

Proximate and phytochemical composition of *Phyllanthus amarus*

CE Achikanu *, II Ujah and MO Ezenwali

Department of Applied Biochemistry, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria.

World Journal of Advanced Research and Reviews, 2022, 15(01), 041–047

Publication history: Received on 13 May 2022; revised on 22 June 2022; accepted on 24 June 2022

Article DOI: <https://doi.org/10.30574/wjarr.2022.15.1.0517>

Abstract

This study aimed to determine the proximate and phytochemical composition of *Phyllanthus amarus*. The results of proximate composition of *Phyllanthus amarus* showed that the plant leaves contains fat 8.40 %, moisture 6.48 %, ash 10.58 %, fibre 2.40 %, protein 23.97 % and carbohydrate 48.02 %. The results of phytochemical composition of *Phyllanthus amarus* revealed Kaempferol 2.3726 ug/ml, Steroid 15.7626 ug/ml, Proanthocyanidin 8.1619 ug/ml, Catechin 23.3542 ug/ml, Anthocyanin 3.8979 ug/ml, Narigenin 13.5721 ug/ml, Dihydrocycytisine 8.0184 ug/ml, Cyanogenic glycoside 8.7028 ug/ml, Aphyllidine 0.2959 ug/ml, Ammodendrine 6.2495 ug/ml, Tannin 9.8090 ug/ml, Flavonoes 8.7210 ug/ml, Cardiac glycoside 14.2203 ug/ml, Flavone 3.60099 ug/ml, Ribalinidine 10.1874 ug/ml, Spartein 4.1965 ug/ml, Phytate 8.1192 ug/ml, Oxalate 9.0074 ug/ml, Epihedrine 1.9501 ug/ml, Sapogenine 18.0269 ug/ml. It may be concluded that the leaves of *Phyllanthus amarus* contain several phytochemicals that could be of good therapeutic purposes.

Keywords: Phytochemical; Proximate; *Phyllanthus amarus*; Steroid; Tanin

1. Introduction

The world is amended with kinds of medicinal plants which have drawn the attention of inquiries due to their myriad benefits to humanity, especially their operation in medicinal usefulness [1]. Over four-quarter of the world's population had been reported using herbal products as necessary medicine [1].

Phyllanthus amarus is a factory of the family *Euphorbiaceae* and has roughly 800 species that are planted in tropical and subtropical countries of the world [2]. The name "*Phyllanthus*" means "splint and flower" and was named so because of its appearance where flower, fruit, and splint appear fused [3]. This plant has been used in traditional drugs for more than three millennium [4].

Phyllanthus is generally employed to reduce pain and expel intestinal gas, to stimulate and promote digestion, as anti-helminths to expel intestinal worms and as a mild laxative. Fruits, vegetable, and medicinal sauces contain a wide variety of free revolutionaries scavenging motives, similar to the phenolic emulsion, vitamins, and some other metabolites that are rich in antioxidant exertions [5]. *Phyllanthus amarus* also have antiseptic, diuretic, antiviral, anti-diabetic, hypertensive, and antipyretic parcels and is used in the treatment of hostility, diarrhea, dysentery, injuries, ulcers, and urogenital conditions [6]. *Phyllanthus amarus* has been classified among plants with a low toxicity with the LD₅₀ averaging 200 mg/ kg/ day [7].

*Corresponding author: CE Achikanu, email: cosmas.achikanus@esut.edu.ng

Department of Applied Biochemistry, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria.

Phytochemicals are non-nutritive-action factory chemicals that have defensive or condition preventative parcels [8]. It's well known that plants produce these chemicals to cover themselves, but recent exploration demonstrates that they can cover humans against conditions. The most important of these bio-active elements of these plants are alkaloid, tannin, flavonoid, and saponin emulsion. Proximate analyses determine the proximate composition of any substance and as well express the nutritive values [9]. The end of the study was to determine the proximate and phytochemical composition of *Phyllanthus amarus*.

2. Methods

2.1. Collection, Identification, Preparation of *Phyllanthus amarus*

Fresh leaves of *Phyllanthus amarus* were collected from the riverbanks of Amuri in Nkanu West Local Government Area in Enugu State Nigeria, in the month of October –November, 2021. The plant was identified and authenticated by Botanist in the Department of Applied Biology and Biotechnology Faculty of Applied Natural Science, Enugu State University of Science and Technology Agbani. The collected plant leaves were air-dried at room temperature (24 °C) for two weeks. The air-dried leaves were then pulverized using mortar and pestle, the fine powder obtained was weighed and the crude powdered leaves were used to prepare the extracts.

2.2. Extraction of *Phyllanthus amarus* leaves

A quantity, 800 g of the ground sample was weighed using analytical weighing balance and soaked in 400 ml of petroleum ether, it was shaken vigorously and left inside the bottle for 24 hours. The ratio of the mixing the sample and solvent is 100 g: 500 ml. The percentage yield of the extract was calculated by the formula:

$$\% \text{ yield} = \frac{\text{Weight of concentration (in grams)} \times 100}{\text{Weight of ground sample (in grams)}} \times \frac{100}{1}$$

The mixture was decanted and filtered into beaker using separating funnel and a filter paper. The weight of the residue was taken after it was dried and the extract was concentrated.

2.3. Proximate analysis

2.3.1. Moisture content

A petri-dish was washed and dried in the oven. Approximately 1-2 g of the sample was weighed into petri dish. The weight of the petri dish and sample was noted before drying. The petri dish and sample was put in the oven and heated at 105 °C for 2 hrs for result noted and heated another 1 hr until a steady result is obtained and the weight was noted. The drying procedure was continued until a constant weight was obtained.

$$\% \text{ moisture content} = \frac{w_1 - w_2}{\text{weight of sample}} \times \frac{100}{1}$$

Where W_1 = weight of petri dish and sample before drying W_2 weight of petri dish and sample after drying

2.4. Carbohydrate determination

Carbohydrate was determined by adding up the whole proximate analysis and subtracting it from hundred 100-(% protein + % moisture + % ash + % fat + % fiber).

2.5. Ash content (AOAC, 1990).

Empty platinum crucible was washed, dried and the weight was noted. Approximately 1-2 g of sample was weighed into the platinum crucible and placed in a muffle furnace at 55 °C for 3 hrs.

$$\% \text{ Ash content} = \frac{w_3 - w_1}{w_2 - w_1} \times \frac{100}{1}$$

Where: W_1 = weight of empty platinum crucible

W_2 = weight of platinum crucible and sample before burning

W_3 = weight of platinum and ash.

2.6. Crude Fiber

A quantity, 2 g of material was mixed with petroleum ether and boiled under reflux for 30 minutes with 200 ml of a solution containing 1.25 g of H₂SO₄ per 100 ml of the solution. The solution was filtered through linen. The boiled water was washed so that the washing is no longer acid. The residue was transferred to a beaker and boiled for 3 minutes with 200 ml of a solution containing 1.25 g of carbohydrate free NaOH per 100 ml. The final residue was filtered through a thin but closed pad of washed and ignited asbestos in a good crucible. It was dried in an electric oven and weighed. It was incinerated, cooled and weighed.

The loss in weight after incineration $\times 100$ is the percentage of the crude fiber.

$$\% \text{ crude fiber} = \frac{\text{weight of fiber}}{\text{weight of sample}} \times \frac{100}{1}$$

2.7. Crude Fat

Soxhlet fat extraction method. This method is carried out by continuously extracting a food with non-polar organic solvent such as petroleum ether for about 1 hr or more. 250 ml clean boiling flasks was dried in an oven at 150-110 °C for about 30 minutes. It was transferred into desiccators and allowed to cool. It was weighed corresponding labeled, cooled boiling flask. The boiling flask was filled with about 300 ml of petroleum ether. The extraction thimble lightly was plugged with cotton wool. The soxhlet apparatus was assembled and allowed to reflux for about 6 hrs. When the flask was almost free of petroleum ether, it was removed and dried at 105-110 °C for 1 hr. It was transferred from the oven into a dessicator and allowed to cooled, then weighed. % fat = Wt of flask + oil wt of sample - Wt of flask $\times 100$.

2.8. Crude Proteins (AOAC, 1984)

Exactly 0.5 g of sample was weighed into a 30 ml Kjehdal flask gently to prevent the sample from touching the walls of the side of each and then the flasks were stopped and shaken. The 0.5 g of the Kjehdal catalyst mixture was added. The mixture was heated cautiously in a digestion rock under fire until a clear solution appeared. The clear solution was then allowed for 30 minutes and allowed to cool. After cooling was up to 100 ml with distilled water was added to avoid caking and the 5 ml was transferred to a kjedahl distillation apparatus, followed by 5 ml of 40 % sodium hydroxide. A volume, 100 ml of receiver flask containing 5 ml of 2 % boric acid and indicator mixture containing 5 drops of Bromocresol blue and 1 drop of methane blue was added under a condenser of the distillation apparatus so that the tap was about 20 cm inside the solution and distillation commenced immediately until 50 drops gets into the receiver flask, after which it was titrated to pink color using 0.01N hydrochloric acid.

$$\% \text{ Nitrogen} = \text{Titre value} \times 0.01 \times 14 \times 4$$

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25.$$

2.9. Phytochemical Analysis

A quantity, 1 g of sample was weighed and transferred in a test tube and 15 ml ethanol and 10 ml of 50 % m/v potassium hydroxide was added. The test tube was allowed to react in a water bath at 60 °C for 60 minutes. After the reaction time the reaction product contained in the test tube was transferred to a separating funnel. The tube was washed successfully with 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water and 3 ml of hexane which was all transferred to the funnel. This extracts were combined and washed three times with 10 ml of 10 % v/v ethanol aqueous solution. The solution as dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000 ul of pyridine of which 200 ul was transferred to a vial for analysis. Quantification was by GC-FID. The analysis of phytochemical was performed on a BUCK M 910 Gas chromatography equipped with flame ionization detector.

A RESTEK 15 meter M \times T-1 column (15 m \times 250 μ m \times 0.15 μ m) was used. The injector temperature was 28 °C with splitless injection of 2 ul of sample and a linear velocity of 30 cms⁻¹, helium 5.0 pa.s was the carrier gas with a flow of rate of 40 mlmin⁻¹. The oven operated initially at 200 °C it was heated to 330 °C at a rate of 3 ucmin⁻¹ and was kept at this temperature of 320 °C. Phytochemicals was determined by the rates between the area and mass of internal standard and the area of the identified phytochemical. The concentration of the different phytochemicals express in ug/g. When the instrument is ready, the NOTREADY, light will turn off, and you can begin your Run. A volume, 1 microliter of the sample was injected onto column A using proper injection technique and the readings was taken.

3. Results

Table 1 Proximate composition of *Phyllanthus amarus*

Contents	Average Value (%)
Moisture	6.48
Ash	10.58
Fat	8.40
Fibre	2.40
Protein	23.97
Carbohydrate	48.02

Table 2 Phytochemical Composition of *Phyllanthus amarus*

Component	Concentration (ug/ml)
Kaempferol	2.3726 ug/ml
Steroid	15.7626 ug/ml
Prothocyanidin	8.1619 ug/ml
Catechin	32.3542 ug/ml
Anthocyanin	3.8979 ug/ml
Narigenin	13.5721 ug/ml
Dihydrocytisine	8.0184 ug/ml
Cyanogenic glycoside	8.7028 ug/ml
Aphyllidine	0.2959 ug/ml
Ammodendrine	6.2495 ug/ml
Tannin	9.8090 ug/ml
Flavonones	8.7210 ug/ml
Cardiac glycoside	14.2203 ug/ml
Flavone	3.6099 ug/ml
Ribalinidine	10.1874 ug/ml
Sparteine	4.1965 ug/ml
Oxalate	9.0074 ug/ml
Ephedrine	1.9501 ug/ml
Sapogenine	18.0269 ug/ml
Phytate	8.1192 ug/ml

4. Discussion

Food fibers have been shown to aid the absorption of dietary minerals as well as reduce the absorption of cholesterol and help to maintain blood sugar [10]. The fibre content was found to be very low. Diets rich in fibre helps to prevent

constipation, support the health of the digestive tract as well as avert colon cancer. This justifies the use of the plant in the prevention and management of diseases such as coronary heart disease, cancer, and diabetes [11].

The crude protein value obtained signifies the healing properties of the plant which are essential for the synthesis/repair of the study tissue and enzyme. Fat content values were 8.66765% and 7.958478%. Fat as biomolecules can serve as an interchangeable source of energy for man. In addition, fat aids the absorption of fat-soluble vitamins and is required for growth, immune formation, and reproduction [12]. The moisture content is a major determinant in the healing, safeguarding and sustenance of food and drug. A high moisture value will promote the activities of spoilage micro-organisms and result in reduced shelf life [13]. The moisture level in the research is suggestive of the potent stability of crude drug against microbial degradation which could lead to the breakdown of important constituents of the sample, and microbial growth during the storage of the sample drug.

The ash content revealed 10.54% and 10.62%, indicating relative purity, freedom from foreign organic matters, sand, and soil adulterants which could alter the quality. Mineral elements are the essential constituents of ash. This study revealed that the leaves of *Phyllanthus amarus* are rich in ash, making it a good source of plant minerals required by man for normal metabolic activity of body tissues as well as the proper assimilation of vitamins [14]. The carbohydrate content of the leaf samples was relatively high. Carbohydrate biomolecules can serve as an interchangeable source of energy for a man [12].

Kaempferol is a natural flavonol, a class of flavonoid found in many fruits, vegetables, and herbs, including grapes, tomatoes, broccoli, tea, and ginkgo biloba leaves. Kaempferol increased intracellular ATP content under hypoxic conditions. It scavenges different types of radicals, inhibits reactive oxygen species (ROS)-generating enzymes, and increases the expression of antioxidant enzymes [15]. A steroid is a group of secondary metabolites derived from cholesterol; it has diverse chemical structure molecules possess the same basic perhydroxycyclopentenophenathrene skeleton [16]. The differences in the basic skeleton and the attachment of different groups result in various classes of steroids [17]. Steroids have many pharmacological applications and research is continuing to find the other applications of the compound in drug design and discovery [18].

Proanthocyanidin is condensed tannin with various pharmacological properties. These phytochemicals are considered as "offense and defense molecules because of their human health benefits. Agricultural wastes and food processing wastes contain an immense amount of proanthocyanidins, exploitation of which can be a sustainable source of dietary supplements and functional ingredients. Proanthocyanidin can impart astringency, bitterness, sourness, sweetness, salivary viscosity, aroma, and color information. Catechin is a natural polyphenolic phytochemical that exists in food and medicinal plants such as tea, legume, and rubiceae. An increasing number of studies have associated with intake of catechin – rich foods with the prevention and treatment of chronic diseases in humans, such as inflammatory bowel disease (IBD). Anthocyanins are a natural compound in plants that comes under the class of the phenolic compound. It is responsible for imparting attractive colors to the plants like red, purple and blue. Anthocyanins are known to possess antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, cardiovascular, and neuroprotective activity [19]. It has been reported that anthocyanins contribute to enhancing learning and memory [20]. Narigenin is one of the naturally occurring flavonoids, predominately found in some edible fruits like citrus species and tomatoes and figs belonging to *Smyrna*- type *ficuscarica* [21]. It occurs in a central position as the primary C15 intermediate in the flavonoid biosynthesis pathway. Narigenin inhibits some drug-metabolizing cytochrome P450 enzymes including CYP3A4 and CYP1A2, which may result in drug-drug interactions [22]. Cyanogenic glycosides are chemical compounds contained in the food that release hydrogen cyanide when chewed or digested. The act of chewing or digestion leads to hydrolysis of the substances, causing cyanide to be released [23]. Cyanogenic glycosides are common in certain families such as the *Fabaceae*, *Rosaceae*, *Leguminosae*, *Linaceae*, and *Compositae*, and identification of their constituents is a useful tool for *informative* taxonomic markers [24].

Tannins are naturally occurring plant polyphenols. Tannins are used as the astringent substance in the treatment of burns. They precipitate the proteins of exposed tissues to form a protective covering. They are used as mild antiseptics in the treatment of diarrhea, and to check small haemorrhages [25]. They are used as healing agents in inflammation, leucorrhoea, gonorrhoea, and piles. Tannins have been found to have antiviral, antibacterial, anti-parasitic, anti-inflammatory, anti-ulcer, and antioxidant properties. Flavonoids are compounds found in fruits, vegetables, and certain beverages that have diverse beneficial biochemical and antioxidant effects. Flavonoids are known to contain a broad spectrum of chemical and biological activities including antioxidant, free radical scavenging, anti-aging, anti-allergic, anti-inflammatory, anti-microbial, anti-leukemic, vasodilator, anticancer, and antibacterial properties, and are reported to be useful for improving blood circulation in the brain of alzheimeric patients [26]. The flavonoids, anthraquinones, and terpenes stimulate glucose uptake in cells. Certain flavonoids exhibit hypoglycemic activity [27] and beta-cell regeneration in pancreas. The antioxidant activity of flavonoids is efficient in tapping superoxide anion(O₂⁻),

hydroxyl(OH), peroxy(ROO), and alcohoxyl(RO) as constituents of fruits and plants. It is also a source of vitamin A, B6, and C which helps maintain vision, and good skin and build immunity against diseases.

Cardiac glycosides are one of the most naturally occurring plant phytoconstituents and are steroid drugs with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered. Cardiac glycosides and catecholamines are the agent of choice in the treatment of congestive cardiac failure. Spartein is a plant alkaloid derived from *Cytisus scoparius* and *Lupinus mutabilis* which may chelate calcium and magnesium. It is a sodium channel blocker so it falls under the category of class 1a antiarrhythmic agents. Spartein is not currently Flavin Adenine Diphosphate(FAD) approved for human use, but a quinolizidine alkaloid [28]. Phytate is the primary storage form of both phosphate and inositol in plant seeds. It forms complexes with dietary minerals, especially iron, and zinc, and causes mineral-related deficiency in humans [29]. It is of major concern for individuals who depend mainly on plant derivative foods. Phytate is of therapeutic use against diabetes mellitus, atherosclerosis, and coronary heart disease and reduces kidney stone formation, HIV-1, and heavy metal toxicity. However, information on the dosage for humans for eliciting beneficial effects is limited. The presence of oxalate in food is known to interfere with the assimilation of nutrients, decrease the nutritive value of the food at high doses and may have adverse effects on human health. Saponin has hypo-cholesterolemic, anti-carcinogenic, anti-inflammatory, antimicrobial, antioxidant activities, anti-haemolytic, and antibacterial activities [30]. It is used in cosmetic making shaving cream, and shampoo making because of its soapy nature. Saponins have been shown to have an inverse relationship with the incidence of renal stones [31]. It binds these cholesterol-like compounds and thus interferes with their growth and division [32].

5. Conclusion

The study revealed that the *Phyllanthus amarus* contain several phytochemicals that could be of great therapeutic purposes.

Compliance with ethical standards

Acknowledgments

This research was sponsored by Tertiary Education Trust Fund (TETFUND), Nigeria.

Disclosure of conflict of interest

There is no conflict of interest.

References

- [1] Qi, (2014) WHO traditional medicine strategy: Background and progress in the last decade.
- [2] Mazumder, A., Mahato, A. and Mazumder, R. (2016). Antimicrobial potentiality of *Phyllanthus amarus* against drug resistant pathogen. *Journal of Natural product research service*, 20(4):323-326..
- [3] Shanker, K., Singh, M., Srivastava, V., Verma, R. K., Gupta, A.K. and Gupto, M.M. (2011). Simultaneous analysis of six bioactive lignans in *Phyllanthus* species by reversed phase hyphenated high performance liquid chromatographic technique, *Acta chromatographica*, 23:321-337.
- [4] Marriott, J. and Lancaster, P.A. (2006). (Handbook of tropical foods). Heavy Technology (Edition), 85-142 Marcel Dekker.
- [5] Usha, R.P.(2001). Human Dietetics and Asian Food Crops. *Horltechnology* 11(14):504-509
- [6] Santos, (1995). Analysis of the mechanisms underlying the antinociceptive effect of the extracts of plants from the genus *Phyllanthus*. *General pharmacology*, 26(7): 1499-1506.
- [7] Krithika, R. and Verma, R.J.(2009). Mitigation of carbon tetra- chloride- induced damage by *Phyllanthus amarus* in Liver of mice . *Actapolpharm*, 66 (4): 439-44
- [8] Achi, N.K., Onyeabo, C. Ekeleme-Egedigwe, C.A. and Onyeonula, J.C. Phytochemical, proximate analysis, vitamin and mineral composition of aqueous extract of *Ficus capensis* leaves in South Eastern Nigeria. *Journal of Applied Pharmaceutical Science*, 2017; 7(3):117-122.
- [9] Jain, A., Katewa, S.S., Galav, P.K. and Sharm, P. (2005). Medicinal plant diversity of Sitamata wildlife Sanctuary, Rajasthan, India. *Journal of Ethnopharmacology*, 102: 143-Mazumder, A., Mahato, A. and Mazumder, R. (2016).

Antimicrobial potentiality of *PhyllanthusAmarus* against drug resistant pathogen. Journal of Natural product research service, 20(4):323-326.

- [10] Dhingra, D., Michael, M., Rajpat, H. and Patil, R.T. (2012). Dietary fiber in foods: A view. Journal of food Science and Technology, 49(3): 255-266.
- [11] Egbon, E.E., Olayioye, E.Y., Olayioye, S.A., Adesuyi, A.T. and Owolabi, T.(2017). Phytochemical screening and proximate composition of *PhyllanthusAmarus*. International Research Journal of Plant Science, 8(1): 9-12.
- [12] Ogbonna, I.L., Ogbonna, P.C. and Ogujiofor, I.B. (2019). Proximate Composition, Vitamin, Mineral and Biologically Active compounds level in leaves of *Mangifera indica* (Mango), *Persea Americana* (Avocado), and *Annona muricata* (Sour-SPP). Journal of Applied Sciences and Environmental Management, 23(1):65-74.
- [13] Unofin, J.O., Otunola, G.A. and Afolayan, A.J. (2017). Essential oil composition, Nutrient and Anti-Nutrient Analysis of *Veroniamesopilifolia* less. Research Journal Botany, 12(2): 38-45.
- [14] Umoh, E.E., Akpabio, U.O. and Udo, I.E. (2013). Phytochemical Screening and nutrient analysis of *Phyllanthus amarus*. Asian Journal of plant science and Research, 3(4): 116-122.
- [15] Calderon -Montano J. M., Burgos-Moron E., Perez-Guerrero, C. and Lazaro M. (2011). A review on the dietary flavonoid *Kampferol*. Mini Reviews Medical Chemistry, 11:298-344.
- [16] Sultan, A. and Rauf Raza, A. (2015) Steroids: A diverse class of secondary metabolites. Medical Chemistry, 5:310-317.
- [17] Marwat, G.A., Khan, A.R., Hassain, I. and Kalsoom, S.A. (2005). Review on naturally occurring steroids. Journal Chemistry Society Pakistan, 27:443-450.
- [18] Sultan, A. and Rauf Raza, A. (2015) Steroids: A diverse class of secondary metabolites. Medical Chemistry, 5:310-317.
- [19] Winter, A.N., Brenner, M.C., Punessen, N., Snodgrass, M., Byars, C., Arora, Y. and Linseman, D.A. (2017). Comparison of the neuro protective and anti-inflammatory effects of the anthocyanin metabolites, protocatechuic effects. Nutrition, 31:1301-1306.
- [20] Kangwan, N.K., Pintha, Preedapirom, W., Tantipaibowong, P., Chumphukam, O. and Suttajit, M. (2015). Learning and Memory enhancing effect of anthocyanin in black rice extract on cerebral Kattunykas tribes of Madumalai Wildlife Sanctuary, Nilgiris district Tamil Nadu, India. Journal of Traditional knowledge, 5:574-578.
- [21] Soltana, H., De Rosso, M., Lazreg, H., Vedova, A.D. and Hammami, M. (2018). Flamin R.LC-QTOF characterization of non anthocyanic flavonoids in four Tunisian fig varieties. Journal Mass spectrom: JMS, 53: 817-823. doi: 10.1002/JMS. 4209.
- [22] Fuhr, U. and Kummert, A. L. (1995). "The fate of naringenin in humans: A key to grape fruit juice-drug
- [23] Wink, M. (1988). plant breeding importance of secondary metabolites for production against pathogens and Herbivores Theoretical and Applied Genetics, 75(2): 225-233.
- [24] Vetter, J. (2000). Plant cyanogenic glycosides. Toxicon, 38(1):11-36.
- [25] Kokate, K.K., Purohit, A.P. and Gokhale, S.B. (2008). Pharmacognosy, 42nd edition. VallubhPrakashan, India, 13-44.
- [26] Sharma, D.K. (2006). Pharmacological properties of flavonoid including flavonolignans-integration of Petro crops with drug development from plants. Journal Science Industry Research, 65:477-484.
- [27] Ahmad, B. and Alam, T. (2003). Components from whole plants of *Phyllanthus amarus*. Indian Journal of chemistry, Section, B: Organic Chemistry including Medicinal Chemistry, 42: 1786-1790.
- [28] Kinghorn, A.D. and Balandrin, M.F. (1984). Quinazolidine alkaloids of leguminosae: structural types, analysis, chemotaxonomy, and biological activities.
- [29] Hurrell, R.F. (2003): Influence of vegetable protein sources on trace element and mineral
- [30] Rao, A. and Gurfinkle, D. (2000). The bioactivity of saponins : Triterpenoid and steroidal glycosides.
- [31] Ajayi, E.O. (2013). Phytochemical and anti-microbial studies of African. Biomedical Research, 3:161-165 American, 102:99-104. bioavailability. The Journal of Nutrition, 133(9):29735-75.
- [32] Okwu, D.E. (2005). Phytochemicals, vitamins and mineral Journal of Molecular Medicine and Advanced Medical Sciences, 9:235-238.