TOXICITY STUDIES AND EMULSION PREPARATION FROM OIL FROM SEED KERNELS OF FOUR CULTIVARS OF *MAGNIFERA INDICA*

Folashade. O. Oyedeji
University of Ibadan
Nigeria
omoeage2002@yahoo.com

Ehimen. A. Erazua
University of Ibadan
Nigeria
erazuaann@gmail.com

Babatunde. B. Adeleke
University of Ibadan
Nigeria
adelekebb46@gmail.com

ABSTRACT

Mango (*Mangifera indica* L.) is one of the most common fruits in Nigeria. The seed kernels usually remain as waste after consumption of the mango fruit. This work was carried out to determine the potential applications of the waste seed kernels. Seed oils of four different cultivars were extracted. Brine Shrimps lethality test was used to study the toxicity of the seed oils. Emulsion capacity of the oils, were determined. Emulsions were produced with the oils, and compared with another vegetable oil (Melon oil), and stabilities of the emulsions over a period of time were monitored. *Mangifera indica* L. seed kernel oil screened for toxicity had 50% lethal concentration (LC_{50}) values ranging from 5122.11ppm to 7663 ppm. Percent emulsion capacity obtained was from 54% to 62.8%, and the emulsion produced from oils were very stable. The study showed that the oils were not toxic, have good percentage emulsion capacity, and form stable emulsion, hence they are all safe for commercial and domestic utilization.

Keywords: *Mangifera indica*, Seed oil, Brine shrimps, Emulsion, Toxicity,

INTRODUCTION

An emulsion is a mixture of two or more liquids that are normally immiscible or partially immiscible. Emulsions are part of a more general class of two-phase system of matter called colloids. In an emulsion, one liquid (the dispersed phase) is dispersed in the other (the continuous phase) (Kumar et al., 2016). Emulsion can be found in electronics, biomedical, aerospace, cosmetic, food pharmaceutical industries as well as in oil production. An emulsion is termed an oil-in-water (o/w) emulsion if the dispersed phase is an organic material and the continuous phase is water or an aqueous solution and is termed water-in-oil (w/o) if the dispersed phase is water or an aqueous solution and the continuous phase is an organic liquid (an "oil") (Slomkowski et al., 2011). Multiple emulsions are complex systems which consist of both water-in-oil (W_1/O) and oil-in-water-emulsions (O/W_2) at the same time (Muschiolik et al., 2006). They combine the properties of both w/o and o/w emulsions (Naveed et al 2010). Oil-in-oil emulsions also exist. A situation where a non-polar oil is dispersed in a more polar oil or vice versa.

Macro-Emulsion is one in which the particles of the dispersed phase have diameters from approximately 1 to 100 μm. It comprises of large droplets and thus are "unstable" in the sense that the droplets sediment or float, depending on the densities of the dispersed phase and dispersion medium. Separation of the dispersed and continuous phases usually occurs within time periods from a few seconds to a few hours, depending upon the viscosity of the fluid medium and the size and density of the droplets. Macro-emulsions usually contain low-molecular-weight or polymeric surfactants that decrease the rates of coalescence of dispersed

droplets. Droplets of the dispersed phase may be also stabilized by adsorption of solid particles onto their surface (so-called Pickering stabilization) (Slomkowski et al., 2011).

Mini Emulsion is one in which the particles of the dispersed phase have diameters in the range from approximately 50 nm to 1 μm. Mini-emulsions are usually stabilized against diffusion degradation (Ostwald ripening) by a compound insoluble in the continuous phase. The dispersed phase contains mixed stabilizers, e.g., an ionic surfactant, such as sodium dodecyl sulfate (*n*-dodecyl sulfate sodium) and a short aliphatic chain alcohol ("co-surfactant") for colloidal stability, or a water-insoluble compound, such as a hydrocarbon("co-stabilizer" frequently and improperly called a "co-surfactant") limiting diffusion degradation (Slomkowski et al., 2011). Mini-emulsions are usually stable for at least several days.

When creating emulsions it is desirable to attain high stability, long shelf life and a pleasant consistency. This can be achieved by changing processing methods, ingredients formula or by the addition of emulsifiers. Emulsifiers are amphiphilic compounds that possess two distinct groups in the same molecule a hydrophobic group, which has an affinity for the oil phase, and a hydrophilic group, which has an affinity for water. Emulsifiers lower the interfacial tension and facilitate droplet disruption, resulting in smaller droplets. An emulsifier determines which phase is the continuous phase and which is the dispersed phase and the use of the wrong emulsifier could result in an inverted emulsion (Benichou et al., 2001, Kanouni et al., 2002). There are a variety of different emulsifiers such as phospholipids, monoglycerides, esters of fatty acids but proteins also can act as emulsifiers and they all have in common the fact that they interact with the interfacial layer.

Mango (*Magnifera indica L.*) belongs to the family Anacardiaceae, it is one of the most cultivated fruits in the world, rich in vitamins and phenolic compounds, with a delicious taste and exotic flavor (Pott *et al*., 2003). Over 1000 mango varieties are available worldwide. Of the available varieties, only a few are grown on commercial scales and traded (Solís-Fuentes *et al.*, 2011). Mango seed consist of about 35-55% of the whole fruit, and is discarded as waste. Food by-products represent a growing problem as the plant material is prone to microbial spoilage, which may cause odours and other environmental problems (Joshi and Attri, 2006). Oil can be extracted from mango seed.

Mango seed oil has not gained industrial attraction in African countries compared with mango fruit. The underutilization could be due to the limited knowledge of the toxicological status of the mango kernel seed oil and appropriate processing technology. Hence the need to test the toxicity, prepare a stable emulsion from the oil and determine the capacity and stability of the emulsion. As emulsion is a viable means of increasing the intake of the oil into the human body, this will increase the usefulness of the mango seed oil both industrially and domestically.

In this article we extracted oil from the seeds of various cultivar of *Mangnifera indica*, studied the toxicity of the seed oil using Brine Shrimps, determined the emulsion capacity of these oils, produced emulsion with the oils, and determined the stabilities of the emulsion over a period of time.

LITERATURE REVIEW

Many researchers have extracted and fractionated mango fats using various solvents and subsequently analysed the fat. Kittiphoom, (2013), Fahimdanesh and Bahram, (2013)

reported that mango seed kernel oil is more stable than many other vegetable oils rich in unsaturated fatty acids. Adejumo et al., (2014) extracted oil from Ogbomoso mango seeds and characterized it to determine its worthiness for both domestic and industrial purpose.

The mango seed kernel has been shown to be a good source of phenolic compounds including microelements like selenium, copper and zinc (Soong and Barlow, 2004).The potential use of phenolic compounds for the development of new skin care cosmetics has been emphasized (Kiken and Cohen, 2002). Phenolic compounds can be used as whitening, sunscreen and anti-wrinkle agents (González, 2008).

Pitchaon *et al.,* (2011) investigated the emulsion-stabilizing properties of extract of mango seed kernel and methyl gallate preparations in cosmetic emulsion using apparent viscosity and total phenolic content. They discovered that the extract of mango seed kernel exhibited the highest degree of free-radical scavenging and tyrosinase-inhibition activities compared with methyl gallate, and that the addition of phenolic compounds from the mango seed kernel and methyl gallate in emulsion affected the stability of the cosmetic emulsion systems. They showed that the higher the temperature storage, the lower the stability of the phenolic compounds found in the cosmetic emulsions. Their results suggested that the interaction between phenolic compounds and the emulsifier in cosmetic emulsion formulations affect the stability of the emulsion system. They concluded that mango seed kernel oil can be used to add chemicals and ingredients to cosmetics and pharmaceuticals.

Nzikou et al., (2010) carried out a study on mango seed kernels to clarify their proximate composition and the characteristics of the extracted oil including unsaponifiable matter and fatty acid composition. They concluded that Mango seed kernels contained a considerable unsaponifiable matter and a low amount of crude protein and ash content of 3.2% (with the presence of following minerals: Ca, K, Na, Mg and P). High unsaponifiable matters content (4.58%) encourages the use oils in cosmetics industry.

METHODOLOGY

All reagents used in this study were of analytical grade and solutions were prepared from distilled water.

Seed Kernel Preparation and Oil Extraction

Fruits of four mango (*M. indica*) cultivars, which are commonly known as Ogbomoso, Alphonso, Lippen, and Saigon were bought from the local market, at Ibadan, Oyo State, Nigeria. The ripe mango fruits were washed and peeled. The mango fruits were then de-pulped and the freshly de-pulped mango stones were washed in a current of water to free them from adhering pulp, fiber and dirt. The mango seed kernels were then dried and de-coated manually. The soft seed obtained had thin outer covers which were peeled off to obtain the seed. These seeds were air dried for weeks, pulverized and stored in an air tight container. Oil extraction was carried out according to the method described by Nzikou et al. (2010) using soxhlet extraction technique. The powdered seeds were packed into the extraction chamber and normal hexane poured into the round bottom flask of the soxhlet extractor. The oil in the seeds was leached for 12 –16 hours in each case until all the powdered seed was extracted. An exhaustive oil extraction was considered to be achieved when no more oil was obtained. After the extraction, the solution was concentrated by distillation method. The seed oil was stored in a refrigerator.

Brine Shrimp Lethality Test

2g of brine shrimps *Artemia salina* eggs with salt mixture was added to 62.5 mL of tap water in a plastic container and allowed to stay for 48 hours. After hatching, the larvae released from the egg shells were collected by using micropipette.

To prepare the stock solution, 0.2mL of the sample was dissolve in 0.2 mL of Dimethyl Sulphoxide (DMSO). The working solution was prepared from the stock solution using serial dilution method. 0.4 mL of the stock solution was made up to 2 mL with sea water; 0.5 mL of this was put in each of the test tube (three test tubes). This gives a concentration of the 1000ppm in triplicate. About 0.5 mL was left out of the 1000ppm prepared. From the remaining 0.5 mL of the 1000ppm, 0.2 mL was taken and put into another bottle containing 1.8 mL of sea water, this gave 100ppm. This was also divided into three portion of 0.5 mL each put into separate test tubes that was well labeled. From the remainder of the 100ppm, 0.2 mL was taken; 1.8 mL of sea water was added and this resulted to 10ppm. A blank sample was prepared and made to 5 mL with sea water and 10 brine shrimps larvae. This served as reference and control for the other concentration.

Using the blank as reference, the other preparation of 1000, 100 and 10ppm were made up to 5 mL mark with sea water and 10 brine shrimps larvae were introduce to each of the test tubes, this was left in the test tube rack for about 24 hours after which the result was taken. The concentration killing 50 percent of the larvae (LC_{50}) was determined using the Finney Probit Computer Program.

Determination of Emulsion Capacity and Preparation of Emulsion

Determination of Emulsion Capacity

5 mL of water and 1mL of sample oil, each heated independently to 75^{o}C, was mixed by stirring and the resulting mixture was homogenized with a blender at 1600 revolution per minute for 5 minutes. The volume of the emulsified layer was measured and recorded. The emulsion capacity was then calculated.

$$Emulsion\ Capacity = \frac{\text{Height of emulsion layer}}{\text{Height of the whole layer}}$$

%Emulsion capacity= emulsion capacity x 100

Determination of Minimum Emulsifier for the Preparation of Emulsion

Emulsions were produced using mango seed oil, and melon oil. Cashew mucilage was used as the emulsifier.

5 mL of water and 5ml of sample oil, each heated independently to 75^{o}C, was mixed by stirring. 1g of Cashew mucilage was rehydrated by adding 10 mL of water. The rehydrated mucilage was added, the volume added was increase per unit volume until complete emulsification of the mixture. The emulsion formed was further homogenized by blending with an electric blender at 1600rpm for 5 minute until a uniform emulsion was obtained.

Preparation of Emulsion and Determination of Shelf life

Emulsions were produced using mango seed oil, paraffin oil and melon oil. Cashew mucilage was used as the emulsifier. The emulsion was prepared with the formulary shown in table1. All oil soluble components were placed in a beaker and heated on the water bath to 75^{o}C, all water soluble component were placed in another beaker and heated to the same temperature. The water phase was added slowly to the oil phase with continuous stirring. The coarse emulsion formed was allowed to cool to 35^{o}C and perfume was added. The emulsion was

allowed to stay for 24 hours at room temperature and then homogenized with a blender at 1600 revolution per minute for 5 minutes. Some quantity of the emulsion was placed in a plastic container with screw cap and placed on a shelf in a room at about 27^{o}C and checked at regular intervals to determine when emulsion breakage and/or spoilage will take place.

Determination of Emulsion Destabilization Pattern of the Emulsions

The emulsion stability was determined by monitoring the volume of emulsion remaining over a period of two hours at regular intervals. A plot of volume of emulsion remaining per time was drawn.

Table 1: Emulsion Formulary

	Chemical description	Percent w/w
	Petroleum jelly	20.00
	Oil	10.55
A	Micro-crystalline wax	3.34
	Emulsifier	2.10
B	Glycerin	3.14
	Water	60.87
	Perfume	q.s

Quantity sufficient (q.s)

RESULTS

Table 2: Brine Shrimps Lethality Test of Alphonso Seed Kernel Oil

Concentration	1000ppm	100ppm	10ppm	Control
Survivor	24	27	29	30
Dead	3	3	1	30
% mortality	10	10	3.33	0
LC_{50} (ppm)	7663.5			

Table 3: Brine Shrimps Lethality Test of Saigon Seed Kernel Oil

concentration	1000ppm	100ppm	10ppm	Control
Survivor	29	28	30	30
Dead	1	2	0	0
% mortality	10	6.66	0	0
LC_{50} (ppm)	6028			

Table 4: Brine Shrimps Lethality Test of Ogbomosho Seed Kernel Oil

Concentration	1000ppm	100ppm	10ppm	Control
Survivor	27	27	29	30
Dead	3	3	1	0
% mortality	10	10	3.33	0
LC_{50} (ppm)	7661.67			

Table 5: Brine Shrimps Lethality Test of Lippen Seed Kernel Oil

concentration	1000ppm	100ppm	10ppm	Control
Survivor	26	27	29	30
Dead	4	3	1	0
% mortality	13.33	10	3.33	0
LC_{50} (ppm)	5122.11			

Table 6: Emulsion Capacity for the Various Vegetable Oil.

Vegetable oil	% Emulsion capacity
Melon oil	54
Ogbomoso seed kernel oil	61.2
Lippen seed kernel oil	59
Saigon seed kernel oil	62.8
Alphonso seed kernel oil	61.5

Table 7: Rate of Destabilization for the Vegetable Oils

Time (Min)	Melon	Saigon	Ogbomoso	Lippen	Alphonso
0	10	9.8	10	9.6	9.4
1	9.0	8.2	9.0	8.5	8.3
2	8.5	8.0	8.5	8.0	8.0
3	8.2	7.9	8.2	7.6	7.2
4	8.0	7.6	8.0	7.2	6.8
5	7.9	7.0	7.6	7.0	6.6
10	6.4	6.7	7.0	6.7	6.4
20	6.2	6.6	6.8	6.3	6.3
30	5.8	6.6	6.4	6.2	6.2
40	5.5	6.4	6.2	6.1	6.0
50	5.4	6.4	6.0	6.0	5.8
60	5.4	6.1	5.9	6.0	5.7
120	5.4	6.1	5.9	6.1	5.7

Oil volumes represent volume of emulsion remaining in mL

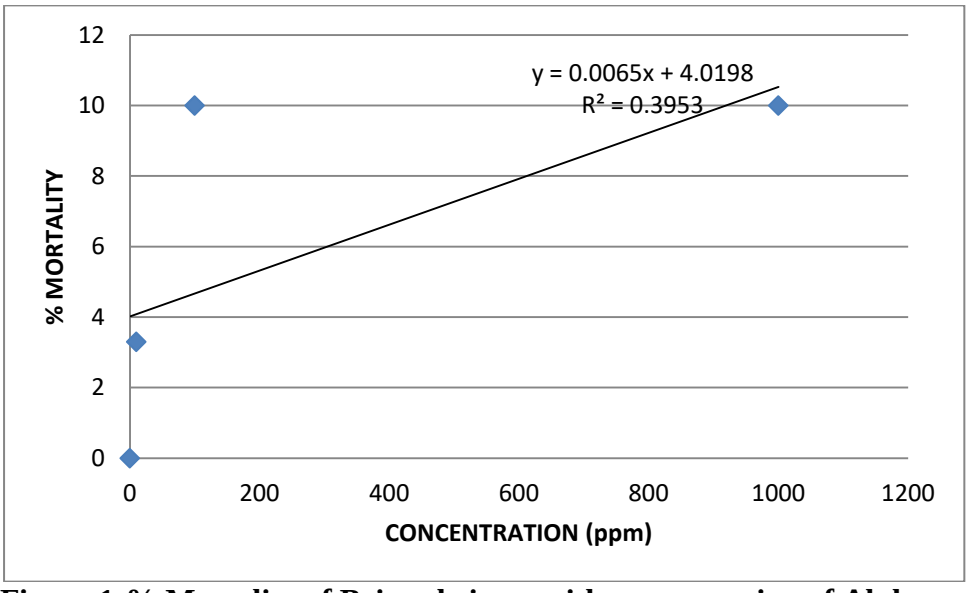

Figure 1:% Mortality of Brine shrimps with concentration of Alphonso seed kernel oil

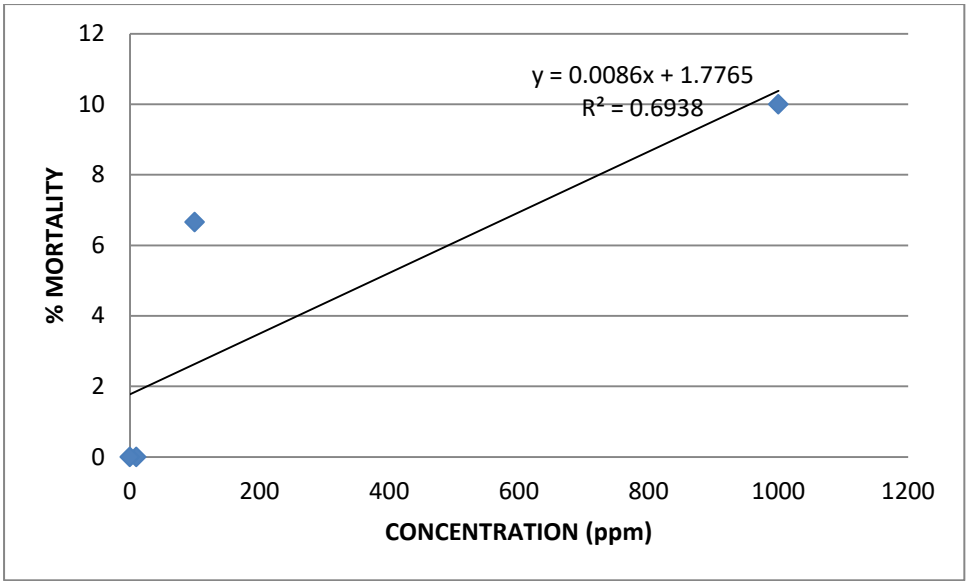

Figure 2:% Mortality of Brine shrimps with concentration of Saigon seed kernel oil

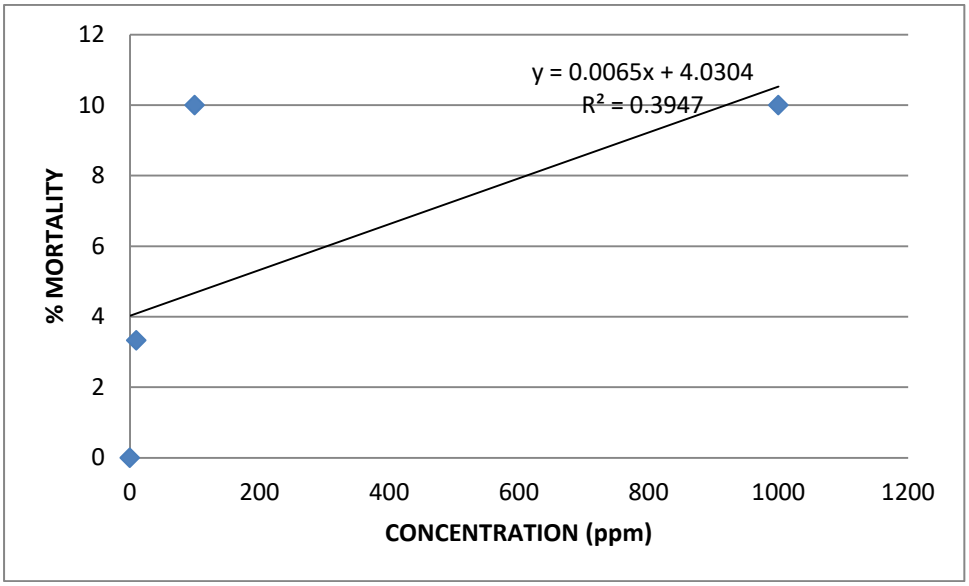

Figure 3: % Mortality of Brine shrimps with concentration of Ogbomoso seed kernel oil

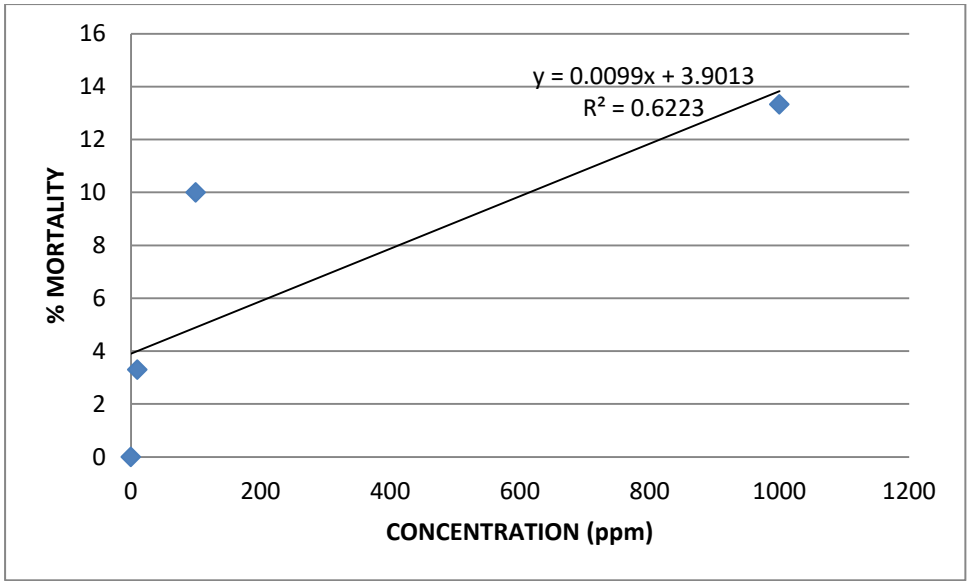

Figure 4: % Mortality of Brine shrimps with concentration of Lippen seed kernel oil

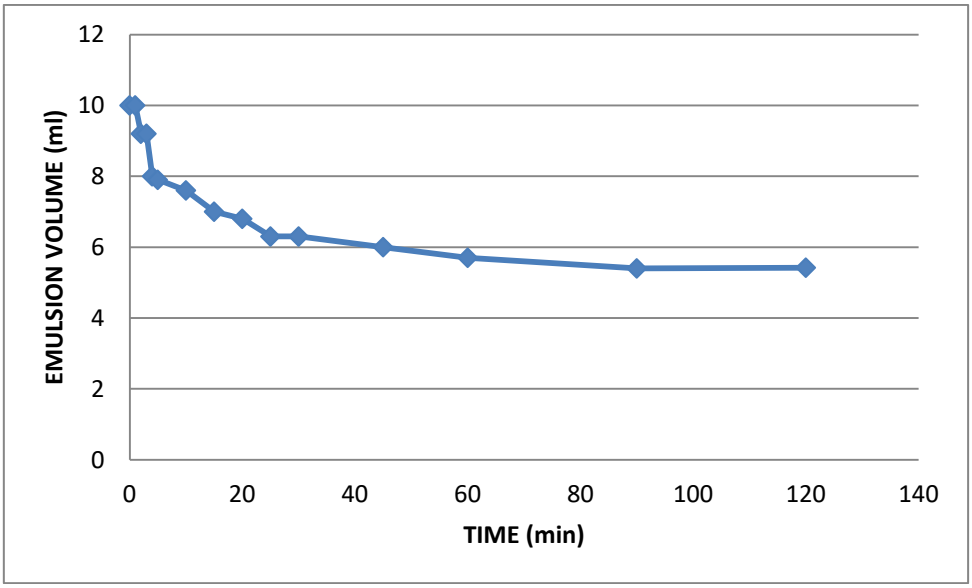

Figure 5: Destabilization of melon seed oil emulsion with time

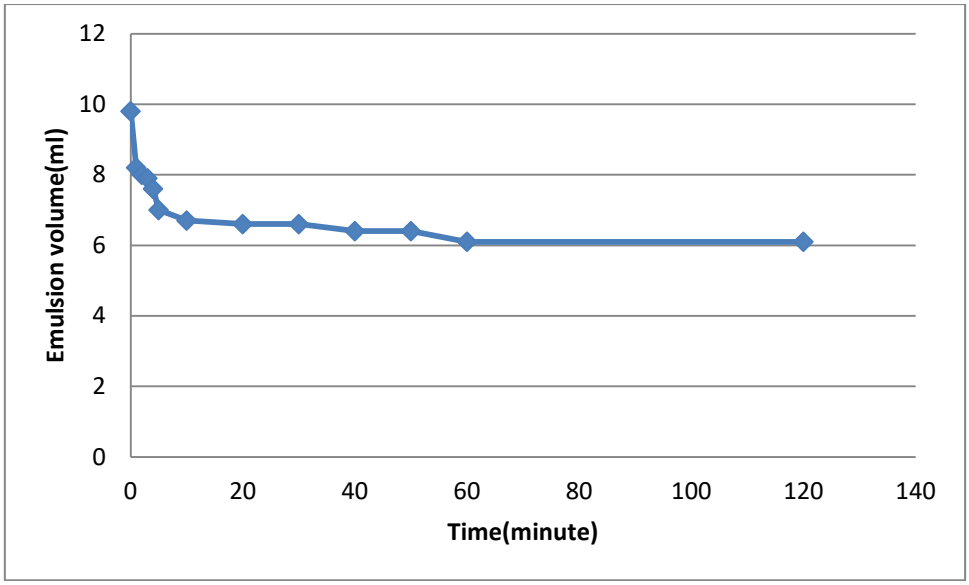

Figure 6: Destabilization of Saigon mango seed oil emulsion with time

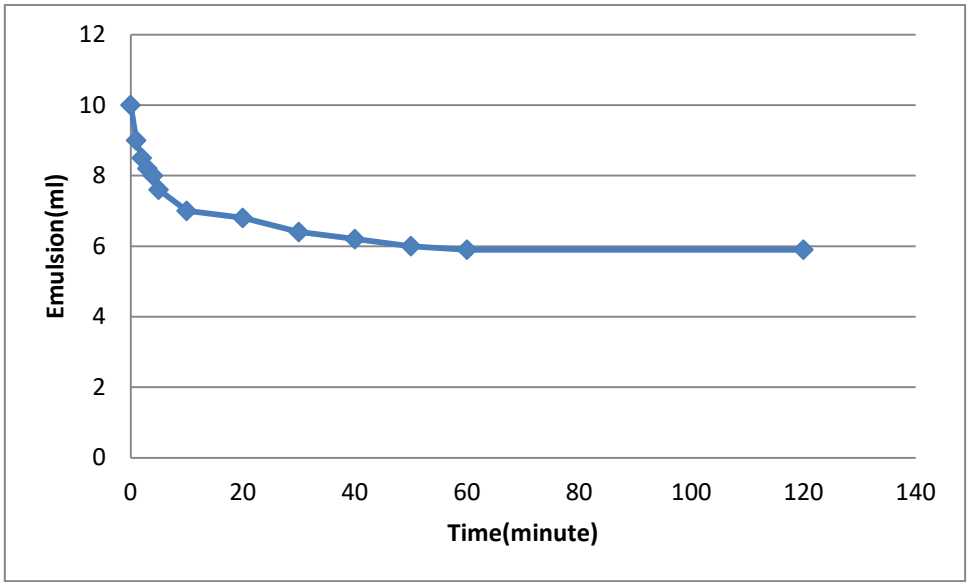

Figure 7: Destabilization of Ogbomosho mango seed oil emulsion with time

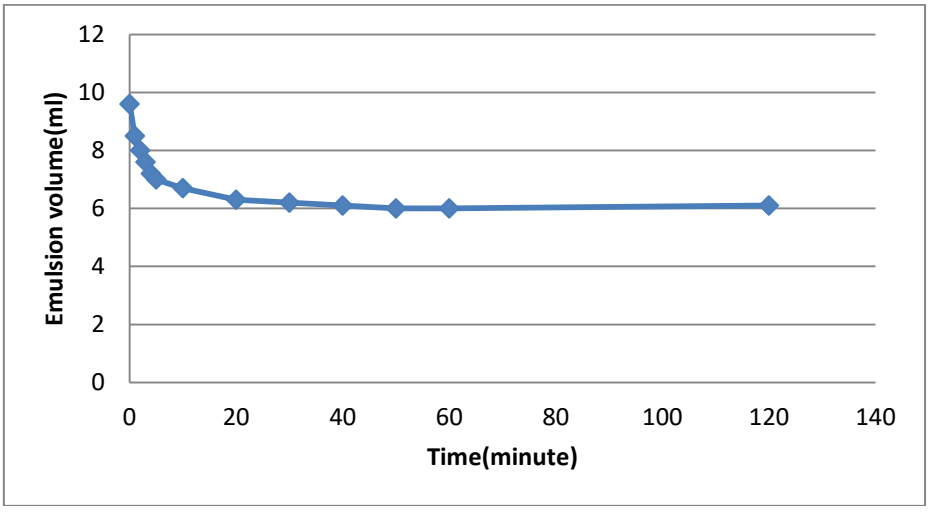

Figure 8: Destabilization of Lippen mango seed oil emulsion with time

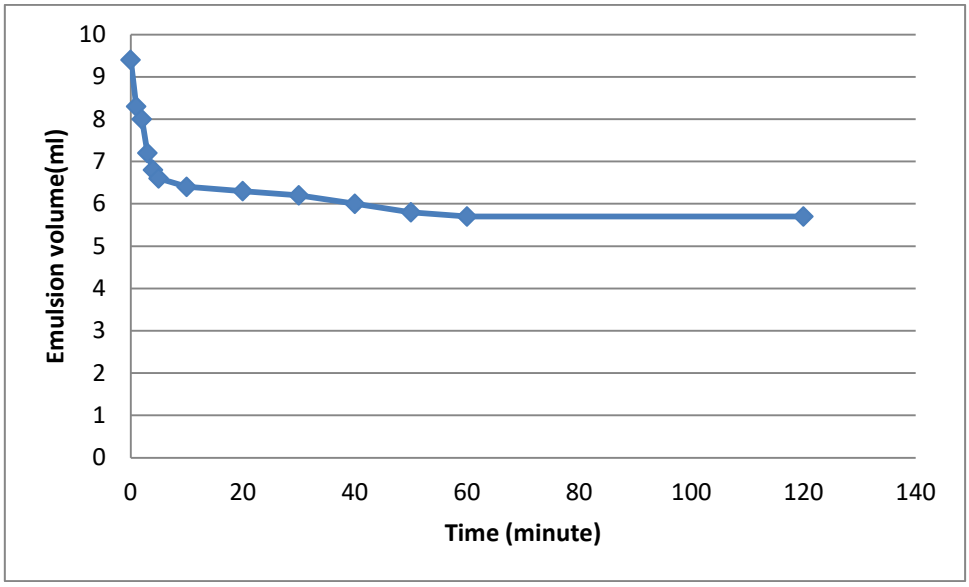

Figure 9: Destabilization of Alphonso mango seed oil emulsion with time

DISCUSSION

Brine Shrimps Lethality Tests

The toxicity of extracts expressed as LC_{50} values is commonly valorized either by comparison to Meyer's or to Clarkson's toxicity index. According to Meyer's toxicity index, extracts with LC_{50} < 1000 µg/mL are considered as toxic, while extracts with LC_{50} > 1000 µg/mL are considered as non-toxic (Meyer et al., 1982). Clarkson's toxicity criterion for assessment of plant extracts classifies extracts in the following order: extracts with LC_{50} above 1000 µg/mL are non-toxic, LC_{50} of 500 - 1000 µg/mL are low toxic, extracts with LC_{50} of 100 - 500 µg/mL are medium toxic, while extracts with LC_{50} of 0 - 100 µg/mL are highly toxic (Clarkson et al., 2004)

The mango seed oil screened for toxicity using brine shrimp had 50% lethal concentration (LC_{50}) values ranging from 7663.5 µg/mL for Alphonso, 6028 µg/mL for Saigon, 7661.67 µg/mL for Ogbomoso and 5122.11 µg/mL for Lippen cultivar (see table 2, 3, 4 and 5). These results confirm that none of the extracts was toxic hence they are all safe for commercial utilization.

Emulsion Capacity

Emulsion was obtained during initial mixing of oil with water by stirring, and the emulsion formed was not very stable. The volume of emulsion created during this stage can be an indication of the possibility of obtaining a stable emulsion. The emulsion capacity obtained for the various vegetable oils, after the emulsion was left for 60 minutes are recorded in Table 6. The emulsion capacity is 61.5% for Alphonso, 62.8% for Saigon, 61.2% for Ogbomosho, 59% for Lippen and 54% for Lippen cultivar. From the result Saigon mango seed oil had the highest emulsion capacity while melon oil had the lowest emulsion capacity

Preparation of Emulsion and Determination of shelf life

The emulsions prepared using cashew mucilage as emulsifier were all creams and off white in colour. They were stable for two years after which they started showing signs of microbial presence. After two years, the emulsions showed the presence of dark coloured patches which are most likely fungal hyphae. When the screw caps of the storage containers were opened, a

sound which showed that gas was escaping from the container was given. The gases could only be attributed to the microorganism's metabolism products or products obtained from the components of the emulsion reacting. The attack of the emulsions by microbial growth may have been as a result of the fact that the muciliage used as emulsifier may have been infected by these microorganisms from source; as the cashew gums were harvested from local trees and no preservatives have been added to the emulsion systems. However no phase separation was observed in any of the samples.

Emulsion Destabilization Pattern of Emulsion

There was a rapid decrease in emulsion volume from t_0-$t_{10,}$ this sharp reduction implies that just a few molecules of the surface active components of the mucilage were arranged at the water and oil interface initially causing a rapid resolution of water. From t_{10}-t_{60} the decrease in emulsion was very steady; the emulsion was trying to stabilize as more of the surfactant molecules in the mucilage were arranged at the water and oil interface. From t_{60}-t_{120}, the emulsion became stable implying that all the surface active molecules in the mucilage had been properly saturated into the emulsion system. This observation was same for all the vegetable oil used with the cashew mucilage emulsifier (see Figure 5, 6, 7, 8, 9 and Table 7).

Mangifera indica seed kernel oil is not toxic, have good percentage emulsion capacity, and form stable emulsion, hence they are all safe for commercial and domestic utilization. *Mangifera indica* seed kernel oil should be utilized for production industrial emulsions in the food, cosmetics and pharmaceutical industries, rather than just discarded as waste.

REFERENCES

Adejumo, O. A., Bolarin F. M and Farounbi. 2014. A Characterization of Ogbomosho Mango Seed Oil. *Proceedings of the International Soil Tillage Research Oganisation (ISTRO) Nigeria Symposium* 144 – 148.

Benichou, A., Aserin, A. and Garti, N. (2001). Polyols, high pressure, and refractive indices qualization for improved stability of W/O emulsions for food applications. Journal *of Dispersion Science and Technology* 22 (2&3): 269-280.

Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, O.M., Pillay, P., Matsabisa, M. G., Bhagwandin, N., Smith, P.J., Folb, P.I., 2004. *In vitro* antiplasmodial activity of medicinal plants native to or naturalized in South Africa. *J Ethnopharm*. 92, 177-191.

González, S., Fernández-Lorente, M. and Gilaberte-Calzada, Y. (2008). The latest on skin photoprotection. *Clinics in Dermatology* 26.6:614–626

Joshi, V. K., and Attri, D. 2006. Solid State Fermentation of Apple Pomace for the Production of Value Added Products. *Natural Product Radiance* 5.4: 289–296.

Kanouni, M., Rosano, H. and Naouli, N.(2002). Preparation of stable double emulsion (W1/O/W2): role of the interfacial films on the stability of the system. *Advances in Colloid and interface Science* 99: 229-254.

Kiken, D. A. and Cohen, D. E. 2002. Contact Dermatitis to Botanical Extracts. *America Journal of Contact Dermatitis* 13:148–152.

Kittiphoom, S., and Sutasinee, S. 2013. Mango seed kernel oil and its physicochemical properties. *International Food Research Journal*, 20.3:1145–1149.

Kumar, H. V., Woltornist, S. J. and Adamson, D. H. (2016). Fractionation and characterization of graphene oxide by oxidation extent through emulsion stabilization. *Carbon*. 98: 491–495. doi:10.1016/j.carbon.2015.10.083

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. and McLaughlin, J. L. A. 1982. Convenient general bioassay for active plant constituents. *Planta Medica 45*:31-34.

Muschiolik., Scherze, I., Preissler, P. and Anita Fechner 2006 Multiple Emulsions Preparation and Stability *Research Gate* DOI: 10.1051/IUFoST:20060043 123-137

Naveed, A., Mahmood, A., Haji, M. S. K., Jawad, A., Gulfishan A.M. and Muhammad, U. 2010 Formulation and Characterization of a Multiple Emulsion Containing 1% L-Ascorbic Acid *Chemical Society of Ethiopia* ISSN 1011-3924

Nzikou, J. M., Kimbonguila, A., Matos, L., Loumouamou, B., Pambou-Tobi, N., Ndangui, C. and Desobry, S. 2010. Extraction and Characteristics of Seed Kernel Oil from Mango (Mangifera indica). *Research Journal of Environmental and Earth Sciences* 2.1:31–35.

Pitchaon, M. and Thepkunya, H. 2011. Characterization and stabilization of the extract from mango seed kernel in a cosmetic emulsion. *Kasetsart Journal of Natural Science* 45:521 – 529

Pott, I., Marx, M., Neidhart, S., Muhlbauer, W. and Carle. R. 2003. Quantitative determination of b-carotene stereoisomers in fresh, dried, and solar-dried mangoes (Mangifera indica L.). *Journal of Agricultural and Food Chemistry* 51:4527–4531

Slomkowski, S., Alemán, J.V., Gilbert, R. G., Hess, M., Horie, K., Jones, R.G., Kubisa, P., Meisel, I., Mormann, W., Penczek, S., Stepto, R.F. T. (2011). "Terminology of polymers and polymerization processes in dispersed systems (IUPAC Recommendations 2011)". *Pure and Applied Chemistry*. **83** (12):2229–2259. doi:10.1351/PAC-REC-10-06-03.

Solís-fuentes, J. A. and Durán-de-bazúa, M. C. 2011. Mango (*Mangifera indica* L.) Nuts and Seeds in Health and Disease Prevention. *Seed and its Fats*. Preedy I. V.and Watson R. R. Eds. Sandiego: Academic press. 741–748.

Soong, Y. Y. and Barlow, P. J. 2006.Quantification of gallic acid and ellagic acid from longan (*Dimocarpus longanLour*.) seed and mango (*Mangifera indica* L.) kernel and their effects on antioxidant activity. *Journal of Food Chemistry* 97.3:524-530.