IN VIVO EVALUATION OF SKIN IRRITATION POTENTIAL, MELASMA AND SEBUM CONTENT FOLLOWING LONG TERM APPLICATION OF SKIN CARE CREAM IN HEALTHY ADULTS, USING NON-INVASIVE BIOMETROLOGICAL TECHNIQUES

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Abstract: The present investigation was conducted to evaluate non-invasively, various functional skin parameters i.e., irritation potential, melasma and sebum contents following long term application of topical cream (w/o) loaded with 2% methanolic extract of Ananas comosus L. versus placebo control (base) in healthy adults. Healthy human volunteers (n = 11, aged 20-30 years) were recruited for investigation and written informed consent was taken from each volunteer. In this single blinded study every volunteer applied formulation on one side of face and placebo on the other side of face twice daily for a period of 12 weeks (three months). Different skin parameters i.e., skin irritancy, melasma, and sebum contents were measured on both sides of face at baseline and after two weeks interval, using photometric device Mexameter and Sebumeter in a draught free room with modulated conditions of temperature (22-25°C) and humidity (55-60%). It was evident from the results that no primary skin irritancy was observed with patch test. Besides, statistical interpretation indicates that treatment with formulation is superior to placebo because it significantly (p ≤ 0.05) reduced the skin irritancy, melasma and sebum secretions throughout the study and reaching maximum -20.76 ± 0.89, -54.2 ± 0.37 and -40.71 ± 0.75%, respectively, at the end of study period. Antioxidant activity of extract was 92% compared to standard antioxidant. Conclusively, active cream loaded with fruit extract was well tolerated by all the volunteers and suitable to treat contact dermatitis, greasy skin, acne and seborrheic dermatitis and augmenting beauty and attraction by depigmentation of human skin. So, in the future, there is need to clinically evaluate these formulations in patients with compromised skin functions i.e., contact dermatitis, melasma, and acne vulgaris in order to explore the actual potential of this fruit.

Keywords: Ananas comosus, acne, erythema, melasma, cream, sebum contents

Exploration of various naturally occurring compounds having depigmenting, anti-inflammatory and sebum reducing properties is an important growing area of research regarding topical treatment of various skin diseases (1). Skin hyperpigmentation affictions i.e., melasma, solar lentigo and post-inflammatory pigmentation were evolved due to excessive fabrication and accumulation of skin melanin pigment and distinguished with aging (2). Commonly used whitening chemicals i.e., hydroquinone and kojic acid, provide depigmentation effects but also have some safety issues upon long-term application while utilization of natural botanical extract in cosmetic products provide new idea for safe and effective remedy with least harmful effects to skin upon long-term exposure (3). Skin care products loaded with natural phenolic extracts may actuate allergic and irritant contact dermatitis and phytophotodermatitis (4).

A majority of world population (66-75%) aged between 15-20 years exhibit an increased production of sebum, which leads toward the inferiority complex and loss of individual self-confidence and disconsolate the quality of life (5). Sebum is secreted by sebaceous glands (6), which are microscopic and lobular glandular structures found in pilosebaceous element of skin and dispersed throughout the skin except palm and soles. It constitute 95% of total skin lipid and consist of mixture of complex lipids i.e., free fatty acids, triglycerides, cholesterol esters, squalene, sterols and glycosphospholipids. Under normal physiological conditions, sebum is fruitful in

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maintaining skin durability, act as lubricant, improve skin barrier function and have intrinsic antimicrobial activity (7). Instead of this, excessive production of sebum is responsible for subservient complexion and unattractive skin appearance, usually designated as oily or greasy skin, and results in pore enlargement which promotes skin pathophysiological conditions called acne and seborrheic dermatitis (8).

Due to these grounds, more attention is given towards investigation of the plants and phytoconstituents having depigmenting and anti-sebum properties. Most important bioactive phytoconstituents of plants include polyphenols, flavonoids, alkaloids, saponins, tannins and essential oils (9). Recently, phenolics are gaining popularity regarding their use in skin care products as: anti-aging, anti-erythema, dry skin conditions, depigmentation, anti-sebum and antioxidant properties (10). Topical formulations are considered appreciable vehicle for delivery of both natural and synthetic compounds to the human skin for cosmetic purpose (11). Peculiarly, the use of botanical extracts with phenolic constituents provides most valuable cosmetic products. There is exorbitant curiosity about the use of herbal extracts containing phytochemical with high polyphenols and flavonoid constituents and play an important role in eviction of various degenerative diseases and modern phytocosmetic preparations (12).

*Ananas comosus* L. is a tropical fruit, belongs to the family Bromeliaceae and is a rich source of phenolics, flavonoids, vitamins A, B and C, several inorganic elements such as calcium, phosphorous and iron and was reported to have free radical scavenging activity (13). Fruit can also be used in eviction of sore throat and seasickness. The antioxidant activity of *Ananas comosus* L. provides the basis for the selection of fruit in skin care topical formulations. The present study was aimed to formulate cream (w/o emulsion) loaded with phenolic rich extract of *Ananas comosus* L. and investigate its effects in vivo for different physiological functions concerning aging process as: melanin pigment, erythema and sebum secretions on human skin.

**EXPERIMENTAL**

**Plant collection and identification**

*Ananas comosus* fruit was purchased during March 2013 from model bazar in Bahawalpur, Pakistan and identified (voucher # 3521/CIDS/IUB) from Cholistan Institute of Desert Study (CIDS), and specimen was deposited in herbarium of Pharmacognosy Department, The Islamia University of Bahawalpur, Pakistan, for future reference.

**Reagents and instruments**

Paraffin oil (h: 110-230, at 25°C) and methanol analytical grade was obtained from Merck (Germany), polysiloxane polyalkyl polyether copolymer (Abil - EM 90 with HLB 5) was purchased from Franken Chemicals (Germany). Distilled water was prepared by using distillation plant (Irmeco GmbH, Germany) and methanolic extract of *Ananas comosus* was prepared in pharmaceutics laboratory of Pharmacy Department, The Islamia University of Bahawalpur, Pakistan. The non-invasive instruments were used to execute the following biometrological measurements in draught free room, with regulated conditions of temperature (22-25°C) and relative humidity (55-60%): erythema and melanin contents were assessed using Mexameter MPA 5; sebum contents were estimated using Sebumeter (MPA 5 Courage + Khazaka, Germany).

**Preparation of *Ananas comosus* extract**

Fruit of *Ananas comosus* was grinded in an electric grinder (National, Japan) to acquire the paste. After that, 100 g of paste was stirred with 300 mL of methanol by using magnetic stirrer for 2 h. Then, the residue was filtered through Whatman no. 41 filter paper. Filtered extract was concentrated and solvent (methanol) was evaporated under reduced pressure at 40°C using rotary evaporator (EYELA, CA-1111, Rikakikai Co. Ltd. Tokyo, Japan). The dried extract was weighed to calculate percentage yield and stored in a refrigerator (4°C) for further use.

**Determination of antioxidant activity of *Ananas comosus* extract**

Free radical scavenging activity of fruit extract was measured using DPPH free radical method. Ten microliters of plant extract was mixed with 90 µL of methanolic solution of DPPH (100 µM) and incubated for 30 min at 37°C. Similarly, 10 µL of standard ascorbic acid (control) was mixed with 90 µL of methanolic solution of DPPH and incubated for 30 min at 37°C. The absorbance was measured at 517 nm at room temperature against the respective blank. A decrease in absorbance indicated an increase of free radical scavenging activity of the solution. The following formula was used to determine percentage inhibition of free radical scavenging activity:

\[
\% \text{ Inhibition} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}}\right) \times 100
\]
In vivo evaluation of skin irritation potential, melasma and sebum content...

Preparation of cream

Active cream was prepared using 14% liquid paraffin, 3.5% Abil® EM 90, 2.0% methanolic extract of *Ananas comosus* and 80.5% of deionized water. Oil and aqueous phases were heated separately up to 75°C, and then aqueous phase containing fruit extract was poured slowly into oil phase with continuous stirring at 2000 rpm using digital homogenizer (IKA Werke, Germany) for 15 min. Then, the stirrer speed was reduced to 1000 rpm for 10 min and then further reduced to 500 rpm and stirred until it cools to room temperature in order to achieve complete homogenization. Base was also prepared by similar method but without containing fruit extract as given in Table 1.

Subjects

Healthy male volunteers (n = 11, aged between 20-30 years) recruited for the present study were devoid of any distinguished skin disease or dermatological problem. All the volunteers signed the written informed consents as documented evidence of terms and conditions for the study. The exclusion criteria for the study included: individuals under treatment for skin infections during the study, individuals hypersensitive to any component of the cream, individuals who does not execute the application rules of the creams, individual with substantial hair on the cheeks, smokers and individuals using dietary supplements including antioxidants. Subjects were instructed to continue their routine diet during the study period to nullify the effects of such changes and avoid using any type of cream or medication used for the skin disorders, which might antagonize or potentiate the effect of research product.

Study protocols

This placebo control and split-face *in vivo* study was accomplished in draught free room with controlled conditions of temperature (22-25°C) and relative humidity (55-60%). Manufacturer’s instructions were followed by authors to execute the instrumental measurements. Two weeks prior to study and during the study period all the volunteers were instructed to use only normal cleansing products. Then, each participant was provided with two creams with 40 g contents, an active cream and other was placebo control, marked “left” and “right” indicating application to the respective cheek. The volunteers were instructed about the proper use of creams i.e., approximately 500 mg of cream was applied two times a day (mornings, 8:00 to 9:00; evenings, 20:00 to 21:00) and area around the eyes was elided. All measurements for skin profilometry were executed at baseline, on day 15, 30, 45, 60, 75 and 90. Prior to the measurements, the volunteers endure in a sitting position in cosmetic lab under environmental conditions of 22 ± 2°C and 40 ± 5% relative humidity, for 15 min to assimilate the inside conditions.

Patch test - analysis for primary skin irritation

Primary skin irritation for creams was assessed by performing patch test at forearms of all volunteers. A 5 × 4 cm area was marked on forearms of all volunteers. Patch for left forearm was drenched with 1 g of active cream and for the right forearm drenched with 1 g of base. Each was applied to marked region separately and covered with surgical dressing for 48 h. After that, the patches were removed and the application area was washed with physiological saline solution. Creams were evaluat-

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Paraffin oil</th>
<th>Abil EM 90</th>
<th>Fruit extract</th>
<th>Distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>14%</td>
<td>3.5%</td>
<td>NIL</td>
<td>q.s.100%</td>
</tr>
<tr>
<td>Active</td>
<td>14%</td>
<td>3.5%</td>
<td>2%</td>
<td>q.s.100%</td>
</tr>
</tbody>
</table>

Table 2. Score given by volunteers to base and formulation on the basis of primary skin irritation/itching.

<table>
<thead>
<tr>
<th>Cream type</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Base</td>
<td>7</td>
</tr>
<tr>
<td>Formulation</td>
<td>10</td>
</tr>
</tbody>
</table>
ed for any type of skin irritation or erythema by using mexameter and assigned different scores to the volunteers according to author’s instructions using 4 points scale ranges from 0 to 3, where 0 value indicate no erythema, 1 stands for mild erythema, 2 stands for moderate erythema and 3 stands for severe erythema. Each volunteer was asked to observe their primary irritancy towards the formulation and base and assign a specific score from selected scale as given in Table 2.

**Panel test - subjective analysis**

For the efficacy perception of creams, a pre-designed questionnaire was provided to every volunteer. It consist of seven parameters i.e., ease of application, spreadability, sense just after application, sense in long term, irritation, shine on skin and sense of softness. Each parameter assigned 11 values ranges from +5 to -5 representing “very bad” to “very good”, respectively. Every volunteer was instructed to fill this questionnaire individually at 90th day of study period.

**Ethical standards**

The acceptance for this study was approved by Institutional Ethical Committee (IEC), Faculty of Pharmacy and Alternative Medicine and Board of the Advanced Study and Research (BASAR), The Islamia University, Bahawalpur, Pakistan. The reference number is 974/AS&RB. This study was preceded by notification of Helsinki and was consistent with Good Clinical Practice guidelines.
Mathematical and statistical analysis

Measured values for different skin parameters such as melanin pigment, erythema and sebum secretion were analyzed statistically using SPSS version 13.0 at PC computer. Paired sample t-test was used to analyze the difference between two preparations and ANOVA was used to analyze the variations at different time intervals with 5% level of significance. The percentage changes of various parameters were determined in all volunteers during respective week and calculated mathematically by using the following formula:

\[
\text{Percentage change} = \left( \frac{A - B}{B} \right) \times 100
\]

where, \( A \) = individual value of any parameter of 2nd, 4th, 6th, 8th, 10th and 12th week. \( B \) = zero hour value of that parameter.

RESULTS

Antioxidant activity

Antioxidant activity of fruit was measured using DPPH free radical method and found to be 92% as compared to standard i.e., ascorbic acid.

Assessment of primary skin irritation

The cosmetic preparations must be devoid of any type of contact dermatitis at the site of application during its use. Skin irritation is caused by direct contact of chemicals or ingredients with skin and different from contact allergy caused by immune response (14). To analyze the creams compatibility for primary skin irritation, patch test was recognized as most appropriate method (15). The patch test was deportmented at forearms of all volunteers for 48 h and it was perceived that erythema levels were reduced moderately (-0.21 ± 0.09%) with base but reduced (-2.67 ± 0.54%) immensely after application of active cream as given in Figure 1. But with paired sample t-test it was manifested that both the base and formulation exhibited insignificant effects on skin erythema levels, so primary skin irritancy was examined with visual scoring only as given in Table 2. The results indicate that no severe erythema was observed in all volunteers, moderate erythema was found in 2 and 1 while mild erythema was occurred in 2 and zero volunteers and no erythema was found in 7 and 10 volunteers with base and for-

Table 3. Percentage of change in the skin erythema, melanin and sebum values after the application of base and formulation.

<table>
<thead>
<tr>
<th>Time(week)</th>
<th>2nd</th>
<th>4th</th>
<th>6th</th>
<th>8th</th>
<th>10th</th>
<th>12th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>-1.11 ± 0.87</td>
<td>-1.08 ± 1.20</td>
<td>-1.23 ± 1.67</td>
<td>-1.02 ± 0.45</td>
<td>-1.34 ± 0.67</td>
<td>-0.97 ± 1.23</td>
</tr>
<tr>
<td>Formulation</td>
<td>-3.76 ± 1.37</td>
<td>-7.95 ± 0.65</td>
<td>-9.32 ± 0.98</td>
<td>-13.11 ± 1.15</td>
<td>-17.43 ± 0.37</td>
<td>-20.76 ± 0.89</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time(week)</th>
<th>2nd</th>
<th>4th</th>
<th>6th</th>
<th>8th</th>
<th>10th</th>
<th>12th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>1.39 ± 0.54</td>
<td>5.58 ± 0.78</td>
<td>4.65 ± 0.86</td>
<td>2.05 ± 1.32</td>
<td>8.94 ± 0.65</td>
<td>13.42 ± 0.12</td>
</tr>
<tr>
<td>Formulation</td>
<td>-4.67 ± 1.03</td>
<td>-12.36 ± 0.22</td>
<td>-20.09 ± 1.11</td>
<td>-31.32 ± 0.85</td>
<td>-39.13 ± 0.45</td>
<td>-54.2 ± 0.37</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time(week)</th>
<th>2nd</th>
<th>4th</th>
<th>6th</th>
<th>8th</th>
<th>10th</th>
<th>12th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>-1.39 ± 0.34</td>
<td>-2.58 ± 0.56</td>
<td>3.35 ± 0.99</td>
<td>5.02 ± 0.26</td>
<td>8.4 ± 0.33</td>
<td>11.07 ± 1.09</td>
</tr>
<tr>
<td>Formulation</td>
<td>-2.96 ± 0.21</td>
<td>-8.15 ± 1.03</td>
<td>-13.43 ± 1.21</td>
<td>-25.01 ± 0.87</td>
<td>-33.43 ± 1.20</td>
<td>-40.71 ± 0.75</td>
</tr>
</tbody>
</table>

Table 4. Average values ± standard error of mean (SEM) for panel test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Average value for base ± SEM</th>
<th>Average value for formulation ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ease of application</td>
<td>4.11 ± 0.01</td>
<td>4.21 ± 0.01</td>
</tr>
<tr>
<td>Spreadability</td>
<td>4.06 ± 0.18</td>
<td>4.16 ± 0.08</td>
</tr>
<tr>
<td>Sense just after application</td>
<td>4.08 ± 0.03</td>
<td>4.21 ± 0.03</td>
</tr>
<tr>
<td>Sense in long term</td>
<td>4.17 ± 0.29</td>
<td>4.23 ± 0.05</td>
</tr>
<tr>
<td>Irritation</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Shine on skin</td>
<td>4.42 ± 0.10</td>
<td>4.04 ± 0.11</td>
</tr>
<tr>
<td>Sense of softness</td>
<td>4.73 ± 0.08</td>
<td>3.94 ± 0.16</td>
</tr>
</tbody>
</table>
ulation, respectively. This kind of assessment, although subjective, can be a careful, authentic and reproducible method. It was concluded from primary irritation test, 48 h semioccluded patch test, that base and formulation exhibit neither erythema nor irritation potential after application and are invulnerable for in vivo studies.

Erythema index for long term analysis

With long term analysis of 12 weeks, it was realized that inappreciable disparity was noted in erythema values for base. Nonetheless, in active cream, a gradual fall in erythema values was observed throughout study period as given in Table 3 and Figure 2. With the help of ANOVA test, it was interpreted statistically that insignificant decrease occurred in 2nd week but significant (p ≤ 0.05) reduction in erythema values were produced by formulation from 4th week onward reaching maximum up to 20.76 ± 0.89% at 12th week, but insignificant (p > 0.05) changes were observed with base at different time intervals throughout study period. It was evident from paired sample t-test, that both creams i.e., active and base, exhibit significant discrepancy in erythema values except after 2nd week.

Acute exposure to UV radiations exhibit inflammatory responses while long term exposure to UV radiations leads towards various degenerative diseases or skin cancer due to excessive fabrication of reactive oxygen species (ROS) and disruption of normal antioxidant barricade of body (4, 16). In recent few years, phenolics have been identified to rehabilitate many dermatological ailments owing to skin exposure to UV radiations (17). Several pheno-
nolic compounds were recognized in Ananas comosus extract, namely, flavonoids, iso flavones, flavones, anthocyanins, catechin and other phenolics (18-20). Phenolic contents in Ananas comosus quench the free radicals or reactive oxygen species (ROS) originating due to subjection to UV radiations, pollution, smoking and prevent the degeneration of collagen as they possess reported anti-collagenase, anti-elastase activities and molecular damage leads to photoprotection and diminish the inflammatory processes. It was concluded that the reduction in erythema values after application of active cream was due to presence of phenolic contents and it can safely be used in cosmetic preparation without causing any significant skin irritation. The results were also confirmed by efficacy assessment of each individual at the end of study period, which showed no irritation of skin and formulation was invulnerable for human volunteers.

Assessment of melanin pigment of skin

In the present study, melanin concentration of skin was increased irregularly after application of base but systematic decline in melanin pigment was observed after the application of formulation during 12 weeks study period. Melanin contents were measured at constant time intervals and percentage changes observed are given in Table 3 and Figure 3. It was apparent from ANOVA that base shows insignificant (p > 0.05) increase on melanin up to 8th week but shows significant (p ≤ 0.05) increase in 10th and 12th week, while significant (p ≤ 0.05) reduction in skin melanin was observed with formulation from 4th week onward and reaches maximum up to -54.2 ± 0.37% at the end of study period. It was manifested by applying the paired sample t-test where active form and base showed significant (p ≤ 0.05) variations regarding skin melanin contents throughout study period.

The topology and color of skin in human beings largely depends upon the presence of specialized melanin containing organelles i.e., melanosomes within epidermis. Melanin is pigmented biopolymer and is synthesized by polyphenol oxidase or tyrosinase enzyme within dendritic melanocytes and dispersed at dermo-epidermal junction (21). Tanning of the skin due to subjection to UV radiations results in elevated levels of melanin within the epidermis (22). Phenolic compounds, especially gallic acid and catechins, have reported anti-tyrosinase activity or inhibit the process of melanogenesis within the melanocytes without melanocytotoxicity (2, 23). The Ananas comosus extract is thriving in phenolics i.e., p-hydroxybenzoic acid, p-hydroxybenzoic aldehyde, gallic acid, syringic aldehyde, vanillic aldehyde, 3,4-dihydroxybenzoic aldehyde, 3-coumaric acid, ferulic acid, caffeic acid, flavonoids and catechin (18, 24). Thus it could be plausible that the decline in skin melanin after long term application can be ascribed to the catechins and gallic acid present in active cream, which inhibits the process of melanogenesis within the melanocytes.

Assessment of skin sebum contents

Skin sebum contents were measured using a special opalescent film, which becomes transparent when it is in contact with sebum lipids. The skin sebum secretions were assessed for formulation and placebo on cheeks of each volunteer throughout 12 weeks of investigation. It was found that base decreases the sebum contents in 2nd and 4th week i.e., -1.39% and -2.58%, respectively, but increases during 6th, 8th, 10th and 12th weeks, whereas the formulation continuously decrease the sebum contents at regular intervals from 2nd up to the 12th week study period as given in Table 3 and Figure 4. When ANOVA was applied, it was apparent that base shows insignificant reduction in 2nd and 4th weeks but significant increase in the sebum contents occurs from 6th week onward up to end of study period. Active formulation significantly reduced skin sebum contents from the 2nd up to 12th week study period. With paired sample t-test it was evident that significant variations were observed among base and formulation regarding the sebum contents of skin.

It is depicted from the results, that an elevation of skin sebum contents after application of base was due to oleaginous nature of creams (w/o emulsion) carrying thick viscous oil i.e., paraffin oil as continuous phase (25). Sebaceous glands in coordination with androgens (male sex hormones), estrogens (female sex hormones) and corticosteroids (adrenal cortex hormones) are responsible for the sebum secretion in the body. Androgen receptors and 5-α reductase are responsible for converting testosterone into dihydrotestosterone i.e., an active form that stimulates the sebum secretion from sebaceous glands. Androgen receptors are widely distributed throughout the skin as reported in the literature (7). Any substances which inhibit 5-α reductase have the potential to inhibit synthesis of dihydrotestosterone and sebum secretion. Phenolic compounds i.e., myrecetin, kempferol, queretin, rutin, toxisol, emodin and caffeic acid have potential to inhibit the 5-α reductase and ultimately reduce the sebum secretion as reported in the literature (26).
Ample quantity of phenolics, namely, caffeic acid, myrecetin, anthocyanins, catechin, isoflavones and flavones is present in *Ananas comosus* extract (23). In this study, the continuous reduction in sebum contents at regular intervals in human cheeks by applying the active cream was due to the existence of these phenolics in formulation. Reported total phenolic contents in *Ananas comosus* fruit is 34.7 to 54.7 GAE/100 g (27). So, topical application of active cream causes reduction in skin sebum contents owing to inhibition of 5α-reductase enzyme. Moreover, *Ananas comosus* extract is rich in vitamin A (13) and one of the important metabolite of vitamin A is isotretinoin, which also has reported anti-inflammatory and anti-sebum activity and reduces the size of pilosebaceous duct and chances of colonization of propionibacterium acnes and is therefore used to treat vital etiological aspects incriminated in acne and seborrheic dermatitis (28).

**Efficacy assessment**

The efficacy assessment of both creams i.e., base and active formulation was performed at the last day of investigation; average points for each parameter are given in Table 4. Average values for the points i.e., easy of application, spreadability, sense just after application, sense in long term was 4.11 ± 0.01, 4.06 ± 0.18, 4.08 ± 0.03 and 4.17 ± 0.29 for base and 4.21 ± 0.01, 4.16 ± 0.08, 4.21 ± 0.03 and 4.23 ± 0.05 for formulation, respectively, indicating better results for active formulation than base. No irritation was found with both formulation on skin of all volunteers and assigned zero (0.00 ± 0.00) value. Basic formulation shows more shine on skin and sense of softness i.e., 4.42 ± 0.10 and 3.94 ± 0.16, respectively. This is because of the presence of excessive oily contents in basic formulation compared to active formulation. By applying paired sample t-test, it was obvious that base and formulation show insignificant (p > 0.05) variations between the average points for each parameter, which indicates that there was no immense efficacy difference between the base and active formulation regarding sensorial assessment.

**CONCLUSION**

In conclusion, the current study manifested that *Ananas comosus* fruit furnish a valuable source of natural phenolics. Creams loaded with 2% extract of *Ananas comosus* L. significantly reduced the skin irritation or erythema, melanin pigment and sebum contents *in vivo* and used topically to treat contact dermatitis, melasma or hyperpigmentation and reduced the sebum contents and treated various pathophysiological conditions of skin i.e., acne, seborrheic dermatitis and greasy or oil skin. Due to these reasons, *Ananas comosus* extract is considered fruitful as an ingredient in skin care products used for cosmetic purpose. Moreover, creams are better tolerated by all volunteers and fortified its acceptance as topical antioxidants after insertion in suitable and secure topical bases. In the future, there is a need to clinically evaluate these creams in patients with melasma, psoriasis or itching and acne, in order to explore the actual potential of this fruit.

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