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RESEARCH ARTICLE

Physicochemical Analysis of *Nephelium Lappaceum* Seed Fat and its Application in Topical Formulations

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Abstract

Nephelium lappaceum L. (Family- Sapindaceae), popularly known as 'Rambutan', is well known for its edible fruits with delicious taste and flavour in Southeast Asia. The fruits are deseeded during processing in the canning industry and considered as a waste by-product. The seeds contain good amount of fatty material and has been claimed to be a source of natural edible fat. The objective of the study was to report the physicochemical parameters of the seed fat together with formulation of few topical preparations such as creams and lotions containing the seed fat that would offer new prototype formulations containing natural ingredients. The physicochemical analysis of the seed fat such as melting point, saponification value, acid value, ester value and peroxide value were studied. The GC-MS analysis of the seed fat was carried out to find its chemical composition. Further, two topical preparations such as creams and lotions were prepared using the seed fat and evaluated using standard recommended procedures. The seed fat was compatible to be used as an ingredient in the bases of creams or lotions.

Keywords: *N. lappaceum seed fat, Physicochemical analysis, GC-MS analysis, Topical formulations*

Introduction

Topical formulations are referred to the preparations that are applied on the surface of the skin and the mucous membrane for desired therapeutic activity. Excipients are inert substances used in the preparation of pharmaceutical and cosmetic formulations and are intended to provide physical, chemical and biopharmaceutical properties to the formulations. Search for new excipients is as important as the search for new drug candidates. The excipients are required to fulfil their ideal requirement characteristics for their inclusion in any pharmaceutical or cosmetic products. Fats and lipids are widely used in the manufacturing of several topical pharmaceutical and cosmetic products.

They are used as lubricants, emulsifiers, emollients in emulsions, ointments, creams, lotions etc. Many times, topical preparations are reported contain synthetic ingredients as excipients which are believed to cause unwanted effects on the skin after their application [1]. The situation further worsens when the topical preparations such as cosmetics are used several times a day and particularly, the women continue for the whole lifetime where there is a chance of potential threat to their health on long run. Few noteworthy examples of commercial cosmetics such as creams and lotions that often contain propylene glycol or butylene glycol, synthetic waxes, BHA (butylated hydroxyl anisole) and BHT (butylated hydroxyl toluene) etc which are also major concerns for the health [2, 3]. Therefore, researchers are now looking towards natural resources in search of suitable excipients for their inclusion in formulation bases as they are nontoxic, cost effective, easily available and biocompatible to the skin [4].

Nephelium lappaceum L. (Family-Sapindaceae), popularly known as 'Rambutan' (Fig. 1), is an evergreen tree, native to Malaysia but grown in other parts of the world. The plant is commonly grown for its fruits which are believed to be the potential source of minerals and other nutrients [5].

The fruits are consumed fresh, canned, or processed, and appreciated for its delicious taste and flavour. The fruits are deseeded during processing in the canning industry and considered as a waste by-product. The seeds contain good amount of fatty material and has been claimed to be a source of natural edible fat [6]. In the light of the above, the present proposal was sought to validate the fundamental physicochemical properties of *N. lappaceum* seed fat for its suitability as an ideal excipient for inclusion in to topical formulations.

Fig. 1: *N. lappaceum* fruit and seed

Our earlier studies demonstrated that the lipstick prepared from seed fat of N. lappaceum was good enough to meet the general characteristics for ideal lipsticks with sufficient hardness and appreciable lustre to the formulation [7]. We further concluded that the seed fat can serve as an ingredient in the preparation of natural lipsticks. Few other reports on the seed fat of *N. lappaceum* is available in the literature. Harahap et al. [8] reported that the seeds contain fat (38.9%), protein (12.4%) and carbohydrate (48%) respectively. The seed oil contains arachidoyl-dioleoylglycerol as the major component (49.84%).

Manaf et al. [9] reported presence of almost equal proportion of saturated (49.1%) and unsaturated (50.9%) fatty acids in the seed fat of *N. lappaceum* together with their composition. In another study, Lourith et al. [6] confirmed presence of oleic and arachidic acids as the major fatty acids together with their composition and stated that the seed fat can be a promising unconventional source of specialty fat for cosmetics.

Materials and Methods

Plant Material

The fruits of *N. lappaceum* that already ripened were purchased from local markets around Ipoh, Perak and authenticated by the botanist. A voucher specimen (herbarium) was prepared and preserved in the department for future references.

The fruits were deseeded and seeds were collected. The collected seeds were washed with water, cut in to small pieces, shade dried, and pulverized to coarse powder. The dried seed powder was preserved in a refrigerator until further use.

Extraction of the Seed Fat

Extraction of the seed fat was performed using petroleum ether (40-60°C) as the solvent. The seed powder (500 g) was extracted with 1.5 L petroleum ether (40–60°C) at 40°C for 1 h. Following extraction, the liquid extract was filtered, the solvent recovered by vacuum distillation and reused for the seed powder for further extraction. The extraction process was repeated 3 times. The seed fat was pooled from three extractions and stored in a well-closed container until further use [7].

Gas Chromatography-Mass Spectroscopy (Gc-Ms) Analysis of the Seed Fat

The GC-MS analysis of the seed fat was performed as suggested by Sandanasamy, Nour, Tajuddin & Nour, 2013 [10]. Briefly, about 100 mg of the seed fat was dissolved in 10 ml of n-hexane in a test tube. The solution was treated with 1 ml of 2M methanolic potassium hydroxide solution and the mixture was vortexed for 15 min. The n-hexane phase was separated, washed twice with distilled water and finally dried over an anhydrous sodium sulfate.

The sample was used for the GC-MS analysis using a Gas chromatography system coupled with Mass spectrometry (Agilent Technologies 7890).

The sample solution was injected into the GC system by auto sampler. Helium was used as carrier gas at the flow rate of 1 ml/min. Separation of the components was achieved on a capillary column (nonpolar capillary DB-1 of 100% dimethylpolysiloxane) with 30 meter in length, 0.25 mm diameter and 0.25 µm thicknesses. The oven temperature was initially set to 60°C for 3 min and then increased to maximum of 240°C at the rate of 3°C/min.

The temperature was maintained constant for 10 min at 240°C. The inlet temperature was set at 250°C. The mass detector conditions were set as split less mode, injector temperature of 250°C and ion-source temperature of 230°C. The library search for the peaks was carried out NIST Library Chem. Station software.

Physicochemical Analysis of Seed Fat

The saponification value, acid value, ester value and peroxide value of the seed fat was performed as per the procedures laid down in British Pharmacopeia, 2012 [11]. The melting point of the seed fat was recorded using Techne Stuart Automatic Melting Point Apparatus (SMP40). The results are presented in Table 1.

Saponification Value

Accurately weighed 40 g of potassium hydroxide was dissolved in 20 ml of water and sufficient ethanol (96%) was added to produce 1000 ml. The solution was left to stand overnight. Accurately weighed 2 g of *N. lappaceum* seed fat was taken into a 250 ml flask; 25 ml of ethanolic solution of potassium hydroxide was added and boiled under a reflux condenser for 1 h while rotating constantly. After 1 h, the solution that was still hot was titrated with 0.5 M hydrochloric acid together with 1 ml of phenolphthalein solution as indicator. The saponification value was calculated from the expression 28.05 v/w where v is the difference, in ml, between the titrations and w is the weight in g for the substance taken.

Acid Value

Accurately weighed 10 g of seed fat was dissolved in 50 ml of a mixture containing 25 ml of 96% ethanol (previously neutralized together with 0.1 M potassium or sodium hydroxide using 0.5 ml of phenolphthalein) and 25ml and petroleum ether, The solution was then titrated against 0.1 M potassium hydroxide until pink colour was obtained for about 15 sec Acid value was calculated as follows:

$$\text{Acid value} = \frac{5.610n}{m}$$

Where

m – Weight of the seed fat

n - Volume (ml) of titrant

Ester Value

The ester value was calculated by subtracting the acid value of fat with the saponification value of the fat.

Ester value **= Saponification** value – Acid value

Peroxide Value

Accurately weighed 5g of the seed fat was placed into a 250 ml conical flask fitted with a ground-glass stopper. Thirty milliliter of a mixture of 2 volumes of chloroform and 3 volumes of glacial acetic acid were added. The flask was shaken and 0.5 ml of saturated potassium iodide solution was added. The mixture was titrated against 0.01 M sodium thiosulphate with vigorous shaking until the yellow colour was discharged (n1). The blank test was carried out under the same conditions (n2). Peroxide value was calculated using the formula given below.

$$\text{Peroxide value} = \frac{10\,(n1 - n2)}{m}$$

Where:m – Weight of the seed fat and n1 – volume (ml) of 0.01 M sodium thiosulphate for sample

n2 – volume (ml) of 0.01 M sodium thiosulphate for blank determination

Formulation of Cream and Lotion

Formulation of Creams

Five different formulations (NC1, NC2, NC3, NC4, NC5) were prepared by taking different ingredients such as seed fat, palm oil, olive oil, beeswax and wool fat as the oil phase, while rose water was used as the water phase in different proportions as listed in Table 2.

Both the phases were separately heated to a temperature of about 75°C. The contents were mixed together in a mortar while still in hot condition with continuous stirring until the creams were formed.

Formulation of Lotions

Six different formulations (NL1, NL2, NL3, NL4, NL5 and NL6) were prepared by taking different ingredients such as seed fat, palm oil, olive oil and coconut oil as the oil phase while distilled water, potassium hydroxide, glycerine and triethanolamine were used as the water phase in different proportions as listed in Table 3. Both the phases were separately heated to a temperature of about 75°C. Once both phase reached 75°C, the content were mixed together in a mortar with continuous stirring. The mixture was then diluted with sufficient distilled water to make up the volume.

Evaluation of Topical Formulations

The prepared formulations (Creams and lotions) were evaluated for homogeneity, physical appearance, type of smear and skin irritation test [12]. The results are presented in Table 4.

Results and Discussion

GC-MS Analysis of Seed Fat

GC-MS analysis of seed fat (Fig. 2 to Fig. 6) revealed presence of several components such as two fatty acids; n-Hexadecanoic acid and oleic acid, two ester; Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester and 9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester and 2-Undecenal, which also known as a component with fruity odour [13].

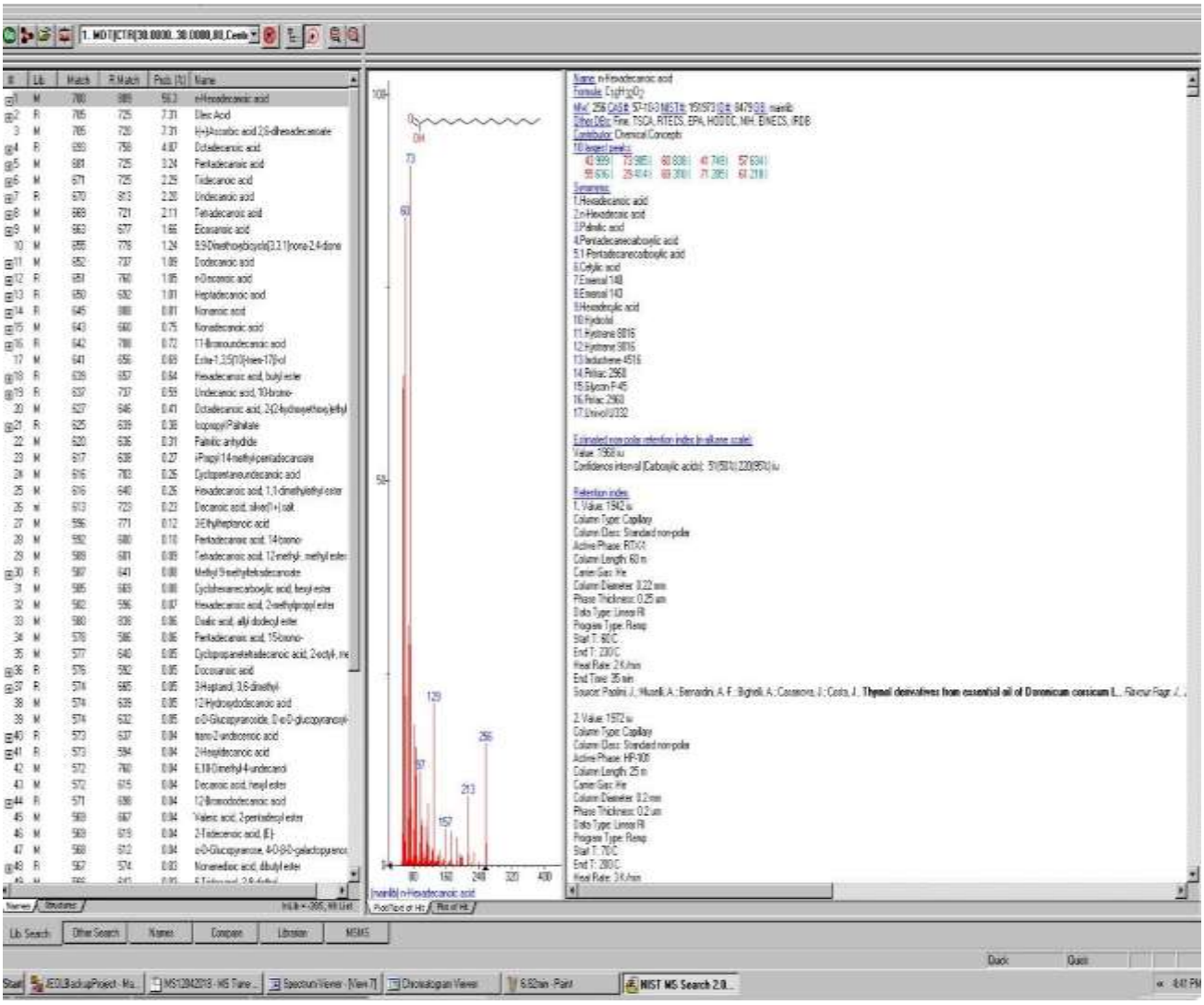

Fig. 2: Composition of seed's fat (2-Undecenal)

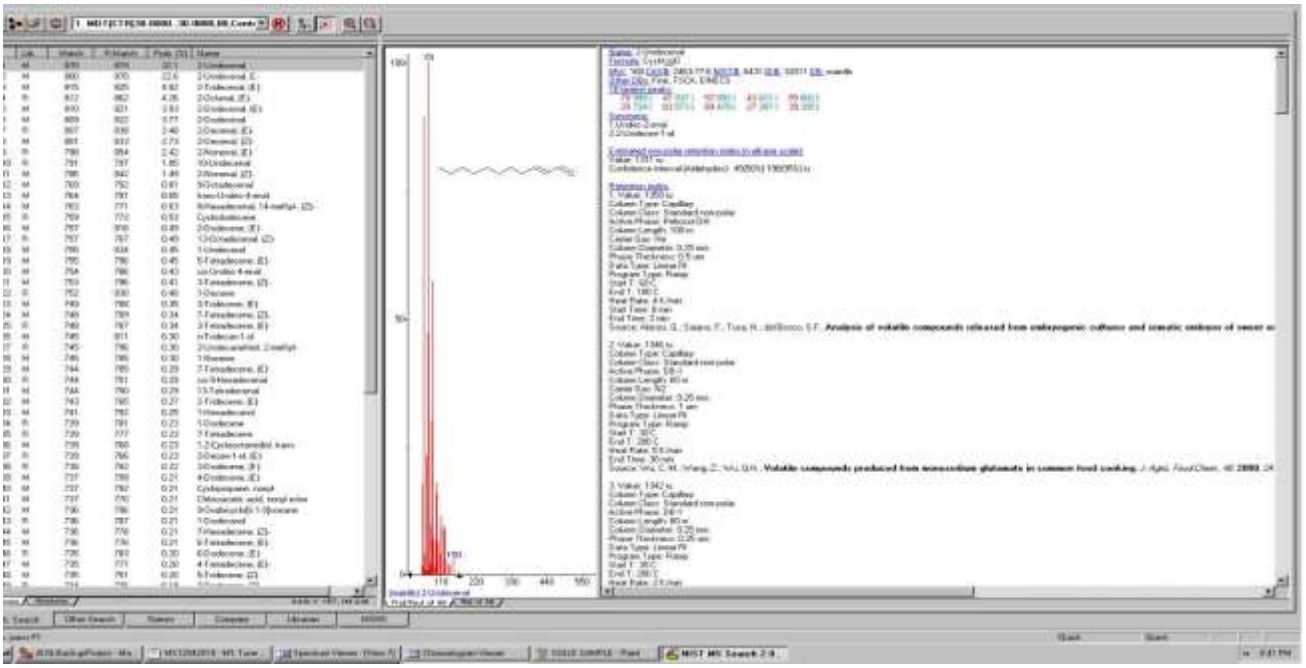

Fig. 3: Composition of seed's fat (n-Hexadecanoic acid)

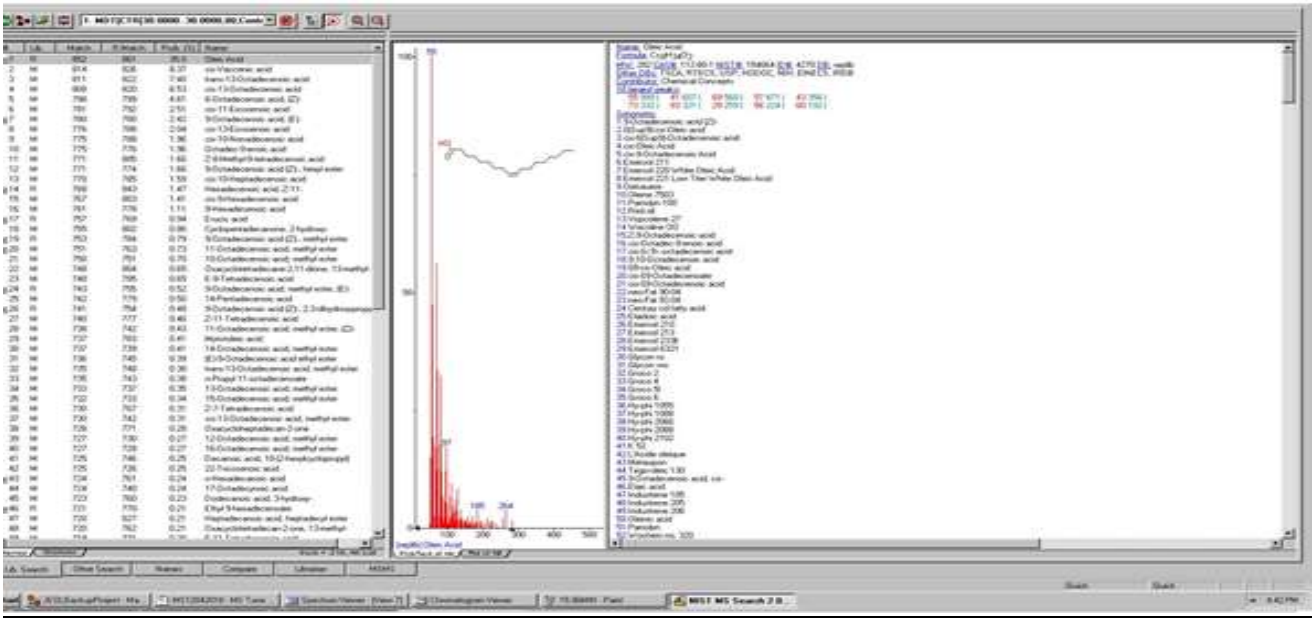

Fig. 4: Composition of seed fat (Oleic acid)

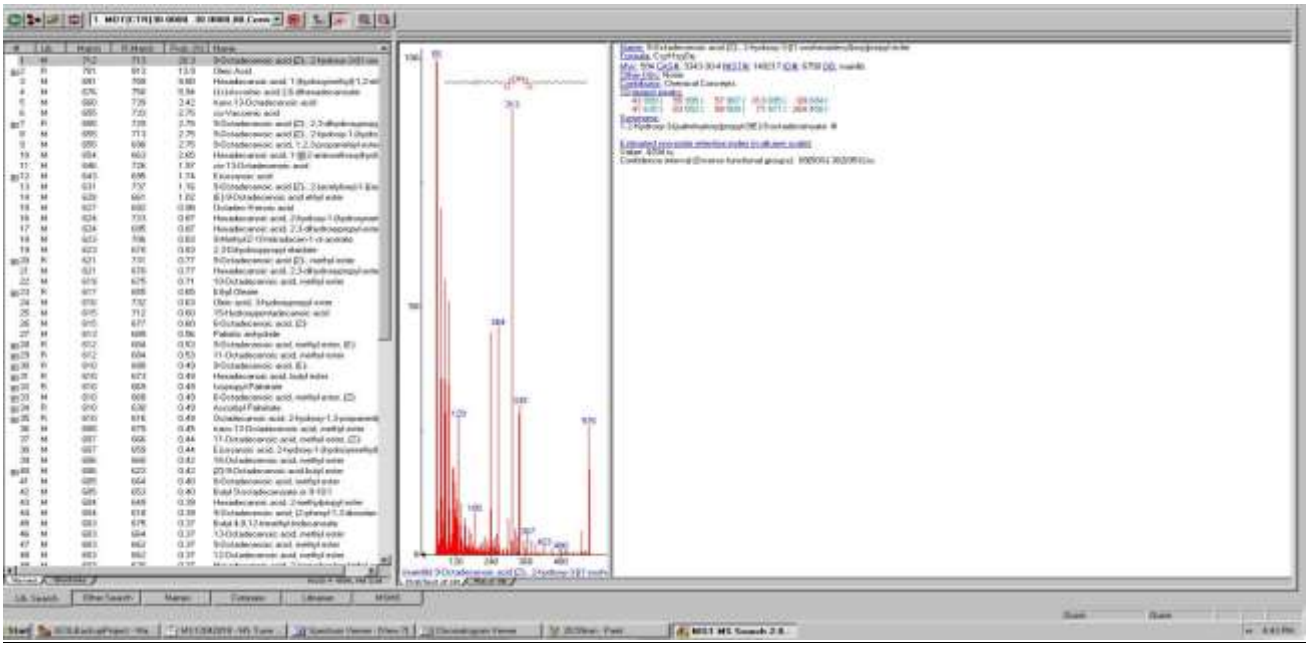

Fig. 5: Composition of seed fat (9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl) oxy] propyl ester

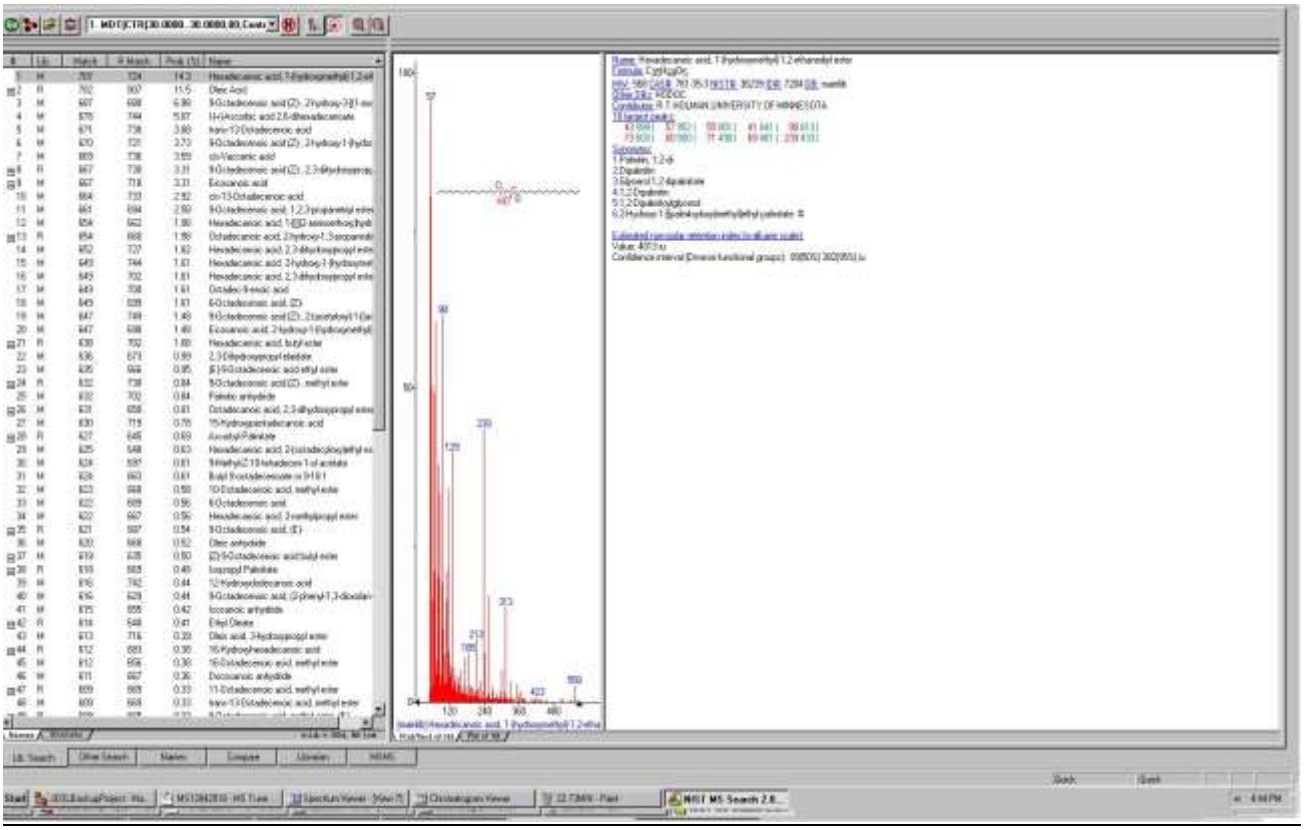

Fig. 6: Composition of seed fat (Hexadecanoic acid, 1-(hydroxymethyl) 1, 2-ethanediyl Ester)

Physicochemical Analysis

The physicochemical parameters of the seed fat such as saponification value, acid value, ester value, peroxide value and melting point are presented in Table 1.

Table 1: Physicochemical analysis of *N.lappaceum* seed fat

Analysis	Value
Saponification value	227.14
Acid value	18.63
Peroxide value	6.60
Ester value	208.51
Melting point	40.7°C

The physicochemical parameters are always useful in quality profile of crude drug evaluation. Melting point of the excipients used in topical preparations is one of the important parameters which affect the spread ability of the preparations. The other parameters such as saponification value, acid value and ester value are related to the amount of fatty acids present in the sample.

Peroxide value is a measure of the extent to which the fat or oil is susceptible to autoxidation and rancidity upon storage conditions [14]. Lower peroxide value refers to lesser sensitivity to oxidation. The results of the study demonstrated melting point: 40.7°C, saponification value: 227.14, acid value: 18.63, ester value: 208.51 and peroxide value: 6.60 respectively. GC-MS analysis revealed presence of 2-Undecenal, n-Hexadecanoic acid, Oleic acid, 9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl) oxy] propyl ester and Hexadecanoic acid, 1-(Hydroxymethyl)-1, 2-ethanediyl ester in the seed fat.

Formulation and Evaluation of Creams and Lotions

Formulation of Creams

Five different formulations of creams (NC1, NC2, NC3, NC4 and NC5) were prepared by taking different ingredients such as seed fat, palm oil, olive oil, beeswax and wool fat as the oil phase, while rose water was used as the water phase in different proportions as listed in Table 2. From among all these five formulations, the formulation NC5 was found to be the ideal one (Fig.3).

Table 2: Composition of cream formulations

Ingredients	NC1	NC2	NC3	NC4	NC5
Seed fat	12.51 g	8.50 g	10.01g	12.52 g	8.50 g
Palm oil	7.52 g	4.26 g	7.08 g	-	1.75 g
Olive oil	-	-	-	7.50 g	1.75 g
Beeswax	5.04 g	-	-	5.11 g	-
Wool fat	-	6.05 g	5.10 g	5.33 g	6.00 g
Rose water	5 ml	10 ml	10 ml	5 ml	10 ml

Fig 7: Cream formulation (NC5)

Formulation of Lotions

Six different formulations of creams (NL1, NL2, NL3, NL4, NL5 and NL6) were prepared by taking different ingredients in different proportions as listed in Table 3. From among all these six formulations, the formulation NL6 was found to be the ideal one (Fig.4)

Table 3: Composition of lotion formulations

Ingredients	NL1	NL2	NL3	NL4	NL5	NL6
Seed fat	5.05 g	6.10 g	5.03 g	8.11 g	7.14 g	7.00 g
Palm oil	3.03 g	-	-	-	3.00 g	1.5 g
Coconut oil	-	-	3.11 g	2.51 g	-	-
Olive oil	-	-	-	-	-	1.5 g
Triethanolamine	0.75 g	-	0.78 g	0.77 g	0.77 g	0.75 g
Potassium hydroxide	-	0.34 g	-	-	-	-
Glycerin	-	1.69 g	-	-	-	-
Distilled water q.s.	10 ml	10 ml	10 ml	25 ml	25 ml	25 ml

Fig. 4: Lotion formulation (NL6)

The prepared formulations NC5 and NL6 were evaluated for physical appearance, colour, odour, homogeneity, type of smear, phase separation and skin irritation tests. The formulations were opaque, white in colour, odourless, non-greasy and there was no phase separation in any of the formulations. Further, the formulations showed good results in homogeneity, type of smear and did not cause any irritation to the skin. From the above studies it may be concluded that the seed fat of *N. lappaceum* is compatible to be used as an excipient in the bases of topical formulations. Further research would be recommended to introduce a whole new safer formulation using the seed fat in topical formulation.

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