of genetic materials between microorganisms through transformation, conjugation or transduction processes or by mobile genes (transposons) has been proposed as a major contributor in the rapid evolution of microorganisms resistant to antibiotics.

Table 2. Antimicrobial susceptibility pattern of test organisms to the crude extracts of *Capsicum annuum* (Cayenne), *Curcuma longa* (Turmeric) and *Piper guineense* (Black pepper)

Organism	50 % (^{v}/v) (± SD)			Control
	Cayenne	Turmeric	Black pepper	E 50 % (^{v}/v)
A. baumannii	09±0.3	-	12±0.6	-
P. aeruginosa	-	10±0.2	11.5±0.5	-
E. agglomerans	10±0.2	14±0.3	14±0.8	-
E. coli	-	-	10.5±1.0	-
K. pneumoniae	-	-	12±0.9	-
B. subtilis	16±0.3	11±0.7	14±0.3	-
Staphylococcus sp.	-	-	13±1.2	-

NOTE: Zones of inhibition (mm) are average of triplicate experiments and standard deviation

Table 3. Minimum inhibitory concentration of extracts of *Capsicum annuum* (Cayenne), *Curcuma longa* (Turmeric) and *Piper guineense* (Black pepper) against selected isolates

Organism	Concentration (% ^{v}/v)							
	5	2.5	1.25	0.625	6.25	0.312	0.156	MIC
Cayenne								
E. coli	+	+	+	+	+	+	+	>5
K. pneumoniae	+	+	+	+	+	+	+	>5
Staphylococcus sp.	-	+	+	+	+	+	+	5
Turmeric								
E. coli	+	+	+	+	+	+	+	>5
K. pneumoniae	+	+	+	+	+	+	+	>5
Staphylococcus sp.	+	+	+	+	+	+	+	>5
Black pepper								
E. coli	-	-	+	+	+	+	+	2.5
K. pneumoniae	-	+	+	+	+	+	+	5
Staphylococcus sp.	-	-	+	+	+	+	+	2.5

+ = *growth,* **-** = *no growth*

The activity of the ethanolic extract of *P. guineense* being the most active extract against the isolates is in accordance with work of [38] as it contains various phytochemicals [39]. The variation in results of different researches on the quality, quantity and composition of extracted product may be due to many factors such as, the effect of temperature, soil composition, age and vegetation cycle stage and different bacterial strains [42].

4. CONCLUSION

All the tested extracts showed varying spectra of inhibitions of the indicator organisms with *Piper guineense* the most active. The tested *Klebsiella pneumoniae* showed resistance to the tested antibiotics thus, the most resistant pathogen of the tested isolates. The resistance pattern observed by the Gram negative bacteria isolates and *Staphylococcus* species indicates that they are multi-drug resistant organisms. The resistance pattern may be due to inherent attributes or acquired on the basis of their chromosomes, plasmids or transposons. Medicinal plants used in pharmaceutical industry are an inexhaustible source of natural drugs that may be employed in combating ailments and inconveniences resulting from microbial attacks. It is therefore recommended to assess the therapeutic potentials of plants from the traditional African system of medicine as it could insight us as to how best these plants can be used in the treatment of diseases. Combination studies are also recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Farrell KT. Spices, condiments, and seasonings. 2nd ed. New York: Van Nostrand Reinhold; 1990.
2. Walker JRL. Antimicrobial compounds in food plants. *In*: Dillon, V. M., Board, R. G. (eds.) Natural Antimicrobial Systems and Food Preservation. Wallingford (UK): CAB International. 1994;181–204.
3. Bulduk S. Food technology. 2nd edition, Detay Publishing, Ankara, Turkey; 2004.
4. Sung-Eun L, Bruce CC, Russell JM, Shin H, Hoi-Seon L. Inhibitory effects of naturally occurring compounds on aflatoxin B_1 biotransformation. J. Agric. Food Chem. 2001;49(11):5171–5177.
5. Aizpurua-Olaizola, Oier, Ormazabal, Markel, Vallejo, Asier, Olivares, Maitane, Navarro, Patricia, Etxebarria, Nestor, Usobiaga, Aresatz. Optimization of supercritical fluid consecutive extractions of fatty acids and polyphenols from Vitis vinifera grape wastes. J. of Food Sci. 2015;80(1):E101–107.
6. Gislene GF, Juliana L, Paulo CF, Giuliana LS. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian J. of Microbiol. 2000;31: 247–256.
7. David OK, Emma LW. Herbal extracts and phytochemicals: Plant secondary metabolites and the enhancement of human brain function. Adv. Nutr. 2011;2(1):32–50.
8. Mallappa KS, Mohd SA, Uma RS. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review. Evidence-Based Complementary and Alternative Medicine. 2016;2016:Article ID 3012462:21.
   DOI: 10.1155/2016/3012462.
9. Sonia M. Biotechnology and plant secondary metabolites production. J. Biotechnol. Biomater. 2016;6(5):40.
10. Al-Bayati FA. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. J. Ethnopharmacol. 2008;166(3):403-406.
11. Amber K, Aijaz A, Immaculata X, Luqman KA, Nikhat M**.** Anticandidal effect of *Ocimum sanctum* essential oil and its synergy with fluconazole and ketoconazole. Phytomed. 2010;17:921–925.
12. Pereira FO, Wanderley PA, Viana FAC, Lima RB, Sousa FB, Santos SG, Lima EO. Effects of *Cymbopogon winterianus* Jowitt ex Bor essential oil on the growth and morphogenesis of *Trichophyton mentagrophytes*. Braz. J. Pharm. Sci. 2011;47:145-153.
13. Oliveira WA, Pereira FO, Luna GCD, Lima IO, Wanderley PA, Lima RB, Lima EO. Antifungal activity of *Cymbopogon winterianus* Jowitt Ex Bor against *Candida albicans*. Braz. J. Microbiol. 2011;42:433 – 441.
14. Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan LA, Manzoor N. Fungicidal

activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against *Candida*. Eur. J. Clin. Microbiol. Infect. Dis. 2011;30:41–50.

15. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. Ethnopharmacol. 2001;74:113–123.
16. Lopez A, Hudson JB, Towers GHN. Antiviral and antimicrobial activities of Colombian medicinal plants. J. Ethnopharmacol. 2001;77:189–196.
17. Pokhrel NR, Adhikari RP, Baral MP. *In-vitro* evaluation of the antimicrobial activity of *Bauhinia variegate*, locally known as Koiralo. World J. of Microbiol. & Biotechnol. 2002;18:69–71.
18. Mustofa VA, Benoit-Vical F, Pelissier Y, Kone-Bamba D, Mallie M. Antiplasmodial activity of plant extracts used in West Africa traditional medicine. J. Ethnopharmacol. 2000;73:145–155.
19. Vermani K, Garg S. Herbal Medicines for Sexually Transmitted diseases and AIDS. J. Ethnopharmacol. 2002;80:49–66.
20. Oyedeji OA, Adeniyi BA, Ajayi O, Konig WA. Essential oil composition of *Piper guineense* and its antimicrobial activity. Another chemotype from Nigeria. Phytotherapy Res. 2005;19:362 – 365.
21. Adeniyi BA, Lawal TO, Olaleye SB. Antimicrobial and gastro-protective activities of *Eucalyptus camaldulensis* crude extracts. J. Biol. Sci. 2006a;6(6): 1141–1145.
22. Adeniyi BA, Odufowoke RO, Olaleye SB. Anti-bacterial and gastro- protective properties of *Eucalyptus torelliana* F. Muell crude extract. Int. J. Pharmacol. 2006b;2(3):362–365.
23. Adeniyi BA, Onwubuche BC, Ekundayo O, Mahady GB. *In vitro* anti-*Helicobacter pylori* activities of a methanol extract of *Eucalyptus grandis.* In: The Proceedings of the 47th Annual American Society of Pharmacognosy Meeting, 31July to 7 August; 2006c.
24. Gaus K, Huang Y, Israel DA, Pendland SL, Adeniyi BA, Mahady GB. Standardized ginger (*Zingiber officinale*) extract reduces bacterial load and suppresses acute and chronic inflammation in *Mongolian gerbils* infected with *cagA*+ *Helicobacter pylori*. Pharmaceu. Biol. 2009;47(1):92–98.
25. Adeniyi BA, Grooves MJ, Gangadharam PRJ. *In-vitro* anti mycobacterial activities of three species of *Cola* plant extracts (Sterculiaceae). Phytoth. Res. 2004;18: 414–418.
26. Lawal TO, Adeniyi BA, Wan B, Franzblau SG, Mahady GB. Anti-*Mycobacterium tuberculosis* activities of *Eucalyptus camaldulensis* Dehnh. and *Eucalyptus torelliana* F. Muell. and isolated compounds. Pharmaceut. Biol. 2011;52.
27. Pinners R, Teutsch S, Simonsen L, Klug L, Graber J, Clarke M, Berkelman R. Trends in infectious diseases mortality in the United States. J. Amer. Med. Assoc. 1996;275:189–193.
28. Iwu MM, Duncan AR, Okunji CO. New Antimicrobials of plant origin. In: J. Janick (ed.), Perspectives on new crops and new uses. ASHS Press, Alexandria, VA. 1999;457–462.
29. Anne MH; 2017. Available:https://www.thoughtco.com/how-to-purify-alcohol-using-distillation-608263
30. Redfern J, Kinninmonth M, Burdass D, Verran J. Using soxhlet ethanol extraction to produce and test plant material (Essential Oils) for their antimicrobial properties. J. of Microbiol. and Biol. Edu. 2014;15(1):45-46.
31. Solomon CU, Arukwe UI, Onuoha I. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker**.** Asian J. Plant Sci. Res. 2013;3(3):10-13.
32. Trease GE, Evans WC. Pharmacognosy (15th ed.) Springer, Berlin; 2002.
33. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
34. Adeniyi BA, Fong HHS, Pezzito JM, Luyengi L, Odelola HA. Antibacterial activity of diospyrin, isodiospyrin and bisisodiospyrin from *Diospyros piscatorial* (Gurke) (Ebenaceae). Phytothe Res. 2000;14:112–117.
35. Lawal TO, Ogudo BU, Oyedemi TO, Bamidele TA, Adeniyi BA. Antibacterial potentials of three common spices against selected pathogens. J. Nat. Sci. Res. 2014;4(18):78-84.
36. Nathisuwan S, Burgess DS, Lewis JS. Extended-spectrum β-Lactamases:

Epidemiology, detection, and treatment. Pharmacother. 2001;21(8):920–928.
37. Hudson C, Bent Z, Meagher R, Williams K. Resistance determinants and mobile genetic elements of an NDM-1- Encoding *Klebsiella pneumoniae* Strain; 2014.
38. Dorman PM, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Appl. Microbiol. 2000;88(2):308–316.
39. Masood N, Chaudhry A, Tariq P. Bactericidal activity of black pepper, bay leaf, anise seed and coriander against oral isolates. Pak. J. Pharmaceut. Sci. 2006;19(3):214-218.
40. Clinical and Laboratory Standards Institute (CLSI). Updated Susceptibility Interpretation Guidelines; 2014. (Accessed on 10th June, 2015)
41. Hamada TA Mahmood AR, Ahmed IB. Antibiotic resistance in pathogenic bacteria isolated from utis in tikrit province. Tikrit Med. J. 2008;14(1):203-210.
42. Ababutain IM. Antimicrobial activity of ethanolic extracts from some medicinal plant. Aust. J. Basic Appl. Sci. 2011;5(11): 678-683.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/22160