ELEMENTAL, PHYTOCHEMICAL AND ANTI-MICROBIAL ANALYSIS OF THE PODS EXTRACT OF *Bauhinia purpurea* FROM MICHIKA NIGERIA.

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ABSTRACT

Laboratory investigations were carried out to evaluate the minerals, phytochemical and biological efficacy of the ethanol extract of the pods of *Bauhinia purpurea (Caesalpiniaceae*) on some disease causative microbes. The result of the elemental concentration using (AAS) reveals the presence of Mn (2±1.4), Na (BDL), Mg (12±2.1), Ca (5.2±1.2), Pb (0,0096±0.097), Fe (2±1.4), Cu (9±1.8). The phytochemical analysis showed the presence of saponins, terpenoids, glycosides, alkaloids, flavonoids but with tannins and resin below detectable levels (BDL). Antimicrobial activity against the microbes, *Staphylococcus aureus*, *Cyst Entamoaba histolitica*, *Escherichia coli*, and *Helicobacter pylori* showed moderate activity on all of them. These findings are in consonance with literature of plants and lend credence for the use of the pods of *Bauhinia purpurea*, as a potential natural drug source for the treatment of some ailments in the Michika environs.

KEYWORDS: Phytochemistry, Biological Efficacy, Bauhinia purpurea, Microbes, Minerals and Ailments.

INTRODUCTION

Herbs are now very popular in developing countries on account of proven knowledge about their safety, efficacy and quality assurance of ethno – medicine, (Mann, *et al*; 2011b). Medicinal plants are used for therapeutic purposes or are precursors for the synthesis of useful drugs used in forms of decoction, concoctions, infusion, sedative etc , (Bulus., 2009). The time- tested ethnic knowledge when supplemented with the latest scientific insights can offer new models of economic development that are both eco-friendly and socially acceptable (Croom, 1983). In fact, there is a consensus among the scientific community that natural products have been playing a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases (Newman, *et al*; 2003). Indeed, the vast majority of the existing chemotherapeutic agents are based on natural products, and this fact anticipates that new leads would certainly emerge from the tropical plant sources, since biological chemo-diversity continues to be an important source of molecular templates in the search for human and animal cure, (Ziegler, *et al*; 2002,

Kalauni, *et al;* 2006, Portet, *et al*; 2007 and Ronan *et al*; 2009). Worthy of note is the fact that, the Nomadic Fulani's of Adamawa highlands in North Eastern Nigeria choose faith healing first, traditional herbal medicine next and modern / orthodox medicine only when the first two have failed (Shariff, 2001, Sudhakar, 2007).

The bitter truth is that the robotic approach in discovering drugs from plants has proven, to date, its inability to excavate the hundreds of molecules that will contribute to the health progress of Man. However, one can reasonably see that the last patches of primary rainforest on earth hold still hundreds of spectacularly active drugs that await discovery. The successful isolation of these drugs will depend on rational and selective collection of plants, heightened powers of observation, creation of original concepts, and formulation of new hypotheses to attain the sudden insight of which will be born new theories to extend the frontier of knowledge (Christophe Wiart, 2006).

Scientists running research projects must make herculean efforts to mentor a new generation of other research and development projects with co- allies to facilitate the development of pilot biotechnology projects for when theories are refined, developed and proven and lead to the discovery of wonder drugs, the real therapeutic usefulness, will be identified as a result of serendipity, (Bernard, *et al;* 2001).

*Bauhinia purpurea (*Caesalpiniaceae) is used in India, Srilanka and Pakistan folk medicine to treat ulcer and diarrhea and by the Malays to treat glandular swellings and stomach tumour (Kurian, 2004). The stem bark is astringent, tonic and anthelmentic. It is used to cure ulcer and leprosy (Pawar; 2007). Its decoction orally taken twice a day is very effective in asthma and other respiratory diseases as an inflammatory, anticancerous, naphra protective and thyroid hormones regulating activity, (Patil *et al*; 2008). In Michilka LGA, Adamawa State Nigeria, the leaves and pods are used as plaster for old and fresh wound treatment, (Per. Comm, 2014).

Provoked by the plight of the inhabitants this research delves into analyzing the elemental composition, investigating their phytochemicals (non nutritive plant chemicals that have protective or disease preventive properties that are produced to protect the plant and can as well protect human diseases), and sourcing extracts from this medicinal plant and testing their antimicrobial activity in order to authenticate the claims of the traditional healers with the view of confirming or disproving the acclaimed curative properties of the pods of *Bauhinia purpurea.*

MATERIALS AND METHOD

Collection, preparation and treatment of sample

The pods of *Bauhinia purpurea* were plucked in Michika LGA Adamawa State on 10th February 2010 after the identification of the plant at the Ministry of Forestry, Mubi, Adamawa State. A specimen of the plant was kept in their herbarium with FHI number 60. The pods were laboratory dried and then pounded into a fine powder using pestle and mortar.

Analysis of mineral constituents

Analysis of mineral constituents (Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn) was carried out on about 2 g of each sample of the pods. This was ashed in the oven at about 400° C for 2 h. The elements in the ash were quantitatively taken up with 20 mL concentrated HNO_3, and filtered into 50 mL volumetric flasks. The volumes were made up to the mark with distilled water. Metals were determined by atomic absorption spectrophotometry (AAS) using Pye Unicam SP9 spectrometer (Pye Unicam Ltd., Cambridge, England). Phosphorus was determined by UV spectrophotometry using Spectronic 20 (Thermo Electron Scientific Instruments LLC, USA). The standard calibrations method was used as described by Vogel (2000).

Solvent extractions

The powdered sample (50 g) of the pods were charged in thimbles and extracted with 400 mL of 98% ethanol, using a Soxhlet extractor for 48 h. The extracts were concentrated using a rotary flash evaporator and preserved at 5°C in airtight, well labeled bottles until further use. The extracts were subjected to phytochemical analysis and antibacterial activity assay.

Phytochemical screening

Phytochemical analysis of all the evaporated solvent extracts was conducted in accordance with a standard procedure (Harborne, 1973). Tests for saponins terpenoids, glycosides alkaloids, flavonoids, tannins and resins were carried out in all the fractions.

Antibacterial activity assay

Four bacteria species: *Staphylococcus aureus*, *Cyst Entamoaba histolitica*, *Escherichia coli*, and *Helicobacter pylori* stock cultures were collected from New Life Specialist Hospital Laboratory Mubi Adamawa, Nigeria. The antibacterial assay was by the cup diffusion method on nutrient agar medium (BSAC 1997). Cups were made in nutrient agar plate using a cork borer (5 mm) and inoculums containing 10^6 CFU/mL of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Each solvent extract (50 µl) were placed in the cups made in inoculated plates. Treatments also included 50 µl Gentamicin (5 µg/mL) on separate plates, to serve as positive control for comparison and distill water as the negative control. All the plates were incubated for 24 h at 37°C and the zone of inhibition around the wells was measured in mm. Each treatment was conducted in triplicate.

Data obtained were subjected to statistical analysis using coupled MSExcel-Analyse-it® (2006). Independent Student's *t*-test at $p<0.05$ was considered significant in results of both mineral contents and antibacterial assay.

RESULTS

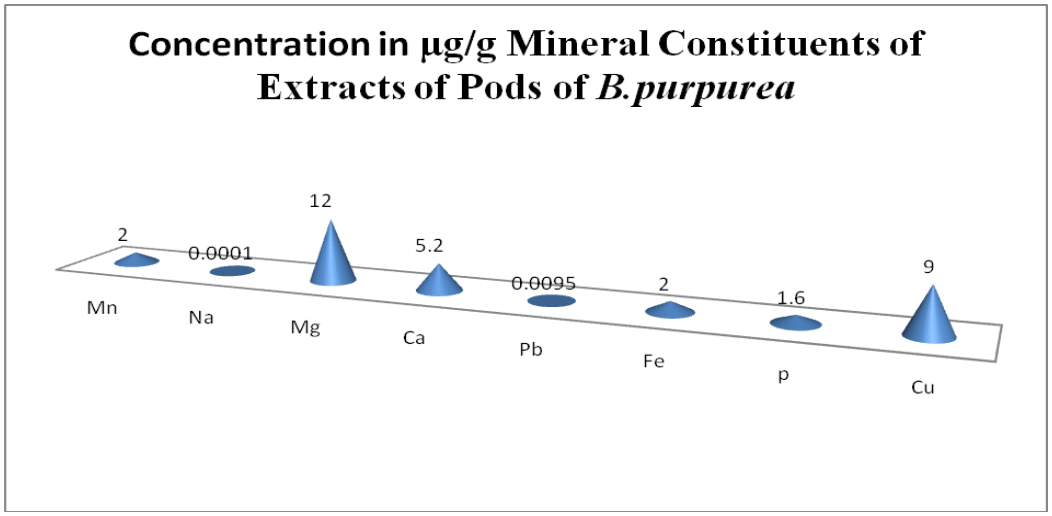

Figure.1: Results of Elemental Analysis.

Table 1: Results of Phytochemical Analysis of pods of B. *purpurea*

Phytochemicals	***B.Purpurea* Pod Extract**
Saponins	+
Terpenoids	+
Glycosides	+
Alkaloids	+
Flavonoids	+
Tannins	-
Resins	-

Key: + = present. - = Below Detectable Levels

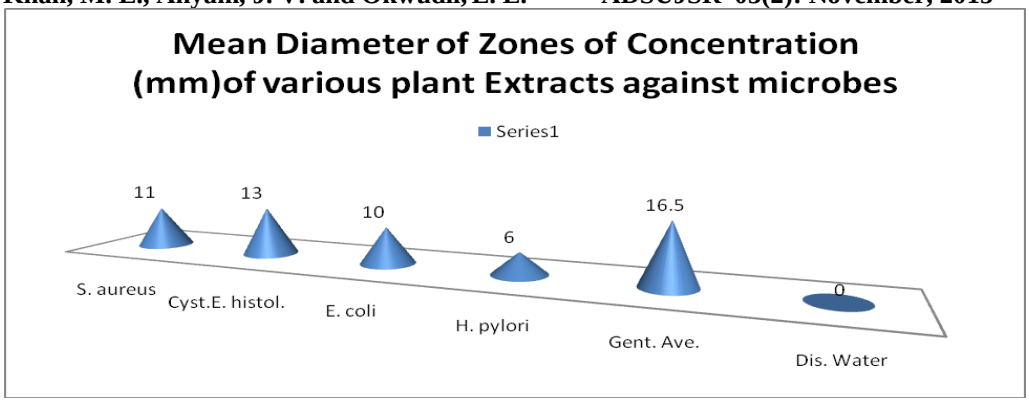

Figure 2: Results of Antimicrobial Investigation.

DISCUSSION

From Fig (1) it was observed that magnesium (Mg) had the highest concentration in the mineral. This could be as a result of the rocks around the zone where the plant was harvested containing this mineral. It plays a vital role in muscle formation, prevents high blood pressure and depression in enzyme activities. Its deficiency interferes with transmission of nerve and muscle impulse, causing irritability and nervousness, thus leading to heart diseases, (Siddhuraju & Becker; 2001, Shivraj, *et al*; 2009). Sodium (Na) was below detectable level. Calcium (Ca) was present in moderate concentration. It plays an important role in building and maintaining strong bones and teeth, large part of human blood and extra cellular fluids. It is also necessary for normal functioning of cardiac muscles blood coagulation, milk clotting and regulation of cell permeability. Its deficiency causes rickets, back pain, indigestion, irritability, premenstrual tension cramping of the uterus, (Shivery, *et al*; 2009). Copper (Cu) was detected and it is an important compound of many enzyme systems such as cytochrome oxidase, lysyl oxidase and ceruloplasmin; an iron oxidizing enzyme in blood. Deficiency has been associated with cardiac abnormalities in human and animals such as anaemia and Neutrogena, (Siddhuraju & Becker; 2001). Zinc (Zn) is important for wound healing and tissues building. The presence of Fe (iron) plays an important role in the formation of blood haemoglobin and cytochromes. Deficiency leads to anaemia, and hyperthyroid, (Siddhuraju and Becker. (2001). Lead (Pb), was present but in a level that could not be detected. It leads to toxicity in the body; causing respiratory problems and restricts the flow of blood into the body system. The Environmental Protection Agency (EPA) has detected and declared that there is no safe level of lead intake. It can cause high blood pressure and reduce haemoglobin production, necessary for O_2 transportation, thus interfering and can interfere with normal cellular Ca metabolism, (Montague & Peter; 2010).

The phytochemistry table (1) indicates the presence of

pharmacologically useful classes of compounds, saponnins terpenoids, glycosides, alkaloids, and flavovoids. Resins and tannins were below detectable levels. These secondary metabolites have been shown to have therapeutic activities of plants and function in a synergistic or antagonistic fashion for the treatment of diseases, (Trease and Evans, 1983). Saponins, a special class of glycosides, have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in plants are cardiotonic in nature and are reported to have anti-diabetic and anti-fungal properties (Kamel, 1991, Gupta, 1994). Alkaloids are reported to have analgesic, anti-inflammatory and adatogenic activities which help to alleviate pains, develop resistance against diseases and endurance against stress (Rauha *et al*; 2000). This confirms with literature as these secondary metabolites are used to treat malaria, asthma, dysentery / diarrhea and other hay fevers (Burkill, 1994, Rahila *et al*; 1994). The results of the above phytochemistry confirms with literature (Hassan *et al*; 2004, Faruq *et al*; 2004 and Olafimihan 2004). Thus, the present investigation clearly reveals the antibacterial nature of this plant and portrays it as a potential source of useful drug thereby suggesting that it could be exploited in the management of diseases caused by these bacteria in human and Plant systems (Raghavendra *et al;* 2006).

The result obtained for antimicrobial activity Fig. (2), showed moderate zone of inhibition for the tested organisms. The pods of *B. purpurea* were active against nearly all the microbes. *Cyst Entamoeba histolitica,* has the highest inhibition zone. This microbe causes stomach problems like gastrointestinal illnesses, dysentery/diarrhoea etc. This is proving of the effectiveness of the pod extracts on curing these ailments. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections (Bowersox, John, 1999). It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections, *E. coli and H. pylori*.cells are major components of serious food poisoning, feces, and fecal-oral transmission and urinary tract infections (Feng *et al;* 2002, Thompson, Andrea 2007). The plant is active against these microbes that cause fever and enteric pains (Machkaire, *et al*; 2011). This explains their usage traditionally as a good therapeutic agent for numerous diseases: ulcer, wounds etc, in Michika L.G.A. Adamawa state Nigeria.

CONCLUSION

The results of this investigation suggest that the pods of this indigenous plant, *B. purpurea* contain active ingredient(s) and could be a potential source for drug

development. This proclamation is confirmed, as their extracts indicate a relatively moderate number of minerals, phytochemicals and were also active against most of the disease causative microbes. More work could be conducted to further isolate, identify, characterize and elucidate the structure(s) of the bioactive compound(s) and possible mechanism(s) involved so that the use of this plant in ethno medical practice is further ascertained.

REFERENCES

Analyse-it® (2010) *General and Clinical Laboratory Analyses Software (Version 1.73),* Analyse-it Software Ltd., Leeds, England.

Bernard P, Scior T, Didier B, Hibert M, Berthon J. Y (2001). Ethno-pharmacology and bioinformatic combination for leads discovery: application tophospholipase A(2) inhibitors. *Phytochemistry;* 58:865–874.

Bowersox, J. (27 May 1999)."Experimental Staph Vaccine Broadly Protective in Animal Studies"(http://web.archive.org/web/20070505050641/http://www3 .niaid.nih.gov/news/newsreleases/19 99/staph.htm) NIH. Archived from the original (http://www3.niaid.nih.gov/news/newsreleases/1999/staph.htm) on 5 May 2007.http://web.archive.org/web/20070505050641/http://www3.niaid.nih.gov/news/newsreleases/1999/staph.htm. Retrieved 28 July 2007.

BSAC (British Society for Antimicrobial Chemotherapy) (1997) A Guide to Sensitivity Testing: Report of working party on antibioticsensitivity testing of the British Society for Antimicrobial Chemotherapy, *Journal of Antimicrobes Chemotherapy* **27**, 1-50

Bulus, I., (2004). Pyhtochemical Constituents and Anti-microbial Activity of two Medicinal plants, (*Ximenia Americana* and *Acacia nilotica*), unpublished project, Department of Chemistry Adamawa state University, Mubi.

Burkill, H. M (1994): The usefull plants of West Tropical Africa Vol 1. Families J –L. Royal Botanical Garden Kew: pp.522.

Christophe, W and Pharm, D. (2006). Ethnopharmacology of Medicinal Plants: *Asia and the Pacific* Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1 1-59745-160-6 (e-book)

Croom, E. M. (1983). Documenting and evaluating herbal remedies. *Eco. Bot*., 37: 13 -27

Faruq, U. Z, Adul-Malik, M and Dabai, Y. U (2004). Antibacterial activity of crude anthraquinone extract of *Sennaitalica* leaves. *Biosci. Res. Comm*.16: 7 -13.

Feng, P. Weagant, S. Grant, M. (2002-09-01). "Enumeration of *Escherichia coli* and the Coliform Bacteria" (http://www.cfsan.fda.gov/~ebam/bam-4.html) Bacteriological *Analytical Manual (8th ed.)*. FDA/Center for Food Safety & Applied Nutrition. http://www.cfsan.fda.gov/~ebam

/bam-4.html. Retrieved 2007-01-25.

Gupta, S. S. (1994). Prospects and prospective of natural products in medicine. *Indian J. Pharmacol*: 26:1 -12.

Harbone, J. B. (1973). Phytochemical Methods. Chapman and Hill Ltd, London. PP: 49 – 188

Hassan, M. M., Oyewale, A. O., Amupitan J. O., Abdullahi, M. S and Okonkwo, B.(2004). Prelimenary phytochemical and antibacterial investigation of crude extracts of the roots of *Detarium microcapum. J. Chem. Soc. Nig*.: 29: 26 -29

Kalauni, S. K., Awale, S., Tezuka, Y., Banskota, A. H., Linn, T. Z., Asih, P. B., Syafruddin, D. Kadota, S (2006). Antimalarial activity of cassane- and norcassane-type diterpenes from *Caesalpinia crista* and their structure-activity relationship. *Biol. Pharm. Bull.29*, 1050-1052.

Kamel, J. M. (1991). An extract of the mesocarps of fruits of *Balanite aegyptiaca* exhibited prominent anti-diabetic properties in Mice. *Chem. Pharmaco*. Bull: **39**: 1229 - 1233.

Kurian, J. C. (2004): plants that heal 7th edition, oriented Watchmen, Publishing House, Pune, Pp 31.

Machkaire, V., Turner, A. D. and Chivige, O. A. (2011): International Society for Horticultural Science. www.pubhort.org.

Mann, A, Ajiboso O, Ajeigbe S, Gbate M, and Isaiah S (2011b). Evaluation of the wound Healing activity of ethanol extract of *Terminalia avicennioides* root bark on two wound models in Rats. *Int. J. Med. Arom. Plants* 1(2):95-100.

Montague and Peter. (2010). Hearth Effects of lead, Online. http://www.welohart.net/lead/lead health

Newman, D. J., Cragg, G. M. Snader, K. M (2003). Natural products as sources of new drugs over the period 1981-2002. *J. Nat. Prod.*, *66*, 1022-1037.

Olafimihan, C. A. (2004). Effects of seasonal variation on the antibacterial activity of aqueous extract of *Azadirachta indica* fresh stem bark. *Biosci. Res.Comm*. 16: 13 -16.

Patil, G. G, Mali, P.Y, and Bhadone, V. V (2008). Folk remedies used against respiratory disorders in Jalgoon district, Maharastra. *Nat prod. Radiance J*, 354 – 368.

Pawar, S and Patil, D. A (2007). Ethno medicine uses of galgoon district, *Nat. Prod. Rodrance* **6**: 34 – 346.

Personal discussion with Pa. Lamurde on the traditional uses of the plant in their locality, 27th MAY 2014.

Portet, B., Fabre, N., Roumy, V., Gornitzka, H., Bourdy, G., Chevalley, S.; Sauvain, M., Valentin, A., Moulis, C (2007). Activity-guided isolation of antiplasmodial dihydrochalcones and flavanones from *Piper hostmannianum* var. *berbicense*. *Phytochemistry 68*, 1312-1320.

Raghavendra, M. P., Satish, S. Raveesha, K. A (2006). Phytochemical analysis and

antibacterial activity of *Oxaliscorniculata*; a known medicinal plant Agriculture Microbiology Laboratory, Department of Studies in Botany, University of Mysore,
Manasagangotri, Mysore, 570 006, India. (My SCIENCE 1(1), 72–78 ℃ University of Mysore http://myscience.uni-mysore.ac.in

Rahila, T., Rukhsandra, N., Zaidi, A A., Shamishilla, R. (1994). Phytochemical Screening of medicinal plants belonging to *Euphorbiaceae pak Vet. J*. 14: 160 -162.

Rauha, J. P., Remes S., Herinonen, W., Hopia, M., Kgjala, T., Pitinlaja, K., Vaorelam H and Vaorela, P. (2000). Antimicrobial effects of finished plant extract containing flavanoids and other phenolic compounds. *Int. J. Food Microbiol*: 56: 3 -12

Ronan, B., Ademir de Jesus, S. Jnr., and Alaíde, B. (2009). Plant-Derived Antimalarial Agents: New Leads and Efficient Phytomedicines. Part II. Non-Alkaloidal Natural Products; *molecules*, www.mdpi.com/journal/molecules; ***14***, 3037-3072;

Shariff, Z. U., (2001). Modern Herbal Therapy for Common Ailments. Nature pharmacy series, Spectrum Books Limited, Ibadan, Nigeria in Association with Safari Books (Export) Limied, United Kingdom, pp:9 -84

Shivraj, H. and Khobragade, C. N. (2009). Determination of nutritive value and mineral elements of some medicinal plants, **8.**

Siddhuraju and Becker, (2001). Effects of Minerals and their prospects; Accessed Available:http://www.unix-oit.umissedu/// ecclmen/581minerals htm

Sudhakar, R. C, Reddy, K. N, Thulsi, R. K and Chiranjibi, P (2007). Ethnobotanical Studies on Medicinal plants Used by the Chenchus of Nallamalais in Kunool Distridicct, Andhra Pradesh, India.Research *journal of medicinal plants*. (4):128 -133

Trease, G. E. and Evans, W. C. (1983): Textbook of Pharmacognosy. 12th Ed., Balliere, Tindall, London:

Thompson, A. (2007). "E. coli Thrives in Beach Sands" (http://www.livescience.com/health/070604_beach_ecoli.html) . Live Science.

Vogel, A. I. (2000). *Vogel's Textbook of Quantitative Chemical Analysis* 6th Ed. Revised by Mendham, J., Denney, R. C., Barnes, J. D. and Thomas, M. J. K. Pearson Edu. Ltd. India pp 104-107; 602-607.

Ziegler, H. L., Staerk, D., Christensen, J., Hviid, L., Hagerstrand, H., Jaroszewski, JW.(2002). *In vitro Plasmodium falciparum* drug sensitivity assay: inhibition of parasite growth by incorporation of stomatocytogenic amphiphiles into the erythrocyte membrane. *Antimicrob. Agents Chemother. 46*, 1441-1446.