Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Botany

Elixir Appl. Botany 39 (2011) 4951-4953

ISSN: 2229-712X

Morphological studies of Neem *(Azadirachta indica* A. Juss.) seed and physicochemical properties of its oil extracts collected in Accra metropolis of Ghana

Crentsil Kofi Bempah*, Archibold Buah-Kwofie and Jacob Asomaning

Nuclear Chemistry and Environmental Research Center, National Nuclear Research Institute, Ghana Atomic Energy Commission, P.O. Box LG 80, Legon, Accra-Ghana.

ARTICLE INFO

Article history:
Received: 29 July 2011;
Received in revised form:
22 September 2011;
Accepted: 29 September 2011;

Keywords
Azadirachta indica,
Seed oil,
Physicochemical,
Morphological,
Accra,
Ghana.

ABSTRACT

The seeds of *Azadirachta indica* A. Juss. popularly known as neem collected from five cities of Accra metropolis was studied. Trees with wide girth and different seed weight were observed. Maximum residual oil content was noticed in trees from Haatso. Weight of the seeds had no effect on the oil yield. Seed oil content in most of the cities was not significantly correlated with morphological parameters of seeds. *A.indica* seed oil extracted was analyzed for their physicochemical properties such as viscosity at 28 ^{o}C (0.07 kg/ms), pH (5.7), acid value (1.102 ml/g), iodine value (71.0 gI_2/100g) and free fatty acid value (48.35 ml/g). The maximum mean percentage oil obtained (52.5 %) makes the commercialization of the seeds of *Azadirachta indica* in Ghana a possible and profitable venture. The result also confirms the oil to be good quality and can find application in industrial purposes.

Introduction

The neem tree *Azadirachta indica* A. Juss. (Meliaceae) is a tropical evergreen related to mahogany. Native to east India and Burma, it grows in much of Southeast Asia and West Africa. They were brought to Africa, back in the 19th century. The first African countries where the tree was planted include Cameroon, Ghana, Kenya, Tanzania, and Ethiopia among others. The evergreen tree is large, reaching 12 to 18 meters in height with a girth of up to 1.8 to 2.4 meters.

Its blossoms are small, white flowers with a very sweet, jasmine-like scent. Its fruit is about 3/4 of an inch (2 cm) long, with white kernels. A neem tree generally begins bearing fruits at three to five years of age, and can produce up to 50 kg (110 lbs.) of fruit annually when mature. The shiny dark green pinnately compound leaves are up to 30 cm long. Each leaf has 10–12 serrated leaflets that are 7 cm long by 2.5 cm wide. It will grow where rainfall is as little, and thrives in very dry and arid conditions. (Muñoz-Valenzuela et al., 2007).

Neem has been extensively studied for its pesticidal and medicinal properties in Asiatic countries. Despite almost every part of the tree having a bitter taste, parts such as leaves, bark, flower, fruit, seed and root have advantages in medical treatment and industrial products. Its leaves can be used as drug for diabetes, eczema and reduce fever. Barks of Neem can be used to make toothbrush. Neem roots has an ability to heal diseases and against insects (Liauw et al., 2008).

Of the entire product from this tree, oil extracted (oil fraction approximately 40-58.9% weight compared to the other parts of the tree) from kernels is the most commercially important, because it has a variety of users ranging from insecticides to pharmaceuticals (Kaura et al., 1998). In Ghana, neem tree can be found in almost all the regions, however, this tree has many potential applications; the utilization of this tree in Ghana for industrial purposes still limited, as a canopy tree.

Neem seed is a part of Neem tree which has high concentration of oil. Neem oil is widely used as insecticides, lubricant, drugs for variety of diseases such as diabetes and tuberculosis. Considerable quantities of the seed oil are employed in cosmetic preparations. This oil could also prolong leather goods when it is applied on them insects (Liauw et al., 2008).

Given the wide range of uses associated with extracts from the neem tree, the objectives for this study was to investigate oil content of neem trees in Greater Accra region of Ghana search for oils from non conventional sources, because of increasing needs for industrial applications, and to determine whether oil yield is related to seed and tree morphology.

Materials and methods

Neem seed processing for Nuclear Magnetic Resonance (NMR) analysis

Neem seeds from matured neem trees growing in the Greater Accra metropolis of Ghana were collected in the months of October 2009 to June 2010. Within 24 hours of collection, the seeds were thoroughly cleaned and washed using water to remove dirt and carefully dried in the open air at room temperature (23±2 ^{o}C) for 3 days prior for analysis.

Neem seeds were analyzed for moisture and oil content using low resolution Nuclear Magnetic Resonance (NMR) technique. NMR is quick and easy to perform, simple to calibrate and not dependent on the sample matrix. 40 seed weight of neem was simply loaded into pre-tared glass vials, weighed, conditioned, and then inserted into the instrument which detected the sample, automatically starting the NMR

Tele: +233244364456
E-mail addresses: crentbempah@fastmail.fm

analysis. The instrument returns the water and moisture content values in less than one minute.

Neem seed preparation for oil extraction

The cleaned seeds were sun dried in the open, until the casing splits and sheds the seeds. The seeds were further dried in the oven at 60°C for 7hrs to a constant weight in order to reduce its moisture content, which was initially at about 5 to 7%. The separation of the shell from the nibs (cotyledon) was carried out using tray to blow away the cover in order to achieve very high yield. Mortar and pestle were used to crush the seeds into a paste (cake) in order to weaken or rupture the cell walls to release castor fat for extraction.

Oil extraction

150ml of normal hexane was poured into round bottom flask. 20 g of the sample was placed in the thimble and was inserted in the centre of the extractor. The Soxhlet was heated at 60 ^{o}C. When the solvent was boiling, the vapour rises through the vertical tube into the condenser at the top. The liquid condensate drips into the filter paper thimble in the centre, which contains the solid sample to be extracted. The extract seeps through the pores of the thimble and fills the siphon tube, where it flows back down into the round bottom flask. This was allowed to continue for 30 minutes. It was then removed from the tube, dried in the oven, cooled in the desiccators and weighed again to determine the amount of oil extracted. Further extraction was carried out at 30 minutes interval until the sample weight at further extraction and previous weight becomes equal. The experiment was repeated for different weights of the sample, 35, 40 and 50 g. The weight of oil extracted was determined for each 30 minutes interval. At the end of the extraction, the resulting mixture *(miscella*) containing the oil was heated to recover solvent from the oil.

Determination of pH and Viscosity of Neem Oil

The pH was selected and the electrodes were lowered into buffer solution. The temperature control was adjusted until the meter indicates exact pH of buffer. The electrodes were rinsed with buffer, followed with the oil. The temperature control was adjusted to temperature of Neem seed oil (24 %). The electrodes were then lowered into the Neem seed oil for the reading to be taken. A little quantity of the oil was poured into a test tube and a viscometer was used to measure the viscosity.

Determination of Acid Value

2g of the test portion was dissolved in some neutral solvent (toluene/ethanol mixture) the solution was thoroughly mixed and then titrated with 0.1 KOH using 1ml of phenolpthalein indicator or solution. The end point was reached when pink colour persisted for 30 seconds. Two determinations were carried out on the same test sample. The acid value is given by the expression: Acid value = V·C·56.1/M, where V is volume of potassium hydroxide (ml), C is concentration of potassium hydroxide, M is mass of the test portion (g), and 56.1 - Molar mass of potassium hydroxide.

Determination of iodine value

2 g of the sample was weighed into a conical flask and 20 ml of carbon tetrachloride was added to dissolve the oil. Then 25ml of Dam's reagent was added to the flask using a safety pipette influenced chamber. A stopper was then inserted and the content of the flask was vigorously swirled. The flask was then placed in the dark for 1 hour and 30 min. At the end of this period, 20 ml of 10% aqueous potassium iodide and 150 ml of water were added using a measuring cylinder. The content was titrated with 0.1 M sodium thiosulphate solution until the yellow due to iodine has almost disappeared. A few drops of 1% starch indicator were added and the titration continued by adding thiosulphate drop-wise until blue coloration disappeared after vigorous shaking. The same procedure was used for the blank test and for other samples. The iodine value (*IV*) is given by the expression:

$IV = 12.69c(V_1 - V_2)/M$,

where: *c* – concentration of sodium thiosulphate used,

*V*1 – volume of sodium thiosulphate used for the blank,

*V*2 – volume of sodium thiosoulphate used for determination,

m – mass of the sample.

Determination of Free Fatty Acid Value

25ml of toluene was mixed with 25ml of ethanol in a beaker the solution was poured on to 2.0 g of neem seed oil in a flask and 1ml of phenolphthalein was added. The mixture was titrated against 0.1M NaOH with constant shaking for which a dark pink colour was observed and the volume of 0.1M NaOH used (V_b) was noted.

The free fatty acid value was calculated as follows:

ffav = $V_b \times 2.82 \times 100$ %; where 100ml of 0.1NaoH = 2.82 g of oleic acid.

Results and discussion

The neem tree plantations located in Accra metropolis in Ghana showed significant contrast in morphology and oil content as well as moisture content. In the 10 selected trees from each city, maximum average oil production potential was observed in seeds from Hatso from which a value of 52.5 % oil was observed (Table 1). Seeds from the same city exhibited largest girth at breast height (5.0 to 64 cm). However, the highest oil yield (60.5 %) was observed from a tree of different city (Achimota). The high yield may be as a result of environmental factor which enhance the growth and productivity of the seed (Abitogun et al., 2009). This yield makes the industrial practice of the oil recovery a profitable venture in Ghana. Least average oil production was exhibited in the tree grown in the Mc Cathy Hill with a mean value of 49.5 %. Seed oil content in most of the cities was not consistently and significantly correlated with morphological parameters of seeds.

The results obtained for the percentage moisture content, varies within a range of 6.2-7.9 %. Neem seeds from University of Ghana campus exhibited higher moisture content (7.9 %), followed by McCarthy Hill and Spintex with a moisture content of 7.1 % each, Haatso (6.6 %) and Achimota (6.2 %). Mean value of 6.98 % shows a variation from a similar study by Salunke and Desai, (1941) who reported a moisture content of the range of 5 to 7 percent.

Fruit weights showed significant variability. Average fruit weight ranged from 10.2 g (Mc Cathy Hill) to 13.6 g (Achimota). This results corroborate the findings of Kaura et al., (1998), who found that seed morphology (seed length and seed weight), as well as its tree height had no significant effect on oil yield.

Based on the physical properties of the oil (Table 2), the pH was 5.7±0.0, the low level was an indicative of the presence of reasonable quantity of free fatty acid in the oil, which is a good indicator of the advantageous utilization of the oil in soap making.

The viscosity was determined at 28 ^{0}C using viscometer. The value obtained was 0.07 kg/ms. The low value might be as a result of the absence of suspended particles in the neem oil sample. All this physical parameters is an attribute of the oil to be used for industrial purposes.

The iodine value is a measure of the degree of unsaturation and it an identity characteristic of native (Hamilton, 1999). The value determined for neem seed oil was 71.0 (gI_2/100g) (Table 3). This value could be used to quantify the amount of double bond present in the oil which reflects the susceptibility of oil to oxidation. Also, it enables us to classify the oil in non-drying groups which can be regarded as liquid oil. Thus, the oil may find its application in the manufacturing of lubricants, hydraulic fluids and coating (Ibiyemi et al, 1992).

The free fatty acid and acid values were determined to be 48.35 ml/g and 1.102 ml/g, respectively. This therefore gives us the indication to determine oleic acid and oleic acid respectively which will therefore allow us to check the level of oxidative deterioration of the oil by enzymatic or chemical oxidation (Table 5).

Conclusion

Based on the analysis of the experimental result obtained, the quantity of oil extracted from neem seed was found to be (52.5 %). The high oil content of the neem seeds obtained in this study strongly indicates its prospects for commercial extraction. The chemical analysis carried out on the oil produced from *Azadirachta indica* have the following properties: acid value of 1.102 ml/g, iodine value of 71.0 gI_2/100g, free fatty acid value of 48.35 ml/g viscosity at 28 ^oC of 0.07 kg/ms and pH of 5.7.

From the correlation analysis, it can be deduced that, oil yield has no consistent/significant correlation with seed morphology. The oil is of good quality and could be recommended suitable for industrial usage.

Reference

Abitogun AS, Alademeyin OJ, Olove DA. Extraction and characterization of castor seed oil. The Internet J Nutri Well. 2009. Vol. 8, No. 2.

Hamilton S. Extraction of lipids and derivative formation. In: Hamilton RS, Hamilton S, eds. Lipid analysis. A practical approach. Oxford: IRL Press. 1999; 13–*64*.

Ibiyemi SO, Adepoju TO, Okanlawon SO, Fadipe VO. Emulsion preparation and stability, Journal of nutritional science.1992; 13 (1-2): 31-34. (s)

Kaura SK, Gupta SK, Chowdhury JB. Morphological and oil content variation in seeds of Azadirachta indica A. Juss (Neem) from northern and western provenances of India. Department of Genetics, CCS Haryana Agricultural Univ. Hisar, India.1998.

Liauw MY, Natan FA, Widiyanti P, Ikasari D, Indraswati N, Soetaredjo FE. Extraction of neem oil (azadirachta indica a. juss) using n-hexane and ethanol: studies of oil quality, kinetic and thermodynamic. ARPN J Eng App Sci. 2008. Vol. 3, No. 3,

Muñoz-Valenzuela S, Arnoldo A, Ibarra-López, Rubio-Silva LM, Valdez-Dávila H, Borboa-Flores J. Neem Tree Morphology and Oil Content. Issues in new crops and new uses. J. Janick and A Whipkey (eds.). ASHS Press, Alexandria, VA.2007.

Salunke DK, Desai BB. Post-harvest Biotechnology of Oil Seeds, CRC Press, 1941. p. 161-170,

Table1: Morphological parameters and nuclear magnetic resonance (NMR) measurement of moisture and oil content of seeds of *Azadirachta indica* A. Juss. collected from five towns in Accra metropolis of Ghana

Sampling location	No. of trees studied	Morphology parameter		Oil content (%)		Moisture content (%)	
		Average GBH (cm) fruit wt for 20 seeds (g)	Range	Mean±SE	Range	Mean±SE	Range
McCathy Hill	10	10.2	24-64	49.5±2.6	43.6-52.7	7.1±3.2	6.8-7.3
Achimota	10	13.6	11-37	49.9±4.2	44.6-60.5	6.2±4.1	5.8-6.6
Spintex	10	13.0	5-25	50.2±1.6	47.3-52.2	7.3±2.9	7.1-7.5
University of Ghana Campus	10	12.2	19-30	51.9±2.7	46.6-55.3	7.0±3.0	7.9-7.2
Haatso	10	11.1	30-38	52.5±2.6	47.8-57.4	6.9±2.5	6.6-7.1

GBH- Girth at breast height
SE- Standard error

Table 2: Physical Properties of the Extracted Oil

Physical property	Value	Units
Odour	Garlic-like	-
Colour	Golden yellow	-
pH	5.7	-
Viscosity	0.07	kg/ms
Percentage yield of oil	52	%

Table 3: Determination of Iodine Value

Parameter	Mass (g)	1st titre (g)	2nd titre (g)	Mean value (g)	Iodine value (gI_2/100g)
Neem oil	2.00	85.45	85.45	85.45	71.0
Blank	-	200	201	201	-

Table 4: Acid Value Determination

Parameter	Volume (ml)	1st titre (ml)	2nd titre (ml)	Mean value (ml)	Acidity value (ml/g)
Neem oil	2.00	0.35	0.35	0.35	1.102

Table 5: Determination of Free Fatty Acid

Parameter	Mass (g)	1st titre (ml)	2nd titre (ml)	Mean value (ml)	FFA (ml/g)
Neem oil	2.0	0.40	0.40	0.40	48.35

FFA-Free fatty acid