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Physicochemical analysis of natural herbal medicated ointment enriched with *Cymbopogon nardus* and virgin coconut oil

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Abstract. Natural compounds from herbal plants have been extensively used for the application of various natural care products, including ointment. The essential oil of *Cymbopogon nardus* has been widely used in multiple applications such as mosquito repellent, perfumery and soap making. Besides, a small dose of this plant's volatile oil could be applied to comfort the stomach and aid digestion. Therefore, this study aims to formulate herbal ointments with varying *Cymbopogon nardus* oil concentration and investigate different oil concentration effects on the ointment properties. In this study, five ointment formulations of ratio virgin coconut oil to herb, 1:2, 1:4, 1:6, 1:8 and 1:10, were evaluated. At the same time, five infused oil ratios to beeswax (5:95, 10:90, 15:85, 20:80 and 25:75) were used. Preparation of *Cymbopogon nardus* oil involved the soaking of the prepared plant in virgin coconut oil. The infused oil then was mixed with the beeswax before it was heated and left to be semi-solid (ointment). The ointment then was tested for physicochemical properties, including hardness, viscosity, pH, turbidity, and colour. The developed ointment showed a good result within pH 6-7.5. Ointment from *Cymbopogon nardus* showed light green as visually observed. However, the colour became darker when the ratio of *Cymbopogon nardus* increased. The developed ointments' viscosity was almost the same as the commercial which the values were within 19.3-25 Pa.s. The comparison of the physicochemical properties of the developed and commercial ointments was almost similar. Thus, the developed ointment in this study can be considered safe and natural. However, the microbial and clinical tests must be conducted in further study to ensure the product's safety.

1. Introduction

Citronella grass, also commonly known as serai wangi in Malaysia is a perennial plant with long, thin leaves cultivated in parts of tropical and subtropical areas of Asia, Africa and America for its essential oils [1]. This plant is bisexual, where the flowers are produced during the matured stages of growth [2]. Interestingly, *Cymbopogon nardus* has been well known as aromatic grasses. Its genus species are aromatic and yield volatiles oil that is important for commercial values [3]. Therefore, these plants have been widely planted commercially by entrepreneurs to extract of its essential oil [4,5]. The active compounds of this oil such as citronellal, trans-geraniol, carvone and limonene are believed to have an anti-microbial effect [6]. In contrast, menthone, trans-geraniol and citronellal have been viewed as strong inhibitory effects [7].

A study by Weng et al. [8] reported that citronellal and citronellol's high content was found in the younger leaves of *Cymbopogon nardus*. In comparison, older leaves were found to have higher content



in geraniol. Besides, a high percentage of citral was found in the older leaves of *Cymbopogon nardus*, where the differences in smell and appearance might be due to this main compound [8]. The citral content had been claimed as a valuable compound that produces strong lemon odour and serves as an aroma compound, mostly in perfumery and flavouring, antimicrobial and vitamin A synthesis.

Throughout the world, herbal traditions have favoured the simple infusion and decoction of medicinal plants as their predominant form of extraction [9]. Infusion or decoction is a straightforward chemical process used with plants, typically dried herbs, flowers, or berries. The infusion releases the plants active constituents easily in water, oil, or alcohol. The infusion process is suitable for light structure substances, and soft tissues remarkably like leaves, flowers, and delicate stems. An infusion is a liquid preparation made by treating fresh or dried plant substances with either hot or cold liquid solvent to extract the medicinal and nutritional principles. Oil infusion is an infusion method of medicinal plants in a fixed oil solvent volume. It can be done using either dried or fresh herbs, roots, bark, and leaves by soaking or steeping into a solvent such as water, alcohol, or oil to extract the plant's soluble compounds. Herbal infused oil absorbs the selected herb's soluble compounds that were soaked or macerated in the warmed oil for a certain time. The herbs then strained and removed from the liquid solvent where the liquid will be applied in the future processes. The quantities used of herbs and liquid will vary according to the herb and may influence the strength of infusion required [10]. Herbal oil extract using the infusion method has been proven stable and safe when the proper and effective preparations of bioactive herbal ingredients applied [11]. This method was used back thousands of years ago for herbal medicine and anti-ageing cosmetic purposes [11].

Medicated topical preparation can be classified as solid in dusting powder form; semi-solids such as creams, gel, ointments, pate and other; liquids in the form of a solution, emulsion, liniments, suspension, soaps, collides, lotion, paints and others [12]. Ointments are semi-solid and greasy preparation for external application to the skin, rectum or nasal mucosa [13]. Ointments can be defined as a composition of fluid hydrocarbons meshed in a matrix of higher solid hydrocarbons [14]. Herbal ointment consists typically of plant materials either in finely sifted or extracted form before incorporating into the solid greasy base [15] such as hard and soft paraffin, natural petroleum jelly, cetyl alcohol, lanolin, magnesium with or without modifying materials [16]. Besides, medicated ointments contain a medicament or medicaments dissolved, suspended, or emulsified in an ointment base, known as vehicles [17]. The ointment base is usually hydrophobic and immiscible with skin secretions [18]. The ointment product is usually packed indefinitely in small beauty containers or cream jars with lids [13]. An ointment can be applied as emollients or to apply suspended or dissolved medicaments to the skin [19]. It also consists of nutritional value that gives the required nutrients to the skin [16]. Besides, the product can also be applied for several purposes such as protectants, antiseptics, antipruritic, keratolytic and astringents [20]. The ointment products can cling to the surface of the application prior washed or worn off within the reasonable duration is one of the product's common properties [16].

All medicines produced from plant original or even synthetic should meet the requirements of safe and effective [21]. The safety and quality of medicinal plant materials and finished herbal medicinal products have become the main concern for health authorities, pharmaceutical industries and the consumers as the increasing the usage of the herbal medicines worldwide and the rapid expansion of the global market for these products [22]. Several reports documented that not all herbs are safe and could contribute to severe adverse effects [23,24]. Instead of that, it has also been reported that some products are not of the desired quality due to the absence of a regulatory standard. Moreover, quality tests and production standards tend to be less rigorous or controlled, and, in some cases, traditional health practitioners would not be certified or licensed [25].

Skin and skin related diseases are ranked top among the various indications where traditional herbal medicines are used [26]. The unavailability of rigid quality control profiles for herbal materials and their formulations is one of the herbal industry's crucial problems [27]. Thus, an assessment of the quality of herbal formulations is highly recommended to establish their acceptability in the modern medicine system. Worst, the inadequate and accepted research methodologies for evaluating natural products cause the lack of data on quality, safety and efficacy [28,29]. There are two classifications of categories

on the quality issues of herbal medicine which are external and internal. External cases involve contamination, adulteration, and misidentification, while internal issues are complexity and non-uniformity of the ingredients in herbal medicines that influence the quality of the herbal medicines [30]. On the other hand, potentially hazardous contamination and residues may occur in herbal medicines. Contaminants in herbal medicines are classified into physicochemical pollutants and biological contaminants [22]. Pesticide residues are one of the examples of physicochemical contaminants while microorganisms, including fungi, is the example of biological contaminant [28].

Recently, home-made medicine like ointment (purposely to sell the product) has been widely formulated without undergoes any properties test which follows the standard. Therefore, the current study aims to formulate herbal ointments with different concentration of *Cymbopogon nardus* to virgin coconut oil as well as to assess the quality status of natural herbal medicated ointment enriched with *Cymbopogon nardus* and virgin coconut oil based on the physiochemical properties including colour, hardness, viscosity, pH and turbidity.

2. Materials and Methods

2.1 Sample preparation

Fresh leaves of *Cymbopogon nardus* was collected from the local area of Jeli, Kelantan, Malaysia. Collected leaves were cleaned by removing desired stems and waste materials. The damage and black leaves were removed manually. The leaves then were cut into small pieces before subjecting to drying methods. The leaves were dried in a hot air oven at a temperature of 65°C for three days. After that, the dried leaves were ground into a coarsely powdered form using a mechanical grinder and preserved in a plastic bag to avoid contamination and surrounding moisture.

2.2 Plant extraction

The extraction of *Cymbopogon nardus* was carried out using the hot infusion method with an oven's aid. The dried leaves were mixed with virgin coconut oil according to the set ratios of herbal infusion oil. In this study, five ointment formulations were evaluated which the ratios of virgin coconut oil to herb were 1:2, 1:4, 1:6, 1:8 and 1:10. The mixture was heated at 65°C for 5 hours using an oven. The mixture then was strained into the beaker by cotton muslin cloth to separate the herbs from the oil infusion. Finally, the herbal infusions of *Cymbopogon nardus* were ready for the preparation of ointment. Figure 1 shows the changes in virgin coconut oil colour before and after the extraction of *Cymbopogon nardus*.

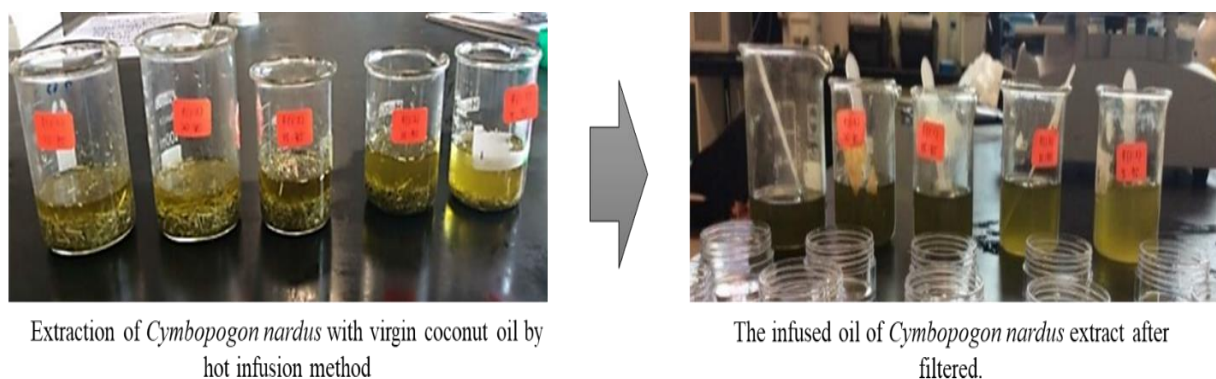


Figure 1. Changes of virgin coconut oil colour before and after the extraction of *Cymbopogon nardus*.

2.3 Formulation of natural herbal medicated ointment

In this study, beeswax was used as a base for the ointment. Five infused oil to beeswax ratios which are 5:95, 10:90, 15:85, 20:80 and 25:75 were used. In the ointment preparation, herbal oil infusion and beeswax mixture was heated up to 60°C using hot plate until the wax was fully melted before removing the mixture from the heat. The mixture was poured into ointment containers while the mixture was still warm and flowing smoothly. The ointment was then let to cool and harden at room temperature before evaluating the physicochemical properties' analysis.

2.4 Physicochemical properties evaluation

All formulated natural herbal medicated semi-solid ointments were tested for physicochemical properties including colour, hardness, viscosity, pH and turbidity. In this study, the properties of the developed ointment were compared with the commercial ointment product brand Tiger Balm (Red) as a benchmark.

2.4.1 Colour. Colour of all formulated natural herbal medicated ointments were analysed by visual observation.

2.4.2 Hardness. The hardness of the ointments was determined using Ametek Brookfield CT-3 Texture Analyzer by using cylinder probe (2mm diameter, 20mm length) as shown in Figure 2. The ointment sample was poked by the cylinder probe twice for each formulation. The reading was viewed at the monitor and recorded. The value of hardness was measured at room temperature ($25 \pm 2^\circ\text{C}$) and repeated three times.

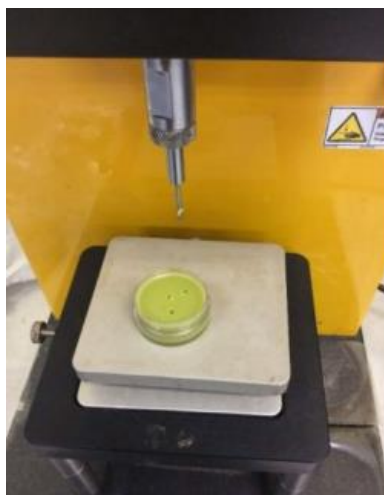


Figure 2. Texture analysis of *Cymbopogon nardus* ointment.

2.4.3 Viscosity. The measurement of viscosity of ointments was carried out using Sine-wave Vibro Viscometer SV10/SV100. 20g of ointment sample was taken out and filled into the provided glass container and put on the stand. The value of each formulation was measured in triplicate and expressed in a mean.

2.4.4 pH. 2.5g of the sample was taken out into 100 ml beaker and added with 50 ml of distilled water. The beaker was heated using a water bath maintained at about 60°C to 70°C, and then let it cool at room temperature before centrifuged at 3000 rpm for 30 minutes. The pH measurement of the water extract was observed using a pH meter. The pH value obtained was repeated in triplicate. All test were carried out at room temperature [31,32].

2.4.5 Turbidity. The turbidity of each ointment formulation was analyzed using UV-VIS Spectrophotometer Spectroquant Pharo 300 at 280 to 290 nm. 0.5 g of sample was weighed and diluted with 20 ml of distilled water before heating on the hot plate until the sample was completely melted. The mixture then was filled in a cuvette using the dropper, and the turbidity of the sample was analyzed. The turbidity test for each ointment sample was carried out in triplicate

3 Result and Discussion

3.1 Hardness, viscosity, pH and turbidity.

Table 1 presents the results of hardness, viscosity, pH and turbidity for 25 natural herbal medicated ointments formulation (F1-F25) enriched with *Cymbopogon nardus* and virgin coconut oil. The hardness, viscosity, pH and turbidity of the commercial ointment, was also measured for comparison purpose.

As stated in Table 1, F5 shows the highest hardness value (435.00 g) while the lowest value goes to F25 (11.00 g). The formulation of F5 contains 3.33 g of *Cymbopogon nardus* and 10 ml of virgin coconut oil while F25 formulation comprises of 6.81 g of *Cymbopogon nardus* and 21 ml of virgin coconut oil. The apparent reason between these two comparisons is due to the amount of virgin coconut oil used in F25 is higher than F5, which act as a solvent to dilute the phytochemical of *Cymbopogon nardus*. The higher volume of solvent applied in the formulation, the lower the hardness value of ointment formulation as presented in Table 1. Beeswax, which acts as a thickener [33] also influences the hardness values. As the ratio of beeswax increases in the ointment formulation, the hardness readings also increase according to the ratio of infused oil to the beeswax of 1:2, 1:4, 1:6, 1:8 and 1:10. The hardness of ointment also increases with increasing the content of solid matter in it [34]. Thus, it can be concluded that the hardness of ointment depends on the amount used of beeswax and virgin coconut oil utilised in the ointment formulation.

The viscosity of the ointment formulations is also recorded in Table 1. The viscosity of the formulated ointments is also influenced by the amount of beeswax used in the ointment formulation. The higher saturated fatty acid fraction of the wax structure, the more viscous the ointments as can be seen for the formulation of F21 (1:10 of infused oil to beeswax), with the viscosity value is 1830 cP while F11 (1:6 of infused oil to beeswax), with the viscosity value, is 8260 cP. The result of viscosity gives an idea about the measurement of strength which the prepared formulations readily spreads on application to the skin or affected part [34]. Besides, viscosity is related to spread ability, where the viscosity of the formulation increases as the spread ability decreases and vice versa. The highest viscosity was achieved in F4 (42300 cP) and the lowest in F22 (1510 cP). These indicate that F22 shows better spread ability as it also composes of higher virgin coconut oil volume (18 ml) as compared with F4 (11 ml of virgin coconut oil). Therefore, F22 can be considered as a highly spreadable ointment that can easily cover the surface of the skin or affected area. Thus, it enhances the diffusion of substances from a dosage form to the external compartment.

From Table 1, the pH values of all the prepared formulations ranged from 5.22 to 7.59, which can be considered as acceptable to avoid the risk of irritation upon application to the skin. The pH values also show a relatively similar pH with the pH of the normal skin except for formulations of F1 (7.13), F3 (7.36), F4 (7.15) and F5 (7.59) which show a bit alkaline condition. pH range from 3 to 7 is suitable for colouring from sour to neutral foods and cosmetic products [35] while skin surface pH is on average between 5.0 and 6.0 [36]. Previous studies on topical herbal formulation from *Dodonaea viscosa L Jacq.* as reported by Shanti [37] showed that the pH range of the formulations was around pH 6. Monitoring the pH value is essential for determining the stability of ointment because pH changes indicate the occurrence of chemical reactions that can compromise the quality of the final product [38].

Table 1: Analysis of physicochemical properties of *Cimbopogon nardus* ointment

Formulation Code	<i>C. nardus</i> : VCO	Infused oil: Beeswax	Physicochemical Analysis			
			Hardness (g)	Viscosity (cP)	pH	Turbidity
Tiger Balm	-	-	49.33	19300	6.06	3.78
F1	5:95	1:2	217.00	30100	7.13	0.903
F2	10:90		228.33	41300	6.88	0.512
F3	15:85		333.00	25500	7.36	0.947
F4	20:80		410.00	42300	7.15	0.983
F5	25:75		435.00	23700	7.59	1.213
F6	5:95	1:4	146.33	15300	5.43	0.071
F7	10:90		163.33	17250	5.95	2.762
F8	15:85		172.00	22570	5.53	1.381
F9	20:80		193.67	27900	5.59	0.104
F10	25:75		176.33	21600	5.51	0.095
F11	5:95	1:6	57.67	8260	5.91	0.011
F12	10:90		36.67	1530	5.71	0.081
F13	15:85		48.33	1820	5.46	0.689
F14	20:80		46.33	5220	5.45	0.944
F15	25:75		55.00	31730	5.48	0.037
F16	5:95	1:8	36.67	2600	5.22	0.384
F17	10:90		80.33	7530	5.30	0.448
F18	15:85		114.00	11630	5.32	2.708
F19	20:80		47.67	2330	5.36	0.384
F20	25:75		42.67	2720	5.35	0.054
F21	5:95	1:10	20.67	1830	5.45	0.749
F22	10:90		34.00	1510	5.63	2.299
F23	15:85		64.67	1680	6.09	0.952
F24	20:80		39.67	2350	6.14	2.255
F25	25:75		11.00	5450	5.50	0.740

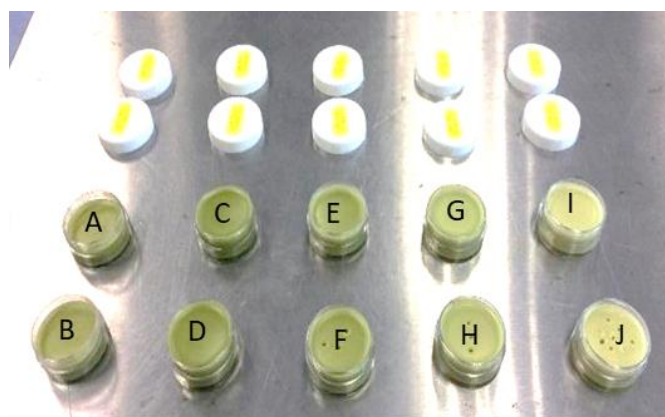
The turbidity of the ointment was measured to observe the turbidity level of the ointment at different concentration of ingredients. The value of F7 (2.762) and F11 (0.011) show the highest and lowest turbidity values of the ointment. It gives a meaning that the ointment absorbs most of the light in F7 and

only the small amount of the light detected for F11 due to its highly turbid formulation. In contrast, the commercial ointment (Tiger Balm Red) turbidity value is 3.780. This value may be due to the presence of several active ingredients' composition such as menthol, camphor, clove oil, cajuput oil, hard paraffin, white paraffin, peppermint oil, cassia oil and robust ammonia solution. The formulated ointment in this study presents a lower value of turbidity because the formulation only includes Citronella nardus oil, virgin coconut oil and white beeswax.

Optimum formulation of ointment has been successfully identified where F9 showed the closest measured physicochemical properties with the commercial ointment with the values of hardness, viscosity, pH and turbidity were 199.10 g, 36299 Pa.s, 5.74 and 0.5714, respectively.

3.2 Colour.

The colour of the formulated ointments is presented in Figure 3. This figure illustrates that the gradual changes of *Cymbopogon nardus* ointment from light green to dark green as visually observed. The green colour is produced due to the natural green colour pigment from the *Cymbopogon nardus* plant during the infusion process. The colour became darker when the ratio of *Cymbopogon nardus* increased. It can be concluded that the higher amount of *Cymbopogon nardus* applied in the formulation, the darker the green colour of the ointment.



A, B, C, D- dark green,
E, F, G, H- medium green
I, J- light green

Figure 3. Colour changes of ointment

4. Conclusion

The present study has successfully formulated the natural herbal medicated ointment enriched with *Cymbopogon nardus* and virgin coconut oil. Physicochemical parameters of ointment have been successfully analyzed in terms of hardness, viscosity, pH, turbidity, and colour. The value of hardness was ranging from 533.00 to 11.00 g while turbidity was 0.011 to 2.762. The viscosity of the developed ointments was almost the same with the commercial which the recorded values were within 19.3-25 Pa.s. The pH values for all ointment range within 6-7.5. The colour became darker as increase the amount of *Cymbopogon nardus* in the ointment formulations. Optimum formulation of ointment has been successfully identified where F9 showed the closest measured physicochemical properties with the commercial ointment with the values of hardness, viscosity, pH and turbidity were 199.10 g, 36299 Pa.s, 5.74 and 0.5714, respectively. Thus, this F9 formulation can be considered as the optimum ointment

formulation in this study. Further microbial and clinical tests are warranted to commercialize this product.

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