



Phytochemical, Heavy Metal Analysis and Phytotoxic Activities of Crude Extract of *Annona senegalensis* Stem Bark

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ABSTRACT

The phytochemical, heavy metals analysis and phytotoxic activities of *Annona senegalensis* extracts was determined. The fresh stem bark of *A. senegalensis* was collected, dried under air at room temperature, pounded into a powder and extracted by serial extraction. The Phytochemical screening was conducted, the concentrations of ten (10) heavy metals in *A. senegalensis* stem bark extracts were evaluated by atomic absorption spectroscopy (AAS) while the phytotoxic activity was studied using radish seeds in various concentrations of *A. senegalensis* stem bark (1000, 500, 250 and 125mg/mL). The result indicates that alkaloids, flavonoids, saponins and tannins, glycosides, terpenoids and anthraquinones were detected while Steroids were not detected. Heavy metals analysis showed that the concentrations of Pb, Mg, Mn, Fe, Zn, Cu, Ca, Ni, Hg and Cd were 3.03 ± 0.01 , 289.86 ± 2.51 , 44.03 ± 0.99 , 12.89 ± 0.36 , 30.48 ± 0.03 , 5.29 ± 0.07 , 88.78 ± 2.01 , 0.63 ± 0.05 , 0.02 ± 0.01 and 0.07 ± 0.03 respectively which were all below maximum permissible levels set by different standard organizations like WHO, FAO and FDA. For the phytotoxic activities, the result shows that the root and shoot lengths of the crude methanol extracts were generally higher than the control. However, there was higher % seed inhibition in the different concentrations of the crude methanol extracts (66.67%, 53.33%, 46.67%, and 80.00%), unlike the control which had only 40.00% seed inhibition. These results have proven the medicinal significances of *A. senegalensis* and a promising plant product based natural herbicides.

Keywords: *Annona senegalensis*, heavy metals, phytotoxic, AAS, radish seeds

INTRODUCTION

Nigeria is naturally blessed with an abundance of natural resources due to its different environmental conditions. The phytochemicals derived from these are been utilized by humans for sustaining their good health (Ahmad *et al.*, 2014). These phytochemicals derived from plants are been used in pharmaceutical discoveries since past century. Various numbers of effective drugs have been manufactures, but traditional medication is adopted in many developing countries (Ahmad *et al.*, 2014). Almost 80% of therapies from primary health care are coming from natural plants all over the world (Abbasi *et al.*, 2010). Therefore, the utilization of the therapeutic efficacy of these plants to gain new novel, cheapest, effective and safer phytodrugs is very vital (Moghadamtousi *et al.*, 2015). One such natural source with higher therapeutic efficacy is *A. senegalensis*.

Phytochemicals are classified into two groups, known as primary and secondary metabolites. The common sugars, amino acids, proteins and chlorophyll are the primary

metabolites whereas the phenolic compounds, alkaloids, flavonoids, essential oils, saponins, terpenoids and tannins are the secondary metabolites (Krishnaiah *et al.*, 2009). Almost all the bioactive compounds in plants are designated to possess high therapeutic potential, which make the plants to possess their medicinal significance due to such phytochemicals in them (Price *et al.*, 1988).

Recently, researchers have shown increasing interest in phytotoxic medicinal plants (Wang *et al.*, 2013; Yang *et al.*, 2012). These could be as a result of the easier screening process of phytotoxic plants from medicinal plants (Chevallier, 1996), (ii) the ability to have more phytochemicals in medicinal plants. Some of these medicinal plants are used in many forms to

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managed weeds, e.g. (i) transplanting the weeds as cover crops to main crops, (ii) as bioherbicides by applying the plant extracts, or (iii) using their active substances to develop new bioherbicides upon isolation and characterization (Islam and Hisashi, 2014).

Annona senegalensis (Wild Custard Apple or Wild Soursop) is a small tree with about 2-6 m in height. The species is habitat around river banks, fallow land, forests (Orwa *et al.*, 2009). The plant is highly distributed in Northern Nigeria, especially, Nasarawa, Kaduna, Kano, Plateau, Niger States and also Federal Capital Territory, Abuja. The plant is popularly known as Numgbere in Nupe (Mustapha, 2013). All parts of *A. senegalensis* plant have been found useful for traditional medicine applications. The leaves of the plant are been used by traditional herbalists to treat yellow fever, tuberculosis, and smallpox (Aiyelaja & Bello, 2006; Mustapha & Uthman, 2013) whereas the stem bark has been used in treating snakebite and hernia (Dambatta & Aliyu, 2011). The ailments like; difficulty in swallowing, gastritis, snake bites, male sexual impotence, erectile dysfunction, tuberculosis, and an antidote for necrotizing toxins are treated by herbalists using the root bark (Ofukwu & Ayoola, 2008; Noumi & Afiatou, 2015; Jiofack *et al.*, 2009). The treatment of chickenpox is effective using juice from tree (Faleyimu & Akinyemi, 2010; Ahombo *et al.*, 2012).

Apart from ethnomedicinal uses of *A. senegalensis*, it serves as food and food additives. The leaves can be prepared as vegetables. In Nigeria and Tanzania, the uses of *A. senegalensis* as pest control have been demonstrated (Igoli *et al.*, 2005; Innocent *et al.*, 2014). The application of spread of fresh leaves on the floor of poultry houses until dried for the control of fleas and lice have been adopted in the Republic of Benin (Salifou *et al.*, 2012).

The works on phytochemical and biological activities of medicinal plants have been reported, but only little information as regard to the heavy metal concentrations in the medicinal plants and herbal drugs have demonstrated (Karayil & Vivek, 2014). The presence of toxic metals (Nickel, Lead, Cadmium, Manganese and Mercury) in herbs can cause health risks (Peter & Smet, 2002). The trace concentrations of heavy metals in the soil and plants growing on it face some heavy metal stress and causes changes in the production of

secondary metabolites. High levels of heavy metal contamination in medicinal or other plants may suppress secondary metabolite production. It is essential to maintain safety quality and efficacy of the plants and their products to avoid serious health problems (Karayil & Vivek, 2014).

World Health Organization (WHO) recommends that such products must be evaluated for the extent of heavy metals and phytotoxic potentials before use in human beings (Ayaz *et al.*, 2014). Thus, according to these backgrounds of *A. senegalensis* multimedicinal uses, the present study was conducted to evaluate specifically stem bark methanol extracts for phytochemical and heavy metal analysis and its phytotoxic properties.

METHODOLOGY

Chemicals

Paraquat (a positive control) in the phytotoxic assay was purchased from Sigma-Aldrich EC 231-791-2 and other chemical (Methanol) were analytical grade

Collection of Sample

The *A. senegalensis* stem bark was identified, harvested, and collected in Lapai town environs, Lapai Local Government, Niger State, Nigeria on May 2019. It was reidentified and authenticated in the Biological Sciences Department, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria.

Preparation of plant materials

The *A. senegalensis* stem bark collected was washed to remove dirties using water, air dried at room temperature, pounded in to powder, weighed, kept in a plastic bags and stored at ± 4 °C.

Extraction Method

From the dried sample (powder) prepared, treatment extract was produced. Briefly, fifty (50 g) grams of measured sample powder was soaked and shaken independently in 500 mL of 70% (v/v) methanol for 2 days at room temperature. This was repeated three times and filtered using filter paper (Number 2, Advantec Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The dried and concentrated filtrate was obtained at 40°C through dryness by a rotary evaporator (Heidolph Laborota 4000, Schwabach,

Germany). The filtrate was resuspended in methanol to produce a 50 mg/ml crude stock fraction (Islam and Hasashi, 2014). The working concentrations (1000, 500, 250, 125 and 0 mg/mL) were prepared by further dilution of crude stock fraction.

Phytochemical Analysis

Qualitative phytochemical assay in plant extracts was performed using previous method of Meriga *et al.* (2012) to ascertain the presence of bioactive components (alkaloids, flavonoids, glycosides, anthraquinones, saponins, terpenoids, sterols and tannins). The Dragendorff's reagent was used to confirm the presence of Alkaloids, steroids and triterpenoids were assessed using Liebermann Burchard test. The "Keller Killian" Test was performed for the presence of glycosides. A few drops of glacial acetic acid and Ferric chloride solution were added and properly mixed up. In the mixture, a few drops of concentrated H₂SO₄ were added and the formation of two layers was observed. A reddish-brown and acetic acid at the lower and upper layer, respectively, that turns bluish-green signifies the presence glycosides. Saponins detection was assessed following the formation of froth after vigorous shaking using diluted samples. The presence of Anthraquinones was conducted by boiling the test sample with 1 ml of H₂SO₄ in a

test tube and then filter. Allow the filtrate to cooled and shaken with an equal volume of chloroform and dilute ammonia until the lower layer was separated. The formation of rose-pink to red color ammoniacal layer signifies the presence of anthraquinone glycosides. The presence of tannins was detected by treating the test sample with gelatin solution. The appearance of white precipitate signifies the existence of tannins.

Heavy Metals Analysis

For heavy metal analysis, the methanol extract was prepared using graded chemicals. Briefly, 2 g of sample stock fraction was taken in a crucible and ignited at 550°C for 6 hrs using muffle furnace. In 5 mL of conc. H₂SO₄, the ash from crucible was digested, evaporated on a hot plate and in to digested residue, 1 mL of distilled water was added. The mixture was filtered, volume raised to 30 ml using distilled water and the resulted solution was analyzed using atomic absorption spectrophotometer (model 1100; Perkin Elmer, Waltham, Massachusetts, USA) to read the presence of heavy metals such as iron (Fe), lead (Pb), zinc (Zn), manganese (Mn), magnesium (Mg), copper (Cu), calcium (Ca), nickel (Ni), mercury (Hg) and cadmium (Cd) (Akram *et al.*, 2015, Abera *et al.* 2015).

Table 1: Working Parameters of Atomic Absorption Spectrophotometer

S/N	Heavy Metal	Wavelength (nm)	Slit Width (cm)	Reference(s)
1	Iron (Fe)	248.3	0.7	Akram <i>et al.</i> (2015).
2	Zinc (Zn)	213.9	0.7	Akram <i>et al.</i> (2015)
3	Magnesium (Mg)	285.2	0.7	Akram <i>et al.</i> (2015)
4	Lead (Pb)	283.3	0.7	Akram <i>et al.</i> (2015)
5	Manganese (Mn)	279.4	0.7	Akram <i>et al.</i> (2015)
6	Copper (Cu)	324.7	0.7	Abera <i>et al.</i> (2015)
7	Calcium (Ca)	422.7	0.7	Akram <i>et al.</i> (2015)
8	Nickel (Ni)	232	0.7	Abera <i>et al.</i> (2015)
9	Mercury (Hg)	253.6	0.7	Abera <i>et al.</i> (2015)
10	Cadmium (Cd)	228.8	0.7	Abera <i>et al.</i> (2015)

Determination of Phytotoxic Activity

Phytotoxic effects were evaluated based on bioassay diaspore germination and early growth of seedlings. The radish seed (*Raphanus sativus* L. was used as the target species.

Germination Bioassay

This was carried out in petri dishes of 90 mm diameter containing two filter papers wetted with 5 mL of methanol as plate 1 (negative control). The same volume of various working concentrations (1000, 500, 250, 125 mg/mL) of the extracts obtained from the powder of *A. senegalensis* stem bark with 25 radish seeds per

petri dish were placed at sufficient distance using sterilized forceps as plate 2, 3, 4 and 5, respectively, (treatment plates). All treatments and control plates were replicated three times, and the dishes were capped and sealed with plastic film. Completely randomized treatment was used as the experimental design. At 0.5 days to day-4 (time no further germination occurred and radical emerges), the germination was examined (Islam and Hisachi (2014). Other germination indices measured include the average root and shoot lengths and number of seeds inhibition (Zebet *et al.*, 2014). After 5 days of incubation of all plates at 25°C in dim light, the following germination indices (number of seeds germinated and percent inhibition of root and shoot lengths) were calculated using formula:

$$\text{Root inhibition\%/shoot length} = \frac{\text{Root/shoot length in test sample}}{\text{Root/shoot length in control}} \times 100$$

Data Analysis

All data recorded were analyzed using Analysis of Variance (ANOVA). The separation of means at a 95% confidence interval ($p < 0.05$) was performed using Duncan Multiple Range Test (DMRT).

RESULTS

The Phytochemical present in *A. senegalensis* extracts are shown in table 1. Alkaloids, flavonoid, phenols, and tannins, Saponins, glycoside, anthraquinone, and terpenoids were detected in *A. senegalensis*

extracts, but steroids were not detected (Table 2).

The concentrations of heavy metals found in the crude powder of *A. senegalensis* stem bark are presented in table 3. The results show that the concentrations of Pb, Mg, Mn, Fe, Zn, Cu, Ca, Ni, Hg, and Cd were 3.03 ± 0.01 , 289.86 ± 2.51 , 44.03 ± 0.99 , 12.89 ± 0.36 , 30.48 ± 0.03 , 5.29 ± 0.07 , 88.78 ± 2.01 , 0.63 ± 0.05 , 0.02 ± 0.01 , and 0.07 ± 0.03 respectively. All the values were below maximum permissible levels of the respective heavy metals in medicinal herbs/products as set by different standard organizations like World Health Organization (WHO), Food and Agricultural Organization (FAO) and Federal Drug Administration (FDA).

The results of the phytotoxic effect of *A. senegalensis* stem bark extracts are shown in Table 4. It reveals that the root and shoot lengths of the crude methanol extracts were generally higher than the control. However, there was higher % seed inhibition in the different concentrations of the crude methanol extracts (66.67%, 53.33%, 46.67%, and 80.00%), unlike the control which had only 40.00% seed inhibition. More so, there was a statistically significant difference ($p < 0.05$) in the results for shoot lengths, unlike the root length that was not.

Table 2. Phytochemical screening of *A. senegalensis* stem bark extracts

Phytochemical	Test Performed	Observation	Result
Alkaloids	Dragendorff's test	Turbidity/precipitation	+
Flavonoids	Ferric chloride test	The yellow color which to change to colorless	+
Anthraquinone	Bontrager's test	Red violet in an aqueous layer formed	+
Phenols & Tannins	Gelatin test	White precipitate appeared	+
Saponins	Froth test	Stable froth formed	+
Glycoside	Test of Keller Kelliani	Red and brown color at lower & acetic layers that changed to blue-green color at upper	+
Terpenoids	Liebermann Burchard test	The deep red-brown color observed	+
Steroid	Liebermann Burchard test	Green-pink color was not observed	-

Key: (+) indicates Phytochemical detected, (-) Indicates Phytochemical not detected.

Table 3: The concentrations of heavy metals in *A. senegalensis* stem bark crude powder

S/N	Metal	Conc. (mg/mL)	PL (mg/mL)	Reference
1	Pb	3.03±0.01	10	Hussain (2006)
2	Mg	289.86±2.51	2000	Khan <i>et al.</i> (2013)
3	Mn	44.03±0.99	200	Khan <i>et al.</i> (2013)
4	Fe	12.89±0.36	20	Khan <i>et al.</i> (2013)
5	Zn	30.48±0.03	50	Khan <i>et al.</i> (2013)
6	Cu	5.29±0.07	10	Maobe <i>et al.</i> (201)
7	Ca	88.78±2.01	614	Khan <i>et al.</i> (2013)
8	Ni	0.63±0.05	1.5	Khan <i>et al.</i> (2013)
9	Hg	0.02±0.01	0.1	Maobe <i>et al.</i> (2012)
10	Cd	0.07±0.03	0.3	Khan <i>et al.</i> (2013)

Values are presented in means ± standard deviations of triplicate analyses. PL: Permissible limits.

Table 4: Phytotoxic effect of crude methanol extract of *A. senegalensis* stems bark

S/N	Conc. (mg/ml)	ARL (cm)	% RLI	ASL (cm)	% SLI	% SI
1	Control	5.60±0.90 ^a	-	5.17±0.87 ^a	-	40.00 ^a
2	1000	4.06±0.99 ^a	72.05	5.00±0.91 ^a	96.71	66.67 ^b
3	500	5.01±0.83 ^a	91.07	5.07±1.14 ^a	98.07	53.33 ^c
4	250	4.09±0.71 ^a	73.04	4.98±1.20 ^a	96.32	56.67 ^c
5	125	4.93±1.20 ^a	88.04	5.10±1.45 ^a	98.65	50.60 ^c

Means with the same superscripts in the same column are not significantly different ($p < 0.05$). All values in the table are means of total no of seeds that germinated in each treatment ± standard error of the mean (SEM). ARL: average root length, RLI: root length inhibition, ASL: average shoot. Length, SLI: shoot length inhibition and SI: seed inhibition

DISCUSSION

The present of phytochemicals; alkaloids, flavonoid, phenols, and tannins, Saponins, glycoside, anthraquinone, and terpenoids in *A. senegalensis* extracts could signify its medicinal potentials as acclaimed by traditional herbalists in the treatment/management of various ailments associated with oxidative stress (Table 2). The medicinal values of these phytochemicals in the management of ailments such as diabetes, microbial diseases, Atherosclerosis, cancer and inflammatory diseases have been reported (Chukwuka *et al.*, 2011; Ruffo *et al.*, 2002). Different portion extracts of *A. senegalensis* can be used to manage diseases such as anaemia, gonorrhoea, dysentery, jaundice, and liver disorder (Babalola, 1993; James *et al.*, 2010). The presence of these phytochemicals in *A. senegalensis* can be

attributed to its various medicinal properties as it is being used traditionally (Ruffo *et al.*, 2002). The presence of flavonoids, saponins, and tannins could be attributed to the anti-allergic; antioxidants and anti-cancer property possess by *A. senegalensis* (Chukwuka *et al.*, 2011; Emmanuel *et al.*, 2005; Ali *et al.*, 2017).

In addition, it was observed the results that crude powder of *A. senegalensis* stem bark recorded lower concentrations of heavy metals (Table 3) than the permissible limit (Table 2), which signified that the extracts are heavy metals toxic free. This finding is similar to the reports of Karayil & Vivek (2014) and Akram *et al.* (2015). Some of these heavy metals, although very toxic at high levels, play some crucial physiological and metabolic roles. For instances, Copper (Cu) deficiency can cause anemia and

congenital inabilities (Maobe *et al.*, 2012) while Nickel (Ni) is required by the body in very small quantity for insulin production in the pancreas. Zinc acts as co-factor for over 200 enzymes and functions in different pathway of metabolism (Khan *et al.*, 2013). WHO (1989) and FAO (1996) have documented the permissible limits of most heavy metals (Table 2).

Germination bioassay is the widely used method to determine the phytotoxic activity (Rice, 1984, Putnam & Tang, 1986) and the early seedling growth is the most sensitive stage to evaluate the phytotoxicity (William & Hoagland, 1982; Wardle *et al.*, 1991; Gong *et al.*, 2001). Several indices have been introduced by previous researchers to study the phytotoxic activities of phytotoxic substances on the germination process (WHO, 2005; Maobe *et al.*, 2012). To investigate the phytotoxic potential of *A. senegalensis* stem bark extracts on germination process, we determined some important germination indices: % seed germination inhibition (%SI), root length (RL), % root length inhibition (%RLI), shoot length (SL), and % shoot length inhibition (%SLI). High significant % seed germination inhibitions were recorded in all extract concentrations used compared to control. However, no significant differences were observed in root and shoot length of germinated seeds in all tested concentrations compared to control while the % RLI and SLI were significantly greater than that of control (Table 3). These signify that crude methanolic extract of *A. senegalensis* stem bark could be a good source of bioherbicidal. Different weeds are amongst the most key factors responsible for the significant reduction in crops yield (Hussain *et al.*, 2008, Ayaz *et al.*, 2014). To counteract these unwanted weeds, different chemicals are used. However, such chemicals are frequently associated with environmental pollution, residual toxicity, carcinogenesis, high cost and hence their use is restricted (Khuda *et al.*, 2012; Zeb *et al.*, 2014). Consequently, the search for alternative herbicides which are safe and

cost-effective is needed. Natural herbicides may be among these alternatives (Anjum and Bajwa, 2005, Ayaz *et al.*, 2014).

The sensitivity of early growth of seedling (% RLI and SLI) to *A. senegalensis* stem bark extracts as compare to % seed germination inhibition as indicated in Table 3, probably could be due to (1) the presence of seed coat that serves as a barrier between the seed embryo and its environment (Araujo & Monteiro, 2005), (2) the seed coats selective permeability that may prevent the inhibitory activity of phytotoxic extract/substances to penetrate the seed coats (Wierzbicka & Obidzinska, 1998), (3) the protrusion of the root through the seed coat (parameter used to measure germination) which does not necessarily mean growth by cell division (Salvatore *et al.*, 2008).

Conclusion

Conclusively, the methanolic extract of *A. senegalensis* could be relatively not toxic and safe for use in the preparation of medicinal products. The results also indicated that *A. senegalensis* stem bark extracts have phytotoxic properties and could contain substances that are phytotoxic. Hence, *A. senegalensis* could be a promising medicinal plant and plant product based natural herbicides.

REFERENCES

- Abbasi, A., Khana, M. A., Mushtaq, A., Muhammad, Z., Sarwat, J., Shahzia, S. (2010). Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province. *Ethnopharmacol.* 128:322–335.
- Abera, M. (2015). Determination of Levels of Some Heavy Metals (Pb, Cr & Cd) in Three Commercially Available Brands of Milk Powder Found in Harar Town, Eastern Hararge, Ethiopia. A M.Sc. Research Project submitted to Haramaya University, Ethiopia.
- Ahmad, M., Sultana, S., Hadi, S., Hadda, T., Rashid, Z. M., Khan, M., Khan, M., Yaseen, G. (2014). An ethnobotanical study of medicinal plants in high mountainous

- region of Chail valley (District Swat-Pakistan) *J Ethnobiol Ethnomed.* 10:36.
- Akram, S., Najam, R., Rizwani, G.H. & Abbas, A. S. (2015). Determination of heavy metal contents by atomic absorption spectroscopy (AAS) in some medicinal plants from Pakistani and Malaysian origin. *Pakistani Journal of Pharmaceutical Sciences*, 28(5): 1781-1787
- Putnam, A. R. and Tang, C. S. (1986). Allelopathy: state of science, in *The Science of Allelopathy*, Eds., pp. 1–19, John Wiley & Sons, New York, NY, USA.
- Araujo, A. S. F. and Monteiro, R. T. R. (2005). "Plant bioassays to assess toxicity of textile sludge compost," *Sciences in Agriculture*, vol. 62, pp. 286–290.
- Ahombo, G., Ampa, R., Diatewa, M., Mpati, J., Abena, A. & Ouamba, J. (2012). Investigating on related diabetes therapeutic plants used in traditional medicine at Brazzaville. *Journal of Medicinal Plant Research*, 6(44): 5630-5639.
- Ali, M., Aminu, F., and Ibrahim, S. (2017). In vitro Assessment of antibacterial activity and Phytochemical screening of *Vitex doniana* on clinical isolate of *salmonella typhi*. *International journal of Advance Academic research/science, Technology and Engineering* /ISSN: 2488-9849. Vol: 3.
- Aiyelaja, A. & Bello, O. (2006). Ethnobotanical Potentials of Common Herbs in Nigeria: A Case Study of Enugu State. *Edu. Res. Rev.*, 1(1):16-22.
- Anjum, T. and Bajwa, R. (2005). Importance of germination indices in interpretation of allelochemical effects. *International Journal of Agriculture and Biology*, 7, 417–419
- Ayaz, M., Junaid, M., Subhan, F., Ullah, F., Sadiq, A., Ahmad, S., Imran, M., Kamal, Z., Hussain, S. & Shah, S.M. (2014). Heavy metals analysis, phytochemical, phytotoxic and anthelmintic investigations of crude methanolic extract, subsequent fractions and crude saponins from *Polygonum hydropiper* L. *BMC Complementary and Alternative Medicine*, 14:465-473.
- Babalola, O, E.O, (1993) The persistence of African traditional medicine in contemporary Nigeria society. *African Marburgenesia*, 26:4.
- Chevallier, A. (1996). *The Encyclopedia of Medicinal Plants*. London: Dorling Kindersley; p. 185.
- Chukwuka, K.S., Ikhelola, J.O., Okonko, I.O., Moody, J. O., Mankinde, T.A. (2011). The antimicrobial activities of some medicinal plants on E.coli on agents of diarrhea in livestock. *Advance Appl. Sci. Res.*, 2:37-48.
- Dambatta, S. & Aliyu, B. (2011). A Survey of Major Ethno medicinal plants of Kano North, Nigeria, their Knowledge and Uses by Traditional Healers. *Bayero Journal of Pure & Applied Science*, 4(2):28-34
- Emmanuel, I.O. Agbafor, J. I. and Ede, R. J. (1996). Physicochemical and Antimicrobial screening of stem bark extracts of *Vitex doniana*. *American-Eurasian journal of scientific research*. 10(4): 248-250.
- Faleyimu, O. & Akinyemi, O. (2010). Herbal Approach to the Treatment of Chicken pox: a case study of Giwa Local Government, Kaduna State. Nigeria. *International Journal of Biomedical Health Science*, 6(1):13-18.
- FAO, (1996). Some medicinal forest plants of Africa and Latin America, *Journal of Science*, 67, 75–78
- Gong, P., Wilke, B.-M., Strozzi, E., and Fleischmann, S. (2001). Evaluation and refinement of a continuous seed germination and early seedling growth test for the use in the ecotoxicological assessment of soils. *Chemosphere*, vol. 44, no. 3, pp. 491–500
- Hussain, M. I., Gonzalez-Rodriguez, L., and Reigosa, M. J. (2008). "Germination and growth response of four plant species to different allelochemicals and herbicides," *Allelopathy Journal*, 22(1).101–110.
- Igoli, J., Ogaji, O., Tor-Anyin, T. & Igoli, N. (2005). Traditional Medicine Practice amongst the Igede People of Nigeria. Part II. *Afr. J Trad CAM*. 2(2):134-152.
- Innocent, E., Hassanali, A., Kisinza, W., Mutalemwa, P., Magesa, S. & Kayombo, E.

- (2014). Anti-mosquito plants as an Alternative or Incremental Method for Malaria Vector Control among Rural Communities of Bagamoyo District. *Tanzanian Journal of Ethnobiology & Ethnomedicine*, 10:56.
- Islam, A. K. M. and Kato-Noguchi, H. (2014). Phytotoxic Activity of *Ocimum tenuiflorum* Extracts on Germination and Seedling Growth of Different Plant Species. *The Scientific World Journal*, vol.2014, 8 pages.
- James, D.B., Owolabi, O.A., Bisalla, M, M. and Jassium, H. (2010). Effects of aqueous extract of (leaves and stem) of *Vitex doniana* on carbon tetrachloride induced liver injury in rats. *Journal of Pharmacol: Toxicol*; 1(1):1-5
- Jiofack, T., Fokunang, C., Guedje, N., Kemeuze, V., Fongnzossie, E., Nkongmeneck, B., Mapongmetsem, P., *et al.* (2009). Ethnobotanical Uses of some Plants of Two Ethnoecological Regions of Cameroon. *African Journal of Pharmacy and Pharmacology*, 3(13):664-684.
- Karayil, S. & Vivek, B. (2014). Heavy Metal Analysis from Traditionally used Herb *Ceropegia juncea* (Roxb.). *IOSR Journal of Pharmacy*, 4(12): 7-11.
- Khan, S.U., Khan, A U, Shah, A. H.A., Shah, S.M., Hussain, S., Ayaz, M. & Ayaz, S. (2013). Heavy metals content, phytochemical composition, antimicrobial and insecticidal evaluation of *Elaeagnus angustifolia*. *Toxicol. Ind. Health*.
- Khuda, F, Iqbal, Z, Zakiullah, K. A, Nasir, F. N. M (2012). Metal analysis, phytotoxic, insecticidal and cytotoxic activities of selected medicinal plants of Khyber Pakhtunkhwa. *Pak J Pharm Sci*. 25(1):51–58.
- Krishnaiah, D, Devi, T, Bono, A, Sarbatly, R. (2009). Studies on phytochemical constituents of six Malaysian medicinal plants. *J Med Plants Res*. 3(2):67–72
- Meriga, B, Mopuri, R, Krishna, T. (2012). Insecticidal, antimicrobial and antioxidant activities of bulb extracts of *Allium sativum*. *Asian Pac J Trop Med*. 5(5):391–395.
- Maobe, M. A. G., Gatebe, E., Gitu, L., and Rotich, H. (2012). Profile of heavy metals in selected medicinal plants used for the treatment of diabetes, malaria and pneumonia in Kisii Region southwest Kenya. *Global Journal of Pharmacology*, 6(3):245–251.
- McGrath, S. P, Smith, S. (1990). Chromium and nickel in heavy metals in soils. In: Alloway BJ, editor. Blackie, Glasgow. 125.
- Moghadamtousi, S., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H. & Kadir, H. (2015). *Annona muricata* (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities. *International Journal of Molecular Science*, 16: 15625-15658.
- Mustapha, A. A. (2013). *Annona senegalensis* Persoon: A multipurpose shrub, its phytotherapeutic, phytopharmacological and phytomedicinal uses. *International Journal of Science & Technology*, 2(12): 862-865.
- Mustapha, A. & Uthman, I. (2013). Plant Remedies Practiced by Keffi People in the Management of Dermatitis. *Journal of Medicinal Plants Studies*, 1(5):112-118.
- Noumi, E. & Safiatou, M. (2015). Some Investigations on the Traditional Pharmacopoeia about Venomous Bites and Stings from Scorpions, Snakes and Spiders in the Hina Subdivision, Far North, Cameroon. *British Journal of Pharmacological Research*. 344-358.
- Ofukwu, R., Ayoola, A. & Akwuobu, C. (2008). Medicinal Plants Used in the Management of Tuberculosis in Humans and Animals by Idoma Tribe of North Central Nigeria. *Nigerian Veterinary Journal*, 29(2):25-30.
- Peter, A.G. & De Smet M, (2002). Herbal Remedies. *New England Journal of Medicine*, 347: 2046-2056.
- Price, K. R, Eagles, J., Fenwick, G. R. (1988). Saponin composition of 13 varieties of legume seed using fast atom bombardment mass spectrometry. . 42:183–193
- Rice, E. L. (1984). *Allelopathy*, Academic Press, Orlando, Fla, USA, 2nd edition, 1984.

- Ruffo, C. K; Birnie, A. and Tengas, B. (2002) Edible wild plants of Tanzania. Relma Technical handbook serial 27. Nairobi, Kenya, pp: 766
- Salvatore, M., Carafa, A. M., and Carrat`u, G. (2008). Assessment of heavy metals phytotoxicity using seed germination and root elongation tests: a comparison of two growth substrates, *Chemosphere*. 73(9), 1461–1464.
- Salifou, S., Offoumon, O., Gouissi, F. & Pangui, L. (2012). Endogenous Recipes for Controlling Arthropod Ectoparasites of Domestic Poultry *Rev. Bras. Parasitol. Vet. Jaboticabal*, 22(1):119-123
- Shukla, S., Mehta, A. (2015) Anticancer potential of medicinal plants and their Phytochemicals; a review. *Braz. J. Bot.* 38(2): 199-210.
- Wang, K-W, Zhu, J-R, Shen, L-Q. (2013). A new lignan with anti-tumour activity from *Polygonum perforfoliatum* L. *Nat Prod Res.* 27(6):568–573
- Wardle, D. A., Ahmed, M., and Nicholson, K. S. (1991). Allelopathic influence of nodding thistle (*Carduus nutans* L.) seeds on germination and radicle growth of pasture plants, *New Zealand Journal of Agricultural Research*, vol. 34, no. 2, pp. 185–191.
- Wierzbicka, M. and Obidzińska, J., (1998). The effect of lead on seed imbibition and germination in different plant species. *Plant Science*, 137(2), 155–171.
- Williams, R. D. and Hoagland, R. E. (1982). The effects of naturally occurring phenolic compounds on seed germination. *Weed Science*, 30, 206–212.
- World Health Organization (WHO) (2005). Quality Control Methods for Medicinal Plant Materials, World Health Organization, Geneva, Switzerland.
- World health Organisation (WHO) (1989). Evaluation of Food Additives and Contaminants. *WHO Technical Report Series 776*, Geneva: World Health Organization
- Yang, Y., Yu, T., Jang, H-J., Byeon, S. E., Song, S-Y., Lee, B-H., Rhee, M.H., Kim, T. W., Lee, J., Hong, S. (2012). In vitro and in vivo anti-inflammatory activities of *Polygonum hydropiper* methanolextract. *J Ethnopharmacol.* 139(2):616–625.
- Zeb, A., Sadiq, A., Ullah, F., Ahmad, S. & Ayaz, M. (2014). Phytochemical and toxicological investigations of crude methanolic extracts, subsequent fractions and crude saponins of *Isodon rugosus*. *Biological Research*, 47:57.