©IDOSR PUBLICATIONS
International Digital Organization for Scientific Research ISSN: 2579-0811
IDOSR JOURNAL OF BIOCHEMISTRY, BIOTECHNOLOGY AND ALLIED FIELDS 4(1): 21-29, 2019.

Evaluation of Chemical Analysis of Terpenoid Fraction of Root Extract of *Physalis angulata*

[1]Bawa A., [2]Njoku O.U. and [2]Nwodo O.F.C.

[1]Department of Science Laboratory Technology Federal Polytechnic, Mubi, Adamawa State, Nigeria.
[2]Department of Biochemistry, University of Nigeria, Nsukka.

ABSTRACT

This study was carried out to evaluate the chemical analysis of terpenoid fraction of root extract of *physalis angulata*. This was done using well known standard methods. The phytochemical analysis of *Physalis angulata* showed that the phytoconstituents appeared in the following order: alkaloids (14.96±0.83 %), terpenoids (13.79±0.04 %), saponins (12.78±2.4 %), flavonoids (0.05±0.00 %) and glycosides (0.05±0.03 %). The proximate content of the extract showed the presence of moisture (81.91±1.33 %), ash (9.00±1.92 %), carbohydrates (3.88±0.00 %), crude fibre (5.20±0.19 %) and protein (0.01±0.00 %) while the mineral composition evaluation revealed that it had very high content/amounts of magnesium (1101.50±2.12 ppm), sodium (407.20±0.42 ppm) and calcium (76.96±0.79 ppm). Zinc (4.95±0.07 ppm), potassium (2.05±0.07 ppm), manganese (1.84±0.05 ppm), copper (1.10±0.14 ppm), cobalt (0.63±0.04 ppm), and selenium (0.20±0.01 ppm) were present in lower quantities. The vitamin content evaluation of the extract revealed the presence of vitamin C (0.75±0.03 mg/100g), vitamin A (0.36±0.01 mg/100g) and vitamin E (0.07±0.00 mg/100g). These results showed that this plant is endowed with many active principles, minerals and vitamins that can be found useful in medicinal/therapeutic purposes.

Keywords: *Physalis angulata,* Phytochemical, Proximate, Vitamins and Minerals

INTRODUCTION

Plants have been used as sources of food and medicine. The most ancient approach to curing sicknesses and diseases is herbalism [1]. Medicinally and even toxic plants have the potentials of helping in the search for more potent and less toxic medicinal agents when thoroughly investigated. Although many plants have been credited with curative and/or toxic attributes in Nigeria and many other African countries, only few are of proven status and even less are clinically useful [2]. The investigation into the proximate and mineral composition as well as the phytochemical screening will serve as a prelude to the mechanism of action and other possible applications of the material.

Physalis angulata is a plant of the family Solanaceae, widely distributed throughout the tropical and sub-tropical region of the world. It is distributed as a weed in gardens, waste lands, along roads, in the forest, along sea levels and in cultivated fields [3]. All over the world, *Physalis angulata* is used as herbal medicine and for the treatment of various human ailment like malaria, hepatitis, asthma, dermatitis and rheumatism [4]; [5]. Infusions of *Physalis angulata* have been used to treat earache and postpartum infection. *P. angulata* leaf has been reported for CNS depressant action and it also possesses an antitumour activity. In addition, the constituent of antitumour glycoside myricetin -3 – 0

neohespenidoside of *Physalis angulata* has been reported [6]. This Study was aimed at Evaluating the Chemical Composition of Terpenoid Fraction of Root Extract of *Physalis Angulata.*

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh roots of *Physalis angulata* were collected from Yola South Local Government Area of Adamawa State, Nigeria. The plant material was identified and authenticated by Mr. Usman Gala of Botany Department, Ahmadu Bello University, Zaria Nigeria. The plant was assigned the voucher number: ABU2051. The root sample was washed and cut into smaller pieces and dried under direct sunlight. The sample was later pulverized to coarse powder using a hammer mill (Gallenkamp, U.S.A.).

Extraction of Plant Material

A known weight (6.952 kg) of the air-dried root powder was extracted with analytical grade ethanol in a soxhlet at 65°C. The mixture was vacuum-filtered through Whatman No 1 filter paper and concentrated using a vacuum rotary evaporator (Eyla N-1000, Japan) to afford 52.503g (0.755% w/w) of the extract.

Fractionation of *Physalis angulata* Root Extract

The extract (52.50 g) was subjected to solvent-guided fractionation in a silica gel (60-120 mesh size) column (2 × 70 cm) successively eluted with 20% ethyl acetate in n-hexane, followed by 30, 40, 50, 60, 70, 80 and 100% ethyl acetate. The solvent fractions were collected in 100 ml volumes and screened for the presence of terpenoid using qualitative phytochemical test. Fractions that gave positive reaction to terpenoids were pulled together and concentrated in rotary evaporator under vacuum to yield (E: nH-F; 14017.50 mg: 26.70% w/w) fraction. A small quantity of the fraction (E: nH-F) was developed using percolated silica trial thin layer chromotography plates in a mixture of n-hexane: ethylacetate: methanol in different ratios, but (3:2:1) ratio which gave the best resolution (showing three distinct terpenoids T_1, T_2, T_3 and one steroid S_1 chromatographic spots) served as the solvent mixture for the final elution in the second column. The third terpenoid chromatographic spot (T_3) which appeared insoluble in n-hexane: ethylacetate: methanol (3:2:1) mixture, was eluted with 20% acetic acid in ethyl acetate. Consequently, the E:nH-Fraction (14017.50 mg) was subjected to further separation in silica gel (60-120 mesh size) column eluted with mixture of n-hexane : ethylacetate : methanol (3:2:1), followed by 20% acetic acid in ethyl acetate. The sub-fractions were collected in 100 ml volumes and screened for the presence of terpenoid using qualitative phytochemical test. The first 300 ml sub-fraction contained a mixture of T_1 and S_1, while the rest of n-hexane: ethyl acetate: methanol (3:2:1) sub-fractions contain only T_2 the largest amount of terpenoid. However, 20% acetic acid in ethyl acetate was used to elute T_3 and trace T_2. The sub-fractions were concentrated in rotary evaporator under vacuum to yield (T_2; 3.15 g: 22.471% w/w), (T_3 and T_2; 0.938 g: 6.69% w/w), sub-fractions.

Qualitative Phytochemical Screening of the Extract

The extract, fractions and sub-fractions were subjected to both quantitative and qualitative screening using standard phytochemical methods as outlined by [7], [8], [9].

Quantitative Phytochemical Analysis

The quantitative phytochemical analysis of the dried and powdered *Physalis angulata* was determined using standard methods described by [10], [11], [12], [13], [14]

Proximate Analysis of *Physalis angulata* Root

These were analysed using the method described by the Association of Official Analytical Chemists [15].

Mineral Analysis of *Physalis angulata* Root

The mineral contents namely sodium, calcium, iron, zinc, potassium, magnesium, copper, and manganese were estimated by the use of atomic absorption spectrophotometer while phosphorus content was determined according to the method of [16].

Determination of Vitamin Content of *Physalis angulata* Root

Vitamins A, C and E, riboflavin, thiamine, and niacin contents were estimated using the methods described by [17].

Statistical Analysis

The statistical analysis was carried out using Statistical Product and Service Solution (SPSS 15.0) version. Statistical differences were evaluated using a one way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test to detect significant differences among the mean values of the dfferent groups.

RESULTS

Qualitative and Quantitative Phytochemical Constituents of Root Extract of *Physalis angulata*

The results of both the qualitative and quantitative phytochemical analysis of the root extract of the plant are shown in table below. The results revealed high contents of alkaloids and terpenoids. The phytoconstituents appeared in the following order: alkaloids (14.96±0.83%), terpenoids (13.79±0.04 %), saponins (12.78±2.4 %), flavonoids (0.05±0.00 %) and glycosides (0.05±0.03 %). The result also revealed that tannins, steroids, phenols and resins were not detected in the sample.

Table 1 Phytochemical Constituents of *Physalis angulata* Extract

Constituents	Qualitative Root Extract	Quantitative % Composition
Alkaloids	++	14.96±0.83
Terpenoids	++	13.79±0.04
Saponins	++	12.78±2.40
Glycosides	+	0.05±0.03
Flavonoids	+	0.05±0.00
Tannins	ND	-
Steroids	ND	-
Phenols	ND	-
Resins	ND	-

n=3. ND = Not detected.

The results of proximate composition of the root extract are presented in Tables 2. The proximate content of the extract showed the presence of moisture (81.91±1.33 %), ash (9.00±1.92 %), carbohydrates (3.88±0.00 %), crude fibre (5.20±0.19 %) and protein (0.01±0.00 %). Other qualitative tests for polysaccharides, pentose sugar, ketose sugar, protein, amino acid, aromatic amino acid and oil showed negative result (i.e not detected).

Table 2: Qualitative and Quantitative Proximate Constituent of Root of *Physalis angulata*

S/N	Constituents	Amount	Amount (%)
1	Moisture	++++	81.91±1.33
2	Ash	+++	9.00±1.92
3	Carbohydrates	++	3.88±0.00
4	Crude fiber	++	5.20±0.19
5	Protein	+	0.01±0.00
6	Reducing Sugar	ND	ND
7	Polysaccharides	ND	ND
8	Pentose Sugar	ND	ND
9	Ketose Sugar	ND	ND
10	Protein	ND	ND
11	Amino acid	ND	ND
12	Aromatic Amino acid	ND	ND
13	Oil	ND	ND

Key:
++ = Present in high concentration
+ = Present in moderate concentration
ND = Not detected
n=3.

Mineral Contents of Root Extract of *Physalis angulata*

As shown in Table 4, mineral content analysis of samples of the root of *Physalis angulata* revealed high content/amounts of magnesium (1101.50±2.12 ppm), sodium (407.20±0.42 ppm) and calcium (76.96±0.79 ppm). Zinc (4.95±0.07 ppm), potassium (2.05±0.07 ppm), manganese (1.84±0.05 ppm), copper (1.10±0.14 ppm), cobalt (0.63±0.04 ppm), and selenium (0.20±0.01 ppm) were present in lower quantities. Cadmium, arsenic and chromium were not detected.

Table 3: Mineral Contents of Root Extract of *Physalis angulata*

S/N	MINERAL	ROOT (ppm)
1	Magnesium	1101.50±2.12
2	Sodium	407.20±0.42
3	Calcium	76.96±0.79
4	Zinc	4.95±0.07
5	Potassium	2.05±0.07
6	Manganese	1.84±0.05
7	Copper	1.10±0.14
8	Cobalt	0.63±0.04
9	Selenium	0.20±0.01
9	Cadmium	ND
10	Chromium	ND
11	Arsenic	ND

Values are expressed as mean±SD. n=3. ND = Not detected.

Vitamin Content of Root Extract of *Physalis angulata*

The results of vitamin content evaluation of the root extract are presented in Table 5. The vitamin content evaluation of the extract revealed the presence of vitamin C (0.75±0.03 mg/100g), vitamin A (0.36±0.01 mg/100g) and vitamin E (0.07±0.00 mg/100g).

Table 4: Vitamin Contents of Root Extract of *Physalis angulata*

S/N	VITAMINS	AMOUNT (mg/100g)
1	Vitamin A	0.36±0.01
2	Vitamin C	0.75±0.03
3	Vitamin E	0.07±0.00

Values are expressed as mean±SD. n=3.

DISCUSSIONS

The extraction of pulverized *Physalis angulata* root with ethanol gave a percentage yield of 4.7 %. The amounts of phytochemicals found in the root extract were quantitatively determined using standard procedures. The results revealed that the phytochemical contents of the root extract appeared in the following order: alkaloids (14.96±0.96 %), flavonoid (0.05±0.00), saponins (12.78±2.40 %), glycosides (0.05±0.03 %) and terpenoids (13.79±0.04 %). Flavonoids have been

reported to partake in reduction of ischemia-reperfusion injury by interfering with inducible nitric-oxide synthase activity. Terpenoids has been identified to possess antioxidant activity against reactive oxygen species [17]. Thus the relative abundance of the terpenoids indicates the fraction might exert antioxidant effects.

[18] reported that trace elements in medicinal plants can have a substantial influence on the therapeutic value of herbal remedies: a positive contribution as a source of essential nutrients or even as active principles, or a negative effect because of the accumulation of high concentrations of potentially toxic elements. Medicinal plants can be rich in trace elements [19]; [20]; [21]. In addition, a number of researches have suggested that all essential and trace elements play vital role in medicine and therapy in health and disease, therefore, it is essential to analyze all parts of plants for determination of the excellent power of healing for human being in numerous ailments and disorders [22]. Enhanced intake of minerals like Ca, K, Na and P can be useful in patients with chronic renal failure [23]. In chronic renal disease there is a decrease of manganese and iron levels in the kidney [24]. However, the evaluation of mineral content of *Physalis angulata* root extract showed that the root contains magnesium (1101.50±2.12), sodium (407.20±0.42), calcium (76.96±0.79), zinc (4.95±0.07), potassium (2.05±0.07), manganese (1.84±0.05), copper (1.10±0.14), cobalt (0.63±0.04) and selenium (0.20±0.01) while cadmium, chromium and arsenic were absent. The terpenoids would be important in attenuating manganese deficiency disorder. Excessive sweating and chronic liver disorders are also causes of manganese deficiency. Selenium deficiencies have been reported to cause asthma while its existence helps to utilize vitamin E [25]. The intracellular levels of calcium ions aid excitation-contraction in muscles, excitation-secretion coupling in glands and excitation-release coupling in nerves. Together with phosphorus, magnesium and some other ions, it is involved in blood formation [26]. Its function in bone formation, blood clotting and transduction are well established. Energy needed for muscles contraction is produced in the presence of calcium, so that ATPase activated to hydrolyze ATP, which provide energy for muscles functioning.

According to [27], magnesium has also same role to produce energy but this energy is required for the muscles of chest wall and diaphragm for breathing. It also helps in healthy lungs function by acting as a bronchodilator and stops bronchial routes from going in to tremor. In addition, [28] reported that iron and zinc are cofactors needed for synthesis and proper functioning of catalase (CAT) and superoxide dismutase (SOD). The permissible limit set by FAO/WHO (1984) in edible plants is 27.4µg/g. According to [29], the concentration of zinc in agricultural produce should be between 15 and 200 ppm. Zinc is essential for growth and development. It is also needed for the function of the cells of the immune system [30]. Also, transition metals are essential elements which, under certain conditions, can have prooxidant effect. Redox active transition metals have ability to induce and initiate lipid peroxidation through the production of oxygen radicals, mainly hydroxyl radical through Fenton's/Haber-Weiss reactions [31]; [32]. Transition metals such as copper [33], [34], cadmium [35], [36], [37], [38] have been reported to induce lipidperoxidation *in vivo* in mammalian model.

Vitamins such as vitamins A, C and E; minerals like Zn and Se all of which are antioxidants which have been reported to be essential to immune system for the health [39]. The vitamin contents of *Physalis angulata* showed the presence of vitamin C (0.75±0.03), vitamin A (0.36±0.01) and vitamin E (0.07±0.00). According to [17], vitamin E, also called α-tocopherol is the major powerful membrane bound antioxidant employed by cells. Pryor (2000) reported that the main function of vitamin E is to protect

against lipid peroxidation. Vitamin C has been reported to be responsible for the regeneration of α -tocopherol from α-tocopherol radicals in membranes and lipoproteins. [22] also reported that vitamin C raises intracellular glutathione levels thus playing an important role in protein thiol group protection against oxidation. This study indicates that *Physalis angulata* contain principles that may be of medicinal and or therapeutic importance.

REFERENCES

1. Apata, L. (1979). The practice of herbalism in Nigeria: in African medicinal plants, University of Ife press, Ile-Ife, Nigeria. 13-19.
2. Kaul, A. K. (1975). Agronomic Comparism of dwarf and tall napier grass in Puerto Rico. *Journal of Agriculture of University of Puerto Rico.* **81: (**1/2) 9-18.
3. Smith, A. R., Shenvi, S. V., Widlansky, M., et al. (2004). Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. *Current Medicinal Chem*istry, **11**: 1135–46.
4. Soares, J. R., Dinis, T. C. P., Cunha, A. P. and Almeida, L. M. (1997). Antioxidant activities of some extracts of *Thymus zygi*. *Free Radical Research*, **26**:469–478.
5. Rengifo-Salgado, E and Vargas-Arana, G. (2013). *Physalis angulata* L. (Bolsa Mullaca): A review of its traditional uses, chemistry and pharmacology. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, **12** (5): 431 – 445.
6. Ismail N, Alam M (2001) A novel cytotoxic flavonoid glycoside from *Physalis angulata.* Fitoterapia 72: 676-679.
7. Harborne, J. B. (1998). 'Phytochemical Methods: A Guide to Modern Technique of Plant Analysis: Champman and Hall, London, pp. 21-72.
8. Trease, G. E. and Evans, W. C. (2002). Pharmacognosy. 15th Ed. Saunder Publishers, London pp. 42 - 44, 221 -229, 246 – 249, 404 -306, 331-332, 391-393.
9. Siddiqui, A. A. and Ali, M. (1997). Practical Pharmaceutical Chemistry. Ist Edn., CBS Publishers and Distributors. New Delhi. pp. 126-131.
10. Browne, R. W. and Armstrong, D. (2000). "HPLC analysis of lipid-derived polyunsaturated fatty acid peroxidation products in oxidatively modified human plasma." *Clinical Chemistry*, **46**(6) (1): 829–836.
11. AOAC (2003). Official Methods of Analysis. (17th edition). Association of Official Analytical Chemists. Virginia U.S.A. pp. 408-426.
12. Obadoni, B. O. and Ochuko, P. O. (2001). Phytochemical studies and comparative efficacy of the extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*, **8**:203–208.
13. Nabavi, S. M., Ebrahimzadeh, M.A., Nabavi, S. F., Hamidinia, A. and Bekhradnia, A. R. (2008). Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. *Pharmacologyonline,* **2**: 560-567
14. AOAC (1990). Official Methods of Analysis. (17th edition). Association of Official Analytical Chemists. Virginia U.S.A. pp. 408-426.
15. Grassmann, J. (2005). Terpenoids as plant antioxidants. *Vitamins and Hormones*, **72**:505-535.
16. Prasad, M. N. V. (2008). Trace elements as contaminants and nutrients: Consequences in ecosystems and human health. *Wiley*, **2008**: 108-137.
17. Abou-Arab, A. A. and Abou-Donia, M. A. (2000). Heavy metals in Egyptian species and medicinal plants and the effect of processing

on their levels. *Journal of Agricultural and Food Chemistry*, **48**(6): 2300-2304.
18. Basgel, S. and Erdemoglu, S. B. (2006). Determination of mineral and trace elements in some medicinal herbs and their infusion consumed in Turkey. *Science of the Total Environment*, **359**(1): 82-89.
19. Nookabkaew, S., Rangkadilok, N. and Satayavivad, J. (2006). Determination of trace elements in herbal tea products and their infusions consumed in Thailand. *Journal of Agricultural and Food Chemistry*, **54**(18): 6939-6944.
20. Sahito, S. R., Memon, M. A., Kazi, T. G. and Kazi, G. H. (2003). Evaluation of mineral contents in medicinal plant *Azadirachta indica* (Neem). *Journal of Chemical Sciences*, **25**: 139-143.
21. Archana, K., Xu, H. and Millikan, M. (2011). Determination and comparison of mineral elements in traditional Chinese herbal formular at different decocotion time used to improve kidney function chemometric approach. *African Journal of Traditional, Complementary and Alternative Medicines*, **8**(5): 191-197.
22. Bogden, J. D. and Klevay, L. M. (2000). Clinical nutrition of the essential trace elements and minerals: The guide for health professionals. *Humana Puerto Inc*., 277.
23. Sahito, S. R., Memon, M. A., Kazi, T. G. and Kazi, G. H. (2013). Evaluation of mineral contents in medicinal plant *Azadirachta indica* (Neem). *Journal of Chemical Sciences*, **25**: 139-143.
24. Fleck, H. (1976). Introduction to Nutrition. 3rd Edn. Macmillan, New York, USA, pp. 11-29.
25. Jimoh, F. O., Babalola, S. A. and Yakubu, M. T. (2009). Assessment of the antioxidant potential of *Cnidoscolous chayamansa. Pharmaceutical Biology,* **47**(9): 903-909.
26. Food and Agricultural organization/World Health Oragnization (FAO/WHO) (1984). FAO/WHO: Food contaminants. In: *Codex Alimentarius*, vol. XVII, edn. 1, FAO/WHO, Codex Alimentarius Commission, Rome.
27. Ghani, A., Ali, Z., Ishtia, M., Maqbool, M. and Parveen, S. (2012). Estimation of macro and micro nutrients in some important medicinal plants of Soon valley district Khushab, Pakistan. *African Journal of Biotechnology,* **11**(78): 14386-14391.
28. Valko, M., Leibfritz, D., Moncol, J., Cronin, M., Mazur, M. and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry and Cell Biology,* **39**(1): 44–84.
29. Jomova, K and Valko, M. (2011). Advances in metal-induced oxidative stress and human disease. *Toxicology*, **283**(3): 65-87.
30. Boveris, A., Musacco-Sebio, R., Ferraroti, N., Saporito-Magrina, C., Torti, H., Massot, F. and Repetto, M. G. (2012). The acute toxicity of iron and copper: Biomolecule oxidation and oxidative damage in rat liver. *Journal of Inorganic Biochemistry*, **116**: 63-69.
31. Palozza, P., Serini, S. and Di Nicuolo, F. (2003). Prooxidant effects of β-carotene in cultured cells. *Molecular Aspect of* Medicine, **24**: 353–362.
32. Karaca, S. and Eraslan, G. (2013). The effects of flaxseed oil on cadmium-induced oxidative stress in rats. *Biological Trace Element Research*, **155**(3): 423-230.
33. Amudha, K. and Pari, L. (2011). Beneficial role of naringin, a flavonoid on nickel induced nephrotoxicity in rats. *Chemico-Biological Interactions*, **193**(1): 57-64.
34. Pari, L. and Amudha, K. (2011). Hepatoprotective role of naringin

on nickel-induced toxicity in male wistar rats. *Pulmonary, Gastrointestinal and Urogenital Pharmacology*, **650**(1): 364-370.
35. Cartea, M. E., Marta, F., Pilar, S. and Pablo, V. (2011). Phenolic compounds in Brassica vegetables: A review. *Molecules,* **16:** 251-280.
36. Hensley, K., Benakass, E.J., Bolli, R., Philip, C., Paula, G. and Ladan, H. (2004). New perspectives on vitamin E: γ-tocopherol and carboxyethyl hydroxychroman metabolites in biology and medicine. *Free Radical Biology and Medicine*, **36**: 1–15.
37. Pryor, W. A. (2000). Vitamin E and heart disease: Basic science to clinical intervention trials. *Free Radical Biology and Medicine*, **28**: 141–164.
38. Naziroglu, M. and Butterworth, P. (2005). Protective effects of moderate exercise with dietary vitamin C and E on blood antioxidative defense mechanism in rats with streptozotocin-induced diabetes. *Canadian Journal of Applied Physiology*, **30**: 172–185.
39. Wedro, B. and Stoppler, M. C.(Ed). (2016). Homocysteine. http://www.emedicine health.com.