Proximate Analysis and Antibacterial Activities of *Allium cepa, Allium sativum Capsicum annum* and *Zingiber officinale*

'Umeaku, C.N., [2]Chris-Umeaku, C. I., 'Ukoha, C.C., and'Ezeanaekwu, R.O.

'Department of Microbiology, Chukwuemeka Odumcgwu Ojukwu University, Uli, Anambra, Nigeria

Department of Biochemistry, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra, Nigeria

Abstract: The extracts of *Allium cepa, Allium sativum, Capsicum annum and Zingiber officinale* were analysed for their antimicrobial properties. The presence of some phytochemical components (Flavonoids, alkaloids, phenols, glycosides, tannins, saponins and amino acids) were confirmed. The antimicrobial activities of the extracts were evaluated against four bacterial isolates; *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis and Enterobacter aerogenes.* Growth inhibition was evaluated by agar well diffusion and tube dilution methods. All the extracts possessed antimicrobial properties in varying degrees. The zones of inhibition were in the range; *Allium cepa* 9-12 mm, *Allium sativumA* - 11mm, *Capiscum Minum* 9 mm and *Zingiber officinaleW* - 22mm. Ethanolic extracts were more active owing to the inherent solubility of the phytochemical compounds in the solvent. The highest antibacterial activity was exhibited by the ethanolic extract of *Zingiber officinale* against *Enterobacter aerogenes* producing mean zone of inhibition of 15mm while ciprofloxacingave28 mm against *Proteus mirabilis.* The plant extracts showed promising applications in the control of some bacterial agents.

Keyword: Proximate Analysis, Antbacteria.l Activity. Allium cepa, Capsicum auum, Zanziber officiale

Introduction

A spice is any dried, fragrant, aromatic substance of vegetal origin used mainly for flavouring and/ or preserving food (Ram *et al.*, 2010, Abera *et al.*, 2011). Species which include plant materials of medicinal importance have been used for the treatment of human ailments as far back as prehistoric times. (Ekwenye and Elegalam, 2005).

Spices have unique aroma and flavours which are derived from compounds known as phytochemical or secondary metabolites (Ram *et al.*, 2010). The phytochemicals are antimicrobial substances present in the spices which are capable of attracting benefits. These compounds from medicinal plants may be potential natural antimicrobials for treating infections (Ekwenye and Elegalam, 2005; Butkhup and Samapito, 2011;). Numerous classes of phytochemicals are found to be associated with spices (Melvin *et al.*, 2009).

In Nigeria, spices like the garlic, ginger, onions and pepper are extensively used as condiments and ingredients in foods. Garlic (*Allium sativum*) has a long folklore history as a treatment for cold, cough, and asthma, and is reported to strengthen the immune system (Ekwenye and Elegalam, 2005). *Allium sativum* is broadly classified into two sub varieties, ophioscordon (hard neck garlic) and sativum (soft neck garlic). Garlic has many medicinal effects such as lowering of blood cholesterol level, anti-platelet aggregation, anti-inflammatory activity (Nelson *et al.*, 2007), as well as the inhibition of cholesterol synthesis (Abdul *et al.*, 2010). Garlic has long been known to have antibacterial properties (Ekwenye and Elegalam. 2005).

*Corresponding author:
chimeaku@yahoo.com; [1]Umeaku, C.N

The main antimicrobial constituent of garlic is the oxygenated sulphur compound, thio-2-propene-1-sulfinic acid called allicin.

Ginger (*Zingiber officinale*) belong to the family Zingiberaceae, it is a perennial herb with thick tuberous rhizomes. The ginger plant originated from China, and then spread to India, South East Asia, West Africa and Caribbean (Christian, 2008). Mature ginger roots are fibrous and nearly dry, and can be cooked as an ingredient in many dishes. The main constituents are sesquiterprenoids with zingberene as the main component. The pungent of ginger is due to non-volatile phenylpropanoid-derived compounds, gingerols and shogoals. The shogoals are formed from gingerols when ginger is dried or cooked. Zingerone is also produced from gigerols during this process, and it is less pungent and has sweet aroma (Nelson *et al.*, 2007). The characteristic odour and flavor of ginger root is caused by a mixture of gingerone, shogoales, and gingeroles, volatile oils that make up about 1-3% of the weight fresh ginger. Medically, ginger is used as a stimulant and it has a sialagogue action, stimulating the production of saliva. It promotes the release of bile from the gall bladder, as well as decrease joint pain from arthritis, may have blood thinning and cholesterol lowering properties and may be useful for the treatment of heart and lungs diseases (Abdul *et al.*, 2010). It is also effective for treating nausea caused by seasickness, morning sickness and chemotherapy (Nelson *et al.*, 2007). Also ginger is effective for the treatment of inflammation, rheumatism, cold, diabetes (Nelson *et al.*, 2007).

Onion (*Allium cepa*) is a bulbous plant cultivated in many countries of the world including Nigeria, with leading production in China. India and United States. The onion consists of its herbaceous plant part and its edible bulb part. The leaves are bluish-green and hollow. The bulbs are large, fleshy and firm.

There are three main varieties-white, red and purple skinned (Nelson *et al.*, 2007). Onion is rich in proteins, carbohydrates, sodium, potassium and phosphorus. Traditionally, onion has been used to treat intestinal infections. It has been reported to be an antibacterial, anti-inflammatory and antioxidant activity (Abdul *et al.*, 2010). The relatively pungency of onion has both genetic and environmental components. Sulphuric compounds in onions have also been shown to be anti-inflammatory both inhibiting formation of thromboxanes and by inhibiting the action of platelet activating factor (PAF). The onion has both anti-thrombotic benefits and antioxidant activity. This later effect is important for cardiovascular health by reducing the probability that platelets aggregate in the blood, a major cause of heart attack (Nelson *et al.*, 2007).

Red pepper (*Capsicum annuum*) is predominantly cultivated in the Northern part of Nigeria with a particular reference to Gombe State. *Capsicum annuum* extracts have various antibacterial activity against *Klebsiella pneumonia* but has inhibiting activity against *Pseudomonas aeruginosa* (Delik and Sevil, 2011). Different extraction mechanism might affect the antimicrobial activity of *Capsicum annuum*. This research tends to evaluate the antibacterial activities of some local spices; *Allium cepa, Allium sativum, Capiscum annuum and Zingiber officinale* against the named bacteria.

Materials and Methods

Collection of the plant materials

The spices were purchased from Nkwo Ogbe market, Ihiala in Ihiala Local Government Area of Anambra State (South-East), Nigeria. They were authenticated by Dr C. Chigor of the Department of Botany, University of Nigeria, Nsukka.

Preparation and Extraction

The outer covering of the garlic, ginger and onion were manually peeled off. The spices were thoroughly washed with clean sterile water. This was done to reduce the microbial load of the plant materials due to handling and transportation. They were sliced into cutlets. 20g of each of the spices were separately weighed with digital weighing balance and ground with electric blender. Each spice was soaked in 200ml of distilled water and 200ml of 80%, ethanol to prepare the aqueous and ethanol extracts respectively. They were kept at room temperature for 48hrs, a time necessary for enough dose of the extraction to take place, after which they were filtered in 250ml conical flasks using the Whatman No.1 filter paper. The extracts (filtrates) were covered with aluminum foil and stored in the refrigerator until they were needed.

Phytochemical evaluation

Phytochemical analyses of the extracts were carried out to determine the active chemical constituents of the spices. However, the presence of a particular phytochemical depends on the solvent of extraction. All the phytochemical analyses were evaluated using the methods described by Prashant *et al.* (2011).

Qualitative evaluation of the phytochemical constituents

Detection of alkaloids: Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). A yellow colored precipitate was formed indicating the presence of alkaloids.

Detection of flavonoids: extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicated the presence of flavonoids.

Detection of proteins and amino acids: This was done by the xanthoproteic test. The extracts were treated with few drops of concentrated nitric acid. Formation of yellow color indicated the presence of proteins.

Detections of glycosides: The extracts were hydrolyzed with diluted hydrochloric acid, and then subjected to test for glycosides. Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammoniac layer indicated the presence of anthranol glycosides.

Detection of phenols: This was evaluated using the ferric chloride test. Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicated the presence of phenols. Filtrate was added to 5ml of ethylacetate and 5ml of 1% ammonia solution. The absorbance was taken at 520nm (Akinyemi, 2010).

Saponins: Standard for saponin solution was prepared by dissolving 10mg of diosgenin in mixture of methanol and distilled water. To the aliquots for each tube, Vanillin reagent (8%, 0.25ml) was added and sulphuric acid (72% v/v, 2.5ml) was slowly on the inner side of the wall. The solutions were mixed well and the tube was transferred to a 60^0C water bath. After 10 minutes incubation, the tubes were cooled in ice for 3-4 minutes. The absorbance was measured at 544nm. 0.1g of the sample was dissolved in aqueous methanol (80%, 0.1ml). 0.25ml of aliquot was taken for spectrophotometric determination for total saponins at 544nm (Akinyemi, 2010).

Alkaloids: Akinyemi's method was used to confirm alkaloid content. 0.5g of the sample was dissolved in 96% ethanol-20% H_2SO_4 (1:1). 1ml of the filtrate was added to 5ml of 60% H_2SO_4 and allowed to stand for 3

hours. The reading was taken at absorbance of 565nm (Akinyemi, 2010).

Sterilization of glassware and media preparation

The glassware (including Petri dishes and test tubes) were washed thoroughly with clean water and detergent. They were dried and sterilized with a hot oven at 150^{0}C for 2h. The media used in this work were nutrient agar, Mac Conkey agar and MRS agar and buffered peptone water. The media were prepared according to the manufacturer's direction. They were sterilized with an autoclave at 121^{0}C at 15psi for 15min.

Isolation and identification of test organisms

The source of inocula for this study was urine. The urine samples were diluted using ten-fold serial dilution. 1.0ml of different dilution of the urine (100, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 1and 10^{-5}) were aseptically plated on the media using pour plating method. The plates were incubated at 37^{0}C for 24h. The colonies grown after incubation were subcultured on a nutrient agar using streaking method. The test organisms were identified using their cultural characteristics, Gram staining and biochemical tests (Cheesbrough, 2000).

Storage and maintenance of the test organisms

The isolated microorganisms were used for the antibacterial sensitivity testing. Prior to use, the organisms were sub-cultured on nutrient agar slant and stored in refrigerator at 4^{0}C.

Preparation of the test microorganisms

This was done according to the method described by Christian, (2008). The test microorganisms (*Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia* and *Proteus mirabilis*) stored in the refrigerator were resuscitated in peptone water medium and incubated at 37^{0}C for 24hr.

Antibacterial bioassay

The extracts were diluted to different concentrations using the double-fold serial dilution technique. The test organisms that were previously enriched with buffered peptone water were diluted in test tubes using the ten-fold serial dilution technique.

The antibacterial activities of the extracts were conducted using the Agar well diffusion method (Christian, 2008). A 20ml of molten nutrient agar was seeded with 1ml of broth cultures of the test organisms in sterile Petri dishes. The Petri dishes were rotated slowly to ensure even distribution of microorganisms. The nutrient agar was left to solidify in the dish. A sterile cork borer was used to make 5mm diameter hole on the agar plates and labeled according to the different concentration of the extracts. 0.2ml of the extracts was inoculated into their corresponding wells with the aid of a sterile pasture pipette. The dishes were allowed to stand for a minimum of 30 min. at room temperature to allow for proper diffusion of the extracts into media to take place. Ciprofloxacin and distilled water were used as positive and negative controls respectively. The plates were inverted and incubated at 37^{0}C for 24hr. At the end of the incubation period, the zones of the diameter of inhibition formed on the medium were measured and recorded in mm.

Determination of Minimum Inhibitory Concentration (MIC)

This was carried out using the agar dilution method previously described by Odelola and Okorosobo (1996). A colony from each stock were sub-cultured into 5 ml of nutrient broth and incubated at 37^{0}C for 18 hours. 0.1ml of the overnight broth of each organism was pipette into 9.9 ml of the broth to yield a 10^1 dilution. The procedure was continued to obtain a final dilution of 10^3 (Smith *et al.*, 2000). A 2cm streak of bacterial strains were made on an oven-dried nutrient agar plates containing increasing concentrations (25 – 200 mg/ml) of the extracts. The lowest concentration that gave no visible growth after overnight incubation at 37^{0}C was taken as the Minimum Inhibitory Concentration (MIC) of each extract. Also this made use of s made use of classic method of successive dilution. In twelve numbered screw tubes (10 x 100 mm), 1 mL of TSB (trypticase soy broth) medium was distributed for every tube, except for the tube number 1. The tubes were submitted to autoclave under constant pressure and temperature of 121 °C. For the first and the second tubes of the series, 1 mL of tested sanitizing agent was added; tube 2 was stirred and 1 mL was withdrawn and transferred for tube 3. This successive transference was repeated until tube 11. It was added to all flasks, except for flask number 11, 0.1 mL of inoculation (tested microorganism) at known concentration. Incubation at optimal temperature was developed for 24 and 48 hours (Figure 1). After this period, the reading was developed; the MIC is the concentration of the higher dilution tube in which the absence of bacterial growth occurred. Tubes 11 and 12 are positive (TSB + inoculation) and negative (TSB + antimicrobial) controls (Mazzola et al., 2001, 2003).

Determination of Minimum Bacterial Concentration (MBC)

- A pure culture of the test microorganism grown overnight, then diluted in growth-supporting broth (typically Mueller Hinton Broth) to a concentration between 1 x 10^5 and 1 x 10^6 cfu/ml.
- A stock dilution of the extract test substance is made at approximately 100 times the expected MIC (if known).
- Further 1:1 dilutions are made in test tubes or 96 well microtiter plates.
- All dilutions of the test product(s) are inoculated with equal volumes of the specified microorganism.

- A positive and negative control tube or well is included for every test microorganism to demonstrate adequate microbial growth over the course of the incubation period and media sterility, respectively.
- An aliquot of the positive control is plated and used to establish a baseline concentration of the microorganism used.
- The tubes or microtiter plates are then incubated at the appropriate temperature and duration.
- Turbidity indicates growth of the microorganism and the MIC is the lowest concentration where no growth is visually observed.
- To determine the MBC, the dilution representing the MIC and at least two of the more concentrated test product dilutions are plated and enumerated to determine viable CFU/ml.
- The MBC is the lowest concentration that demonstrates a pre-determined reduction (such as 99.9%) in CFU/ml when compared to the MIC dilution.

Results

Table 1 shows the phytochemical components in the extacts. *A. cepa* contained the six phytochemical components tested. Both *A. sativum* and *C. annum* lacked saponin, while *Z. officinale* contained all the phytochemicals tested but lacked flavonoids Table 2 presents the authentication of the bacteria used for the study . Table 3 shows antibacterial activities of the aqueous extract against the test organisms. *Z. officinale* gave the highest zone of 15mm. In Table 4 antimicrobial activities of ethanolic extract of *Z. officinale* gave 22mm. Table 5 describes the minimum inhibitory concentration (MIC) and the minimum bacteriocidal concentration (MBC).

Table1:Phytochemical constituents of *Allium cepa, Allium sativum, Capsicum annuum and Zingiber officinale*

Phytochemical	*A.cepa*	*A. sativum*	*C. annuum*	*Z.officinale*
Amino acid	+	+	+	+
Tannins	+	+	+	+
Alkaloids	+	+	+	+
Saponins	+	-	-	+
Phenolics	+	+	+	+
Flavonoids	+	+	+	-

Key: + = positive, - = negative

Table 2: The characterization and identification of the test microorganisms

Parameter	*E. coli*	*Enterobacter spp.*	*Klebsiella spp.*	*Proteus spp.*
Colour of colony	Colourless	Red	Grey-white	Colourless
Morphology	Rod	Rod	Rod	Rod
Gram reaction	-	-	-	-
Catalase	+	+	+	+
Oxidase	-	-	-	-
Nitrate	+	+	+	+
Citrate	+	-	+	-
H_2S	-	-	-	+
Methyl red	+	+	+	+
VP test	-	+	-	-
Urease	-	-	+	+
Coagulase	-	-	-	-
Indole	+	-	-	-
Glucose	A/G	A/G	A/G	A
Maltose	A/G	A/G	A	
Lactose	A/G	A/G	A/G	A
Sucrose	A/G	A/G	A/G	A
Mortility	+	+	-	+

Keys: + = positive, - = negative, A = acid production, A/G = acid and gas production.

Table 3: Antibacterial activities (mm) of aqueous extracts of the samples on *Enterobacter aerogenes, E.coli, Proteus mirabilis, K.pneumoniaae* Zones of inhibition

Extract	Concentration	*E.aerogenes*	*E.coli*	*P.mirabilis*	*K.pneumoniaae*
Allium cepa	200	8	12	9	10
	150	5	10	8	9
	100	-	9	7	-
	50	-	8	4	-
	25	-	4	4	-
Allium sativum	200	4	11	-	4
	150	-	10	-	3
	100	-	4	-	3
	50	-	3	-	-
	25	-	2	-	-
Capsicum annuum	200	-	-	9	9
	150	-	-	9	7
	100	-	-	8	6
	50	-	-	7	-
	25	-	-	5	-
Zingiber officinale	200	15	11	11	11
	150	9	-	-	-
	100	8	-	-	-
	50	8	-	-	-
	25	5	-	-	-
Ciprofloxacin	500	24	19	28	25
Distilled water	200	-	-	-	-

Key: - = no inhibition

Table 4: Antibacterial activity (mm) of ethanolic extracts of the samples on *Enterobacter aerogenes, E. coli, Proteus mirabilis, K. pneumonia* Zones of inhibition

Extracts	Concentration	*E. aerogenes*	*E.coli*	*P.mirabilis*	*K.pneumoniae*
Allium cepa	200	9	11	10	10
	150	8	10	9	9
	100	7	9	7	8
	50	8	7	6	7
	25	6	4	3	6
Allium sativum	200	18	16	18	20
	150	16	14	16	17
	100	11	13	10	12
	50	9	10	7	11
	25	6	7	5	9
Capsicum annuum	200	10	10	10	21
	150	9	9	9	4
	100	8	9	-	4
	50	6	8	-	3
	25	5	6	-	2
Zingiber officinale	200	22	20	20	21
	150	20	19	18	17
	100	17	14	16	14
	50	9	10	11	9
	25	7	9	9	6

Key: - = No inhibition

Table 5: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts of *Zingiber officinale* on bacterial isolates.

Isolates	Concentrated extract dilution/ml					
	200	150	100	50	25	MBC
E. aerogenes	+	+	+	-	-	200
E. coli	+	+	+	-	-	200
P. mirabilis	+	+	+	-	-	200
K. pneumoniaae	+	+	+	-	-	200

Key: – indicates no growth
+ indicates growth

Discussion

Allium cepa showed antimicrobial activity against *E. aerogenes, E. coli, P. mirabilis* and *K. pneumonia* as seen in Table 3. This is in accordance with the study of Tagoe *et al.*, (2011) in their observed that all the organic plant extract studied inhibited the growth in a marked significant difference. They ginger had the highest antifungal activity. They slated that phytochemicals present in medicinal plants have health benefits and antimicrobial activity against some pathogenic bacteria. All the medicinal plants used in this study shared antimicrobial activities against the test organisms in various degree. Table 3 compared the antibacterial activity of the plant extract with that of commercially available antibacterial agent ciprofloxacin. Ciprofloxacin however gave better clearance than the plants extracts.

In this study, *Allium sativum* had the highest against *Escherichia coli*, followed by *Klebsiella pneumoniae*. This is similar to the results obtained by Buthup and Samappito, 2011. In their study, they recorded that *Allium sativum* the greatest inhibitory effect on *Bacillus cereus, E. coli*. bacteria. The findings of this study could support the view that Nigerian medicinal plants are promising sources of potential antibacterial agents, which may be efficient as preventive agents in the pathogenesis of some diseases.

Higher concentrations of ethanolic extracts of *Allium sativum, Zingiber officinale* (200mg 1ml) gave good clearance in all the test organisms of this (16mg 1ml-21mg 1l). They compared favorably with the clearance zone obtained from ciprofloxacin 19-28 mg1l. Although ciprofloxacin gave better clearance. Higher concentrations of extracts of *Allium sativum* and *Ginziber officinale* could be used as alternatives. Nagori and Solanki (2011) in their study of medicinal plants in wound healing pointed categorically that many medicinal plants are found useful in treating wounds. This is because of the antibacterial effects of the extracts of the implicated bacteria. According to Ekwenye and Elegalam, 2005, Garlic has long been known to have antibacterial proper.

Conclusion and Recommendation

All medicinal plants used in this study showed antimicrobial activities against the test organisms. This study suggests that concerted efforts should be geared towards conducting more researches in order to ascertain the required dosage that could be administered for the treatment of infectious diseases caused by these test organisms.

References

Abdul, H., Muhammad, B.H., Muhammad, Y., & Sumayya, S. (2010). *In vitro* antibacterial activity of *Allium cepa* against clinical isolates of *Vibriocholerae. Journal of Ayub Medical College Abbottabad* 22. 160-163.

Abera, A., Lemessa, F., and Muleta, D. (2011). The antifungal activity of some medicinal plants against coffee berry disease caused by *Colletotrichum kahawae. International Journal of Agricultural Research 6*: 268- 279.

Akinyemi, M., Yahaya, Y., Banso, A., & John, F. (2010). Phytochemical and antimicrobial of *Terminalia avicennoides* extracts against some bacterial pathogens associated with patients suffering from complicated respiratory tract diseases. *Journal of Medicinal Plant Research* 2: 94-97.

Akram, M.,Shah, M.I., Usmanghan, K., Mohiuddin, E., and Sami *et al.*, A. (2011). *Zingiber, officinale* roscoe. (Amedicinal plant). *Pakistan Journal of Nutrition* 10: 399 – 400.

Aseervatham, J., Palanivelu, S., and Panchanadham, S. (2011).*Semecarpus anacardium* (Bhallataka) alters the glucose metabolism and energy production in diabetic rats. Evid. Based Complement. *Alternative Medicine* 10. 1155/ 2011/ 142978.

Asha, B., Krishnamurthy, K.H., and Devaru, S. (2011). Evaluation of anti hyperglycaemic ginger activity of *Zingiber officinale* (Ginger) in

albino rats. *Journal of Chemical Pharmacology Research*3: 452-456.

Butkhup, L., and Samappito, S. (2011). *In vitro* free radical Scavenging and antimicrobial activity of some selected Thai medicinal plants. *Research Journal of Medicinal Plant*5: 254 - 265.

Cheesbrough, M. (2000). District laboratory practice in tropical countries. Fourth edition. Cambridge University Printing Press, CB2 2RU, UK, 62-70.

Christain, A. (2008). Antibacterial activity of aqueous and ethanolic extracts of nine Nigerian spices against four food borne bacteria. *Journal of Food Safety* 10: 77-80.

Dadji, G.A.F., Tamesse, J.L., and Boyom, F.F. (2011). Adulticidal effects of essential oils extracts from *Capscium annuum* (Solanaceae) *Piper nigrum* (piperaceae) and *Zingiber* ginger , *Capscium annumofficinale* (zingiberaceae) on *Anopheles gambiae* (Diptera- Culicidea), vector of malaria. *Journal of Entomology* 8:152-163.

Delik, K., & Sevil, T. (2011). Antimicrobial activities of solvent extracts of different spices. *Journal of Environmental Biology* 32: 251-256.

Ekwenye, U.N. & Elegalam, N.N. (2005). Antibacterial activity of *Zingiber officinale* and *Allium sativum* extracts on *Escherichia coli* and *Salmonella typhi*. *International Journal of Molecular Medicine and Advance Science*1: 411-416.

Hemalatha, R., Babu, K.N., Karthik, M., Ramesh, R., Kumar, B.D., and Kumar, P.U. (2011). Immunomodilatory activity and Th1/ Th2 cytokine response of *Ocimum sanctum* in myclosuppressed Swiss Albino mice. *Trends in Medical Research* 6:23-31.

Hiral, C., ,Shipra, B., & Sumitra, V.C. (2004). Comparison of antibacterial activities of selected species of Zingiberaceae family and some synthetic compounds. *Journal of Turk Biology* 29:83-97.

Ige, S.F., Akhigbe, R.E., Adewale, A.A., Badmus, J.A. and Olaleye *et al.*, S.B. (2011). Effect of *Allium cepa* (onion) extract on cadmium induced nephrotoxicity in rats. *Kidney Research Journal* 1: 41- 47.

Joseph, B., and Raj, S.J. (2011). An overview: Pharmacognostic properties of *Phyllanthus amarus*. *Linn International Journal of Pharmacology*, 7: 40 – 45.

Joshi, B., Sah, G.P., Basnet, B.B., Bhatti, M.R., and Sharma *et al.*,D. (2011). *Phytochemical* extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). *Journal of Antimicrobial Agents* 3: 1- 7.

Kansal, L.,Sharma, V., Sharma, A., Lodi, S., and Sharma, S.H. (2011). Ameliorating effect of *Coriandrum sativum* extracts on hematological and immunological variables in an animal model of lead intoxication. *Journal of Pharmacy and Allied Health Sciences*1 (1): 16-29

Khan, S., Singla, R.K., and Abdin, M.Z. (2011). Assessment of Phytochemical diversity in *Phyllanthus amarus* using HPTLC Fingerprints. *Indo Global Journal of Pharmaceutical Sciences* 1:1-12.

Kolawole, O.T., Abiona, F.E., Kolawole, S.O., Ayankunle, A.A., and Olaniran, O.I. (2011). Effect of *Momordica* charantia fruit Extract on normal and alloxan-diabetic rats. *International Journal of Pharmacology* 7:532–535

Mehri, A., Hasani-Ranjbar, S., Larijani, B., and Abdollaahi, M. (2011). A systematic review of efficacy and safety of Urtica dioica in the treatment of diabetes. *International Journal of Pharmacology* 7:161-170.

Melvin, M., Joe, J.J., & Vijayapriya, M. (2009). Antimicrobial activity of some common spices against certain human pathogens. *Journal of Medicinal Plants Research* 3:1134-1136.

Mishra, P., and Mishra, S. (2011). Study of antibacterial activity of *Ocimum sanctum* extract against gram positive and gram negative bacteria. *American Journal of Food Technology* 6:336 – 341.

Mmereole, F.U.C., (2011). Evaluation of the dietary inclusion of aloe vera as an alternative to antibiotic growth promoter in broiler production. *Pakistan Journal of Nutrition* 10: 1 – 5.

Nagori, B.P., and Solanki, R. (2011). Role of medicinal plants in wound healing. *Research Journal of Medicinal Plants* 5: 392 – 405.

Nelson, C.A., Reginald, A.O., Okro, N., & Janet, K. (2007). Antibacterial activity of *Alliumcepa*, and *Zingiber officinale* on *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from high vaginal swab. *Journal of Tropical Medicine* 3:55-80.

Prashant, T., Bimlesh, K., Gurpreet, K., & Harleen, K. (2011). Phytochemical screening and extraction. *International Journal of Pharmaceutical Science* 1:98-106.

Ram, K.P., & Pranay, J. (2010). Comparative studies on the antimicrobial activity of *Piper nigrum* and *Curcuma* longa extracts. *International Journal of Applied Biotechnology* 1:492-501.

Ramanathan, T., Gurudeeban, S., and Satyavani. (2011). Local anesthetic effect of *Citrullus Colocynthis* on rana hexadactyla. *Research Journal of Medicinal Plants* 5 (3): 338-342

Shobana, S., Vidhya, V.G., & Ramaya, M. (2009). Antibacterial activity of *Allium ophioscordon* and *Allium sativum* on enteric pathogens. *Journal of Biological Sciences* 1: 123-126.

Tagoe, D.N.A., Nyarko, H.D. and Akpaka. (2011). A comparison of the antifungal properties of onion (*Allium cepa*), Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) against *Aspergillus flavus, Aspergillus niger* and *Cladosporium herbarum. Research Journal of Medicinal Plants* 5: 281 -287.