SAR Journal of Medical Biochemistry

Abbreviated Key Title: *SAR J Med Biochem*

Home page: https://www.sarmedjournals.com/sjmb

OPEN ACCESS

ISSN 2707-7721 (P)

Research Article

Stabilization of edible oils with bitter leaf *(Vernonia amygdalina)* and water bitter leaf (*Struchium sparganophora)* extracts

Arawande Jacob Olalekan*, and Aderibigbe Akeem Shina

Department Of Chemistry, University of Medical Sciences, PMB.536, Ondo-City, Ondo-State, Nigeria

***Corresponding Author**
Arawande Jacob Olalekan

Article History: | Received: 05.02.2020 | Accepted: 14.02.2020 | Published: 28.02.2020 |

Abstract: The studies on ant oxidative effects of bitter leaf and water bitter leaf extracts on edible oils were evaluated during fifteen weeks of storage of refined soybean oil (RSO) and crude groundnut oil (CGO) in a transparent plastic container at room temperature. The bitter leaf and water bitter leaf were obtained, dried, milled, sieved and separately extracted with five different solvents (methanol, water, chloroform, acetone, and ethyl acetate). The water extract of bitter leaf and methanol extract of water bitter leaf were separately added at varying concentrations (200 ppm – 1000 pm) to RSO and CGO stored in transparent plastic containers. Another set of the edible oils which contained no additive (0ppm) and 400 ppm butylatedhydroxytoluene were equally set up for comparison. The refractive index, free fatty acid and acid value of the oil samples were monitored every three weeks using standard methods for a period of fifteen weeks. The solvent extractive value showed that methanol had the highest yield of extract for bitter leaf (8.710%) and water had the highest yield of extract for water bitter leaf (7.315%).The lowest yield of extract was obtained with chloroform for bitter leaf (2.570 %) and water bitter leaf (2.210 %).The result revealed that methanol extracts of water bitter leaf was more effective against hydrolytic rancidity of CGO than RSO while the water extract of bitter leaf was more effective against hydrolytic rancidity of RSO than CGO. The extracts of both plants had little or no effect on the refractive index of the edible oils.

Keywords: Bitter leaf extract, water bitter leaf extract, BHT, Free Fatty Acid, Acid value, edible oils.

INTRODUCTION

In Nigeria and some other African countries where soya bean oil and groundnut oil are common, the oil merchants purchase it when it is relatively cheaper (that is during their season) and store them for a period of about four to six months or more and later sell them at higher price when the oil seeds are off-season with primary target of making much profit without considering the deterioration that might have probably occurred during the period of storage thereby posting health risk to the consumers of such oils (Arawande and Akinnusotu, 2018). Hence there arises the need to prevent oil deterioration by adding antioxidants that will impede the oil rancidity.

The use of synthetic antioxidant such as butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA), propylgallate (PG) and citric acid to prevent lipid oxidation have been established (Ullah *et al*., 2003; Ruger *et al*., 2002; Cuvelier *et al*., 1992). But it has been discovered that some of these synthetic antioxidants especially butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) are carcinogenic thereby they are being discouraged in international market as food additives (Tomovic, *et al*., 2017;Indrajit *et al*., 2013; Vivek and Surendra, 2006, Carrasquero *et al*., 1998). This leads to provoking interest in seeking for safer means of natural antioxidant of plant origin that will effectively serve the same purpose of preventing oil rancidity (Said *et al*., 2018; Enrol *et al*., 2004, Emmanuel and Mudiakeoghene, 2008; Tian and White, 1994). The use of plant extracts such as bananas, plantain, wild lettuce and cabbage star leaf to inhibit oil deterioration has been reported by Arawande and Komolafe, 2010. Arawande and Abitogun, 2009 reported the comparative studies on antioxidative potential of citric acid and methanoic extract of cabbage star leaf in crude palm kernel oil. Arawande *et al*., 2012 also reported antioxidative effect of wild lettuce on stability of refined soyabean oil.

Bitter leaf and water bitter leaf are plants which are in everywhere in large quantity and they are only use as medicine (such as curing of diabetes, fever, gonorrhea and tumor related ailment), animal feeds and human consumption

(Akinwunmi and Amadi, 2019; Akani *et al*., 2017; Francis, 2015; Oboh, 2006; Godwin *et al*., 1994). The phytochemical composition and effect of its aqueous extract induced air-way inflammatory response in Wistar rats has been reported by Eko *et al*., 2008. Also, antioxidant properties and inhibitory effect of ethanol extract of Struchium sparganophora leaf on α - amylase and α- glycosidase activities has been reported by Oboh. *et al*., 2012. The nutritive, antioxidant, antimicrobial and antimalarial activities of the leaves have been reported by many researchers (Martini, *et al*., 2019; Adeiza and Abdul Malik, 2017; Adesanoye and Farombi, 2014; Ebenezer and Olatunde, 2011). Owing to the potency of the plant in preventing diabetes and tumor- related ailments suggest that they contain certain antioxidants which can be extracted with suitable solvents.

The aim of this research was to determine the extractive values of bitter leaf and water bitter leaf using different solvents, to investigate the antioxidant potential of highest solvent yield extracts of each of the plants at varying concentration (200 ppm – 1000 ppm) on refined soybean oil and crude groundnut oil, to determine the effect of the extracts on refractive index of the oils as well as to compare the antioxidant activities of the extracts with that of 400ppm butylatedhydroxytoluene (BHT) by monitoring their free fatty acid (FFA), acid value (AV) and peroxide value (PV) for a period of fifteen weeks at interval of three weeks.

MATERIALS AND METHODS

Sources of materials

The refined soya bean oil was obtained before being fortified with vitamin A from JOF Ideal Family Farm along Owo/Benin express way in "Owo" Local Government Area of Ondo state, while crude groundnut oil was obtained from 'Ore' in Odigbo Local Government of Ondo State.

Water bitter leaf (Struchium sparganophora) was obtained from Boyo camp via Ute, Ose Local Government, Ondo State and Bitter leaf (*Vernonia amygdalina)* was obtained from Laje road, Ondo city, Ondo state. Butylatedhydroxytoluene was obtained from Human Nutrition Department, University of Ibadan. Oyo state.

Preparation and extraction of bitter leaf and water bitter leaf.

The leaves of the two plants were plucked by hand, separately rinsed in water, cut into smaller pieces for easy drying and sun-dried. The dried leaves were grounded using electric blending machine and they were sieved with 40mm mesh size. The powdery samples were packed into two plastic bottles separately and kept appropriately prior to extraction.

Twenty grams (20 g) of each dried powdery were weighed into cleaned and dried reagent bottles and 200 mL of each solvent (methanol, acetone, ethylacetate, chloroform and water) was separately added to each bottle and left for 72 hours during which it was intermittently shaken on a shaking orbit machine. The mixture was filtered through 0.45 µm nylon membrane filter. The extracts were evaporated to dryness under reduced pressure at 40^0C by rotary evaporator. The obtained extract was weighed and the percentage extractive value of each solvent was calculated thus;

$$\%\, Extractive\ value\ of\ solvent = \frac{Weight\ of\ extract}{Weight\ of\ sample} \times 100$$

(Arawande and Akinnusotun, 2018).

Addition of additives to edible oils

Methanol extract of bitter leaf and water extract of water bitter leaf at varying concentrations (200 ppm – 1000 ppm) was added to 100 mL of edible oils (refined soya bean oil and crude groundnuts oil) contained in transparent plastic containers of equal capacity (500 mL) and they were thoroughly shaken for proper mixing. The oil samples containing 400 ppm butylatedhydroxytoluene (BHT) and one without additive, 0 ppm (control) were also set up. Each container was properly labeled and stored in an open place at room temperature ranging from 27 ^{0}C to 34 ^{0}C.

Physical and chemical analysis of edible oils

The refractive index, the free fatty acid and the acid value of each oil samples were determined every three weeks for a period of fifteen weeks using standard method of analysis (AOCS, 2004).

Determination of refractive index

Abbe's refractometer was used for refractive index determination. Warm water was circulated into the cavity around the prism and a thermometer was fitted in a hole in the cavity for reading the temperature at which the refractive index reading was taken (25^0C). The prism was opened cleaned with acetone. Then a drop of oil sample was put on one part of the prism and it was closed. The mirror below the eyepiece was adjusted and the reading was taken up to four decimal places where the sharp demarcation of light and shade appeared and the temperature on the thermometer was noted. The refractive index was reported as $\cap_D^{25}$.

Determination of free fatty acid (FFA)

25 mL of ethanol was poured into conical flask, 2 drops of phenolphthalein indicator was added and the mixture was titrated against 0.1M of KOH solution until faint pink colour was obtained. Thereafter about 5 g of the oil sample was weighed into the mixture and the mixture was heated until it boiled. Two drops of phenolphthalein was added to the mixture. The initial burette was noted and recorded and the mixture was titrated against 0.1M KOH until a faint pink colour was obtained that persisted for 15seconds.

$$FFA = \frac{\text{Titre value X Conc. KOH X Z}}{\text{Weight of the sample}}$$

Z = (28.2 for oleic acid for soya bean oil and groundnut oil)
It is expressed in % Oleic acid. (AOCS, 2004)

Determination of acid value

25mL of ethanol was poured into conical flask, 2 drops of phenolphthalein indicator was added and the mixture was titrated against 0.1M of KOH solution until faint pink colour was obtained. Thereafter about 5 g of the oil sample was weighed into the mixture and the mixture was heated until it boiled. Two drops of phenolphthalein was added to the mixture. The initial burette was noted and recorded and the mixture was titrated against 0.1M KOH until a faint pink colour was obtained that persisted for 15seconds.

$$\text{Acid value (AV)} = \frac{\text{Titre value X Conc. KOH X 56.11}}{\text{Weight of the sample}}$$

It is express in (mg KOH/g oil) (AOCS, 2004)

RESULTS AND DISCUSSIONS

The result of the physico-chemical analysis confirmed rationale for the use of important nutritional plants which have been neglected. These plants have being extracted with five different solvents (water, methanol, acetone, ethylacetate and chloroform). The extract of the highest yield solvent for each plant (methanol for bitter leaf and water for water bitter leaf) was introduced into the edible oils (refined soybean oil and crude groundnut oil) together with synthetic antioxidant (Butylatedhydroxytoluene) at varying concentration (200 ppm, 400 ppm, 600 ppm, 800 ppm, 1000 ppm) and that of BHT is 400ppm.

Table 1: Percent yield of bitter leaf and water-bitter leaf extract using different solvents.

	Percent yield of extract	
Solvent	**Bitter Leaf**	**Water Bitter Leaf**
Methanol	8.710	7.115
Water	4.935	7.315
Ethylacetate	3.640	3.510
Acetone	3.645	3.240
Chloroform	2.570	2.210

Table 1 shows the percentage of the extractive value (% yield) of bitter leaf and water bitter leaf extracted with five different solvents (water, methanol, ethylacetate, acetone and chloroform). The result shows that the percentage yield of bitter leaf extract was 8.71% in methanol, 4.935% in water, 3.645% in acetone, 3.640% in ethylacetate and 2.570% in chloroform. While the percentage yield of water bitter leaf extract was 7.315% in water, 7.115% in methanol, 3.15% in ethylacetate, 3.24% in acetone and 2.21% in chloroform.

Concentration of Additive	Refractive Index at 40^0C	
	Refine Soybean Oil (RSO)	Crude Groundnut Oil (CGO)
200 ppm MBLE	1.4694	1.4647
400 ppm MBLE	1.4686	1.4645
600 ppm MBLE	1.4693	1.4640
800 ppm MBLE	1.4683	1.4643
1000 ppm MBLE	1.4692	1.4637
200 ppm WWLE	1.4698	1.4632
400 ppm WWLE	1.4687	1.4633
600 ppm WWLE	1.4685	1.4642
800 ppm WWLE	1.4692	1.4632
1000 ppm WWLE	1.4701	1.4638
400 ppm BHT	1.4690	1.4635
0 ppm (No additive) control	1.4694	1.4642

Table 2: Refractive index of refined soybean oil and crude groundnut oil stored with varying concentrations of plant extracts and 400 ppm BHT

NOTE: MBLE= Methanol Bitter Leaf Extract; WWLE= Water Bitter Leaf Extract, BHT= Butylated hydroxytoluene

The higher value obtained for methanol in bitter leaf may be attributed to the polarity of methanol over water and other solution (Arawande *et al*., 2012). While the higher value obtained for water in water bitter leaf may be attributed to the affinity of water for water bitter leaf water coupled with polarity of water over other solvents used for extraction. The extractive value of solvents is a function of the ability of the solvent to obtain bioactive ingredient from a material of organic origin.

Table 2 indicates the refractive index of edible oils stored with different levels of methanol extract of bitter leaf, water extract of water-biter leaf and 400ppm BHT. Generally, the presence of the additive in the edible oils causes slight changes in the refractive index of the oils. RSO stored with methanol bitter leaf extract had refractive index between 1.4683 and 1.4694 while RSO stored with water water-bitter leaf extract had refractive index between 1.4685 and 1.4701.This shows that RSO containing WWLE have slightly higher refractive index than RSO stored with MBLE.RSO without additive has refractive index of 1.4694 while RSO containing BHT has refractive index of 1.4690. The refractive index of CGO stored with varying concentration of MBLE are within 1.4637 and 1.4647 while the refractive index of CGO containing varying concentration of WWLE is between 1.4632 and 1.4642. CGO without any additive has refractive index of 1.4642 and CGO stored with BHT has refractive index of 1.4635. The refractive index of edible oils containing additives are within the recommended values given by Standards Organization of Nigeria (SON). Refractive index of edible oils is used to ascertain the level of purity or adulteration of edible oils. The refractive index of both RSO and CGO with and without additives were acceptable and hence the additives did not post any risk of adulteration to the oils.

Table 3: Mean value of Free Fatty Acid (% oleic acid) of refined soybean oil and crude groundnut oil stored with varying concentration of plant extracts and 400 ppm BHT for fifteen weeks

	* Free Fatty Acid (% oleic acid)	
Concentration of Additive	Refine Soybean Oil (RSO)	Crude Groundnut Oil (CGO)
200 ppm MBLE	0.2350±0.0588	1.3969±0.0898
400 ppm MBLE	0.2468±0.0643	1.5981±0.2093
600 ppm MBLE	0.2468±0.0492	1.4087±0.1059
800 ppm MBLE	0.2585±0.0526	1.5745±0.1990
1000 ppm MBLE	0.2468±0.0492	1.4087±0.2345
200 ppm WWLE	0.2468±0.0965	1.5863±0.3776
400 ppm WWLE	0.2350±0.0719	1.6218±0.2277
600 ppm WWLE	0.2350±0.0000	1.5981±0.2093
800 ppm WWLE	0.2115±0.0322	1.5745±0.1899
1000 ppm WWLE	0.2115±0.0670	1.4206±0.2330
400 ppm BHT	0.2233±0.0965	1.4087±0.2882
0 ppm (No additive) control	0.2350±0.0525	1.4107±0.2098

NOTE: MBLE= Methanol Bitter Leaf Extract; WWLE= WaterWater- Bitter Leaf Extract, BHT= Butylated hydroxytoluene *Mean value±standard deviation

Table 3 reveals the mean value of free fatty acid (% oleic acid) of refined soybean oil and crude groundnut oil stored with varying concentration of plant extracts and 400 ppm BHT for fifteen weeks. The free fatty acid of refined soybean oil containing 400 ppm to 1000 ppm methanol bitter leaf extract were higher than RSO containing the same concentration of water water-leaf extract. However the free fatty acid of RSO containing 200 ppm of methanol bitter leaf extract had lower value than RSO containing water water-bitter leaf extract. It was observed that the free fatty acid of RSO containing 800 ppm and 1000 ppm water water-bitter leaf extract were lower than RSO stored with 400 ppm BHT and RSO without any additive (0 ppm). This suggests that 800 ppm and 1000 ppm of water water-bitter leaf extract was effective in lowering the free fatty acid of RSO than 400 ppm BHT. The free fatty acid of crude groundnut oil containing varying concentration of methanol bitter leaf extract had lower values with CGO containing water water-bitter leaf extract. CGO containing 200 ppm, 600 ppm and 1000 ppm had lower free fatty acid than CGO containing no additive (0 ppm). The free fatty acid of edible oils is a measure of hydrolytic rancidity index caused by moisture, lipase enzymes and heat (Rossell, 1994) and that the higher the free fatty acid value of edible oils, the more the extent of hydrolytic rancidity that set in (Onwuka, 2005; Rossell, 1986; Ihekoronye and Noddy, 1985). Therefore, it is noted that 800 ppm and 1000 ppm water water-bitter leaf reduced the degree of hydrolytic rancidity of RSO than 400 ppm BHT whereas 200 ppm of methanol bitter leaf extract reduced the extent of hydrolytic rancidity of CGO than 400 ppm BHT.

Table 4: Mean value of Acid value (mgKOH/g oil) of refined soybean oil and crude groundnut oil stored with varying concentrations of plant extracts and 400ppm BHT for fifteen weeks

Concentration of Additive	*Acid value (AV) mgKOH/goil	
	Refine Soybean Oil (RSO)	Crude Groundnut Oil (CGO)
200 ppm MBLE	0.4676±0.1169	2.7796±0.1786
400 ppm MBLE	0.4910±0.1281	3.1801±0.4164
600 ppm MBLE	0.4910±0.0978	2.8032±0.2107
800 ppm MBLE	0.5144±0.1046	3.1329±0.3959
1000 ppm MBLE	0.4910±0.0978	2.8032±0.4667
200 ppm WWLE	0.4910±0.1921	3.1565±0.7514
400 ppm WWLE	0.4676±0.1432	3.2272±0.4531
600 ppm WWLE	0.4676±0.0000	3.1801±0.4164
800 ppm WWLE	0.4208±0.0640	3.1130±0.3780
1000 ppm WWLE	0.4208±0.1333	2.8267±0.4637
400 ppm BHT	0.4442±0.1921	2.8032±0.5734
0 ppm (No additive) control	0.4676±0.1046	2.8071±0.4175

NOTE:
MBLE= Methanol Bitter Leaf Extract; WWLE= Water Water- Bitter Leaf Extract, BHT= Butylated hydroxytoluene
*Mean value±standard deviation

Table 4 reveals mean value of acid value (mgKOH/g oil) of refined soybean oil and crude groundnut oil stored with varying concentrations of plant extracts and 400ppm BHT for fifteen weeks. The acid value trend is similar to free fatty acid trend discussed in Table 3 above.

The acid value of refined soybean oil RSO containing 400 ppm to 1000 ppm of water water-leaf extract were lower than RSO containing the same concentration of methanol bitter leaf extract. It was equally noted that the acid value of RSO containing 200 ppm of methanol bitter leaf extract had lower value than RSO containing water water-bitter leaf extract. It was obvious that the acid value of RSO containing 800 ppm and 1000 ppm water water-bitter leaf extract were lower than RSO stored with 400 ppm BHT and RSO without any additive (0 ppm). This implies that 800 ppm and 1000 ppm of water water-bitter leaf extract was effective in lowering the acid value of RSO than 400 ppm BHT. The acid value of crude groundnut oil containing varying concentration of water water-bitter leaf extract had higher values with CGO containing methanol bitter leaf extract. CGO containing no additive (0 ppm) had higher acid value than CGO containing 200 ppm, 600 ppm and 1000 ppm. Acid value of any oil is an indices of hydrolytic rancidity and it is one of the quality parameters of oil. The lower the acid value of an oil the better is the quality of the oil, hence the less the extent of hydrolytic rancidity. The plant extracts are more effective in combating hydrolytic rancidity in refined soyabean oil than crude groundnut oil. This pronounced effect for soybean oil might be due to the fact that soybean has undergone refining process which crude groundnut oil has not undergone

CONCLUSION

The varying concentration of methanol extract of bitter leaf and water extract of water bitter leaf incorporated into the edible oils (refined soyabean oil and crude groundnut oil) had almost same effect on hydrolytic rancidity as a result of antoxidative activity of the two plant (bitter leaf and water bitter leaf) extracts when compare with that of 400ppm BHT.

The two extracts were effective against hydrolytic rancidity. Therefore, in this research work the natural antoxidants reduced lipid peroxidation or rancidity like that of synthetic antioxidant and can be used to replace synthetic antioxidant since research has revealed that synthetic antioxidant is carcinogenic. The effect of methanol extract of bitter leaf and water leaf extract of water bitter leaf at varying concentration on edible oils for longer period (at least 10 months) should be investigated.

The effect of both natural and synthetic antioxidant activity on refined soyabean oil, palm kernel oil and crude groundnut oil stored in a dark condition at room temperature can further be investigated when the oil is stored in a dark bottle or metal container. Other solvent apart from methanol and water can also be used for extraction of the plant before incorporation into the edible oils together with propylgallate (PG) or butylatedhydroxyanisole (BHA) as synthetic antioxidant.

REFERENCES

1. Adeiza, S. S., & Abdulmalik, B. S. (2017). Assessment of the phytochemical constituents and in – vitro antibacterial activity of *Vernonia amygdalina* extracts on someclnical isolates. *The Pharmaceutical and Chemicals Journal*. *4*(4),123 – 128.
2. Adesanoye, O. A., & Farombi, E. O. (2014). in Vitro Antioxidant properties of methanolic leaf
3. Akani, N. P., Jumbo, B., & Nwankwo, C. E. (2017). Effect of drying temperatures on minerals composition and bacterial populations of Vernonia amygdalina (Bitter leaf). *Research Journal of Food Science and Quality Control 3*(2), 39 – 49.
4. Akinwunmi K.F., & Amadi C. V. (2019). Assessment of antioxidant and antidiabetics properities of *Picralima nitida* seed extracts. *Journal of Medicinal plants Research* 13(1):9 – 17
5. AOCS. (2004). Official and Tentative Method of American Oil Chemist Society Champaign. U.S.A, Method.8:553.
6. Arawande, J. O., & Akinnusotu, A. (2018). Comparative study on extraction and characterization of castor seed and oil from three different state capitals in Nigeria. *American Journal of Food Science and Nutrition 5* (2), 37-42.
7. Arawande, J. O., Komolafe, E. A., & Ijitona, O. O. (2012). Antioxidative effect of wild lettuce (*Launaea taraxaciafolia*) on stability of refined soyabean oil. *Journal of Agricultural Technology 8*(7), 2283-2295.
8. Arawande, J.O., & Abitogun, A.S. (2009). Comparative studies of antioxidant potential of citric acid and methanolic extract of cabbage star leaf on crude palm kernel oil. *Journal of Chemical Society of Nigeria*. *34*(1), 54-57.
9. Arawande, J.O., & Komolafe, E. A. (2010). Antioxidative potenetials of banana and plantain
10. Carrasquero, A., Salazar, M., & Nava, P. B. (1998). Antioxidant activity of grape seed extract on
11. Cuvelier, M. E., Richard, H. and Berset, C. (1992). Comparison of antioxidant activity of some acid phenols: Structure activity relationship. *Biosci. Biol. Tech. Biochem*., 56,324-326.
12. Ebenezer, O.F., & Olatunde, O. (2011). Antioxidative and chemopreventive properities of *Vernonia amygdalina* and *Garcinia biflavonoid*. *International Journal of Environmental Research and Public Health*.2533 – 2555.
13. Eko, M., Eteng, M., & Eyong, E. (2008).Phytochemical composition and effect of aqueous extract of *Struchium sparganophora* on cockroach crude extract-induced airway inflammatory responses in Wistar rats. *Global Journal Pure & Applied Sciences* 14(4):29-35
14. Emmanuel, O. A., & Mudiakeoghene, O. (2008).The use of antioxidants in vegetable oil-A
15. Enrol, D., Mechmet, U., Ferda, C., Dimitra, D., Gulhan, V.U. Mosschos, P., & Atalay, S. (2004). Antimicrobial and antioxidantive activities of essential oils and methanol extract of *Saliva cryptantha* and *Saliva multicaulis*(Vahl), *Journal of Food Chemistry*, 84: 519-525
16. extract of *Vernonia amydalina*. *Nigeria Journal of Physiological Science* 29:91 – 101.
17. Francis, M. (2015). The amazing health benefits of Bitter leaf (*Vernonia amygdalina*). The Nature's Farmacy.Retrieved from the Nature's Farmacy website
18. Godwin, O., Wieslaw, O., Merian, J., Stanislaw, B., Michael, F., & Adetunde, A. (1994). Flavonoid from *Vernoniaamygdalina* and their antioxidants activities.*Journal of Agriculture and Food Chem*istry *42*, 2445-2448.
19. Ihekoronye, A.I., & Ngoddy, P.O. (1985). Integrated Food Science and Technology for the Tropics, Macmillian Publisher Limited, London, 67-78.
20. Indrajit, D.,Thorat, D. D., Jagtap, D. M., Joshi, D.C., Sutar, R. F., & Kapdi, S. S. (2013).Antioxidants, their properities, uses in food products and their legal implications. *International Journal of Food Studies 2*:81 – 104.
21. Martini, M. Y.,Nursuhaili. A.B., Nurafigah, S. P., Azizah, M., & Mahomed, T. .M. .M. (2019). A review: Medicinal values agronomic practices and postharvest handlings of *Vernonia amygdalina*. *Food Research 3* (5), 380 – 390.
22. Oboh, G. (2006). Nutritive value, antioxidants and antimicobial properties of *Struchium sparganophora* leaves. *Journal of Nutrition and Health*. *18*(2),151-160.
23. Oboh, G., Ayodele J. A. and Adedayo O. A. (2012). Antioxidant properties and inhibitory effect of ethanoic extract of *Struchium sparganophora* ("Ewuro odo") leaf on α– amylase and α – glucosidase activities. *African Journal of Traditional Complementary and Alternative Medicine* 9(3):342 - 349.
24. Onwuka, G. I. (2005). Food analysis and instrumentation: Theory and practice. *Naphthali Prints, Lagos, Nigeria* 134-138
25. peel extracts on crude palm oil". *Ethnobotanical Leaflets* 14: 559-569.
26. review.*African Journal of Biotechnology 7*(25), 4836-4842.
27. Rosell, J.B. (1986). Classical analysis of oils and fats. In: Hamilton, R.J. and Rossell, J.B. (Eds.). Analysis of oils and fats. *Elsevier Applied Sciences, England* 1-32.
28. Rossell, J. B. (1994).Measurement of rancidity In: Rancidity in Food. Allen, J.C. and Hamilton, R.J. (Eds). 3rd ed. Blackie UK 22-53
29. Ruger, C.W., Klinker, E.J., & Hammond, E.G. (2002). Abilities of some antioxidants to stabilize soybean oil in industrial use conditions. *Journal of American Oil Chemist Soc*iety *79*(7), 733-736.
30. Said, E., Metwally, E. A., & Ahmed M. M. (2018). Role of natural antioxidant in treatment of toxicity. *Journal of Toxicological Analysis 1*(1), 3- 10.

31. Tian, L. L., & White, P.J. (1994). Antioxidant activity of Oat extracts in soybean and cottonseed *Journal of American Oil Chemist Soc*iety 71: 1079-1086.
32. Tomovic, V., Jokanovic, .M., Sojic, .B., Skaljac, S., & Ivic, M. (2017). Plants as natural antioxidants for meat products. IOP cont. Series: *Earth and Environmental Science* 85, 12- 30
33. Ullah, J., Hamayoun, M., Ahmed, T., Ayub, M., & Zarafullah, M. (2003).Effect of light, natural and synthetic antioxidants on edible oils and fats. *Asian Journal of Plant Sci*ence *2*(17-24), 1192-1194.
34. vegetable oils, *Journal of Science, Food & Agric*ulture 77:436-467.
35. Vivek K.G., & Surendra K.S. (2006). Plants as natural antioxidants. *Natural Product Radiance 5*(4), 326 – 334.