Colour and spreadability of Neem (Azadirachta Indica A. juss) ointment and cream formulations

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Abstract. Herbal plants are a major source of raw material for traditional medicines. Recently there has been an increase of interest to study the therapeutic potential of herbal plants as herbal care products. In this study, a preliminary study on the formulation of neem (Azadirachta Indica) ointment and cream have been conducted. The neem leaves were extracted and formulated into ointment and cream. The raw neem extract is added into the ointment and cream bases at four different concentrations (0% w/w, 0.5% w/w, 1% w/w and 2% w/w) and stored at three different storage temperatures (4°C, 25°C and 45°C). The semambu ointment and cream formulated were evaluated in terms of their colour and spreadability. It has been found that the extract content and storage temperature influence the colour and spreadability of the formulated neem ointment and cream.

1. Introduction
Herbal plants are popularly used for their therapeutic, medicinal, aromatic or savory qualities. One or more parts of herbal plants contain and produce a variety of chemical substances and unique bioactive ingredients that can be advantageous to the human body. It has been reported that not less than 80% of the world’s populations use herbal medicine for some part of their primary health care [1]. The demand of herbal medicine that is derived mainly from herbal plants is growing exponentially as the productions of these health care products require a great amount of natural ingredients from herbal plants. This also brings up the interest in the design of formulations which contain active natural herbal ingredients that acts as vehicle to deliver the drugs to specific targets in the body.

Pharmaceutical semisolid such as ointment and cream are the examples formulation which can be use as the vehicle to deliver the herbal medicament. The herbal plants extract which acts as the active ingredient is added to the semisolid to give the therapeutic effect. Natural extract of aloe vera, hena, tomato and cucumber are some of example for the most commercial and well known pharmaceutical semisolid products. Neem or semambu (Azadirachta indica A. Juss) as it is known in Malaysia, is one of the most useful traditional medicinal plant which is well known in India (Neem) and neighbouring countries [2]. In Malaysia, semambu leaves are traditionally used to cure mild skin diseases such as measles and scabies. Besides medicinal potential, this plant has also other potential such as for use as a pesticide [3] and pollution control properties [4, 5].

There were a number of recent works that studied the formulation of azadirachta indica based substances into ointments and creams but some important properties such as colour and spreadibility were not investigated and quantified [6, 7] especially at different storage temperatures. Hence, the objectives of this present work are to formulate semambu topical ointments and cream, and to evaluate their physical sensorial properties in terms of colour and spreadability when stored at different storage temperatures.
2. Material and methods

2.1 Preparation of semambu leaves
Fresh healthy semambu leaves (Azadirachta Indica A. juss) were collected from the Faculty of Design and Architecture, Universiti Putra Malaysia. The semambu leaves were destalked. Then the leaves were spread evenly and allowed to dry in an oven at 45°C for 15 hours, where this method had been reported that the semambu extraction leave can still preserved relatively high Total Phenolic Content (TPC) values [8]. The dried semambu leaves were then ground to fine powder and stored in air tight containers.

2.2 Preparation of semambu leaf extract
The dried semambu powders were weighed for approximately 5 g. Then, the powder was extracted with 200 ml methanol solution for 5 hours by using Soxhlet apparatus (Electrothermal, U.K). Then the extract solutions were filtered using a Whatman No.1 filter paper. The resulting semambu leaves extracts were evaporated at 45°C temperature using a rotary evaporator (Laborota 4001 Efficient, Heidolph, Germany) to obtain the respective extracts. The concentrated extracts were stored in a refrigerator at -4°C until used for further analysis [8].

2.3 Formulation of semambu ointment and cream
For semambu ointment formulation, the required quantities of petroleum jelly were placed in the water bath and allowed to melt at 70°C. After melting, the liquid petroleum jelly was stirred gently at the temperature of 70°C and the semambu leave was added. The mixture were then cooled and solidified forming the semambu ointment. Meanwhile, for semambu cream, the semambu leave extract was directly mixed with the aqueous cream without heating. The semambu leave extract were added at four different concentrations (0% w/w, 0.5% w/w, 1% w/w and 2% w/w). The different concentrations of semambu ointment and semambu cream prepared were stored at 4°C, 25°C, and 45°C and the physical tests were conducted after 1 week of storage period.

2.4 Physical analyses
2.4.1 Colour
The colour of the samples was measured by using a Colour Reader (Konica Minolta Sensing, Japan). L*, a*, and b* values were measured for each sample. The L* value represent the relative brightness, ranging from total black (L*=0) to total white (L*=100) and a* and b* are the chromaticity coordinates. Where, the chromaticity coordinate a* represents the different between red (positive value, +a*) and green (negative value, -a*) and the chromaticity coordinate b* represents the difference between yellow (positive value, +b*) and blue (negative value, -b*). The meter was calibrated against a standard white plate before each measurement. This method for evaluating the colour of materials has been previously used for herbs and food products [8, 9].

2.4.2 Spreadability
The objective the spreadability characterization for the different concentrations of the semambu ointments and semambu creams is to mimick the process of applying them onto the human skin. The spreadability of the samples was measured using a texture analyzer (Stable Micro Systems TA XT Plus, UK). TTC Spreadability Rig was used to evaluate the spreadability in terms of the firmness properties of the samples. The TTC Spreadability Rig is a set of precisely matched male and female perspex 90° cones. Tests were carried out by fillong the sample in the female cones sample holders immediately after removal from storage. Then the tested samples in female cones were locked into the base holder precisely centred under the matching upper male cone probe. During the experiment, the male cone probe is programmed to penetrate the samples in the female cones holder and the sample is squeezed out from the female cones. A force versus time data of penetration of the male probe through the ointment or cream in the female probe was obtained. When the male TTC Spreadability Rig probe
penetrated into the female probe which contains the sample, the force applied increases up to the point of maximum penetration depth. The maximum force value can be taken as the 'firmness' at the specified depth.

3. Results and discussions
3.1 Colour
Figure 1 illustrates the lightness (L* value) for the semambu ointments. Pure petroleum jelly exhibit relatively higher lightness in comparison to ointments containing semambu extracts. There are no apparent differences in terms of lightness between the semambu ointments with 0.5%, 1% and 2% concentrations. There is a slight increase in the L* value when the storage temperature increases from 4°C to 45°C for ointments containing 0.5% and 2% semambu extracts.

Figure 2 illustrates the chromaticity coordinate a* value for semambu ointment where this value represented the differences between red (positive value) and green colour (negative value). It can be observed that as the storage temperature increases, the a* value becomes more positive, indicating that the ointment is more towards the red colour.

As can be observed in Figure 3, all four different concentrations of semambu ointments give a positive b* value. This indicates that the semambu ointment is toward the yellow colour. Increasing semambu extract concentration decreases the b* value. As the temperature increases from 4°C to 25°C, the b* values decreases. But as the temperature is further increased to 45°C, the changes were not apparent. This shows that the b* value are affected by low storage temperature changes between 4°C and 25°C.

In general, the lightness of the semambu cream is decreasing as the added semambu extract concentration increases as shown in Figure 4. There are generally no apparent differences in terms of lightness between semambu creams with 0.5% and 1% semambu extract concentrations except at the highest storage temperature of 45°C. The lowest lightness is observed for the cream with 2% semambu extract concentration at 45°C storage temperature.

Figure 5 represents the chromaticity a* for semambu cream. All the four different concentrations of the semambu creams give negative a* values indicating towards green colour when stored at 4°C and 25°C. A higher storage temperature of 45°C produces semambu creams that exhibit higher redness in their colours; positive a* values. Hence, storage temperature also affects the a* value where the a* value generally increases (becomes more positive) with increasing storage temperature.

In terms of the colour lightness or L* value, the obvious differences between the semambu ointment and semambu cream are that the effect of semambu extract concentrations is more pronounced for the semambu cream formulations depicted by the higher L* value fluctuations (Figure 6) compared to semambu ointments (Figure 1). For the a* value, semambu ointment gives positive values at all the storage temperatures but for semambu cream only the semambu cream at storage temperature of 45°C gives a positive (red colour) result. Meanwhile, for the b* value which represent the differences between yellow (positive value) and blue colour (negative value), both semambu ointment and semambu cream yield positive b* value results but the semambu creams exhibit relatively higher positive b* value (yellow colour) then the semambu ointments.
Figure 1. $L^*$ value versus storage temperature for semambu ointments containing 0% w/w, 0.5% w/w, 1% w/w and 2% w/w semambu extracts.

Figure 2. $a^*$ value versus storage temperature for semambu ointments containing 0% w/w, 0.5% w/w, 1% w/w and 2% w/w semambu extracts.
Figure 3. $b^*$ value versus storage temperature for semambu ointments containing 0% w/w, 0.5% w/w, 1% w/w and 2% w/w semambu extracts.

Figure 4. $L^*$ value versus storage temperature for semambu creams containing 0% w/w, 0.5% w/w, 1% w/w and 2% w/w semambu extracts.
Figure 5. $a^*$ value versus storage temperature for semambu creams containing 0% w/w, 0.5% w/w, 1% w/w and 2% w/w semambu extracts.

Figure 6. $b^*$ value versus storage temperature for semambu creams containing 0% w/w, 0.5% w/w, 1% w/w and 2% w/w semambu extracts.

3.2 Spreadability
The spreadability properties can be determined by measuring the firmness of the sample. The firmness of the semambu ointments and semambu cream at three different storage temperatures for four different semambu extract concentrations are presented in Figure 7 and Figure 8.

As the storage temperature of the semambu ointment increases, the firmness of the semambu ointments decrease exponentially as illustrated in Figure 7. At storage temperatures of 25°C and 45°C, the firmness of the semambu ointments are lower compared to the semambu ointments stored at 4°C. The ointment network structure degrades as the temperature increases which resulting the semambu ointment firmness decreases as the storage temperature increases [10]. Figure 7 also illustrates that the
firmness of the semambu ointments is inversely proportional to the added semambu extract concentrations, where the higher the concentration of the semambu extract, the lower the firmness of the ointment formulation. This shows that the spreadability of the formulated semambu ointments increases with increasing semambu extract concentration. The amount of aqueous semambu extract added will influence the properties of the ointment as the network structure of the ointment base has been disrupted [11]. Hence, the semambu ointment that exhibit the highest spreadability is the ointment which has the highest amount of semambu extract added, 2% w/w and being stored at highest storage temperature of 45°C. There is also a relatively higher difference in the ointment firmness when the storage temperature increases from 4°C to 25°C in comparison to those observed between the storage temperature increases from 25°C to 45°C.

Figure 8 illustrates that the firmness for semambu creams containing 0.5% w/w semambu extract exhibit a relatively linear decrease as the storage temperature increases. The effect of increasing storage temperature on the spreadability of the semambu creams is more pronounced as the concentration of the semambu extract increases. Higher semambu extract concentrations and storage temperatures lowers the semambu creams firmness hence enhances their spreadabilities. Thus, the semambu cream exhibiting the highest spreadability contains 2% w/w of semambu extract stored at 45°C.

The firmness of the semambu ointments are relatively higher compared to semambu creams at lower storage temperatures of 4°C and 25°C. This shows that the semambu ointment has a firmer structure and thus lower spreadability relative to the semambu cream at these storage temperatures. Both formulations yield similar trends where the spreadability increases with the increase in the added semambu extract concentration and storage temperature.

![Figure 7](image_url)

**Figure 7.** The firmness of the semambu ointments.
4. Conclusions
The physical properties of the semambu ointment and semambu cream formulations were evaluated in terms of their colour and spreadability. In terms of colour, semambu ointment shows a more stable formulation compared to semambu cream when subjected to a variation in the storage temperature and semambu extract concentration. From the observation, it can be concluded that the firmness for the semambu ointment and semambu cream decreases as the storage temperature increases. In addition, the semambu ointment has a firmer structure and low spreadability properties compared to the semambu cream.

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