STUDY ON THE PREPARATION OF PAIN RELIEF BALM USING KAN-ZAW SEED OIL

Hnin Wai Lin[1], Nwe Nwe Aung[2] and Khin Thet Ni[3]

Abstract

In this research work,the dried Kan-zaw seeds were collected from Myoe Haung Village, Palaw Township, Myeik District, Tanintharyi Region. Firstly the phytochemical investigation of dried Kan-zaw seed was carried out. Secondary plant metabolites such as flavonoids, tannins, carbohydrates, saponins, phenols, glycosides, reducing sugars, terpenoids, steroids and alpha-amino acids were found to be present in Kan-zaw seed. The oil was extracted from dried Kan-zaw seed by using oil press machine. The physico-chemical characteristics of expressed Kan-zaw seed oil such as acid value, peroxide value, iodine value and free fatty acids were studied. FTIR analysis of functional groups present in the Kan-zaw seed oil was also conducted. Fatty acid profile of expressed Kan-zaw seed oil was evaluated by Gas Chromatography Flame Ionization Detection (GCFID) method. Based on the expressed Kan-zaw seed oil, pain relief balm was formulated using D-optimal mixture design of Design Expert Software trial version 11.0. Optimum formulation of pain relief balm was judged by its properties such as pH, viscosity and spreadability. Pain relief balm with pH 6.52, viscosity 11613.33 cP and spreadability 29.56 gcm/s was found as optimum composition. Skin irritation test for prepared pain relief balm was conducted in rabbit model and there were no skin rashes, inflammation and itching or redness on applied portions for 96 hours. Consumer acceptance for pain relief balm with optimum composition was studied by 9 point-hedonic scale. Total score for pain relief balm was 6.7 and it was between like slightly and like moderately.

Keywords: Kan-zaw, D-optimal mixture design, skin irritation test, hedonic scale test

Introduction

Medicinal plants have provided the modern medicine with numerous plant derived therapeutic agents (Evans, 2000). The therapeutic value of the plant depends on the active constituents present inside the different parts of the plant, which may be present in the small or large quantity. The secondary metabolites are the important substance responsible for the main medicinal properties in the crude drugs (Patel *et al*, 2018). The bioactivity of natural products is associated with the effects of various phytochemicals such as tannins, terpenoids, cardiac glycosides, saponins, flavonoids among others (Njerua *et al*, 2013).

A variety of herbs and essential oils can be used for pain and inflammation associated with sports and exercise, as well pain and inflammation associated with rheumatism, arthritis, surgery, or other medical conditions. Herbal pain relief oil is a perfect blend of oils like sesame oil, essential oils and herbs. It gives quick relief from any type of ache in the body after rubbing on the body, as a result no strains persist in the body (Chauhan *et al*, 2016).

Madhuca indica J. F. Gmel. (English Name: Indian Butter Tree, Family Sapotaceae, locally known as Kan-zaw in Myanmar. It is a tremendous therapeutic plant growing throughout the subtropical region of the Indo-Pak subcontinent and also in Tanintharyi Region, Myanmar. *Madhuca Indica* has several pharmacological activities, and potential to provide health to the

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society. It is used as anti-diabetic, antiulcer, hepatoprotective, antipyretic, antifertility, analgesic, antioxidant, swelling, inflammation, piles, emetic, dermatological, laxative, tonic, anti-burn, anti-earth worm, wound healing headache and many more problems. Madhuca oil contains fatty acids such as palmitic 21.3%, stearic 24.3%, oleic 36.7%, linoleic 15.2% and arachidic 1.3% (Dhara , 2010). Kan-zaw seed oil, obtained from plant origin, has been used as versatile oil throughout Myanmar. The present study was aimed to extract the Kan-zaw seed oil by expression method and to analyze the characteristics of the Kan-zaw seed oil and finally to formulate the pain relief balm using Kan-zaw seed oil.

Materials and Methods

Materials

Kan-zaw seeds were purchased from Myoe Haung Village, Palaw Township, Myeik District, Tanintharyi Region.

The raw materials such as beeswax, paraffin wax, vaseline, camphor and menthol were purchased from Empire Chemical Store, Pabedan Township, Yangon Region.

Methods

Extraction of Kan-zaw Seed Oil by Mechanical Expression Method

The dried Kan-zaw seeds were dehulled by hand to remove the seed-coats and then air-dried for three days and stored in air-tight plastic bag. The dried Kan-zaw seeds were expressed by using expeller (Taiwan made Best Oil Press Machine with Heater, Model No.02) between 45-50^{o}C to obtain Kan-zaw seed oil and oil cake residues separately. The expressed oil was settled and filtered to remove the residual solids.

(i) (ii) (iii)

Figure 1 (i) Kan-zaw Seed with Seed Coat (ii) Kan-zaw Seed without Seed Coat (iii) Expressed Kan-zaw Seed Oil

Identification of Kan-zaw seed and Analysis of Kan-zaw Seed Oil

Phytochemical characteristics of Kan-zaw seed powder were firstly investigated. The physico-chemical characteristics of Kan-zaw seed oil such as acid value, peroxide value, iodine value and free fatty acid were also studied. Functional group of Kan-zaw seed oil was also investigated. Fatty acid profile of Kan-zaw seed oil was evaluated by Gas Chromatography.

Preparation of Pain Relief Balm using Kan-zaw seed Oil

Basic formulation of pain relief balm was selected from the preliminary investigation of characteristics of balm before implementing the experimental design. Formulation of pain relief balm was based on the levels as shown in Table (1) and 25 samples were formulated according to the D-optimal mixture design of the Design expert software trial 11. Firstly 8.63g of beeswax, 4.76g of paraffin wax, and 54.15g of vaseline were mixed and heated with stirring in water bath at 75-80°C for 15min to get the phase I. Secondly, 11.76g of Camphor, 15.76g of menthol and 4.93g of Kan-zaw seed oil were mixed and stirred thoroughly at room temperature to obtain the phase II. The two phases were mixed at room temperature and continuously stirred until the mixture became thick. Finally, the balm formed were filled into the sterilized glass bottles during flowable condition.

Table 1 Level of Components in the Formulation of Pain Relief Balm

Sr. No.	Component	Low level (g)	High level (g)
1.	Beeswax	7	10
2.	Paraffin wax	3	7
3.	Vaseline	50	60
4.	Kan-zaw seed oil	4	6
5.	Camphor	8	16
6.	Menthol	14	18

Analysis of Prepared Pain Relief Balm

pH, viscosity, spreadability, stability, skin irritation test and consumer acceptance of prepared pain relief balm were also studied.

Results and Discussion

From the results of phytochemical investigations shown in Table(2), it can be seen that Kan-zaw seed powder contains flavonoids, glycosides, phenols, reducing sugars, tannins, saponins, carbohydrates and α - animo acids except alkaloid and cyanogenic glycoside.

Calixto *et al.,reported that* recently discovered analgesic substances include, alkaloids, flavonoids and terpenoids. According to Table (3), it was known that acid value and peroxide value of Kan-zaw seed oil were in accordance with Codex Standard. Table (4) and Figure (2) show the functional groups present in the Kan-zaw seed oil which could be assigned. According to these results, Kan-zaw seed oil may contain alkane groups, carbonyl groups, methyl groups and carboxylic acid groups. The fatty acids, retention times (RT) and peak area (%) of Kan-zaw seed oil were analysed by GCFID and the respective chromatogram and fatty acid profile are illustrated in Figures (3) , (4) and Table (5). From these results, it was known that Kan-zaw seed oil contained 56.375% oleic acid, 23.137% palmitic acid, 12.205% stearic acid and 7.299% linoleic acid as major components and trace amount of other acids such as arachidic, palmitoleic and linolenic acid. Myristoleic, palmitoleic, oleic, linolenic, linoleic acids are polyunsaturated omega fatty acids. Because of their beneficial properties on the skin it is used for anti-inflammatory, acne reductive, skin ligthtening and moisture retentive properties of the skin. Table (6) shows the formulae of 6 components and responses of pain relief balm. The properties

of pain relief balm such as pH, viscosity and spreadability were judged for the optimum formulation. Table (7) shows the optimum composition of pain relief balm predicted by design expert. This composition was judged by pH, 6.52±0.03, viscosity, 11613.33±32 and spreadability 29.56±0.65 of prepared pain relief balm. Table (8) represents the summary of ANOVA (analysis of variance) for pH, viscosity and spreadability. In fit summary of response pH, design expert suggested for quadratic model. The sequential F-test by design expert indicated that the quadratic model and F value were significant. In this case, p-value was significant and lack of fit was not significant. Similarly, for responses, viscosity and spreadability, the sequential F-test by design expert indicated that the quadratic model and F value were significant and p-value was significant and lack of fit was not significant respectively. Figures (5), (6) and (7) indicate the residual plots, contour graphs and 3D plots for pH, viscosity and spreadability of pain relief balm. From the results, it can be seen that, viscosity and spreadability were affected by beeswax, paraffin wax and vaseline. On studying the stability of pain relief balm, there was no distinct change in pH, viscosity and colour of the products during two months and the results are shown in Table (9). Skin irritation test for pain relief balm was conducted with rabbit model. There were no skin rashes, inflammation and itching or redness on applied portions for 96 hr. Investigation of consumer acceptance for pain relief balm with optimum composition was carried out by 9-point hedonic scale and results are shown in Table (10). Total score for pain relief balm was 6.7 and it was between like slightly and like moderately.

Table 2 Phytochemical Screening of Kan-zaw Seed

Sr. No.	Phytoconstituent	Test	Results
1	Alkaloid	Mayer's reagent	-
		Wagner's reagent	
		Dragendorff's reagent	
		Hager's reagent	
2	Carbohydrate	10% α naphthol &	+
		conc:H_2SO_4	
3	Glycoside	10% lead acetate solution	+
4	Phenols	1% $FeCl_3$ solution	+
5	α- amino acid	Ninhydrin reagent	+
6	Saponin	Frothing test	+
7	Tannin	1% gelatin & 10% NaCl solution	+
8	Flavonoid	Mg ribbon & conc:HCl	+
9	Steroid	Acetic anhydride & conc: H_2SO_4	+
10	Terpenoid	Acetic anhydride & conc: H_2SO_4	+
11	Reducing sugar	Fehling solution	+
12	Starch	Iodine solution	+
13	Cyanogenic glycoside	H_2O, conc: H_2SO_4	-

(+) present (-) absent

Table 3 Physico-chemical Properties of Kan-zaw Seed Oil

Sr. No.	Properties	Kan-zaw Seed Oil	Literature value*	Sr. No.	Properties	Kan-zaw Seed Oil (oil press machine)	Literature value*
1.	Relative density (at 20˚C)	0.910	0.856-0.870	6.	Acid value (mg KOH/g)	3.565	3.5
2.	Refractive index	1.464	1.452-1.462	7.	Peroxide value (mleq/peroxide oxygen/kg)	2.641	2.78
3.	Saponification value (mg KOH/g)	190.569	187-196	8.	Moisture (%w/w)	0.155	-
4.	Unsaponifiable matter (%)	1.016	1.00-3.00	9.	Aflatoxin	-	not detected
5.	Iodine value (mgI_2/g)	63.294	58.00-70.00	10.	Yield of oil content (%w/w)	32.7	-
				11.	Yield of residual oil (%w/w)	2.7	-

*R.S.Kureel and et-al (2009)

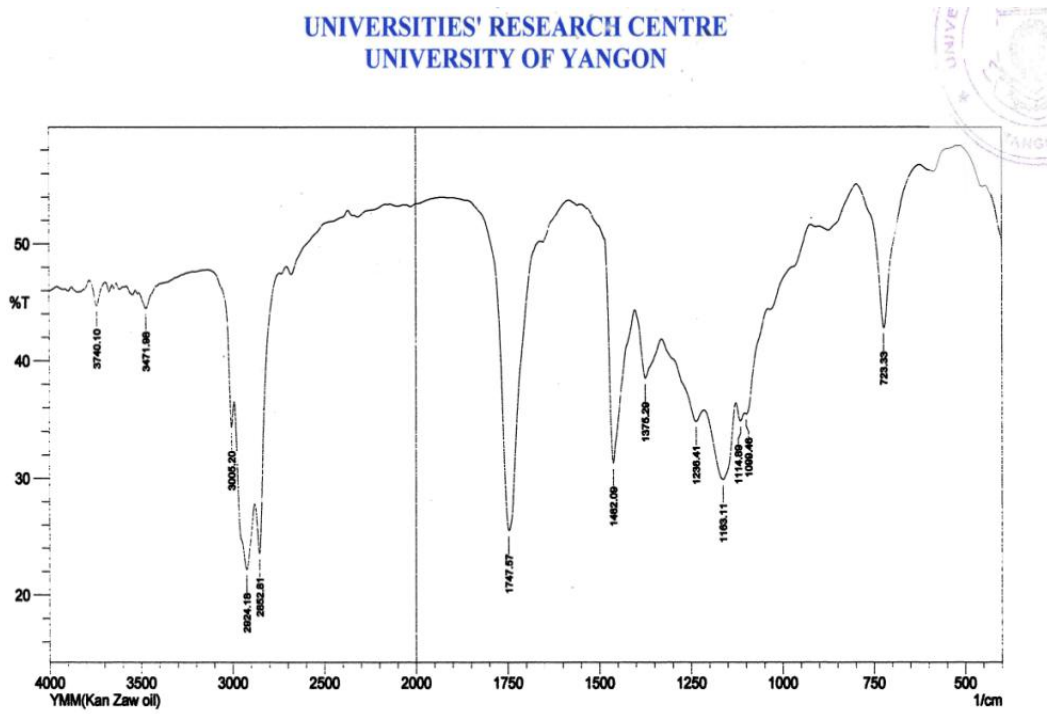


Figure 2 FTIR Spectrum of Kan-zaw Seed Oil

Table 4 Functional Group of Kan-zaw Seed Oil Analysed by FTIR

Wave number, cm^{-1}		Functional group	
Kan-zaw Seed Oil	**Literature***		
3740.10	3650	ν-OH	Stretching vibration of hydroxyl compound
3471.96	3550-3450	ν -OH	Stretching vibration of hydroxyl compound
3005.20	3100-3020	ν-CH	Stretching vibration of olefinic C-H
2924.18	2930	ν -CH	Stretching vibration of alkane
2852.81	2850	ν-CH	Stretching vibration of alkane
1747.57	1870-1650	ν-C=O	Stretching vibration of carbonyl group
1462.09	1460	δ- CH	Asymmetric bending vibration of methyl group
1375.29	1380	δ-CH	Symmetric bending vibration of methyl group
1236.41	1255-1210	δ-OH	OH in plane bending vibration
1163.11 1114.89	1200 and 1410-1310	δ-OH	OH in plane bending vibration
1099.46	1180-1080	ν -C-O	Stretching vibration of monomeric carboxylic acid
723.33	750-650	δ-OH	OH out of plane bending vibration

*Mohan, (2000)

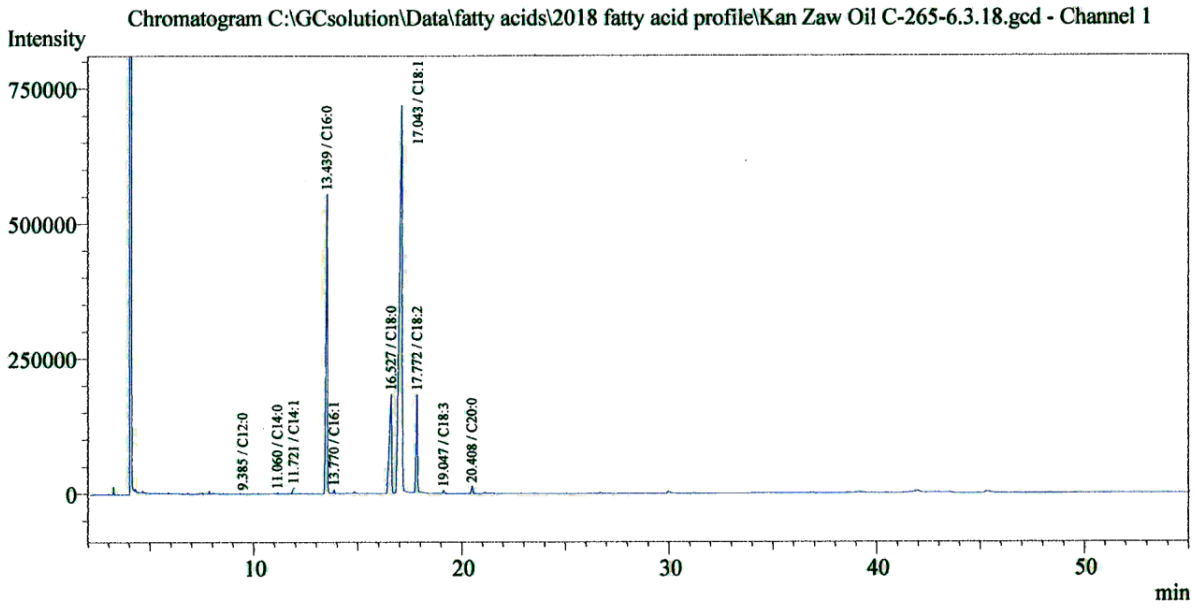


Figure 3 GC Chromatogram of Kan-zaw Seed Oil

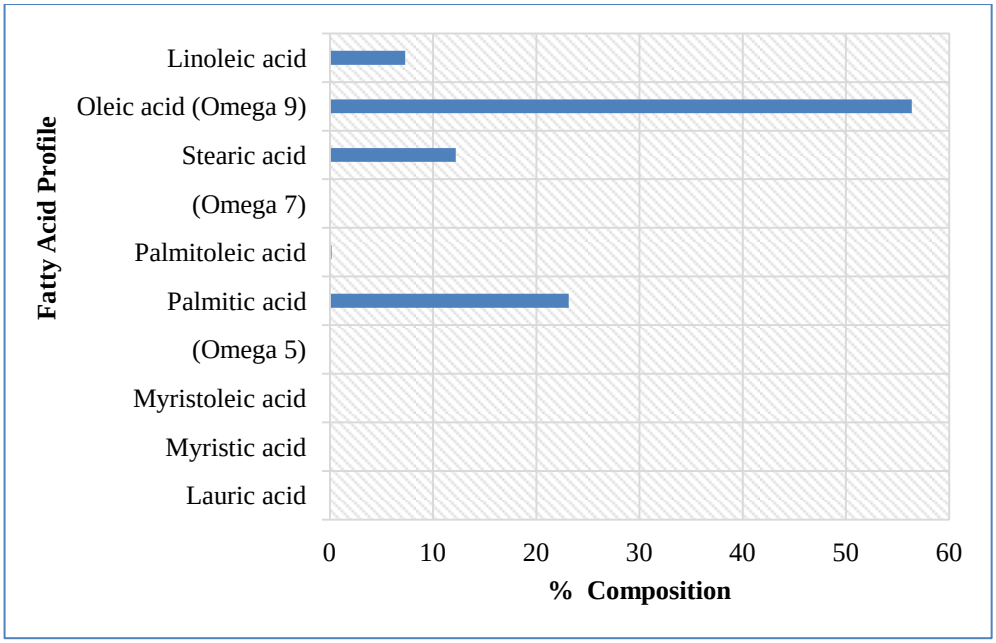


Figure 4 Fatty Acid Profile of Kan-zaw seed oil

Table 5 Fatty Acid Profile of Kan-zaw Seed Oil Analysed by GC

Sr. No.	Fatty Acid Profile	Molecular weight (g/mol)	Molecular formula*	Retention Time (min)	% Composition
1.	Lauric acid	200.322	$C_{12}H_{24}O_2$	9.385	0.01
2.	Myristic acid	228.37	$C_{14}H_{28}O_2$	11.060	0.047
3.	Myristoleic acid (Omega 5)	226.36	$C_{14}H_{26}O_2$	11.721	0.025
4.	Palmitic acid	256.43	$C_{16}H_{32}O_2$	23.137	23.137
5.	Palmitoleic acid (Omega 7)	254.41	$C_{16}H_{30}O_2$	13.439	0.162
6.	Stearic acid	284.48	$C_{18}H_{36}O_2$	13.770	12.205
7.	Oleic acid (Omega 9)	282.47	$C_{18}H_{34}O_2$	16.527	56.375
8.	Linoleic acid (Omega 6)	280.45	$C_{18}H_{32}O_2$	17.043	7.299
9.	Linolenic acid (Omega 3)	278.436	$C_{18}H_{30}O_2$	19.047	0.204
10.	Arachidic acid	312.54	$C_{20}H_{40}O_2$	20.408	0.607

*https://pubchem.ncbi.nlm.nih.gov>…

Table 6 Formulation of Pain Relief Balm

Run	Variable						Response		
	Beeswax (g)	Paraffinwax (g)	Vaseline (g)	Kan-zaw oil (g)	Camphor (g)	Menthol (g)	pH	Viscosity (cP)	Spreadability (gcm/s)
1	10.00	7.00	50.00	4.00	15.00	14.00	6.50	12560	27.77
2	10.00	3.00	51.00	6.00	16.00	14.00	6.20	11675	28.40
3	10.00	3.00	60.00	4.00	9.00	14.00	6.40	10975	27.85
4	7.00	7.00	50.00	4.00	14.00	18.00	5.90	11854	28.50
5	7.00	3.00	60.00	4.00	8.00	18.00	6.30	10650	29.20
6	10.00	4.87	56.23	4.94	8.00	15.96	6.10	11980	28.12
7	10.00	7.00	50.00	5.28	9.72	18.00	6.70	12780	27.30
8	7.97	3.00	55.28	4.79	10.96	18.00	6.60	10956	29.50
9	8.69	7.00	50.00	6.00	12.06	16.25	5.90	10675	30.45
10	7.00	3.00	56.00	4.00	16.00	14.00	6.30	12120	27.85
11	7.00	7.00	54.15	4.00	13.13	14.73	5.80	12050	27.00
12	7.00	7.00	59.56	4.44	8.00	14.00	6.40	11950	27.95
13	10.00	7.00	55.00	6.00	8.00	14.00	6.10	12750	26.25
14	7.00	6.38	54.62	6.00	8.00	18.00	5.70	12560	25.59
15	9.01	7.00	53.99	4.00	8.00	18.00	6.10	12655	26.65
16	7.00	3.00	57.36	6.00	11.52	15.12	6.50	11876	26.41
17	10.00	7.00	55.00	6.00	8.00	14.00	6.20	12945	25.75
18	7.00	3.00	50.00	6.00	16.00	18.00	5.50	10395	30.56
19	8.53	3.48	59.99	6.00	8.00	14.00	6.80	10655	30.23
20	10.00	3.21	51.55	6.00	11.24	18.00	5.60	11765	27.40
21	7.00	5.00	58.59	4.00	11.41	14.00	6.70	11654	27.90
22	10.00	3.00	50.00	4.00	15.00	18.00	6.40	10565	31.30
23	7.00	7.00	50.00	6.00	16.00	14.00	5.20	11786	28.25
24	10.00	3.00	60.00	4.00	9.00	14.00	6.10	10987	27.95
25	10.00	3.00	55.00	6.00	8.00	18.00	5.50	11905	28.20

Table 7 Predicted Formula of Pain Relief Balm

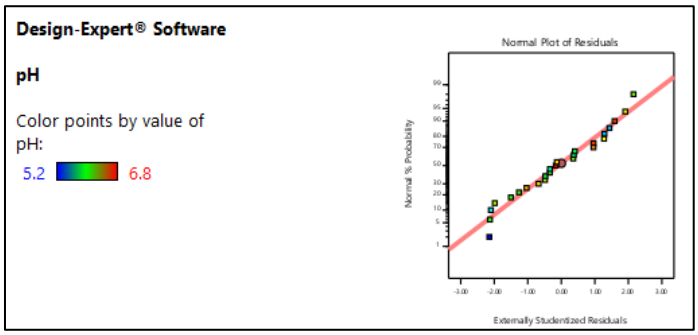
Sr. No.	Component	Composition (g)	pH	Viscosity (cP)	Spreadability (gcm/s)
1	B	8.63	6.52 ± 0.03	11613.33 ± 32	29.56 ± 0.65
2	P	4.76			
3	V	54.15			
4	KZO	4.93			
5	C	11.76			
6	M	15.76			

B = Beeswax, P = paraffin wax, V = Vaseline, KZO = Kan-zaw seed oil, C = camphor, M = menthol

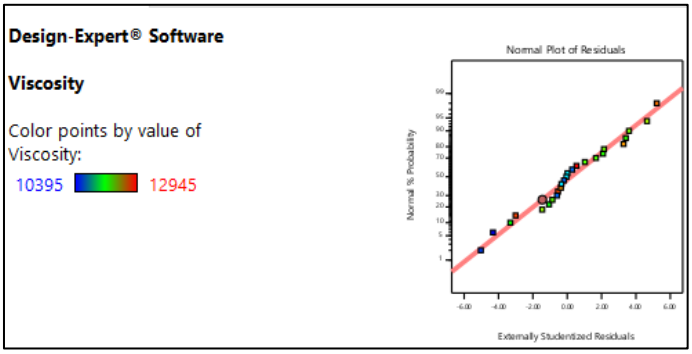
Table 8 Analysis of Variance (ANOVA) for Pain Relief Balm

Response	pH	Viscosity (cP)	Spreadability (gcm/s)
R-squared	0.9695	0.9852	0.9686
Mean squared	0.2046	2.054	2.55
P-value	0.042	0.011	0.044
F-value	6.35	11708	6.17
Pred R-squared	-8.2849	-5.6935	-13.0448

Figure 5 Pain Relief Balm



(a)



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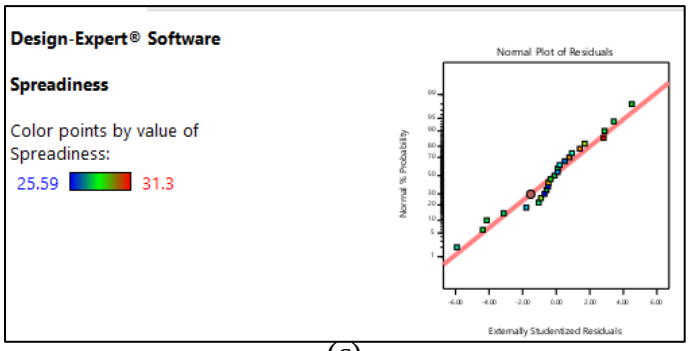
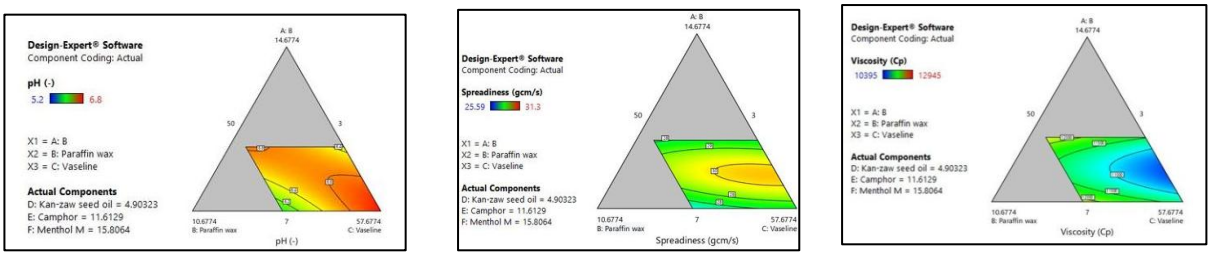


(c)

Figure 6 Residual Plots of (a) pH (b) Viscosity (c) Spreadability of Pain Relief Balm



(a) (b) (c)

Figure 7 Contour Graph of (a) pH (b) Viscosity (c) Spreadability of Pain Relief Balm

Table 9 Physico-chemical Properties of Pain Relief Balm (after 2 months)

Sr. No.	Formulation	pH	Viscosity (cP)	Colour & Appearance
1.	Pain relief balm	6.5±0.03	11578.67±17	pale yellow & non greasy

Table 10 Investigation of Consumer Acceptance for Pain Relief Balm by 9- Hedonic Scale

Panelist	1	2	3	4	5	6	7	8	9	10	Total score	Average	Overall acceptability
Odour	1	6	6	7	5	6	6	6	8	8	59	5.9	6.7
Sensation	5	7	6	8	7	7	7	8	8	8	71	7.1	
Relief of pain/aches	5	7	6	8	7	7	7	8	8	8	71	7.1	

9 =like extremely 6 = like slightly 3 = dislike moderately
8 = like very much 5 = neither like nor dislike 2 = dislike very much
7 = like moderately 4 = dislike slightly 1 = dislike extremely

Conclusion

In this research, Kan-zaw seed oil expressed by oil press machine was used to prepare the pain relief balm. The pain relief balm was formulated using D-optimal mixture design from Design Expert Software Trial version 11.0. By using design expert software, the robustness of the quality products can be obtained. The prepared pain relief balm showed significant effects in anti-inflammable activities. From this research, it can be concluded that Kan-zaw seed oil can be used safely in indigenous medicinal products and it can be a valuable potential source of new drugs due to the presence of many bioactive secondary metabolites in it.

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