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Phytochemistry and Antibacterial Activity of Black Pepper (Piper Nigrum) Seeds Extracts on Some Food Borne Pathogens

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Abstract:

The study was aimed to investigate the phytochemical constituents and antibacterial efficacy of Piper nigrum extracts against some food borne pathogens. Aqueous and methanol extracts from Piper nigrum were prepared, screened for phytochemical properties and tested for antibacterial activity against 6 pathogenic bacteria (Klebsiella pneumoneae, Salmonella typhi, Shigella spp, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus). Phytochemical screening of the extracts showed that Piper nigrum extracts contain Alkaloid, Anthraquinone, saponin, tannin, phenol, steroid, flavonoid and terpenoid. Alkaloid was found to be the most abundant constituent making about 13.10%, followed by flavonoid, tannin and steroid constituting 5.25%, 2.20 % and 1.27% respectively. Statistical analysis of the result showed that methanol extract demonstrated highest antibacterial efficacy with average zone of inhibition of 14.09±2.47 mm among the isolates than aqueous extracts (12.28±1.82 mm). Based on the susceptibility of the organisms to the extracts, E. coli was found to be the highest susceptible organisms with average zone of inhibition of 14.68±1.97 mm, followed Salmonella typhi (13.84±2.52 mm), S. aureus (13.68±1.65 mm), Shigella (13.31±1.12 mm), Pseudomonas (12.19 ±0.98mm) while least average zone of inhibition is shown by Klebsiella (11.42±1.60 mm). The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts range from 3.125 to 50 mg/ml. There is no significant difference on the susceptibility of the organisms against the extracts at $p < 0.05$. The results of present study have provided the justification for therapeutic potential of Piper nigrum and also its use as dietary supplement for food flavoring and preservation.

1. Keywords: Antibacterial activity; Extract; Pathogenic bacteria; *Piper nigrum*; Phytochemicals

2. Introduction

Spices are plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Herbs and spices have been used during the middle Ages for flavoring, food preservation or medicinal purposes **[1]**. Spices have been used for centuries by many cultures to enhance flavor, aroma and as preservative and medicinal agents **[2]**. Spices are widely used as condiments and ingredients in food preparation. In Nigeria, some spices are useful in the preparation of certain soups which are delicacies and also recommended for rapid relief of ailments such as cold, malaria fever, etc **[3]**. These spices are also said to be therapeutically useful in the management of stomachache, leprosy, cough, and loss of appetite, rheumatoid pain, convulsion and

inflammation **[4]**. Black pepper (*Piper nigrum* L.) is a flowering vine of the Piperaceae family. It is a valuable medicinal plant and one of the most commonly used spices and considered as ''The King of spices'' among various spices **[5]**. Black pepper is used as medicinal agent, a preservative, and in perfumery. Whole Peppercorn of *Piper nigrum* or its active components are being used in different types of foods and as medicine. It contains major pungent alkaloid Piperine which is known to possess many interesting pharmacological actions. It is widely used in different traditional systems of medicine like Ayurvedic and Unani System of medicines **[6]**. Piperine exhibits diverse pharmacological activities like antihypertensive and antiplatelets **[7]**, antioxidant, antitumor **[8]**, anti-asthmatics **[9]**, antipyretic, analgesic, anti-inflammatory, anti-diarrheal, antispasmodic, anxiolytic, antidepressants **[10]**, hepato-protective **[11]**, immuno-modulatory, antibacterial, antifungal, anti-thyroids, anti-apoptotic, anti-metastatic, antimutagenic, anti-spermatogenic, anti- Colon toxin, insecticidal and larvicidal activities etc. Piperine has been found to enhance the therapeutic efficacy of many drugs, vaccines and nutrients by increasing oral bioavailability by inhibiting various metabolizing enzymes **[12]**.

It is also known to enhance cognitive action and Fertility **[13]**. The phytochemical investigations of *P. nigrum* revealed that it contains variety of phytochemicals. Piperine was the first pharmacologically active compound isolated from different members of Piperaceae family. Many investigators isolated different types of compounds viz Phenolics, flavonoids, alkaloids, amides and steroids, lignans, neolignans, terpenes, chalcones etc and many other compounds **[5]**. Khan and Siddiqui **[14]** evaluated the antibacterial potential of aqueous decoction of *Piper nigrum* L. (black pepper) against different bacterial isolates from oral cavity of two hundred individual volunteers. Black pepper (aqueous decoction) showed strongest antibacterial activity at a concentration of 10µL/disc. In a study conducted by *Ganesh et al.* **[15]** on antibacterial activity of Pepper (*Piper nigrum* L.) against some human pathogens (*Staphylococcus aureus, Salmonella typhi, Escherichia coli, Proteus sp.* and *Pseudomonas aeruginosa*) using Agar well diffusion method, and found that all the bacteria showed sensitive to methanol and chloroform extract of pepper except Pseudomonas aeruginosa which showed resistant to both the extracts. The aim of the present study was to investigate the phytochemical constituents and antibacterial activity of *Piper nigrum* extracts against some pathogenic bacteria responsible for food spoilage.

3. Materials and Methods

3.1. Sample Collection and Identification of Plant Materials

Black pepper *Piper nigrum* were used in this study was purchased from Rimi market in Kano city, Nigeria. Identification and authentication of the plant material was done at compounding laboratory in the Department of Pharmaceutical Technology, School of Technology Kano with the following voucher number SOT/PCT/01/083 with voucher specimen deposited for future reference.

Figure 1: *Piper nigrum* seeds.

3.2. Test Organisms

Six (6) Bacterial isolates responsible for food spoilage including *Klebsiella pneumoneae, Salmonella typhi, Shigella sp, Pseudomonas aeruginosa, Escherichia coli,* and *Staphylococcus aureus* were obtained from Laboratory of Science Lab Technology, School of Technology Kano. The bacteria were isolated from spoiled food and identified to the species level by using different laboratory procedures including; Gram's stain, cultural characterization and Biochemical tests include (Indole, Methyl red, Vougues Proskeaur, Catalase, Citrate utilization and coagulase tests) according to method described by Holt *et al.* **[16]**. The isolates were maintained on Nutrient agar slants before use.

3.2.1. Indole test

Tryptophan broth was inoculated with an isolate of the test organism and incubated at 37^0C for 24 hours. About 0.5 ml of Kovack's reagents was added to the broth culture.

3.2.2. Methyl red test

MR-VP broth was inoculated with an isolate of the test organism using sterile inoculating loop and incubated at 37^0C for 24 hours. About 5 drops of Methl-red reagent was added to the broth culture.

3.2.3. Voges Proskauer

MR-VP broth was inoculated with an isolate of the test organism using sterile inoculating loop and incubated at 37^0C for 24 hours. Six milliliters (6ml) of 5% alpha naphthol was added followed by 0.2 ml of KOH. The tube was shaken gently and remained undisturbed for 5 minutes.

3.2.4. Citrate utilization test

Simmon's citrate agar was streaked back and forth with inoculums of the test organism and incubated aerobically at 37^0C for 24 hours.

3.3. Preparation of Extracts

Aqueous and methanol extracts of *Piper nigrum* were prepared separately. The fresh Seeds of *Piper nigrum* were washed and air dried for two weeks. After drying, the seeds were grounded to fine powder using sterile pestle and mortar under aseptic condition. Fifty grams (50 g) powder of the plant material was soaked in 500 ml of distilled water and methanol respectively. The flasks were kept at room temperature for 3 days with intermittent manual shaking after which filtration was done using Whatman filter paper. The methanol extracts was evaporated at 50°C using rotary evaporator while the aqueous extract was evaporated at 40°C in water bath. All dried extract samples were dissolved in 10% Dimethylsulphoxide (DMSO) separately to the final concentration of 200 mg/ml as a stock concentration. The extract solutions were stored at 4°C before use to avoid contamination **[17]**.

3.4. Qualitative Phytochemical Screening

The phytochemical screening of the plant materials for various phytochemical constituents such as terpenoids, flavonoids, alkaloids, reducing sugars, steroid, glycoside, phenol, Anthraquinones, saponin and tannin was conducted using standard methods as described by Sofowora **[3]** and Trease and Evans **[18]**.

3.5. Quantitative Phytochemical Analysis

Different methods were employed in evaluating the quantity of phytochemical constituents of the plant material used. Spectrophotometric method was used to determine Terpenoids, tannins, steroids, anthraquinone, and glycosides. Folin-Ciocalteu procedure was used to determine phenol content. Flavonoids, alkaloids and saponin were determined by the methods described by Adeniyi *et al*. **[19]**.

3.6. Antibacterial Susceptibility Test

The sensitivity of each extracts was determined using the agar well diffusion method as described by Ali and Yahaya **[20]** with modifications. The prepared bacterial suspension equivalent to 0.5 McFarland Standard (1.5×10^6 CFU) was inoculated into sterile Mueller- Hinton agar medium in a sterile Petri-dish. A sterile 6 mm diameter sterile cork borer was used to bore 5 wells into the agar medium. The wells were then filled up with approximately 0.1ml of the extract solution at a concentration of 25, 50, 75 and 100 mg/ml taking care to prevent spillage onto the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 hour to allow proper diffusion of the extract into the medium after which the plates were incubated at 37^0C for 24 hours, and thereafter the plates were observed for zones of inhibition and measured **[21]**. Ciprofloxacin 50 mg/ml from Micro Lab limited was used as a positive control.

3.7. Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was determined using broth dilution technique. Two fold serial dilutions of the extracts were prepared by adding 2ml of 100mg/ml of the extract into a test tube containing 2ml of Nutrient broth, thus producing solution containing 50mg/ml of the extract. The process continue serially up to test tube No. 5, hence producing the following concentrations; 50, 25, 12.5, 6.25 3.125 mg/ml. Test tube No. 6 do not contain extracts and serve as negative control. Exactly 0.5 ml of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 37^0 C for 24 hours. After incubation the test tubes were observed for growth by checking for turbidity **[22]**.

3.8. Determination of Minimum Bactericidal Concentration (MBC)

From each tube that did not show visible growth in the MIC, 0.1ml was aseptically transferred into extract free Mueller Hilton agar plates. The plates were incubated at 37°C for 24 hours. The MBC was recorded as the lowest concentration of the extract that had less than 99% growth on the agar plates **[22]**.

3.9. Statistical Analysis

The data of average zone of inhibition produced by the isolates against the extracts used was analyzed using One-Way ANOVAs and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the means ± standard deviation. Significance level for the differences was set at $p<0.05$.

4. Results

4.1. Phytochemical Screening

The qualitative and quantitative phytochemical screening of *Piper nigrum* extract is presented in Table 1. The result indicated the presence of Alkaloid, terpenoids, flavonoids, steroid, phenol, Anthraquinones, saponin and tannin while reducing sugars and glycoside are absent. Quantitatively, Alkaloid was found to be the abundant constituent making about 13.10%, followed by flavonoid, tannin and steroid constituting 5.25%, 2.20 % and 1.27% respectively.

S/N	Phytochemical	Qualitative analysis	Quantitative analysis (%)
1	Alkaloids	+	13.10±0.11
2	Flavonoid	+	5.25±0.10
3	Glycosides	-	0.00±0.00
4	Reducing sugar	-	0.00±0.00
5	Saponin	+	0.06±0.00
6	Steroids	+	1.27±0.01
7	Phenols	+	0.70±0.01
8	Terpenoid	+	0.17±0.01
9	Anthraquinones	+	0.90±0.01
10	Tannin	+	2.20±0.03

Table 1: Qualitative and quantitative phytochemical screening of *Piper nigrum* extract.

4.2. Antibacterial Activity of Aqueous Extract

The antibacterial activity of aqueous *Piper nigrum* extract is presented in Table 2. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by *E. coli* (16.8 mm) at 100 mg /ml. The zone of inhibition of the control (Ciprofloxacin 50 mg/ml) ranges from to 19-22 mm.

Concentration (mg /ml)/zone of inhibition (mm)					
Isolates	**25**	**50**	**75**	**100**	**Control**
Klebsiella pneumoneae	09.20 ±0.00	09.80 ±0.00	10.50 ±0.11	12.10 ±0.13	22
Salmonella typhi	11.30 ±0.15	12.40 ±0.13	13.70 ±0.22	16.50 ±0.26	21
Shigella sp	09.70 ±0.17	11.20 ±0.20	13.80 ±0.09	13.60 ±0.31	22
Pseudomonas aeruginosa	08.30 ±0.00	10.20 ±0.26	12.40 ±0.14	12.60 ±0.21	20
Escherichia coli	12.30 ±0.20	13.50 ±0.12	14.70 ±0.17	16.80 ±0.36	22
Staphylococcus aureus	09.30 ±0.15	11.80 ±0.20	14.30 ±0.23	15.00 ±0.12	19

Table 2: Antibacterial activity of *Piper nigrum* aqueous extract.

4.3. Antibacterial Activity of Methanol Extract

The antibacterial activity of methanol extract is presented in Table 3. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by *Shigella sp* (18.6 mm) at 100 mg /ml. The zone of inhibition of the control (Ciprofloxacin 50 mg/ml) ranges from to 19-22 mm.

Concentration (mg /ml)/zone of inhibition (mm)					
Isolates	**25**	**50**	**75**	**100**	**Control**
Klebsiella pneumoneae	10.80 ±0.20	11.40 ±0.12	13.20 ±0.17	15.40 ±0.17	22
Salmonella typhi	12.80 ±0.12	13.50 ±0.17	14.40 ±0.25	16.20 ±0.20	21
Shigella sp	11.70 ±0.32	12.80 ±0.25	15.10 ±0.32	18.60 ±0.37	22
Pseudomonas aeruginosa	12.0± 00.12	12.20 ±0.36	13.90 ±0.15	16.00 ±0.23	20
Escherichia coli	13.3± 00.32	14.20 ±0.20	15.80 ±0.12	16.90 ±0.32	22
Staphylococcus aureus	12.3± 0.017	12.50 ±0.32	16.10 ±0.20	18.30 ±0.47	19

Table 3: Antibacterial activity of *Piper nigrum* methanol extract.

4.4. MIC and MBC of the Extracts

Minimum inhibitory concentration of aqueous and methanol extract of *Piper nigrum* is represented in Table 4. The result showed dilutions of various concentrations of aqueous and methanol extracts can inhibit and/or kill the isolates. Lower MIC (6.25 mg/ml) was shown by methanol extract than aqueous extract. MBC of methanol extract ranges between 12.5 - 50mg/ml

	Aqueous extract		**Methanol extract**	
Isolates	**MIC (mg/ml)**	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**
Klebsiella pneumoneae	12.5	50	6.25	25
Salmonella typhi	6.25	12.5	6.25	25
Shigella sp	12.5	25	6.25	12.5
Pseudomonas aeruginosa	12.5	25	12.5	50
Escherichia coli	6.25	25	3.125	12.5
Staphylococcus aureus	12.5	25	6.25	12.5

Table 4: Minimum inhibitory concentration (MIC) and MBC of the extracts.

5. Discussion

The results of the present study suggested that several phytochemicals were present in *Piper nigrum* seeds extract. The presence of the phytochemicals can be correlated with the fact that solvent extracts showed antibacterial activity against the bacterial isolates. Phytochemicals give plants their colour, flavor, smell and are part of a plant`s natural defense system and protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites **[23]**. The phytochemicals alkaloid, terpenoids, flavonoids, steroid, phenol, Anthraquinones, saponin and tannin were present in *Piper nigrum* extracts according to this study. The results are in accordance with the findings of other authors who have studied this plant **[24-26]**. According to this study, Alkaloid is present in the extracts. Alkaloids comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents, anaesthetics and Central Nervous Stimulants **[27]**. Alkaloids are known to play some metabolic roles and control development in living system **[28]**. It also interferes with cell division, hence the presence of alkaloids in clove could account for their use as antimicrobial agents. Aboaba *et al*. **[29]** had reported that the antimicrobial properties of substances are desirable tools in food spoilage and food safety. This suggests that the *P. nigrum* extracts which have been confirmed to contain alkaloids may also be useful as preservatives in food. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory and immunomodulatory properties **[30]**. Flavonoids are also present in the extract as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity **[31]**. It also helps in managing diabetes induced oxidative stress. Steroids are importance in pharmacy as they possess compounds like sex hormones and can be used for drug production **[32]**. Tannin and saponin were present in the extract. Saponins protect against hypercholesterolemia and

antibiotics properties **[33]**. In addition, it has been found that saponins have antitumor, antioxidant and anti-mutagenic activities and can lower the risk of human cancers by inhibiting the growth of cancer cells **[34,35]**. The growth of many fungi, yeast, bacteria and viruses was inhibited by tannins **[36]**. The Alkaloid was found to be the most abundant constituent making about 13.10%, followed by flavonoid, tannin and steroid constituting 5.25%, 2.20 % and 1.27% respectively.

The results of antibacterial activity of *P. nigrum* extracts against food borne pathogens are given in Table 2 and 3 which shows that the methanol extracts is more effective against the tested isolates than aqueous extracts. The better efficacy of the ethanol extract as against the aqueous extract may be because different solvents have different polarities, hence different degrees of solubility for the various phyto-constituents **[37]**. *E. coli* and *Salmonella* were also more susceptible to the extracts in comparison with the rest 14.97 and 13.84 mm respectively. The result of antimicrobial activity of *P. nigrum* in this study was inconformity with the study conducted by many researchers **[24-26]**. Based on the susceptibility of the organisms to the extracts, *E. coli* was found to be the highest susceptible organisms with average zone of inhibition of 14.97±1.97 mm, followed *Salmonella typhi* (13.84±2.52 mm), *S. aureus* (13.7±1.65 mm), *Shigella* (13.44±1.12 mm), *Pseudomonas* (12.27 ±0.98mm) while least average zone of inhibition is shown by *Klebsiella* (11.55±1.16 mm). The result of this study revealed that Gram negative bacteria are more susceptible towards the black pepper extracts than gram positive bacteria. The variation in the inhibition among the gram positive and gram negative bacteria is due to the cell wall and cell membrane compositions. The antibacterial activities of the extracts are expected due to the presence of compounds such as alkaloid, flavonoids and tannin. The results obtained in this study corroborate with the report of Khan and Siddiqui **[14]** who evaluated the antibacterial potential of aqueous decoction of *Piper nigrum* L. (black pepper), *Laurus nobilis* L. (bay leaf), *Pimpinella anisum* L. (aniseed), and *Coriandum sativum* L. (coriander) against different bacterial isolates from oral cavity of two hundred individual volunteers. Black pepper (aqueous decoction) showed strongest antibacterial activity comparable to aqueous decoction of *Laurus nobilis* and *Pimpinella anisum* at the concentration of 10µL/disc. The result of MIC and MBC of the extracts showed that dilutions of various concentrations of aqueous and methanol extracts of *P. nigrum* can inhibit and/or kill the isolates. Lower MIC (3.125 mg/ml) was shown by methanol extract than aqueous extract. MBC of methanol extract ranges between 12.5 - 50mg/ml while the MBC of *Shigella sp* and *Pseudomonas aeruginosa* was not found in aqueous extract.

6. Conclusion

In conclusion, this study revealed that *P. nigrum* extracts possess medicinal properties and antibacterial activity that inhibit bacterial growth. The results of the present study show that *P. nigrum* methanol extracts are more effective against all tested bacterial strains than aqueous extracts. *E. coli* and *Shigella* were also more susceptible to the extracts while *Klebsiella* was the least susceptible. The antibacterial activities of the extracts are expected perhaps due to the present of bioactive compounds like Alkaloid, Terpenoid, Saponin, Tannin, flavonoids and Anthraquinones which were dissolved in the solvents. The results of present study have provided the justification for therapeutic potential of *p. nigrum* and also used as dietary supplement for food preservation.

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References

1. Harsha N, Sridevi V, Lakshmi C, Rani K ,Vani S (2013) Phytochemical Analysis of Some Selected Spices. International Journal of Innovative Research in Science, Engineering and Technology 2: 6618-6621.
2. Ene-Obong HN, Onuoha NO, Aburime LC, Mbah O (2015) Nutrient composition, phytochemicals and antioxidant activities of some indigenous spices in Southern Nigeria. 11TH International Food Data Conference, Hyderabad, India 1-31.
3. Sofowora A (1993) Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd. 2nd Edn 26-100.
4. Valko M, Leibfritz D, Moncol J, Cronin M T, Mazur M, et al. (2007) Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry and Cell Biology 39: 44-84.

5. Damanhouri Z A, Ahmad A (2014) A Review on Therapeutic Potential of Piper nigrum L. (Black Pepper): The King of Spices. Medicinal and Aromatic Plants 3: 161.

6. Ahmad N, Fazal H, Abbasi BH, Farooq S, Ali M, et al. (2012) Biological role of Piper nigrum L. (Black pepper): A review. Asian Pacific Journal of Tropical Biomed: S1945-S1953

7. Taqvi SI, Shah AJ, Gilani AH (2008) Blood pressure lowering and vasomodulator effects of piperine. J CardiovascPharmacol 52: 452-458.

8. Manoharan S, Balakrishnan S, Menon V, Alias L, Reena A (2009) Chemopreventive efficacy of curcumin and piperine during 7,12-dimethylbenz[a] anthracene-induced hamster buccal pouch carcinogenesis. Singapore Medicinal Journal 50:139-146.

9. Parganiha R, Verma S, Chandrakar S, Pal S, Sawarkar HA, et al. (2011) In vitro anti-asthmatic activity of fruit extract of Piper nigrum (Piperaceae). International Journal of Herbal Drug Research 1: 15-18.

10. Li S, Wang C, Wang M, Li W, Matsumoto K, et al. (2007) Antidepressant like effects of piperine in chronic mild stress treated mice and its possible mechanisms. Life Science 80: 1373-1381.

11. Matsuda H, Ninomiya K, Morikawa T, Yasuda D, Yamaguchi I, et al. (2008) Protective effects of amide constituents from the fruit of Piper chaba on D-galactosamine/TNF-alpha-induced cell death in mouse hepatocytes. Bioorganisms Medicinal and Chemical Letter 18: 2038-2042.

12. Johnson JJ, Nihal M, Siddiqui IA, Scarlett CO, Bailey HH, et al. (2011) Enhancing the bioavailability of resveratrol by combining it with piperine. Molecular and Nutrition Food Research 55: 1169-1176.

13. Wattanathorn J, Chonpathompikunlert P, Muchimapura S, Priprem A, Tankamnerdthai O (2008) Piperine, the potential functional food for mood and cognitive disorders. Food ChemToxicol 46: 3106-3110.

14. Khan M, Siddiqui M (2007) Antimicrobial activity of Piper fruits. Natural product Reseach 6: 111-113.

15. Ganesh P, Suresh Kumar R, Saranraj P (2014) Phytochemical analysis and antibacterial activity of Pepper (Piper nigrum L.) against some human pathogens. Central European Journal of Experimental Biology 3: 36-41.

16. Holt JG, Krieg NR, Senath PHA, Staley JT, Williams ST (1994) Bergey's Manual of Determinative Bacteriology 9th Ed. Baltimore. Williams and Wilkins publishers.

17. Yahaya A, Ali M, Idris A (2017) Antibacterial Activity and Phytochemical Screening of Carica papaya on some Enteric Bacterial Isolates of Public Health Importance. Greener Journal of Biological Sciences 7: 001-007.

18. Yahaya A, Ali M, Idris A (2017) Antibacterial Activity and Phytochemical Screening of Carica papaya on some Enteric Bacterial Isolates of Public Health Importance. Greener Journal of Biological Sciences 7: 001-007.

19. Trease GE, Evans WC (2002) Phytochemicals. In: Pharmacognosy. 15th ed. Saunders Publishers, London 42-44, 221- 229, 246- 249, 304-306,331-332, 391-393.

20. Adeniyi SA, Orjiekwe CL, Ehiagbonare JE (2009) Determination of alkaloids and oxalates in some selected food samples in Nigeria. African Journal of Biotechnology 8: 110-112.

21. Ali M, Yahaya A (2016) Antibacterial activity and phytochemical screening of Carica papaya leaf extract on Staphylococcus aureus recovered from patients with urinary tract infections. International Journal of Applied Research and Technology 5: 34-39.

22. Anibijuwon, II, Udeze OA (2009) Antimicrobial Activity of Carica papaya (Paw-paw Leaf) on Some Pathogenic Organisms of Clinical Origin from South Western, Nigeria. Ethno Botanical Leaflets. 13: 850-864.

23. Ahmed I, Beg AZ (2001) Antimicrobial and phytochemical studies on 45 Indian Medicinal plants against multi-drug resistance human pathogens. Journal of Ethnopharmacol 74: 113-123.

24. Ibrahim TA, Dada IBO, Adejare RA (2010) Comparative phytochemical properties of crude ethanolic extracts and physicochemical characteristics of essential oils of Myristical fragrans (nutmeg) seeds and Zingiber officinate (ginger) roots. Electronic Journal of

Environment, Agriculture, Food and Chemical 9: 1110-1116.

25. Aneja KR, Joshi R, Sharma C, Aneja A (2010) Antimicrobial efficacy of fruit extracts of two Piper species against selected bacterial and oral fungal pathogens. Brazil Journal of Oral Science 9: 421-426.

26. Akrayi HFS, Abdulrahman ZFA (2013) Evaluation of the Antibacterial Efficacy and the Phytochemical Analysis of some Plant Extracts against Human Pathogenic Bacteria. Journalof Physics Conference Series 29-39.

27. Shiva Rani SK, Neeti Saxena, Udaysree (2013) Antimicrobial Activity of Black Pepper (Piper nigrum L.). Global Journal of Pharmacology 7: 87-90.

28. Madziga HA, Sanni S, Sandabe UK (2010) Phytochemical and Elemental Analysis of Acalypha wilkesiana Leaf. Journal of American Science 6: 510-514.

29. Edeoga HO, Omobuna G, Uche LC (2006) Chemical composition of Hyotissu aveoleus and Ocimum gratissium hybrids from Nigeria. African Journal of Biotechnology 5: 892-895.

30. Aboaba OO, Ezeh AR, Anabuike CL (2011) Antimicrobial activities of some Nigerian spices on some pathogens. Agriculture and Biology Journal of North America 2: 1187-1193.

31. Rabi T, Bishayee A (2009) Terpenoids and breast cancer chemoprevention. Breast Cancer Research Treatment 115: 223-239.

32. Rio DA, Obdululio BG, Casfillo J, Marin FG, Ortuno A (1997) Uses and properties of citrus flavonoids. Journal of Agriculture, Food and Chemical 45: 4505-4515.

33. Okwu DE (2001) Evaluation of the chemical composition of indigenous spices and flavoring agents. Global Journal of Pure and Applied Sciences 7: 455-459.

34. Amin MM, Sawhney SS, Jassal MS (2013) Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale. Wudpecker Journa of Pharmaceutical and Pharmacology 2: 001-005.

35. Roa RR, Sung MK(1995) Saponins as anti-carcinogens. The Journal of Nutrition 125: 717S-724S.

36. Prohp TP, Onoagbe IO (2012) Determination of phytochemical composition of the stem bark of triplochiton scleroxylon k. schum. (sterculiaceae). International Journal of Applied Biology and Pharmaceutical Technology 3: 68-76.

37. Doughari JH, El Mahmud AM, Manzara S (2007) Studies on the antibacterial activities of root extract of Carica papaya L. African Journal of Microbiology Research 037-041.

38. Gao X, Ohlander M, Jeppsson N, Björk L, Trajkovski V (2000) Changes in antioxydant effects and their relationship to phytonutrients in fruits of sea buck- thorn (Hippophaerhamnoides L) during maturation. Journal of Agricultural and Food Chemistry 48: 1485-1490.