Nigerian Research Journal of Chemical Sciences **(ISSN: 2682-6054)**
Volume 9, Issue 1, 2021

DETERMINATION OF TOTAL FLAVONOID AND ANTIOXIDANT ACTIVITIES OF LEAVES AND PEELS OF *MANGIFERA INDICA* AND *CITRUS SINENSIS*

*M.I. Shago[1], Abba Mohammed[1], G. Suleiman[2] and K. Abdullahi[2]

[1] Department of Biochemistry, Faculty of Science, Yobe State University, Damaturu, Yobe State, Nigeria

[2] Department of Chemistry, Faculty of Science, Yobe State University, Damaturu, Yobe State, Nigeria

*Corresponding Author: abbamm122@gmail.com

ABSTRACT

All parts of the *Mangifera indica* and *Citrus sinensis* plant contain secondary metabolites that possess several beneficial properties. Total flavonoid content and antioxidant activities of the *Mangifera indica* and *Citrus sinensis* leaves and peels' extracts were determined. Antioxidant activities of these extracts were determined by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) while the total flavonoid was determined by using quercetin as a standard. All the leaves and peels extracts of *Mangifera indica* showed good antioxidant activities. The methanolic extract of the leaves showed IC_{50} values of 10.03 µg/ml as compared to the standard ascorbic acid where the IC_{50} of the standard is 90.47 µg/ml. However, the leaves and peels of *Citrus sinensis* showed poor antioxidant activities with the IC_{50} values of 158.78ug/ml for leaves and 145.38 ug/ml for peels. All the leaves and peels of *Mangifera indica* and *Citrus sinensis* showed the presence of flavonoid in which the *Mangifera indica* leaves had higher total flavonoid content than *Citrus sinensis* leaves, *Mangifera indica* peels and *Citrus sinensis* peels.

Keywords: Antioxidant, *Citrus sinensis*, Flavonoid, *Mangifera indica*, Methanolic

INTRODUCTION

Mangifera indica, which is the botanical name for Mango, is considered as one of the main tropical fruits in the world. It is believed to be originated from Asia [1]. Based on documented reports, China, India, Brazil, Nigeria, Pakistan, Mexico, Thailand, and Philippine are well-known for mango cultivation while India is the country with highest cultivation of mango [2]. World

production of mango is approximately 42 million tons per year which is second only to banana production. There are about 1000 mango varieties grown all over the world [2]. Mango is known by various names around the world. For example, *Manja* in Arabic, *Mannko* in Greek, *Am* or *Ambi* in Hindi, *Amba* in Sinhala, *Mangue* in French, Mango in Finnish, Mango in Dutch, *Mangue* in German, *Mángguǒin* in Chinese, and *Mampalam* in Tamil [3]. Both ripe and unripe mango are used in pickles, juice, oils, nectar, powder, sauce, cereal flakes, and jam [4]. Mango fruit, peel and flesh are reported to be a rich source of fibre, vitamins C, vitamin A, essential amino acids, and polyphenols [5]. Mango seed has also been reported as a rich source of polyphenols [6]. The common use of mango fruit is as a food item, while various parts of mango tree have also been used for medical purposes since ancient times, mostly in Southeast Asian and African countries [7].

The World health organization (WHO) estimates that about 80% of the population still depends upon herbal medicines for the treatment of various diseases due to easy availability, economic reasons and fewer side effects [8]. Herbal remedies have formed the basis of traditional systems of medicine for ages and have formed the foundation of modern pharmacology. Herbal medicines have long history of popularity, better patient tolerance as well as acceptance [8]. Availability of medicinal plants is not a problem especially in developing countries like India, which is having rich agroclimatic, cultural and ethnic biodiversity.

Orange, the tasty, juicy fruit, belonging to the family *Rutaceae* is botanically known as *Citrus sinensis*. *Citrus sinensis* is one of the most important and widely grown fruit crops with total global production reported to be around 120 million tons [8]. Orange fruit is cultivated in more than 130 countries including India, UK, France, Germany, Holland, Nigeria, Brazil, China, USA and Spain. Oranges are generally available from winter through summer with seasonal variations depending on the variety [8].

Flavonoids are low molecular weight secondary metabolites that are produced by plants and generally are described as non-essential for plant survival, unlike primary metabolites. Secondary metabolites products are biologically active in many ways, and over 10,000 structural variants of flavonoid, have been reported by Williams and Graver [9]. Their synthesis appears to be ubiquitous in plant and evolved early during land plant evolution aiding in plant protection [10]. Due to their physical and biochemical properties, flavonoids also are able to interact with many diverse targets in subcellular location, to elicit various activities in microbes, plants and

animals [11]. Although flavonoids have many roles in plants, including their influence on the transport of auxi [12], they also play important role in modulating the levels of reactive oxygen species (ROS) in plant tissues [13], and provide colouring to various tissues including flowers [14]. In addition, they are required for signaling symbiotic bacteria in the legume rhyizobium symbiosis [15] and are important in root and shoot development [16].

Antioxidants are chemicals (both naturally occurring and man-made) that can prevent or slow cell damage. Fruits and vegetables contain many antioxidant compounds including phenolic compound, anthocyanins and tocopherols [17]. Antioxidants can also be produced artificially and consumed in form of supplements. Antioxidants are one of the first lines of defense that the body employs to keep free radicals in check and prevent them from causing domino effect of damage on other cells. Antioxidant compounds can "donate" electrons to unstable free radicals so they do not have to snatch electrons from unsuspecting nearby cells. Antioxidants can also help repair cell damage caused by free radicals.

Studying the relationship between antioxidant status and disease has proven to be a highly profitable line of research. It has expanded our knowledge concerning the etiology of numerous diseases and the means by which they might be prevented. But it is essential to take a balanced perspective and avoid the danger of over-enthusiasm for the potential of antioxidants [18].

The aim of this study is to determine the total flavonoid and antioxidant activities of leaves and peels of *Mangifera indica* and *Citrus sinensis*. The objectives of this study include:

- To determine the quality of flavonoid in the leaves and peels of *Mangifera indica* and *Citrus sinensis*
- To ascertain the quantity of flavonoid in the leaves and peels of *Mangifera indica* and *Citrus sinensis*
- To determine the antioxidant activities of leaves and peels of *Mangifera indica* and *Citrus sinensis*

MATERIALS AND METHODS

Materials

Test tube, dropper, measuring cylinder, round bottom flask, condenser, heating mantle, beaker, conical flask, mortar and pestle.

Equipment

Weighing balance (model/PA214) made by Chaus corporation, UV UNICO Spectrophotometer (model No.UV 2150), S/N KP 12111212018.

Chemicals

Methanol, Ethanol, Sodium hydroxide, Sodium nitrite, Aluminium chloride, Distilled water, 2,2-diphenyl-1-picryl hydrazyl (DPPH), Quercetin

Sample collection

The plant samples, (Cit*rus sinensis* and *Mangifera indica*) leaves, were collected from Yobe State University botanical garden during rainy season while the peels were collected from local market in Damaturu and brought to laboratory for analysis.

Sample preparation

The plant samples (*Citrus sinensis* and *Mangifera indica*) were washed with distilled water immediately after collection from the field and dried under shade in the laboratory at ambient temperature after which they were crushed with a crusher into small particles using mortar and pestle. The leaves and peels were grinded separately and stored in a drying cabinet (set at 30% humidity).

Extraction

The plant parts (leaves and peels) were separately extracted with methanol using soxhlet extractor. About 50 g of each plant part was weighed and transferred into 500 ml soxhlet extraction chamber. About 200 ml of methanol was added to the boiling flask. The set up was mounted over a heating mantle and then coupled with condenser, to which cold water was allowed to circulate. The extraction lasted for about 5 hours.

Qualitative test of flavonoids

The extract (0.5 g) was dissolved in ethanol. About 5 ml of the solution was added to concentrated sulphuric acid (1 ml) and 0.5 g of magnesium metal. A pink colouration indicates the presence of flavonoids.

Measurement of antioxidant activities

The antioxidant activities of *Mangifera indica* and *Citrus sinensis* leaves and peels were determined on the basis of their scavenging activity of stable 2,2-diphenyl-1-picryl hydrazyl

(DPPH) free radical as follows: Exactly 1 ml of each solution of different concentrations (1-500 µg/ml) of the extracts was added to 3 ml of 0.004% ethanolic DPPH free radical solution. After 30 minutes the absorbance of the preparations were taken at 517 nm by UV spectrophotometer which was then compared with the corresponding absorbance of standard ascorbic acid concentrations (1-500 µg/ml). The method described by Hatano *et al* [19] was used to measure the absorbance with some modifications. Then the % inhibition was calculated by the following equation:

$$\% \text{ radical scavenging activity} = \frac{(\text{Absorbance of blank} - \text{Absorbance of sample})}{(\text{Absorbance of blank})} \times 100\%$$

From the calibration curves obtained from different concentrations of the extracts, the inhibitory concentration (IC_{50}) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals [20].

Procedure of carrying out antioxidant activities

At first, 12 test tubes were taken to make aliquots of 12 concentrations (1, 5, 10, 25, 50, 75, 100, 150, 200, 300, and 500 µg/ml) with the samples.

Extracts and ascorbic acid were accurately weighed and dissolved in ethanol to make the required concentrations by dilution technique. The ascorbic acid was taken as the standard.

DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To dissolve homogeneously, vortex mixer was used. After preparing the desired concentrations, 3 ml of 0.004% DPPH solution was added to each of the test tubes by means of a pipette. The room temperature was recorded and the test tubes were kept for 30 minutes in light to complete the reactions.DPPH was also added to the blank test tube at the same time where only ethanol was taken as blank.After 30 minutes, the absorbance of each test tube was measured using a UV spectrophotometer.IC_{50}were measured from the graphs of % Inhibition vs Concentration [20].

Quantitative determination of total flavonoid

Total flavonoid content was determined by Aluminum chloride method using quercetin as a standard. About 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). However, 0.3 ml of 5% Sodium nitrite was added then 0.3 ml of 10% Aluminum Chloride was added after 5 minutes. The reaction was then allowed for 6 minutes for incubation

at room temperature. Exactly 1 ml of 1M Sodium hydroxide was added to the reaction mixture. Afterwards, the final volume was made up to 10 ml with distilled water. Absorbance of sample was measured against the blank at 510 nm using a spectrophotometer. All the experiments were repeated three times for precision and values were expressed in mean ± standard deviation in terms flavonoid content (Quercetin equivalent, QE) per g of dry weight.

RESULTS AND DISCUSSION

Table 1 shows the results of qualitative analysis of total flavonoid of methanolic extract of *Citrus sinensis and Mangifera indica* (leaves and peels), where the leaves and peels of both *Mangifera indica and Citrus sinensis* show the presence of flavonoid.

Table 1: Results for qualitative tests of flavonoid

Plants	**Tests reagent**	**Extracts**	
		Leaves	Peels
Mangifera indica	Sodium hydroxide	+	+
Citrus sinensis	Sodium hydroxide	+	+

Table 2 shows the results of quantitative analysis of total flavonoid in which the *Mangifera indica* leaves have the higher flavonoid content followed by *Citrus sinensis* leaves, *Mangifera indica* peels and *Citrus sinensis* peels.

Table 2: Results of the total flavonoid tests

Sample	Absorbance	A-B	Sample weight (g)	Total volume	Total Flavonoids Content (mgQ/Kg)

Mangifera indica Peels	0.29	0.275	0.05	10	1755.36
Mangifera indicaLeaves	0.64	0.625	0.05	10	3784.35
Citrus sinensis Peels	0.25	0.235	0.05	10	1523.48
Citrus sinensis Leaves	0.253	0.51	0.05	10	3117.68

Blank=0.015

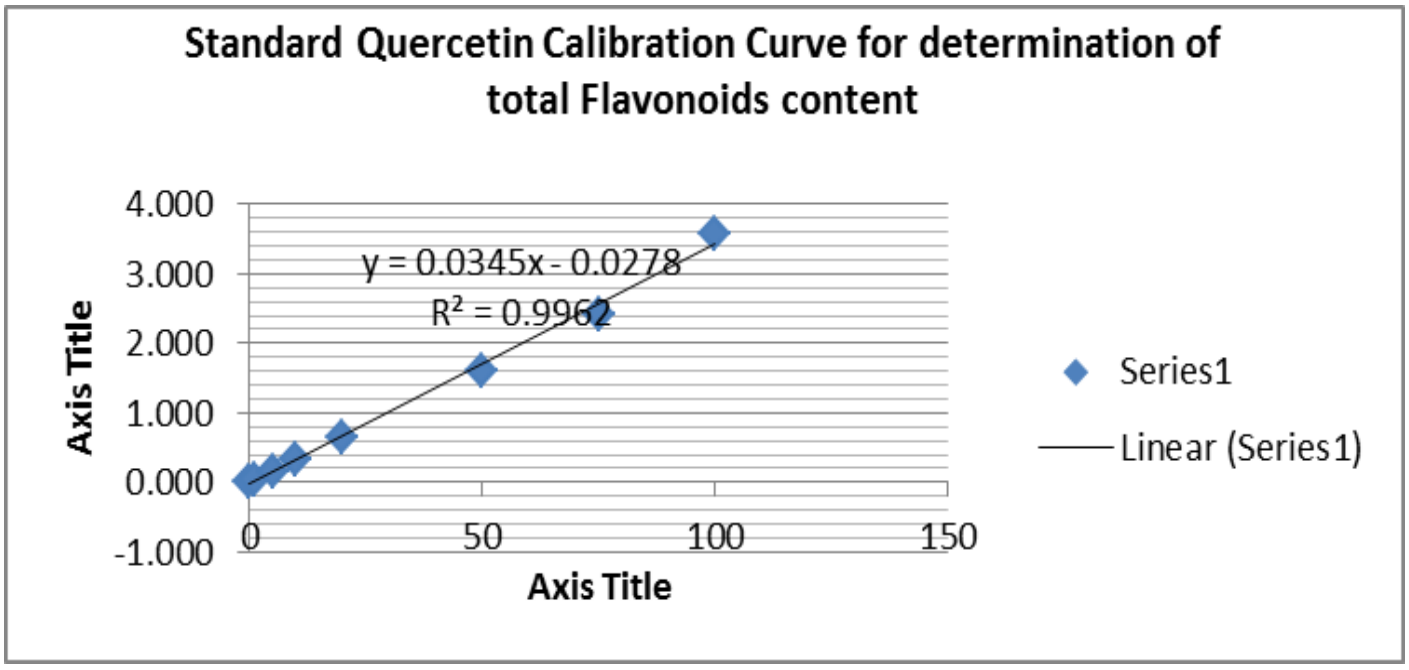

Figure 1: Standard quercetin calibration curve for determination of Total flavonoid content.

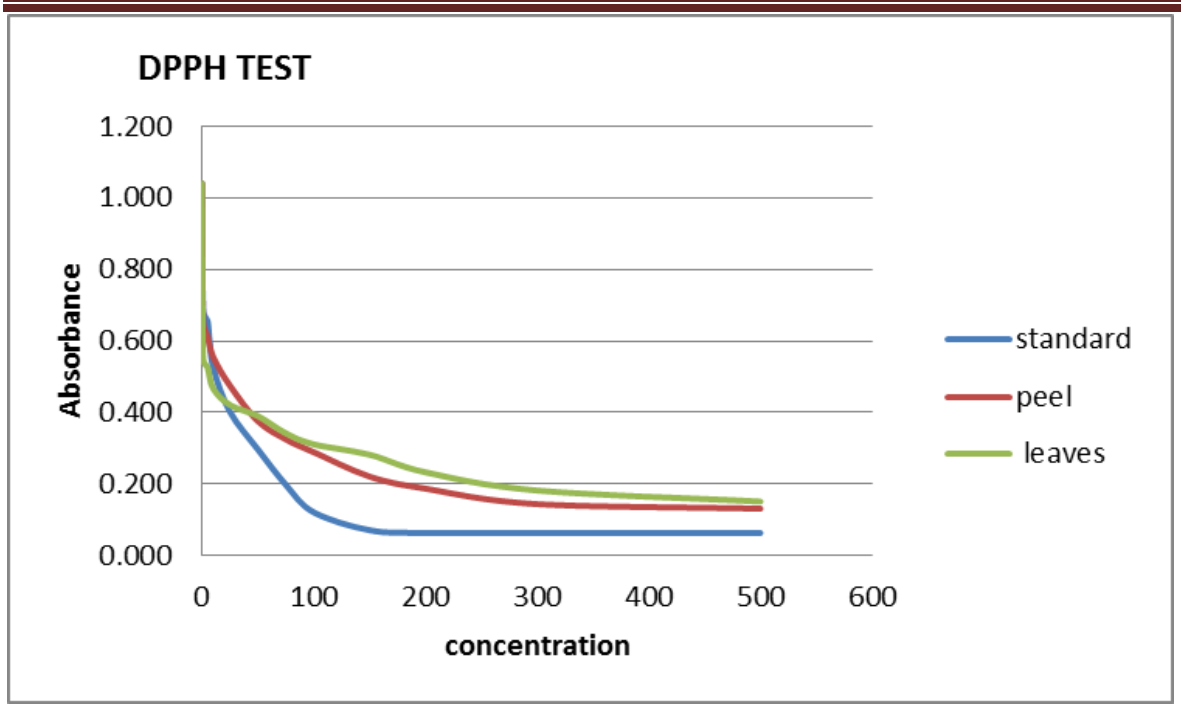

Figure 2: DPPH scavenging assay of leaves and peels extract of *Mangifera indica* compared with the standard ascorbic acid.

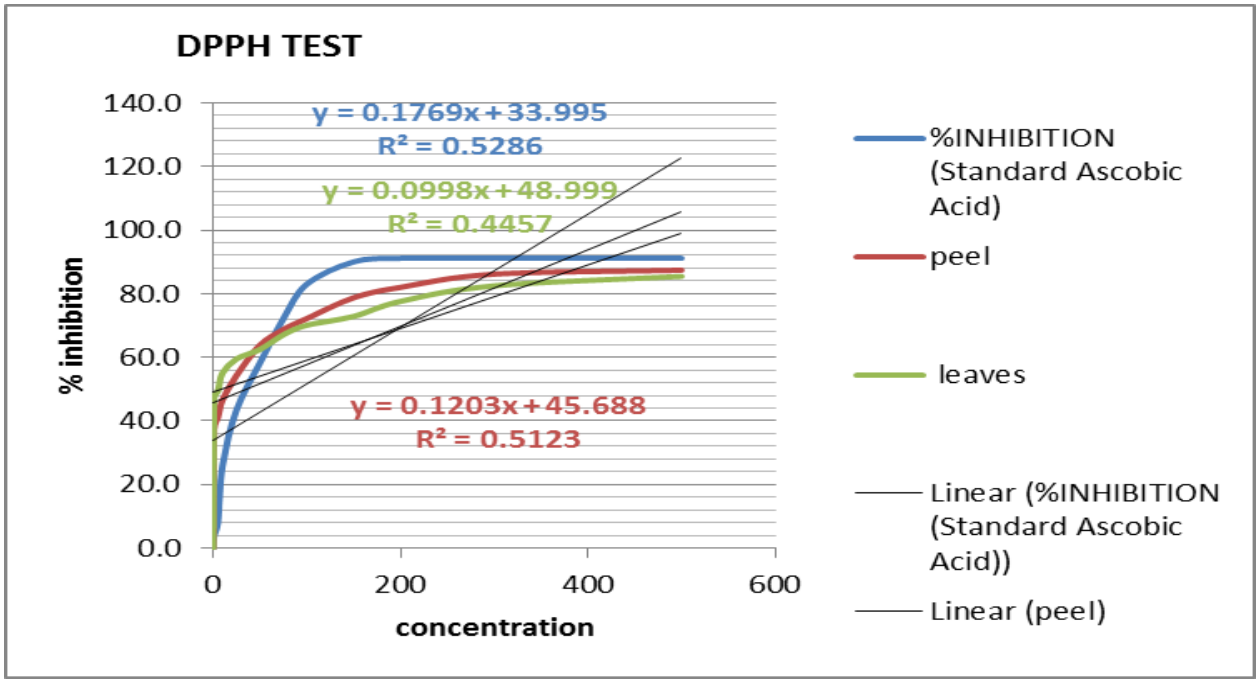

Figure 3: Evaluation of IC_{50} of leaves and peels extract of *Mangifera indica* and standard ascorbic acid.

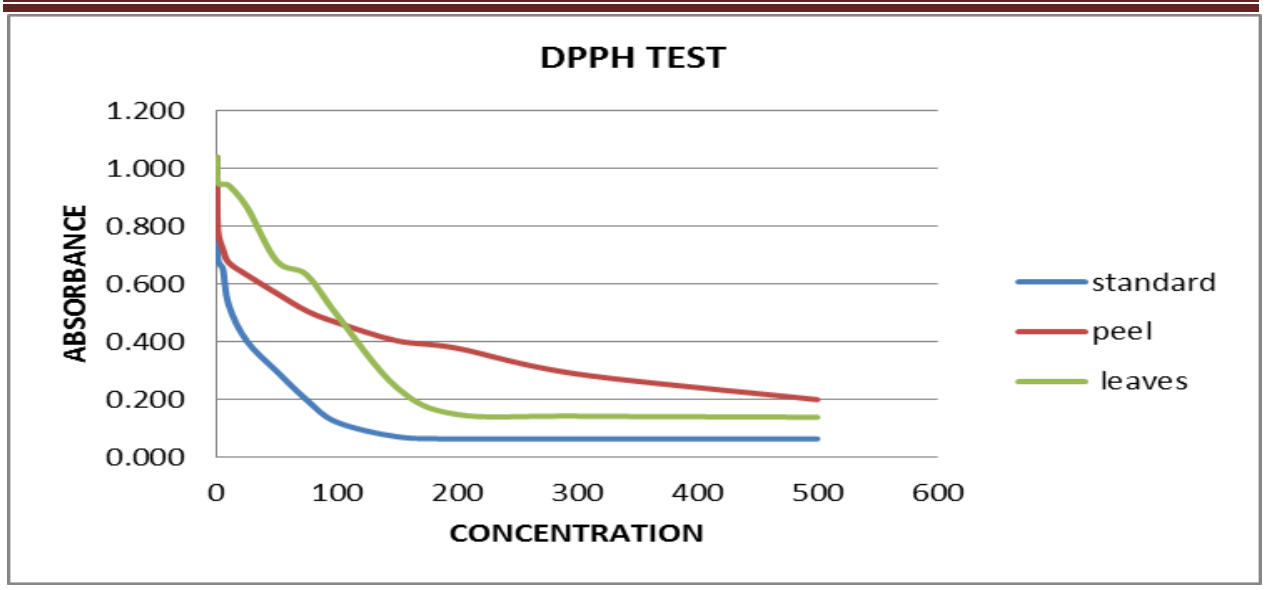

Figure 4: DPPH scavenging assay of leaves and peel extracts of *Citrus sinensis* compared with the standard ascorbic acid.

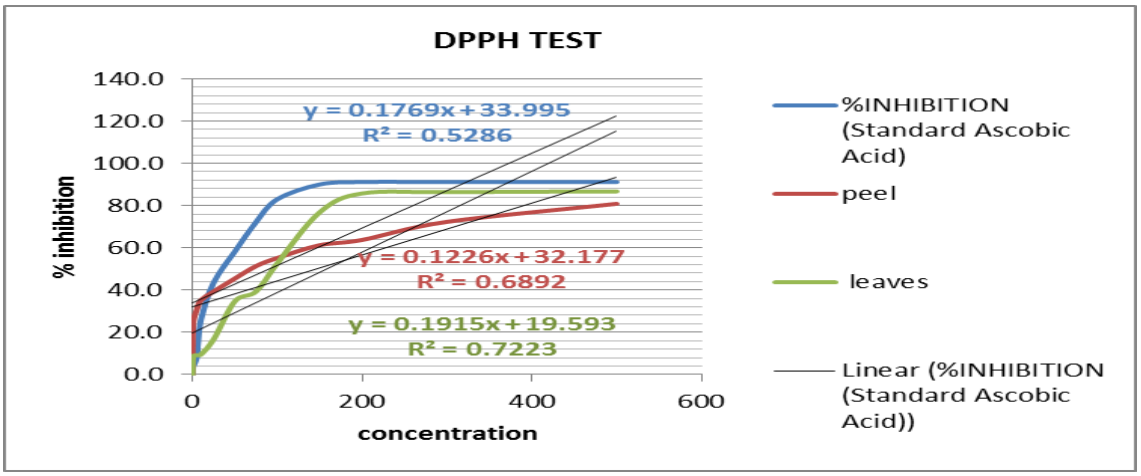

Figure 5: Evaluation of IC_{50} of leaves and peels extracts of *Citrus sinensis* and standard ascorbic acid.

Having reported contents of flavonoids in the leaves of *Mangifera indica* and *Citrus sinensis* in this work which are higher than in the peels of the both plants, it entails the ethnomedicinal quality preference of the leaves to the peels. Experimental evidences have shown that flavonoids as obtained from plant extract may modify allergens, viruses and carcinogens, and also have potential biological response modifier effects such as anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities as reported from *in vitro* studies [21]. Flavonoids (both

flavonols and flavanols) are most commonly known for their antioxidant activities. Food manufacturers have become interested in flavonols for their medical properties especially their potential roles in prevention of cancer and cardiovascular disease [22]. Many flavonoids also possess the ability to decrease the level of urea and residual nitrogen in the blood in hyper-azotaemia and induce diuresis. Epicatechin improve blood flow and therefore seem good for cardiac health. It may also help to prevent some types of cancer [23]. DPPH is widely used for testing preliminary radical scavenging activity of a compound. In this study, methanolic extract of *Mangifera indica* and *Citrus sinensis* (both leaves and peels) showed potential free radical scavenging activity.

Figure 2 shows that the standard ascorbic acid has the highest inhibition which is then followed by leaves and peels of *Mangifera indica* respectively. These were all deduced from eventual arithmetic of the absorbance. Figure 4 also shows that the standard ascorbic has the highest activity which is then followed by peels and leaves of C*itrus sinensis* respectively. Based on the $IC_{50,}$ Figure 3 shows that the leaves of *Mangifera indica* are 9 times as potent in comparison with the standard ascorbic acid with the IC_{50} value of 10.03µg/ml. The methanol extract of the *Mangifera indica* peels is twice in activity than the standard ascorbic acid with the IC_{50} value of 35.84 µg/ml.

From the graphs in Figure 5, the IC_{50} of the peels and leaves extract of *Citrus sinensis are* 145.38 µg/ml and 158.78 µg/ml respectively. This indicates a lower antioxidant activity as compared to the standard ascorbic acid evidenced by its higher IC_{50} compared to the standard ascorbic acid. The stronger antioxidant activity of the *Mangifera indica* leaves than its peels and that of the peels and leaves of *Citrus sinensis* could be due the its higher content of flavonoid than the aforementioned as discovered in this research work. There is however a high impact of the flavonoid in agitating antioxidant performances as reported in documented experiments [22]. More so, the general and natural richness in nutrients of a plant leaves than the peels could also be the impinging stuff to the antioxidant quality.

CONCLUSION

The determination of total flavonoid showed that the *Mangifera indica* leaves has higher total flavonoid followed by *Citrus sinensis* leaves, *Mangifera indica peels* and *Citrus sinensis* peels in that order. This however hints that eating mango and orange with their peels can contribute in

giving the body prevention against several ailments like pathological diseases, and cardiovascular problems. The good antioxidant capacity of leaves and peels *of Mangifera indica* ascertained in this work is of no doubt paramount to its several medicinal properties as it has been in immense utilization by many human communities especially in the Africa.

REFERENCES

[1] Hirano, R., Htun Oo, T., & Watanabe, K.N. (2010). Myanmar mango landraces reveal genetic uniqueness over common cultivars from Florida, India, and Southeast Asia. *Genome*, *53*(4), 3, 21–330.

[2] Saúco, V.G. (2004). Mango production and world market: Current situation and future prospects. *Acta Hortic*, 645, 107-116.

[3] Ghuniyal, J. (2015) Ethanomedical, chemical, pharmacological, toxicological properties of *mangifera indica*: a review. *International Journal of PharmacyResearch & Review*, 4(10), 51–64.

[4] Siddiq, M., Akhtar, S. & Siddiq, R. (2012). Mango processing, products and nutrition. Tropical andSubtropical Fruits: Postharvest, *Physiology, Processing and Packaging*, 277-297.

[5] Ajila, C.M., Bhat, S.G. & Rao, U.J.S.P. (2007) Valuable components of raw and ripe peels from two Indian mango varieties. *Food Chemistry*, 102(4), 1006-1011.

[6] Ignat, I., Volf, I. & Popa, V.I. (2011). A critical review of methods for characterization of polyphenolic compounds in fruits and vegetables. *Food Chemistry*, 126 (4), 1821-1835.

[7] Mukherjee, S. K. (1953). The mango-its botany, cultivation, uses and future improvement, especially as observed in india. *Economic Botany*, 7 (2), 130–162.

[8] WHO, (2008). Traditional medicine. *Fact sheet*, No. 134.

[9] Williams, C. A. & Grayer, R. J. (2004). Anthocyanins and other flavonoids. *Natural product reports*, *21*(4), 539–573.

[10] Delaux, P.M., Xie, X., Timme, R.E., Puech-Pages, V., Dunand, C., Lecompte, E., Delwiche, C.F., Yoneyama, K., Bécard, G. & Séjalon-Delmas, N. (2012). Origin of strigolactones in the green lineage. *The New phytologist*, *195*(4), 857–871.

[11] Butler, V.S., Monday, G.K. & Djordjevic, M.A. (2000). Flavonoid new role for and molecule J. Integ. *Plant Biol*, 52, 98 – 111.

[12] Peer, W.A. & Murphy, A.S. (2007). Flavonoid and Auxin Transport: Modulators or Regulators. *Trends Plant Science*, 12: 556 – 563.

[13] Agati, G., Pollastri, S. & Tattini, M. (2012). Favonoid as antioxidant in plant location and functional significance. *Plant Science,* 196, 67 – 76.

[14] Davies, K.M., Albert, N.W. & Schwinn, K.E. (2012). From landing lights to mimicry: the molecular regulation of flower colouration and mechanisms for pigmentation patterning. *Functional plant biology, FPB, 39*(8), 619–638.

[15] Djorjevic, M.A., Rebmind, J.W., Batley, M. & Rolfe, B.G. (1987) Clovers secrete specific phenolic compounds which either stimulate or repress nod gene expression in Rhizobium trifolii. *EMBOJ* 6, 1173–1179.

[16] Buer, C.S. & Djordjevic, M.A. (2009). Architectural phenotypes in the transparent testa mutants of Arabidopsis thaliana. *Journal of experimental botany*, *60*(3), 751–763.

[17] Bartosz, G. (1997). Oxidative stress in plants. *Acta Physiol Plant.* 19, 47–6.

[18] Temple, N.J. (2000). Antioxidant and Disease: More questions than answers. *Nutrition Research,* 20 (3), 449 – 459.

[19] Hatano, T., Kagawa, H., Yasuhara, T. & Okuda, T. (1988). Two new favonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. *Chem. Pharm. Bull.,* 36, 1090-2097.

[20] Gupta, M., Mazumdar, U.K., Sivakumar, T., Vamis M.L., Karkis, S., Sambathkumar, R. & Mainkandan, L. (2003). Antioxidant and Ant-inflammatory activities of *Acalypha fructicasa. Nig. J. Nat. Prd. Med.,* 25-29.

[21] Galeotti, F., Barile, E., Curir, P., Dolci, M. & Lanzotti, V. (2008). Flavonoid from Carnation (Dianthus caryophyllus) and the antifungalactivity. *Phytochemistry letter,*1(1), 44-48, ISSN 1874-3900.

[22] Lotito, S.B. & Frei, B. (2006). Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? *Free radical biology & medicine*, 41(12), 1727–1746.

[23] Schuier, M., Sies, H., Illek, B. & Fischer, H. (2005). Cocoa-related flavonoids inhibit CFTR-mediated chloride transport across T84 human colon epithelia. *The Journal of Nutrition*, 135(10), 2320–2325.