

Full Length Research Paper

Phytochemical screening of the leaf extracts of *Hyptis spicigera* plant

Z. Ladan^{1*}, J. O. Amupitan², O. A. Oyewale², R. G. Ayo³, E. Temple² and E. O. Ladan⁴

¹National Research Institute for Chemical Technology, Private Mail Bag 1052, Zaria, Nigeria.

²Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.

³Division of Agricultural Colleges, Ahmadu Bello University, Zaria, Nigeria.

⁴National Agricultural Extension and Research Liaison Services, Ahmadu Bello University, Zaria, Nigeria.

Received 4 March, 2014; Accepted 7 May, 2014

The present study reports the screening of phytochemical constituents of the leaf extracts of *Hyptis spicigera* using hexane, ethylacetate and methanol and the leaf powder of the plant. Qualitative analysis of phytochemical constituents showed the presence of the following secondary metabolites vitamins, carbohydrates, saponins, flavonoids, steroids, alkaloids, quinones, coumarin, terpenoids, resins and cardiac glycosides. The quantitative analysis of total phenolics, alkaloids, saponins, terpenoids and flavonoids carried out using standard protocols revealed the presence of flavonoids (8.82%), saponins (6.23%), terpenoids (16.10%), alkaloids (7.55%) and phenolics (20.75%) respectively. Phenolics showed the highest content (20.75%) while saponins (6.23%) gave the least content. The high content of phenolics in the plant showed that *H. spicigera* plant may contain antioxidant properties and could be a good source of natural antioxidants. Also, the richness in flavonoids, saponins, alkaloids and terpenoids in this plant can be correlated with its medicinal properties used by traditional herbal healers in Northern Nigeria.

Key words: *Hyptis spicigera*, phytochemical screening, secondary metabolites.

INTRODUCTION

Plants have been the subject of human curiosity and use for thousands of years (Ram et al., 2004) and have played important roles in many countries of the world for centuries by providing food, shelter, clothing, agrochemicals, flavours and fragrances and more importantly, medicines (Gurib-Fakim, 2006). Traditional people have relied on medicinal plants to combat various

ailments caused by microorganisms such as bacteria, fungi and viruses that infect the body system. Plants have indeed formed the basis of sophisticated traditional medicine systems which will continue to provide mankind with new remedies for all forms of ailments (Gurib-Fakim, 2006).

Bioactive natural products have enormous economic

*Corresponding author. E-mail: zakariladan@gmail.com

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](http://creativecommons.org/licenses/by/4.0/)

importance as specialty chemicals as they can be used as drugs, lead compounds, biological or pharmaceutical tools, feed stock products, excipients and nutraceuticals (Pieters and Vlietinck, 2005). In recent times, focus on plant research has increased all over the world and a large body of evidence and knowledge has accumulated in the literature to show immense potential of medicinal plants used in various medical, pharmaceutical, cosmetic, agrochemical applications. An advantage of natural bioactive molecule is that they have a milder side effect on the body in comparison to chemically synthesized drugs (Badisa et al., 2003). With the increasing acceptance of herbal medicines as alternative form of health care delivery, the screening of medicinal plants for bioactive compounds is imperative (Masoko et al., 2005; Cowan, 1999).

Hyptis spicigera belongs to the family Lamiaceae and is commonly known as Black beniseed, or Black sesame. It is an erect aromatic herb, up to 1 m in height, with a terminal inflorescence in which the seeds are packed in quadruplets or more in the flowers. The plant is found around Senegal to western Cameroon, possibly native to Brazil, now widely naturalized in tropical Africa and Asia as well as Nigeria. It grows naturally and commonly as a weed. It prefers roadsides, waste places, cultivated places and often damp places (Burkill, 1995). Generally, the whole plant is used in traditional stores to protect cowpea against damage by *Callosobruchus* species (Lambert et al., 1985). The Bajju and Atyapp people of Kaduna state, northern Nigeria, make use of the inflorescence (where the seeds are packed) to cure headaches by sniffing it and also crushing the leaves and applying to the head to relieve head colds and headaches (Dalziel, 1937).

This paper reports the phyto constituents of the leaf extracts of *H. spicigera* and their potential medicinal applications.

MATERIALS AND METHODS

Collection of the plant

About 500 g of the leaf part of *H. spicigera* was collected in Basawa Village, Zaria, Kaduna state, Nigeria on the 26th November, 2013. It was taxonomically identified and authenticated by Mallam U. S. Gallah of the Herbarium Section, Department of Biological Science, Ahmadu Bello University Zaria, Nigeria, and a sample Voucher No.528 was deposited at the Herbarium section of the Department of Biological sciences.

Extraction and isolation

The plant was dried in the shade for 14 days and pulverized to powder using pestle and mortar in the Laboratory. Approximately 450 g of the powdered plant material was macerated sequentially with hexane (1 L), ethyl acetate (1 L) and methanol (1 L) at room temperature (27°C) and concentrated *in vacuo* to afford the various crude extracts which were stored in the refrigerator (4°C) until needed for further analysis.

Phytochemical screening

Phytochemical analysis of the crude extracts was carried out according to standard methods (Harborne, 1998; Sofowora, 1993; Farnsworth, 1996; Rangari, 2002).

Salkowski reaction test for phytosterols

To 0.5 ml each of the extracts in a test tube was added 1.0 ml of concentrated H₂SO₄ (conc.) from the sides of the test tube and then 1.0 ml chloroform. Appearance of reddish brown colour in chloroform layer indicates the presence of phytosterols.

Liebermann-Burchard's test for triterpenoids

Extracts were treated with few drops of acetic anhydride, boil and cool. Conc. sulfuric acid was added from the sides of the test tubes which showed a brown ring at the junction of two layers, and formation of deep red color indicated the presence of triterpenoids.

Foam test for saponins

Small amount (0.1 g) of the extracts were taken in test tubes with little quantity (1.0 ml) of water and shaken vigorously. Appearance of foam persisting for 10 min indicated presence of saponins.

Dragendroff's test for alkaloids

About 0.5 g each of hexane, ethyl acetate and methanol extracts were dissolved in 1.0 ml chloroform and evaporated. The residue was acidified by adding few drops of Dragendroff's reagent (Potassium bismuth iodide). Appearance of orange red precipitate indicated presence of alkaloids.

Molisch's test for carbohydrates

About 0.5 g each of the extracts was mixed with Molisch reagent, and then added H₂SO₄ conc. along the sides of the test tube to form layers. Appearance of reddish violet ring the interference indicated the presence of carbohydrates.

Lead acetate test for flavonoids

To 0.1 g each of the extracts were dissolved in ethanol and few drops of 10% lead acetate solution were added. Appearance of yellow precipitate indicated presence of flavonoids.

Legal's test for lactones

To 0.1 g each of the extracts 1.0 ml sodium nitroprusside and 1.0 ml pyridine were added in test-tubes. The mixtures were treated with 0.01 mol dm⁻³ NaOH. Appearance of deep red colour indicated the presence of lactones.

Ferric chloride test for phenolic compounds and tannins

About 2.0 ml of each extract was measured in a test tube and 0.01 mol dm⁻³ Ferric chloride solution was added drop by drop. Appearance of bluish black precipitate indicated presence of phenolic compounds and tannins.

Ninhydrin test for proteins

Few drops of ninhydrin were added to the extracts. Appearance of blue colour indicated presence of amino acid. Proteins may rarely give positive result with this test.

Keller-Killiani test for glycosides

About 1 ml of glacial acetic acid, few drops of 0.01 mol dm^{-3} Ferric chloride solution and H_2SO_4 (Conc) slowly through the sides of the test tube) were added to the extracts. Appearance of reddish brown ring at the junction of the liquids indicated the presence of de-oxy sugars.

Quantitative determination of phytochemical constituents

Determination of total phenolic compound (TPC)

Total phenolic content of the hexane, ethylacetate and methanolic extracts was determined by standard method (Makkar et al., 1993) with little modifications, using tannic acid as a standard phenolic compound. The extracts were diluted with distilled water to a known concentration in order to obtain the readings within the standard curve range of 0.0 to 600 μg of tannic acid/ml. 250 μl of diluted extract or tannic acid solution was mixed with 1 ml of distilled water in a test tube followed by the addition of 250 μl of Folin-Ciocalteu reagent. The samples were mixed well and then allowed for 5 min at room temperature in order to allow complete reaction with the Folin-Ciocalteu reagent. Thereafter, 2.5 ml of 7% sodium carbonate aqueous solution was added and the final volume was made up to 6.0 ml with distilled water. The absorbance of the resulting blue colour solution was measured at 760 nm using spectrophotometer after incubating the samples for 90 min. All the experiment was conducted in three replicates.

Determination of alkaloids

About 5.0 g of the dried powdered plant was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. The mixture was covered and allowed to stand for 4 h. This was filtered and the extract concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide solution was added dropwise to the extract until the precipitation was complete. The solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and finally filtered, dried, weighed and the percentage alkaloid was calculated (Harborne, 1998).

Determination of total terpenoids

About 2 g of the plant leaf powder was weighed and soaked in 50 ml of 95% ethanol for 24 h. The extract was filtered and the filtrate extracted with petroleum ether (60 to 80°C) and concentrated to dryness. The dried ether extract was treated as total terpenoids (Ferguson, 1956).

Determination of saponins

About 15 g of each sample was placed into a conical flask and 100 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 200 ml and 20% ethanol. The combined extracts were reduced to 40 ml

over water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml 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 constant weight and the percentage saponin was calculated (Obdoni and Ochuko, 2001).

Determination of flavonoids

About 5.0 g of the plant sample was weighed and extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 41. The filtrate was evaporated into dryness over a water bath and weighed to a constant weight. The percentage flavonoids was then calculated (Soni and Sosa, 2013).

RESULTS AND DISCUSSION

The extracts of the leaves of *H. spicigera* were screened for the presence of the following secondary metabolites: alkaloids, glycosides, flavonoids, carbohydrates, tannins, steroids, terpenoids and resins, coumarins, saponins and quinines. The results of the phytochemical screening showed the presence of all the secondary metabolites analyzed in ethylacetate and methanol extracts while hexane extract showed only the presence of alkaloids, glycosides, carbohydrate and resins. Other secondary metabolites such as flavonoids, tannins, steroids, terpenoids, coumarins, saponins and quinones were absent in the hexane extract.

The phytochemical content was found to be similar to that obtained by other authors (Onayade et al., 1991) with different extracts revealing the different partitioning abilities of the different solvents used. The presence of these phytochemicals in all the extracts is quite instructive as this lends credence to the use of the plant for medicinal purposes. A lot of plants contain non-toxic glycosides that can be hydrolyzed to give phenolic compounds that are toxic to microbial pathogens (Abaoba and Efuwape, 2001). The saponin content in the ethylacetate and methanol extracts were found to be present in these extracts, respectively. Saponins possess the property of precipitating and coagulating red blood cells (Sodipo et al., 1991). It also foamed in aqueous solution and has hemolytic effect and can also bind on cholesterol sites. These properties make saponins present in the plant to exhibit medicinal properties (Sodipo et al., 1991) and this therefore supports the findings in this present study that extracts of the plants may be useful in chemotherapy of mycotic infections which the antimicrobial studies revealed (Ladan et al., 2009). Alkaloids were found present in hexane, ethylacetate and methanol extracts and this can be corroborated with literature reports which indicate that naturally occurring alkaloids and their synthetic derivatives

Table 1. Qualitative phytochemical analysis of the leaf extracts of *Hyptis spicigera*.

Phytochemical constituents	Leaf extracts		
	Hexane	Ethylacetate	Methanol
Tannins	-	+	+
Saponins	-	+	+
Steroids	-	+	+
Flavonoids	-	+	+
Alkaloids	+	+	+
Terpenoids	-	+	+
Glycosides	+	+	+
Coumarins	-	+	+
Carbohydrates	+	+	+
Quinones	-	+	+
Resins	+	+	+

have analgesic, antispasmodic and bactericidal activities (Okwu and Okwu, 2004). They exhibit marked physiological activity when administered to animals. Classes of alkaloids are among the major powerful poisons known and despite being poisonous, some of the alkaloids are known to be useful in correcting renal disorders (Konkwara, 1979). The use of some plants for medicinal purpose, in the traditional treatment of diseases is due to the presence of flavonoids and saponins (Zwadyk, 1992; Othira et al., 2009), hence the use of *H. spicigera* for the treatment of diarrhea, dysentery, colds and several other diseases by local herbalists or traditional healers is not surprising. The presence of flavonoid was evident in methanol and ethylacetate extracts, flavonoid containing plants have been used as diuretic, laxative, emollient and poultice (Baba-Mousa et al., 1999) therefore; the use of *H. spicigera* rich in saponins and other *Hyptis* species in traditional medicine lent credence to the medicinal potentials of the plant. Tannins in some medicinal plants have been found to be responsible for the antiviral and antibacterial activities exhibited by such plants (De-Ruiz et al., 2001; Elegani et al., 2002). Therefore, *H. spicigera* with high tannin content in ethylacetate and methanol extracts could probably be a source of phytochemicals for the treatment of bacterial infections. Phenolic compounds like tannins present in plant cells are inhibitors of many enzymes (proteolytic and hydrolytic) used by plant pathogens. Other compounds such as saponins have antifungal properties (Abaoba and Efuwape, 2001; Mohanta et al., 2007). Therefore, these phytochemicals detected in this study may be responsible for the antimicrobial potency of the leaf extracts of *Hyptis spicigera* and also lend credence to the claims of traditional application of the plant as remedies for various ailments.

Quantitative phytochemical analysis

Results of the quantitative analysis data of the plant

material revealed significant levels of phytochemical constituents present in the leaf as evident in the qualitative analysis data (Table 1). Phenolic content (20.75%) is the highest followed by terpenoids content (16.10%) while flavonoid (8.82%), saponins (6.23%) and alkaloids (7.55%) followed respectively. Subhashini et al. (2013) and Soni and Sosa (2013) have reported various phyto constituents in the leaves of *Ecbolium viride* (Forks) Merrill plant and the methanolic and ethyl acetate extracts of the leaves of *Anogeissus leiocarpus* and found the following values: terpenoids (0.3034 w/w), saponins (0.1100 w/w), alkaloids (0.1340 w/w), flavonoids (0.0884 w/w) and phenols (0.03045) for the *E. viride* (Forks) while alkaloids (152.0 ± 0.1 mg/g), phenolics 1294.81 ± 3.0 mg/g, flavonoids (330.7 ± 3.0 mg/g) in the methanol extract and alkaloids (80.20 ± 0.0 mg/g), phenolics (616.5 ± 4.4 mg/g), flavonoids (202.5 ± 4.0 mg/g) in the ethyl acetate extract of the *A. leiocarpus* plant have been reported. The quantitative values of these metabolites reported in the leaf part of *H. spicigera* are higher (Table 2) than those reported for *E. viride* (Forks) Merrill and *A. leiocarpus* plants. Phenolic compounds are one of the most important constituents of plant secondary metabolites with marked physiological properties.

The phyto constituents found in the plant may be responsible for its biological properties such as anti-oxidative, anti-inflammation, anti-carcinogenic, anti-hypertensive, anti-diabetic, anti-cancer, cardiovascular protection and improvement of endothelial function (Han et al., 2007). Several studies have described the anti-oxidant properties of different parts of various medicinal plants which are rich in phenolic compounds (Brown and Evans, 1998; Krings and Berger, 2001; Malencic et al., 2007). Natural anti-oxidants mainly come from plants in the form of phenolic compounds, such as flavonoids, phenolic acids, tocopherol etc (Ali et al., 2008) and used for the treatment of degenerative diseases. The anti-oxidative properties of flavonoids are due to several

Table 2. Quantitative analysis of the leaf part of *Hyptis spicigera*.

Phytochemical constituents	Yield (g)	Yield (%)
Total Flavonoids	0.44	8.82
Terpenoids	0.32	16.10
Total Saponins	0.94	6.23
Total Alkaloids	0.38	7.55
Total Phenolics	0.62	20.75

different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation (Akinmoladun et al., 2007; Benavente-Garcia et al., 1997). This plant (*H. spicigera*) will provide the natural anti-oxidant needed to enhance good living by scavenging free radicals that cause ill health in humans.

Conclusion

Phytochemical screening of the leaf part of *H. spicigera* revealed the presence of tannins, carbohydrates, saponins, flavonoids, steroids, alkaloids, quinones, coumarin, terpenoids, resins and cardiac glycosides which are important secondary metabolites. The richness of the plant in phenolic contents and other secondary metabolites affirmed its medicinal efficacy and potentials. The finding from this study therefore suggests that the leaf could be a potential source of natural anti-oxidant that could have great importance as therapeutic agents in preventing or slowing ageing associated with oxidative stress and related degenerative diseases. It is recommended that further investigation on the isolation and characterization of the bioactive constituents of the leaf leading to structural elucidation is necessary.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are grateful to the Management of National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria for providing necessary chemicals and equipment for the conduct of this research work.

REFERENCES

Abaoba OO, Efuwape BM (2001). Antibacterial properties of some Nigerian species. *Biol. Res. Comm.* 13:183-188.
 Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola BL, Onibon TR, Akinboboye AO (2007). Chemical constituents and antioxidant

activity of *Alstoniaboonei*. *Afr. J. Biotechnol.* 6(10):1197-1201.
 Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahuand A (2008). Indian medicinal herbs as source of antioxidants. *Food Res Int.* 41:1-15. <http://dx.doi.org/10.1016/j.foodres.2007.10.001>
 Baba-Mousa F, Akpagana K, Bouchet P (1999). Antifungal activities of seven West African Combretaceae used in traditional Medicine. *J. Ethnopharm.* 66:335-338. [http://dx.doi.org/10.1016/S0378-8741\(98\)00184-6](http://dx.doi.org/10.1016/S0378-8741(98)00184-6)
 Badisa RB, Tzakou O, Couladis M, Pilarinou E (2003). Phytotherapy Research "Cytotoxic Activities of some Greek labiatae Herbs" 17(5). Wiley and sons Ltd. U.S.A. pp. 472-475.
 Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del-Rio JA (1997). Uses and properties of Citrus flavonoids. *J. Agric. Food Chem.* 45(12):4505-4515. <http://dx.doi.org/10.1021/jf970373s>
 Brown JE, Rice-Evans CA (1998). Luteolin rich artichoke extract protects low density lipoprotein from oxidation *in vitro*. *Fr. Rad. Res.* 29:247-255. <http://dx.doi.org/10.1080/10715769800300281>
 Burkill HM (1995). The useful plants of West Tropical Africa Royal Botanic Gardens Kew 4(2):88-144.
 Cowan MM (1999). Plant products as antimicrobial agents. *Clin. Microb. Rev.* 12(4):564-582. PMID:10515903, PMCID:PMC88925
 Dalziel JM (1937). Useful plants of West Tropical Africa. Crown Agent for Overseas Governments, London. pp. 462-463.
 De-Ruiz REL, Fusco RMD, Angela S, Sohar OR (2001). Isolation of flavonoids and anthraquinones of *Amaranthus muricatus* (Moquin) ex Hicken (Amarathaceae). *Acta Farm. Bon.* 20:9-12.
 Elegani AA, El-nima MSEI, Muddathir AK (2002). Antimicrobial activity of some species of the family Combretaceae. *Phytoth. Res.* 16:551-561.
 Fansworth NR (1996). The ethno-botanical approach to drug discovery: strengths and Limitations, In: Prance G.T.9Eds), *Ethno-botany and the search for new drugs*. In: Ciba. pp. 100-150.
 Gurib-Fakim A (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Mol. Asp. Medi.* 27:1-93. <http://dx.doi.org/10.1016/j.mam.2005.07.008>, PMID:16105678
 Han X, Shen T, Lou H (2007). Dietary polyphenols and their biological significance. *Int. J. Mol. Sci.* 9:950-988.
 Harborne JB (1998). *Phytochemical methods*. Chapman and Hall Ltd., London. pp. 100-200.
 Konkware JO (1979). *Medicinal plants of East Africa*. Literature Burea, Nairobi. pp. 3-15.
 Krings U, Berger RG (2001). Antioxidant activity of roasted foods. *Food. Chem.* 72:223-229. [http://dx.doi.org/10.1016/S0308-8146\(00\)00226-0](http://dx.doi.org/10.1016/S0308-8146(00)00226-0)
 Ladan Z, Amupitan JO, Okonkwo EM, Aimola IA, Habila N (2009). Antimicrobial potency of *Hyptis spicigera* leaf extracts against some pathogenic microorganisms. *J. Med. P. Res.* 3(11):905-908.
 Lambert J, Arnason JT, Philoge-Ane BJR (1985). Bruchid control with traditionally used insecticidal plants *Hyptis spicigera* and *Cassia nigricans*. *Ins. Sci. Appl.* 6:167-170.
 Makkar H, Bluemmel M, Borowy N, Becker K (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J. Sci. Food Agric.* 61:161-165. <http://dx.doi.org/10.1002/jsfa.2740610205>
 Malencic D, Popovic M, Miladinovic J (2007). Phenolic content and antioxidant properties of soybean (*Glycine max*, L. Merr Seeds. *Mol.* 12:576-581. <http://dx.doi.org/10.3390/12030576>
 Masoko P, Picard J, Eloff JN (2005). Antifungal activities of six South

- African Terminalia species. J. Ethnopharmacol. 99:301-308.
<http://dx.doi.org/10.1016/j.jep.2005.01.061>
- Mohanta TK, Patra JK, Rath SK, Pal DK, Thatoi HN (2007). Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semecarpus anacardium* L. Sci. Res. Ess. 2(11):486-490.
- Obdoni BO, Ochuko PO (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. Glob. J. Pure Appl. Sci. 8b:203-208.
- Okwu DE, Okwu ME (2004). Chemical composition of Spondias mombin plant parts. J. Sus. Agric. Environ. 6(2):140-147.
- Onayade OA, Looma A, Scheffer JJ, Svendsen AB (1991). Composition of the herb essential oil of *Hyptis spicigera* Lam. Flav. Frag. J. 5:101-105. <http://dx.doi.org/10.1002/ffj.2730050209>
- Othira JO, Onek LA, Deng LA, Omolo EO (2009). Insecticidal potency of *Hyptis spicigera* preparations against *Sitophilus zeamais* L and *Tribolium castaneum* (herbst) on stored maize grains. Afr. J. Agric. Res. 4(3):187-192.
- Pieters L, Vlietinck AJ (2005). Bio-guided isolation of pharmacologically active plants components, still a valuable strategy for the finding of new lead compounds. J. Ethnopharmacol. 100:57-60.
<http://dx.doi.org/10.1016/j.jep.2005.05.029>
- Ram AJ, Bhakshu LM, Raju RRV (2004). *In vitro* antimicrobial activity of certain medicinal plants from Eastern Ghats India used for skin diseases. J. Ethnopharm. 90:353-357.
- Rangari VD (2002). Pharmacognosy and phytochemistry. Nasik:Carrier Pub. pp. 100-150.
- Sodipo OA, Akanji MA, Kolawole FB, Adetuga OO (1991). Saponins is the active antifungal principle in *Garcinia kola*, heckle seed. Biol. Sci. Res. Comm. 3:171.
- Sofowora A (1993). Medicinal plants and traditional medicine in Africa, New York, John Wiley and Sons, pp. 191-300.
- Soni A, Sosa S (2013). Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. J. Pharm. Phytochem. 2(4):22-29.
- Subhashini S, Poonguzhali TV Madha VS (2013). Quantitative phytochemical analysis of *Ecbolium viride* (Forks) Merrill and *Justicia gendarussa* Burm F. Int. J. Cur. Tr. Res. 2(1):34-37.
- Zwadyk P (1992). Enterobacteriaceae in Zinsser Microbiology, 20th Ed. Gerogthieme Verlag, Stuttgart. P. 87.