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Antibacterial Activity of Gallic (Allium Sativum) Extracts on Food Borne Pathogens

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ABSTRACT

The study was aimed to investigate the phytochemical constituents and antibacterial activity of Allium sativum extracts against some food borne pathogen. Aqueous and methanol extracts from Allium sativum bulbs were prepared, screened for phytochemical analysis 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 Allium sativum bulb extract contain Alkaloid, Anthraquinone, saponin, tannin, phenol, steroid, flavonoid, terpenoid, glycoside and reducing sugar. Alkaloid was found to be the most abundant constituent making about 7.2 %, followed by Tannin, saponin and flavonoid constituting 4.8 %, 4.3 % and 2.8 % respectively. Statistical analysis of the result showed that methanol extract demonstrated highest antibacterial activity with average zone of inhibition of 14.12±1.51 mm among the isolates than aqueous extracts (12.00±1.06 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 14.29±1.12 mm, followed S. aureus (13.61±1.23 mm), Salmonella typhi (13.56±1.89 mm), Shigella (13.22±1.41 mm), Pseudomonas (12.36 ±1.38mm) while least average zone of inhibition is shown by Klebsiella (11.33±0.80 mm). The MIC and MBC of the extracts ranges from 3.125 to 50 mg/ml There is no significant different on the susceptibility of the organisms against the extracts at $p<0.05$. The results of the present study have provided the justification for therapeutic potential of Allium sativum and also used as dietary supplement for food flavoring and preservation.

Keywords: *Allium sativum*; Pathogenic bacteria; Antibacterial activity; Phytochemicals

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]. Kao et al. [3] had shown that the colouring, flavouring, aromatic and pungent properties of spices were due to the rich presence of essential oils and oleoresins. 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 [4]. 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 [5].

Garlic (*Allium sativum* L.) is one of those plants that were seriously investigated over several years and used for centuries to fight infectious diseases [6]. It is belong to family Amaryllidaceae [7]. Therapeutic use of garlic has been recognized as a potential medicinal value for thousands of years to different microorganisms. For example; antifungal, antiviral, antibacterial antihelmantic, antiseptic and anti-inflammatory properties of garlic are well documented. Moreover, garlic extracts exhibited activity against both gram negative (*E. coli, Salmonella* sp. and *Citrobacter Enterobacter, Pseudomonas Klebsiella*) and gram positive (*S. aureus, S. pneumonia, streptococcus*

and *Bacillus anthrax*) all of which are cause of morbidity worldwide [8].

In Africa, particularly in Nigeria, it is used to treat abdominal discomfort, diarrhea, otitis media and respiratory tract infections [9]. In Europe and India, it was used to treat common colds, hay fever and asthma [10]. Many researches had demonstrated its effectiveness and broad spectrum antimicrobial activity against many species of bacteria, viruses, parasites, protozoan and fungi [9]. Garlic extract inhibits the growth of Gram positive and Gram negative bacteria, such as *Staphylococcus*, *Streptococcus*, *Micrococcus*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Lactobacillus*, *Pseudomonas*, *Shigella*, *Salmonella*, *Proteus*, and *Helicobacter pylori* [11]. The objective of the present study was to investigate the phytochemical constituents and antibacterial activity of Gallic extracts against some food borne pathogens.

Materials and Methods

Sample Collection and Identification of Plant Materials

Gallic (*Allium sativum*) bulbs were used in this study and were 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/01/082. Voucher specimen has been deposited there for future reference.

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 diagnosed 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). The isolates were maintained on Nutrient agar slants at 4^0C.

Preparation of Extracts

Aqueous and methanol extracts of *Allium sativum* were prepared separately. The fresh bulbs of *Allium sativum* were washed and air dried for two weeks. After drying, the bulbs were grounded to fine powder using sterile pestle and mortar under laboratory condition. Fifty grams (50 g) powder of the plant material was soaked in 500 ml each of distilled water and methanol respectively. The flasks were kept at room temperature for 3 days with intermittent 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 until dried extract samples were obtained. All the dried extract samples were dissolved in 10% DMSO separately to the final concentration of 200 mg/ml as a stock concentration. The extract solutions were stored at 4°C before use.

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 [4] and Trease and Evans [12]

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*. [13].

Antibacterial Susceptibility Test

The sensitivity of each extracts was determined using the agar well diffusion method as described by Ahmed and Beg [14] with modifications. The prepared bacterial suspension equivalent to 0.5 McFarland Standard (1.5 x 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. Ciprofloxacin 50 mg/ml (Micro Lab limited) was used as a positive control.

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 [14].

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 [14].

Statistical Analysis

The data of average zone of inhibition produced by the isolates against the antibiotics 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 $p<0.05$.

Results

Characteristics of the Isolates

The biochemical characterization of the isolates from spoiled food is presented in Table 1. The isolates were tested based on Gram staining, Indole, Methyl red, Vogues Proskauer, Citrate utilization, catalase, oxidase, and coagulase tests. Lactose and mannitol fermentation were also employed.

Table1. *Characteristics of the isolates*

Isolates	GS	IN	MR	VP	CI	CA	OX	CO	LF	MF
Klebsiella	-	-	-	+	+	+	-	-	+	NA
Salmonella typhi	-	-	+	-	+	+	-	-	-	NA
Shigella sp	-	+	+	-	-	+	-	-	-	NA
Pseudomonas	-	-	-	-	+	+	+	-	-	NA
Escherichia coli	-	+	+	-	-	+	-	-	+	NA
S. aureus	+	NA	NA	NA	NA	+	-	+	NA	+

Key: *GS = Gram Staining, IN=Indole, MR=Methyl Red, VP=Vogues Proskauer, CI=Citrate, CA=Catalase, OX=Oxidase CO=Coagulase, LF=Lactose Fermentation, MF=Mannitol Fermentation. NA = Not applicable.*

Phytochemical Screening

The qualitative and quantitative phytochemical screening of *Allium sativum* extract is presented in Table 2.

The result indicated the presence of Alkaloid, terpenoids, flavonoids, steroid, phenol, Anthraquinones, saponin and tannin, reducing sugars and glycoside.

Quantitatively, Alkaloid was found to be the abundant constituent making about 7.2 %, followed by Tannin and saponin constituting 4.8 % and 4.3 % respectively.

Table2. *Qualitative and quantitative phytochemical screening of Allium sativum extract*

S/N	Phytochemical	Qualitative analysis	Quantitative analysis (%)
1	Alkaloids	+	7.20±0.05
2	Flavonoid	+	2.18±0.03
3	Glycosides	+	0.05±0.00
4	Reducing sugar	+	0.08±0.01
5	Saponin	+	4.30±0.02
6	Steroids	+	0.50±0.00
7	Phenols	+	0.80±0.00
8	Terpenoid	+	0.40±0.01
9	Anthraquinones	+	1.40±0.03
10	Tannin	+	4.80±0.03

Antibacterial Activity of Aqueous Extract

The antibacterial activity of aqueous *Allium sativum* 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 *E. coli* (18.23±0.36 mm) at 100 mg /ml. The zone of inhibition of the control (Ciprofloxacin 50 mg/ml) ranges from to 19-22 mm

Table3. *Antibacterial activity of Allium sativum aqueous extract*

	Concentration (mg /ml)/zone of inhibition (mm)				
Isolates	**25**	**50**	**75**	**100**	**Control**
Klebsiella pneumoneae	8.00±0.00[a]	8.84±0.00[a]	10.56±0.11[a]	12.17±0.13[a]	22
Salmonella typhi	8.73±0.15[a]	11.46±0.13[a]	13.70±0.22[a]	15.45±0.26[b]	21
Shigella sp	9.40±0.17[a]	10.28±0.20[a]	13.82±0.09[b]	14.71±0.31[b]	22
Pseudomonas aeruginosa	8.55±0.00[a]	10.77±0.26[a]	12.49±0.14[a]	12.65±0.21[a]	20
Escherichia coli	10.33±0.20[a]	12.59±0.12[a]	14.88±0.17[b]	18.23±0.36[b]	22
Staphylococcus aureus	9.37±0.15[a]	10.55±0.20[a]	14.83±0.23[b]	15.70±0.12[b]	19

Key: *Values having different superscript on the same row are considered significantly different at p<0.05*

Antibacterial activity of Methanol Extract

The antibacterial activity of methanol extract is presented in Table 4. 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* (18.87±0.37) at 100 mg /ml. The zone of inhibition of the control (Ciprofloxacin 50 mg/ml) ranges from to 19-22 mm

Table4. *Antibacterial activity of Allium sativum methanol extract*

	Concentration (mg /ml)/zone of inhibition (mm)				
Isolates	**25**	**50**	**75**	**100**	**Control**
Klebsiella pneumoneae	10.28±0.20[a]	12.64±0.12[a]	13.62±0.17[a]	14.54±0.17[b]	22
Salmonella typhi	12.58±0.12[a]	13.98±0.17[b]	15.44±0.25[b]	17.20±0.20[b]	21
Shigella sp	10.76±0.32[a]	12.85±0.25[a]	15.10±0.32[b]	18.87±0.37[b]	22
Pseudomonas aeruginosa	11.60±0.12[a]	12.82±0.36[a]	14.29±0.15[b]	15.54±0.23[b]	20
Escherichia coli	12.38±0.32[a]	13.92±0.20[b]	15.18±0.12[b]	16.87±0.32[b]	22
Staphylococcus aureus	12.33±0.17[a]	12.50±0.32[a]	15.71±0.20[b]	17.93±0.47[b]	19

Key: *Values having different superscript on the same row are considered significantly different at p<0.05*

MIC and MBC of the Extract

Minimum inhibitory concentration of aqueous and methanol extract of *Allium sativum* is represented in Table 5. The result showed dilutions of various concentrations of aqueous and methanol extracts 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

Table5. *Minimum inhibitory concentration (MIC) and MBC of the extracts*

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

Key:*NF = Not found*

DISCUSSION

The results of the present study suggested that several phytochemicals are present in *Allium sativum* bulb extracts.

The presence of the phytochemicals can be correlated with the fact that solvent extracts showed antibacterial activity against the bacterial strains.

Phytochemicals give plants their colour, flavour, smell and are part of a plant`s natural defense system and protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites [15]. The phytochemicals alkaloid, terpenoids, flavonoids, steroid, phenol, Anthraquinones, glycoside, reducing sugar, saponin and tannin were present in *Allium sativum* extracts according to this study.

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 [16]. Alkaloids are known to play some metabolic roles and control development in living system [17]. It also interferes with cell division, hence the presence of alkaloids in clove could account for their use as antimicrobial agents. Aboaba *et al.* [18] had reported that the antimicrobial properties of substances are desirable tools in food spoilage and food safety. This suggests that the *Allium sativum* 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 [19]. 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 [20, 21]. 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 [20, 22]. Tannin and saponin were present in the extract. Saponins protect against hypercholesterolemia and antibiotics properties [23]. 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 [24, 25]. The growth of many fungi, yeast, bacteria and viruses was inhibited by tannins [26]. The phytochemical content of the extract of *S. aromaticum* revealed that the Alkaloids was found to be the most abundant phytochemical (7.2 %) followed by tannin (4.8 %), saponin (4.3 %) and flavonoids (2.18 %).

The results of antibacterial activity of *Allium sativum* extracts against food borne pathogens are given in Table 3 and 4 which shows that methanol extract demonstrated highest antibacterial activity with average zone of inhibition of 14.12±1.51 mm among the isolates than aqueous extracts (12.00±1.06 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 14.29±1.12 mm, followed *S. aureus* (13.61±1.23 mm), *Salmonella typhi* (13.56±1.89 mm), *Shigella* (13.22±1.41 mm), *Pseudomonas* (12.36 ±1.38mm) while least average zone of inhibition is shown by *Klebsiella* (11.33±0.80 mm). The result of antimicrobial activity of *Allium sativum* in this study was inconformity with the study conducted by many researchers [8, 9, 11].

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 Deresse [8] who found that garlic extracts exhibited activity against both gram negative (*E. coli, Salmonella* sp. and *Citrobacter Enterobacter, Pseudomonas Klebsiella*) and gram positive (*S. aureus, S. pneumonia, streptococcus* and *Bacillus anthrax*). This study supported a study conducted by Tsao and Yin [11] who found that Garlic extract inhibits the growth of Gram positive and Gram negative bacteria, such as *Staphylococcus, Streptococcus, Micrococcus, Enterobacter, Escherichia, Klebsiella, Lactobacillus, Pseudomonas, Shigella, Salmonella, Proteus,* and *Helicobacter pylori*.

The result of MIC and MBC of the extracts showed that dilutions of various concentrations of aqueous and methanol extracts of *Allium sativum* 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.

CONCLUSION

In conclusion, this study revealed that *Allium sativum* extracts possess medicinal properties and antibacterial activity that inhibit bacterial growth. The results of the present study show that *Allium sativum* ethanol extracts are more effective against all tested bacterial strains than aqueous extracts. *E. coli* and *S. aureus* were the 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 *Allium sativum* and also used as dietary supplement for food preservation.

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REFERENCES

[1] Harsha N, Sridevi V, Lakshmi C, Rani K. and Vani S. Phytochemical Analysis of Some Selected Spices. International Journal of Innovative Research in Science, Engineering and Technology. 2013; 2, (11) 6618-6621

[2] Ene-Obong HN, Onuoha NO, Aburime LC, Mbah O. Nutrient composition, phytochemicals and antioxidant activities of some indigenous spices in Southern Nigeria. 2015;11

[3] Kao SH, Hsu CH, Su SN, Hor WT, Chang WH, Chow LP. Identification and immunologic characterization of an allergen, alliin-lyase, from garlic (*Allium sativum*). J. Allergy Clin. Immunol. 2004; 113(1):161-168.

[4] Sofowora A. (1993) *Medicinal plants and traditional medicine in Africa*. Spectrum Books Ltd. 2nd Edn;. 1993; 26-100.

[5] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. The *International Journal of Biochemistry and Cell Biology*, 2007; 39, 44-84.

[6] Onyeagba, R.A., O.C. Ugbogu, C.U. Okeke and O. Iroakasi. Studies on the antimicrobial effects of garlic (*Allium sativum linn*), ginger (*Zingiberofficinale roscoe*) and lime (*Citrus aurantifolia linn*). Afr. J. Biotechnol., 2004; 3(10): 552-554.

[7] Friesen N, Fritsch RM, Blattner FR. Phylogeny and new intrageneric classification of *Allium* L. (Alliaceae) based on nuclear ribosomal DNA its sequences. Aliso. 2004; 22:372-395.

[8] Deresse D Antibacterial Effect of Garlic (*Allium sativum*) on *Staphylococcu aureus*: An *in vitro* Study Asian Journal of Medical Sciences 2010; 2(2): 62-65

[9] Jaber MA, Al-Mossawi A Susceptibility of some multiple resistant bacteria to garlic extracts. Afr. J. Biotechnol. 2007; 6(6):771-776.

[10] Timbo BB, Ross MP, McCarthy PV, Lin CT. Dietary supplements in a national survey: Prevalence of use and reports of adverse events. Am. Diet Assoc.2006; 106(12):1966-1974.

[11] Tsao SM, Yin MC. *In vitro* antimicrobial activity of four diallyl sulphides occurring naturally in garlic and Chinese leek oil. J. Med. Microbiol. 2001; 50:646-649.

[12] Trease GE, Evans WC. Phytochemicals. In: Pharmacognosy. 15th ed. Saunders Publishers, London, 2002; pp. 42-44, 221- 229, 246- 249, 304-306,331-332, 391-393.

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

[14] Ahmed I and Beg AZ. Antimicrobial and phytochemical studies on 45 Indian Medicinal plants against multi-drug resistance human pathogens. J Ethnopharmacol. 2001 74, 113-123.

[15] Ibrahim TA, Dada IBO, Adejare RA. Comparative phytochemical properties of crude ethanolic extracts and physicochemical characteristics of essential oils of Myristical fragrans (nutmeg) seeds and Zingiber officinate (ginger) roots. Electronic J Environ Agric Food Chem 2010; 9(6): 1110-1116.

[16] Madziga HA, Sanni S and Sandabe UK. Phytochemical and Elemental Analysis of Acalypha wilkesiana Leaf. *Journal of American Science*, 2010; 6(11), 510-514.

[17] Edeoga HO, Omobuna G and Uche LC. Chemical composition of *Hyotissu aveoleus* and *Ocimum gratissium* hybrids from Nigeria. African Journal of Biotechnology, 2006; 5(910), 892-895.

[18] Aboaba OO, Ezeh AR and Anabuike CL. Antimicrobial activities of some Nigerian spices on some pathogens. *Agriculture and Biology Journal of North America*, 2011; 2(8), 1187-1193.

[19] Rabi T and Bishayee A. (2009) Terpenoids and breast cancer chemoprevention. Breast Cancer Res Treat. 2009; 115, 223-239.

[20] Salah N, Miler NJ, Pagange G, Tijburg L, Bolwell GP and Rice E. *et al*. Polyphenolic flavonoids as scavenger of aqueous phase radicals as chain breaking antioxidant. Arch Biochem Broph 1995; 2, 339-46.

[21] Rio DA, Obdululio BG, Casfillo J, Marin FG and Ortuno A. Uses and properties of citrus flavonoids. J Agric Food Chem 1997; 45, 4505-4515.

[22] Okwu DE. Evaluation of the chemical composition of indigenous spices and flavoring agents. *Global Journal of Pure and Applied Sciences*, 2001; 7(3), 455-459.

[23] Amin MM, Sawhney SS and Jassal MS. Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale.

Wudpecker J. Phar. and Pharmaco. 2013; 2(1), 001-005.

[24] Roa RR, Babu RM and Rao MRV. Saponins as anti-carcinogens. The Journal of Nutrition, 1995; 125, 717-724.

[25] Prohp TP and Onoagbe IO. Determination of phytochemical composition of the stem bark of *triplochiton scleroxylon k. schum.* (sterculiaceae). *International Journal of Applied Biology and Pharmaceutical Technology*, 2012; 3(2), 68-76.

[26] Chung KT, Wong TY, Wei CL, Huang YW and Lin Y. Tannins and human health. A review, Criti. Rev. Food. Sci. Nutri., 1998; 6: 421-446.