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13

CHARACTERIZATION AND EVALUATION OF MEDICINAL QUALITIES OF PHARMACEUTICAL GRADE NEEM OIL

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Abstract : Pharmaceutical Neem oil was produced from the crude Neem oil sample obtained at NARICT, Zaria by refining process namely: degumming, alkali refining, decolourization and deodourization processes at 60°C. The results of the physicochemical analyses of the pharmaceutical oil sample produced were specific gravity 0.9114, refractive index 1.4632, viscosity 27Centipoise, acid value 0.02 MgKOH/g, free fatty acid value 0.01 MgKOH/g, Saponification value 185.8 KOH/g, peroxide value 5.25M Eq/Kg and ester value of 185.75 KOH/g. The physicochemical and comparative analysis of the dark brown coloured neem oil and produced light yellow coloured pharmaceutical Neem oil confirm that purification was achieved as indicated by the result of the physicochemical analyses. Investigation of the medicinal efficacy of the pharmaceutical oil shows that it is effective against fungi skin infections like ringworm, eczema, skin rashes and it is an excellent anti-dandruff. Result also shows that it is safe for use without any adverse effect.

INTRODUCTION

The neem or mangosa tree, also called Indian Liliac belongs to the family 'Meliaceae' and is botanically known as 'Azdirachta Indica', it originated from Southern Asia, today, it grows in tropical and sub-tropical areas of Africa, America and Australia, it resembles an English Oak in appearance and has leaves similar to those of a white cedar, it is an evergreen deciduous fast growing tree often reaching a height of 25-30 meters, it thrives well mainly in tropical climates that have an annual rainfall of 400 to 800mm. (Ruskin, 1992)

All parts of the neem tree, leaves, fruits, seeds, bark, roots and neem oil extracted from the kernel have several applications in medicine, pharmaceuticals, toiletries, cosmetics, public health, livestock products, health, agriculture, horticulture, pest control soap and non edible products. The kernel of the neem seed contain about 40% to 50% oil, the average yield of the neem oil is about 1000cm^3 per 2.5Kg neem seed. Statistical data on the country's export potential indicate that Nigeria can generate about five billion naira annually. Scientific research had confirmed that neem seed oil is very effective organic antiseptic, antifungal, antibacterial, antiviral, dermatological and dental agents. Neem oil can effectively get rid of over 400 pests and diseases from plants and animals. It has been used to reverse desertification and to reduce erosion and desertification, making it an important weapon in the fight against global warming. (Ruskin, 1992)

Neem leaves has been mixed with stored grains to prevent insect infestation, it is a natural alternative to pesticides, it is been used to reverse desertification, making it an important weapon in the fight against global warming, (http://www.pharmacult.com.au)

Keywords : Crude neem oil, pharmaceutical grade neem-oil, refining process and physicochemical analysis.

Neem oil is a very important commercial product, in composition, it is much like other vegetable oils composed primarily of triglycerides of oleic, stearic, lino-oleic, palmitic acid. (Ruskin, 1992)

Neem oil has moisturizing and regenerative properties, it contain vitamin E, has essential fatty acids and glycerides together with its healing properties provide an excellent natural moisturing base for skin care. Research has shown that neem extract "Nim 76", acts as powerful spermicidal. When isolated and refined, it could be used as a new birth control drug without any side effects. It has gained particular attention from scientists seeking a cure for AIDS, because of its antiviral properties and ability to boosts immune system at all levels without destroying beneficial bacteria, unlike synthetic antibiotics. Pharmaceutical oils are refined herbal or animal oil, the oil could be nourishing and medicinal in nature or it could possess either of the above mentioned properties, they are very useful in drug, cosmetics and some food processing, medicine and dermatological industries. Crude neem oil is greenish or yellow/brown in colour with repulsive garlic odour, which necessitates refining to a pharmaceutical oil grade. (http://wwwmaintainroseherbs.com)

Neem seed oil can be extracted using solvent extraction method employing suitable solvent; it uses less neem seed oil and takes longer time compared to the solvent extraction method, which gives higher yield and shorter processing time. However, most active ingredient of the oil is not completely retained in the mechanically expressed oil. Whereas, most active ingredient in the solvent extracted oil is retained. It is quite effective against rice pests, neem oil also destroys bean seed beetle, a variety of insects mostly attacking legumes at the egg stage itself. (Ruskin, 1992)

Neem oil can influence nearly forty species of insects, it is significant that some of these pests are resistant to pesticides or are inherently difficult to control with conventional pesticides. It work by intervening at several stages of the insect's life, they may not kill instantly but incapacitates it in several ways. It is very subtle, employs effects such as repellcnce, feeding and ovi-positional deterrence, growth inhibition, mating disruption, chemo-sterilization, etc. A mixture of neem leaves, clay and cow dung develops pest resistant property so it can be used to make bins for storage of grains. (http://www.pharmacult.com.au)

The aim of this research work is to produce, characterize and evaluate the medicinal qualities and potentials of pharmaceutical grade neem oil. This will be of great benefits to drugs, cosmetics, soap, agrochemicals and toiletries manufacturing industries. It is a renewable source of various useful products, it will provide employment opportunities in Nigeria and also since the demand for neem oil and other neem products are very high and always increasing, the production of the oil will be of immense economic potentials and also a future foreign exchange earner for Nigeria.

Globally, different products had been produced from neem oil and more research is ongoing. Presently, in Nigeria, neem oil insecticides is one of the main product produced from neem oil, hence the urgent need to research into the production of more useful and beneficial products from neem oil, e.g., pharmaceutical grade neem oil from crude neem oil.

EXPERIMENTAL

The crude neem oil sample was obtained from National Research Institute for Chemical Technology (NARICT), Zaria, and Kaduna State, Nigeria. Refining operation: Refining of crude neem oil for upgrading it to pharmaceutical grade involves the following processes namely: degumming, alkali refining, decolourization and deodourization respectively. Degumming: The crude neem oil was degummed by the addition of hot water at about 60°C and vigorous shaken of the mixture in a separating funnel, which was allowed to separate by gravity for about five

to ten minutes after which the aqueous layer containing lecithin otherwise known as gum was discarded. This procedure was repeated five times to ensure that most of the gum was removed; finally, the percentage of oil recovered was determined and recorded. Alkali refining: Neutralization of the free fatty acid was carried out using three different concentrations of NaOH solution - 1.0M, 2.0M and 3.0M respectively, then $10cm^3$ of 1.0M and 2.0M NaOH respectively were used to neutralize $90cm^3$ of each sample of oil and $25cm^3$ of 3.0M NaOH was used for 225cm of oil sample at 60°C with the addition of $2cm^3$ NaCl to enhance the precipitation of the soap stock, neutralized oil samples were then washed with hot water at about 60°C to remove the soap formed and the yields of the three different oil samples were determined and noted. Decolouration: In this process, $80cm^3$ of the alkali refined oil was heated at a temperature of 50°C. 10% weight of the fuller's earth was added and stirred for fifteen to twenty minutes, the mixture of oil and bleaching agent were filtered using filter paper in an oven at about 60°C until the filtration was complete, the above procedure was repeated using activated carbon alone for the second run, then both activated carbon and fuller's earth were combined in equal ratio of 5% weight each for the third run of the above procedure and the yield of oil determined.

Deodourization: Deodourization of the above oil was done to strip off the odour using an essential oil, the essential oil was added to the refined neem oil to give the desired smell or odour, the amount of essential oil added depends on when the desired odour is attained.

The physicochemical properties of both the crude and the refined oil were conducted and the results were compared, the analyses were carried out under the same condition.

The specific gravity bottle was cleaned with acetone, ether and dried in an oven at 60°C, the weight of the empty bottle was taken, after which the bottle was filled with the oil sample and properly covered, the weight was then taken using a weighing balance, after which the sample was removed from the bottle, the bottle was properly washed and filled with distilled water, after which the weight was taken, this procedure was conducted for both the crude and the refined neem oil, finally, the specific gravity was computed using the relationship below. (Olaniyi, 1998)

$$\text{Specific Gravity} = \frac{W_o - W}{W_1 - W}$$

Where, W = Weight of empty bottle, W_0 = Weight of bottle and oil content, W_1 = Weight of bottle and water content.

Viscosity: Four hundred cm^3 of oil sample was poured into the cup of "Clandon Viscometer, Model VT - 03 Viscometer", the lowest number spindle was selected and screwed into the underside of the viscometer, the cup containing sample was carefully locked into position so that the spindle cone would be completely immersed in the sample, the machine was switched on and pointer deflection on the machine scale was observed for about ten seconds and allowed to stabilize, after which the position of the pointer on the scale was read off, this gives the value of viscosity of the oil sample in centipoises. (Olaniyi, 1998)

Refractive Index determination: The refractive index was determined using Abbey refractometers, Number 00836, the glass prism of the refractometers was thoroughly cleaned with alcohol to ensure that it is free from dust, a drop of oil sample was placed on the lower prism and smeared, then closed with the other covering prism, the light source of the refractometers was switched on, while viewing through the telescope, the coarse adjustment knob was rotated until the black shadow appears central in the cross wire indicator, while still viewing through the telescope, the fine knob adjustment was made until the rainbow-coloured fringe which appeared on the black dividing line disappeared, the coarse knob was rotated to give fine adjustment and make the black shadow appear exactly central in the cross wire indicator, the

reading under the telescope and that of the fine adjustment knob were noted and divided by 10,000, this value was then added to the value obtained through the telescope field to give the value of the refractive index of both crude and upgraded oil at room temperature. (Olaniyi, 1998)

Colour Determination: Lovibond comparator was used, the sample was placed in the special plastic cuvetted up to the $10cm^3$ mark, then the special matching colour disk holder were placed in the apparatus compartment side by side with the oil sample containing cuvetted in an upright position and the colour was matched with the colour disc (match) until a suitable colour disc matched with the colour of the oil sample by visual observation, the value on the matching colour disc indicates the colour value of the oil sample under test. Analyses carried out on the chemical properties were listed below: Acid value determination: Two grams of sample was dissolved in $50cm^3$ of mixed neutral solvent ($25cm^3$ diethyl ether with $25cm^3$ ethanol carefully neutralized with 0.1M NaOH using 1% phenolphthalein solution), the mixture was titrated with 0.1M NaOH aqueous solution with constant shaken to faint pink colour. (Dora, 1991)

$$\text{Acid value} = \frac{\text{Titre value} \times 5 \times 61 \times 0.00282}{\text{Weight of sample(g)}} = \text{mgKOH / g}$$

Free fatty acid: The amount of free fatty acid (FFA) was calculated as being equivalent to half the value of acid value, that is,

$$\text{FFA} = \frac{\text{Acid value}}{2} = \text{mgKOH / g}$$

Saponification value : 0.5M KOH was prepared in 95% ethanol, 2g of oil sample was weighed and 25cm of the KOH was added, $25cm^3$ of the blank solution was also measured into a conical flask, the two samples were then connected to a reflux apparatus and allowed to boil for an hour until the reflux is completed, $1cm^3$ of phenolphthalein was added to the mixture and the resulting mixture was titrated while hot against 0.5M HCI acid solution, the volume of the acid used to attained the end point was recorded, the blank determination was carried out using the same procedure described above until the colour changes from blue to transparent white, then the volume of acid used was also noted, the Saponification value was determined using the relationship below. (Dora, 1991)

$$\text{Saponification value, (S.V)} = \frac{56.1 \times T(V_o - V_1)}{M}$$

Where, T = Molarity of the standard KOH solution used, V_0 = Volume of acid used for the first titration with oil sample, V_1 = Volume of acid used for the second titration of the blank solution, M = Mass of the oil sample used. Peroxide Value: Two grams of sample was weighed into clean dried boiling tube, 1 gram of potassium iodide (KI) powder was added to the liquid oil and $20cm^3$ of the solvent mixture (i.e., glacial acetic acid and chloroform in the ratio 2:1), then the boiling tube was placed in boiling water bath so that the liquid mixture boils within 30 seconds and allowed to boil vigorously for not more than 30 seconds , the content after boiling was quickly poured into a flask containing $20cm^3$ of 5% potassium iodide, (KI) solution and the tube was washed out twice with $25cm^3$ of water, then the mixture was titrated with 0.002M sodium thiosulphate using fresh 1% starch solution, a blank titration was carried out at the sample time, the peroxide value was calculated using the relationship below. (Dora, 1991)

$$\text{Peroxide value} = \frac{\text{Sample titre} - \text{Blank titre} \times 2}{\text{Weight of Sample}} = \text{mEq / Kg}$$

Determination of Ester value :

$$\text{Ester Value} = \frac{\text{Saponification Value}}{\text{Acid Value}}$$

RESULTS AND DISCUSSION

From the experimental work conducted in this research work the results obtained were presented in Tables-1 to 4

Table-1 : Decolonization Recovery of the produced Pharmaceutical grade Neem oil

Adsorbent used (10%)	Quantity of oil used	Quantity of oil Recovered	% of oil Recovered
Activated carbon	80cm³	53.3cm³	66.5
Fuller's earth	80cm³	62.32cm³	77.9
Activated carbon and Fuller's earth In ratio 1 : 1	80cm³	67.68cm³	84.6

Table-2 : Alkali Refining of Crude Neem oil at 60°C

NaOH Concentration	Amount of oil used	Amount of NaOH used	% of oil Recovered
1.0M	90cm³	10cm³	96
2.0M	90cm³	10cm³	93
3.0M	225cm³	25cm³	88

Table-3 : Results of the physicochemical analyses of the crude and pharmaceutical neem oil

Parameters	Crude neem oil	Pharmaceutical Neem oil
Colour	Dark brown	Light yellow
Refractive Index	1.4722	1.4682
Viscosity (Cp)	80	27
Specific Gravity	0.9218	0.9114
Acid Value (mg/KOH/g)	0.21	0.02
Free Fatty Acid (mgKOH/g)	0.11	0.01
Saponification Value (mg/KOH/g)	178.3	185.8
Peroxide Value (mEq/Kg)	18.5	5.25
Ester Value	178.09	185.78

Table-4 : Physicochemical Value of Typical Pharmaceutical oil

Parameters	Virgin Linseed oil	Refined Olive oil	Sweet Orange oil
Colour	Brownish Yellow	Clear Colourless	Pale Yellow
Solubility	Slightly soluble in Alcohol	–	–
Specific Gravity –0.850	0.931	–	0.842 – 0.850
Refractive Index	1.480	–	1.470 – 1.476
Acid Value	0.2 – 4.5	0.5	–
Iodine Value	160-200	-	–
Peroxide	<15.0	<10.0	<20
Saponification Value	188-195	-	-

Source : British Pharmacopoeia, 2004

Table-1 shows the results of decolourization recovery of the produced pharmaceutical oil, Table-2 shows the results of Alkali refining of the crude neem oil at 60°C and Table 3 shows the results of the physicochemical analyses of the crude oil and pharmaceutical oil produced while Table-4 shows the physicochemical values of typical pharmaceutical oil. The upgrading of neem oil to pharmaceutical oil was conducted in this research work, the major parameters for the assessment of a pharmaceutical oil are the purity, the efficacy of the oil, its safety when used or applied for treatment and other such characteristics. (Olaniyi, 1998)

This research work focus mainly on the purification of the crude neem oil and its efficacy for the treatment of some skin diseases and hair treatment, degumming process indicated that about 71.7% of the oil was recovered, this implies that about 17.0cm^3 of the gum or lecithin and other impurities were removed from the oil, degumming process enhanced the quality of the oil produced. Neutralization of the free fatty acid was carried out using different concentrations of NaOH to determine the effects of concentrations of the alkali on the free fatty acid, (FFA) and other absorbable impurities.

Based on the obtained results, the lowest concentration of alkali (1.0M NaOH) gave the highest quality oil, which was 96% yield of oil, and 3.0M NaOH gave 88%, the lowest yield of oil, this was due to the fact that part of the neutral oil, free fatty acid and other absorbable impurities got saponified to form soap stock and the soap stock formed was more for the NaOH with higher concentration, neutralization of the FFA in the oil prevented it from becoming rancid in a short time. From Table-3, it was observed that the free fatty acid (FFA) of the neem oil was 0.11 while that of the purified neem oil was 0.01; this shows that the free fatty acid content of this particular neem oil was low and was reduced to a negligible level or completely removed during purification process.

The alkali refined oil was bleached using three different types of adsorbents. Fuller's earth, activated carbon and their combination in equal ratio and the oil obtained after bleaching were 77.9%, 66.5% and 84.6% respectively, after the bleaching, the colour of the crude neem oil sample changed from dark brown to light yellow colour, this shows that bleaching had occurred, also, based on different quantities of oil obtained and colour of refined oil sample, it was observed that activated carbon has a higher adsorptive property but poor filterability and high oil retention, combination of the two adsorbents in equal ratio gave the best filterability and colour adsorption than Fuller's earth.

From Table-3, chemical properties determined were Saponification value, acid value and peroxide value, Saponification value of the crude neem oil increased from 178.3 to 185.8 for the upgraded neem oil, this value compared favourably with the standard values of 178.23 for neem species found in Nigeria, based on this results, the oil can be used for soap, cosmetics, medicinal applications and formulation of pesticides and other uses. (Okonkwo, 2006) Also, from Table-3, the acid value and the peroxide values for the crude neem oil were 0.21 and 18.5 but reduced to 0.02 and 5.25 for the refined neem oil namely: viscosity, refractive index and density were also determined, viscosity of the crude neem oil after refining changes from 80 to 27 Centipoises, specific gravity changed from 0.9218 to 0.9114 for crude neem oil and upgraded oil, refractive indices were 1.4722 for crude neem oil and 1.4682 for upgraded pharmaceutical neem oil, all the above values of physical properties compared well with the average standard values. (Olaniyi, 1998)

The refined oil, that is, the pharmaceutical neem oil when administered on patients suffering from ringworm, eczema and skin rashes (the skin disease) were cured in less than a week, that is, about five days, when applied on hair to treat hair dandruff, it was cured under a week, when tested on head hair lice, it was eliminated within three days, the above results and observations confirmed that the pharmaceutical neem oil is effective against the above mentioned skin and hair infections without any side effects, for the pharmaceutical oil to be used for medicated soap it should be blended with other vegetable oil of higher Saponification value, the results of the refining process and the physicochemical analysis show that the refined neem oil satisfy the standards for pharmaceutical oil.

CONCLUSION

Neem pharmaceutical grade oil was developed from crude neem oil by refining process, the physicochemical analyses of the pharmaceutical grade neem oil properties show that it was properly refined, safe for use externally and satisfies the standard for a typical pharmaceutical oil, the light yellow pharmaceutical grade oil produced from the dark brown crude neem oil possess the following properties: specific gravity 0.9114, refractive index 1.4632, viscosity 27 Centipoises, acid value of 0.02mg KOH/g, Saponification value of 185.8 KOH/g, free fatty acid value of 0.01mgKOH/g, peroxide value of 5.25mEq/Kg and ester value of 185.75 KOH/g.

The results obtained from the investigation of the medicinal qualities of the pharmaceutical neem oil show that it was effective for the treatment of fungi infections of the skin namely: ringworm, skin rashes, and eczema and hair dandruff, based on the above results it can be concluded that refining enhances the quality of the pharmaceutical grade neem oil produced and it can be used in the production of drugs, cosmetics, medicated balm and soap, it can also serve as additive to existing medicated cream to improve its efficacy. Based on the economic analyses of the manufacturing process, this study when commercialized will be economically viable, cost effective and profitable.

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