Journal of Biotechnological Research

Vol. 1, No. 1, 1-10, 2016

Antibacterial and Phytochemical Properties of Crude Extract of Zingiber Officinale

Idongesit Ambrose[1] --- Joseph Peter Essien[2] --- Ubong Ekong[3] --- Wesley Braide[4] --- Gloria Chidiebere[5] --- Dennis Edem[6]

[1]*Ministry of Environment and Mineral Resources, Uyo, Akwa Ibom State*
[2]*Department of Microbiology, University of Uyo, Uyo, Akwa Ibom State – Nigeria*
[3]*Department of Pharmaceutical Microbiology, University of Uyo, Uyo.*
[4,5]*5Department of Microbiology, Federal University of Technology, Owerri, Imo State – Nigeria*
[6]*Dennis Edem is Department of soil science, university of Uyo, Nigeria*

(*Corresponding Author)*

ABSTRACT

This research was carried out on the antibacterial and phyto-chemical properties of aqueous and methanol extracts of the rhizome of Zingiber officinale. The methanol and aqueous extracts of the spice demonstrated high antibacterial potency against Staphylococcus aureous, Bacillus subtilis and Escherichia coli. The effect of the extracts on the test organisms varied with the dilution level used, and methanol extract was generally more inhibitive than the aqueous extract. Phyto-chemical analysis of the extracts confirmed the presence of saponins, flavonoid, cardiac glycosides, polyphenols and anthraquinones. These are anti-oxidative compounds which possibly might have contributed to the high antibacterial potency of the spice.

Keywords: Antibacterial effect, Phyto-chemistry, Aqueous extract, Methanol extract, Anti-oxidative compounds.

DOI: 10.20448/805.1.1.1.10

Citation | Idongesit Ambrose; Joseph Peter Essien; Ubong Ekong; Wesley Braide; Gloria Chidiebere; Dennis Edem (2016). Antibacterial and Phytochemical Properties of Crude Extract of Zingiber Officinale. Journal of Biotechnological Research, 1(1): 1-10.

Funding : This study received no specific financial support.

Competing Interests: The authors declare that they have no competing interests.

History : **Received:** 25 May 2016/ **Revised:** 8 June 2016/ **Accepted:** 14 June 2016/ **Published:** 20 June 2016

Publisher: Online Science Publishing

1. INTRODUCTION

Various plants and plant parts have been in use for a very long time as medicinal preparations. These plant parts are highly accepted due their effectiveness in treating various ailments and are also considered as safe [7]. Many effects have geared towards discovering new antimicrobial compounds from various kinds of sources such as plants, animals and micro-organisms. A large number of plant products have long been utilized as a source of therapeutic agents globally [13, 16]. Among the plants used as herbal medicine is ginger (*Zingiber officinale*). Ginger is a perennial herb which grows from underground rhizomes, and is often mistakenly called the “roots”. It is a perennial herb, with leafy stem up to 60 cm. The rhizome is horizontal, branched, fleshy, aromatic, white or yellowish to brown. Leaves are narrowly or linear-lanceolate, up to 20 cm

long and 1.5-2 cm wide.Flowers are produced in a dense spike, yellow green with purple endings. This plant is widely distributed in South-Eastern Asia [19]. The rhizome of *Zingiber officinale* comprises water (82%), protein (2.5g), fat (0.8g), carbohydrate (11g), fibre (2.1g), calcium (20mg), iron (2.5mg), vitamin A potency (negligible), thamine (0.02mg), riboflavin (0.04mg), nicotinamide (0.8mg) and ascorbic acid (4mg) [17, 3]. In addition to its proximate constituents, ginger also contains about 2% essential oil which is principally made up of zingiberene; the rhizome is rich in the secondary metabolites such as phenolic compounds (gingerol, paradol and shogaol), volatile sesquiterpenes (zingiberene and bisabolene) and onoterpenoids (curcumene and citral) [3]. In the fresh ginger rhizome, the gingerols were identified as the major active components and gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one is the most abundant constituent in the gingerol series. The powdered rhizome contains 3-6% fatty oil, 9% protein, 60-70% carbohydrates, 3-8% crude fiber, about 8% ash, 9-12% water and 2-3% volatile oil [3]. In dried ginger powder, shogaol a dehydrated product of gingerol, is a predominant pungent constituent up to biosynthesis3-5. Oleoresin, which is isolated by acetone and ethanol extraction, contains 4-7.5% of dried powder, pungent substances namely gingerol, shogaol, zingerone and paradol [14]. The pungency of ginger could be destroyed by prolonged contact with 5% sodium hydroxide (NaOH) [12]. Active constituent of ginger is shogaol, a component of the oil and represents a compound formed by loss of water from gingerols, since it does not appear to be present in the fresh rhizome and is possibly an artifact of extraction [12]. Ginger is known to have medicinal values and are used for the treatment of certain bronchial ailment because of the present of high content of essential oils, which act as expectorants loosening sticky mucous and having antiseptic actions [15]. Ginger has also been found to have antimicrobial properties as it contains extremely high levels of phytochemicals [10, 7]. In addition to its beneficial effects on the heart and its anti-cancer activities, ginger has been reported for its anti-inflammatory effects and has high contents of anti-oxidant properties [4]. Several researches have been conducted on the antimicrobial potentials of Nigerian medicinal plants [1, 7]. The complexity and variation in plant bioactive constituents necessitates frequent studies on individual properties of plants of common usage to ascertain their efficacy and safety. This study was designed to reveal the phytochemical and antimicrobial properties of *Zingiber. officinale* (ginger).

2. MATERIALS AND METHODS

2.1. Collection of Sample

Fresh and mature rhizome of *Zingiber officinale* was obtained from farm located at Obio Imoh Street, Uyo L. G. Area of Akwa Ibom State, Nigeria. The sample was identified by the taxonomist in the Botany and Ecological Studies, University of Uyo, Uyo.

2.2. Preparation of Sample for Extraction

Fresh samples were rinsed with clean water and drained. The samples were dried under sunshine and subsequently milled into powder with a heavy duty blender, then sieved through a 2mm mesh and then stored in an air tight container for subsequent analysis.

2.3. Methanol Extract

This was carried out by the Soxhlet's extraction technique using methanol as the extraction solvent. 20g of dried sample ginger was put into a small calico bag and tied at the mouth. This was done to avoid it being easily opened and to prevent particles of the sample from floating during extraction. The folded sample was carefully transferred into an extraction thimble whose mouth was plugged with cotton wool. The thimble with the sample was dropped into 100ml Soxhlet's extractor. The extractor apparatus was then connected to the receiver flask ¾ filled with 200ml of the extraction solvent (methanol). The assembly was connected to David's double surface condenser and placed on an electrothermal heater and heated at 55^{o}C to extract. After extraction process, the thimble was removed, the apparatus reassembled and distillation carried out to recover the solvent. The residue left contained only water and the extract. The water removed by first of all, concentrating the extract on a hot plate at low temperature to evaporate to dryness at 70^{o}C in water bath. The dry extract was reconstituted in a known volume of distilled water and stored for use in the refrigerator.

2.4. Aqueous Extract

30g of the ginger powder was weighed and poured into a 200ml of distilled water. This was heated using hot plate for one hour. The mixture was stirred at interval. It was the filtered and extract (filtrate) obtained was stored in a refrigerator for further analysis

2.5. Phytochemical Analysis

Freshly prepared extract of ginger was chemically tested for the presence of the active components using standard procedure by Evans, [12]. Analyses were carried out to determine the presence of alkaloids, tannins, saponins, cardiac glycosides, anthraquinones, flavonoids, polyphenols, phlobatannins hydroxy-methyl antaraquinones.

2.6. Test for Tannins

2g of the powdered sample was boiled with 25ml of distilled water for the following tests.

- $FeCl_3$ Test: A few drops of $FeCl_3$ were added to the 3ml of the filtrate. A greenish black precipitate indicates the presence of tannins.
- Acid Test: A few drops of diluted HCl acid were added to 20ml of the filtrate and then heated. A reddish brown colour shows the presence of tannins.

2.7. Test for Saponins

- Fehling's test: 3ml of the extract was mixed with 5ml of Fehling's solution and heated. A reddish brown precipitate indicates saponins.
- Frothing Test: To 1ml of the aqueous extract in a test tube, 5ml of distilled water added and thoroughly shaken. The presence of a steady froth indicates the presence of saponins.

2.8. Test for Flavonoids

The alcoholic extract of ginger was added to a few pieces of magnesium metal, then conc. HCl acid was added. The formation of orange, red, crimson and magenta was taken as an evidence for the presence of flavonoids.

2.9. Test for Phlobatannins

0.5g of the sample was mixed with 5.0ml of water and boiled with 1% aqueous HCl acid for 2 minutes. A red precipitate shows the presence of phlobatannins.

2.10. Test for Cardiac Glycosides

0.5g of the extract was dissolved in 2ml of chloroform, and conc. H_2SO_4 acid

was carefully added to form a lower layer. A reddish brown colour at the interface indicated the presence of a glycore portion of the cardiac glycosides.

2.11. Test for Polyphenols

2g of a well ground sample was heated with 10ml of distilled water for 30 minutes. Then a mixture of 1% $FeCl_3$ and 1% potassium iodide was added to the solution. This was the filtered, the formation of a green-blue colour indicates the presence of reducing compound.

2.12. Test for Hydroxyl-methyl Anthraquinones

2ml of aqueous extract was treated with a solution of 5% ammonia. The formation of red colour or precipitate indicates the presence of hydroylmethyl anthraquinones.

2.13. Test for Anthraquinones

5g of the extract was shaken with 10ml of benzene, then filtered and 5ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the lower phase indicates the presence of the free anthraquinones.

2.14. Test for Alkaloids

2.0g of the sample was boil in 25ml of 5% H_2SO_4 in 50% ethanol for 5 minutes in a water bath, then allowed to cool before filtering. The filtrate was used for the following tests.

- Dragendorff's test: A few drops of Dragendorff's reagent were added to 3ml of the filtrate and allowed to stand for sometimes. The presence of alkaloids was detected by an orange precipitate.
- Wagner's test: To a small portion of the filtrate, 2 drops of Wagner's reagent were added to the filtrate. A reddish precipitate indicates the presence of alkaloids.

2.15. Test Organisms

The test organisms namely *Staphylococcus aureus* was obtained from air, *Escherichia coli* from sewage sample and *Bacillus subtilis* from soil sample. The identities of these organisms were confirmed by Gram staining, cultural characteristics and biochemical tests as described by Aneja, [5] and Bashir et al., [8].

2.16. Antibacterial Assay of Ginger Extract

Antibacterial activity of ginger was done using well diffusion technique (2004). Mueller-Hinton agar plates were prepared and inoculated with a standardized test organism using sterile swab sticks [8]. The plates were allowed to stand for 30 minutes after which wells were made using 6mm diameter cork borer. With help of sterile pipettes, 100µl each of the extract concentrations were introduced into each of the wells. The plates were allowed to stand for a pre-diffusion time of 15 minutes after which they were incubated at 37^{o}C±1^{o}C for 24 hours. After incubation, zones of inhibition were measured. Antibacterial activity was expressed as mean diameter of the zone of inhibition.

3. RESULTS

The results of the antibacterial assay of *Zingiber officinale* revealed a high killing effect against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. This however, varied with different dilution levels. The methanol extract was observed to be generally better than aqueous extract. The aqueous extract of the rhizome was more effective against the test organisms at higher dilution levels (10^{-6}), while a good antimicrobial potency, the methanol extract was effective at dilution level of 10^{-4}. *Escherichia coli* was sensitive to *Zingiber officinale* at a very concentrated dilution level. *Staphylococcus aureus* was sensitive to both the methanol and the aqueous extracts of *Zingiber officinale* while *Bacillus subtilis* was resistant to the ginger extract. Phytochemical analysis confirmed the presence of flavonoids, saponins, cardiac glycosides, polyphenols and anthraquinones.

Table-1. Phytochemistry of Rhizome of *Zingiber officinale*

Constituent	Test Method	Observation	Aqueous Extract	Methanol Extract
			Ginger	
Alkaloids	(i) Wagner's	No ppt.	-	-
	(ii) Mayer's	Light greem	-	-
	(iii) Dragendorff's	Orange but no precipitate (ppt)	-	-
Saponin	(i) Frothing	Persistent foam	+	+
	(ii) Fehling's	Reddish brown ppt	+	+
Flavonoids	(i) Conc. HCl acid	Crimson colour formed	+	+
Phlobatannins	1% aq. HCl	No coloration	-	-
Cardiac Glycosides	(i) Conc. H_2SO_4	Greenish solution	-	-
	(ii) Distilled water	Brick-red ppt.		
	(iii)		+	+
Hydroxymethyl Anthraquinones	5% Ammonia	Yellow	-	-
Anthraquinones	10% Ammonia	Pink colour	+	+
Polyphenols	Mixture of 1% $FeCl_3$ and KI	Green-blue	+	+

Table-2. Inhibition of the Bacteria by Various Dilutions of *Z. officinale* Extract

Test Organism	Solvent	Inhibition Zone in mm^2					Control	
		Extract Dilution					Solvent	Distilled Water
		10^{o}	10^{-2}	10^{-4}	10^{-6}	10^{-8}		
Staph. aureus	Methanol	3.4	4.6	6.2	4.8	3.2	0.2	0
	Aqueous	2.4	3.8	5.6	7.2	2.5		
Bacillus subtilis	Methanol	2.8	3.6	4.8	4.8	4.0	0	0
	Aqueous	1.8	3.6	4.3	4.4	4.4	0	0
E. coli	Methanol	3.3	4.4	6.1	4.7	3.2	0	0
	Aqueous	2.5	3.9	5.7	7.4	5.2	0	0

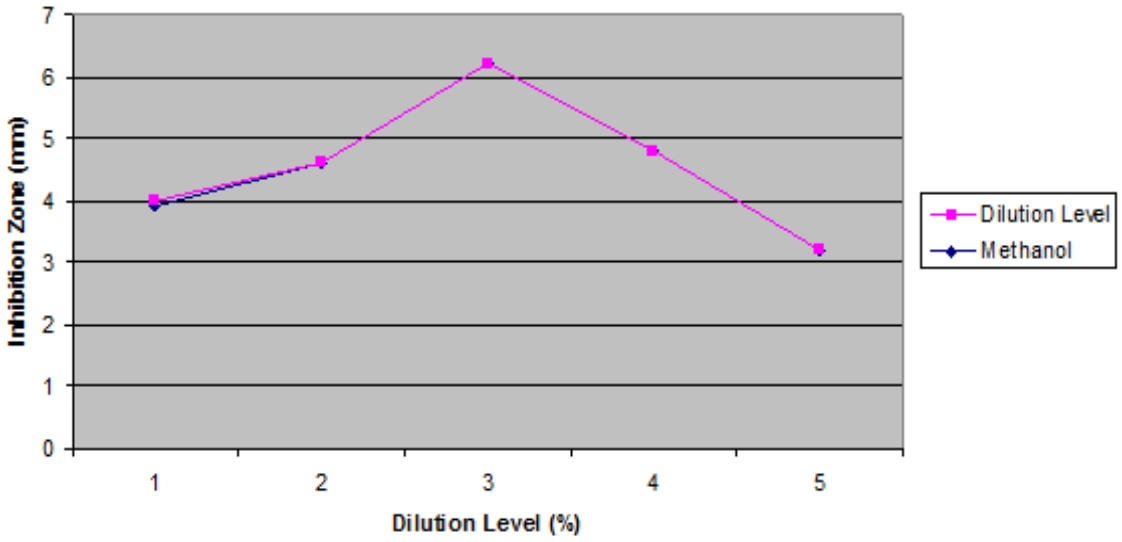

Fig-1. Antibacterial Effect of Methanol Extract of *Z. officinale* against *Staphylococcus aureus*

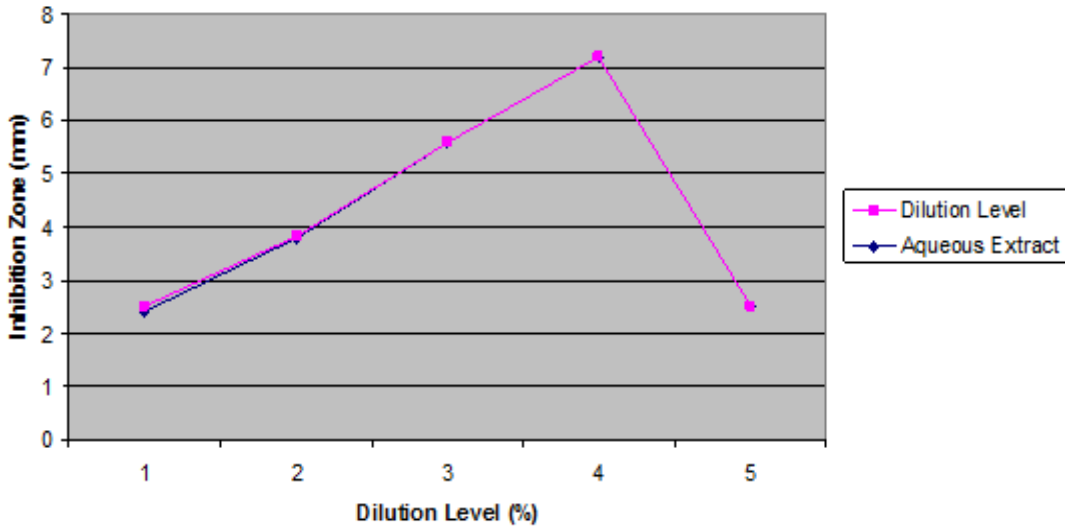

Fig-2. Antibacterial Effect of Aqueous Extract of *Z. officinale* against *Staphylococcus aureus*

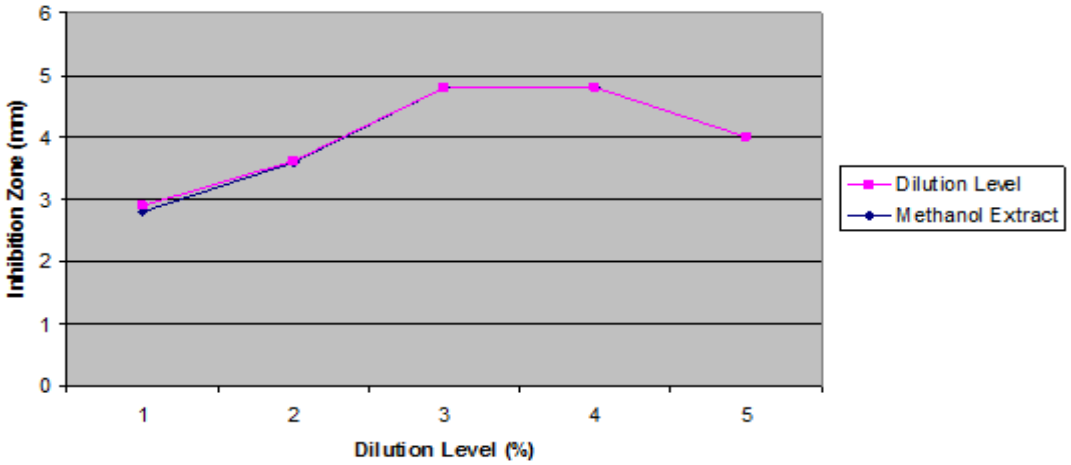

Fig-3. Antibacterial Effect of Methanol Extract of *Z. officinale* against *Bacillus subtilis*

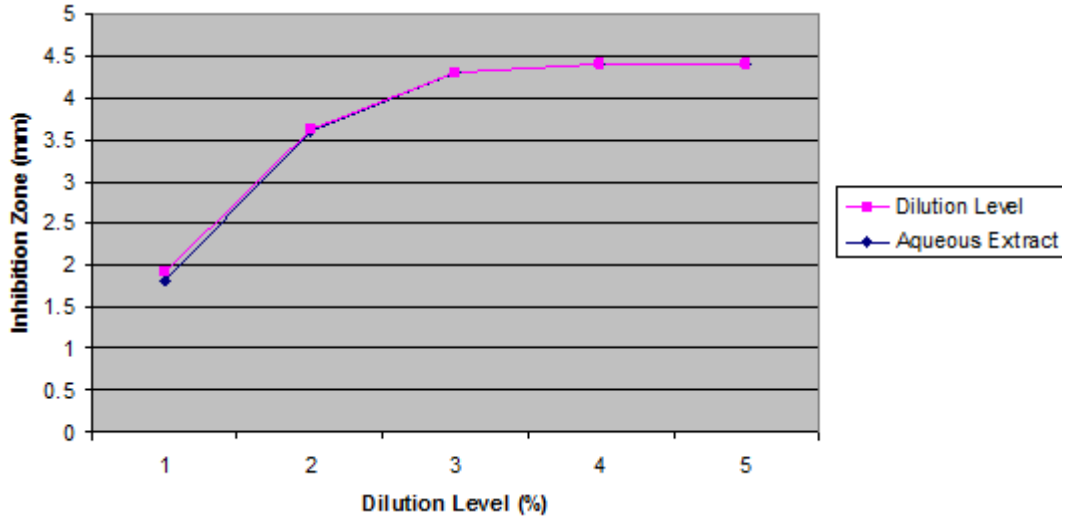

Fig-4. Antibacterial Effect of Aqueous Extract of *Z. officinale* against *Bacillus subtilis*

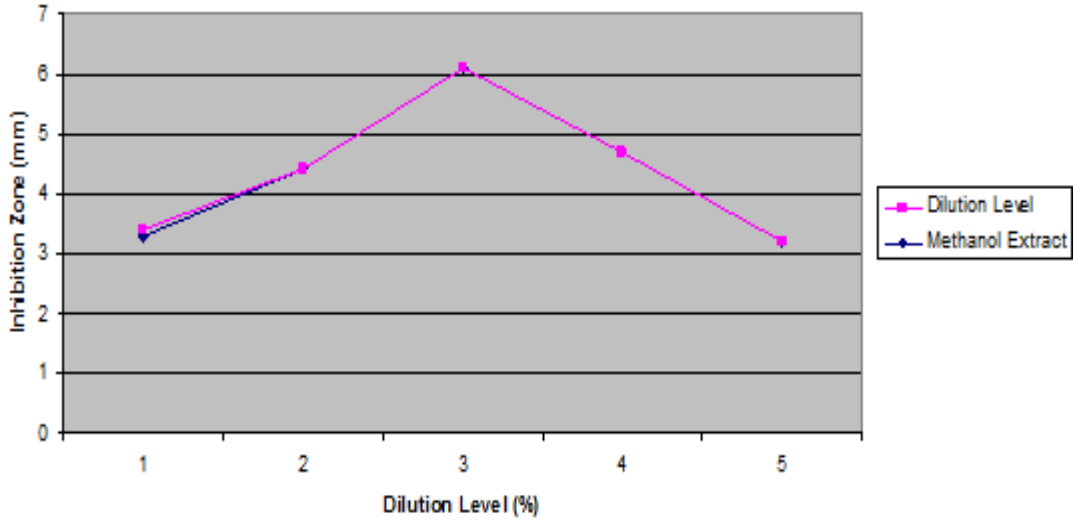

Fig-5. Antibacterial Effect of Methanol Extract of *Z. officinale* against *Escherichia coli*

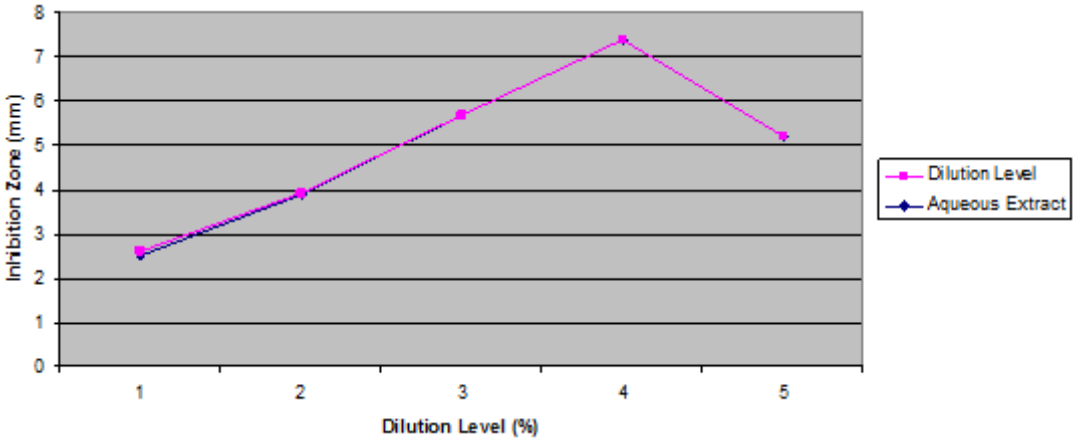

Fig-6. Antibacterial Effect of Aqueous Extract of *Z. officinale* against *Escherichia coli*

4. DISCUSSION

The present study has provided evidence for the antibacterial activity of aqueous and methanol extracts of *Zingiber officinale*. At 37^{o}C, methanol extract of *Zingiber officinale* showed a higher level activity against the test organisms than the aqueous extract. Both the methanol and aqueous extracts lost their potency at high dilution levels. Although both extracts followed a similar trend, the aqueous extract was more effective at 10^{-6} diluent, while the methanol extract gave better results at 10^{-4} dilution level.

The bacterial isolates tested showed indication of increased susceptibility to higher dilution levels for aqueous extract (table 2). It might be possible that dilutions permitted faster diffusion of the active agent(s) present in the extract within the medium and caused more damage. It is also possible that higher dilutions allow easy transport of the active antimicrobial molecules into the microbial cells where the agents might have exerted control on the productive mechanism of the organism. The low dilutions might have been transported at relatively slower rate or the active molecules of the extract might have aggregated instead of being dispersed resulting in lesser transport into cell and subsequent damage to the microbial organisms. The antimicrobial potency of the methanol extract was observed to be higher than the aqueous extract. This may be attributed partly to the high solvent property of methanol and partly due to its inherent antibacterial potential. On the other hand, variation in the extracts effect in the test organisms might be due to the difference in the percentage concentration of the active principles and their stability at the storage and incubation. The individual genetic properties of the test organisms also have a major role to play in the organism's resistance to the extract effects. Figures 1 to 6 show the individual species response to both the methanol and aqueous extracts of *Zingiber officinale*. Slight variations in species responses was noticed between the plant material and between the aqueous and methanol solvent. *Staphylococcus aureus* was more susceptible to methanol and aqueous extracts of *Zingiber officinale* while high susceptibility was observed in methanol extract of *Capsicum acuminatum* by Bashir et al., [8]. *Bacillus subtilis* showed less susceptibility to both methanol and aqueous extracts of ginger. Higher susceptibility of *Escherichia coli* had been recorded in aqueous extract of *Zingiber officinale*. Phytochemical analysis showed that extract from *Zingiber officinale* was effective against the test organisms. The presence of saponins, flavonoids, cardiac glycosides, polyphenols and anthraquinones were detected in *Zingiber officinale*. The presence of antinutrient compounds in plants

have earlier been reported in some leguminous plant [2, 21, 12]. Bertram and Magobb, [9]; Stoilova et al., [20] reported that the basic lipid components of leaves are associated with naturally occurring antioxidants. These include alpha tocopherol lecithin, quercetin and some polyphenolic compound such as flavonoids, flavones cinnamic acid and hydroxybenzoic acid. However, the absence of anthraquinones, glycosides and phenols was unexpected as these compounds have been encountered in some leguminous plants [1]. For example Rhein, an anthraquinones belonging to a group of bioactive compound known as anthracene has been identified specifically in *Zingiber officinale* [23, 22, 6]. Preuss et al., [18] reported that the concentration of bioactive components in plant varies among different plants species with age and with the environmental conditions. The presence of these antioxidative compounds in ginger extract has provided evidence for the antimicrobial of the aqueous and methanol extracts [6]. The antimicrobial activity of the extracts against the test organisms further confirmed the therapeutic efficacy of *Zingiber officinale* [11].

5. CONCLUSION

In our present state of knowledge of the phyto-chemistry of *Zingiber officinale* has been satisfactorily used by alternative medicine practitioners (popularly known in Nigeria as herbalists or native doctors) for the treatment of various bacterial related infections. Their efficacy may be attributed to the presence of certain pharmacologically active compounds known to possess anti-oxidative properties. This is important to enable us cope with the proliferation of resultant strains of these organisms. It is not possible to determine with certainty the most effective concentrations of the extracts. Further investigations are however very necessary to ascertain the appropriate concentration and microbial specificity for higher efficacy and safety.

Funding: This study received no specific financial support.

Competing Interests: The authors declare that they have no competing interests.

Contributors/Acknowledgement: All authors contributed equally to the conception and design of the study.

REFERENCES

[1] Aboaba, O. O., Ezeh, A. R. and Anabuike, C. L. (2011). Antimicrobial activities of some Nigerian spices on some pathogens. Agric. Biol. J. N. Amer, 2(8): 1187-1193.

[2] Aboaba, O. O. and Efuwape, B. M. (2001). Antibacterial properties of some Nigerian species. Bio. Res. Comm., 13: 183 – 188.

[3] Ali, B. H., Blunden, G., Tanira, M. .O.. and Nemmar, A.(2008). Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. Food Chem Toxicol., 46(2): 409-20.

[4] Aliyu, B. S. (2006). Common ethno-medicinal plants of the semi-arid regions of West Africa: their descriptions and phytochemicals. Triumph publishing company Limited, Gidan Sa'adu Zungur, Kano, Nigeria.

[5] Aneja, K. R. (2012). Experiment in Microbiology (4th ed.). New Delhi, New Age International Limited. pp: 57-71.

[6] Aziz, D. M., Wsoo, M. A. and Ibrahim, B. M. (2015). Antibacterial and antioxidant activities of extracts from medicinal plant ginger (Zingiber officinale) and identification of components by gas chromatography. Afri. J. Plan. Sci., 9(10): 412 -420.

[7] Bashir, S. F., Kawo, A. H., Bala, J. A. and Dubai, Y. U. (2013). In vitro antibacterial activities and preliminary phytochemical screening of the aqueous and ethanolic extracts of Zingiber officinale. Ife J. Sci., 15(1): 93 – 99.

[8] Bashir, S. F., Gurumayum, S. and Kaur, S. (2015). In vitro antimicrobial activity and preliminary phytochemical screening of methanol, Chloroform and hot water extracts of Ginger (Zingiber officinale). As. J. Pharm. Clin. Res., 8(1): 176 – 180.

[9] Bertram, J. F. and Magobb, J. E. O. (1980). Naturally occurring antioxidants in leaf lipids. J. Sci. F. and Agric., 31: 646 – 650.

[10] Cakit, A., Kordali, S., Zengin, H., Izumi, S. and Hirata, T. (2004). Composition and antifungal activity of essential oil isolated from Hypericum bussopifolium and Hypericum heterrophyllum. Flav. Frag., J. 19: 62-68.

[11] Ernest, E. and Pittler, M. H. (2000). Efficacy of ginger for nausea and vomiting. A systematic review of randomized clinical trials. Br. J. Annesth., 84: 367.

[12] Evans, W. C. (2009). Trease and Evans' Pharmacognosy (16th ed.). Published by W. B. Saunders Ltd. London. pp: 320 – 333.

[13] Gibbons, S. (2005). Plant as a source of bacterial resistance modulators and anti- infective agents. Phytochem. Rev., 4: 63 – 78.

[14] Hoffman, T. (2007). Antimicrobial activity of some medicinal plants from India. Hawaii Med. J., 66: 326-327.

[15] Kawo, A. H., Suleiman, Z. A. and Yusha'u, M. (2011). Studies on the antibacterial activities and chemical constituents of Khaya senegalensis and Ximenia Americana leaf extracts. Afri. J. Microbiol. Res., 5(26): 4562-4568.

[16] Khan, R., Islam, B., Akram, M., Shakil, S., Ahmed, A., Ali, S. M., Siddidui, M. and Khan, A. U. (2009). Antimicrobial activity of five herbal extracts against multi-drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecule, 14: 586 – 597.

[17] Tindull, W. H. (1984). Vegetable growing in the tropics (6th ed.). NOS Vastrand, U. S. A. pp: 87 – 106.

[18] Preuss, H. G., Echard, B., Enig, M., Brook, I. and Elliot, T. B. (2005). Minimum inhibitory concentrations of herbal essential oils and monolaurin for gram- positive and gram-negative bacteria. Mol. Cell Biochem, 272: 29 – 34.

[19] ROSS, I.A. (2005): Medicinal Plants of the World. Humana Press, Totowa, New Jersey. pp: 507-560.

[20] Stoilova, I., Krastanov, A. Stoyanova, A. Denev, P. and Gargova, S. (2007). Antioxidant activity of a ginger extract (Zingiber officinale). Food Chem., 102: 764 – 770.

[21] Suffredini, I. B., Sarder, H. S. and Goncalves, A. G. (2004). Screening of antibacterial extracts from plants native to Brazilian Amazon rainforest and Atlantic forest. Braz. J. Med. And Bio. Res., 37: 379 – 384.

[22] Taura, D. W., Lawan, S., Gumel, S. M., Umar, S. and Sadisu, U. F. (2014). Antibacterial activity of ethanolic extract of Zingiber officinale and Pipper nigrum against some clinical isolates. Commun. Appl. Sci., 2: 52-64.

[23] Yahya, M. F. Z. R., Saifuddin, N. F. H. A. and Hamid, U. M. A. (2013). Zingiber officinale ethanolic extract inhibits formation of Pseudomonas aeruginosa biofilm. Int. J. Pharm. Bio. Sci., 3: 46 – 54.