Research Article
Journal of Research and Review in Science, 168-173
Volume 4, December 2017
DOI:10.36108/jrrslasu/7102/40(0142)

ORIGINAL RESEARCH

Phytochemical Screening And Biocidial Studies Of Stem Bark *Chrysophyllum Albidum* (Linn) And Straw *Aristolochia Ringens.*

Moses S. Owolabi[1*], Omowonuola, A. Adenike[1], Oladipupo A. Lawal[1], Avoseh N. Opeyemi[1], Ogunwande I. Ajani[1], William N. Setzer[2]

[1]Department of Chemistry, Lagos State , University, PMB 001, Ojo, Lagos, Nigeria

[2]Department of Chemistry, University of Alabama in Huntsville Huntsville, Alabama 35899, USA.

Correspondence
**Moses S. Owolabi*
Department of Chemistry, Lagos State , University, PMB 001, Ojo, Lagos, Nigeria
Sunnyconcept2007@yahoo.com

Abstract:

Introduction: Medicinal plants play a significant role in the health maintenance in underdeveloped countries, herbs and spices continue to serve as a new sources for herbal medicines .

Aims: The objective was to investigate phytochemical screening and biocidal activity on stem bark *Chrysophyllum albidum* (Linn) and straw *Aristolochia ringens* using chloroform.

Materials and Methods: Chloroform extracts of *C. albidum* and *A. ringens* were obtained and screened for phytochemical constituents. The colour intensity or the precipitate formation was used as analytical responses to different tests. The Antimicrobial activities of the crude extracts were evaluated against some microorganisms with broth microdilution test. The extracts were screened for in-vitro cytotoxic activity against liver tumour and breast tumour cells.

Results: The results of the phytochemical screening revealed the presence of alkaloids, saponins, steroids, anthraquinone and glycosides. *C. albidum* and *A. ringens* chloroform extracts showed high quantities of alkaloid and flavonoid respectively. While cardiac glycoside and anthraquinone were found with low quantity in *C. albidum* and *A. ringens* respectively. *C. albidum* showed promising bacterial activity against *Escherichia coli* and cytotoxic activity to liver tumour cells. While *A. ringens*, showed excellent bacterial activity against *Bacillus cereus* and good fungal activity against *Candida albicans*. *A. ringens* also showed good cytotoxic to breast tumour and liver tumour cells. The observed antimicrobial and cytotoxic effects may be due to the antagonist or synergistic effect of the secondary metabolites identified in the chloroform extracts. .

Conclusion: The secondary metabolites of *C. albidum* and *A. ringens* shows promising potential which support their consistent use in herbal medicines.

Keywords: *Chrysophyllum albidum, Aristolochia ringens, Chloro- form,* Antimicrobial activity, cytotoxicity.

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

Ever since ancient days, medicinal plants constitute one of the major sources to obtain a wide range of biological and pharmacological active compounds which have played a significant role in human society for treatment of wide array of ailments in various communities. Thus, extracts from herbs and spices have continue to play a very important role in all divisions of human population either directly as folk medicines or indirectly in the preparation of recent drugs used as antioxidant, antibacterial, antiparastic, antifungal, cytotoxic, anti-*Fusarium* actiivites for food safety and consumption [1-7].

Chrysophyllum albidum (Linn) commonly known as white star apple is a Tropical forest fruit tree belongs to the Sapotaceae family with about 800 species [8]. *C. albidum* is native to West and Central Tropical Africa [9-11] . In Nigeria, it is found along forest savannah transitional and coaster savannah zones. The plant often grows to a height of 25-37 m in height with a mature girth varying from 1.5 to 2 m [12]. The tree has dark green, pale tawny with silver-white when mature, flowers appear on the leaf axils and fruit spherical in diameter, greenish-grey when unripe, turning orange-red when matured. Locally the plant is named 'Agbalumo', (Yoruba) 'Udara' (Igbo) according to the specific area. *C. Albidum* has been used traditional in herbal medicine to treat malaria and yellow fever, while the leaf is used as an emollient and for the treatment of skin eruption, stomach ache and diarrhea [13]. Studies have reported that seeds and roots extracts of *C. albidium* have demonstrated good antimicrobial, anti-inflammatory, anti-diarrheal and anti-hemorrhoidal properties [14].

Aristolochia ringens Vahl, one of the 500 aromatic species of *Aristolochiaceae* family, native to Brazil, Central America Mediterranean countries and the Caribbean [15]. The plant is commonly known by the Yoruba in the south western part of Nigeria as '*akogun'*. It is a rhizomatous aromatic climbing shrub. *Aristolochia* species are known to contain alkaloids and aristolochic acid [16]. Various Aristolochia species have been reported in herbal medicines since antiquity in obstetrics and in treatment of snakebite venom, festering wounds, and tumours, and they are still in use majorly in Chinese herbal medicine [17-19]. *A. ringens* is used to treat cholera, fever, bowel troubles, ulcers, leoprosy, and poisonous bites [20,21]. The leaf, stem, and floral essential oil compositions of *A. ringens* have been previously reported [22-24]. The leaf oil was dominated by sesquiterpenoids including (*E*)-caryophyllene (11.4%), *trans*-4(14),5-muuroladiene (13.0%), bicyclogermacrene (12.8%), spathulenol (8.0%), as well as the diterpenoid methyl copalate (10.3%). The study aimed at ascertaining the phytochemical constituents, antimicrobial and cytotoxic activities of chloroform extracts of *C. albidum* and *A. ringens* as an ingredient in the production of herbal medicine in Nigeria.

2. MATERIAL AND METHODS

2.1 Plant Collection

The stem bark of *C. albidum* (Linn) and straw *A. ringens* were purchased from a local market at Idi Oro market, Lagos State, and authenticated at Botany Department, University of Lagos.

2.2 Plant Extraction

All the plant materials were washed thoroughly with distilled water and air-dried.

Prior to crude extract, the plants were washed thoroughly with distilled water and air-dried for five days. Later, the plants were pulverized using an electric grinder and stored in a polythene bags until ready for use. 650 g of each pulverized plants of *C. albidum* and *A. ringens* respectively were soaked in 1.5 litres of chloroform for 24 hours. Thereafter, each crude extract were filtered using Whatman filter paper No 42 (125 mm). The filtrate were freeze dried, concentrated under reduced pressure of 300-500 mmHg at 50-60°C and preserved in refrigerator at 4°C for further use. The yield of the chloroform extract of C*. albidum* and *A. ringens* were determined and recorded as percentage of dry weight of the plants materials. All the chemicals used were of AR grade and were purchased from Olaolu Chemicals, Igbo Elerin, (Lagos).

2. 3. Phytochemical screening

Phytochemical screening was performed on the extracts using standard procedures to identify chemical constituents.

2.3.1. Screening for Alkaloids

0.5 g of the extract was mixed with 5 ml of 1% HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1 ml of the filtrate was treated with a few drops of Wagner's reagent. A reddish brown precipitate indicates the presence of alkaloids [25].

2.3.2. Screening for Flavonoids

2mL 10 % Sodium hydroxide was added to 5mL of distilled water containing 0.2 g of the chloroform extract . The appearance of a yellow colour indicates the presence of flavonoids [26].

2.3.3. Screening for Phenols

The chloroform extract (0.2 g) was diluted with 50% ethanol and added 3 drops of $FeCl_3$.. Appearance of deep bluish green color indicates presence of phenols [26]

2.3.4. Screening for Tannins

0.2 g of the extract was dissolved in 5mL distilled water and filtered, few drops of 10% ferric chloride solution were then added to the filtrate. The appearance of a bluish black colour indicates the presence of tannins [26].

2.3.5. Screening for Anthraquinones

Crude extract (0.5 g) was mixed thoroughly with benzene (10 mL), and filtered. Followed by addition of few drops of 10% NH_3 solution shake and filtrated. The appearance of a pink, a reddish or violet color shows the presence of anthraquinones [25].

2.3.6. *Screening for Cardiac Glycosides*

Crude extract (0.5 g) dissolved in 2 mL glacial acetic acid containing few drops of 10% $FeCl_3$ solution followed by

addition of 2 mL of conc. sulfuric acid and filtered. Appearance of brown ring separation in between the mixture, indicates the presence of cardiac glycosides [26].

2.3.7. *Screening for Phlobatannins*

1 mL of (0.2 g) extract dissolved in 10 mL of water was added a few drops of 1% HCl and boiled. Appearance of red precipitation shows the presence of phlobatannins [26].

2.3.8. *Screening for Terpenoids*

Acetic anhydride (0.5 mL) was shaken thoroughly with 1 mL of (0.2 g) chloroform extract dissolved in 50% ethanol, followed by addition of few drops of conc. H_2SO_4. Appearance of bluish green precipitate shows the presence of Terpenoids [25].

2.3.9. *Screening for Steroids*

Crude extract (0.2 g) was mixed thoroughly with glacial acetic acid (2 mL) and the solution was heated, cooled, and then filtered. 0.5 mL of acetic anhydride was added to the residue, followed by the addition of few drops of conc. H_2SO_4. Appearance of violet to blue or bluish-green colour indicates the presence of steroids [25].

2.4 Quantitative determination of the phytochemical screening

2.4.1 Preparation of fat free sample

2 g of the sample were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 h.

2.4.3. Determination of total alkaloids

200mL of 10% acetic acid dissolved in ethanol was added to (5 g) the crude extract in a 250ml beaker, covered and allowed for 4 h, and filtered, concentrate on a water bath until one quarter of the original volume was obtained. Aqueous ammonia solution was added dropwise to the mixture until all the crude extract was precipitated, washed, filtered and collected over aqueous ammonia solution. The solid alkaloid obtained was dried and weighed [27].

2.4.4. Flavonoid Determination of total flavonoid

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight [28].

2.5. Antimicrobial Screening

The chloroform extract was screened for antimicrobial activity against Gram-positive bacteria, *Bacillus cereus,*

Staphylococcus aureus, Staphylococcus epidermidis; Gram-negative bacteria, *Pseudomonas aeruginosa, Serratia marcescens* and *Escherichia coli.* Minimum inhibitory concentrations (MIC) were determined using the microbroth dilution technique [29]. Dilutions of the solid extracts of *C. albidum* and *A. ringens* respectively were prepared in cation-adjusted Mueller Hinton broth (CAMHB) beginning with 50 µL of 1% w/w solutions of each crude extract in DMSO plus 50 µL CAMHB. The extracts were serially diluted (1:1) in CAMHB in 96-well plates. Organisms at a concentration of approximately 1.5×10^8 colony forming units (CFU)/mL were added to each well. Plates were incubated at 37°C for 24 hr; the final minimum inhibitory concentration (MIC) was determined as the lowest concentration without turbidity. Gentamicin was used as a positive antibiotic control; DMSO was used as a negative control. Antifungal activity was determined as described above using *Candida albicans* in yeast-mold (YM) broth and infected leaves of tomato with approximately 7.5×10^7 CFU/mL. Antifungal activity against *Aspergillus niger* and *Botrytis cinerea* were determined as above using potato dextrose broth inoculated with *A. niger* hyphal culture and grey mould horticulture respectively diluted to a McFarland turbidity of 1.0. Amphotericin B was the positive control.

2.6. Cytotoxicity Screening

Hepatocarcinoma cell line of human being marked (HepG2, CRL-11997™) cells grown in complete medium marked (DMEM: F12 made up of L-glutamine and sodium bicarbonate, 10% of FBS, with 1% penicillin/streptomycin) were kept warming at 37°C in a 5 % CO_2 of the environment. Once about 90% of confluent, the cells were risen with phosphate buffered saline (PBS), containing about 0.25% (w/v) of Trypsin/EDTA, estimated and suspended in fresh complete media. 5×10^5 cells/well (100 µL) seeded in a 96- well plates and warmed for 24 h to attach. Cells were then treated with the chloroform extracts at a concentration of 10 and 50 µg per mL in 200 µL medium for 72 hours. Thereafter, the medium was gently removed, and DMEM:F12 media with MTT (5 mg/mL in PBS) were added to the cells and warmed for 1 hr. The MTT medium was gently replaced with DMSO (200 µL/well), plate was then mixed carefully to allow the dissolution of formazan crystals. Absorbance was measured at 550 nanometer. All extracts were tested in triplicate.

MCF-7 (human breast tumor marked ATCC No. HTB-22) cells with a size of RPMI 1640 supported with 10% Fetal bovine serum (FBS), 30 mM HEPES, sodium bicarbonate, 100,000 units penicillin/streptomycin (10 mg/L) at pH=7.35 were prepared. MCF-7 cells plated in a 96-well cell, culture plates at a concentration of 1.2×10^4 cells/well with a volume of 100 µL in each well were warmed at 37°C of 5% CO_2 within 2 days. After 2 days, the cells developed to about 80% growth. The supernatant solution was gently removed and resettled with 100 µL growth medium containing 1 µL of the chloroform extract (1% in DMSO), to give a final conc. of 100 µg per mL (100 ppm). The plate was warmed at 37°C and 5% CO_2 for 2 days. After 2 days, the liquid was gently removed from the well. The absorbance were measured using spectrophotometric technique at 570 nm (using a Molecular Devices SpectraMax Plus 384 microplate reader) from each well at 100 µL of the MTT solution containing a pre-read absorbance. Formazan crystals were precipitated for a period of 4 h at 37°C and 5% CO_2. After 4h, the formed Formazan crystals were dissolved in a DMSO solution to give a purple crystal. Growing medium, DMSO, and tingenone (100 µg/mL) were used as negative and positive control compounds,

respectively. Eight replicate solutions were added to wells. The percent kill ratios (% $kill_{compound}$ / % $kill_{control}$) were determined and average absorbance, standard deviations were also calculated.

3. RESULTS AND DICUSSION

The results of chloroform extracts of *C. albidum* and *A. ringens* were shown in (Table 1). Chloroform extracts values were higher in *C. albidum* indicating the possibility of considerable amount of secondary metabolites in the leaves of *C. albidum* compared to the stem of *A. ringens* respectively. The phytochemical screening of *C. albidum* revealed the presence of alkaloid, flavonoid, cardiac glycoside and steroid while the extracts of *A. ringens* revealed the presence of alkaloid, flavonoid, steroid and anthraquinone as the only secondary metabolites (Table 2). Quantitative assessment of the percentage chemical constituents of the chloroform extracts were summarized in (Table 3). Both extracts of *C. albidum* and *A. ringens* revealed the presence of Alkaloid and Flavonoid respectively with *C. albidum* having higher quantities, while cardiac glycoside were found only in *C. albidum* with low quantity. Polar phytochemicals constituents such as Phenol and Tannins were absent in both crude extracts. Antimicrobial screening (Table 4) revealed that the stem bark extract of *C. albidum* exhibited antibacterial activity against *Escherichia coli* (MIC = 19.5 µg/mL) while the straw of *A. ringens* inhibited *B. cereus* (MIC = 19.5 µg/mL) but both *C. albidum* and *A. ringens* showed marginal activity against any of the tested fungi (MIC = 625 µg/mL). The results of cytotoxicity for the chloroform extracts of *C. albidum* and *A. ringens* revealed that *A. ringens* is cytotoxic to two different cell lines while *C. albidum,* were selectively cytotoxic to liver tumor cells, but not to breast tumor cells. This indicated that Nigerian *A. ringens* showed notable in-vitro cytotoxicity and this could be attributed the secondary metabolites present in these medicinal plants.

Phytochemical screening of the chloroform extracts of *C. albidum* and *A. ringens* revealed some differences in their constituents. The results support the idea that the *C. albidum* and *A. ringens* possesses active medicinal agents as well as revealing their physiological activities [25]. The antimicrobial actions of *C. albidum* and *A. ringens* are primarily due to the presence of active components in a concerted synergistic or antagonistic activities. Studies had revealed that mixtures of these chemical constituents showed a broad spectrum of biological effects and pharmacological efficacy [30,31]. The antimicrobial activities could likely be attributed to the presence of alkaloid, flavonoids, cardiac glycosides, steroid and anthraquinone present in the chloroform extracts. This study provides the basis for their pharmacological use as a cure for some human ailments and diseases. Cardiac glycosides have been found to be effective in congestive heart failure [32].The presence of alkaloids may be responsible for their antimalaria efficacy. Possession of analgesic properties of these medicinal plants confirmed their use in treatment of stomach disorder, a view supported by [33]. Flavonoids are potent water soluble antioxidants and free radical scavengers' agents which prevent oxidative cell damage and have strong anti cancer activity [34]. Studies had shown that flavonoid in intestinal tracts lower the risk of heart failure [33,35]. The presence of the above metabolites in the studied medicinal plants confirmed their use for the treatment of various diseases conditions to their antimicrobial, anti-inflammatory, and anti-carcinogenic effect. However, further research work is ongoing towards bio-guided column chromatography and in-vivo studies of these plants.

Table 1. Nature and yield of chloroform crude extracts from the *C. albidum* and *A. ringens*

Plant Extract	Extract	Extract Texture	Extract Yield	Percentages Recovery (%)
C. albidum	Dark Green	Semi Sticky Powder	120.00 g	10.0
A. ringens	Dark Brown	Sticky Semi Solid Powder	84.00 g	7.0

Table 2. Qualitative analysis of secondary metabolites found in *C. albidum* and *A. ringens*

Secondary metabolites	*C. albidum*	*A. ringens*
Phenol	-	-
Tannins	-	-
Phlobatanin	-	-
Alkaloid	+++	+++
Flavonoid	++	++
Cardiac glycoside	++	-
Steroid	+	+
Terpenoids	-	-
Anthraquinone	-	-

+++ = Test strongly positive ; ++ = Positive;
+ = Weakly positive; - = No effect

Table 3.
Quantitative analysis of the relative amount of secondary metabolites in *C.albidum* and *A. ringens*

condary metabolites	*C. albidum*	*A. ringens*
Phenol mg/100g	-	-
Tannins mg/100g	-	-
Alkaloid mg/100g	52.50 ± 0.50	45.82 ± 0.70
Flavonoid mg/100g	26.60 ± 0.80	13.53 ± 0.30
Cardiac glycoside mg/100g	27.10 ± 0.80	-

Table 4. Antimicrobial activity (MIC, µg/mL) of *C.albidum* and *A. ringens*

Sample	*C. albidum*	*A. ringens*
Bacteria		
P. aerug	312.5	625
E. coli	< 19.5	> 2500
S. aureus	2500	> 2500
B. cereus	312.5	< 19.5
S. epi	> 2500	> 2500
S. mar	> 2500	1250
Fungi		
A.niger	625	625
Candida	625	156
Botrytis	625	625

4. Conclusion: The results of the study support the idea that herbal medicines is still relent as health care substitute in developing countries and provide new chemotherapeutic agents for development.

ACKNOWLEDGEMENTS
The authors are thankful to Jaiyeola Elizabeth, Suenu Tijani and Sambo Taofikat for the collection and identification of the plant sample. Mrs. Helen Owolabi is gratefully acknowledged for her useful comments.

AUTHORS' CONTRIBUTIONS
Owolabi M.S conceived and designed the experiments; Omowonuola A.A, Avoseh N.A and Jaiyeola Elizabeth, performed the experiments on phytochemical screening, Lawal O.A and Ogunwande I.A contributed reagents/analysis tools; Setzer W.N. designed the experiment on antimicrobial and cytotoxicity; Setzer W.N and Owolabi M.S analyzed the data and wrote the paper.

COMPETING INTERESTS

The author(s) declare no conflict of interest.

References

1. Akter, R., Uddin, S.J., Grice, I.D. And Tiralongo, E., 2014. Cytotoxic Activity Screening Of Bangladeshi Medicinal Plant Extracts. J. Nat. Med. 68(1), 246–252
2. Bhagwata, M.K., Datara, A.G., 2014. Antifungal Activity of Herbal Extracts Against Plant Pathogenic Fungi. Arch. Phytopathol. Plant Prot. *47*(8), 959–965.
3. Horn, D., Duraisingh, M.T., 2014. Antiparasitic Chemotherapy: From Genomes To Mechanisms. Annu. Rev. Pharmacol. Toxicol. *54*, 71–94.
4. Hoseini Alfatemi, S.M., Sharifi Rad, J., Sharif Rad, M., Mohsenzadeh, S., Teixeira Da Silva, J.A., 2014. Chemical Composition, Antioxidant Activity And In Vitro Antibacterial Activity Of *Achillea Wilhelmsii* C. Koch Essential Oil On Methicillin-Susceptible And Methicillin-Resistant *Staphylococcus Aureus* Spp. Biotech , (3) 1–6
5. Sharifi Rad, J., Hoseini Alfatemi, S.M., Sharifi Rad, M., Iriti, M., 2014. Free Radical Scavenging And Antioxidant Activities Of Different Parts of *Nitraria Schoberi* L. Tbap 4(1), 44–51.
6. Tangarife-Castaño, V., Correa-Royero, J.B., Roa-Linares, V.C., Pino-Benitez, N., Betancur-Galvis, L.A., Duráncd, D.C., Stashenko, E.E., Mesa-Arango, A.C., 2014. Anti-Dermatophyte, Anti-*Fusarium* And Cytotoxic Activity of Essential Oils And Plant Extracts Of *Piper* Genus. J. Essent. Oil Res. 26(3), 221–227.
7. Znati, M., Jannet, H.B., Cazaux, S., Bouajila, J., 2014. Chemical Composition, Biological And Cytotoxic Activities of Plant Extracts and Compounds Isolated from *Ferula Lutea*. Molecules 19(3), 2733–2747.
8. Ehiagbonare, J.E., Onyibe, H.I., Okoegwale, E.E., 2008. Studies On The Isolation Of Normal and Abnormal Seedlings of *Chrysophyllum Albidum*: A Step Towards Sustainable Management of The Taxon In The 21st Century. Sci. Res Essay. 3(12), 567-570.
9. Adebayo, A.H., Aliyu, R., Gatsing, D., Garba, I.H., 2006. The Effects Of Ethanolic Leaf Extract Of *Chrysophyllum Albidum* G. On Biochemical And Haematological Parameters of Albino Wistar Rats. Afr. J. Biotechnol. (9), 2145-2150.
10. Adewusi, H.G., Bada, S.O., 1997. Preliminary Information On The Ecology of Chrysophyllum Albidum In West And Central Africa: In Proceedings of A National Workshop On The Potentials Of The Star Apple In Nigeria. 16-25.
11. Amusa, N.,A., Ashaye, O. A., Oladapo, M. O., 2003. Biodeterioration Of The African Star Apple (Chrysophyllum Albidum) In Storage And The Effect on Its Food Value. Afr. J. Biotech., (2) 56 – 59.
12. Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., Simons .A., 2009. Agro -Forestry Database: A Tree Reference And Selection Guide, (Version 4.0 Db) Pearson, D. 1976: Chemical Analysis Of Foods . (7 Th Edition) London: Church Hill Livingstone, 488 -496.
13. Idowu, T.O., Iwalewa, E.O., Aderogba, M.A., Akinpelu, B.A., Ogundaini, A. O., 2006. Biochemical And Behavioural Effects of Eleagnine From *Chrysophyllum Albidum*. J. Biol. Sci., (6) 1029-1034.
14. Okoli, B..J., Okere, O.S., 2010. Antimicrobial Activity Of The Phytochemical Constituents of *Chrysophyllum Albidum* G.Don_Holl. (African Star Apple) Plant Journal of Research In National Development (8), 1.

15. Watson, I., Dallwitz, M. J., 1992. The Families of Flowering Plants; Description, Illustrations, Identification And Information Retrieval. The Plant Book (Revised Version).
16. Mabberley, D .J., 1997 *The Plant-Book*, 2nd Ed., Cambridge University Press, UK.
17. Parameshwari, G., Subbraj, T. M., 2009. A Studies On Anntivenom Activity of *Andrographis Paniculata* And *Aristolochia Indica* Plant Extracts Against *Echis Cainatus* Venom.The Internet Journal Of Toxicology. 6 (1) 1559- 3916.
18. Balachandran, P., Wei, F., Lin R., Khan, I., Pasco, D., 2005. Structure Activity Relationships Of Aristolochic Acid Analogues: Toxicity In Cultured Renal Epithelial Cells. Journal Of Kidneyinternational. (67), 1797-805.
19. NTP., 2008. (National Toxicological Programme), United States Department of Health And Human Services Research Triangle Park, NC 27709.Final Report On Carcinogens Background Document For Aristolochic Acids;1-12.
20. Krishnaraju, A.V., Rao, T. V.N., Sundararaju, D., Vanisree, M., Tsay, H, S., Subbaraju, G. V. 2005. Assessment Of Bioactivity of Indian Medicinal Plants Using Brine Shrimp (Artemia Salina) Lethality Assay. Int. J. Appl. Sci. Eng. (3), 125-134.
21. Kanjilal, P. B., Kotoky, R., Couladis, M., 2009. Chemical Composition of *Aristolochia Indica* L. J. Essent. Oil Res. (21), 1-2.
22. Stashenko, E.E., Ordóñez, S.A., Marín, N.A., Martínez, J.R.. 2009. Determination of The Volatile And Semi-Volatile Secondary Metabolites, And Aristolochic Acids In *Aristolochia Ringens* Vahl. J. Chromatogr. Sci. (47), 1-6.
23. Larrahondo, J. E., Acevedo, C., 1990. Terpenoids of *Aristolochia Ringens*. An. Asoc. Quim. Argent.. (78), 355-358.
24. Owolabi, M.S., Lawal, A.O., Prajwal, P., Setzer, W. N. and Lajide, L. (2012). Assessment of Essential Oil Composition and Insecticidal Activity of *Aristolochia ringens* from Nigeria. Biopesticide International, 8(1): 26-31
25. Sofowora, A. 1993. Screening Plants For Bioactive Agents. In: Medicinal Plants And Traditional Medicinal In Africa, 2nd Ed., Spectrum Books Ltd., Sunshine House, 134-156.
26. Trease G. E., Evans W. C., 1989. Pharmacognsy. 11th Edn. Brailliar Tiridel Can. Macmillian Publishers.
27. Harborne, J. B., 1973. *Phytochemical Methods*, London. Chapman And Hall, Ltd. 49-188.
28. Boham, B. A., Kocipai, A. C., 1974. Flavonoids And Condensed Tannins From Leaves Of *Hawaiian Vaccinium Vaticulatum* And *V. Calycinium.* Pacific Sci. (48) 458-463.
29. Satyal, P., Woods, K. E., Dosoky, N. S., Neupane, S., Setzer, W. N., 2012. Biological Activities And Volatile Constituents Of *Aegle Marmelos* (L.) Corrêa From Nepal. Journal of Medicinally Active Plants 1
30. Felix, M. T., 1982. Medicinal Microbiology. Churchill Livingstone Publishers, London. 7-8.
31. Kubmarawa, D., Khan, M.E., Punah, A. M., Hassan, M., 2008. Phytochemical ScreeningAnd Antimicrobial Efficiency of Extracts From *Khaya Senegalensis* Against Human Pathogenic Bacteria. African Journal Of Biotechnology. 7(24), 4563-4566.
32. Aboaba, S.O., Smith, S.I., Olide, F.O., 2006. Antimicrobia effect of edibles plant extracts on escherichia coli 0157: H7 Pakistan. Journal of Nutrition. 5(4), 325-327.
33. Okwu, D.E., Emenike, I.N., 2006. Evaluation of the phyto-nutrients and vitamins content of the citrus fruits. International Journal of Molecular Medicine and Advance Science 2(1), 1-6.
34. Stauth, D., 1993. Studies force new view on biology of flavonoids, Eureka alert!. Adapted from a news release issued by Oregon State University. URL accessed.
35. Orijajogun , O.J., Olajide, O.O., Fatokun ,A.O., Orishadipe, A.Y.,. Batari, M.L., 2013. The preliminary chemical constituent of free radical scavenging activity of the exocarp of the fruit extract of African star apple (Chrysophllum Albidum). International Journal of Pharma Science and Research, 3(3): 72-80.