# Phytoformulation of *Sassurea lappa* plant extract: A Single blind, non-invasive and split face study of cream on various skin parameters

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**Abstract**: *Saussurea lappa* (SL) has been reported for its antioxidant and anti-ageing properties. Due to this reason it can be incorporated in a stable phytoformulations for cosmetic use. The objective of the study was to evaluate the anti-aging potential of cosmetic o/w emulsion containing the botanical extract of SL. An emulsion (o/w) was prepared using TEGO® Care 450 (Polyglceryl-3-Methyl Glucose Distearate) emulsifier and final emulsion was loaded with 4 % extract of SL in aqueous phase. This emulsion evaluated for its antioxidant and anti-ageing properties on healthy human subjects using a non-invasive technique called surface evaluation of living skin (SELS). The formulation containing SL extract showed significant ($p \leq 0.05$) changes in Skin roughness (SEr) as -3.13%, -6.26%, -9.39%; Skin Scaliness (SEsc) as -4.19%, -8.39%, -12.58%; Skin wrinkles (SEw) as -0.5%, -1.08%, -1.63%; and Skin smoothness (SEsm) as 3.28%, 6.57%, 9.85%, respectively, after 30, 60 and 90 days of continous use. Topical application of the cosmetic cream containing SL extract exerts have a significant anti-aging effects, perhaps due to the presence of Kaempferol, gallic acid, Caffeic acid and other essential phenolics.

**Keywords**: *Saussurea lappa*, surface evaluation of living skin (SELS).

## INTRODUCTION

Skin aging is the rapidly growing problem of human skin. UV rays are the continuous source of skin aging causing the issues of sunburn, immune suppression and cancer (Narayanan *et al.*, 2010). The repeated exposure of skin to the dangerous UV rays, cause photoaging and skin cancer. In the United States, the number of over age people is expected to double by 2025 (Farage *et al.*, 2008). The structure and physiological integrity of the skin are lost as a result of a number of intrinsic and extrinsic factors (Hwang *et al.*, 2011). The Human skin is the most affected organ system by these factors. Human skin is a prominent indicator of age, as it serves as the main barrier between the harsh external environment and the internal organs. The aged skin seems to be thin and have reduced elasticity with unblemished marks and varied smoothness (Hwang et al., 2011). Clinically the aged skin is identified by wrinkles, hyper pigmentation, dryness and scaliness. Most of these signs are prominent on visible areas of skin such as the face, neck, upper chest, forearms and hands (Helfrich *et al.*, 2008).

In recent years, the research is going on to discover the cosmeceutical agent which effectively slows down the process of photoaging. Zouboulis (2012) reported that botanicals can significantly slow down the process of photoaging by incorporation into the cosmetics products (Zouboulis and Makrantonaki, 2012). Studies are going on to discover the cosmetic product which revitalizes the skin and protect it from harmful UV radiations. There is a wide range of cosmetic products available in the market as antiaging products. Most of these products contain botanical extracts and show sustained anti-aging activity. *Saussurea lappa* (SL) is a member of the *Asteraceae* family and is a perennial herb (Madhuri *et al.*, 2012). It widely distributed in the Himalayan region of the subcontinent. It has been used by the local people in traditional medicines. No obvious adverse effects are reported by the use of *Saussurea lappa*. It has been proposed that *Saussurea lappa* can effectively slow down the aging process and it contains the antioxidant constituents. One of the studies revealed that SL root contains phenolic and flavonoid contents which can prevent the antioxidant stress (Chang *et al.*, 2012). The antioxidant stress leads to wrinkling, scaliness, dryness and lack of smoothness in the skin.

The aim of this study was to study the impact of a topical cream containing the root extracts of *Saussurea lappa* (SL) on the surface evaluation of living human skin (SELS) (Arshad *et al.*, 2015a) especially on the parameters including scaliness, wrinkles, roughness, and smoothness.

## MATERIALS AND METHODS

### *Identification of the plant*

The plant material, *Saussurea lappa* was purchased from Bahawalpur local market and submitted to Prof. Dr. Iqbal Azahr, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan for authentication. The voucher specimen is UK-FPH-DP-AG-16-01.

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***Material***

TEGO® Care 450 (Polyglyceryl-3 Methylglucose Distearate) was purchased from Evonik (Goldschmidt Essen, Germany) while stearyl alcohol, paraffin oil, and alcohol (Ethyl alcohol) were purchased from Merck KGaA (Darmstadt Germany). Synergy HT BioTek® USA microplate reader. Distilled water was prepared in the cosmetics laboratory of the Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan. *Saussurea lappa* was purchased from the local market of Bahawalpur, Pakistan.

***Preparation of extract***

About 100 g fresh roots of *Saussurea lappa* were taken, crushed and extracted three times by maceration with 80% hydroalcoholic solution of water and methanol. An acidifying condition obtained with a 0.25ml of 1% hydrochloric acid by keeping it at room temperature for 24 hours. The extract residue was removed by filtration through multiple layers of muslin cloth to get a coarse filtrate. The coarse filtrate was then filtered through a Whatman No.1 filter paper to get the particle-free extract. The methyl alcohol was removed under vacuum at 40°C. Highly concentrated botanical extract of *Saussurea lappa* was collected through rotary evaporator and stored under refrigeration at 4°C for future analysis and/or characterization.

***Evaluation of antioxidant activity of plant Extract***

Antioxidant potential was determined according to the method of Baysal and Demirdoven (Baysal and Demirdöven, 2007). Various extracts alone and emulsions containing SL extracts were subjected to the analysis. According to this method, 10µL of the solution was taken in 96-wells plate trailed by the addition of 90µL of 100 µM Methanolic DPPH solution in a total volume of 100 µL. The solution was mixed well and kept at 37$^{o}$C for half hour. Microplate reader (Synergy HT BioTek® USA) was used to determine any decline in the absorbance which was taken at 517nm. Vitamin C (Ascorbic acid) was used as standard solution. All experiments were conducted in triplicates to take the mean and ensure accuracy (Arshad *et al.*, 2015b). Test solutions were assayed at various dilutions such as 0.015, 0.0313, 0.0625, 0.125, 0.25, 0.5 mM for the determination of $IC_{50}$ values.

$$\text{Inhibition (\%)} = \frac{\text{(Abs of control - Abs of test solution)}}{\text{Absorbance of control}} \times 100$$

***Preparation of Cosmetic Cream***

Two separate phases A and B, oil phase (A) containing Polyglyceryl-3 Methylglucose Distearate (TEGO® Care 450) (5%,) stearyl alcohol (3%) and paraffin oil (8%)) and aqueous phase (B) containing *Saussurea lappa* extract 4% w/w (2mg/ml) were heated prior to preparation of the cream (O/W emulsion), at 80±5$^{o}$C and then mixed A into B by starring at 2000rpm for 10min. The formed emulsion gradually cooled and stirring speed reduced to 1500 rpm for 5 min. Further speed was reduced to 1200 rpm for 15 min until a uniform cream obtained.

***Cosmetic properties of formulation***

Formulation was kept at different storage conditions in order to perform accelerated stability studies. The formulation was observed for phase separation at high temperature (Anchisi *et al.*, 2001), any variation in globules size using optical microscope, any difference in consistency index, flow index and plastic behavior of herbal cosmetic formulation (Gaspar and Campos, 2003).

*In-vivo* Study procedure

*In-vivo* study has been carried out during the months of April 2015 to June 2015. The split-face *In-vivo* study was accomplished in draught free room with controlled conditions of temperature (22-25$^{O}$C) and relative humidity (55-60%). Manufacturer's instructions were followed by authors to execute the instrumental measurements (Arshad *et al.*, 2015b). Two weeks prior to study and during the study period all the volunteers were instructed to use only normal cleansing products, thirteen healthy males age (20-25) were selected. All instruments were used according to manufacturer's instructions. Volunteers were asked to use normal cleansing products two weeks prior to the start of experiments. Each volunteer was then given two types of creams, the active cream (active formulation) contains the SL extract and the control (placebo) having no SL extract. The volunteers were trained about the proper use of the creams. Measurements of skin parameters called surface evaluation of living skin (SELS) were used to evaluate four clinical parameters (SEsm, SEr, SEsc and SEw) to quantitatively and qualitatively describe the skin surface as an index (Mahmood and Akhtar, 2013). This practice was done every month for 3 months. Approximately, 500 mg of both active cream and control were to be applied to the cheeks twice daily (mornings, 7:00-9:00; evenings, 19:00-21:00) over a period of 3 months. The area around the eyes was omitted. Volunteers were kept in the experimental room for at least 30 min in order to tolerate skin adjustment to room temperature before taking the readings.

***Ethical approval***

Ethical approval for this study (Ref No 62-2014/PREC) was taken from the Pharmacy Research Ethics Committee (PREC), the Islamia University of Bahawalpur, Pakistan.

***Instrumental assessment***

Visioscan® VC 98 is a particular UV-A light video camera with high resolution to study the skin parameters. (Kanlayavattanakul *et al.*, 2016). All measurements were taken in controlled temperature (20-25°C) and relative humidity (45-60%).

**Table 1**: Composition of Control and *Saussurea lappa* o/w emulsion

| Formulation | Phase A (Oily Phase) | | | Phase B (Aqueous Phase) | |
|---|---|---|---|---|---|
| | Tego Care-450 | Stearyl Alcohol | Paraffin Oil | Water | *Saussurea lappa* Extract |
| Control | 5% | 3% | 8% | Q.s to make 100% | None |
| Formulation SL | 5% | 3% | 8% | Q.s to make 100% | 4% |

**Table 2**: Antioxidant activity of *Saussurea lappa* extract

| Concentration of extract | Antioxidant activity of extract | Antioxidant activity of standard ascorbic acid |
|---|---|---|
| 2mg/ml | 80.90% | 93.00 % |

**Table 3**: Globules size measurements of phytoformulations at various thermal conditions

| Temperature | 8°C | 25°C | 40°C | 40°C+75% RH |
|---|---|---|---|---|
| Fresh | 2.45 µm | 2.45 µm | 2.45 µm | 2.45 µm |
| 60 days | 2.26 µm | 2.05 µm | 1.92 µm | 1.74 µm |
| 90 days | 2.22 µm | 1.95 µm | 1.78 µm | 1.56 µm |

**Table 4**: Formulation rheological attribute at various thermal conditions

| Temperature | 8°C | | | 25°C | | | 40°C | | | 40°C + 75 % RH | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh | 60 Days | 90 Days | Fresh | 60 Days | 90 Days | Fresh | 60 Days | 90 Days | Fresh | 60 Days | 90 Days |
| Flow index | 0.28 | 0.3 | 0.45 | 0.06 | 0.6 | 0.24 | 0.66 | 0.15 | 0.4 | 0.38 | 0.05 | 0.28 |
| Consistency index | 1799 | 5395 | 2925 | 6374 | 97064 | 5184 | 435.3 | 5517 | 37036 | 1639 | 12360 | 508018 |

## STATISTICAL ANALYSIS

Values for various skin parameters were calculated at 0 h, 1st, 2nd and 3rd month. IBM SPSS Statistics V-21 Premium was used for data analysis on the computer by using the two-way ANOVA for variation at different time intervals and the paired sample *t*-test for the variation between the two formulations. $p<0.05$ (5%) was considered as the level of significance.

Percent changes shown in the tables specify the difference from baseline values (0 hr) at different time intervals and were calculated by the following formula:

$\% \text{ Change} = [(Dx - D0)/D0] \cdot 100.$

## RESULTS

### *Free radical scavenging activity*

At concentration of 2mg/ml extract exhibit high free radical scavenging potential i.e. 80.90% against ascorbic acid as standard (93.00%). The high antioxidant activity of extract was due to various essential phenolic acids.

### *Cosmetic attribute of phytoformulations*

The droplet size of emulsion systems was determined after 0 hours of development and then after 60 and 90 days storage at various conditions. Droplets size, of fresh formulation was 2.45µm, while 1.74 µm and 1.56 µm after 60 and 90 days respectively at 40°C+75% RH. Emulsion droplets size was confirmed after 60 and 90 days at higher temperatures and the mean droplet size of formulation was decreased (table 3). Rheological behaviors of cosmetic phytoformulations were evaluated at 0 hour after preparations and then during 60 and 90 days at various conditions. With Power law Model the value of flow index and consistency index were calculated (table 4).

### *In vivo response of topical cosmetic emulsion*

*Skin roughness*

In fig. 1, all the absolute values and the percent changes in the SEr parameter is presented along with the statistical evaluation of the change in SEr from baseline to 30 days, from 30 days to 60 days and from 60 days to 90 days, after the treatment with the topical cream formulation containing *Saussurea lappa* botanical extract. The formulation of botanical extract significantly reduced the roughness of the skin after 30, 60 and 90 days. The control side of the face also had reduced roughness, but the reduction was insignificant. The change in skin roughness produced by the *Saussurea lappa* extract after the 90-days completion of the intervention was observed -9.39%.

### *Skin scaliness*

All the absolute values and percent changes in SEsc parameter for three months intervention are shown in fig. 2. The results showed are duction in skin scaliness over the course of 90 days. Significant improvement was observed after the period of 90 days and reduction in

scaliness was sustained. There were slight changes in the scaliness produced by the control which is not statistically significant. The change in skin scaliness produced by the *Saussurea lappa* botanical extract after the 90-days completion of intervention was observed -12.58%.

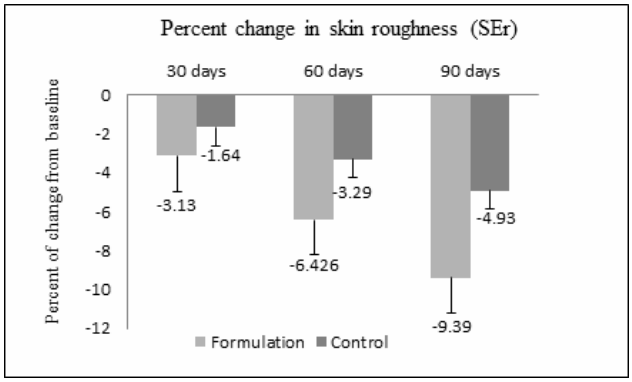


**Fig. 1**: Results and Statistical Evaluation of the Skin Roughness (SEr) Parameter

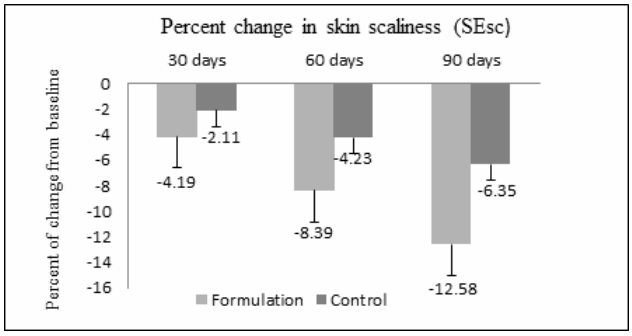


**Fig. 2**: Results and Statistical Evaluation of the Skin Scaliness (SEsc) Parameter

***Skin smoothness***

The smoothness of skin showed an increase, after the intervention of topical cream containing *Saussurea lappa* botanical extract as shown in fig. 3. The increase in smoothness was significant at the end of 90 days. The control also produced an increase in the smoothness of the skin, but that increase did not sustain for a long time and was associated with the control base. The change in skin smoothness produced by the *Saussurea lappa* botanical extract after the 90 days completion of intervention was observed 9.85%

***Skin wrinkling***

Fig. 4 shows the absolute values and percent change in the SEw parameter of the skin after the intervention of *Saussurea lappa* extract cream. The results of wrinkling are quite interesting. The skin wrinkling decreased slightly after the intervention of topical cream of *Saussurea lappa* extract. The side of the face where a control was applied showed very little decrease in skin wrinkles. The result shows that change in skin wrinkling produced by the *Saussurea lappa* extract after the 90-days of intervention was observed -1.63%

## DISCUSSION

The ultraviolet radiations cause metalloproteinases in the human skin (Degrade the collagen in the dermis). The cosmetic products are available to protect the human skin from these damaging effects of the UV rays. These rays also cause oxidation in the human skin. The present study was conducted to evaluate the impact of a topical cream containing botanical extract of *Saussurea lappa* (SL). The 90 days intervention of SL revealed significant improvements in the SELS parameters. All these significant improvements are mentioned in the above figs. (1-4). *Saussurea lappa* has been used in different traditional medicines in the Himalayan region. The root of SL contains several useful constituents which are vital antioxidants and significantly protect from the oxidants. The characterization of SL revealed that it contain total phenolic contents (27.62±0.07µGAE/g extract) and flavonoid contents (50.75±0.11µGAE/g extract) (Chang *et al.,* 2012). These constituents are essential antioxidants and can revitalize the photoaged human skin. Godic (2014), confirmed the prevention of lipids oxidation of human skin by the application of botanical extracts in the form of topical agent (Godic *et al.*, 2014). One another study demonstrated that SL has the highest antioxidant activity in DPPH scavenging method (Saha *et al.*, 2013). Bradley (2015) also studied the antioxidant activity of botanical extracts (Bradley *et al.*, 2015). The antioxidant activity of *Saussurea lappa* is also confirmed by *In-vitro* pharmacological activities (Thara and Zuhra, 2012). In this study, DPPH method showed the strong antioxidant activity of *Saussurea lappa* in the presence of ascorbic acid as a control. These findings suggest that *Saussurea lappa* has significant activity when used in the topical agents to prevent oxidation in human skin.

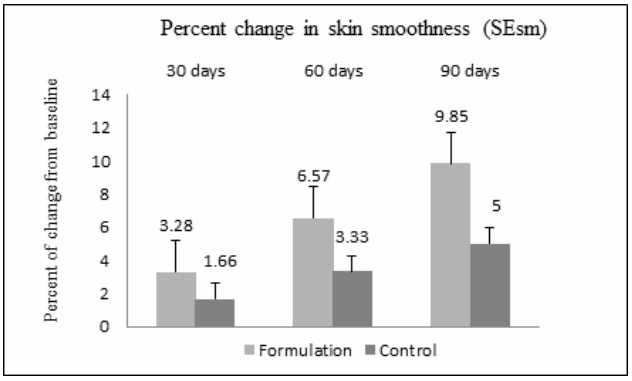


**Fig. 3**: Results and Statistical Evaluation of the Skin Smoothness (SEsm) Parameter

One another study conducted to evaluate the different medicinal plants in topical agents as antioxidants found that the roots extract of *Saussurea lappa* (SL) can be used in several chronic skin diseases (Thara and Zuhra, 2012). This shows the potential application of *Saussurea lappa* in dermatological products. The SELS parameter of skin

shows significant improvement after the application of a topical cream containing the botanical extracts of *Saussurea lappa*. There are four different SELS parameters including SEr (roughness of skin), SEsm (smoothness of skin), SEsc (scaliness of skin), SEw (wrinkles of skin). All these parameters determine different features of skin such as the amount of dark pixels, depth of wrinkles, dryness. These show the aging of human skin. In the present study, control produced statistically insignificant ($p \leq 0.05$) effects on the roughness parameter of skin and the formulation produced significant effects at all reading intervals when two ways ANOVA was performed. When paired sample *t*-test was applied, significant effects were observed for active formulation.

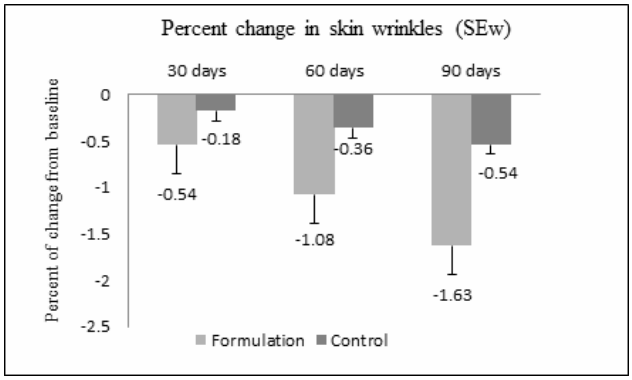


**Fig. 4**: Results and Statistical Evaluation of the Skin Wrinkling (SEw) Parameter

The percentage change in the SEr (skin roughness) after the 90 days intervention of active formulation (*Saussurea lappa*) and its control showed there was significant (-9.39) decrease in the roughness of skin with the active formulation as compared to control (-4.93). The percentage change in the values of SEsc and SEw of active formulation and control revealed to be (-12.58, -6.35) and (-1.63, -0.54) respectively. This shows that active formulation of *Saussurea lappa* had a significant reduction in scaliness and wrinkles formation as compared to control. These results show the presence of antioxidant constituents in the roots of *Saussurea lappa*. The results of the present study are similar to the previous studies which show antioxidant potential of *Saussurea lappa*. The SEsm (skin smoothness) also increase and found to be (9.85), control also increased the smoothness of skin up to (5.00) but this change is not sustained for a longer period of time.

These results verify the use of *Saussurea lappa* in skin preparation to slow down the process of aging and revitalize the human skin. The results of the current study reveal that better skin protective dermatological products can be produced by incorporation of a botanical extract of *Saussurea lappa*. Further research is required to formulate the products for specific SELS parameters.

## CONCLUSION

From the results of this study, it appears that *Saussurea lappa* possesses potential antiaging properties when applied topically and stable topical cream containing *Saussurea lappa* botanical extracts can be formulated. The formulation showed a decrease in skin roughness, scaliness, and wrinkles, while there was an increase in the skin smoothness. This study also depicts that noninvasive biophysical techniques are a valuable tool for assessment of anti-aging effects of topical skin applications.

In this study, we have determined the impact of a topical cream containing *Saussurea lappa* extracts on the skin surface evaluation parameters. The results of this study show that when this topical cream is applied and used for a period of 90 days, superior results are obtained as compared to control. The smoothness of skin increase, the roughness of skin decrease, scaliness of skin also decreases and there is also a reduction in skin wrinkling. The results of present study lead the pathway for further research on this topic and the development of more potent topical agent with fruit extracts.

## ACKNOWLEDGEMENT

Authors are thankful to Prof. Dr. Khan Usmanghani for identification of plant. Authors are also thankful to Mr. Abdul Hameed for his technical support.

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