

Extraction and Utilization of Essential Oil from Neem Seeds in Toothpaste Formulation

Aye Aye Mar¹, Chaw Su Mon Mon Oo²

Abstract

The present research work focused on the extraction of essential oil from neem seeds and its utilization in herbal toothpaste formulation. Firstly, the physico-chemical properties of neem seeds (*Azadirachta indica* A. Juss) were determined and the essential oil was extracted from them by using solvent extraction process. Effect of particle size of neem seeds, extraction temperature and time, types of solvent such as n-hexane and ethanol on the yield percent of essential oil were investigated. The physico-chemical characteristics and fatty acid profile of extracted essential oil were also determined using American Oil Chemists' Society (AOCS) official method and Gas Chromatography (GC). The extracted essential oil was utilized in toothpaste formulation. The characteristics such as pH, alkalinity, viscosity, density, lead content, arsenic content, foaming power and physical appearance of formulated herbal toothpastes were also determined and compared with commercial product.

Keywords: essential oil, physico-chemical properties, solvent extraction, herbal toothpastes

Introduction

Neem (*Azadirachta indica* A. Juss) tree is belonged to Meliaceae family, and grows rapidly in the tropic and semi-tropic climate. It is also observed that this tree could survive in very dry and arid conditions. Neem tree is an evergreen tree, but it may become leafless for a short period in certain conditions. All parts of neem plant such as leaves, barks, flowers, fruits, seeds and roots have advantages in medical treatment and industrial products (Janick and Whipkey, 2007). 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. There are several methods to obtain neem oil from the seeds like mechanical pressing, supercritical fluid extraction, and solvent extraction. Mechanical extraction is the most widely used method to extract neem oil from neem seeds. Neem oil is generally light to dark

¹ Professor and Head, Dr., Department of Industrial Chemistry, University of Mandalay

² MSc Student, Department of Industrial Chemistry, University of Yangon

brown, bitter and has rather strong odour. It comprises mainly triglycerides and large amounts of triterpenoid compounds, which are responsible for the bitter taste (www.arnjournals.com).

Toothpaste is used with a toothbrush as an accessory to clean maintain the oral health and improve the esthetics. Most of the toothpastes contain the same basic functional ingredients, all of which have a specific role to play within the formulation (jpda.com.pk/images/.../08-kefi-Iqbal.pdf). The regular formulation of modern toothpastes contains abrasive agents, detergents, humectants, thickening, flavouring, colouring and antimicrobial agents. Therapeutic toothpastes which have incorporated in them some drug or chemical which by reason of its bactericidal, bacteriostatic, enzyme-inhibiting or acid neutralizing qualities reduce the incidence of dental caries or aid in the control of periodontal disease (Balsam and Gershon, 1974). The main purposes of this study were to extract and characterize the essential oil from neem seeds and to utilize the extracted essential oil in herbal toothpaste formulation.

Materials and Methods

Materials

In this research work, neem seeds were collected from Myanmar Golden Crop. Co. Ltd., Pakokku Township, Magway Region. 95% ethanol and normal hexane (analytical grade, British Drug House Co. Ltd., England) were used for the extraction of essential oil from neem seeds. Sodium lauryl sulphate, calcium carbonate, glycerine, xanthan gum, sodium fluoride, sorbitol, methyl paraben, titanium dioxide, clove oil and peppermint oil were used for the formulation of herbal toothpaste.

Methods

Preliminary Preparation of Neem Seeds

Neem seeds were washed with water to remove dirt and other impurities and then sun dried at 30°C to 32°C for 4 hours. Then, the dried neem seeds were ground and screen to get three different particle sizes (-10 + 35 mesh size, -35 + 60 mesh size and -60 +100 mesh size).

Physico-chemical Properties of Neem Seeds

The properties of neem seeds such as moisture content, ash content, fat content, crude fiber content and protein content were determined by Association of Official Analytical Chemists (AOAC) methods.

Extraction of Neem Oil

Neem seeds powder 25 g (-10 + 35 mesh size) and 50 mL of n-hexane were placed into the 2.5 L of round bottomed flask and heated at 80°C for 150 min. After extraction, the miscella was then separated from the solids by filtration and the neem oil was recovered by distillation at 90°C for 120 min. The last trace of normal hexane from extracted neem oil was removed by drying in an oven at 105°C for 30 min. The yield percent of extracted neem oil and the recovery of n-hexane were calculated and the results are recorded. The same procedure was repeated for the extraction of neem oil from neem seeds powder using ethanol as solvent. The yield percent of extracted neem oil and the recovery of ethanol were determined and the results are recorded.

Determination of the Maximum Yield Percent of Extracted Neem Oil

Effect of Amount of Solvent

Neem oil was extracted from neem seeds powder with different amounts of solvent (n-hexane / ethanol) ranging from 50 mL to 150 mL based on 25 g (-10+35 mesh size) of neem seeds powder. The yield percent of neem oil and recovery of solvent were determined and the results are recorded.

Effect of Particle Size of Neem Seeds Powder

Neem oil was extracted from different particle sizes of neem seeds powder (-10+35 mesh size, -35+60 mesh size and -60+100 mesh size) using n-hexane / ethanol as solvent based on 25 g of neem seeds powder and 100 mL of solvent (weight of neem seeds powder to volume of solvent ratio 1:4). The yield percent of neem oil and recovery of solvent were determined and the results are recorded.

Effect of Extraction Temperature

Neem oil was extracted from neem seeds powder using different extraction temperatures ranging from 40°C to 80°C for 150 min based on 25 g of neem seeds powder and 100 mL of solvent (weight of neem seeds

powder to volume of solvent ratio 1:4). The yield percent of neem oil and recovery of solvent were determined and the results are recorded.

Determination of the Physico-chemical Properties and Fatty Acid Profile of Extracted Neem Oil

The physico-chemical properties such as acid value, refractive index, peroxide value, saponification value, unsaponifiable matter and iodine value of extracted neem oil were determined by AOCS Official Method Cd 3a-63, 1973 and colour of extracted neem oil was determined by Lovibond Tintometer (Model-E). The fatty acid profile of extracted neem oil was tested by Gas Chromatography. The respective results are recorded.

Formulation of Herbal Toothpaste

In the formulation of herbal toothpaste, firstly, 2 g xanthane gum as binder, 33 g of glycerine as humectant and 10 mL of distilled water were added in a mortar and pastel and stirred thoroughly at room temperature for 60 min to prepare a liquid dispersion. And then, the liquid dispersion was stored for overnight where the binder is slightly swollen. Secondly, 0.5 g of sodium fluoride, 2 g of sodium lauryl sulphate, 0.3 g of sorbitol, 0.05 g of methyl paraben as preservative and 15 mL of distilled water were added into a beaker and heated at 70°C for 5 min and stirred with 200 rpm using a magnetic stirrer to obtain the additive solution. Finally, the prepared slightly swollen binder, additive solution and 30 g of calcium carbonate as abrasive were gradually added in a mortar and pastel stirring with one way direction for 30 min to obtain the binder is completely swollen and dissolved. When a uniform paste was formed, 1 g of neem oil as therapeutic agent, 0.3 g of clove oil, 0.3 g of peppermint oil and 5 g of titanium dioxide were added and stirring with one way direction for 30 min until the paste was homogenously. After that the formulated herbal toothpaste was filled into a tube and stored at room temperature.

Physico-chemical Properties of Formulated Herbal Toothpaste

The physico-chemical properties such as pH, foaming power, viscosity, density, alkalinity, lead content and arsenic content of formulated herbal toothpaste were determined and the results are recorded.

Organoleptic Properties of Formulated Herbal Toothpaste

The physical appearance such as texture was determined on the basis of 9 point Hedonic scale by a panel of 10 semi-trained judges.

The overall acceptability of the formulated herbal toothpaste was taken as the average score of all these organoleptic properties and the results are recorded.

Results and Discussion

The physico-chemical characteristics such as moisture content, ash content, fat content, crude fibre content and protein content of neem seeds are shown in Table (1). From the results in Table (2), it can be seen that 32.96 %w/w of neem oil were obtained using 100 mL of ethanol based on 25 g (-10 + 35 mesh size) of neem seeds powder at extraction temperature 80°C for 150 min. The results in Table (3) indicate that 47.4 %w/w of neem oil was obtained using 25 g (-35+50 mesh size) of neem seeds powder at extraction temperature 80°C for 150 min. The effect of extraction temperature on the yield percent of neem oils are shown in Table (4). The highest yield (47.45 %w/w) of neem oil could be obtained using 100 mL of ethanol and 25 g (-35+50 mesh size) of neem seeds powder at the extraction temperature 60 °C for 150 min.

The physico-chemical characteristics such as acid value, peroxide value, refractive index, saponification value, unsaponifiable matter, iodine value and colour of extracted neem oils are shown in Table (5). The properties such as acid value, saponification value and iodine value of extracted neem oils are quite different with the properties of neem oil in literature value. The structural formula and composition of fatty acids in extracted neem oil are shown in Table (6).

Herbal toothpaste was formulated using extracted neem oil, abrasive, foaming agent, binder, humectants and preservative as shown in Table (7). T care toothpaste (made in Myanmar) was chosen to evaluate the properties of formulated herbal toothpaste because the ingredients used in T care toothpaste are the same as the formulated herbal toothpaste. The physico-chemical properties of formulated herbal toothpaste were determined and the results are shown in Table (8). From the results in Table (8), it was found that the viscosity of formulated herbal toothpaste lower than the viscosity of T care toothpaste and the alkalinity of formulated herbal toothpaste higher than the alkalinity of T care toothpaste. The properties of formulated herbal toothpaste and T care toothpaste were found to be within the respective limits of toothpaste specifications (<http://www.brookfieldengineering.com./AR>).



Figure (1) Neem Seeds with Husk Figure (2) Neem Seeds without Husk



Figure (3) Neem Oil using n-Hexane Figure (4) Neem Oil using Ethanol

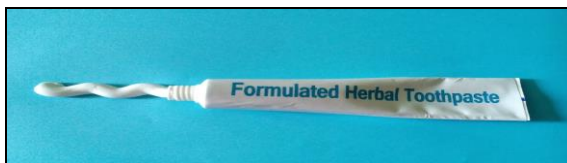


Figure (5) Formulated Herbal Toothpaste

Table (1) Physico-chemical Properties of Neem Seeds

Sr. No.	Properties	Neem Seeds, (% w/w)	
		Experimental Value	Literature Value*
1	Moisture content	8.38	9.66
2	Ash content	3.308	5.08
3	Fat content	8.74	23.10
4	Crude fibre	61.00	30.40
5	Protein content	20.6	12.90

* <http://www.hort.purdue/.../munoz126-128>

Table (2) Effect of Amount of Solvent on the Yield Percent of Neem Oil

Particle size of neem seeds = -10 + 35 mesh

Extraction temperature and time = 80°C for 150 min

Sample No.	Neem Seeds (g)	Solvent (mL)		Neem Oil (% w/w)		Solvent Recovery (mL)	
		n-Hexane	Ethanol	n-Hexane	Ethanol	n-Hexane	Ethanol
I	25	50	50	21.76	27.18	30	31
II	25	75	75	25.16	31.68	50	55
III	25	100	100*	26.32	32.96	60	78
IV	25	125	125	26.25	32.60	75	95
V	25	150	150	26.29	32.58	110	120

* The suitable amount of solvent

Table (3) Effect of Particle Size of Neem Seeds on the Yield Percent of Neem Oil

Amount of solvent (n-hexane / ethanol) = 100 mL

Extraction temperature and time = 80°C for 150 min

Sample No.	Neem Seeds (g)	Size of Neem Seeds (mesh)	Neem Oil (% w/w)		Solvent Recovery (mL)	
			n-Hexane	Ethanol	n-Hexane	Ethanol
I	25	-10 + 35	26.32	32.96	60	78
II	25	-35 + 60*	35.66	47.40	62	76.5
III	25	-60 + 100	35.81	47.50	61	77.5

* The suitable particle size of neem seeds

Table (4) Effect of Extraction Temperature on the Yield Percent of Neem Oil

Particle size of neem seeds = - 35 + 60 mesh

Amount of solvent (n-hexane / ethanol) = 100 mL

Sample No.	Neem Seeds (g)	Extraction Condition		Neem Oil (% w/w)		Solvent Recovery (mL)	
		Temp. (°C)	Time (min)	n-Hexane	Ethanol	n-Hexane	Ethanol
I	25	40	150	27.60	28.80	65	70
II	25	50	150	26.32	32.96	65	70
III	25	60*	150	35.68	47.45	63	75
IV	25	70	150	35.65	47.43	60	75
V	25	80	150	35.66	47.40	62	76.5

* The suitable extraction temperature

Table (5) Physico-chemical Properties of Extracted Neem Oil

Particle size of neem seeds = -35 + 60 mesh

Ratio of neem seeds to solvent = 25g : 100 mL (1:4 w/v)

Extraction temperature and time = 60 °C for 150 min.

Sr. No.	Characteristics	Amount	
		Experimental Value	Literature Value*
1	Acid value, (mg KOH/g)	22.44	2.88
2	Peroxide value, (meq peroxide/kg)	5.452	10.20
3	Refractive index	1.469	0.889
4	Saponification value, (mg KOH/g)	73.63	210.0
5	Unsaponifiable matter, (% w/w)	0.393	-
6	Iodine value,(Wijs)	71.2	36.54
7	Colour	R 10.2, Y 9.9	Light yellow

* <http://www.sciencedomain.org/review-history.php?iid=176&id=22&aid=137>**Table (6) Structural Formula and Compositions of Fatty Acids in Extracted Neem Oil**

Test parameter and method = Fatty acid profile, Gas chromatography

Sr. No.	Fatty Acid	Carbon & Double Bonds	Chemical Structures	Compositions (% w/w)
1	Lauric	C _{12:0}	CH ₃ (CH ₂) ₁₀ COOH	15.539
2	Myristic	C _{14:0}	CH ₃ (CH ₂) ₁₂ COOH	0.076
3	Palmitic	C _{16:0}	CH ₃ (CH ₂) ₁₄ COOH	18.632
4	Palmitoleic	C _{16:1}	CH ₃ (CH ₂) ₅ CH=CH(CH ₂) ₇ COOH	48.183
5	Stearic	C _{18:0}	CH ₃ (CH ₂) ₁₆ COOH	15.418
6	Oleic	C _{18:1}	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₇ COOH	0.399
7	Linoleic	C _{18:2}	CH ₃ (CH ₂) ₂ CH=CHCH ₂ CH=CH(CH ₂) ₇ COOH	1.426
8	Linolenic	C _{18:3}	CH ₃ (CH ₂) ₂ CH=CHCH ₂ CH=CHCH ₂ CH=CH(CH ₂) ₇ COOH	0.263

Table (7) Compositions of Formulated Herbal Toothpaste

Amount of formulated herbal toothpaste = 100 g

Sr. No.	Ingredients	Formulated Herbal Toothpaste (g)	Literature Value* (g)
1	Sodium lauryl sulphate	2.0	0.1-7
2	Glycerine	33.0	-
3	Xanthan gum	2.0	1-2.5
4	Sorbitol	0.3	0.005-0.25
5	Sodium fluoride	0.5	-
6	Methyl paraben	0.05	0.1-5
7	Titanium dioxide	5.0	-
8	Calcium carbonate	30.0	5-60
9	Distilled water, (mL)	25.0	8-20
10	Clove oil	0.3	0.3-2
11	Peppermint oil	0.3	0.3-2
12	Neem oil	1.0	1-5

* <http://www.freepatentsonline.com/4168.html>**Table (8) Physico-chemical Characteristics of Formulated Herbal Toothpaste**

Sr. No.	Characteristics	Toothpastes		Literature Value*
		Formulated	T care	
1	Physical appearance	Smooth, moist	Smooth, moist	-
2	pH	7.35	8.1	7-8
3	Foaming power, (mL)	170	180	> 150
4	Viscosity, (cP)	187000	279000	170000-200000
5	Density, (g cm ⁻³)	1.3	1.28	1.3
6	Alkalinity, (ppm)	28	16	20-40
7	Lead content	Nil	Nil	-
8	Arsenic content	Nil	Nil	-

* <http://www.freepatentsonline.com/4168.html>

Conclusions

In the present research work, neem oil was extracted from neem seeds powder by solvent extraction method and the extracted neem oil was utilized in herbal toothpaste formulation. Based on the results of experiments, it can be concluded that the most favourable conditions for extraction of neem oil from neem seeds using n-hexane and ethanol were as follows: 25 g of neem seeds powder (-35 +60 mesh size) and material-to-solvent ratio 1:4 (w/v) at extraction temperature 60°C for extraction time 150 min.

Toothpaste contains active and inactive ingredients which have their own importance. An active ingredient such as sodium fluoride may act as anticaries. Inactive ingredients may help provide the texture, mouthfeel, cleansing activity, colour and flavour, as well as preservatives. In the formulation of herbal toothpaste, 0.5 g of sodium fluoride, 30 g of calcium carbonate were used as active ingredients and 2 g of sodium lauryl sulphate, 33 g of glycerine, 2 g of xanthane gum, 0.3 g of sorbitol, 0.05 g of methyl paraben, 5 g of titanium dioxide, 25 mL of distilled water, 0.3 g of clove oil, 0.3 g of peppermint oil and 1 g of neem oil were used as inactive ingredients.

Acknowledgements

We are grateful to Dr Thida Win, Rector, Dr Aung Kyaw Thin and Dr Tin Tun Aung, Pro-Rectors, University of Mandalay, for their permission to submit this article. We would like to express deepest gratitude to Dr Khin Thet Ni, Professor and Head (Retd.) of the Department of Industrial Chemistry, University of Yangon for her valuable suggestions, helpful support and advice in this research work.

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