

## Investigation of a New Sebum Control Cream Containing Apple Juice Extract

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(Received: 27 April 2010;

Accepted: 7 October 2010)

AJC-9169

Some polyphenols are considered useful to reduce sebum production. The objective of this study is to evaluate of stable water in oil skin caring cream (W/O emulsion), containing 3 % apple juice extract (rich in polyphenols), on sebum contents of skin in healthy male volunteers and compared it with base (without apple juice extract). Concentrated apple juice extract (*Malus domestica*) was entrapped in the inner phase of water in oil emulsion. Formulation containing apple juice extract and base containing no extract was formulated and subjected to different storage conditions to check their stability for a period of 4 weeks. Then both the formulation and base were applied to the cheeks of healthy human volunteers (n = 12) twice in a day for a period of 10 weeks. Sebum level on the both cheeks was determined with device (Sebumeter) before application of cream and during the study period. Every volunteer was asked to fill the performa about sensory evaluation of base and formulation at the end of study. A cream (base and formulation) was very well accepted by all volunteers. There was a significant reduction in sebum level. Statistically significant ( $p \leq 0.5\%$ ) result was found for skin sebum production by the formulation containing apple juice extract (3 %). This result shows the efficacy of sebum tested cream to reduce the greasiness and improve the appearance of oily facial skin. The new topical product was ideal in all aspects and can be used in skin disorders like acne for further investigation.

**Key Words:** *Malus domestica*, Skin sebum, Formulation, Abil EM 90, ANOVA.

### INTRODUCTION

Acne vulgaris is an inflammatory disease in sebaceous glands and pilosebaceous units in the skin and is characterized by excess sebum production, hypercornification of the follicular wall epidermis, local infection with propionibacterium acnes and local inflammatory events such as the augmentation of proinflammatory cytokine production and the formation of papules and pustules<sup>1</sup>. Treatments for acne include: benzoyl peroxide, retinoic acids such as tretinoin, isotretinoin and adapalene; antibiotics are also widely prescribed<sup>2</sup>. In addition, herbal medicines are well known to be effective for the prevention and/or remission of various diseases including acne<sup>3</sup>. Flavonoids from medicinal plants have also been reported to possess various pharmacological effects including antiinflammatory and anti-ageing effects against skin disorders<sup>4</sup>.

Two days herbal extract are used in cosmetic preparations for beauty and attractiveness<sup>5</sup>. Herbal cosmetics are classified on the basis of dosage forms as cream (emulsion), powder, soaps, solutions, etc. and according to the part or organ of the body where it is to be applied as cosmetics for skin, hair, nail, teeth, mouth, etc.<sup>6</sup>. The use of cosmetics is determined by both

their efficacy as well as the minimal risk of skin irritation/skin sensitization that they cause. This is influenced by their formulation, nature of their use, quantity and quality of ingredients<sup>7</sup>.

Flavonoids have many pharmacological actions *in vivo* and *in vitro*. They are antioxidants, free radical scavengers and have antiinflammatory, antitumourigenic and antitumour metastatic activities<sup>8</sup>. In the regulation of lipid synthesis, a common dietary flavonoid, quercetin, has been reported to exhibit the suppressive action of de novo triacylglycerol synthesis in human intestinal cells<sup>9</sup>. In addition, a citrus flavonoid, hesperetin, has been shown to inhibit triacylglycerol synthesis in rat hepatocytes (Fig. 1). The formulation of herbal medicines has been effective in improving the constitution of acne patients<sup>10</sup>.

### EXPERIMENTAL

*Malus domestica* fruits were purchased from local market, Bahawalpur, Pakistan and Abil<sup>®</sup>EM90 was purchased from Franken Chemical (Germany). Paraffin oil was purchased from Merk KGaA Darmstadt (Germany). Methanol was taken from BDH England.

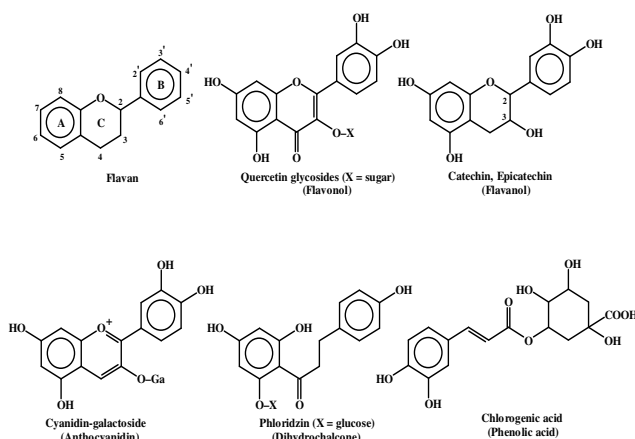


Fig. 1. Structure of Polyphenolic compounds present in apple

Centrifuge machine (Hettich EBA 20, Germany), cold incubator (Sanyo MIR-153, Japan), conductivity-meter (WTW COND-197i, Germany), sebumeter MPA 5 (Courage + Khazaka, Germany), digital humidity meter (TES Electronic Corp, Taiwan), electrical balance (Precisa BJ-210, Switzerland), homogenizer (Euro-Star, IKA D 230, Germany), hot incubator (Sanyo MIR-162, Japan), pH-meter (WTW pH-197i, Germany), refrigerator (Dawlance, Pakistan), rotary evaporator (Eyela, Co. Ltd., Japan) were used in this study SPSS (12.0).

**Extract preparation and formulation:** Raw juice of *Malus domestica* fruit was obtained by washing and inspection, crushing, milling or slicing to apple pulp<sup>11</sup> and extract of *Malus domestica* fruits juice was prepared by maceration. Phenolic compounds of fruit juice were extracted with methanol formic acid -double distilled H<sub>2</sub>O (MFW; 70; 2; 28,) for 24 h at 4 °C. Filtered extract was evaporated at 45 °C under vacuum, using a rotary evaporator. Extract was stored at 8 °C for further analysis.

For a formulation paraffin oil (16 %), surfactant Abil®EM90 (2 %) and bees wax (3 %) was heated up to 80 ± 1 °C. At the same time, aqueous phase consisting of water (quantity sufficient to make 100 %) was heated to 80 ± 1 °C and then the apple juice extract (3 %) was added in this aqueous phase. After that, aqueous phase was added to the oil phase drop by drop stirring was continued at 2000 rpm by the mechanical mixer for ca. 10 min until complete aqueous phase was added, 2 to 3 drops of lemon oil were added during this stirring time. After the complete addition of the aqueous phase, the speed of the mixer was reduced to 1000 rpm for homogenization, for a period of 5 min and then the speed of the mixer was further reduced to 500 rpm for 5 min for complete homogenization until the emulsion cooled to room temperature. Similar procedure was followed for the base formulation except no apple juice extract was added in it.

**Study design:** One sided blind study was designed with placebo control in the month of February-March. For application of formulation and base, 12 volunteers were chosen whose ages were in between 25 and 30 years. Only male volunteers were included in this work. Volunteers were examined for any serious skin disease or damage especially on cheeks and forearms. Every volunteer was provided with a volunteer protocol before the study. This protocol stating every volunteer signed the terms and conditions of the testing individually.

Volunteers were not informed about the contents of the formulations. Skin tests were performed at 25 °C and 40 % relative humidity conditions. Before application of formulation a patch test was performed on forearms of the volunteers for 48 h to check any irritation in the formulation or base.

Each volunteer on the second day was provided with two formulations *i.e.* base and active formulation and volunteers were instructed properly about the application of formulation. Every individual was instructed to come for measurements of readings for skin sebum production on week 1, 2, 3, 4, 6, 8 and 10.

**Ethical standards:** This study was approved by the Board of Advance Study and Research (BASR), the Islamia University, Bahawalpur and Institutional Ethical Committee, Faculty of Pharmacy and Alternative medicine, The Islamia University, Bahawalpur.

**Burchard test (Patch test):** Patch test was performed on the forearms of each volunteer on the first day of skin testing. A 5 cm × 4 cm region was marked on forearms. The patch (Bandage discs) for the right forearm is saturated with 1.0 G of base, while the patch for the left forearm is saturated with 1.0 g of formulation. each of these was applied to the 5 cm × 4 cm marked region separately. The regions were covered with surgical dressing after dressing. The patches were removed after 48 h and forearms were washed with physiological saline<sup>12</sup>. After 48 h, scores were recorded for the presence of erythema (skin redness), using a scale for 4 points, from 0 to 3, where 0 stand for absence, 1 for mild, 2 for moderate and 3 stands for severe erythema, respectively. Each volunteer was asked to note their irritation/itching towards the patches and then assign the score from the same scale. Each score with respect to volunteers is given in Table-1.

Score	0	1	2	3
No. of volunteers				
Base	7	2	1	0
Formulation	8	1	1	0

**Panel test:** Every individual was provided with a performa prepared previously to test the sensory values of creams. This consisted of seven parameters to be evaluated and every parameter was assigned 11 values from -5 to +5 indicating very bad to very good, respectively. This performa was to be completed independently by each individual at the end of the study period. From the average reply of volunteers it was concluded that base and formulation were felt well on the skin, produced a pleasant feeling on application to the skin and produced no irritation on the skin in both the cases, *i.e.*, base and formulation, as these were assigned 0.00 point for irritation by all the volunteers. Shine on skin was more for formulation. This was expected since the formulation contained essential fatty acids. Similarly, the formulation led to more softness of the skin than base. It was found from paired sample t-test that there was an insignificant difference between the average points of sensitivity for base and formulation. It was concluded that there was no immense variation between base and formulation regarding the sensory evaluation. Both the creams have similar performance from the sensory point of view (Table-2).

TABLE-2  
AVERAGE VALUES  $\pm$  SEM FOR PANEL TEST BY  
12 VOLUNTEERS FOR BASE AND FORMULATION

	Average points for Base $\pm$ SEM	Average points for Formulation $\pm$ SEM
Ease of application	4.10 $\pm$ 0.06	4.16 $\pm$ 0.10
Spreadability	4.22 $\pm$ 0.05	4.47 $\pm$ 0.06
Sense just after application	3.87 $\pm$ 0.07	4.05 $\pm$ 0.08
Sense in long term	4.18 $\pm$ 0.08	4.13 $\pm$ 0.09
Irritation	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Shine on skin	4.07 $\pm$ 0.09	4.24 $\pm$ 0.04
Sense of softness	4.21 $\pm$ 0.06	4.41 $\pm$ 0.07

**Mathematical and statistical analysis:** The percentage changes for the individual values of different parameters, taken every week, of volunteers were calculated by the following formula:

$$\text{Percentage change} = [(A - B) / B] \times 100$$

here; A = individual value of any parameter (from 1st to 10th week); B = zero hour value of that parameter.

The measured values obtained for different parameters skin sebum production were analyzed using SPSS 12.0 on the PC computer (paired samples t-test for variation between the two formulations; two-way ANOVA for variation between different time intervals and level of significance was 0.5 %).

## RESULTS AND DISCUSSION

**In vitro evaluation of creams:** Stability of base and the formulation was evaluated using different conditions of storage *i.e.*, 8, 25, 40 and 40 °C + 75 % RH (relative humidity). In this study no liquefaction and phase separation was observed in the formulation samples throughout the study period of 4 weeks even at elevated temperatures, however slight liquefaction was observed in the base after 3rd week. Abil<sup>®</sup>EM90 is a lipophilic surfactant<sup>12</sup> and it has been found that lipophilic surfactants are more stable at elevated temperatures<sup>13</sup>. Furthermore pH of the formulation was 5.67, considered as normal skin pH range *i.e.* 4 to 6.5<sup>14</sup>.

**In vivo characterization of base and the formulation for skin sebum effects:** Type 1- $\alpha$  reductase converts testosterone into more potent dihydrotestosterone, which results in the enlargement in sebaceous glands, leading to secretion of high level of sebum<sup>15</sup>. The polyphenol plant extract regulates the extreme sebum secretion.

The three most important groups of flavonoids present in apple and apple products are flavanols or catechins, flavonols and anthocyanins with the main representatives (-)- epicatechin, quercetin glycosides and cyanidin galactoside, respectively. These compounds all belong to the group "polyphenolics", together with procyanidins, which consist of oligomeric and polymeric catechins phloridzin and phloretin xyloglucoside (dihydrochalcones) and chlorogenic acid and p-coumaroylquinic acid (phenolic acids), which are also present in apple. Topical application of  $\alpha$ -linoleic acid and EGCG ((-)-epigallocatechin-3-gallate) in animal model have shown to inhibit sebum production. This activity was due to selective inhibition of 5 $\alpha$ -reductase<sup>16</sup>, an enzyme found in sebaceous gland<sup>17</sup>.

In this study, the creams were evaluated for skin sebum productions. The effect of base and formulation on skin sebum production was noted in volunteers and results for average

percentage changes are present in Fig. 2. It was found that formulation decreased the sebum contents through out the study period while the base.

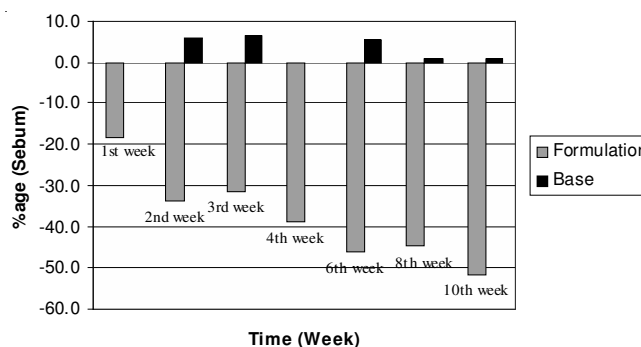


Fig. 2. Percentage of change in skin sebum production after application of base and the formulation

With the help of ANOVA test, it was found that formulation produced highly significant effect on sebum of skin ( $P = 0.000$ ) while base produced insignificant effect on sebum of skin.

When the paired sample t test was applied it was found that the base and formulation showed significant variations ( $P < 0.05$ ) with regard to skin sebum secretion except 1st week.

**Panel test:** For sensory evaluation of formulation a feedback form containing seven questions was prepared and volunteers were asked to give their opinion that what they felt about the base and formulation. Average points were calculated from the points assigned by each volunteer for each question about base and the formulation and results were compiled. It was found from average score assigned by the volunteers that the formulation was aesthetic in all aspects of sensory evaluation by the volunteers.

## Conclusion

Stable water in oil emulsion containing extract of apple juice (3 %) can be formulated. It was found that formulation produced a pronounced decreased the sebum secretion of skin as excess sebum is one of the most contributing cause of acne.

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