Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.94, 2022

Purification and Identification of the Active Principles in Ethanol Extract Fractions of Phyllanthus Amarus Leaves

Yakubu Ojochenemi Ejeh Otitoju Olawale Isaac John Umaru Michael Sunday Abu
Moses Adondua Abah* Ezekiel Terhemba
Department of Biochemistry, Faculty of Pure and Applied Sciences,
Federal University Wukari, PMB 1020, Taraba State, Nigeria

Abstract
All organisms have their own antioxidant defense system that protects them against free radical damage by enzymes such as superoxide dismutase and catalase or compounds such as ascorbic acid, tocopherol and glutathione. However, our body system sometimes cannot work efficiently to mop up these free radicals thus, leading to unavoidable damages caused by oxidation. As a result, consumption of additional antioxidants through herbs such as *Phyllanthus amarus*, foods or supplements (phytochemicals) are beneficial to the body system, defending it against these harmful ROS. This research is aimed at ascertaining the presence of various antioxidants in ethanolic extract fractions of *Phyllanthus amarus* leaves. *Phyllanthus amarus* leaves were collected from Federal University Wukari premise, Taraba State, Nigeria and dried. The dried leaves were crushed into a fine powder manually using mortar and pestle. The ethanolic extract of *Phyllanthus amarus* leaves was placed under fan to evaporate the ethanol before subjecting it to further assays. The results obtained from this study revealed that for beta carotene inhibition assay, fraction 4b (445.9459%) with the solvent combination Chloroform:ethyl acetate (50:50) has the highest bleaching inhibition capacity while fraction 4a (81.08109%) has the lowest bleaching inhibition capacity. Fraction 2a (56.9565 mg/mL) has the highest phenolic content while fraction 6b (7.608669 mg/mL) has the lowest phenolic content. The assay for total flavonoid content showed that fraction 3a (145.71428 mg/mL) has the highest flavonoid content while fraction 8a (121.42857 mg/mL) has the lowest flavonoid content. The results for anti-lipid peroxidation increased with increasing extract concentration. Fraction 3b (55.732 mg/mL) with the solvent combination (chloroform 100:00) has the highest antioxidant capacity while fraction 6a (2.775 mg/mL) with the solvent combination of ethyl acetate: ethanol (50:50) has the lowest antioxidant activity. The correlation between total antioxidants capacity and total Phenolic content and that of antioxidant and flavonoid was found to be weak negative (r^2 =-2.058, r^2=-0.024 respectively), while the correlation between phenolics and Betacarotene and that of the total antioxidant capacity and total flavonoid content was found to be weak positive (r^2=0.245 and r^2=0.313 respectively). The results of the analysis revealed that *Phyllanthus amarus* leaves contain significant nutritional components that are good for human health, such as flavonoids which is a subclass of the phytochemical, polyphenols. It is reasonable to conclude that fractions 2a and 2b has the highest antioxidant activities which have good potentials for drug development, especially against diseases of oxidative stress origin.

Keywords: Antioxidant, Flavonoids, *Phyllanthus amarus,* Ethanol extract
DOI: 10.7176/ALST/94-01
Publication date: August 31st 2022

Introduction

The study of phytochemicals has been instrumental in the discovery of new plant natural products which are of commercial values in various industries such as the traditional and complementary medicine systems, pharmaceutical industries, nutraceuticals, and dietary supplement industries [1]. Owing to the consistent threat of microorganisms, environmental hazards to public health, the significance of phytochemistry in the medical and pharmaceutical industries for the quest for the discovery of new drugs has overshadowed their essence in other industries. According to [2], about 80% of 122 plant-derived drugs are related to their original traditional uses. Reportedly, as at the dawn of 21st century, 11% of the 252 drugs considered as basic and essential by the World Health Organization (WHO) were exclusive of flowering plant origin [1].

Different parts of *Phyllanthus amarus* are being used for therapeutic purposes in Nigeria by rural dwellers in the treatment/management of various diseases [3]. The genus, Phyllanthus belongs to Euphorbiaceae which is a large family of upright or prostrate herbs or shrubs, often with milky acrid juice. *Phyllanthus* plants are either monoecious or homogenous. Their leaves are simple, alternate or opposite. Their flowers are very small and diclinous, clustered in cup-shaped structures, greenish and often with glands. *Phyllanthus* fruit is a three-lobed capsule extending from the cup and the commonly long stalk [4]. Different classes of organic compounds of medicinal interest such as alkaloids, flavonoids, lactones, steroids and terpenoids have been reported to be present in this plant. However, lignans, triterpenes, alkaloids, and tannins, are the most abundant compounds so far found in this genus [1].

Reactive oxygen species (ROS) such as superoxide radical anion, hydroperoxyl radicals are generated in

cells from byproducts of metabolism and are responsible for the development of a wide number of degenerative diseases such as cardiovascular disease (CVD), diabetic, cirrhosis, cancers among others [5]. All organisms have their own antioxidant defense system to protect them against free radical damage by enzymes, such as superoxide dismutase and catalase or compounds such as ascorbic acid, tocopherol and glutathione [6]. However, our body system sometimes cannot work efficiently that lead to unavoidable damage caused by oxidation. As a result, consumption of additional antioxidants through herbs, foods or supplements (phytochemicals) are beneficial to the body system, defending it against these harmful ROS [7]. Although there have been some reports on the health benefits of *Phyllanthus amarus,* information regarding the antioxidant content of this species is limited. This study was aimed at ascertaining the presence of various antioxidants in ethanolic extract fractions of *Phyllanthus amarus* leaves.

Material and methods

Plant collection

Phyllanthus amarus leaves were collected at Federal University Wukari premise Taraba State, Nigeria. The dried leaves were crushed into a fine powder manually, using mortar and pestle as described by [8].

Figure 1: *Phyllanthus amarus*

Preparation of ethanolic extract

Exactly 415g of the pulverized sample was soaked in about 1150ml of 99.8 % ethanol after which the post ethanolic residue was soaked in 99.8% chloroform for 48hours using the same measurements. The extracts were first sieved using a mesh of which the juice obtained was further filtered using whatman number 1 filter paper to obtain the filtrate. The ethanolic extract was placed under fan to evaporate the ethanol before subjecting it to further experiment.

Fractionation of ethanolic extract

Packing of the column and elution was done according to the method of [9]. The ethanolic extract was subjected to column chromatography to separate the extract into its component fractions. Silica gel was used in packing the column while varying solvent combinations of increasing polarity were used as mobile phase. The eluted fractions were collected in aliquots of 100ml in each of the beakers.

Determination of total antioxidant capacity

The experiment was carried out in duplicate according to the procedure of [10]. 0.02g of DPDH was dissolved in 100ml of methanol then 2ml of DPPH solution was added into test tubes and 100µL of each fraction was added. The mixture was shaken vigorously and the absorbance read within 30 seconds at 517nm in a UV-visible spectrophotometer. Methanol was used to zero the reading and the absorbance of DPPH solution was measured and recorded as blank.

Determination of total flavonoid content

Total flavonoid content was determined using the aluminum chloride colorimetric method of [11]. Quercetin standard was used for the derivation of the calibration curve and total flavonoid content was expressed as mg/ml quercetin equivalent (QE).

Determination of total phenolic content
Total phenolic content (TPC) of the extract was estimated following the procedure of [12]. Garlic acid was used as standard and the data was calculated as garlic equivalence in μg.

β-*carotene bleaching inhibition assay*
The method of [13] was used in assaying for β-carotene inhibition. In this assay, antioxidant activity was determined by measuring the inhibition of conjugated dienehydroperoxides arising from linoleic acid oxidation.
The bleaching capacity was calculated in percentage:
% Anti Linoleic acid peroxidation = $[(AS_{120} - AC_{120}/AC_0 - AC_{120})] \times 100$
Here, AS_{120}= Absorbance of test sample after incubation for 120 minutes.
AS_{120}= Absorbance of control after incubation for 120 minutes. AC_0= Absorbance of control before incubation for 120 minutes.

Anti-lipid peroxidation inhibition assay
This assay was used to estimate the lipid Peroxidation of the plant extract by following the methods of Mandal and [14]. Anti-Lipid Peroxidation was assessed using the formula:
% Lipid Peroxidation inhibition= $[(Ai-AS)/(Ai-Ac)] \times 100$
Here, Ai =Absorbance of Fe^{2+} induced peroxidation; As = Absorbance of test sample; Ac =Absorbance of control.

Result and Discussion
Total antioxidant capacity (TAC)
The results for total antioxidant capacity revealed that F1a has the highest antioxidant capacity, followed by F3b and then F2a with the values 61mg Trolox/ mL of n-hexane fraction of the chloroform extract, 55.732mg Trolox/mL of chloroform fraction of the chloroform extract and 55mg Trolox/mL n-hexane:chloroform (50:50 solvents combination) respectively. On the other hand, F6a (2.775mg/mL) has the lowest antioxidant capacity. Fraction 1 had a solvent combination of chloroform (100:00), fraction 3 with a solvent combination of chloroform (100:00) while fraction 2 with a solvent combination of N-hexane: chloroform (50:50). These results are represented in figure 2.

The total antioxidant capacity of the fractions of chloroform extract of *Phyllanthus amarus* varied greatly from one another. This is in line with the studies of [15]. This implies that it has an appreciable amount of bioactive compounds which could be polyphenols (Flavonoids) which are known for their antioxidant activity [16,17].

This is in consonance with some studies that medicinal plants used in traditional and healing are good sources of antioxidants.

The result also revealed that F3b has the highest antioxidant value indicating that the chloroform solvent combination is more efficient in the extraction of antioxidant phytochemicals from *Phyllanthus amarus* solvent combination of ethyl acetate: ethanol 50:50 with the lowest extraction ability as evident in F6a, thus the efficiency of chloroform in this extraction may be as a result of the non-ploarity of some of the antioxidant phytochemicals (Betacarotene).

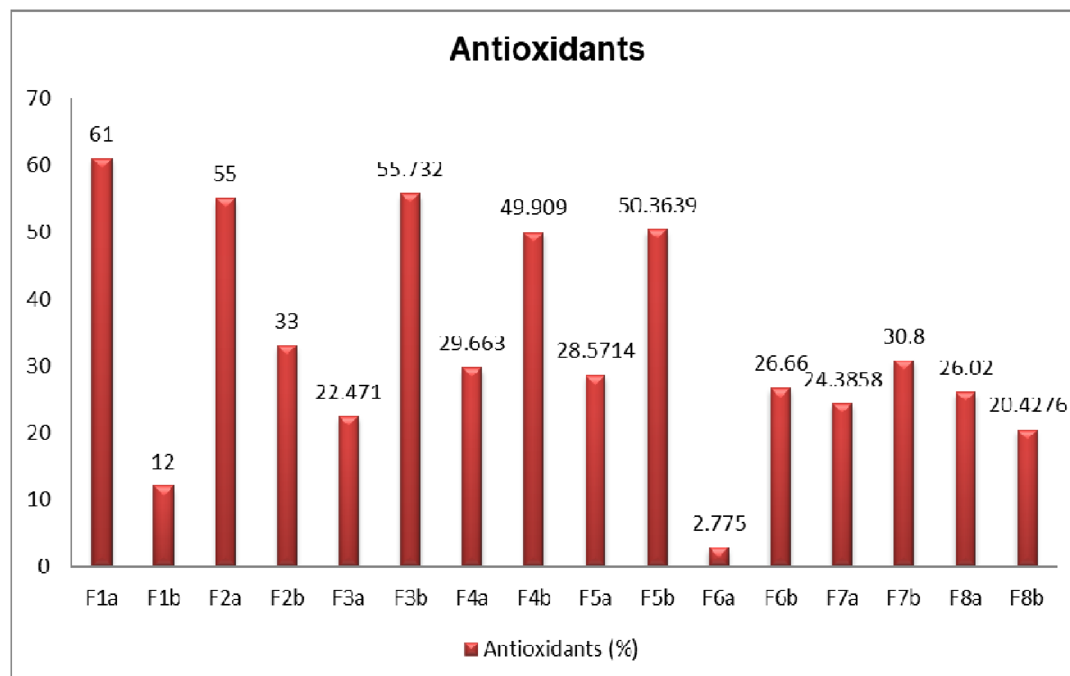

Figure 2: Total antioxidant capacity

F1a=n-hexane 100:00, F1b= n-hexane 100:00, F2a= n-hexane: chloroform 50:50, F2b= n-hexane: chloroform 50:50, F3a= chloroform 100:00, F3b= chloroform 100:00, F4a=Chloroform: Ethyl acetate 50:50, F4b=Chloroform: Ethyl acetate 50:50, F5a=Ethyl acetate 100:00, F5b=Ethyl acetate 100:00, F6a=Ethyl Acetate: Ethanol 50:50, F6b=Ethyl Acetate: Ethanol 50:50, F7a=Ethanol 100:00, F7b=Ethanol 100:00, F8a=Ethanol: Methanol 50:50, F8b=Ethanol: Methanol 50:50.

These findings indicate that the difference in the concentration and the partition of the phytochemicals in the different solvents from *P. amarus* leaves could significantly be affected by the extraction solvents. These findings are in tandem with previous studies on *Limnophila aromatica* [3] and *Phoenix dactylifera* [18], whereby the variation can be explained by the difference in solubility of the different compounds in the sample.

The cellular defense for antioxidation is mediated by the physiological antioxidant enzymes (GPX, SOD, CAT, GSH). Antioxidants are responsible for the defense mechanisms of the organism against the pathologies associated to the attack of free radicals. Thus, the intake of plant derived antioxidant is involved in the prevention of degenerative diseases that may lead to oxidative stress such as cancer, Parkinson, Alzheimer or atherosclerosis [19, 20].

Total phenolic content (TPC)

The results for total phenolic content indicate that F2a (56.9565mg QAE/mL of the chloroform extract) has the highest value followed by F1b (35.652mg QAE/mL of the chloroform extract) and then F4a (29.1304mg QAE/mL chloroform extract). While F6b (7.60869mg QAE/mL chloroform extract) has the lowest phenolic content. F1b has the solvent combination of N-hexane (100:00), F2a with a solvent combination of N-hexane: chloroform (50:50), F4a chloroform: ethyl acetate (50:50), F6b with ethyl acetate: ethanol (50:50), F4b chloroform: ethyl acetate (50:50) and fraction 5b with ethyl acetate (100:00). This is represented in figure 3.

The term phenolic acid generally describes the phenolic compounds having one carboxylic group. Phenolics or phenol carboxylic acids (a type of phytochemical called polyphenol) are one of the main classes of plant phenolic compounds [1]. Different types of soluble polyphenols, such as ferulic acid, gallic acid, or flavonoids, have been found to either stimulate or inhibit spore germination and hyphal growth of saprotrophic fungi. Plant mycorrhizal infection, nutrient uptake, and plant growth can be impaired by specific phenolics released by competitors in a process referred to as allelopathy [21].

The total phenolic content in the different fractions of the chloroform extract of *Phyllanthus amarus* using Folin–Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: y=0.0064, r^2=0.9845). The values obtained for the concentration of total phenols are expressed as mg of GA/ml of extract as shown in figure 3. Total values for total phenolic content range from 9.3478mg GAE/mL N-hexane (100:00) fraction to 56.9565mg GAE/ mL ethanol: methanol (50:50) fraction. These results were in agreement with previous studies that nonpolar solvents have low extraction ability for poly phenolic compounds compared to solvent combinations of ethanol and methanol [22]. The high concentration of phenolic compounds was as a result that the genus,

Phyllanthus amarus is known for its high phenolic content [23]. The presence of phenol is a clear indication that the *Phyllanthus amarus* can be exploited in pharmaceuticals for the treatment of many disease conditions. The presence of phenols makes the plant a potential cancer therapy because phenols are well known for their enormous ability to combat cancer. Antioxidants are derivatives of phenolics and their derivatives particularly phenolic acids, flavonoids and other phenolics [24]. Phenolic compounds are known to exhibit direct antioxidant activity by inducing endogenous protective enzymes and positive regulatory effect on signaling pathways [25]. Phenolic acids act as antioxidants due to their phenol moiety [24].

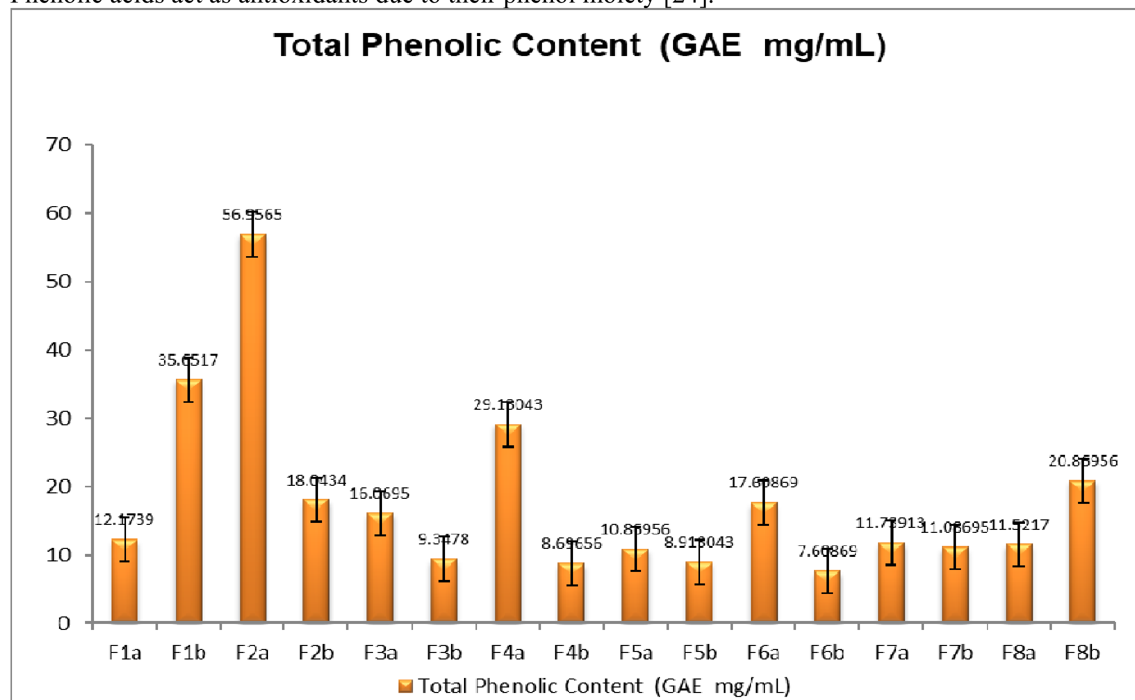

Figure 3: Total phenolic content
F1a=n-hexane 100:00, F1b= n-hexane 100:00, F2a= n-hexane: chloroform 50:50, F2b= n-hexane: chloroform 50:50, F3a= chloroform 100:00, F3b= chloroform 100:00, F4a=Chloroform: Ethyl acetate 50:50, F4b=Chloroform: Ethyl acetate 50:50, F5a=Ethyl acetate 100:00, F5b=Ethyl acetate 100:00, F6a=Ethyl Acetate: Ethanol 50:50, F6b=Ethyl Acetate: Ethanol 50:50, F7a=Ethanol 100:00, F7b=Ethanol 100:00, F8a=Ethanol: Methanol 50:50, F8b=Ethanol: Methanol 50:50.

Total flavonoid content (TFC)

The results for total flavonoid content shows that F5a (147.142857mg QE/mL the chloroform extract) with the solvent combination ethyl acetate 100:00 has the highest flavonoid content followed by F3a (145.71428mg QE/mL chloroform extract) with solvent combination of chloroform 100:00 and then F2b (145.0mg QE/ mL chloroform extract) with the solvent combination of n-hexane: chloroform 50:50. While F8a (121.42857mg QE/mL chloroform extract) with the solvent combination of Ethanol: Methanol 50:50 has the lowest flavonoid content followed by F7b (122.14285mg QE/mL chloroform extract) with the solvent combination of Ethanol 100:00 and then F8b (124.28571mg QE/mL chloroform extract). These results are represented in figure 4.

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables and certain beverages. They have miscellaneous favorable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease (AD), atherosclerosis, etc [26]. They belong to a class of low-molecular-weight phenolic compounds that are widely distributed in the plant kingdom. They constitute one of the most characteristic classes of compounds in higher plants [24]. Aluminum chloride forms acid stable complexes with C-4 keto groups and either the C-3 and C-5 hydroxide group of flavones and flavonoid. This study demonstrated that F5a has the highest flavonoid concentration compared with F5b. This observation is an indication of the possibility that F5a could exhibit antioxidant activity as asserted by the GCMS and FTIR analysis of *Phyllanthus amarus* methanol extract [11], where flavonoid presence was confirmed.

This study showed that the extraction solvents had a significant effect on the extraction of flavonoids. The high flavonoids content in F5a could be attributed to the high extractability potentials of the solvent (ethyl acetate 100%). Absolute ethyl acetate extracted the highest flavonoid levels from *P. amarus.* These findings were supported by previous studies on *S. chinensis* fruit pulp, *Limnophila aromatic* and *Macadamia tetrapylla*

skin waste which reported that extraction solvents significantly affected flavonoids [3, 27, 28]. This variation can be also explained by the different polarities of the compounds which were selectively more soluble in different solvents. The difference in the total phenolic levels of the fractions further confirmed that extraction of phenolic compounds from the sample, and solvent combination of chloroform and N-hexane (50:50) was the best solvent for maximum extraction of TPC. These findings were supported by previous studies which also found that different extraction solvents significantly affect the extraction yield of TPC [3, 27, 28].

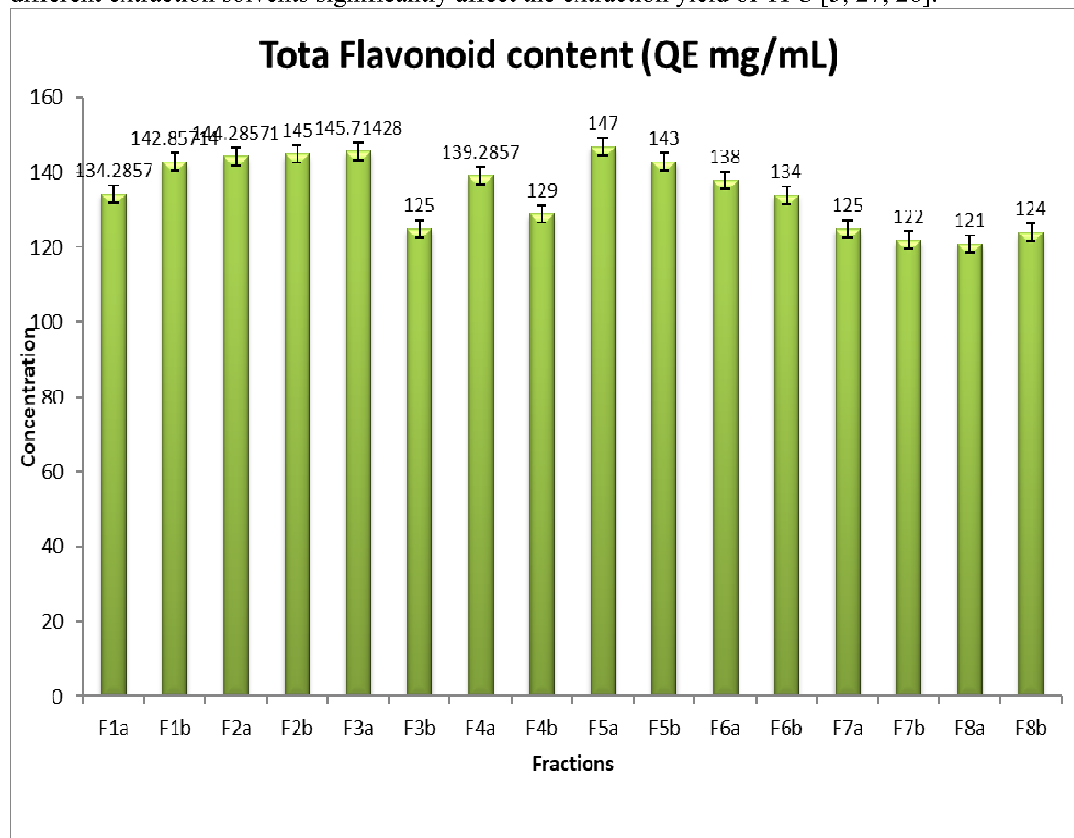

Figure 4: Total flavonoid content

F1a=n-hexane 100:00, F1b= n-hexane 100:00, F2a= n-hexane: chloroform 50:50, F2b= n-hexane: chloroform 50:50, F3a= chloroform 100:00, F3b= chloroform 100:00, F4a=Chloroform: Ethyl acetate 50:50, F4b=Chloroform: Ethyl acetate 50:50, F5a=Ethyl acetate 100:00, F5b=Ethyl acetate 100:00, F6a=Ethyl Acetate: Ethanol 50:50, F6b=Ethyl Acetate: Ethanol 50:50, F7a=Ethanol 100:00, F7b=Ethanol 100:00, F8a=Ethanol: Methanol 50:50, F8b=Ethanol: Methanol 50:50.

Beta- carotene bleaching inhibition assay

The results for beta-carotene bleaching inhibition as represented in figure 5 revealed that F4b (445.9459%) with the solvent combination of Chloroform: Ethyl acetate 50:50 has the highest percentage bleaching inhibition followed by F2a (427.027%) with the solvent combination of n-hexane: chloroform 50:50 and then fraction 5b (417.2297%) with the solvent combination of Ethyl acetate 100:00. While F4b (81.10%) with the solvent combination Chloroform: Ethyl acetate 50:50 has the lowest bleaching inhibition activity, followed by F7a (82.10%) with the solvent combination of Ethanol 100:00 then F8b (84.12%) with the solvent combination of Ethanol: Methanol 50:50.

Beta carotene is a fat soluble and lipophilic terpenoid. A free radical which is controlled in the biological system by some essential enzymes that possesses antioxidant activities such as superoxide dismutase. In the absences of antioxidants, beta carotene undergoes rapid bleaching and decolorization from the formation of hydroperoxides by linoleic acid oxidation. The beta carotene content of *Phyllanthus amarus* showed good antioxidant activity of the plant which ranges from 445.9-79.05 mg/ml. F1b and F4a with the concentration of 445 mg/mL and 427 mg/mL has the highest beta carotene activity followed by F2a and F4b with 322 mg/mL and 344 mg/mL respectively. F8b (79 mg/mL) was observed to have the lowest beta carotene bleaching inhibition activity follow by F3b (81mg/mL). Above all the solvent combination used, chloroform extracted a better content of the beta carotene. This is in line with the works of [4, 27, 28] which posits that different extraction solvents significantly affect the extraction yield of different phytochemicals. Betacarotene is a nonpolar solute and chloroform is nonpolar, nonpolar solvents extracts a better proportion of nonpolar solutes. This observation

indicates that chloroform is the best solvent in the extraction of betacarotene because of its ability in the extraction of nonpolar solutes.

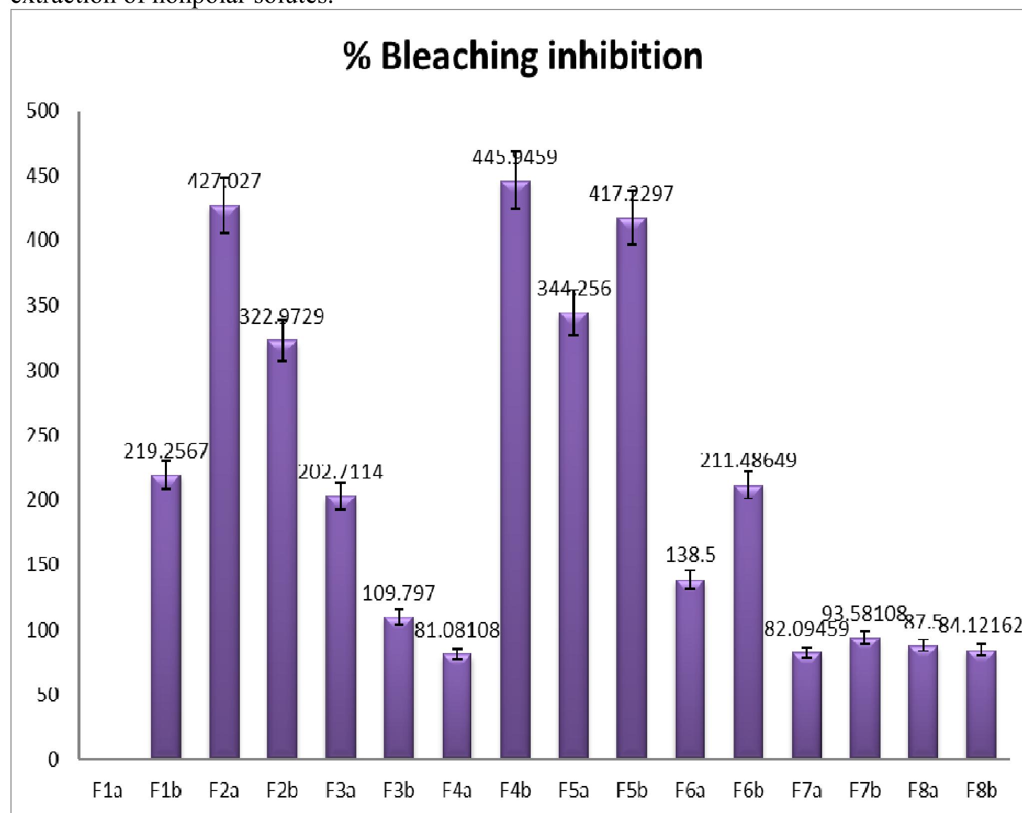

Figure 5: β-Carotene bleaching inhibition assay
F1a=n-hexane 100:00, F1b= n-hexane 100:00, F2a= n-hexane: chloroform 50:50, F2b= n-hexane: chloroform 50:50, F3a= chloroform 100:00, F3b= chloroform 100:00, F4a=Chloroform: Ethyl acetate 50:50, F4b=Chloroform: Ethyl acetate 50:50, F5a=Ethyl acetate 100:00, F5b=Ethyl acetate 100:00, F6a=Ethyl Acetate: Ethanol 50:50, F6b=Ethyl Acetate: Ethanol 50:50, F7a=Ethanol 100:00, F7b=Ethanol 100:00, F8a=Ethanol: Methanol 50:50, F8b=Ethanol: Methanol 50:50.

Anti-lipid peroxidation assay

The results for anti-lipid peroxidation as represented in figure 6 with different concentrations of the ethanolic extract indicates that the extract's ability to inhibit peroxidation increased progressively as the concentration of the extract increased.

Due to the interface function between the body and the environment, the skin is chronically exposed to both endogenous and environment pro-oxidants agent, leading to the harmful generation of reactive oxygen species (ROS). There compelling evidence that oxidative stress is involved in the damage of cellular constituent such as DNA, cell membrane lipid or proteins [29]. The percentage lipid inhibition of the extract at the concentration of 1000µg/mL was observed to have the highest anti-lipid peroxidation activity while the concentration of 125µg/mL has the lowest activity. This is generally in agreement with other research studies that have investigated the effect of plant extract on meat [23, 30].

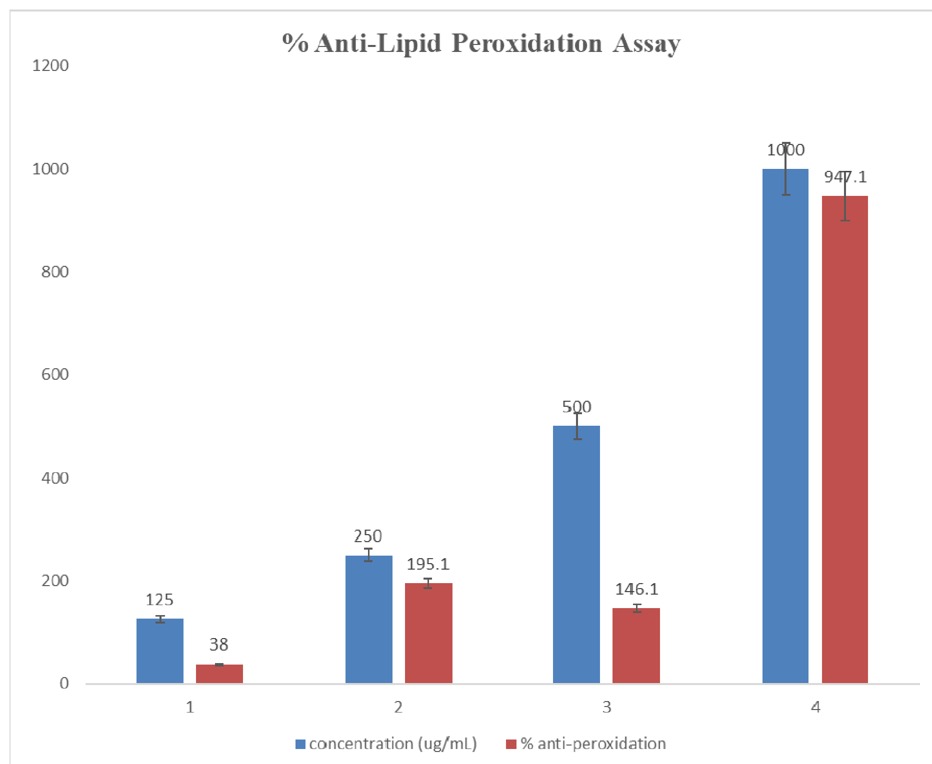

Figure 6: Anti-lipid peroxidation assay

Correlation of results

Pearson's correlation coefficient (r) was obtained from bivariate correlation analysis and used to describe the correlation between the antioxidant activities and the content of antioxidant components (TAC, TFC, TPC Beta-Carotene). The correlation between total antioxidants capacity and total Phenolic contents and that of antioxidant and flavonoid was found to be weak negative (r^2 =-2.058, r^2=-0.024 respecitviely), while the correlation between phenolics and Betacarotene and that of the total antioxidants and total flavonoids was found to be weak positive (r^2=0.245 and r^2=0.313 respectively). These results are represented in the figures below.

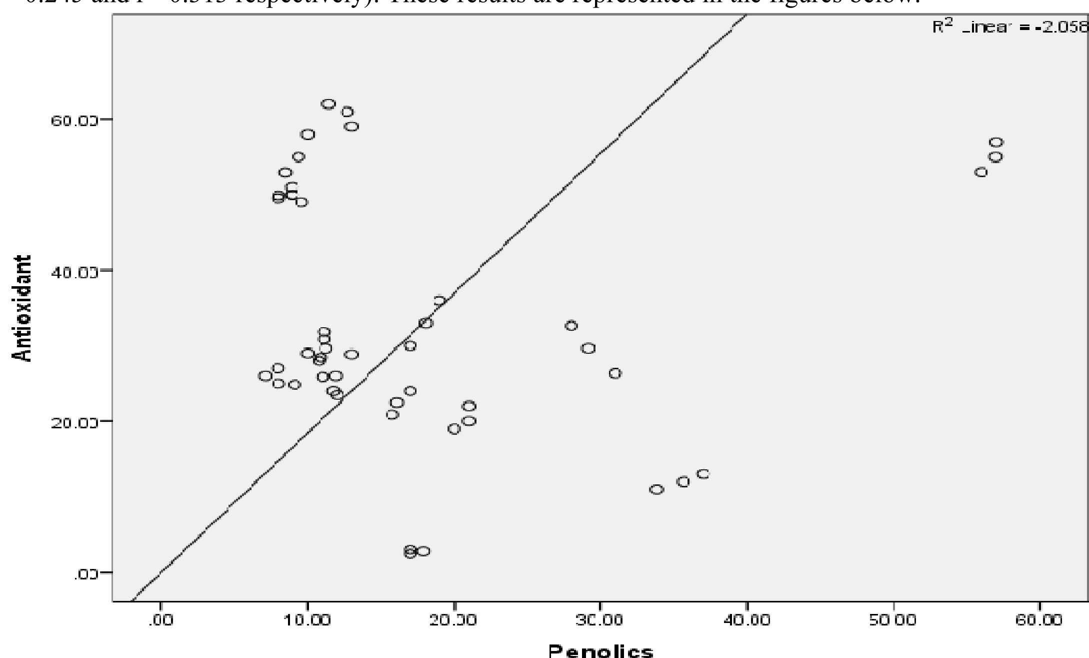

Figure 7: Correlation Between total Antioxidants and Total Phenolic Content (r^2=-2.058)

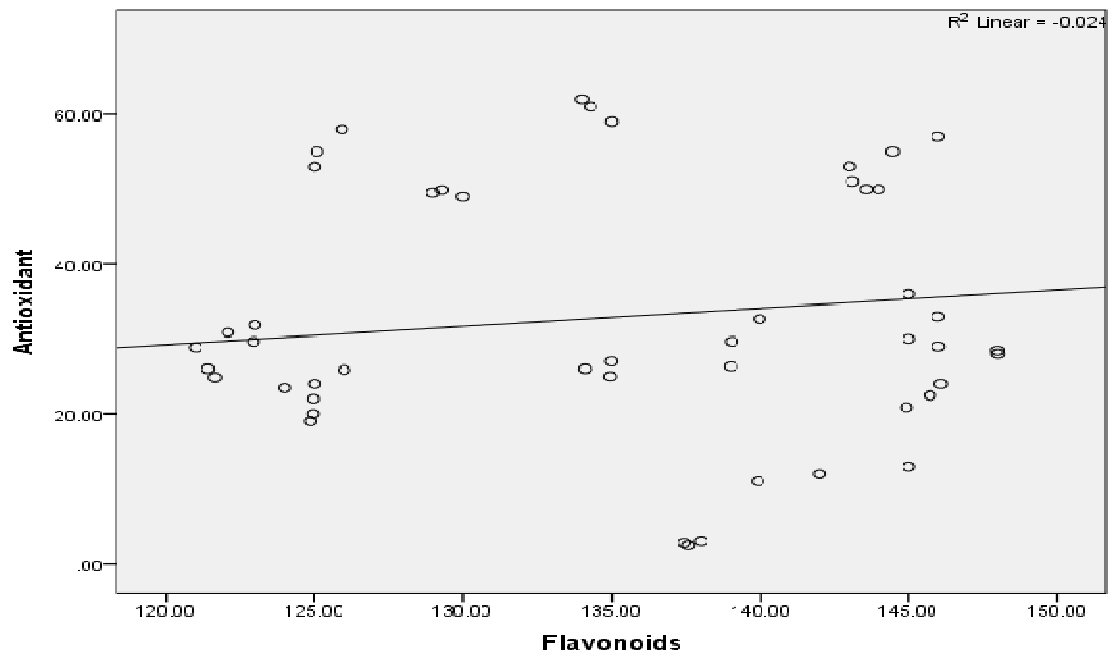

Figure 8: Correlation between antioxidants and flavonoids (r^2= -0.024)

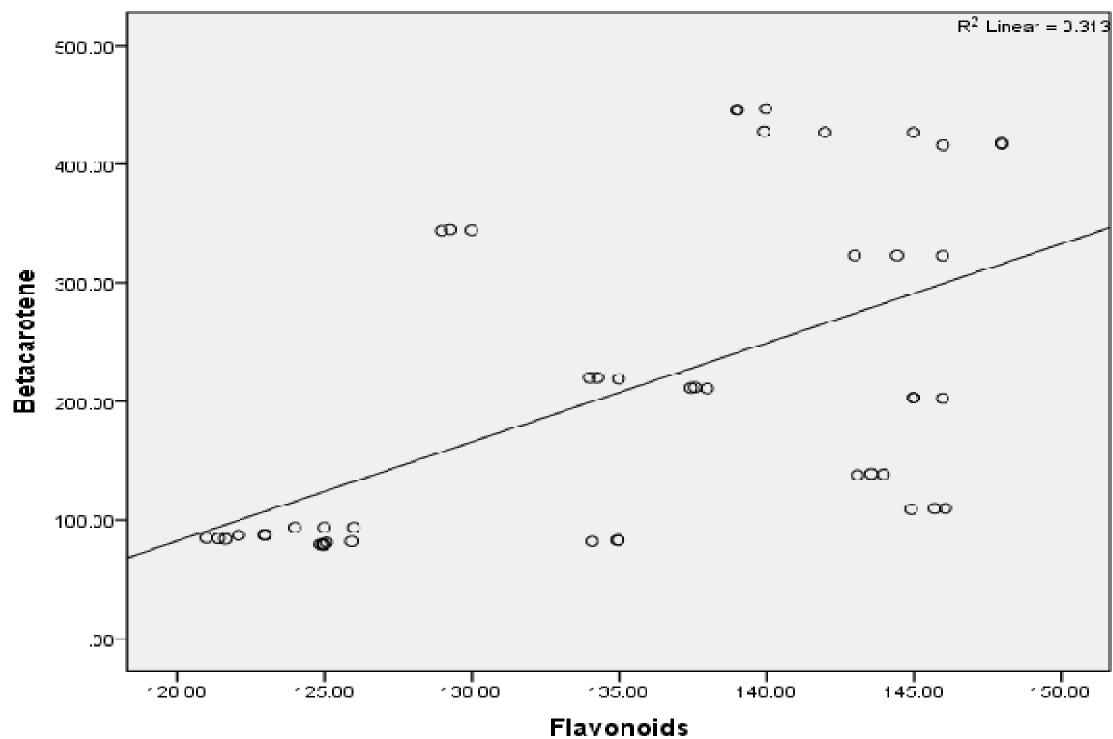

Figure 9: Correlation between total phenolics and betacarotene

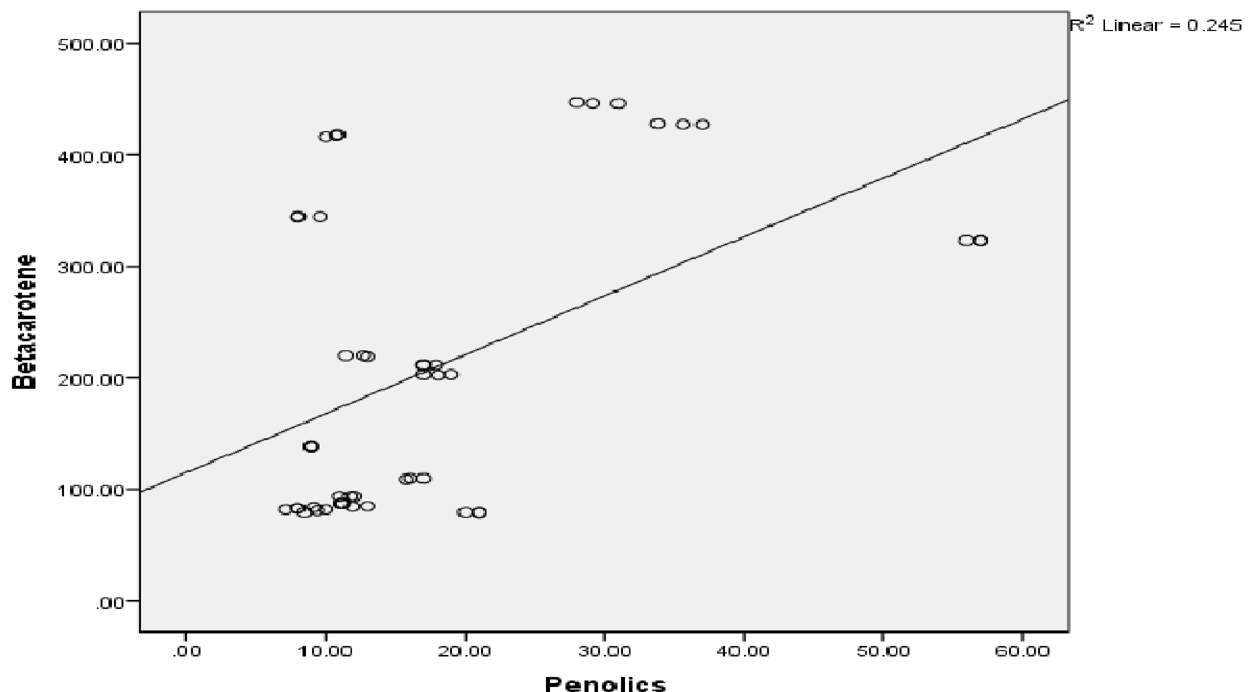

Figure 10: Correlation Between Beta-Carotene and Phenolics (r^2 =0.245)

The correlation coefficient between total phenol content and b-carotene bleaching test of *Phyllanthus amarus*, was significant significant (r2= 0.313). This is in line with the studies of [31], which further added that the presence of antioxidants will minimize the oxidation of b-carotene. This indicates that the flovonids contents of *Phyllanthus amarus* were responsible for the antioxidant activity of the extract.

Conclusion

The results of the analysis revealed that *Phyllanthus amarus* leaves contain significant nutritional components that are good for human health such as flavonoids which is a subclass of the phytochemical, polyphenols. The correlation that exists between beta carotene bleaching inhibition assay and the total flavonoids (r^2 = 0.313) is an indication that the flavonoid content of *Phylanthus amarus* extract earlier demonstrated using DPPH is the consequence of the inherent flavonoid content of the plant. It is reasonable to conclude that F3b with the solvent combination (chloroform 100:00) and F2a with solvent combination (n-hexane: chloroform 50:50) with the highest antioxidant activity have good potentials for drug development, especially against disease of oxidative stress origin.

Acknowledgments

We would like to thank Dr. Yakubu Ojochenemi Ejeh for initiating the concept of this research work and spearheading it.

Funding

This research did not receive any specific grant.

Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

References

[1]. Vincenzo, L. (2013). Phenolic compounds: Introduction. DOI: 10.1007/978 3-642-22144-6_57.

[2]. Fabricant, D. S., Farnsworth, N. R. (2001). The Value of Plants Used in Traditional Medicine for Drug Discovery. *Environ. Health Perspect.*, **109**, 69–75.

[3]. Do, Q.D., Angkawijaya, P.L., Tranguyen *et al.,* (2014). Effect of Extraction Solvent on Total phenol Content,

Total Flavonoids, and Total Antioxidant Activity of *Limnosphila aromatic. Journal of Food and drug analysis*, **22**(3):296-302.

[4]. Sulaiman, C.T., Thushar, K.V., George, S. and Balachandran, L. (2014). Phenolic characterization of selected Salacia species using LC-ESI-MS/M/S analysis. *Natural product Research,* **28** (13):1021-1024.

[5]. Halliwell, B. (2015). Antioxidant in human health and disease. Annual Review of Nutrition **16**: 33-50.

[6]. Mau, J. L., Lin, H. C. and Song, S. F. 2002 Antioxidant properties of several specialty mushrooms. Food Research International, **35**: 519-526.

[7]. Shi, H. L., Noguchi, N. and Niki, E. 2001 Introducing natural antioxidants. In J. Pokorny et al., Antioxidants in Food: practical applications, (eds.), Woodhead Publishing Ltd. and CRC Press.

[8]. Taofeeq, O., Abubakar, A.A.M., Fatimah, A., Mardhiyyah, T. and Shehu, O.O. (2018). Hepatotoxic Assessment of *Phyllanthus amarus* extract in wistar rats. *European Journal of Medicine*, **23**(4); 1-11.

[9]. Yakubu, O.E., Nwodo, C.F.O., Joshua, P. and Nnaemeka, U.M. (2014). Fractionation and determination of total antioxidant capacity, total phenolic and total flavonoids contents of aqueous, ethanol and n-hexane extracts of Vitex doniana leaves. *African Journal Of Biotechnology*, **13**(5):693-698.

[11]. Chang, C., Yang, M., Wen, H. and Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Analaysis,* **10**: 178-182.Chavan, J.J., Jagap, U.B., Gaikwad, N.B., Dixit, G.B. and Bapat, V.A. (2013). Total phenolic, Flavonoid and Antioxidant Activity of Saptarangi (*Salacia chinensis* L) Fruit Pulp. *Journal of Plant Biochemistry and Biotechnology*, **22**(4):409-413.

[12]. Velioglu, Y.S., Mazza, G., Gao, L., Oomah, B.D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J. Agric. Food Chem.* **46**: 4113-4117.

[13]. Jayaprakash, G.K., Singh, R.P., Sakariah, K.K. (2001). Antioxidant activity of grape seed extracts on peroxidation models *in-vitro J. Agric. Food Chem.,* **55**: 1018-1022.

[15]. Kim, J.S. (2014). Comparison of antioxidant properties of chloroform extract. *Food Nutri Sci*, **3**:1307-1320.

[16]. Dos-Reis, L. C., R., De-Oliveira, V. R., Hagen, M. E. K., Jablonski, A., Flôres, S. H., De-Oliveira, A. (2015). Carotenoids, Flavonoids, Chlorophylls, Phenolic Compounds and Antioxidant Activity in Fresh and Cooked Broccoli (Brassica Oleracea var. Avenger) and Cauliflower (Brassica Oleracea Var. Alphina F1). LWT–Food Sci. Technol, **63**(1): 177–183.

[17]. Roca, M., Chen, K., Pérez-Gálvez, A. (2016). Chlorophylls; In Handbook on Natural Pigments in Food and Beverages; *Woodhead Publishing: Elsevier*, pp 125–158.

[18]. Kachaou, W., Abbes, F., Blecker, Attia, H. and Besbes, S. (2013). Effect of extraction solvents on antioxidant activity, and phenolic content of Tunisian date varieties (*Phoenix dactylifera* L). *Industrial crops and products*, **45**:262-269.

[19]. Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M. & Teiser, J. **(2007).** Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology*, **39**: 44-84.

[20]. Pisochi, A.M. and Negulescu, G.P. (**2011)**. Methods for total antioxidants activity determination. A. *Review, Biochemistry and Analytical Biochemistry*, **1**:1-10.

[21]. Veitch, N.C. (2010). Flavonoid chemistry of the Leguminosae. In: Santos-Buelga C, EscribanoBailon MT, Lattanzio V (eds) Recent advances in polyphenol research, vol II. WileyBlackwell, Oxford, UK, pp 23–58.

[22]. Jagajothi, A., Manimekalai, G., Evangelene, V.K., Nirmala, A. (2013). Antimicrobial activity and Phytochemical analysis of *Phyllanthus amarus. J Today's Biol Res Rev*., **2**:55-62.

[23]. Baba, S.A., Malik, S.A. (2018). Evaluation Of Antioxidant And Antimicrobial Activity Of The Methanolic Extracts Activity Of *Gentiankurrooroyle*. *Saudi J Biol Sci*, **21**:493-498.

[24]. Kumar, N. and Goel, N. (2019). Phenoli acids: natural versatile molecules with promising therapeutic applications. *Biotechnology report*, **24**:1905-1915.

[25]. Stevenson, D.E., Hurst, R.D. (2007). Phenolic Phytochemical-jusst antioxidants or much more? *Cell. Mol.Life Sci*., **64** :2900-2916.

[26]. Panche, A.N., Diwan, A.D. and Chandra, S.R. (2016). Flavonoids: and overview. *Journal of Nutritional Science*, **5**:1-15.

[25]. Dailey, A. and Voung, Q.V. (2015). Effect of extraction solvent on recovery of bioactive compound and antioxidant properties from *Macadamia tetraphylla. Cogent food and Agriculture,* Article ID: 1115646.

[30]. Ebrahimzadekh, M., Nabavi, S., Bahrmian, F., Bekhradnia, R. (2008). Antioxidants And Free Radical Scavenging Activity. *Pak J Pharm Sci*, **23**:29-34.

[31]. Hlila, M. B., Omri, A., Jannet, H.B., Lamari, A., Aouni, M. & Selmi, B. (2013). Phenolic composition, antioxidant and anti- acetylcholinesterase activities of the Tunisian Scabiosa arenaria. Pharm Biol, **51**(5): 525–532.