Total Antioxidant Activity, Total Phenolic and Total Flavonoid Content of Some Plant leaves in South-West Nigeria.

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Abstract— Folk medicine reportedly uses the leaves *of Carica papaya (Caricaceae), Magifera and Moringa oleifera* as herbal remedies for the management of some diseases. This study was carried out to screen the leaf extracts of these plants and determine the total antioxidant activity, total phenolic content and total flavonoid content using standard chemical and chromatographic procedures. Phytochemical screening confirmed the presence of antioxidant, flavonoids and phenols. The result further showed that total flavonoid, total phenolic content and antioxidant activity of the dried leaves of *Moringa oleifera, Mangifera L and Carica papaya L* are significantly higher than those of the fresh leaves. The highest values of antioxidant activity (3278±0.02 µmol/g), total flavonoid content (0.80±0.02mg quercetin eq/g) and total phenolic content (5.275±0.015mg gallic acid/g) were found in the dry leaf extracts of *Moringa oleifera* while the least values of total antioxidant activity (1034.58±0.02 µmol/g), total flavonoid content (0.275±0.0015mg quercetin eq/g) and total phenolic content (2.35±0.01 mg gallic acid/g) were obtained in fresh leaves of *Carica papaya L.* This study also revealed that there is a strong positive correlation between the total antioxidant activity and the total flavonoid content of the leaves of the three plants studied. Also a strong positive correlation between the total flavonoid content and the total phenolic content and the strong positive correlation also existed between the antioxidant activity and total phenolic content of the leaves of the three plants studied.The results indicated that the previously reported anti-sick ling properties of these plants may be due to their inherent antioxidant nutrient composition, thus supporting the claims of the traditional healers and suggests a possible correlation between the chemical composition of these plants and their uses in traditional medicine.

Index Terms— Anti-sickling, Carica papaya L, Folk medicine, Magifera L, Moringa oleifera L, Total antioxidant, Total flavonoid content, Total phenolic content,

1 INTRODUCTION

Herbal medications have been used for relief of symptoms induced by diseases since very old times. Despite the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Much interest, in medicinal plants however, emanates from their long use in folk medicines as well as their prophylactic properties, especially in developing countries [1]. Large number of medicinal plants has been investigated for their antioxidant properties and their chemical constituents have been discovered to be very effective in preventing destructive processes caused by oxidative stress [2].

Most antioxidants in plants are phenols which act as chain-breaking antioxidants. Phenols sometimes have additional mechanisms of antioxidant action, e.g., by chelating transition metal ions [3]. Although the toxicity profile of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts [4,5]. In the south-west region of Nigeria, *Carica papaya L* (Pawpaw leaves), *Mangifera L* (Mango leaves) and *Moringa oleifera L* are being used as remedies for a number of ailments. However, very few empirical evidence has been shown to support the claims of those who use these plan t leaves for antisickling or curative purposes. This study therefore seeks to provide empirical qualitative and quantitative evidence as to the chemical constituents of these leaves in terms of total flavonoid, toatal phenolic and antioxidant activities that could be responsible for the use of these plant leaves as herbal medications.

1.1 Flavinoids, Phenols and Antioxidant Activities

According to Nunes et al. (2012), antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells. Moreover, the role of free radical reactions in disease pathology is well established and is known to be involved in many acute and chronic disorders in human beings, such as diabetes, atherosclerosis, aging, immunosuppression and neurodegeneration [6]. Flavonoids, a class of phenolic compounds has been shown to possess anti-inflammatory, antiviral, anticarcinogenic, antithrombotic, antiallergic and hepatoprotective [7]. Thus, diets rich in fruits and vegetables and other green leaves are believed to play an important role in preventing diseases but human choices of diet are driven by necessity and economy. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity. Phenolic compounds are secondary metabolites in fruits and vegeta-

bles. They have been reported to exhibit antioxidant activity which allows them to scavenge both active oxygen species and electrophiles, to inhibit nitrosation and to chelate metal ions, to have the potential for autoxidation and the capability to modulate certain cellular enzyme activities [8].

1.2 Carica papaya L (Cariceae) pawpaw leaves

Carica papaya plants produce natural compounds (annonaceousacetogenins) in leaf bark and twig tissues that possess both highly anti-tumour and pesticidal properties. The high level of natural self-defence compounds in the tree makes it highly resistant to insect and disease infestation [9]. *Carica papaya L.* leaf tea or extract has a reputation as a tumour-destroying agent [10].

Fresh, green leaf is an antiseptic, whilst the brown, dried pawpaw leaf is the best as a tonic and blood purifier [11]. The tea, prepared with the green papaya leaf, promotes digestion and aids the in treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart [12]. *Carica papaya L.* leaf tea or extract has a reputation as a tumour-destroying agent [13].

1.3 *Magifera indica L(mango leaves)*

The mango leaves are being used traditionally to treat malaria fever among other ailments [14]. Moreover, in a qualitative screening, Vinso et. al (2010) reported the presence of tannins, saponins and flavonoid ,but no trace of alkaloids or anthraquinones.

1.4 *Moringa oleifera* L (moringa leaves)

Moringa has been used as a traditional medicine around the world, for anemia, skin infections, blackheads, anxiety, bronchitis, catarrh, chest congestion, asthma, blood impurities, cholera, glandular, swelling, headaches, conjunctivitis, cough, diarrhea, eye and ear infections, fever, abnormal blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis, for intestinal worms, lactation ,diabetes and pregnancy [16]. The leaves possess remarkable nutritional and medicinal qualities. Another important point is that *Moringa* leaves contain all of the essential amino acids in a good proportion, which are the building blocks of proteins. These leaves could be a great boon to people who do not get protein from meat. *Moringa* even contains argenine and histidine.

Many companies across the world manufacture various products of *Moringa*leaves such as *Moringa*Tea, *Moringa*Tablets, *Moringa*Capsules, *Moringa*leaf Powder, *Moringa*Soaps and *Moringa*Face wash. Some beverages are also available in market prepared by *Moringa* leaves. It is against this background that this study seeks to investigate and establish with empirical evidence, the total flavonoid and phenolic content together with the anti oxidant activities of pawpaw, mango and moringa leaves in both the dried and wet (fresh) samples of each.

1.5 AIM AND OBJECTIVE OF THE STUDY

The study aimed at providing an empirical evidence as a basis for advising the traditional medicine practitioners, herb users, herb sellers, health institutions and farmers on the health and economic importance of the plant leaves (pawpaw, mango and moringa).

The objective of this study is to compare the total flavonoid and phenolic contents of different plant leaves (pawpaw, mango and moringa) in both the dried and wet (fresh) samples and also correlate their anti oxidant activities with the chemical content of the leaves in terms of the total flavonoids and phenolic content.

2 MATERIALS AND METHOD

2.1 SAMPLE COLLECTION

The leaves were collected from various farms at Ipetumodu, Osun state. They were then taken to the herbarium at Obafemi Awolowo University for identification. The leaves to be used as dried samples were firstly rinsed under running tap to remove sand and other dirt and afterwards sundried for about 7days, and then oven dried at 100°C, then blended, sieved and stored in air-tight containers in cool and dry place ready for further analysis. . The leaves to be used as wet (fresh) samples were also collected from various farms at the same location and then rinsed and placed under room temperature so as to dry off the moisture from the leaves. Both the wet and dried samples were packed and labeled and then taken to the laboratory.

2.2 EXTRACTION PROCEDURE OF ANTIOXIDANTS

10g of the powdered sample were transferred into a 250ml beaker and 100ml of 90% methanol added to extract all the antioxidants. The mixture was thoroughly mixed on a magnetic stirrer to obtain a homogenous solution which was consequently filtered through a Whatman No 1 filler paper into a 100ml beaker. The solution in the 100ml beaker was evaporated to 5ml on a rotary evaporator and used for the assay.

2.3 DETERMINATION OF TOTAL ANTIDOXANT ACTIVITY (TAOA)

0.3ml aliquot of sample solution [100µg/ml] was combined with 2.7ml of the regent solution [0.6M H_2SO_4 2.8mM sodium phosphate and 4mM ammonium molybdate]. The mixture were capped and incubated in boiling water bath at 95°C for 90min. The samples extract were then cooled to room temperature and the absorbance measured at 695nm.

A blank solution containing 2.7ml of the reagent solution and 6ml ethanol was incubated like samples above. Stock solutions of α-tocopherol were prepared in methanol (range 0-10 μmol/ml) and treated as sample above. The antioxidant activity of the extracts was exposed as equivalents of α-tocopherol using an extinction coefficients of 4 x 10^3m-^{1}cm^{-1} on a Jenway 6000 UV-Visible spectrophotometer.

Total Antioxidant Activity (μmol α-tocopherol eq/g) or TAOA(μg/g) =
Absorbance of sample X Gradient Factor from Standard Curve x Dilution Factor.

2.4 DETERMINATION OF TOTAL PHENOLIC CONTENT.

0.1ml of sample extract was mixed with 46ml of distilled water. 1ml of Folin-Ciocalteau reagent was added and mixed thoroughly. 3ml of 2% Na2CO3 was added to the mixture working Gallic acid standards of range (0-100μg/ml) was prepared from 50μg/ml stock gallic acid in ethanol. The absorbance of sample extract as well as Gallic acid working standard solutions were read at a wavelength of 760nm on a UV-Visible Jenway 6000 spectrophotometer.
Concentration of Total phenolic in mg gallic acid/g =
Absorbance x Gradient factor from Standard curve x DF

2.5 DETERMINATION OF TOTAL FLAVONIOD

0.1ml of the sample extract was pipetted into a 100ml test tube; o. ml of of 10% $AlCl_3$.6H2O (hydrated Aluminium Trichloride solution) and 0.1ml of 1M CH3COOK (potassium acetate) were added and mixed thoroughly.
Working Quercetin standard of range 0-100ug/ml were prepared from stock 50ug/ml Quercetin solution. The absorbances of sample extracts as well as working Quercetin solutions were read on a JENWAY 6000 Spectrophotometer at a wavelength of 415nm.

Total Flavoniod Content in mg quercetin equivalents per g.
= Absorbance of sample x Gradient Factor from Standard Curve x DF

3.0 RESULTS AND DISCUSSION

3.1 RESULTS

TABLE 1: Total antioxidant activity, total phenolic content and total flavonoid content in fresh and dried leaves.

	Total Antioxidant Activity µmol/g		**Total Phenolic Content mg gallic acid/g**		**TOTAL FLAVONOID µg/ml**	
	FRESH LEAVES	DRY LEAVES	FRESH LEAVES	DRY LEAVES	FRESH LEAVES	DRY LEAVES
Moringa oleifera L	1257.91 ± 0.02[a]	3278.56 ± 0.02[b]	2.865 ± 0.015[a]	5.275 ± 0.015[b]	0.44 ± 0.01[a]	0.80 ± 0.02[b]
Carica papaya L	1034.58 ± 0.02[a]	2987.93 ± 0.02[b]	2.35 ± 0.01[a]	4.695 ± 0.025[b]	0.275 ± 0.015[a]	0.615 ± 0.025[b]
Magifera L	1238.63 ± 0.02[a]	3187.77 ± 0.00[b]	2.750 ± 0.020[a]	5.095 ± 0.025[b]	0.335 ± 0.015[a]	0.700 ± 0.020[b]

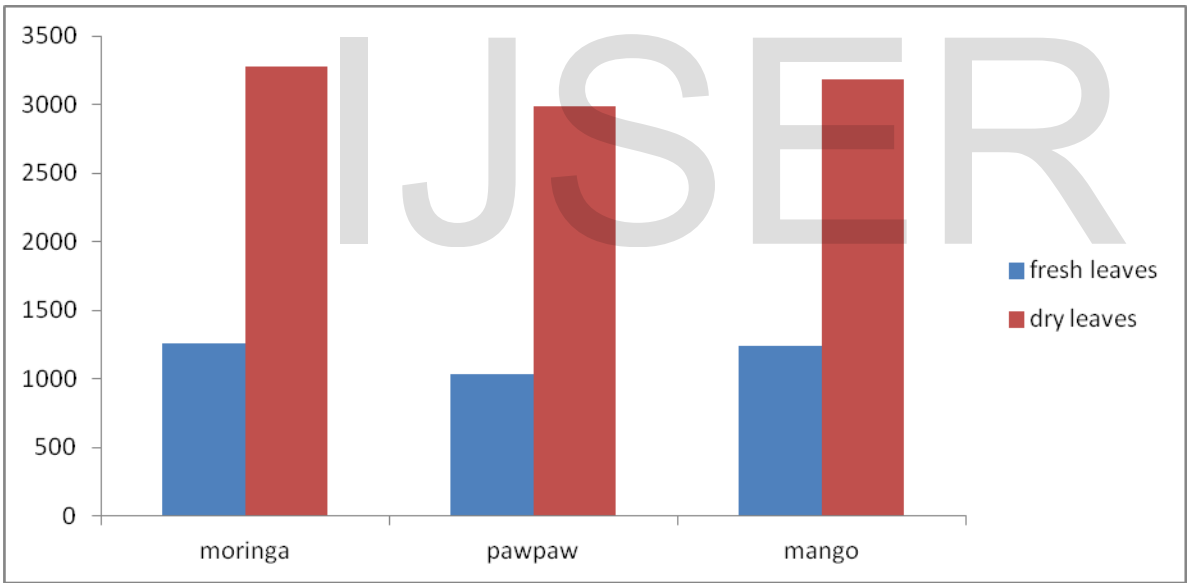

Fig. 1: Total Anti-oxidant Activities (µmoltocopherol/g) of the leaves of the three plants

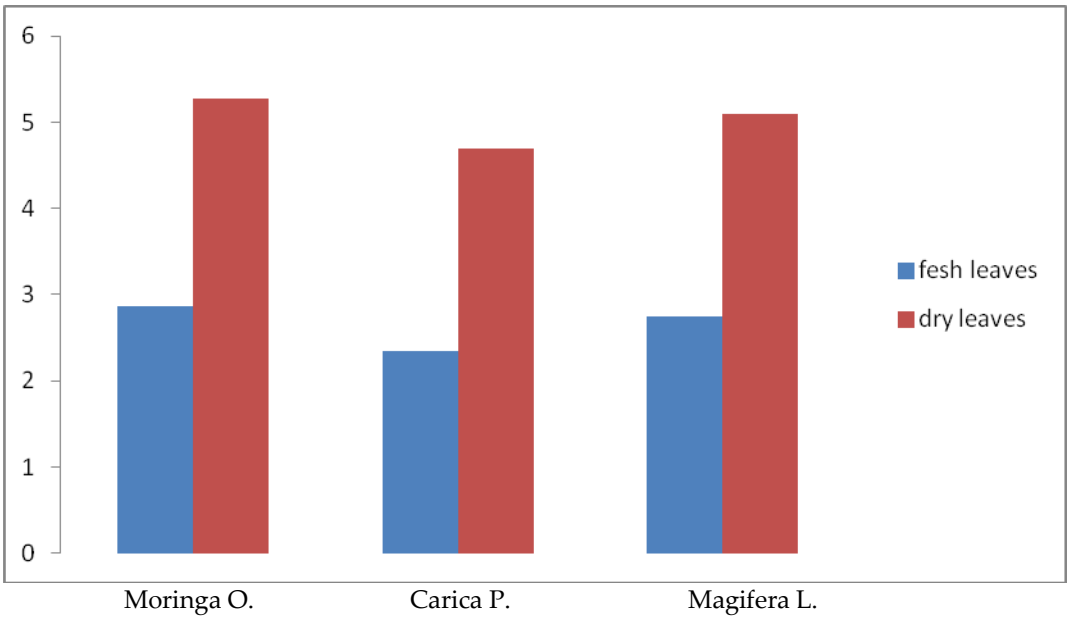

Fig. 2: Total Phenolic Content (mg gallic acid/g) of the leaves of the three plants

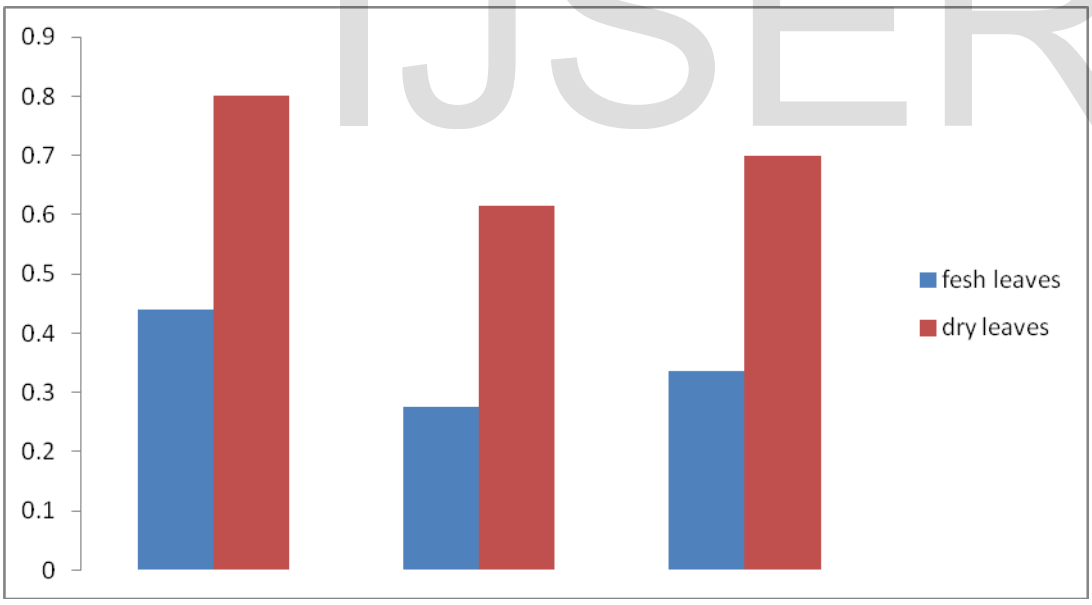

Fig. 3: Total Flavonoid Contents (mg quercetin eq/g) of the leaves of three plants

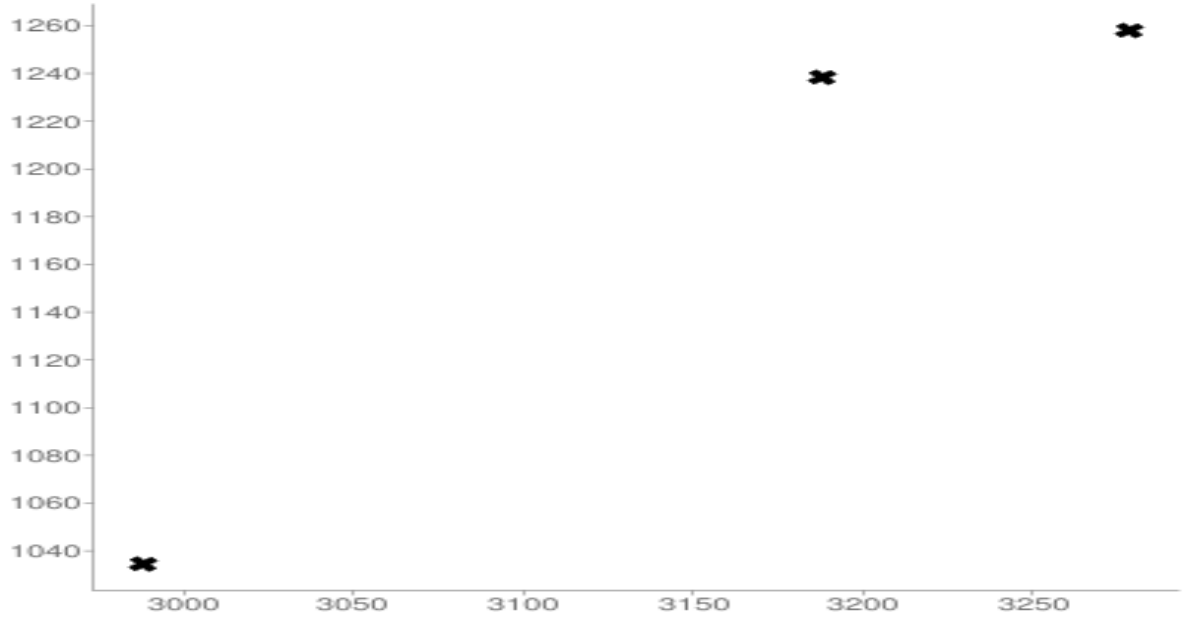

Fig. 4: scattered plot showing the relationship between Total Anti-oxidant Activities of wet and dry leaves of the three plants. The value of R is 0.9731. This is a strong positive correlation between the TAA of the wet and dry leaves

Fig. 5: scattered plot showing the relationship between Total Phenolic Content of wet and dry leaves of the three plants. There is a strong positive correlation between the TPC of the wet and dry leaves (R = 0.9949)

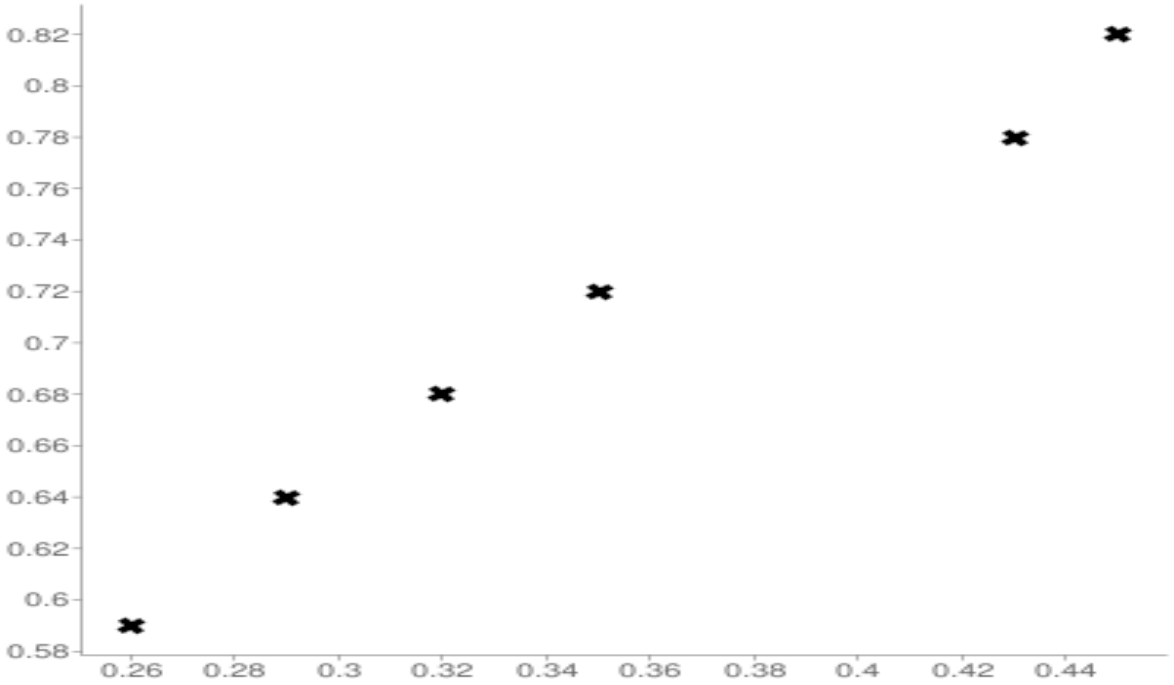

Fig. 6: Scattered plot showing the relationship between the Total Flavonoids Content of the wet and dry leaves of the three plants. There is a strong positive correlation between the TFC of wet and dry leaves (R = 0.9902)

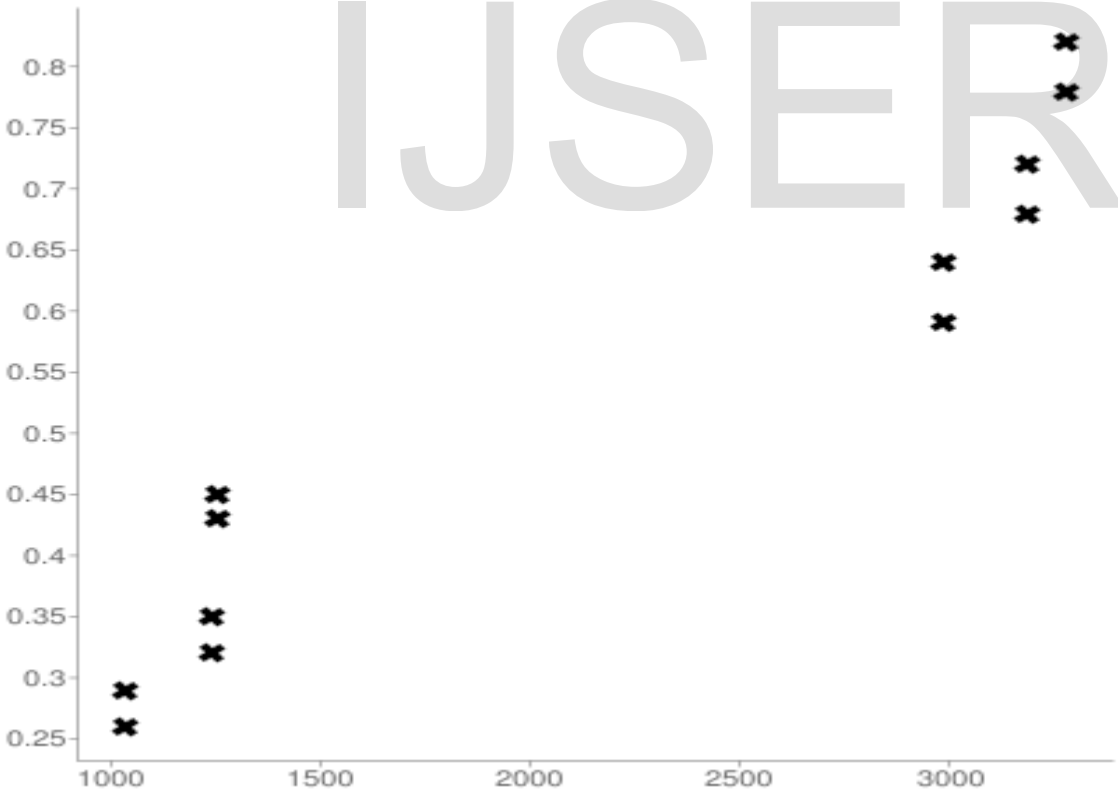

Fig. 7: plot showing a strong positive correlation (R = 0.9548) between the TAA and TFC of the leaves of the three plants

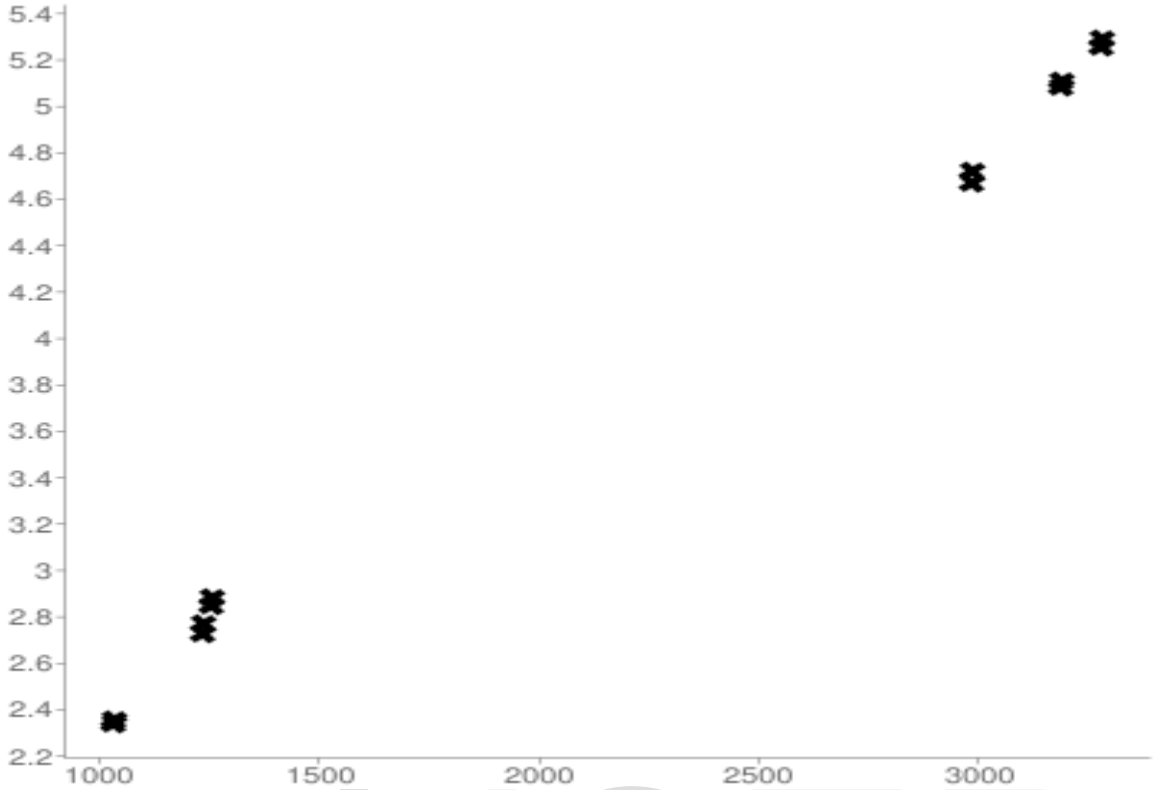

Fig. 8: plot showing a strong positive correlation (R = 0.9965) between the TAA and TPC of the leaves of the three plants

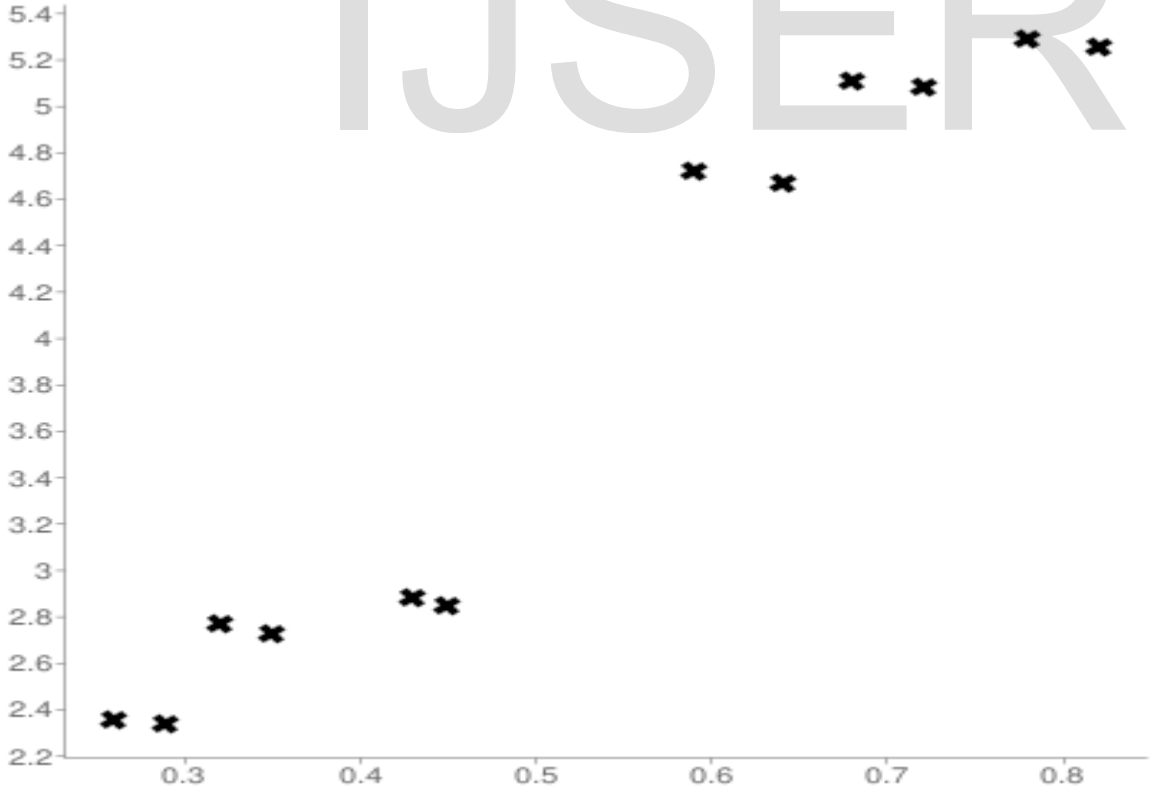

Fig. 9: plot showing a strong positive correlation (R = 0.9711) between the TFC and TPC of the leaves of the three plants

3.2 Discusssion

Total Flavonoid content ,Total Phenolic Content and Total

Antioxidant Activity o of the dried and fresh leaves

The result of this study as shown in table 1 shows that the total phenolic content , the total antioxidant and total flavonoid content of the dry leaves is significantly more than the total phenolic content, total antioxidant activity and total flavonoid content of the fresh leaves of the three plant leaves: Carica papaya L, Moringa oleiferfa and Magifera indica L. This result corroborates the reports of Chang et al. (2002) ; (Onah et al., 2002). Edeoga et al (2005) and (Elekwa et al., 2005) that dried plant leaves contain higher phytochemical contents than the fresh leaves.

Total Antioxidant Activity, Total flavonoid and Total phenolic content of the three plant leaves

The results presented inTable 1 further revealed that dried Moringa oleifera L had the highest flavonoid content (0.80±0.02), phenolic content (5.75±0.015) and antioxidant activity (3278.56±0.02) which are significantly different from the others while the lowest values were found in fresh Carica papaya L.

Total antioxidant and Total Flavonoid

The result of this study, as represented in Fig 7 also showed that there is a strong positive correlation between the total antioxidant activity and total flavonoid content of Carica papaya L, Moringa oleifera and Magifera leaves this supports the report of Hue et al 2012, Sen et al 2013.

Total antioxidant and total phenolic content

Furthermore, the result of this study, as represented in fig 8 shows that there is a strong positive correlation between the total antioxidant activity and total phenolic content of the extract of the plant leaves. This result is in justification of the result obtained by Samineer et al 2010 in their report that, high total phenols content increases antioxidant activity and there was a linear correlation between phenolics content and antioxidant activity in mango leaves extract. However a controversial report was given by Saika and Upadhaya 2011 that there was no relationship between phenolic content and antioxidant activity of ethanolic extract of some plant leaves in india.

4.0 Recommendation and Conclusion

4.1 Conclusion

It can be concluded from this study, that the dried leaves of pawpaw, moringa and mango belonging to the family Moringa oleifera, Carica papaya, and Mangifera respectively are promising groups with rich total antioxidant activity, total phenolic content and total flavonoid content than when fresh and that this dry leaves posses higher potential to cope against oxidative stress and thus act as strong anti-cancerous as well as anti-malaria than when they are fresh.

In conclusion, results obtained from various biochemical indices in this study further establishes the antioxidant status of these plants, a correlation for their local antimalarial properties and usage and an indication of the presence of certain bioactive compounds which may be useful as templates in continuous discovery of potent anti malarial drugs that are cheap and affordable for endemic poor populace of west Africa.

4.2 Recommendation

The use of these plants as either herbal or modern medicine by either boiling and taking in their extract or cooking as vegetables especially moringa leaves or processing them into solid drugs for body intake helps to reduce the risk of malaria, diabetes, cancer and other illness. Intake of these plant especially when dried helps better to reduce diseases due to the presence of the studied phytochemicals found in higher proportion than when fresh.

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REFERENCES

[[1]. Maqsood S, Singh P, Balange A K., Samoon M H. "Effect of Die tary Chitosan on Non-specific Immune Reponse and Growth of Cyprinus carpio Challenged with Aeromanias hydrophilia", Inter. Aqua Res. 2010, vol 2, pp77-85.

[2]. Zengin G, Cakmak Y S, Guter G O, Aktumsek A. "*Antioxidant Properties of Methanolic Extract and Fatty Acid Composition of Centaurea urvillei"*, 2011, vol 5, pp123-132.

[3]Samineh Jafari, Arman Moradi, Ali Salaritabar, AbbasHadjiakhoondi, Mahnaz Khanavi. "Determination of Total phanolic and flavonoid content of Leonurus cardiaca L. in compare withAntioxidant activity", 2010, vol5, no 7 pp484-487. DOI: 10.3923/rjbsci. 2010. 484.487.

[4]. Vongtau H O, Abbah J, Chindo B A, Mosugo O, Salawu A O, Kwanashie H O, Gamaniel K S. *" Central inhibitory effects of the methanol extract of Neorautanenia mitis root in rats and mice".*2005 *J pham Bid* vol 43 pp:113-120.

[5]. Oluyemi K A, Okwuonu U C, Baxter D G, Oyesola T O. (2007). *Toxic effects of methanolic extract of Aspil Africana leaf on the estrous cycle and uterine tissues of wistar rats.* Vol 25, pp: 609-614.

[6]. Nunes P X, Silvia S F, Guedes R J, Almeida S.. "*Biological oxid tions and antioxidant activity of natural products, phytochemicals as nutraceuticals global approaches to their role in nutrition and heaith".* 2012

[7] Batra, P. and Sharma A.K. "Anti Cancer Potential of Flavonoids: Recent Trends and Future Perspective" Biotech December 2013, vol 3, no 6,pp: 439-459. DOI: 10. 1007/s13205-013-0117-5.

[8]. Ghebhardt .E.,Harnly J.M.,Bhajwat S.A.." USDA's Flavonoid Dat base: Flavonoids in Friut". U.S Department of Agriculture (USDA), Agricultural Research Service, Beltsville Human Nutrition Research Centre. Nutrient Data Laboratory and Food Consumption Laboratory, Beltsville, MD.

[9] Peter, R. N. "*Pawpaw (Asimina)". In: J. N. Moore and J. R. Ballington (eds). Genetic Resources of Temperate Fruit and Nut Trees. Acta Hort,*1991. 290, pp:567-600.

[10]. Walter Last. *"Cancer remedies. www.health-science spirit.com/cancer6-remedies" Honolulu*, 2008.

[11]. Ezuruike, U.F., Pietro, P.M., "The Use of Plants in the Traditional Management of Diabetes in Nigeria". Pharmacological and Toxicological Considerations. Journal of Ethnopharmacology. 2014, http://dx, dii.org/10.1016/j.jep204.05.055

[[12]. Mannyar Y. and Bhixavatimath, P. "Antihyprglycemic and Hypolipidemic activities of aqeous extract of Carica papaya L in Alloxan-induced diabetic rats". Journal of Ayurveda and Integrative Medicine. 2012. Vol 3 pp: 70-74.

1[13]. Walter Last. *"Cancer remedies. www.health-sciencespirit.com/cancer6-remedies"* ulu. 2008

[14]. Singh, s. and Singh R. "Herbal Medical Treatment of Malaria in Aliepo Local Government Area, Kebbi, Nigeria". Journal of Medicinal plant Studies. 2014, vol 2, no 2, pp: 117-126.

[[15]. Vinson J.A, Dabbagh V A, Serry M M and Jang J,. "*Plant Flavonoids are Powerful Antioxidants Using an In Vitro Oxidation Model for Heart Disease". J. Agric. Food Chem*., 1995, 43: 208.

[16] Johnson, I.T. "*NewAapproaches to the Role of Diet in the Prevention of Cancers of the Alimentary Tract". Mutat.* Res. 2004, vol 55 no1-2 pp: 9-28.

[17]. Chang C, Yang MH, Wen HM, Chern JC. "*Estimation of Total Flavonoid Content in propolis by Two Complementary Colorimetric Methods".* J. Food Drug Anal. ,2002, vol 10:pp178-182.

[18]. Onah JO, Akubue PI, Okhide GB "The Kinetics of Reversal of Pre-sickled Erythrocytes" 2002. 107: pp 640- 648.

[19] Edeoga H.O., Okwu D.E., Mbaebie B.O. "Phytochemical constituents of some Nigerian Medicinal Plants". Afr. J. Biotech. 2005, 4(7): 685-688.

. [20] Elekwa I., Monanu, M.O., Anosike E.O. "Effects of an Aqueous Extracts of Zanthoxylum macrophylla Roots on Membrane Stability of Human Erythrocytes of Different Genotypes". Biokemistri 2005, vol17, no1 pp: 7-12.

[21]. Hue,S., Boyce, A.N., Somansundram M, "Antioxidant Activity, Phenolic and Flavonoid Contents in the Leaves of Different varieties of Sweet Potato(Ipomea batatas)". Australlian Journal of Crop Science, 2012 vol 6, no. 2, pp: 375-380.

[22] Sen S., De b., Devanna N., and Chakroborty R. "Total Phenolic Content, Total Flavonoid Content and Antioxidant capacity of the leaves ofMeyna spinosa rb, an Indian Medicinal plant. Chin J. Nat. Med. 2013 March vol 11 n0 2 pp; 49-57. DOI: 10.1016/S1875-5364913)60042-4

[23] Saika, L. R. and Uphadhyaya, S. "Antioxidant Activity, Phenol and Flavonoid Content of Some Less Known Medicinal Plants of Assam". International Journal of Parma and Bio Sciences, April; 2011; vol 2, no2.