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PHYTOCHEMICAL SCREENING, PROXIMATE AND MINERAL COMPOSITIONAL ANALYSES OF *PHYLLANTHUS NIRURI LEAVES*

J.A Adebisi[1], N.A Okunloye[1], V.A. Togun[2,Ω], J.I. Okwusidi[3]
[1]Department of Biochemistry, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
[2]Department of Animal Production and Health, Faculty of Agricultural Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
[3]Department of Physiology, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State. Nigeria.
Ω To a fond memory of Prof V.A. Togun who passed on in course of this study. R.I.P. Prof.

ABSTRACT: *Potent antimalarial effectiveness of aqueous leaf extract of P. niruri plant was recently demonstrated in this laboratory. As a prelude to detailed mechanistic studies of this aqueous leaf extract, an in-house phytochemical, proximate and mineral compositional analyses of P. niruri leaf samples, by AOAC procedures were hereto undertaken. Results of phytochemical screening of the leaf samples revealed the presence of alkaloids, tanins, antraquinones, glycosides, saponins, flavonoids, steroids and terpenoids. Proximate analysis showed percentage values of moisture, ash, crude fibre, ether extract, crude protein, nitrogen free extract and carbohydrate contents. Mineral compositional analysis revealed the presence (mg/l) of major and minor elements, Zn, Fe, Na, Mn, K, Mg, Ca, and Cu, in natural blend. These results show potentials of P. niruri leaf samples and suggesting it as a good source for mineral supplementation. These findings may also explain some basis of antimalarial properties of aqueous extract of P. niruri leaves.*

KEYWORDS: aqueous leaf extract, phyllanthus niruri, phytochemical screening, proximate and mineral analyses.

INTRODUCTION

In recent times, focus on plant based research has increased all over the world and a large body of evidence has accumulated to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 15 years period (Pande et al., 2007). *Phyllanthus niruri* L. (Euphorbiaceae) is a small erect herbaceous medicinal plant with pale green leaves having small oblong elliptic glabrous leaves with diverse global naturopathic applications and potentials yet to be fully elucidated. It is widely distributed in the tropics, and grows up to 30-40 cm in height (Bagchi et al., 1992), with little girth. This plant is widely spread throughout the tropics and subtropics in sandy region as weed in cultivated and waste lands (Ross, 1999), and has a high utility in ethnobotanical medicine.

Phyllanthus niruri (P. niruri) leaves contain bitter substances Phyllanthin, Hypophyllanthin, Niranthin, Nirtetralin, Niruretin, Nirurin, Niruriside, Phyltetralin. Quercetin, kaempferol-4- rhamnopytanoside (Bep-Oliver-Bever (1986), Rastogi and Mahrotra, (1991) and De Souza et al., (2002)). *P. niruri* was also found to have anti-

oxidant and heptoprotective properties and antiinflammatory potential (Kiemer et al, 2003). Some of the flavonoids obtained from this plant had shown antinociceptive properties (Santos et al., 2002), latex with oil in Opthalmia. Stem and leaves yield a black dye which possesses antibacterial property (Agarwal, 2003). The plant is popular with rural people because of its immense medicinal properties like antidote against liver diseases, antiviral properties**,** antioxidant, heptoprotective, anti inflammatory and strong inhibitory effect against neurogenic pain (Thyagarajan et al., 1998, Kiemer et al., 2003, Chattopadhyay et al., 2006). Current medicinal knowledge of the activity of plant phenolics indicates that useful drugs may be developed from them in the future, or that they could be used as templates for further research and development.

Minerals are inorganic elemental species or substances that are of neither animal nor vegetable origin (that is, natural compounds generally not containing a C, H, O, or N skeleton). Inorganic elements constitute the major part of dry ash that remains after ignition of organic matter, and consequently dry-ashing techniques are still the main stay way to determine total minerals in foodstuffs (Nielsen, 2010). Classification of minerals can be carried out according to different methods (O'Dell and Sunde, 1997). They can be classified and divided, for instance, into two groups (minerals and trace elements) taking into account their essentiality or well-known nutritional concern, recommended concentration as mg/kg for minerals or μg/kg for trace elements), and maximum limits (MLs) in terms of toxicity. As well, minerals are nutritional elements known as the main cellular and structural building materials taking part in osmotic/oncotic and acid/ base regulation exemplified by, calcium (Ca), potassium (K), sodium (Na), phosphorus (P), magnesium (Mg), and chlorine (Cl).

Trace elements on the other hands are typically known as electrolytes, enzymes, and hormones, constituents of which the biological roles are known for nutritive value-adding and health impact. These typically include iodine (I), iron (Fe), copper (Cu), chromium (Cr), molybdenum (Mo), selenium (Se), manganese (Mn), zinc (Zn), and cobalt (Co). In addition, some trace elements fluorine (F), boron (B), silico (Si), nickel (Ni), vanadium (V), lithium (Li), and antimony (Sb) still have as yet undefined/unknown or limited/restricted beneficial impact on metabolic and physiological functions (O'Dell and Sunde, 1997). Furthermore, some other elements are either regulated additives: aluminum (Al), sulfur (S) as sulfites and sulfates, bromine (Br) as bromides and bromate salts or strictly controlled toxic contaminants with known negative health impact. This latter group includes arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), and tin (Sn) (Codex Alimentarius, 2013, Enzyme Commission Regulation (EC) 1881/2006, 2006).

Finally, several minerals and trace elements can also be part of adulterant compounds intentionally added to foodstuffs for economic and/or other reasons such as authenticity and/or fraudulence and tampering). These inclusions lessen the purity or effectiveness of foodstuffs, causing an undesirable effect with safety risks and damaged integrity by possible loss of beneficial substance properties (Kelly et al., 2005, Moore et al., 2012). These premises formed the basis of necessary prudent physicochemical screening, physicochemical characterization, and compositional analysis of putative drug agent.

A recent study from this laboratory has demonstrated a dose dependent effectiveness and potency of aqueous leaf extract of P. niruri against malaria parasitemia induced by Plasmodium (P) berghei, NK 65, an analog of P. falciparum, the parasite that causes malaria infestation. Malaria is a major public health scourge and a cause of global mortality and morbidity in most tropical and sub-tropical region of the world (Adebisi et al., 2021). This study was thus construed to undertake in-house phytochemical, proximate and mineral compositional analyses of leaf samples of P. niruri prelude to further detailed mechanistic studies of its aqueous extract.

MATERIALS AND METHODS

MATERIALS

Plant Collection, Authentication

The plant, *P. niruri* was collected in its natural habitat in and around Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. It was identified and authenticated by an Angiosperm Taxonomist in the Biology Department, Ladoke Akintola University of Technology, (LAUTECH) Ogbomoso, Oyo state, Nigeria and issued a voucher number LHO374. A specimen of the plant has depicted in Figure 1 was deposited at LAUTECH Herbarium, Ogbomoso, Nigeria.

Figure 1: Phyllanthus niruri L.; (A) whole plant (B) aerial part (C) leaves.

Scientific Description:

Current Plant Name: Phyllanthus niruri Sch.
Synonym(s): P. amarus. Schum and Thonm
Common Names: Stone Breaker, Chanca Pietra plant. Yor: Ehin Olobe/Elebe
Family: Euphorbiaceae
Order: Euphorbiales

Equipments:

Atomic Absorption Spectrophotometer (Agilent Technologies Inc), Mettler Toledo Analytical Balance, Murfle furnace (Meditech), Hot plate(Wincom Company Ltd), Hot

Air oven (Genlab,uk), Desiccator (Fisher Scientific Inc), Soxhlet extractor with reflex condenser and timble(ACE glass Inc), Kjeldahi digestion system and distillation unit(Tecantor Digestion system, 1007 Digestor), Platinum crucibles, Spatulas.

Glassware and Consumables:
Beakers and measuring cylinder 50ml to 500ml, Elemeyer or conical flask 50ml-500ml **(Pyrex),** Cotton wool (Pyrex, Nig), Whatman No 1 filter paper.

Reagents and Chemicals:
Boric acid (H_2BO_3), Sulphuric Acid (H_2SO_4), Hydrochloric acid (HCl), sodium hydroxide (NaOH), mercury oxide(HgO), Selenium oxide, Anhydrous copper sulphate, iron sulphate sodium sulphate, Hexane, Methyl red, Bromocresol green, were supplied by a consortium of University certified and reputable local suppliers/agents of International manufacturers of laboratory research chemicals.
All reagents used in the study were of analytical grade and were prepared in all glass distilled water.

METHODS

Plant Preparation
Leaves of P. niruri plants were plucked and slightly rinsed in cold tap water to remove sand, dirt and dust. The leaves were thoroughly air dried and pulverized to fine powder with the aid of heavy duty laboratory blender (Mahavir Impex, Mumbai, India). The powder was stored in air tight individual zip locked plasticized containers in 20g working portions for subsequent uses.

Phytochemical Screening
Phytochemical screening of P. niruri leaf samples were carried out using standard and other published procedures to test for the presence of alkaloids, tannins, anthranquinone, glycosides, saponin, flavonoids, phlobatanins, steroids, terpenoids and purine alkaloids (Bruce et al., 2019; Harborne, 1993)

Proximate Analysis
Proximate analysis evaluated physicochemical parameters: moisture and ash content as well as crude fiber, ether extract, crude protein, nitrogen free extract and carbohydrate content of the leaf sample of the P. niruri, essentially as described by Tahira et al, 2012.

Compositional Mineral Analysis:
Mineral content of the leaf sample was done basically by ashing method as described in the analytical procedures of AOAC (2005).
The absorbances of the minerals were determined spectrophotometrically with the aid of Atomic Absorption Spectrophotometer (AAS). The absorbance values were read off accordingly at the respective wavelengths of the elements: Fe-248.5nm, Cu-324.7nm, Zn-213.9nm and Mn-279.5nm, K-766.5, Na-589.0, Mg-285.2, and Ca-422.8 (AOAC, 2005; British pharmacopeia, 2011).

Statistical Analyses

Data obtained from the various analyses were subjected to basic descriptive statistics and dispersion around the mean (Daniel, 1983). Values of all experimental variables were given as Mean±SD.

RESULTS

The results of phytochemical screening carried out on leaf samples of P. niruri as well as brief paraphrased details of procedural and analytical protocol are summarized in Table 1.

Table 1: Summary of details of phytochemical analysis of leaf extract of *Phyllanthus niruri* showing the presence (+) or absence (-) of phytochemical constituents

Phytochemical	Brief test procedure/protocol	Observation	Indication
Alkaliods	0.2g of leaf sample + 2% H_2SO_4 for 2 mins + filteration + few drops of Dragencloff reagent	Orange precipitate	+
Tannins	0.5g of leaf sample + 2ml of H_2O + heat + filteration + ferric chloride to filterate	Dark green solution	+
Anthraquinones	0.5g of leaf sample + 2ml of 10% HCl + heat for few minutes + filteration + cooling. Equal volume of $CHCl_3$ + filterate + few drops of 10% NH3+ heat	Rose pink colour	+
Glycosides	0.5g of leaf sample + 2ml HCl + NaoH + FEW DROPS OF FEHLING SOLUTION A and B	Red precipitate	+
Saponins	0.2g of leaf sample + 5ml H_2O, shaking heat	Frothing (appearance of creamy miss of small bubbles)	+
Flavonoids	0.2g of the extract + dilute NaOH + HCl	Yellow solution later turns colourless	+
Phlobatanins	0.5g of leaf sample + H_2O filtration + boiling with 2% HCl	Red precipitate	-
Steroids	2ml acetic anhydride + 0.5g leaf sample + 2ml H_2SO_4	Colour change from violet to green	+
Terpenoids	Salkowski test	Reddish brown colouration	+

Purine Alkaloids	0.5g of leaf sample + 10% sodium carbonate + boiling+ little chloroform + filteration. Residue + few drops of HCl on watch glass + crystal of potassium chlorate + evaporation + cooling + 2 drops of ammonia solution	Purple colour	-

Evident from Table 1, the leaves of P. niruri plant contain alkaloids, tannins, anthraquinones, glycosides, saponins, flavonoids, steroids. Conspicously absent from the phytochemicals present in these leaf samples were phlobatannins and purine type alkaloids.

Determined Proximate parameters and their percent occurrence in leaves of P. niruri are depicted in Table 2.

Table 2: Summary of values (Mean±SD) obtained from proximate and compositional mineral analyses of leaf samples of P. niruri.
N.F.E; nitrogen free extract; % N.F.E = 100 – (% Ash + % Crude fibre + % Ether extract + % Crude protein), Total Carbohydrate = % N.F.E + % Crude fibre (Tahira et al, 2012).

S/N	**ANALYSIS**			
	Proximate		**Mineral composition**	
	Parameter	**% occurrence**	**Mineral**	**Concentration(mg/l)**
1	**Moisture**	9.57 ± 0.127	Zn	146 ± 0.001
2	**Ash**	7.88 ± 0.116	Fe	389.63 ± 0.039
3	**Crude fibre**	14.70 ±0.100	Na	733.75 ± 0.009
4	**Ether extract**	13.23 ±1.283	Mn	179.06 ± 0.001
5	**Crude protein**	17.58 ±0.254	K	13001.25 ± 0.070
6	**N.F.E**	46.61±0.110	Mg	2500.00 ± 0.040
7	**Carbohydrate**	61.31±0.100	Ca	8898.75 ± 0.060
8	–	–	Cu	31.58 ± 0.004

Obvious from Table 2, carbohydrate and nitrogen free non-proteinous extract (NFE) constituted the bulk of the proximate matter at almost 69% and 55% respectively. The former being the summation of % NFE and % crude fiber. The latter, % NFE was computed as 100 less the summed value of % moisture, % ash, % crude fiber and % ether extract (Tahira et al, 2012). Displayed also in Table 2 are the mineral contents of the leaf samples. The detected minerals were Fe, Cu, Zn, Mn, K, Na, Mg and Ca. Contents and their respective values in mg/l indicate that Ca, Mg and K were present at highest concentrations at 8899, 2500 and 13001mg/l respectively. These were followed by Na at 733mg/l, and to a lesser extent Fe and Mn. Both Zn and Cu occurred least at 146 and 32mg/l respectively (Table 2).

DISCUSSION

The phytochemical screening of leaf samples of *P. niruri* revealed the presence of alkaloids, tannins, antraquinones, glycosides, saponins, flavonoids, steroids and terpenoids. Flavonoids are the other form of the two plants phenolic structures. Phytochemicals exhibit different structural characteristics with various pharmacological actions. For example, lignans have excellent hepatoprotective (Chang et al., 2003,Yan et al., 2009) and anti-viral properties (Gnabre et al., 2001), whereas terpenes exhibit anti-microbial activities. Flavonoids from *P. niruri* have been shown to have antioxidant (Hayashi et al., 2012), antileishmanial, and anti-inflammatory properties (Guardia et al., 2001). Phytochemical studies have shown that extracts of genus Phyllanthus contain a variety of components, including gallic acid (Calixto et al., 1998, Patel et al., 2011). Furthermore, studies have demonstrated cytotoxic activity of gallic acid on the human promyelocytic leukemia HL-60 cell lines (Ishihara and Sakagami, 2003, Sakaguchi et al., 1999). Gallic acid has also been shown to induce apoptotic cell death in HSC-2 and HL-60 cells (Pinmai et al., 2008).

The activity might be attributed to the presence of phytochemicals which have been identified in this present work; or even a combined action of more than one metabolite. However, the active principle(s) responsible for this observed activity need to be fully delineated.

Proximate analysis is defined by H. Bennett in the *Concise Chemical and Technical Dictionary* (CCTD, 1947) as the "determination of a group of closely related [bio]components together, e. g. total protein, fat." It conventionally includes determinations of the amount of water, protein, fat (ether extract), ash and fiber, with nitrogen-free extract, NFE (sometimes termed Nifext) being estimated by subtracting the sum of these five percentages from 100. In order to emphasize the group nature of the percentage of protein, fat and fiber, many Chemists use the word "crude" before these three terms.

The current proximate analysis of leaves of P. niruri revealed percentage values of moisture, total ash, crude fibre, ether extract and hence enhancing the drug characteristics of the leaves. For example the current moisture content of 9.5% of the leaf samples falls within the general limit of 8-14% acceptable for crude drugs (British pharmacopoeia, 2011). The moisture level is suggestive of potent stability of crude drug against microbial degradation (Okoye et al., 2020) which could lead to breakdown of important constituents of the drug sample, and microbial growth during the storage of the sample drug. Total ash on the other hand, indexes relative purity: freedom from foreign organic matters, sand, and soil adulterants which could alter the quality of the whole drug (Kunle e tal., 2002). In this instance study, total ash was less than 10% (Table 2). The crude protein, NFE and carbohydrate content of the leaf samples were relatively high. Thus the results of the present study suggest that P. niruri leaves possess tangible nutrichemical value.

Minerals analysis revealed the presence of Zn, Fe, Na, Mn, K, Mg, Ca, and Cu, and quantified in their concentrations, mg/l. The leaf samples of P.niruri by composition present as a good source of mineral supplement. The results obtained from mineral

analysis show that P.niruri leaves contain minerals, zinc, iron, sodium, manganese, potassium, magnesium, calcium and copper, with potassium being present at the highest concentration. Finally, as revealed in this study, P. niruri leaves contained some of these minerals in very high concentrations, suggestive that the leaves of P.niruri could be used as a very good source of minerals.

CONCLUSION

In conclusion, the phytochemicals observed in the present study may explain some of the basis of the antimalarial properties of *P.niruri* as described in various experimental studies. The proximate analysis of *these leaves* samples show that they could be of good dietary benefits. The high concentrations of the minerals both major and minor present in these leaf samples strongly suggest that *P.niruri* leaf extract could serve as potential mineral supplements. Finally, these results of this instant study may also explain some of its naturopathic consumption as mineral decorticate by humans.

Future Research

Malaria continues to be a major public health scourge and a cause of global mortality and morbidity in most tropical and sub-tropical region of the world. Adebisi et al. (2021) as recently demonstrated a potent dose dependent effectiveness and potency of aqueous leaf extract of P. niruri against malaria parasitemia induced by Plasmodium (P) berghei, NK 65, an analog of P. falciparum, the parasite that causes malaria infestation. Detailed mechanistic studies of extracts of these *P.niruri* leaves are thus planned to elucidate their mechanistic role in malaria therapy.

REFERENCES

Adebisi, J.A; Okunloye, N.A.; Togun, V.A. and Okwusidi, J.I. (2021) Evaluating in vivo antiplasmodial potency of aqueous leaf extract of phyllanthus niruri in swiss albino mice. Euro J. Biol. Med. Sci Res 9(1):10-21

Agarwal VS. (2003) Directory of Indian Economic Plants: 377.

AOAC (2005). Methods of Analysis of Association of Official Analytical Chemistry 16th Edition Washington D.C, USA pp600-792

Bagchi GD, Srivastava GM, Singh SC.(1992) Distinguishing Features Of Medicinal Herbaceous Species Of *Phyllanthus* Occurring In Lucknow. District (Up) India. Int.J.Pharmacognosy. 30: 161-168.

Bep-Oliver-Bever.(1986) Medicial Plants of Topical West Africa. Cambridge University Press, Cambridge.

British Pharmacopoeia (2011) Ash values, Acid insoluble Ash, water soluble, Extractive and Alcohol soluble extractive. Appendix xi. Her majesty stationary office London A108, A113.

Bruce, S.O; Onyegbule F.A. and Ezugwu C.O. (2019) Pharmacognostic, Physiochemical and Phytochemical evaluation of leaves of Fadogia denkowskii Schweinf (Rubiaceae). J of Pharmacognosy and Phytotherapy 11(3) 52-60.

Calixto, J.B., Santos, A.R.S., Filho, V.C. and Tunes, R.A. (1998). A review of the plants of the genusPhyllanthus: their chemistry, pharmacology, and therapeutic potential. J. Med. Biol. Res. 31: 225-258.

Chang, C. C., Lien, Y. C., Liu, K. C., Lee, S. S. (2003). Lignans from Phyllanthusurinaria.Phytochemistry 63: 825–833

Chattopadhyay P, Agrawal SS and Garg A. (2006) Liver regenerative effect of *Phyllanthus amarus* Linn. Against alcohol induced liver cell injury in partially hepatectomised Albino Rats. International J. of Pharm. 2(4): 426-430.

Codex Alimentarius (2013) International food standards, general standard for contaminants and toxins in food and feed, Codex Standard 193-1995, Rome, Italy

Commission Regulation (EC) 1881/2006 (2006) *Off. J. Eur. Comm*., L 364, 20.12.2006, 0018-00.20

Daniel, W.W. (1983) Biostatistics: A foundation for analysis in the health sciences, 3rd Ed. New York John Willey and Son. Pp 15-28.

De Souza TP., Holzschuh MH, Lionco MI, Ortega GG and Petrovick PR. (2002) Validation of a LC method for the analysis of phenolic compounds from aqueous extract of *Phyllanthus niruri* aerial parts. J. Pharma. Biomed. Anal.30: 351-356.

Gnabre J.N., Itob Y, Mab Y, Huanga R.C., (1996). Isolation of anti- HIV-l lignansfrom Larrea tridentate by counter-current chromatography. Journal of Chromatography; 353-364

Guardia T, Rotelli AE, Juarez AO, Pelzer L.E., (2001). Anti-inflammatory properties of plant flavonoids.Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat.Farmaco; 56: 683-687

Harborne J.B (1973) Phytochemical methods: A guide to modern techniques of plant analysis. Chapman A & Hail, London. P279

Hayashi Y, Matsushima M, Nakamura T, Shibasaki M, Hashimoto N, Imaizumi K, Shimokata K, Hasegawa Y, Kawabe T. (2012). Quercetin protects against pulmonary oxidant stress via hemeoxygenase-1 induction in lung epithelial cells. BiochemBiophys Res Commun; 417: 169-174

Ishihara M, Sakagami H., (2003). Application of semiempirical method to estimate the cytotoxic activity of gallic acid and its related compounds. Anticancer Res; 23: 2549-2552

Kelly, S., Heatonb, K., & Hoogewerff, J. (2005) *Trends Food Sci. Technol*. **16**, 555–567.doi:10.1016/j.tifs.2005.08.008

Kiemer AK, Hartung T, Huber C and Vollmar AM. (2003) *Phyllanthus amarus* has anti-inflammatory potential by inhibition of iNOS, cox- 2 and cytokine via the NF-KB pathway. *J. Hepatol*. *38*: 289-297.

Moore, J.C., Spink, J., & Lipp, M. (2012) *J. Food Sci*., **77**, R118–R126. doi:10.1111/j.1750-3841.2012.02657.x

Nielsen S. (2010) in *Food Analysis*, S. Nielsen (Ed) Springer, New York, NY. doi:10.1007/978-1-4419-1478-1

O'Dell, B.L., & Sunde, R.A. (1997) *Handbook of Nutritionally Essential Mineral Elements*, Marcel Dekker Inc., New York, NY

Okoye V.O.; Brase S.O. and Onyegbule, F.A (2020) Phytochemical screening and pharmacognostic properties of Peurar phaseoloides leaves (ROXB), Benth. (Fabaceae). Inter J. Pub. Health Pharmacy Pharmacol.5(2):11-24.

Pande PC, Tiwari L, Pande HC, (2007) Ethnovetenary plants of Uttaranchal- A review. Indian J Trad Knowl , 6:444-458.

Patel, Jay Ram; Tripathi, Priyanka; Sharma, Vikas; Chauhan, Nagendra Singh; Dixit, Vinod Kumar, (2011). "Phyllanthusamarus: Ethnomedicinal uses, phytochemistry and pharmacology: A review".Journal of Ethnopharmacology 138 (2).

Pinmai K, Chunlaratthanabhorn S, Ngamkitidechakul C, Soonthornchareon N, Hahnvajanawong C.(2008). Synergistic growth inhibitory effects of Phyllanthusemblica and Terminaliabellerica extracts with conventional cytotoxic agents: doxorubicin and cisplatin against human hepatocellular carcinoma and lung cancer cells. World J Gastroenterol 2008; 14: 1491-1497

Rastogi RP, Mahrotra BN. (1991, 1993,1995 &1998) Compendium of Indian Medicinal Plants. Vol.3, Vol.3, Vol.4 , vol.5, Csir New Delhi, India.

Ross L. (1999) Medicinal Plants of the World. Chemical Constituents, Traditional and Modem Medicinal Uses. Pub: Humana Press Inc., Totowa, New Jersey: 249-254.

Sakaguchi N, Inoue M, Isuzugawa K, Ogihara Y, Hosaka K., (1999). Cell death-inducing activity by gallic acid derivatives.Biol Pharm Bull; 22: 471-475

Santos ARS, Chechinel Filho V, Miguel OG, Siani AC, Campos MM, Yunex RA and Calixto JB. (2002) Antinoceptive properties of extracts of new species of plants of the genus *Phyllanthus* (Euphorbiaceae) J. Ethnopharmacol. 72:229-238.

Tahira M, Ali B, Shah Z, Danish M, Khalid S. Elemental and Nutritional analysis and Ethanomedicinal study of selected wild plant species of district swabi khyber, Pakhtunkhwa, Pakisthan. J Pharm Res 2012; 5;9:4910-3.

Thyagarajan SP, Subremanian S, Thirunalasundari T, Venkateswaran PS and Blumberg BS. (1998) Effect of *Phyllanthus amàrus* on chronic carriers of hepatitis B virus. Lancet .*2*; 764-766.

Yan F, Zhang QY, Jiao L, Han T, Zhang H, Qin LP, Khalid R., (2009).Synergistic hepatoprotective effect of Schisandraelignans with Astragalus polysaccharides on chronic liver injury in rats.Phytomedicine; 16: 805-813.

Concise Chemical and Technical Dictionary.(CCTD)(1947) Edited by H. Bennett.J. Lewis Maynard: *J. Phys. Chem.*, 51, 5, 1217–1218.

Conflict of interests

The authors hereby declare no conflicts of interests