boiled under reflux on a water bath for 30 minutes. This is followed by the addition of 1 ml of phenolphthalein solution and the solution is titrated immediately with 0.5 M hydrochloric acid ("*a*" ml). The operation was repeated by omitting the substance being examined ("*b*" ml).[16] The saponification value was calculated from the expression:
saponification value = 28.05 $(b - a)/w$,
where w is the weight of the substance in grams.

Acid value determination

The acid value is the number which gives in milligrams the amount of potassium hydroxide necessary to neutralize the free acids present in 1 g of the substance. Five grams of the formulation being examined was accurately weighed and dissolved in 50 ml of a mixture of equal volumes of ethanol (95%) and ether, previously neutralized with 0.1 M potassium hydroxide to phenolphthalein solution. Then 1 ml of phenolphthalein solution was added and titrated with 0.1 M potassium hydroxide until the solution remains faintly pink after shaking for 30 seconds. The acid value was calculated from the expression[16] acid value = 5.61 n/w, where n = the number of ml of 0.1 M potassium hydroxide required and w = the weight in grams of the substance.

Nonvolatile content

One gram of each formulation was weighed in a large weighing bottle and heated on a steam bath under a jet of air for 30 minutes. Then heating was continued at 105°C in an oven for 2 hours, then cooled in a desiccator, weighed and reported as the percent of nonvolatile content.[14]

Ash value

Ash measurement is an indicator of the effectiveness of the demineralization (DM) step for removal of calcium carbonate.[13] Five grams of each formulation was weighed in a flat-bottomed silica crucible and heated on a steam bath under a jet of air for 1 hour. Then 1 g of ash less cellulose powder was added to it and mixed with a glass stirring rod. The dish was heated at 600°C in a muffle furnace and the ash obtained was examined.[16]

Clinical Study

In clinical studies, the six marketed herbal moisturizers [Table 1] were evaluated for their ability to improve the skin hydration. *In vitro* evaluation previously performed suggests that all the formulations are suitable for human skin use. *In vivo* study suggests that the moisturizer improves the skin appearance and conductivity.

Clinical protocol

This study was conducted at the University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India. A total of 36 (12 males and 12 females) human volunteers, aged from 22 to 25 years, who were willing to give informed consent, were included in the study. They were asked to refrain from using any cosmetic on their forearm for 5 days prior to and during the study period. A separate group was also prepared from among the above subjects to act as controls (site used was other than the where formulation was applied).

Table 1: Selected formulations and their constituents

Formulation code	Constituents present
A1	Aloe vera extract, olive oil and rose water
A2	Wheat germ oil, olive oil, Aloe vera extract, turmeric extracts and rose water; enriched with vitamins D and E
A3	Wheat germ oil, rose water
A4	Aloe vera, sandalwood, and rose petals
A5	Wheat germ oil, glycerin, rose water
A6	Wheat germ oil, olive oil, rose water

Exclusion criteria

Volunteers having known hypersensitivity reactions to any of the formulation ingredients, having skin wounds or scratches on the volar forearm, and those who are not willing to give informed consent were excluded from the study.[18]

Subjects' inclusion criteria

Written informed consent was taken from all 18 human volunteers (20–25 years) before conducting the study. Their skin prototype and skin nature were determined by questionnaire method.[19] Volunteers having dry and mixed skin were selected for the subjective study to determine the effectiveness and safety of commercial herbal moisturizers with regard to the claims produced about them.

Study recruiting procedure

The information about all volunteers including personal data, a description of symptoms and details of past medical history (family history, history of possible exacerbating factors, etc.) was obtained in order to determine the eligibility for enrolment in the trial. All the volunteers willingly consented to meet at the laboratory between 10 a.m. and 5 p.m. If any of the volunteers experienced any discomfort, they were allowed to withdraw at any time from the study. However, none of them had withdrawn from the entire study procedure.[20] To the control group, water was applied half an hour before taking the readings. Test groups 1, 2, 3, 4, 5 and 6 were applied A_1, A_2, A_3, A_4, A_5 and A_6, respectively. Subjects were applied the moisturizer of the respective group twice a day for 3 weeks.

Hydration measurement

Hydration is directly related to the conductivity of the

skin, which is the inverse of the resistance. Resistance was measured using a Cassio Multimeter in kiloohms. The conductivity was calculated and expressed in microsiemens. Initially, baseline reading of the selected portion was taken. The moisturizer of the respective group was applied twice a day for 3 weeks. The readings were then taken daily for 3 weeks. The percent increase in conductance from the baseline was plotted against the weeks [Figure 1].

Skin appearance

The image of the respective portion of the forearm was taken initially and after the 3rd week of the study period. The visual appearance of the images were compared and the differences were observed (the image was taken by using the digital camera of 8.2 mega pixels).

Glow measurement

The glow was measured using the luxmeter, which gives reading in luxes (unit of light), which actually measures the photons reflected by the skin.

Psychometric analysis

The products were compared based on sensory evaluation and ranking was done as per the score obtained according to the hedonic scale [Table 2].[21] The parameters of psychometric analysis were color, odor, texture, wetness, spreadability, thickness, absorbency, gloss, stickiness, slipperiness and firmness.

RESULTS AND DISCUSSION

Physicochemical parameters are important to collect the information regarding the rheological behavior, stability and skin compatibility of the formulation. Result of their analysis justified the compatibility of selected herbal moisturizers with all type of skins. pH, nonvolatile matter, saponifcation value, acid value, fatty content, spreadability and layer thickness values confirmed the good cosmetologic property of all formulations [Table 3]. All formulations hold the pseudoplastic flow that is a desirable property needed by all creams and lotions for considerable stability. The results were in congruence with the results obtained by Kapoor *et al*. for herbal sunscreens.[22]

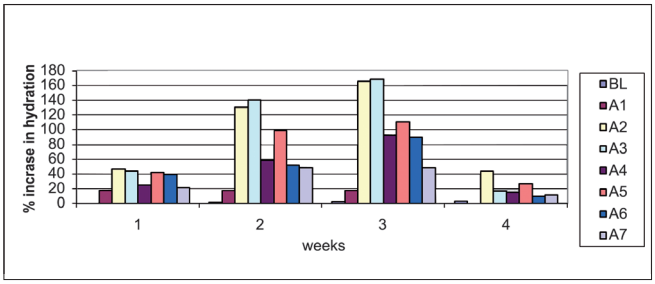

Figure 1: Effects of moisturizers on skin hydration

Table 2: Hedonic scale values for grading the products

Grade	Score
Extremely liking	8–9
Between extremely liking and medium	7
Medium	5–6
Between medium and dislike	4
Dislike	1–3

In the skin hydration study, different moisturizers were compared with each other in terms of conductivity. Each one of them corresponds to an average of six volunteers in a particular group. Skin conductance is a direct measure of the moisture present in the stratum corneum. The increase in conductance can be due to humectants, occlusive agents, exfoliants, emollients or combinations. The overall effect is an increase in the water holding of the stratum corneum. If the skin is wet, the value of resistance is less, which is of only about 1100 Ω. With dry skin, the amount is much higher at around 495,000 Ω. When we measure body resistance by electronic multimeter, the resistance can be about 1 MΩ for dry skin.[24] The purpose of moisturizer is to decrease the resistance of skin. Hence, the percentage increase in conductance (derived from the inverse of resistance) is the skin hydration measurement. Increase in skin conductance on the 3rd week was significant for all the products in a time-dependent pattern. Highest values among those were 168.125 and 165.24% for A2 and A1, respectively ($n = 18$, $P < 0.05$). The effect remaining after the regression period was below 50% for all the products, indicating some sustained effect of moisturizers on skin hydration. Figure 1 shows the percent increase in hydration with respect to time.

The sequence of hydration effect obtained was in the order A2 > A1 > A4 > A3 > A5 > A6. When we compared the constituents of the formulations with the obtained results, it could be seen that A2 formulation contained wheat germ oil, olive oil and Aloe vera extract. So, due to the synergistic effects of wheat germ oil and Aloe vera extract, this formulation produced highest hydration effects. Second in rank was formulation A1 which contained only Aloe vera extract and olive oil. It has been reported by Belo *et al*. that Aloe vera extract improves the skin hydration by humectants mechanism.[13] So, we could also predict that due to hydration mechanism the formulations improved skin hydration. When oil is added to the formulation, the effect is increased as it forms an occlusive layer on the skin and prevents loss of moisturizer.[23] In general, when applied to skin, the vegetable oils are easily absorbed and show great spreadability.[24] In formulation A5, glycerin also produced its softening effect, hence A5 was better than A6.

The dry skin has a parched look caused by its inability to retain moisture. It usually feels "tight" and uncomfortable after washing unless some type of moisturizer or skin cream is applied. It looks dull. Skin seems to be with reduced wrinkles after the 3rd week of moisturizer application. So it can be considered that all the moisturizers improved the skin appearance. Skin pictures taken as baseline and after the end of study period have been shown in Figure 2. Improvement in the appearance of skin supports the data for the increase in hydration.

Psychometric analysis

Psychometric parameters were evaluated to visualize the compliance of moisturizers with skin [Table 4]. Initially, the readings were taken for all the volunteers, before applying the formulations, which were considered baseline values (BL). All the volunteers were asked if they felt any irritation after applying the moisturizer. The moisturizers were then applied to the forearm twice daily and then subsequent readings were taken after each application. The differences in effectiveness of the moisturizers and control were statistically found to be different. The highest score for the product acceptance was scored by A2 (84 ± 6) and lowest by A6 (69 ± 0). The difference is not high; hence, all the products are acceptable to the consumers. The details of the scores obtained by all the products are given in Table 4. Comparisons of products are shown in Figure 3.

Table 4: Psychometric analysis

Property	A1	A2	A3	A4	A5	A6
Color	7 ± 0	7 ± 0	7 ± 1	7 ± 0	7 ± 0	6 ± 0
Odor	8 ± 0	7 ± 1	6 ± 0	6 ± 1	5 ± 1	4 ± 1
Texture	7 ± 1	8 ± 1	7 ± 2	8 ± 0	7 ± 0	6 ± 0
Wetness	7 ± 1	8 ± 1	7 ± 1	7 ± 0	6 ± 1	6 ± 0
Spreadability	7 ± 1	7 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0
Thickness	7 ± 2	7 ± 0	6 ± 0	7 ± 1	7 ± 0	7 ± 0
Absorbency	6 ± 0	7 ± 0	7 ± 0	6 ± 1	6 ± 2	7 ± 1
Gloss	7 ± 0	7 ± 1	6 ± 1	7 ± 0	7 ± 0	6 ± 1
Stickiness	7 ± 0	6 ± 0	6 ± 0	6 ± 1	6 ± 0	7 ± 0
Slipperiness	7 ± 0	6 ± 0	7 ± 0	7 ± 0	6 ± 1	7 ± 0
Firmness	8 ± 0	8 ± 1	7 ± 0	7 ± 0	7 ± 0	7 ± 0
Average score	78 ± 5	84 ± 6	72 ± 5	74 ± 4	70 ± 5	69 ± 0

n: 6

Luxmeter measures the light intensity, which is the number of photons. When the light is incident the on our skin, the skin also reflects some portion of light. The measure of this reflected light is considered here as glow, i.e., the glow is measured in luxes. For the moisturizer to increase the glow of skin, the light reflection from the skin should increase. The readings obtained by keeping the probe of luxmeter a constant distance away and in the same intensity environment range from 5 to 7 luxes. But the difference was not found after the application of moisturizer and after the study period. This shows that the glow when measured in luxes was not affected by the moisturizer application.

CONCLUSION

The test results indicate that the moisturizers improve the skin hydration and appearance on daily use. Significant improvement was seen after the 3rd week of short period of study in the skin conductivity and the method employed seems to be easy and efficient. The statistical analysis of the experimental data was carried out by one-way analysis of variance (ANOVA) and the differences were considered as statistically significant at 95% confidence level. It was

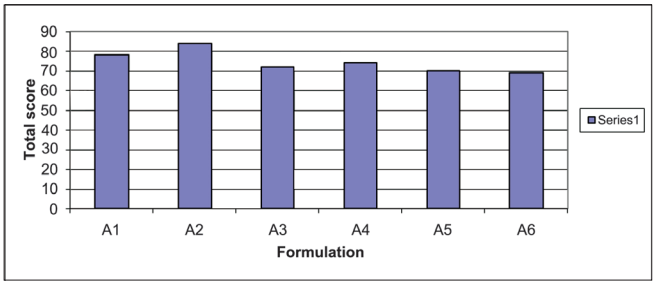

Figure 3: Ranking of different moisturizers on the basis of psychometric analysis

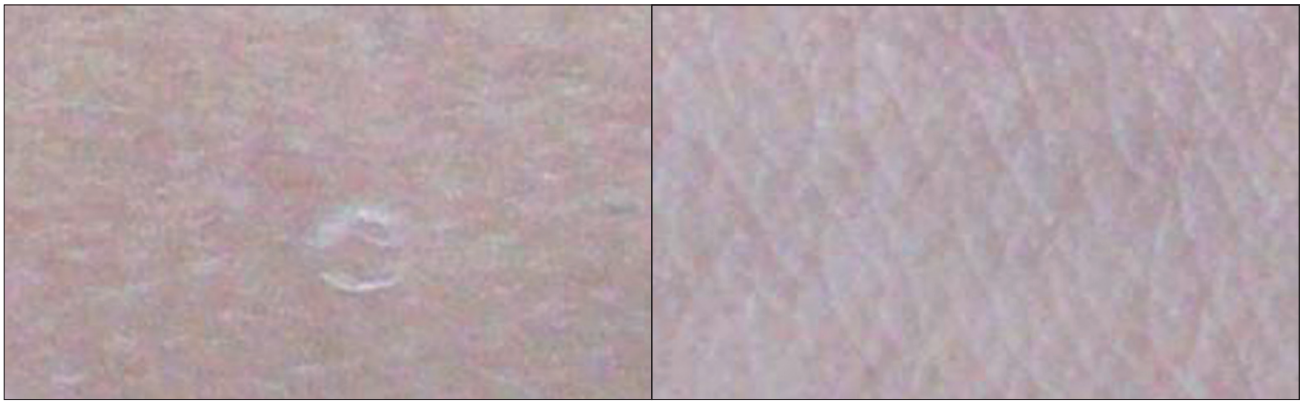

Figure 2: Comparative pictures showing change in skin appearance (Picture taken initially, after 3rd week)

Table 3: Physiochemical data

Parameters	A1	A2	A3	A4	A5	A6
pH	6.15 ± 0.1	6.24 ± 0.13	6.3 ± 0.05	6.6 ± 0.04	6.9 ± 0.02	6.9 ± 0.1
Spreadability (perimeter)	19.468 ± 0.4	17.89 ± 0.5	14.4 ± 0.23	18.212 ± 0.1	18.52 ± 0.3	15.7 ± 0.4
Saponification value	29.45 ± 0.26	16.8 ± 0.48	14.025 ± 0.01	12.62 ± 0.35	12.61 ± 0.3	9.81 ± 0.17
Acid value	2.917 ± 0.06	2.13 ± 0.05	0.8976 ± 0.1	1.23 ± 0.05	6.732 ± 0.12	3.48 ± 0.1
Nonvolatile %	21.6 ± 0.4	13.02 ± 0.53	14.87 ± 0.1	13.85 ± 0.23	7.02 ± 0.6	14.87 ± 0.06
Ash value	—	—	—	—	—	—

found that the formulations containing wheat germ oil and Aloe vera extract produced higher skin hydration as compared to the formulations containing them separately. The mechanism predicted was humectants mechanism along with the occlusive layer formation on the skin by the formulations.

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