PhytochemicalProfile and AntibacterialEffectsof*Zingiberofficinale*Root Extract onSomeEnteric BacterialPathogens

ABSTRACT

The majority of Africans today depend either totally or partially on medicinal plants for the treatment of various diseases. In some rural communities, ethno-medicineissometimestheonlyform of healthcare. Therefore, this work aimed to determine thephytochemicalconstituentsandantibacterialpotentialsof *Z.officinale*, which is one of the medicinal plants used by some people. The active ingredients of the plant were first extracted using water and ethanol as solvents. This was followed by the phytochemical analysis of the extracts. Furthermore, the antibacterial effects of aqueous andethanolic extracts of *Z. officinale* on*Escherichia coli, Salmonella typhi* and *Shigella dysenteriae*isolated from faeces of gastroenteritis patients were evaluated using theagar diffusiontechnique(punch method). Additionally, a two-fold tube dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts.The mean values of zones of inhibition obtained were statistically analyzed using ANOVA. Theleast significant difference was determined according to the LSD test at $P<0.05$. Phytochemicalanalysis revealed the presence of saponin, alkaloids, flavonoids, and tannins. The antibacterial results showed thatboth the aqueous andethanolic extracts have antibacterial effects against all the test organisms but at varying degrees. At the 500mg/ml concentration, the ethanolic extract of *Zingiber officinale* produceda zone of inhibitionof21.00^{b}against*S.dysenteriae*and20.00^{b}against*E.coli* and*S.typhi*.On the other hand, the 500mg/ml concentration of the aqueous extract had a zone of inhibition of 20.00^{b} against *S.dysenteriae*,16.00^{b}against*E.coli*and10.00^{b}against*S.typhi*.The findings from this study lend credence to the claims that*Z. officinale* extracts possess antibacterial potentials. Also, the higher potency in ethanolic than in aqueous extract suggests that the potency might be doseand solvent dependent. Conclusively, owing to the findings from this study, the active ingredients of *Z. officinale* could be harnessed and employed in the development of novel antibacterial therapies.

Keywords:Ginger, *Escherichia coli, Salmonella typhi, Shigella dysenteriae*, Aqueous, Ethanol, Antibacterial.

1. INTRODUCTION

In some parts of the world, traditional medicine is still being practised[1]. This often involves the use of a medicinal herb or plant in the treatment of diseases. According to Abayomi et al. [2], a consultative committee of the World Health Organisation (WHO), a medicinal plant is any plant that contains chemicals that have therapeutic value or that serve as precursors to produce effective pharmaceuticals.One of the therapeutic herbs that has been widely used in folk medicine is*Zingiber officinale* (popularly known as ginger), which belongs to the Zingiberaceae family. There are more than 45 genera and 800 species in this family. *Z. officinale* is a perennial plant that grows upright to a height of one to three feet and is a widely used homeremedy. The roots of this plant (fresh and dried)have been used in ancient China, India, and other countries to treat various diseases likecold-induced illnesses, nausea, asthma, cough, colic, heart palpitations, swelling, dyspepsia, loss of appetite, and rheumatism [3].

Ginger contains a variety of bioactive substances[4]. Recent research has revealed that the biological properties of ginger, including anti-inflammatory, antibacterial, antioxidant, and anti-cancer properties, could be attributed to the bioactive compounds present in it. Furthermore, an increasing body of research has shown that ginger may help prevent and treat severaldiseases, including neurological conditions [5], cardiovascular conditions, obesity, diabetes mellitus, nausea and vomiting and respiratory issues [6].

With the increasing resistance of microorganisms to conventional antibiotics, there is an urgent need to produce novel, more potent, andbroad-spectrum antibiotics that are readily available, affordable, and have low levels of toxicity and resistance. Consequent upon the afore-mentioned properties of this plantand its use in traditional medicine, this research aimed at determining the phytochemicals composition andassessing the antibacterial properties of aqueous and ethanolic extracts of *Zingiber officinale* againstentericbacterialpathogens.

2. MATERIALSANDMETHODS

2.1 CollectionofPlantSamples.

Zingiber officinale was purchased from cemetery markets in Aba South Local Government AreaofAbiaState,Nigeria.It was identified in the Department of Plant Science and Biotechnology, Imo State University, Owerri. Three hundred grams (300gm) of *Zingiber officinale* was washed, sliced, and sun-dried for seven days. It was later crushed using an electric blender. It was thereafter packed into acleanpolythenebagandlabelledaccordingly.

2.2 Isolation of TestOrganisms

The test organisms used in this research were *Escherichia coli, Salmonella typhi* and *Shigelladysenteriae.*Theseorganismswereisolatedfromstoolsamplesofgastroenteritis patientsattending Abia State University Teaching Hospital, Aba. Ethical clearance wasobtained from the hospital's ethical committee. The consent of the patients was sought after a briefdiscussion with the physician. The importance of the research was explained to them. Those who agreed to participate were made to fill out the consent form. The isolation and identification of thebacteriawerecarriedout.Culturalandmorphologicalidentification, besides biochemical characterization of isolates, were carried out using the methods described by Cheesbrough. *S.typhi*was serologically differentiatedfrom other*Salmonella*species bythepresenceof vi antigen[7]. Pure cultures of the bacteria were maintained in aNutrient agar slant andkeptintherefrigeratorforfuture use.

2.3 Extraction

Ninety-eight per cent (98%) of ethanol and distilled water were used for the extraction. The ground leaf was weighed (150 grams each) and dissolved in 500 ml of the solvent.Thesewerestopperedand kept for ten days with intermittent shaking [8]. Afterwards, the mixtureswere filtered with Whatman's number one filter paper.The ethanol extract was concentrated at40°C under reduced pressure using a Rotary evaporator (R100).A hot air oven was then used to concentrate the aqueous extract overnightat40^{o}C[9]. Theconcentratedextractwas collected in screw-capped bottles, labelledand storedat4^{o}Cintherefrigerator.

2.4 PhytochemicalScreening

The method described by Lajubutuet al.[10] was used for this. The extract was tested for alkaloids, Tannins, Saponins, and flavonoids.

2.5 AntibacterialAssay

The in-vitro antimicrobial assay was carried out using theagar-geldiffusion(punchmethod)technique,as described by Osadebe and Ukwueze[11]. In this method, the broth culture of the test isolates(O.1ml) was aseptically inoculated by spreading evenly onto the dried surface of Muller-Hintonagar plates using a bentsterile glass rod. Sixwells (5.0mm diameter) were then madein theplates using a sterile cork borer. The fifth well served as the negative control, while the sixthwell served as the positive control. Sterile distilled water served as the negative control, whileciprofloxacin was used as the positive control. Double dilution of the extracts wasmade togetthe various concentrations as follows: (500mg/ml, 250mg/ml, 125mg/ml and 63mg/ml) that wereused for the antimicrobial assay.The bottom of wells 1-4 were sealed with one drop ofsterile molten Muller-Hinton agar to prevent diffusion of the extracts under the agar. Fixedvolumes (O.lml)of thefourdifferentconcentrations of the extracts were transferredinto wells 1-4 using a sterile Pasteurpipette. The control wellswerefilled with O.lml of distilledwaterandl0µgofciprofloxacin,respectively.

The plates were left on the bench for 40 minutes for pre-diffusion of the extracts to take place[12]. They were then incubated at 37°C for 24hours. Antimicrobial activities of the extracts weredetermined by measuring the resulting zone diameters of inhibition (mm)againsteach testorganism using a ruler. The experiment was carried out in triplicate and the mean values of theresults weretakenasantimicrobialactivity[13, 14].

2.6. DeterminationofMinimumInhibitoryConcentration(MIC)andMinimumBactericidal Concentration(MBC)

Inoculum was prepared by making a direct broth of colonies selected from 24-hour agar plates.The suspensionwasadjustedtomatch0.5mlMac-Farlandstandard usingsterile saline.The MIC and MBC of the potent extracts were determined according to the macro broth dilutiontechnique[15].Doubledilution wasalsodoneheretogetthefourdifferent concentrations of the extracts. Standardized suspensions of the test organisms wereinoculated into a series of sterile tubes of peptone water containing dilutions (500, 250, 125, and63mg/ml) of the extracts. They were incubated at 37°C for 24 hours. The MIC was read as the lowest concentration, which inhibited visible growth (absence of turbidity) inthe test organisms.

For MBC determination, a loopful of the broth from each of the tubes that did not show

anyvisiblegrowth(noturbidity)duringMICdeterminationwassubculturedontoextract-free Muller-Hinton agar plates and further incubated for 24 hours at 37°C. The lowest concentration at which no visible growth was observed was noted as the minimum bacteriocidal concentration (MBC), whereas the lowest concentration at which visible growth occurredwasregardedastheMinimumBacteriostaticConcentration(MBS).

3. RESULTS

3.1. Phytochemical components of *Zingiberofficinale.*

Table 1 shows the phytochemicals or bioactive compounds present in the extracts of Ginger (*Zingiberofficinale*). The phytochemical analysis showed the presence of alkaloids, saponins, flavonoids, and tannins.

Table1:Phytochemicalcomponentsof*Zingiberofficinale.*

Extract	Alkaloids	Saponin	Flavonoids	Tannins
Zingiber officinale	+	+	+	+

Key: Present = (+)
Absent = (+)

3.2. Antibacterial Effects of *Zingiber officinale* root extracts on Some enteric Pathogens

Table 2a below shows the mean diameter of the zones of inhibition produced by the differentconcentrationsof*Zingiber officinale* root ethanolic extract and the control treatments on the testorganisms. There is a concentration-dependent inhibition of the growth of the pathogens. That is to say, the highest zones of inhibition (20.00mm, 20.00mm, and 21.00mm for *E. coli*, *S. typhi*, and *S. dysenteriae*, respectively) were seen in the highest concentration (500mg/mL). However, when compared with the zones of inhibition produced by the positive control (ciprofloxacin), those produced by the latter were higher (24.00mm, 25.00mm, and 27.00mm for *E. coli*, *S. typhi*, and *S. dysenteriae*, respectively). On the other hand, at the lowest concentration (63mg/ml), no zone of inhibition (0.00) was seen. This corresponds with the no zone of inhibition (0.00mm) observed in the negative control (distilled water) for all the pathogens.

Table2a:Mean*diameterofzoneofinhibition(in millimetres) ofdifferentconcentrationsof*Zingiber officinale* root ethanolic extract and the control treatments on the testorganisms.

		Zones of Inhibition [in millimetre (mm)]		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
Z. officinale	500mg/ml	20.00^{b}	20.00^{b}	21.00^{b}
Z. officinale	250mg/ml	16.00^{c}	6.00^{c}	15.00^{c}
Z. officinale	125mg/ml	6.00^{d}	0.00^{d}	0.00^{d}
Z. officinale	63mg/ml	0.00^{e}	0.00^{d}	0.00^{d}
Ethanol	0.1ml	0.00^{e}	0.00^{d}	0.00^{d}
Ciprofloxacin	10µg/ml	24.00^{a}	25.00^{a}	27.00^{a}
LSD		1.485	1.369	1.369

Key: Meansonthesamecolumnwithdifferentlettersuperscriptsaresignificantlydifferent (P <0.05),accordingtothe LSDtest; Ethanol = Negative Control; and Ciprofloxacin = Positive Control.

Table 2b below shows the mean diameter of the zones of inhibition produced by the differentconcentrationsof*Zingiber officinale* root aqueous extract and the control treatments on the testorganisms. There is a concentration-dependent inhibition of the growth of the pathogens. That is to say, the highest zones of inhibition (16.00mm, 10.00mm, and 20.00mm for *E. coli*, *S. typhi*, and *S. dysenteriae*, respectively) were seen in the highest concentration (500mg/mL). However, when compared with the zones of inhibition produced by the positive control (ciprofloxacin), those produced by the latter were higher (22.00mm, 20.00mm, and 26.00mm for *E. coli*, *S. typhi*, and *S. dysenteriae*, respectively). On the other hand, at the lowest concentration (63mg/ml), no zone of inhibition (0.00) was seen. This corresponds with the no zone of inhibition (0.00mm) observed in the negative control (distilled water) for all the pathogens.

Table2b: Mean*diameterofzoneofinhibition (in millimetres) of differentconcentrationsof*Zingiber officinale* root aqueous extractand the control treatments on the testorganisms.

		Zones of Inhibition [in millimetre (mm)]		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
Z. officinale	500mg/ml	16.00^{b}	10.00^{b}	20.00^{b}
Z. officinale	250mg/ml	10.00^{c}	6.00^{c}	12.00^{c}
Z. officinale	125mg/ml	6.00^{d}	0.00^{d}	4.00^{d}
Z. officinale	63mg/ml	0.00^{e}	0.00^{d}	0.00^{e}
Distilledwater	0.1ml	0.00^{e}	0.00^{d}	0.00^{e}
Ciprofloxacin	10µg/ml	22.00^{a}	20.00^{a}	26.00^{a}
LSD		1.627	1.369	0.939

Key: Meansonthesamecolumnwithdifferentlettersuperscriptsaresignificantlydifferent (P <0.05),according to theLSDtest; Distilled water = Negative Control; and Ciprofloxacin= Positive Control.

3.3. Minimum Inhibitory Concentration (MIC)

Table 3a below shows the minimum inhibitory concentration of different concentrations of*Zingiber officinale* root ethanolic extract and the control treatments on the testorganisms. The minimum inhibitory concentration of all the test organisms was (250mg/mL) which is the least concentration that inhibited the growth of all the test organisms.

Table 3a:Theminimum inhibitory concentration of different concentrations of*Zingiber officinale* root ethanolic extract and the control treatments on the testorganisms.

		Bacterial Pathogens		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
Z. officinale	500mg/ml	-	-	-
Z. officinale	250mg/ml	-	-	-
Z. officinale	125mg/ml	+	+	+
Z. officinale	63mg/ml	+	+	+
Ethanol	0.1ml	+	+	+
Ciprofloxacin	10µg/ml	-	-	-

Key: (-) =inhibition(nogrowth); (+) = no inhibition (growth); Ethanol =negativecontrol; Ciprofloxacin= positivecontrol.

Table 3b below shows the minimum inhibitory concentration (MIC) of different concentrations of*Zingiber officinale* root aqueous extract and the control treatments on the testorganisms. The MIC of *E. coli* was 125mg/ml, that of *S. typhi* was 250mg/ml, and that of *S. dysenteriae* was 125mg/ml.

Table 3b: Theminimum inhibitory concentration of different concentrations of*Zingiber officinale* root aqueous extractand the control treatments on the testorganisms.

		Bacterial Pathogens		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
Z. officinale	500mg/ml	-	-	-
Z. officinale	250mg/ml	-	+	-
Z. officinale	125mg/ml	+	+	+
Z. officinale	63mg/ml	+	+	+
Distilledwater	0.1ml	+	+	+
ciprofloxacin	10µg/ml	-	-	-

Key: (-) =inhibition(nogrowth); (+) = no inhibition (growth); Distilledwater=negativecontrol; Ciprofloxacin= positivecontrol.

3.4. Minimum Bactericidal Concentration (MBC)

Table 4a below shows the minimum bactericidal concentration (MBC) of different concentrations of*Zingiber officinale* root ethanolic extract and the control treatments on the testorganisms. The MBC of all the test organisms (*E. coli*,*S. typhi*and *S. dysenteriae*)was 500mg/ml.

Table 4a: The minimum bactericidal concentration of different concentrations of*Zingiber officinale* root ethanolic extract and the control treatments on the testorganisms.

		Bacterial Pathogens		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
Z. officinale	500mg/ml	-	-	-
Z. officinale	250mg/ml	+	+	+
Z. officinale	125mg/ml	+	+	+
Z. officinale	63mg/ml	+	+	+
Ethanol	0.1ml	+	+	+
Ciprofloxacin	10µg/ml	-	-	-

Key: (-) =inhibition(nogrowth); (+) = no inhibition (growth); Ethanol=negativecontrol**;**Ciprofloxacin= positivecontrol.

Table 4b below shows the minimum bactericidal concentration (MBC) of different concentrations of*Zingiber officinale* root aqueous extract and the control treatments on the testorganisms. The MBC of *E. coli* was 500mg/ml, while that of *S. typhi* and *S. dysenteriae* could not be determined.

Table 4b: The minimum bactericidal concentration of different concentrations of*Zingiber officinale* root aqueous extractand the control treatments on the testorganisms.

		Bacterial Pathogens		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
Z. officinale	500mg/ml	-	+	+
Z. officinale	250mg/ml	+	+	+
Z. officinale	125mg/ml	+	+	+
Z. officinale	63mg/ml	+	+	+
Distilledwater	0.1ml	+	+	+
Ciprofloxacin	10µg/ml	-	-	-

Key: (-) =inhibition(nogrowth); (+) = no inhibition (growth); Distilledwater=negativecontrol; Ciprofloxacin= positivecontrol.

4. DISCUSSION

This research was carried out to determine the phytochemicals in *Z. officinale* and evaluate the antibacterial effects of the plant extract on selected enteric pathogens. The phytochemicals that were identified in the ginger (*Z. officinale*) roots used in this study are alkaloids, saponins, flavonoids and tannins. These phytochemicals have long been reported to bepresentin higher plants by Kaufman et al. [16] and were said toconferantibacterialpotentialstoanyplantinwhichtheyare found. Aside from the

antibacterial properties, these compounds are widely believed to be responsible for other medicinal properties the plants in which they are contained possess[16, 17].

Furthermore, the phytochemical result ofthe ethanolic extract of *Zingiber officinale* in this study corroborates the earlier findings byWahab *et al.* [18] and Osaboret al.[19]. Although, tannin, which was found in this study, was not reported by Osabor et al.[19]. This additional component (tannin) found in this study might have contributed significantly to the higher rate of inhibition that was recorded. This variation in phytochemicals and theirconcentrations could be due to the variation in habitats, as opined by Farooq et al.[20]. This might be true as the phytochemical analysis of *Datura stramonium* (Jimson weed) by Dike-Ndudim et al. [21] showed the presence of Tannin, Phenol, flavonoid, alkaloid, phytate, and hydrogen cyanide. However, Walter andNowacki[22] disagreed with that proposition and suggested that the production of phytochemicals could result from plants' responsestothreats. Nevertheless, it could be that the combination of these factors plays a key role in the variation in the composition and concentration of phytochemicals in medicinal plants. Therefore, this area of research requires further studies.

Theresultsobtainedinthisresearchindicatedthatbothaqueous andethanolicextracts of*Zingiber officinale* exhibited antibacterial action against all the test organisms, although withdifferent levels of sensitivities to the extracts. As revealed in this research, the antibacterial properties of *Z. officinale* extract agree with the report by Nassan and Mohamed [23], who reported that *Zingiber officinale* possessesantibacterialproperties. The antibacterial properties observed with both aqueous and ethanolic extracts suggest that water and ethanol could be used as solvents in the extraction process. Reports from previous studies have validated this proposition. The study by Dike-Ndudim et al. [21] reported that the aqueous and ethanolic extracts of Jimsonweed (*Datura stramonium*) possess antibacterial properties. A similar report was obtained from Ndubueze et al.[24] with aqueous and ethanolic leaf extracts of*Gongronemalatifolium.*

Generally,andin thisstudy,the ethanolicextracts of *Zingiber officinale*were more effectivethan the aqueous extract, indicating that ethanol is a better solvent than water. This agrees with the reports of Ogunjobi andNnadozie[25],Ezeifekaet al.[26], andAnyanwu et al.[8]. However, it contradicts the report by Ndubueze et al. [24], in which no statistically significant difference was reported in both aqueous and ethanolic leaf extracts of *Gongronemalatifolium*. Furthermore, since both extracts proved to possess antibacterial properties, future research with medicinal plants should continue exploring these solvents and other possible methods of extraction. Modifications and enhancement of the extraction methods would ensure that the findings from studies of this nature are indisputable.

Conclusion

Thesuccessful inhibition of enteric organisms by *Zingiber officinale*'sextracts offers hope for mitigating the diseases caused by pathogens. Considering the level of multi-resistance these bacteria have developed against conventional antibiotics over the years, harnessing the active ingredients of this medicinal plant might go a long way in treating the infections caused by the organisms.

REFERENCES

1. SofoworaEA.MedicinalPlantsandTraditionalMedicineinAfrica(4th edition).John WileyandSonsLtd.Chichester.1984; Pp.96-106.
2. Abayomi S,Eyitope O, Adedeji O. The Role and Place of Medicinal Plants in theStrategies for

Disease Prevention. African Journal of Traditional Complement AlternativeMedicine.2013;10(5):210–229.

3. FosterS.Ginger,*Zingiberofficinale*–yourfoodisyourmedicine.2011;Pp.30-35.
4. StonerGD.Ginger:Isitreadyforprimetime?.CancerPrevalenceResearch.2013;6:257–262.

5. Ho S, Chang K, Lin C. Anti-neuroinflammatory capacity of fresh ginger is attributedmainlyto10-gingerol.FoodChemistry. 2013; 141:3183–3191.

6. Townsend EA,Siviski ME,ZhangY,Xu C,HoonjanB,EmalaCW.Effectsof gingeranditsconstituentsonairwaysmoothmusclerelaxationandcalciumregulation.American JournalofRespiratory CellMolecules. 2013;48:157–163.

7. Cheesbrough M. District Laboratory Practice in Tropical Countries. Second editionupdatepart2. 2012; P62-70.
8. AnyanwuGO,Dike-Ndudim JN,Ndubueze CW.PhytochemicalandAntibacterial Profileof*Moringaoleifera*lamSeedExtractsonSomeWoundandEntericBacterialPathogens.Journalof ComplementaryandAlternativeMedicalResearch.2022;17(2):26-36.

9. Fatope MO, Ibrahim H, Takeda Y. Screening of higher plants reputed as pesticidesusing the Brime Shrimp Lethality Assay. International Journal of Pharmacognosy.1993;31(4):250-254.
10. Lajubutu BA, Pinny RJ, Robert MF,Odelola HA, Oso BA. Antimicrobial activityofdiosquinoneandplumbaginfrom*D.mespiliformis*(Hostch)(Ebenaceae). PhytotherapyResearch.1995;9:346-350.

11. OsadebePO,UkwuezeSE.ComparativeStudyofthePhytochemicaland antimicrobial properties of the Eastern Nigerian species of African Mistletoe *(Loranthusmicranthus)*sourcedfromdifferenthosttrees.JournalofBiologicalResearchBiotechnology .2004;2(1):18-23.

12. EsimoneCO,AdiukwuMU,OkontoJM.(1998).PreliminaryantimicrobialScreening of ethanolicextractfromthelichen*Usenasubfloridans*.LaboratoryJournalofPharmceuticalResearchand Development. 1998; 3(2):99-01.
13. AbayomiS.TheStateofMedicinalPlantResearchinNigeria.UniversityofIfePress. 1982;Pp.200.

14. JunaidSA,OlabodeAO,OnwuliriFC,OkorosiAEJ,AginaSE.Theantimicrobialpropertiesof*Ocimumg ratissimum*extractonsomeelectedbacterialgastrointestinalisolates.African JournalofBiotechnology.2006;5(22):2315-2321.

15. Boron JE,Fingold SM. Method of testing antimicrobial effectiveness. In Bailey ScottsDiagnostic MicrobiologyMosby.CV (8th edition),Missouri.1990.

16. Kaufman BP, Calson FT, Dayanandan P, Evans LM, Fisher BJ, Parks C, Wells RJ."Plants: their biology and importance". Harper and row publishers, New York. 1989;Pp.681-700.
17. DuttaAC."Botanyfordegreestudents"5th edition.OxfordUniversitypress. 1993;Pp.810–844.

18. WahabGA,IsiakaAA,LabunmiL,MorenikeGA.Phytochemicalsandantioxidant potential of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) extracts. GSCBiologicalandPharmaceuticalSciences,2022;19(01),226-234.
19. OsaborVN,Bassey FI,Umoh UU.(2015).PhytochemicalScreeningandQuantitative Evaluation of Nutritional Values of Zingiber officinale (Ginger). Ameriacn ChemicalScience Journal. 2015;8(4):1-6.

20. Farooq A, Sajid L, Muhammed A, Anwarul HG. *Moringa oleifera:* a food plant withmultiplemedicinaluses.*Phyto*therapy Research.2007;21:17-25.

21. Dike-Ndudim JN,Amadi CN, Ndubueze CW. Antimicrobial and Phytochemical evaluation of *Datura stramonium* (Jimson weed) on selected microorganisms. International Journal of Science and Research Archive.2021;02(02):245-256. https://doi.org/10.30574/ijsra.2021.2.2.0084

22. WalterGR,NowackiEK. Roleofalkaloids.Alkaloidsbiologyand metabolisminplants.Plenus Press,NewYork. 1978;Pp.181-190.
23. NassanMA,MohamedEH.Immunopathologicalandantimicrobialeffectofblack pepper, ginger and thyme extracts on experimental model of acute hematogenous pyelonephritis in albino rats. International Journal of Immunopathology. 2014;27:531-541.
24. Ndubueze CW, Dike-Ndudim JN,Udujih HI. Antibacterial Effect of*Gongronemalatifolium* leaf extracts on selected Gram-Positive and Negative Clinical Bacterial Isolates.European Journal of Botany, Plant Sciences and Phytology. 2020;5(1)**:** 1-12. https://www.eajournals.org/journals/european-journal-of-botany-plant-sciences-and-phytology-ejbpsp/vol-5-issue-1/antibacterial-effect-of-gongronema-latifolium-leaf-extracts-on-selected-gram-positive-and-negative-clinical-bacterial-isolates/

25. OgunjobiAA,NnadozieN.(2004). Comparative effectoftheantimicrobialActivitiesof *Ocimumgratissimum*and*Venoniaamygdalina*.BulletofScienceAssociationofNigeria.2004;25:165-170.

26. Ezeifeka GO, Orji MU, Mbata TI, Patrick AO. Antimicrobial Activities of *Cajanuscajan,Garciniakola*andXylopiaaethiopicaonPathogenicmicroorganisms.Biotechnology. 2004;3(1):41-43.