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THE PHYSICOCHEMICAL AND ANTIOXIDANT POTENTIALS OF *THEOBROMA CACAO* L. SEED OIL (MALVACEAE) OBTAINED FROM SOUTHERN NIGERIA

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ABSTRACT

Theobroma Cacao L. (Malvaceae) is one of the major cash crops of Nigeria that has use in cosmetics, Pharmaceuticals and food industries. While the seed powder is used as herbal medicine for diabetes, cancer and heart disease, the seed oil provides good moisturizing effect (cocoa butter) for cosmetics, suppository base (Theobroma oil) in pharmaceuticals and chocolate in confectionary industries. The aim of this study is therefore to assess the physicochemical and *in vitro* antioxidant properties of the seed oil of *Theobroma cacao* L grown in Southern Nigeria. The yield obtained from the leaching process in a Soxhlet apparatus using n-hexane as solvent of extraction after 6 h of heating was 45.0%. The chemical analysis were mostly titrimetric and the results obtained were acid value (4.10 mg KOH/g oil), hydroxyl value (72.39 mg KOH/g oil), saponification value (187.65 mg KOH/g), iodine value (45.03 g iodine/100 g oil) and peroxide value (1.09 meq. O_2 / Kg of oil). The IC_{50} of 29.12 and 27.11 µg/mL were obtained for theobroma oil and standard antioxidant ascorbic acid respectively by *in vitro* antioxidant studies using 1, 1 diphenyl-2- picyrlhydrazyl (DPPH) radical scavenging assay for comparison. These results reveal that *T. cacao* has excellent natural antioxidant activity and a source of high quality oil.

KEYWORDS: *Theobroma cacao*, physicochemical, antioxidant, extraction, DPPH, titrimetric.

INTRODUCTION

Most countries of the world desire to have local content in all sectors of development by achieving sustainable development through agriculture and its products.[1] This will give incentives to local industries to harness raw materials from their immediate environment and reduce the over-dependence on foreign products and importations and hence revive the ailing economies and create employment.[2]

The country Nigeria is known to grow cocoa in large quantities especially in the South-Western part of the country. Hitherto, this has served the food and confectionary industry as a source of raw material for production of beverages but the need to expand to other sectors such as the pharmaceuticals and cosmetics has caused researchers to investigate how to further harness the potentials of T. cacao seed in the development of raw materials.

The tropical tree *Theobroma cacao* belongs to the family Malvaceae and grows wild in the forests of Central America though it can be cultivated in West Africa and Asia. The botanical name of the cacao tree is *Theobroma cacao* which means 'food of the gods' in Greek language due to its value to the native Americans. There are about 20 species of the genus *Theobroma* but *Theobroma cacao* is the most important because of its commercial value. The cocoa seed remains the source of cocoa powder and cocoa butter from which food (chocolate), cosmetics and ethno-medicine[3] is obtained but recently researchers using the non-edible parts (leaf, bark, root and husks) of the plant unveils more medicinal values and use.[4-6] Research shows that health related benefits of cocoa such as protection from heart disease, cancer and diabetes mellitus may be due to the presence of compounds such as polyphenols, flavonoids and procyanidins.[7-9] of which theobromine is also an antioxidant compounds. The seed oil from *T. cacao* is of great economic importance globally. Seeds, usually contain an abundant reserve of fats and oils more than any other parts of the plant. Fats and oils are triesters formed from glycerol and long chain fatty acids and are thus called triacylglycerol.[10] Some of the fatty acids occurring in nature are stearic, palmitic, linolenic and oleic acids. The difference between fats and oils is that while fats are solids at 25°C and have a larger percentage of saturated fatty acids, oils are liquids at same temperature and have mostly the unsaturated fatty acids. Fats and oils from a chemical point of view contains two reactive sites, the double bond in the unsaturated fatty acid chain and the acid group of the fatty acid chain. They can become rancid on long storage and on exposure to air. These changes which result to an unpleasant odour arise due to chemical reactions such as

hydrolysis of the glycerides, oxidation of saturated fatty acids to ketones and oxidation at the double bonds of the unsaturated fatty acids[11] Physicochemical analysis of fats and oils are essential to determine their identity and to assess their quality since these properties are affected by climate, method of extraction, storage, handling and adulteration.[12] Antioxidant supplements (natural or synthetic) are important in cosmetics and medicine to scavenge or neutralize free reactive oxygen species produced as a result of exposure of skin to ultraviolet B (UVB) light.[13] These free radicals causes accelerated skin damage and photoaging by inducing oxidative stress.[14] This study therefore, aims to characterize the physicochemical and the *in-vitro* antioxidant properties of *Theobroma cacao* seed oil originating from southern Nigeria.

MATERIALS AND METHODS

Sampling Procedure

Fresh and healthy matured pods of *Theobroma cacao* were purchased from Elele market in Rivers State, Nigeria. A sample of the pod was identified and authenticated in the Department of Pharmacognosy of Madonna University, Elele. Rivers State where a voucher specimen was deposited. The seeds were harvested manually from the pods, sorted, washed and oven dried at 50˚C for 12 h. The roasted seeds were then dehusked and pulverized to a fine powder and stored.

Oil extraction and yield determination

The oil was extracted by leaching in Soxhlet apparatus using 200 mL of n-hexane (b. p. 40 - 60 ˚C) as a solvent. The milled dried mature seed sample (25 g) was placed in a thimble and loaded into a Soxhlet extractor. Six runs of the condensing solvent (lasting 6 h) was sufficient to extract all the oil from the sample. The process was stopped when the condensing solvent passing through the sample turned colourless. The solvent/oil mixture was separated at 40 ˚C using a rotary vacuum evaporator Type 349/2 (Buchi, Germany) and finally air oven at 40-50 ˚C. The resulting theobroma oil weight was expressed as a percentage of powdered sample weight. The extracted seed oil was capped in an airtight dark brown vial and placed in a freezer at -2 ˚C until used for further analysis.[15]

$$Percentage\ yield\ of\ oil\ = \frac{weight\ of\ oil\ obtained}{\text{Total weight of material used for extraction}} \times 100$$

Analysis of the physicochemical properties of Theobroma oil

The chemical analysis of the theobroma oil was carried out using the methods of the Association of official Analytical Chemists, AOAC[16] and Olaniyi et al.[17] All determinations were done in triplicates and the values of the parameters expressed as mean ± standard deviation.

Specific gravity determination

The specific gravity bottle of 50 mL was washed, oven dried at 60 ˚C and then finally cleaned with acetone and air dried. The weight of the empty bottle and its fitted stopper (W) was taken, after which the bottle was filled to the brim with distilled water and the stopper also inserted. The weight ($W_{1)}$ was recorded for specific gravity bottle and its water content. The distilled water was then removed and the bottle was again dried at 60 ˚C to remove moisture. The theobroma oil was finally loaded into the bottle and the stopper fitted in, after which the weight (W_o) was taken and finally the specific gravity of the oil was computed using the mathematical formula below.

$$Specific\ gravity\ of\ test\ sample = \frac{Wo - W}{W1 - W}$$

Refractive Index determination

The refractive index was determined using Abbey refractometer model A 80251 (BS). The glass prism of the refractometer was thoroughly cleaned and drops of the oil sample was placed on the lower prism, spread thinly. The equipment was adjusted using both coarse and fine adjustment knob until the fine and thin demarcation between light and black shadow appears on the cross wire indicator. The reading of the telescope were recorded to give the value of the refractive index of the oil at room temperature.

Viscosity

A 20 mL volume of Theobroma oil was placed in a 50 mL beaker and the viscosity was determined using Brookfield viscometer (DV2T, Brookfield Engineering Laboratories, Massachusetts, USA) by inserting the spindle down to a depth of 1 cm in the oil sample. Analysis was done using spindle #62 at 12 rpm for 5 min at 27.5 ^{o} C. Determinations were carried out in triplicates.

Acid Value

Diethyl ether (20 ml), ethanol (20 ml) and 1ml of 1% phenolphthalein solution were mixed in an Erlenmeyer flask and neutralized with 0.1M KOH solution until a pink colour persisted for

30 seconds. The oil sample (2 g) was dissolved in the neutralized diethyl ether/ethanol mixture. This mixture was warmed in a water bath at 40°C and titrated with 0.1M ethanolic KOH using 1 ml of 1% phenolphthalein solution as indicator.

$$Acid\ value = \frac{V \times M \times 56.1}{W}$$

Where; M = molarity of KOH solution (0.1M), V = volume of KOH solution (titre value), and W = weight of oil sample (g).

Saponification value

A 2 g quantity of *T. cacao* oil and 20 ml of 0.5 M KOH solution were placed in a distillation flask fitted with a reflux condenser and boiled for 1h with occasional shaking. The amount of KOH remaining after saponification (refluxing) was determined while still hot by titrating with 0.5 M HCl using 2 drops of phenolphthalein as an indicator. A blank experiment was also carried out by repeating this procedure without the oil sample. The saponification value was derived from this equation.

$$Saponification\ value = \frac{(X - v) \times M \times 56.1}{W}$$

Where; X = volume of HCl used in the blank titration, V = volume of HCl used in the test titration, M = molarity of HCL (0.5M) and W = weight of the oil sample (2g).

Peroxide value

A known weight (2.5 g) of theobroma oil was dissolved in a solvent mixture (glacial acetic acid: chloroform, 3:2). Then 0.5 mL of saturated potassium iodide solution was added. After 1 min, 30 mL of distilled water was added and this was titrated with vigorous shaking with 0.01M sodium thiosulphate ($Na_2S_2O_3$) solution using freshly prepared starch mucilage as indicator. A blank titration was carried out and the peroxide value was calculated using the formula below.

$$Peroxide\ value = \frac{F \times (A - B) \times 10}{\text{weight of oil (g)}}$$

Where F is the factor of 0.01M $Na_2S_2O_3$, A is the titre value of the oil sample and B is the titre value of the blank titration.

Hydroxyl value determination

This is defined as the number of milligram of potassium hydroxide (KOH) equivalent to the hydroxyl content of 1.0g of the substance (oil).[11] The hydroxyl value is determined by

acetylating the substance and titrating the cooled solution with 0.5 M ethanolic KOH using Phenolphthalein as indicator. The hydroxyl value is obtained from the formula.

$$Hydroxyl\ value = \frac{a+28.05 \times V}{\text{Weight of oil}}$$

Where a in the equation is the acid value of the substance and V is the difference in mL between the titration.

Iodine value (Wij's method)

Wij's reagent is a solution of iodine monochloride in glacial acetic acid and is produced by a mixture of iodine trichloride solution and iodine solution. The Wij's reagent was prepared by dissolving 8 g ICl_3 in 200 mL glacial acetic acid in a conical flask, and dissolving 9 g of I_2 in 300 mL Carbon tetrachloride (CCl_4) in another flask. These two solutions were mixed together and diluted to 1 L with glacial acetic acid and kept in a bottle as Wij's reagent. A quantity of *T. cacao* oil (0.5 g) was dissolved in 2 mL of CCl_4, then 20 mL of Wij's reagent was added, mixed and allowed to stand for 30 minutes. Then 15 mL of 10 % KI and 100 mL of water was added. This was mixed thoroughly and titrated with 0.1 M sodium thiosulphate ($Na_2S_2O_3$) using few drops of 1% starch mucilage as an indicator towards the end point. The detection of the end point is the disappearance of blue colouration. Blank titration was also carried out with the omission of the oil. The titre volumes for the determination and blank were noted as Vs and Vb respectively.

$$Iodine\ value = \frac{(Vs - Vb) \times 1.269}{\text{Mass of Oil}}$$

Antioxidant activity test (DPPH radical scavenging capacity test)

This assay compares by *in vitro* method, the DPPH (1, 1- diphenyl-2- picryl hydrazyl) radical scavenging ability of the *Theobroma cacao* oil with that of standard antioxidant ascorbic acid[18] A stock solutions of 100 µg/mL of theobroma oil and standard ascorbic acid (Kermel) were prepared differently in methanol from which further dilutions gave various concentrations (10 -50 µg/mL). The 2.0 mL of the different concentrations of the theobroma oil and standard ascorbic acid respectively were mixed with 2.0 mL of 0.5 mM DPPH solution, mixed vigorously and incubated for 30 min in the dark at room temperature after which the absorbance of the mixture were measured at 517 nm using a UV Visible spectrophotometer (Techmel & Techmel, USA) while using methanol as a blank. A solution of DPPH in methanol was used as control for all the experiment. The free radical scavenging activities of the test samples, expressed as percentage of inhibition were calculated using the

equation below. The IC_{50} values were calculated from linear regression by plotting the graph between concentration and % inhibition (Figure 1).

$$\%\ Inhibition\ of\ DPPH\ activity = [\frac{(Ac-As)}{Ac}] \times 100$$

Where Ac is the absorbance value of the control samples, As is the absorbance values of the test or standard.

RESULT AND DISCUSSION

The physicochemical properties of the seed oil of *Theobroma cacao* were assessed chemically by titrimetric analysis using appropriate standard titrant /reagents while the antioxidant properties was investigated by DPPH radical scavenging method. The results obtained from the chemical and physical analysis of theobroma oil are presented in Table 1. The yield of the oil obtained from the dried seeds of *T. cacao* was 45.0 % of the total seed weight. The extraction of oils usually takes place in an industrial scale by hot or cold press from the milled seeds while on a laboratory scale, while soxhlet extractor was used.[19,20] Traditionally extraction is done by boiling the powdered seed in distilled water for about 4 h. Stability of the oil and free fatty acid content are dependent on factors such as the method of extraction, geo-origin of plant and the specie, temperature changes, storage and handling / refining processes.[21,22] These quality parameters serves as a criteria for quality control, adulteration assessment and grading of raw material such as oils for food, cosmetics and pharmaceutical industry.[12] The colour, taste and odour of the oil is an indication of the authenticity and non- rancidity of the oil. Typically, theobroma oil has chocolate taste and odour. Specific gravity and refractive index are also important physical properties of oils for quality control and detection of the level of adulteration.[20] The specific gravity obtained for the theobroma oil is 0.92 ± 0.14 which is in agreement with the literature value.at 25 ˚C.[22,23]

Table 1: Chemical and Physical Properties of Theobroma oil.

Properties	Value
Colour	Pale yellow
Odour and taste	Chocolate odour and taste
Oil yield (%)	45.0
Specific gravity (g/ml)	0.92 ± 0.14
Refractive index	1.4465 ± 1.24
Viscosity (centipoise)	121.5 ± 1.06
Acid value (mg KOH/ g of oil)	4.10 ± 0.43
Saponification value (mg KOH/ g of oil)	187.65 ± 0.71
Peroxide value (meq. O_2 / Kg of oil)	1.09 ± 0.35
Hydroxyl value (mg KOH/ g of oil)	156 ± 0.78

Iodine value (g of iodine /100 g of oil)	45.03 ± 2.45
Values are means of ± standard deviation for n = 3	

Previous study by Anietie et al[24] showed similar results for the refractive index and specific gravity of theobroma oil at 1.455 and 0.914 respectively.

Viscosity describes the rheological properties of fluids and it is a measure of internal resistance to flow of fluids.[25] The viscosity of theobroma oil from our study shows a high value of 121.5 cp. This high value for viscosity is of pharmaceutical interest because an oil with high viscosity is used as a suppository base and for ointments for drug delivery. Oils usually contain a small quantity of free fatty acid which on exposure to air is responsible for its rancidity and oxidation with resultant unpleasant odour. The acid value has been defined as the number of milligram of potassium hydroxide required to neutralize the free fatty acids in I g of the oil. The acid value determination defines quality and edibility of oils, thereby it helps in rejecting both low grade and rancid oils.[25] The acid value recorded in the current study is 4.10 mg KOH/ g and it is within the value (1-6 mg KOH/ g) accepted for most fresh fats and oils.[17] The peroxide value is the number which expresses in milliequivalent of active oxygen, the amount of peroxide contained in 1 kg of the oil. High concentration of peroxide in oils is an indicator for poor quality. The value of peroxide from this study is 1.09 meq. O_2 / kg of oil and this shows good grade of oil and less liable to oxidative rancidity in an adequate storage system. Saponification value is the number of mg of KOH required to neutralize the fatty acids obtained by complete hydrolysis of 1 g of the oil. It is a measure of the total fatty acids (free and combined) present in the sample. The saponification value from the study is 187.65 mg KOH/g oil, which is also similar to the results obtained by Anietie et al.[24] Though saponification value does not identify the particular oil, but it may be indicative of purity. Hydroxyl value can be defined as the number of mg of KOH required to neutralize the acid combined by acylation in 1g of the oil. The hydroxyl value obtained for theobroma oil is 156 mg KOH/ g of oil. The iodine value is the number of grams of iodine absorbed per 100 g of oil or fat. It is the measure of unsaturation in the oil. The study reveals an iodine value of 45.03 g of iodine /100 g of oil which indicates a high level of saturation and physical appearance as fat or semi-sold state and its important application as suppository base. The iodine value of 35.27 g of iodine /100 g of oil was obtained from a previous study.[24]

The DPPH radical scavenging of theobroma oil was evaluated using ascorbic acid as the reference antioxidant compound. Theobroma oil and ascorbic showed a concentration dependent radical scavenging (Figure 1) with IC_{50} of 29.12 and 27.11 µg/ ml respectively.

The free radical scavenging activity demonstrated by theobroma oil in the study with IC_{50} value comparable to that of the standard ascorbic acid may support their use in cosmetics as antioxidant to neutralize the reactive oxygen species elicited by sun rays. Theobroma cacao has been known to be a rich source of polyphenols, flavonoids, procyanidins which are all natural antioxidant compounds.[3]

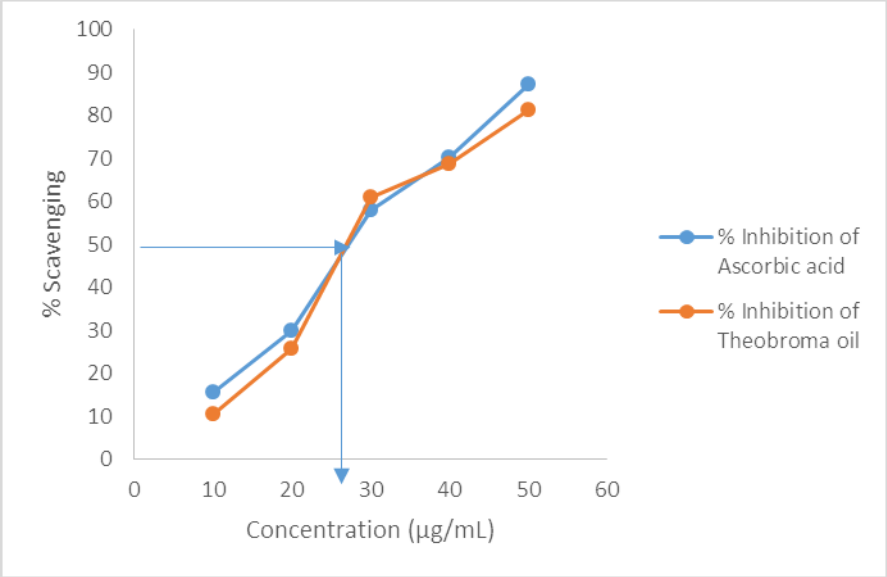

Figure 1: Comparative analysis (% inhibition) of *Theobroma cac*ao oil with standard (ascorbic acid) antioxidant for DPPH free radical scavenging assay.

CONCLUSION

This study shows that the *Theobroma cacao* seed has high percentage yield which makes them a veritable source of raw material for the industry. The oil also has good physicochemical properties which may be profitable for food, cosmetics and pharmaceuticals.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. Local Content Requirements and the Green Economy. New York and Geneva, 2014.
2. Chete LN, Adeoti JO, Adeyinka FM, Ogundele O. Industrial development and growth in Nigeria: Lessons and challenges. WIDER working paper, 2014.
3. Andújar I, Recio MC, Giner RM, Ríos JL. Cocoa Polyphenols and Their Potential Benefits for Human Health. Oxid Med Cell Longev, 2012; 2012: 23 pages.

4. Zainal B, Abdah MA, Taufiq-Yap YH, Roslida AH, Rosmin K, 3. Anticancer Agents from Non-Edible Parts of Theobroma cacao. Nat Prod Chem Res, 2014; 2(4).
5. Ohno M, Sakamoto KQ, Ishizuka M, Fujita S. Crude cacao Theobroma cacao extract reduces mutagenicity induced by benzo[a]pyrene through inhibition of CYP1A activity in vitro. Phyther Res, 2009; 23(8): 1134–9.
6. Santos RX, Oliveira DA, Sodré GA, Gosmann G, Brendel M, Pungartnik C. Antimicrobial activity of fermented Theobroma cacao pod husk extract. Genet Mol Res, 2014; 13(3): 7725–35.
7. Baharum Z, Akim AM, Hin TY, Hamid RA, Kasran R. Theobroma cacao: Review of the Extraction, Isolation and Bioassay of its potential anti-cancer Compounds. Trop Life Sci Res, 2016; 27(1): 21–42.
8. Rimbach G, Melchin M, Moehring J, Wagner AE. Polyphenols from cocoa and vascular health—a critical review. Int J Mol Sci, 2009; 10(10): 4290–309.
9. Corti R, Flammer AJ, Hollenberg NK, Lüscher TF. Cocoa and cardiovascular health. Circulation, 2009; 119(10): 1433–41.
10. Hornback JM. Organic Chemistry. Pacific Grove, CA.USA: Brooks/Cole Publishing Company, 1998; 1244 p.
11. Olaniyi AA, Ayim JK, Ogundaini AO, Olugdade TA. Essential Inorganic and Organic Pharmaceutical Chemistry. 1st editio. Ibadan, Nigeria: Shaneson C.I. Limited, 1998; 533- 537 p.
12. Vermaak I, Kamatou GPP, Komane-mofokeng B, Viljoen AM, Beckett K. African seed oils of commercial importance — Cosmetic applications. South African J Bot [Internet], 2011; 77(4): 920–33. Available from: http://dx.doi.org/10.1016/j.sajb.2011.07.003.
13. Pham-Huy LA, He H, Pham-Huy C. Free radicals, Antioxidants in Disease and Health. Int J Biomed Sci, 2008; 4(2): 89–96.
14. Halliwell B, Gutteridge JMC. Free radicals in Biology and Medicine. 4th ed. Oxford, UK: Clarendon Press, 2007.
15. Reazai M, Mohammadpourfard I, Nazmara S, Jahanbakhsh M, Shiri L. Physicochemical Characteristics of Citrus Seed Oils from Kerman, Iran. J Lipids, 2014; Article ID(174954): 3 pages.
16. Official methods of Analysis of the Association of Official Analytical Chemists, AOAC. Washington, DC, USA, 1999; 16th edition.
17. Olaniyi AA, Ogungbamila FO. Experimental Pharmaceutical Chemistry. 1st editti. Ibadan, Nigeria: Shaneson C.I. Limited, 1998; 57-61.

18. Pandey K, Sharma P, Dudhe R. Antioxidant and Anti-inflammatory Activity of Ethanolic Extract of Parthenium Hysterophorus Linn. Asian J Pharm Clin Res, 2012; 5(4): 28–31.
19. Kluczkovski AM, Martins M, Mundim MS, Simoes HR, Nascimento KS, Marinho AH. Properties of Brazil nuts: A review. African J Biotechnol, 2015; 14(8): 642–8.
20. Mweta DE, Magombo PU. Quality assessment of oil from Parkia filicoidea (Mkundi) seeds. African J Food Sci, 2017; 11(5): 130–3.
21. Hilali M, Charrouf Z, Soulhi AE, Hachimi L, Guillaume D. Influence of Origin and Extraction Method on Argan Oil Physico-Chemical Characteristics and Composition. J Agric Food Chem, 2005; 53: 2081–7.
22. Aminul Islam AKM, Yaakob Z, Anuar N, Primandari SRP, Osman M. Physiochemical Properties of Jatropha curcas Seed Oil from Different Origins and Candidate Plus Plants (CPPs). J Am Oil Chem Soc, 2012; 89: 293–300.
23. Oluba OM, Ogunlowo YR, Ojieh GC, Adebisi KF, Eidangbe GO, Isiosio LO. Physicochemical Properties and Fatty Acid Composition of Citrullus lanatus (Egusi Melon) Seed Oil. J Biol Sci, 2008; 8(4): 814–7.
24. Anietie O V, Ajayi o o. Comparative Study of the Physicochemical Properties of Oils of Sheanut (Vitellaria paradoxa) and Cocoa (Theobroma cacao). Br J Appl Sci Technol, 2015; 7(5): 496–501.
25. Barkatullah IM, Rauf A, Inyat-Ur-Rahman K. Physicochemical Characterization of Essential and Fixed Oils of Skimmia laureola and Zanthoxylum armatum. Middle-East J Med Plants Res, 2012; 1(3): 51–8.