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Development of Neem Oil and Virgin Coconut Oil Based Cream Formulations with Methanolic Extract of *Leucas zeylanica* and *Ophiorrhiza mungos* leaves

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Ultraviolet radiations (UV) cause numerous skin diseases when exposed in excess. *Ophiorrhiza mungos* (OM) and *Leucas zeylanica* (LZ) have identified as plants with high Sun Protection Factor (SPF) values. Neem oil and Virgin Coconut Oil (VCO) are natural oils that have been used in Ayurvedic medicine for several indications. The objective of the study was to evaluate the SPF activity and carry out stability studies in Neem oil and VCO based cream formulations consisted with methanolic extracts of OM and LZ. The Methanolic extracts were incorporated in to selected ratios of VCO and Neem oil bases. Samples were subjected to secondary homogenization. Most stable ratios of the emulsions were identified as 33% VCO: 35% water: 32% $T_{80,}$ and 29% Neem oil: 42% water: 29% $T_{80.}$ Cream formulations were prepared based on above identified ratios. All creams were thermodynamically and kinetically stable more than 120 days at room temperature (28± 2 ^{0}C). The SPF values of creams were above 30 and they showed higher SPF values compared to the pure leaf extracts of OM and LZ. Neem oil based creams had the highest SPF values which is greater than 38. The creams were o/w type and in the acceptable pH range for topical application. Due to the consistency of the creams, they could retain on the skin for longer period. All creams composed with other standard sunscreen characteristics which increase the market value of the product.

Keywords. Sun Protection Factor, Stability evaluation, Characteristics**Introduction**

Ultraviolet (UV) radiation cause sunburns, wrinkles, premature aging and skin cancers (Dutra *et al*., 2004). It is considered that sunscreen agents with the Sun Protection Factor (SPF) value of 15 or greater are suitable for the use against the harmful effects of solar radiation (Ratnasooriya *et al*., 2014). The public prefer to use herbal creams as alternative photo protective agents, because some synthetic ingredients such as Amino Benzoic acid can cause photo sensitivity reactions. This study was done using *Ophiorrhiza mungos* (OM) and *Leucas zeylanica* (LZ) which were previously identified as potential agents with photo protective effect SPF values as 39.2 (+/- 0.92) and 39.8 (+/- 0.35) respectively (Napagoda *et al*., 2016).

For this study, two natural oils, Virgin Coconut Oil (VCO) and Neem oil were selected (Sanjeewani and Sakeena, 2013 and De Silva *et al*., 2018). Tween 80® (Polyoxyethylene sorbitan monooleate - T_{80}), molecular formula $C_{64}H_{124}O_{26}$, was used as the surfactant. The objective of the study was to evaluate SPF activity and to formulate stable cream formulation(s) using Neem oil

Table 01 - Ratios of Oil: water: surfactant used to prepare emulsions for preliminary study

Plant type		Oil		Surfactant (T_{80})	Water
OM	LZ	VCO	Neem oil		
I	I'		3.0	3.0	4.0
J	J'		3.1	3.0	3.9
K	K'		2.9	2.9	4.2
L	L'		3.2	3.0	3.8
S	S'	3.2		3.2	3.6
T	T'	3.3		3.2	3.5
U	U'	3.3		3.1	3.6
V	V'	3.4		3.2	3.4

and VCO based emulsions with methanolic extracts of OM and LZ.

Methodology

Preparation of leaf extracts

Two hundred grams of air dried and grinded plant leaves were extracted by maceration with 800 mL of 99% (v/v) methanol in 1000 mL amber colour bottles for 03 days while occasionally stirring. Resulted solutions were filtered and concentrated by using a rotary evaporator (HAHNSHIN Scientific-Model No: HS-2005V, Sr no V-00449) at 65 ^{0}C and 145 rpm. Concentrated extracts were further evaporated using a water bath at 65 ^{0}C for 02 hours to get solid extracts. The solid extracts were labelled and stored at 04± 2 ^{0}C for further use.

2.2 Determination of SPF values of the leaf extracts

Two sets of dilution series of the leaf extracts were prepared (2.0 mg/ mL, 1.0 mg/ mL, 0.5 mg/mL, 0.125 mg/mL and 0.625 mg/mL) using 99% v/v methanol as the diluent. The absorbance of UV radiation by each extract were tested using UV spectrophotometer (Spectrum instruments-SP-UV-5000DB) in the range from 290 to 320 nm, at 05 minutes intervals. Then the SPF values were calculated using Mansur equation (Mansur *et al.*, 1986).

2.3 Development of secondary emulsions with leaf extracts

Selected ratios were tested as in table 01. Secondary homogenized emulsions were prepared by using a high shear homogenizer (IKA® T25 digital ULTRA-TURRAX®) at 10000 rpm for 05 minutes at RT.

Stability evaluation of secondary emulsions

Creaming index of each secondary emulsion was observed after 24 hours. Long term stability of the emulsions were observed in 7th, 14th, 30th, 60th, 75th and 90th days at RT, 04± 2 ^{0}C and 40± 2 °C.

Five grams of most stable secondary emulsions were centrifuged (VS – 600N 2007) at 1200 rpm for 05 minutes at RT and observed for 30 days for the evaluation of accelerated stability.

Characterization studies of secondary emulsions

pH values were measured using pH meter (Trans Instrument BP 3001) at RT on initial day and 75th day.

SPF evaluation was done only for most stable secondary emulsions as mentioned in 2.2. Viscosities of the most stable secondary emulsions were measured by using BROOKFEILD Viscometer (Model No, LVDV-II+).

Development of topical creams

Each cream was made using the most stable emulsions. Steric acid (10g), Glycerine (10g), Glycerolmonostearic – GMS (4.5g), Triethanolamine – TEA (0.5g) and Methyl paraben (0.1g) were used as excipients.

Dried leaf extract was incorporated to obtain the 0.1% w/w per cream formulation.

Characterization and evaluation of stability of cream formulations

The SPF, pH and viscosity were determined as per in section 2.5. Microscopic analysis was done using an optical microscope at 40 X 10 magnification using methylene blue as the staining dye. The stabilities of cream formulations were tested at different temperatures (RT, 04 ± 2 ºC and 40 ± 2 °C). The long-term stability evaluations were done on 3rd, 14th, 30th, 60th, 75th 90th and 120th days. For the accelerated stability studies were done as per in 2.4 and observed phase separation for 30 days at RT.

3.0 Results and Discussion

3.1 SPF of the leaf extracts

The SPF values of methanolic extracts of OM and LZ leaves in 1mg/mL concentration were 39.78 and 39.73 respectively and in 0.5 mg/mL concentrations were 25.03 and 17.42 respectively. It was shown that both methanoloic extracts of LZ leaves and whole plant has almost similar SPF values.

3.2 Stability of secondary emulsions

Initially no phase separation was observed in the secondary emulsions. Therefore, the creaming indices were zero. Emulsions, T, T', V and V' were stable in RT, 04± 2 ºC and 40± 2 °C for 90 days. K and K' were stable for 90 days in RT, 4± 2 ºC but it was only stable for 75 days in 40± 2 °C. The instability characteristics initially appeared in the emulsions that stored at 40± 2 ^{0}C. It could be due to the effect of high temperature on the constituents of the emulsions (Suryati *et al.*, 2015). Among the secondary emulsions that were subjected to centrifugation, V, K, V', and K' were stable only for 14 days at RT. However, T, T' were stable for 30 days at RT.When the emulsions are under the high speed centrifugation force, they tend to undergo phase separation faster than in the normal conditions (Badolato *et al.,* 2008). Based on the stability studies, the most stable ratios were T/T' and K/K'.

3.3 Characterization studies of the secondary emulsions

The initial pH values of all emulsions were in the range of 6.47 to 6.89 at RT and in 75th day they were between 6.21 – 6.82 at all temperatures. Thus they were in the acceptable pH range for topical application (Lambers *et al.*, 2006). The viscosities of T, T', K and K' were 990K cP, 989K cP, 1010K cP and 1020K cP respectively.

3.4 Stability of creams

All the creams that consisted with both OM and LZ were stable more than 120 days in RT and 04± 2 ^{0}C and however it was stable at 40± 2 ^{0}C. Creams usually are thermodynamically unstable and temperature differences could have been affected the stability and other properties of its emulsifying agents (Anisa and Nour, 2010). However creams that were subjected to accelerated study were stable more than 120 days at RT.

3.5 Characterization of the creams

All the pH values of creams were between 6.06 – 6.76. Thus they were in the acceptable pH range for skin (Lambers *et al.*, 2006). The viscosities of creams prepared from T, T', K and K' were 1020K cP, 1030K cP, 1050K cP and 1060K cP. All creams had high consistency. The microscopic analysis proved that all the creams were in o/w type. All creams had lower SPF values than their relevant emulsions as mentioned table 02

3.6 SPF values of pure secondary emulsions, medicated secondary emulsions and creams

Table 02 - Comparison of SPF in the concentration of 0.5g/ mL

Sample label	SPF		
	Emulsion bases	Emulsions with leaf extract	Creams
T	38.4780	38.1456	31.5151
K	32.1400	40.0080	39.8341
T'	38.4780	38.4560	36.5787
K'	32.1400	40.0080	38.6668

All emulsions and all creams had higher SPF values than their own leaf extracts. All these results could be due to the alteration of the activity of constituents presented in the crude plant and also due to the alteration of intermolecular interactions, rheology and penetration properties (Hamid *et al*., 2015 and Suryati *et al.*, 2015).

In this study maceration was used as the extraction technique because it was the most effective method for thermo liable compounds (Zhang *et al*., 2017). Increasing the temperature of extracts can alter their chemical properties Therefore, methanol is more appropriate in using as a solvent (Zhang *et al*., 2017). However the risk of exposure to methanol was minimized by using the rotatory evaporator until the pure dry extract was obtained. It was found that, the stable ratios for each emulsion consisted of the two separate leaf extracts used in this study were the same. This could be due to the presence of similar types of compounds in both plants (Radhika *et al*., 2018 and Madhavan, 2013).

In cream formulation GMS was used as a stabilizer, emollient and plasticizer. Steric acid was used as an emulsifier and solubilizing agent. Methyl paraben was used as an antibacterial agent. TEA was used as an emulsifying agent to produce stable o/w formulations and as a pH adjuster. Further, Glycerine was used due to its humectant and emollient properties (Raymond *et al*., 1986).

4.0 Conclusion

As the conclusion of the study, two different stable ratios of medicated emulsions has found as 33% VCO: 35% water: 32% T_{80} and 29% Neem oil: 42% water: 29% T_{80}. Creams that were prepared according to these ratios were proven thermodynamically stable more than 120 days at RT and 04 ± 2 ^{0}C, kinetically stable at RT more than 120 days. All final cream formulations had higher SPF values than methanolic extracts of OM and LZ leaves. The SPF values of each cream was above 30. Hence all the creams can be used as effective sunscreen agents.

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