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PHYTOCHEMICAL ANALYSIS OF GARLIC EXTRACTS

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ABSTRACT
This study was aimed at investigating the phytochemical and antibacterial characteristics of Garlic [Allium sativum] extract. 150g of fresh garlic bulbs were purchased from Ekowe market in Southern Ijaw Local Government Area, Bayelsa State was peeled and air dried for five days. Sample was blended and soaked in 200ml of distilled water for 6 hours in a sterile beaker. The crude extract was then filtered using sterile whatman's filter paper. The filtered extract was then heated using heating mantle until water was loosed. After exhaustive extraction, the pure sample was subjected to phytochemical screening. 1.25g of sorbitol agar was weighed and dissolves in 31.25ml of distilled water. 1.968g of salmonella and shigella (SS) agar was dissolved in 31.25ml of distilled water while 3.46g of mannitol was also dissolved in 31.25ml of distilled water using standard procedure. The aqueous extract of Garlic was screened for the presence of the secondary metabolites using the procedure of Sofowora (1993). Two (2) milliliter of each extract was placed in a test tube for each of the tests and concentrated by evaporating the extract in a water bath. Tests were carried out for carbohydrates, tannins, saponins, flavonoids, alkaloids and phenols. The result of the phytochemical screening of garlic plant showed the presence of carbohydrate (80mg/ml) brick red precipitate, tannins (1.1mg/ml), a stable foamy Solution called saponin (20mg/ml), an orange precipitate of alkaloid (0.9mg/ml), phenol (7.32mg/ml) and a pink scarlet coloured solution called flavonoid (6.5 mg/ml), as its chemical constituents. The antibacterial screening also indicates that garlic had the best antibacterial activity against staphylococcus aureus, Escherichia coli, and Salmonella and Shigella species. Therefore, Garlic is recommended as a good antibacterial and antioxidant agent.

KEYWORD: Phytochemical, Antibacterial and Antioxidant.

INTRODUCTION
Allium sativum commonly known as Garlic is a family of Liliaceae, a species in the onion genus. Garlic (*Allium sativum*) is highly regarded throughout the world for both its medicinal and cultural value. Early men of medicine such as Hippocrates, Plato and Aristotle encouraged a number of therapeutic uses for botanicals. Today, garlic is commonly used in many cultures as a seasoning spicy. Garlic also stands as the second most utilized supplement, with its sulfur containing compounds; high trace mineral content, and enzymes (Peter *et al., 2008*). Garlic has shown anti-viral, anti-bacterial, anti-fungal and antioxidant abilities (Peter *et al., 2008*). Diseases that may be prevented by garlic's medicinal actions include Alzheimer's disease, cancer, cardiovascular disease (including atherosclerosis, strokes, hypertension, thrombosis and hyperlipidemias) children's conditions, dermatologic applications, stress, and infections; and possible benefits in diabetes, drug toxicity, and osteoporosis (Peter *et al*., 2008). Garlic is probably one of the earliest known medicinal plants, which used from ancient time to cure different disease conditions in human (Londhe *et al*., 2010). Garlic's principal medicinal uses are to lower blood pressure and cholesterol, fight infections, and prevent cancer.

Modern garlic (A. sativum) is only two species. Viz;
1. Allium sativum, the soft necks
2. Allium ophioscorodon, the hard necks

Taste, storage ability and stability in growing are the critical factors in selecting garlic classes of interest.

The hard necks have more intense flavors but less storage capabilities while the soft necks are excellent keepers but offer milder. Hard necks are generally grown in cooler climates whiles the soft neck grows closer to the equator (Al – zahim *et al*, 1997).

According to Amagase, 2006, garlic has some bioactive components which protect it against bacteria, fungi, viruses. Scientists estimate that there may be as many as 10,000 different phytochemicals having the potential to affect disease such as cancer, Stroke or metabolic syndrome. Phytochemicals in freshly harvested plant foods may be destroyed or removes by modern

processing techniques including cooking. Other animal studies have shown a strong oxidative effect in the gut that can damages intestinal cells, though many of these results were obtained by excessive amount of Allicin, which has been clearly shown to have some toxicity at high amount or by physically injecting the lumen itself with allicin or garlic supplements (Banerjee, 2001 and Amagase, 2003). Phytochemical screening of different extracts showed that garlic contains important compounds such as carbohydrates, reducing sugars, lipids, flavonoids, ketones, alkanoisds, steroids, saponis, alkaline, tannin and phenols e.t.c. alkaloids have been documented to possess analgesic, antispasmodic and bactericidal effect (Okigbo *et al*. 2009b).

Vaidya *et al*. (2009), clarified the mechanism of the antioxidant activity of garlic, such as trapping damaging free radicals. When allicin decomposes, it forms 2-propenesulfenic acid, and this propenesulfenic formed when garlic is cut or crushed has a lifetime of less than one second (Block *et al*., 2010). Allicin has been found to have numerous antimicrobial properties, and has been studied in relation to both its effect and its biochemical interaction (Ankri and Mirelman, 1999).

A screening of allicin against 30 strains of MRSA found high level of antimicrobial activity including against strains that are resistant to other chemical agents of the strains tested, 82% showed intermediate or full resistance to Mupirocin(Cutler and Wilson, 2004). This same study examined use of an aqueous cream of allicin, and found it somewhat less effectively than allicin liquid. At 500mg/l however, the cream was still active against all the organisms tested – which compares well with the 20g mupirocin currently used for tropical application (Cutler and Wilson, 2004). Allicin has antiviral activity both in vitro an in vivo. Among the viruses suspectible to allicin are Herpes simplex type 1 and 2, parainfluenza virus type 3, human cytomegalo virus, influenza B, Vaccina virus, Vesicular stomatitis virus and Human rhinovirus type 2 (Lilie *et al*., 2012). Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular and other metabolic diseases, atherosclerosis, hyperlipidemia, thrombosis, hypertension and diabetes (Sanjay and Subir, 2002). Effectiveness of garlic in cardiovascular diseases was more encouraging in experimental studies, which prompted several clinical trials (Sanjay and Subir, 2002). Epidemiologic studies show an inverse correlation between garlic consumption and progression of cardiovascular disease. Cardiovascular disease is associated with multiple factors such as raised serum total cholesterol, raised LDL and an increase in LDL oxidation, increased platelet aggregation, hypertension and smoking (Khalid and Gordon, 2006). Rapidly growing prevalence of cardiovascular disease is a major threat for the developed as well as developing world warranting urgent need of intervention. Complementary and alteration medicines are gaining popularity among general population because of their safety profile and easy administration. Garlic, in particular, is considered to be one of the best disease-preventing foods because of its potent and widespread effects (Waris and Tabinda, 2013).

According to Sofawaro (1993), Garlic is an African medical plants rank highest among plants use in the investigation of antimicrobial properties. This could be due to their highest traditional medicinal use and also the ease of carrying such test. Among medicinal plant of African origin are Psidum Guajara (Guava), Azadirachtalndica (Neem), Vernomial Amygdalina (Bitter Leaf), Anacardium Accidentals (Cashew), Allium Cepa (Onion), Allium Sativum (Garlic).

According to Johnson *et al*., (2008), biodiversity provides mankind enormous direct benefit and indirect essential services through natural ecosystem, function and stability. Initial report of antimicrobial activity of garlic showed that allicin (Ally-1, 2-propene thiosulfinate), a notable flavonoid in garlic is formed when cloves are crushed (Ross *et al*., 2000). It is well known throughout history that garlic is a safe, natural, remedy for variety of ailments, such as snake bites, parasitic infections, abdominal pains, rheumatism, and hemorrhoids, garlic can be used also to lower blood pressure and cholesterol levels, fight infections, for gastro-intestinal disorders, prevents cancer, and recommended as antimicrobial, anti-inflammatory, antithrombotic, and antitumor (Noman, 2016). Desired medicinal results of garlic are obtained when bulb are chewed and swallowed or mixed with food and eaten (Noman, 2016). Other effective uses of garlic include the hepatoprotective, antihelmentics, anti-inflammatory, and antioxidant, antifungal and wound healing Londhe *et al*., 2010.

The consumption of garlic in any form did not reduce blood cholesterol levels in the patient with moderately high baseline cholesterol level (Gardner, 2007). Garlic produces various sulfur compounds that together with their breakdown products, yield a characteristics pungent taste and odour, which may persist on the breath and body for up to 30 hours as garlic is metabolic (Block, 2010). Garlic preparations are used to treat insect stings and improve scar healing (Block, 2010).

Leyla *et al*., 2014 state that the exact mechanism of all ingredients and their long-term effects are not fully understood. Further studies are needed to elucidate the pathophysiological mechanisms of action of garlic as well as its efficacy and safety in treatment of various diseases.

MATERIALS AND METHODS

Materials used for this study include; weighing balance, inoculation loop, glassware, measuring cylinder, forceps, petri-dishes, distilled water, ethanol, and filter paper. Fehling solution A and B, Ferrichloride($Fecl_3$), heating mantle, hydrochloric acid(Hcl), tetraoxosulphate (vi)

acid(H_2SO_4), water bath, autoclave, incubator, culture media (Sorbitol Agar, Mannitol Agar, Samonelle and Shigella Agar, Nutrient Agar), Magnesium ribbon, sensitivity disc, blender, diethyl ethanol and garlic.

Collection of Specimen
Fresh garlic (Allium sativum) bulbs were purchased from Ekowe market in Southern Ijaw Local Government Area, Bayelsa State. The bulbs of the garlic were peeled and air dried for five days.

Sterilization of Materials
All glassware were washed with detergent and rinsed with distilled water. These materials were then sterilized using autoclave at 121^{o}C for 15mintues. The culture media namely; Salmonella and shigella at 121^{o}C for 15miuntes immediately it was corked using aluminum foil. The inoculating loop was also sterilized by heating to red hot over a flame before and after each inoculation on petri-dishes.

Preparation of Culture Media
1.25g of sorbitol agar was weighed and dissolves in 31.25ml of distilled water. 1.968g of salmonella and shigella (SS) agar was dissolved in 31.25ml of distilled water while 3.46g of mannitol was also dissolved in 31.25ml of distilled water according to the instruction given for the standard (direction for use). The conical flask was covered with cotton wool and aluminum foil was used to mask the mouth of the conical flask to avoid contamination. Thereafter, it was autoclaved at 121^{o}C for 15 minutes.

Collection and Maintenance of Test Organism
The test organisms that were used obtained in Hoffman and Bright Laboratory Hospital, Yenagoa, Bayelsa State from pregnant woman. These isolated organisms were Shigella spp, Salmonella typhi (spp), Staphylococcus aureus, and Escherichia coli purify plates of each of the bacterial isolates were obtained by culturing on their plates and each of the bacterial isolates were obtained by culturing on their respective selective media. Biochemical tests were performed to re-identify and confirm the isolates. Fresh plates of the test bacteria were made from the isolate cultures obtained on agar slants. Discrete colonies of the flash cultures of the different bacterial isolates were then picked and suspended in 5ml Nutrient broth and incubated for 24 hours at 37^{o}C prior to antibacterial susceptibility testing.

Extraction
150g of fresh sample of Allium sativum was obtained, peeled, blended and soaked in 200ml of distilled water for 6 hours in a sterile beaker. The crude extract was then filtered using sterile whatman's filter paper. The filtered extract was then heated using heating mantle until water is loosed. After exhaustive extraction, the pure sample was subjected to phytochemical screening for the examination of the chemical composition.

Phytochemical Screening
The aqueous extract of Allium sativum was screened for the presence of the secondary metabolites using the procedure of Sofowora (1993). Two (2) millimeters of each extract was placed in a test tube for each of the tests and concentrated by evaporating the extract in a water bath. Tests were carried out for carbohydrates, tannins, saponins, flavonoids, alkaloids and phenols.

Test for Carbohyftrate
1ml each of the Fehling solution A and B reagent were mixed together and 2ml of each the crude extracts was added and mixed together in a test tube. Thereafter, was boiled gently and a brick red precipitate was formed showing that carbohydrate is present.

Test for Flavonoids
2ml of the crude extract was put in a test tube and was mixed with few fragment of magnesium ribbon. Thereafter, concentrated hydrochloric acid was dropped gradually and a pink scarlet colour appeared showing the presence of Flavonoids.

Test for Phenols and Tannis
Crude extract was mixed with 2ml of 2% solution of Ferrichloride (Fecl3) in a test tube. Blue-green or black colouration indicated the presence of the phenols and tennis.

Test for Saponnis
Crude extract was mixed with 5ml of distilled water in a test tube and was shaken vigorously. The formation of stable foam showed that saponin is present.

Quantitative Determination of the Chemical Constituent
Determination of total carbohydrate
One milliliter (1ml) of the sample was pipette into a test tube followed by the addition of 1ml of 5% Phenol solution and 5ml of concentrated sulphuric acid H_2SO_4. These were mixed thoroughly and allowed to settle in a water bath set at 25 to 30 for 20 minutes and the absorbance was then measured at 490nm. These results were compared with a calibration curve to calculate the carbohydrates content.

Estimation of Total Flavonoid
One milliliter (1ml) of sample (1mg/ml) was mixed with three 3ml of methanol, 0.2ml of 10%. Aluminum chloride, 0.2ml of 1m potassium acetate and 5.6ml of distilled water and remains at room temperature for 30 minutes. This absorbance of the reaction mixture was measured at 420nm with UV visible spectrophotometer. The concentration of the Flavonoid was expressed in terms of mg/ml.

Determination of Saponin
The samples were grounded and 20g of each were put into a conical flask aqueous and ethanol. The samples were heated over a hot water bath for 4hours with

continues stirring at about 5 another 200ml 2/. The combined extracts were red, hot at about 90°C. The concentrate was transferred into a 250ml reparatory funnel and 20ml of diethyl ether layer was discarded. The purification process was repeated. 60ml was washed twice with 10ml of 5% Aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a cons weight, the Saponin content was calculated as percentage.

Determination of Total by Spectrophotometric Method

The fat free sample was boiled with 50ml of either for the extraction of the phenolic component for 15minutes. 50ml of the extract was pipetted into a 50ml flask, And then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amulet alcohol were also added. The samples were made up to mark and left to react for 30minutes for colour development, this was measured at 505nm.

Determination of Tannin Using Van-Burden Androbinsonmethod

500g of the sample was weighed into 50ml plastic bottle. 50ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtered was pipetted out into a tube and mixed with 2ml of 0.1m $fecl_3$ in 0.1m HCL and 0.008m potassium Ferro cyanide. The absorbance was measured at 120nm within 10 minutes.

Alkaloid Determination Using Harborne Method

5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Quality Assurance of the Method

Quality assurance of a method is a way of measuring, maintaining the quality of the method. These include;

1. Bacterial isolates were cultured on their respective selective media.
2. All glass wares used were acid washed and oven dried before use.
3. All reagents used were of high quality control grade.

RESULTS

Table 1: Test for Various Chemical Compounds.

Chemical Constituent	Tests	Observation of the Equeous	Inference
Carbohydrate	1ml of each solution of felhing A and B reagent + 2ml of the crude extract and boiled	Brick-red Precipitate formed	+++
Saponin	2ml of crude extract + 5ml of distilled water + vigorous shaken	Foamy Solution formed	+++
Tanins and phenol	2ml of crude extract +2ml of 2% solution $FecL_3$	Blue green or black colouration formed	+++
Alkaloids	2ml of crude extract +2ml of 1% Hcl and heated +Mayer's And Wagner's reagent	Turbidity of the orange precipitate formed	+++
Flavonoid	Crude extract + Magnesium Ribbon + concentration Hcl	Pink scarlet coloration formed	+++

Key: +++ presence.

Table 2: Quality Analysis.

Constituent(mg/ml)	Allium Sativum Aqueous Extract
Carbohydrate	80
Saponin	20
Phenol	7.35
Flavonoid	6.50
Tannin	1.1
Alkaloids	0.9

Table 3: Antibacterial Screening Showing Zone of Inhibition (Zone Of Clearance).

Concentration (mg/ml)	Escherichia coli	Shigela specie	Salmonella specie	Staphylococcus aureus
25	3	2.5	3	2.5
50	6.5	4.5	6.5	5.5
100	11.5	7.5	9	8.5
200	16.5	12	13	13.5

DICUSSION

The phytochemical screening of garlic plant has shown that carbohydrate (80mg/ml) and alkaloid (0.9mg/ml), phenol (7.32mg/ml), flavonoid (6.5 mg/ml), were abundantly present as chemical constituents found in garlic extract which corroborates the result of (Okigbo *et al*. 2009b).

From this present result, garlic contains alkaloids which have been documented to possess analgesic, antispasmodic and bactericidal effects, which underpins the result of (Okigbo *et al*. 2009b). The antibacterial screening indicates that garlic had the best antibacterial activity against staphylococcus aureus, Escherichia coli, and Salmonella and Shigella species. This is in line with work done by (El-Mahmood, 2009). Since garlic possess alkaloids which are medically useful by acting on the peripheral nervous system or directly acting on the brain. Prominent among them are the pain receivers morphine and the codeine of which the latter alkaloids is an effective cough suppressant. Other alkaloid like quinine is useful in the reduction of fever (http://science.jrank.org./pages/232/Alkaloid.html" Alkaloid –Role in the plants, Role in Animals, Mediacal use, Alkaloid for pain and pleasure.).

Result also indicates garlic have possess the capacity to regulate and boost the immune system, and also act as an anti-inflammatory and antibacterial agent because it contains the foamy steroid called saponin which tend to exhibit these functional characteristics which depict the findings of (Piicncu, 2018). In addition to these functional traits, garlic promotes cardiovascular integrity by lowering cholesterol and body fats levels and reduce cancer also by keeping blood sugar within normal range which are also a functions of this foamy saponin in garlic which has been stated by (Londhe *et al*., 2010; Sanjay and Subir, 2002; Khalid and Gordon, 2006).

From this present study, garlic have shown to contain flavonoid which function to reduce the risk of stroke, asthma and heart disease which is correlates the findings of (Waris and Tabinda, 2013).

Result also showed that garlic function as an antiseptic and disinfectant agent by possessing phenol as one of its components. The role of garlic in skin maintenance has also been exposed by the action of tannin in garlic.

CONCLUSION

The results of this present research have shown the phytochemical components and antibacterial potentials of garlic plant extract which is useful in combating organisms such as staphylococcus auerus, Escherichia coli, Salmonella and Shigella species. It has also established the presence of carbohydrates, phenol, flavonoids, and alkaloids and amongst others. Thus, the use of garlic as a spice, fruit and nut is safe and medicinal.

We recommend,

1. The use of garlic as dietary meal either in raw (chewing) form or add as a spice for daily food.
2. Regular propagation of the garlic plant.
3. Frequent screening of other plants as a way to discover hidden vegetables plant that might have the potentials in the treatment of infection and disease.

REFERENCES

1. Al-Zahim, N; Newberry, J. H. And Ford-Lloyd, B. V. (1997). Classification of genetic variation in garlic (Allium sativum 1.) Hortscience, 36: 1102-114.
2. Amagase, H. (2006). Clarifying the real bioactive constituents of garlic. J. Nutr; 136: 716 - 725.
3. Amagase, H., Petesch, BI. Matsuura, H., Kasuga, S., Itakura, Y. (2003). "intake of garlic and its bioactive components". J. Nutr, 131(3s): 955s -625. PMID 11238798.
4. Ankri S. and Mirelman D. (1999). Antimicrobial Properties of Allium from Garlic. Microbes and infection, 1(issue 2): 125 - 129.
5. Banerjee, SK; Mukherjee, PK; Maulik, SK (2001). "Garlic as an Antioxidant: The Good, Bad, and the Ugly". Phytotherapy Research, 17(2): 97 - 106.
6. Block, E. (2010). Garlic and other Alliums: the lore and the science. Cambridge, UK: Royal Society of Chemistry.
7. Block, E Dane AJ, Thomas S, Cody RB (2010). Applications of Direct Analysis in real Time-Mass Spectrometry (DART-MS) in Allium Chemistry. 2-propenesulfenic and 2-propene-sulfinic Acid, Dially Journal of Agricultural and Food Chemistry, 58(8): 4617 - 4625.
8. Cutler, RR and Wilson P. (2014). "Antibacterial Activity of a New, Stable, Aqueous Extract of Allicina against Methicilla-Resistant Staphylococcus auerus". British Journal of Biochemical Science, 61(2): 71 - 4.
9. El-mahmood Muhammad. (2009). Efficacy of Crude Extracts of Garlic (Allium sativumlinn) against Nosocomial Escherichia coli, Staphylococcus aureus, Streptococcus pneumonia and Pseudomonas

aeruginosa. Journal of Medical Plants Research, 3(4): 179 - 185.
10. Garder CD, Lawson LD, Block E, et al. (2007). "Effect of Raw Garlicvs Commercial Supplements on Plasma Lipids Concentration in Adults With Moderate Hypercholestrolemia: a Randomized Clinical Trial". Arch. Itern. Hem. Med., 167(4): 346 – 53.
11. http://science.jrank.org./pages/232/Alkaloid.html" Alkaloid –Role in the plants, Role in Animals, Mediacal use, Alkaloid for pain and pleasure.
12. Johnson, M. Maridass, M and Irudayaraj, V. (2008). Preliminary Phytochemical and Anti-Bacterial Studies on Passififlora Edulis. Ethno botanical Leaflets, 12: 425 – 432.
13. Khalid, R. and Gordon, M. L. (2006). Garlic and Cardiovascular Disease: A Critical Review. *Journal of Nutrition.*, 136(3): 736S – 740S.
14. Leyla, B., Peir, H. K. and Ali, G. (2014).Garlic: A review of potential therapeutic effect. Avicenna J Phytomed., 4(1): 1 – 14.
15. Llic, Dusica; Nikolic, Vesna; Ciric, Ana; Sokovic, Marina: Stanojkoic, Tatjana; Kundakovic, Mihajilo; Ljubisa. (2012). "Cytotoxity and antimicrobial activity of Allium and its Tramsformation products" Journal of Medicinal Plants Research, 6(1): 59 – 65.
16. Londhe, V., Gavasane, A. T., Nipate, S.S and Chaudhari P.D. (2011). Role of garlic (Allium sativum) in various disease: an overview.
17. Noman D Salih (2016). The Antimicrobial effects of Mixed Extract of Allium sativum and Syzgium aromaticum Against Pathogenic microbes Triggering Inflammation in Asthma and Sinusitis patients.
18. O' Gara, EA, Hill, DJ, Maslin, DJ. (2000). Activities of garlic oil, Garlic powder, and their diallyl constituent against Helicobacter pylori. Appl. Environ. Microbial, 66: 2269 – 2273.
19. Okigbo, R.N; Anuagasi, C.I. and Amadi, J.E.(2009a). Activities in Selected medicinal and aromatic plants indigeneous to Africa. Journ. Medicinal Plants Research, 3(2): 086 – 095.
20. Okigbo, R.N; Anuagasi, C.I; Amadi, J.E. and U.J. (2009b). Potential Inhibitory Effects of some Africa Tuberous Plant Extracts on Escherichia coli, Staphylococcus aureus and Candida albicans. International Journal of Integartive Biology, 6(2): 91 – 98.
21. Peter B. B., Patrick M, F., and Pina L., Potential Health Benefits of Garlic (Allium sativum): A Narrative Review. *Journal of Complementary and Integrative Medicine.* Volume 5, issue 1 Article 1.
22. Picincu, A. (2018). What are the Health Benefits of Saponin?
23. Ross, Z.M; Maslin, D.J. and Hill, D.J. (2000). The effect of steam Distilled Garlic oil on lactic acid and other enteric bacteria 4th symposium on European Microbiological societies FEMS Microbial. Rev., 12(G43): 137.
24. Snajay, K.B. and Subir, K.M. (2002). Effect of garlic on cardiovascular disorders: A review. *Nutrition journal.* 1:4.
25. Sofowora, A. (1993). Medicinal Plants and Medicine in Africa. Spectrum Books, Ibadan, Nigeria. Pp. 120 – 123.
26. Vaidya, Vipraja; Keith U, in gold; Derek. A. Pratt. (2009). "Garik" Source of the Ultimate Antioxidants-sulfenic Acids". Angeandte Chemic., 121(1): 163 – 6.
27. Waris, Q. and Tabinda, A. (2013). Role of Garlic Usage in Cardiovascular Disease Prevention: An Evidence-Based Complementary and Alternative Medicine. Volume 29.