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Efficacy Study of Sunscreens Containing Various Herbs for Protecting Skin from UVA and UVB Sunrays

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ABSTRACT

Currently, no standard protocols and objective measures are existing in present system for quality analysis of herbal sunscreens. Present work is an attempt to compile rapid, non-invasive technologies to investigate the sunscreens containing various herbs like aloe vera, jojoba, cucumber, wheat germ, olive etc for their efficacy in protecting skin from UVA and UVB sunrays. Commercial herbal sunscreens containing herbs aloe vera, basil, green tea, etc and bearing SPF range 10–40 coded as HS1- HS14, were analyzed by subjective, photostability and other parameters evaluation. All sunscreens shown pH [6.09±0.01 to 8.30±0.03], Saponification value [6.01±0.2 to 207.57±0.3], Acid value [1.56±0.6 to 17.27±0.5], Ash value [0.01±1 to 0.08±2 gm], Spreadibility [96±0.9 to 98±0.9 %], Layer thickness [28.99±1.55 to 32.25±1.00 %]. Viscosity profile showed the pseudoplastic behaviour of all formulations. Phase separation was observed in HS1 to HS4, HS7 & HS9 to HS12 during stability study. None of them were found to be irritant [erythema score = 0] and have microbial count load in the range of to 31±1 to 34±2 CFU/gm. 98±5 % of all sunscreens has shown SPF as per labelled claim by *In-vitro* and *In-vivo* method. HS 6, 9, 11 were found to be unstable in UVA range. HS8, most preferred by volunteers after Psychometric evaluation. Results of the study scientifically verified that herbs are having enough potential to protect skin to protect skin from harmful sunrays and it is worthwhile for consumers to use herbal sunscreens. Overall study is useful to substantiate product claims.

Keywords: Herbal, sunscreens, commercial, efficacy, sun protection factor.

INTRODUCTION

It is a well-known fact that an over exposure of human skin to ultraviolet light may lead to sunburn cells, premature skin aging and an increased risk for skin cancers (1–4). Numbers of conventional and novel herbal cosmetics are useful to treat damaged skin (5–7). The steady increase in the incidence of melanoma, non-melanoma cutaneous neoplasia and preneoplasic disorders has contributed to the demand for more effective protection from the sun (8–10). Although modern sunscreen containing UV-filters are highly efficient to protect the skin from the deleterious effects of the sun (11–13), but herbal sunscreens are rapidly replacing them due to associated side effects with UV filters. Number of herbs like *G.glabra, C.longa, P.corlifolia, C.tora, A.catechu, P. granatum, E. officinale, C.asiatica, C.zeylanicum, A. vera* etc were already explored scientifically for their sun protecting efficacy in literatures. So many herbal sunscreens are available in market in form of creams, lotion and gel having labelled sun protection factor [SPF]. Most commonly used herbs are aloe vera, basil, green tea, almond, olive, jojoba, and cucumber etc, incorporated in herbal sunscreens (14–17). Scientifically these plants are already explored for multipurpose biological activities like antioxidative, anti-aging and anti-scavenging properties etc (18–19).

Quality analysis is needed to ensure that the product has the expected effects. Quality implies certification in

respect of authentication, standardization, composition, stability and safety. It is complicated science with herbal cosmetics because specific constituent responsible for the claimed effects can not be identified (20–21). No doubt, many herbs are found to be miraculous cures for several diseases, but before marketing finished herbal cosmetics must undergo sufficient quality evaluation to establish the strong faith of consumer. Efficacy of sunscreens is important public health issue (22). The growing market of such products also increases safety and efficacy concerns among the consumers. In order to guarantee constant efficacy of sunscreen products, they should be photostable.

Several sunscreen producers claim that their products give good protection against both UVA and UVB radiation; however, the photostability of the product is rarely declared. This is also important for the consumer to know when choosing a sunscreen. Since it has been known for several years that some products may be photounstable, one would have expected a large improvement in the photostability of sunscreen products. Up to now, there is no standard method for determining photostability of a sunscreen (23, 24–25). Several different systems are currently in use which generates the need of unique or uniform international standard method for measuring UVA protection (26–29). Subjective and Photostability evaluation are important parameters for ensuring the efficacy of herbal cosmetics. Therefore, in present work exhaustive investigation has been carried out by evaluating various parameters to provide scientific documentation in support of safety and efficacy of commercial herbal sunscreens. This work is an attempt to increase the faith of consumers on herbals by providing scientific documentation against their claims. The objective of research in herbal cosmetic field is to getting best from the nature for better tomorrow.

MATERIALS AND METHODS

All fourteen commercial herbal sunscreens were purchased from local dealer of Raipur, Chhattisgarh, India and coded as HS1-HS14. Herbal sunscreens having "Low protection" [SPF 6–15], "Medium protection" [SPF 15–30] and "High protection"' [SPF 30–60], defined by the Commission Recommendation 2006/647/EC (30) were selected for present study. Other commonly used chemicals were of analytical grade [SD Fine Chem, Mumbai, India]. Commercial sunscreens containing extract, juices and oils of different herbs as mentioned on the containers is summarised in table 1.

Instruments used for analysis were pH meter [335, Systronic, India], Brookfield viscometer [DV-I, LV-I spindle, Brookfield Engineering Laboratories, USA], Colony counter [M-37, Rolex, India], Muffle furnace [77 S8HT8,Tempo, India], Micro centrifuger [RM-12CDX, Remi, India], Deep freezer [RQF 650, Remi, India] and UV-V spectrophotometer [UV 1700, Shimadzu, Japan].

Table 1: List of herbs present in commercial sunscreens

Sunscreens	Herbs (English name)	Herbs (INCI name)
HS1	Water melon	*Citrullus vulgaris*
HS2	Sandal wood, Winter cherry, Cobras Saffron, Wheat germ, Honey, Red Sandal Wood, Symplocos, Aloe vera	*Santalum album, Withania somnifera, Mesua ferrera, Triticum vulgae, Apis mellifica, Pterocarpus santalinus, Symplocos racemosa, Aloe barbadensis*
HS3	Carrot, Symplocos, Wheat germ	*Daucus carota, Symplocus racemosa, Triticum vulgae*
HS4	Aloe vera, Apple	*Aloe barbadensis, Malus sylvestris*
HS5	Sunfower, Indian madder, Cucumber	*Helianthus annuus, Rubia cardifolia, Cucumis sativus*
HS6	Aloe Vera	*Aloe barbadensis*
HS7	Orange, Vitamin C	*Citrus aurantium, Ascorbic acid*
HS8	Coriander, Vitamin E,	*Coriandrum sativum,Tocopherol*
HS9	Aloe vera, Vitamin E,	*Aloe barbadensis, Tocopherol*
HS10	Aloe vera, Basil, Turmeric	*Aloe barbadensis, Ocimum sanctum,Curcumba longa*
HS11	Sandal wood, Aloe vera, Carrot, Honey, Sunflower	*Santalum album, Aloe barbadensis, Daucus carota, Apis mellifica, Helianthus annus*
HS12	Wheat germ,Vitamin E	*Triticum vulgae ,Tocopherol*
HS13	Aloe vera, Vitamin E	*Aloe barbadensis Tocopherol*
HS14	Cucumber, Jojoba, Orange, Sandal wood, Lavendar, Vitamin A, C, E	*Cucumis sativus, Simmondsia Chinensis, Citrus aurantium, Santalum album, Lavandula vera, Ascorbic acid, Tocopherol*

Study protocol

Twenty eight volunteers [23–29 years], equally distributed in two groups were recruited for subjective evaluation for testing sunscreens efficacy [SPF *In-vitro* determination] and safety [sensitivity test]. Back of the forearm was the chosen site for the study (31). Each sample was tested in two groups of volunteers. For psychometric analysis out of 28, only 10 volunteers of 23–29yrs were selected for study (32–34).

Subjects inclusion criteria

Written informed consent has been taken from all 28 human volunteers [23 to 29 years] before conduction of study. Their skin prototype and skin nature has been determined by questionnaire method (35). Volunteers having untanned, dry, oily, normal, mixed skin and prototype I-III were selected for the subjective study to determine the effectiveness and safety of commercial herbal sunscreens with regards to the claims produced on them.

Subjects exclusion criteria

Volunteers those were any type of allergy and skin wounds or scratches on the back of forearm, has been excluded from study. Although none of the participants involved in the present study were found to be suffering from any of the above mentioned defects.

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] were obtained in order to determine the eligibility for enrolment in the trial. All volunteers willingly consented to meet at the laboratory between 10 am to 5 pm. If any of the volunteers were experienced any discomfort, they were allowed to withdraw any time from the study. However, none of them had been withdraw from whole study procedure.

Quality analysis

All quality parameters were evaluated according to the guidelines of Bureau of Indian Standard (BIS), World health Organization [WHO], European Cosmetic, Toiletry and Perfumery Association [COLIPA] and Scientific Committee of Cosmetics and Non-Food Products [SCCNFP] (36–39).

Physicochemical analysis

Type of emulsion, colour, odor, pH, fatty content, ash value, volatile and nonvolatile content of the commercial herbal sunscreens HS1-HS14 were determined by standard techniques and methods (40–41). Saponification, acid and ester values were determined according to methods discussed in Indian Pharmacopoeia (42). Rheological behavior has a fundamental importance in the formulation of sunscreen, because the formation of an evenly distributed film is critically influenced by the flowing properties of the formulation (43–46). Viscosity profile of each herbal sunscreen was measured using a Brookfield viscometer at 10 to100 rpm (47). Viscosity measurements were made under 25°C, 8 ml samples and using LV-spindle. Spreadability and layer thickness were evaluated according to Multimer (48), spreadability refers to the % area covered by a fixed amount of cream sample after the uniform spread of sample and layer thickness refers to thickness of the layer in microns. Stability of each herbal sunscreen was determined by centrifugation and freeze thaw method (49). During centrifugation study all sunscreens were centrifuged at 3500–13500 rpm at the intervals of 500 rpm for 10 minutes, and further observe for phase separation. In freeze thaw study all sunscreens were kept alternatively at 20°C and 40°C, then observe for color change and phase separation. All evaluations were carried out in triplicate.

Safety analysis

Safety analysis includes determination of microbiological specification and sensitivity profile. Microbial examination of all herbal sunscreens [1 ml] was tested according to COLIPA guidelines and Indian Standards methods (50). Total numbers of viable mesophyllic microrganism were recorded by using a colony counter (51). All samples [1 ml] were determined for the presence or absence of *Pseudomonas aeruginosa, Staphylococcus aureus and Canidida albicans* (51–52). To ensure commercially available herbal sunscreens are free from any adverse effect a sensitivity study using a patch test design was conducted on all volunteers. After 24 hrs volunteers were observed for any irritation, erythema score [redness], and oedema. Sunscreens were applied on the back of forearm with the help of surgical gauze (0.5 mg/cm^2) and the erythema score [redness] was determined using the scale defined in the Indian Standards (53).

Photostability evaluation

Photostability evaluation for 14 herbal sunscreens was carried out by measuring Area under the curve index [AUCI] by measuring absorbance (54) under the wavelength range of interest by using two-beam UV-V spectrophotometer [UV 1700, Shimadzu, Japan].

The sunscreen was weighed and placed between two plates of polished fused silica [quartz] with diameter

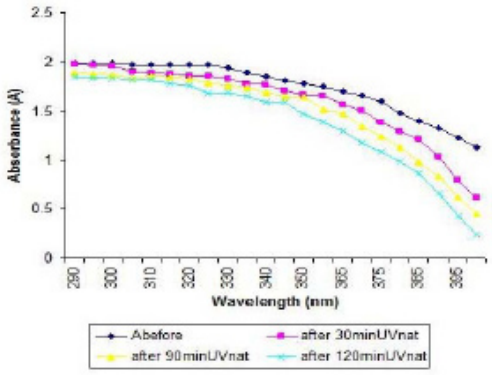

Figure 2a

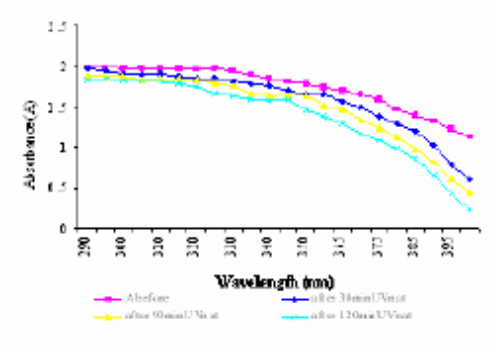

Figure 2b

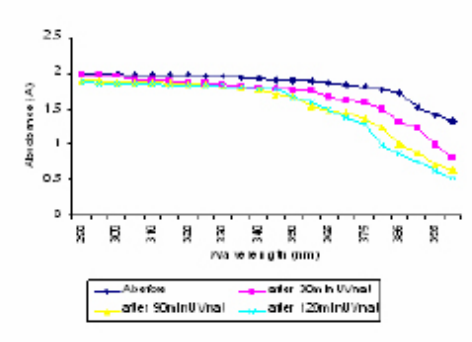

Figure 2c

Figure 2a–2c: Absorption spectra of HS6, HS9 and HS10 after and before UVnat exposure

25 mm and thickness 5 mm. The amount applied was 0.5 mg/cm^2 (55). For UV natural [UV_{nat}], samples were placed horizontally outdoors when the weather was sunny. This was done in early July in Raipur [latitude: 21°15' N, Longitude 81°41' E]. The total exposure time was 120 min with measurements of the absorption spectra [Figure 2a–c] before exposure and after 30 min, 90 min and 120 min of UV_{nat}.

To eliminate the degradation possibility of the photoactive compounds could be caused by a temperature increase, control samples of sunscreen between silica plates were placed on a heating plate for 20 minutes. The temperature was kept at 50°C ± 2°C, this is about 15°C higher than the temperature of the skin. Spectra were recorded prior to and after heating. The temperature did not influence the degradation since the absorption spectra did not change after heating.

The spectra were recorded by UV-V spectrophotometer [UV 1700, Shimadzu, Japan]. The AUC for UVB [290–320 nm], UVA1 [340–400 nm], UVA2 [320–340 nm] was calculated for each spectrum before [AUC_{before}] and after [AUC_{after}] before and after UV_{nat}. Maier et al. used the difference between the spectral transmission before and after a defined UV exposure, ΔT. A product was labeled photounstable if the mean photoinstability was higher than 5% (56). In present study we calculated AUCI [Table 5] to find out the photostability of investigated herbal sunscreens, if the AUCI [AUCI = AUC_{after}/ AUC_{before}] was greater/equal to 0.80, the sunscreen was considered photostable.

The AUC was calculated with the following equation:

$$\sum_{\lambda\ min}^{\lambda\ max} A(\lambda)\Delta\lambda$$

Where A is absorption and λ is wavelength. It was measured in steps of 5 nm. For UVA λ_{max} = 400 nm and λ_{min} = 320 nm. The same calculation was done for each UV range respectively, before and after UV_{nat}.

Efficacy Analysis

Efficacy analysis is an important step to verify the claim produced by finished products. In present study, efficacy of herbal sunscreens has been determined by *In-vitro* and *In-vivo* method [Table 7].

In-vitro method

This method is based on the fact that herbal sunscreens are topical preparations which are soluble in a range of

Table 5: AUCI values for herbal sunscreens

Sunscreens	UVB (290–320 nm)			UVA 2 (320–340 nm)			UVA 1 (320– 340 nm)		
	30min	90min	120min	30min	90min	120min	30min	90min	120min
HS1	0.91	0.90	0.90	0.95	0.93	0.89	0.84	0.84	0.83
HS2	0.93	0.93	0.92	0.99	0.96	0.96	0.95	0.94	0.92
HS3	0.89	0.89	0.87	0.88	0.87	0.85	0.81	0.80	0.80
HS4	0.83	0.83	0.84	0.86	0.85	0.81	0.84	0.83	0.84
HS5	0.87	0.91	0.91	0.90	0.89	0.89	0.93	0.91	0.93
HS6	0.89	0.88	0.88	0.76	0.78	0.77	0.68	0.67	0.65
HS7	0.84	0.83	0.90	0.91	0.89	0.84	0.86	0.84	0.83
HS8	0.87	0.83	0.85	0.93	0.92	0.89	0.89	0.86	0.85
HS9	0.86	0.83	0.82	0.78	0.65	0.64	0.79	0.76	0.76
HS10	0.94	0.93	0.91	1.00	0.98	0.98	0.95	0.95	0.94
HS11	0.81	0.82	0.85	0.84	0.83	0.83	0.76	0.65	0.63
HS12	0.87	0.85	0.84	0.91	0.90	0.89	0.84	0.83	0.83
HS13	0.87	0.85	0.86	0.86	0.85	0.84	0.82	0.81	0.81
HS14	0.94	0.92	0.92	1.00	0.98	1.00	0.98	0.97	0.96

Area under curve index (AUCI), Bold numbers shows when AUCI is <0.80

Table 7: Efficacy evaluation parameter

Sunscreens	SPF		
	In vitro	In vivo	Labelled claim
HS1	9.8±1.6	10.0±1.0	10
HS2	31.7±1.8	29.5±1.1	30
HS3	38.0±2.1	40.6±1.0	40
HS4	15.2±2.0	14.5±1.2	15
HS5	21.4±2.6	19.0±1.6	20
HS6	20.8±2.1	19.9±1.4	20
HS7	24.9±1.0	25.2±1.2	25
HS8	20.9±2.2	20.5±1.1	20
HS9	23.9±1.7	24.5±1.2	24
HS10	31.5±1.7	30.8±1.7	30
HS11	14.4±1.6	15.1±1.6	15
HS12	14.7±1.0	14.9±1.0	15
HS13	20.6±1.6	19.0±1.5	20
HS14	32.6±2.0	29.9±1.3	30

All the values are represented as Mean ± SD (n=3), $p < 0.001$in the column

organic solvents, and which are specifically designed to absorb radiation in the ultraviolet [UV] spectrum (57). The UV-V spectrophotometer [UV 1700, Shimadzu, Japan] was used to carry out for SPF determination. Spectrophotometer set to 400 nm and allowed to warm up for 15 min. 0.10% solution of each sunscreen [HS1-HS14] was prepared in 95% ethanol. Weigh 0.050 gm of sunscreen in 100 ml beaker, add 50.0 ml of ethyl alcohol and stir to dissolve the sunscreen. Now transfer 100 ml of 0.10% prepared solution of each sunscreen in different [fourteen] calibrated volumetric flask (10 ml) record the formulation number and labelled SPF on volumetric flask. Fill the one cuvette [quartz] with test sample (0.10% solution) and other cuvette with ethanol. Now measure the absorbance for each sample [HS1-HS14] at 400 nm to 290 nm at the interval of 5 nm. SPF was calculated by using the equation derived by Mansaur et al (58–59). EE (λ) x I(λ) values determined by Sayre (60–61) was used in below equation. Each sample observed in triplicate.

$$SPF = CF \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times A(\lambda)$$ Equation

CF = 10 (Correction factor)
EE(λ) = erythemogenic effect of radiation of wavelength λ
I(λ) = intensity of solar light of wavelength λ
A(λ)= spectrophotometric value for absorbance of wavelength λ by a prepared solution of test sample.

In-vivo method

This method is based on subjective evaluation of human volunteers. The Sun Protection Factor (SPF) value of a product is defined as the ratio of the Minimal Erythema Dose on product protected skin (MEDp) to the Minimal Erythema Dose on unprotected skin (MEDu) of the same subject [Volunteer].

$$SPF = \frac{MEDp\ [protected\ skin]}{MEDu\ [unprotected\ skin]}$$

The Minimal Erythema Dose (MED) in human skin is defined as the lowest ultraviolet UV dose that produces the first perceptible unambiguous erythema with defined borders appearing over most of the field of UV exposure, 16–24 hrs after UV exposure (31, 62).

The test products [herbal sunscreens] were applied in the amount of 2 mg/cm^2 ± 2.5% on the back of forearm of the 28 volunteers [age 23–28 years, skin: Dry – normal, phototype: I–III], 15 min prior UV solar radiation, natural sunlight was used as a source of radiation. Study was carried out in the month of June [11.30–3.30 am], Institute of pharmacy, Pt. R.S.U, Raipur. According to Raipur Standard sun chart, between this duration most of the UV rays are falling. Study carried under the investigation of dermatologist. The visual assessment of skin reactions [perceptible unambiguous erythema] after UV exposure was noted down. Each sample [sunscreens] was tested in

two groups of total 28 volunteers. Back of the forearm of each volunteer was divided into two subsites of 2 × 2 cm^2 area separated by 0.8 cm by each other. One subsite at which no product was applied (unprotected) and at other subsites [protected], test sample [2 mg/cm^2] was applied. For each subject, the Minimal Erythema Doses on unprotected skin and on skin protected by the test products were recorded. The SPF for the product was calculated as the arithmetic mean of all individual SPF values obtained from all subjects in the test. Product application and MED assessment has been carried out in stable conditions, with the room temperature maintained between 18 and 26°C. To aid the uniform coverage product was deposited with a syringe, and then spread over the whole test site with light pressure, using a finger cot. A new finger cot must be used for each product. Spreading time of products was in the range of 20 to 50 seconds (22).

The selection of volunteers and the test method were carried out in accordance with the ethical principles as set out in the declaration of Helsinki and International Ethical Guidelines for Biomedical Research Involving Human Subjects (60–61). The study was approved by the Ethical Review Committee of the Institute of Pharmacy, Raipur.

The statistically results obtained by vitro and vivo method was compared graphically with SPF claimed on label [Figure 3].

Psychometric evaluation

Different questionnaires regarding herbal sunscreens have been asked from 10 volunteers recruited for study [Table 8]. Their answers regarding attributes decided by evaluator have been converted into scale and then analysed by using

Table 8: Psychometric evaluation parameters

Sunscreens	Appearance	Fragrance	Lathry	Soft-ness	Smooth-ness	After effect	Overall ranking
HS1	5.4±3.5	6.9±0.6	6.3±0.8	7.0±0.0	6.9±0.3	6.2±1.5	38.7±6.7
HS2	7.0±2.8	7.1±2.2	6.5±0.7	7.3±0.8	7.3±0.9	7.7±1.0	42.9± 8.4
HS3	2.6±2.1	2.2±1.9	5.1±2.4	6.4±1.6	6.1±1.6	5.2±2.9	27.6±12.5
HS4	6.2±3.7	7.8±0.4	6.2±0.6	6.9±0.7	6.7±0.8	6.5±1.9	40.3±8.1
HS5	4.2±3.1	3.5±2.9	5.1±2.2	6.3±1.3	6.4±1.2	6.6±1.1	32.1±11.8
HS6	6.2±3.7	6.8±2.5	5.5±1.5	7.0±0.7	6.7±0.6	7.3±1.1	39.5±10.1
HS7	6.6±3.3	6.1±2.4	8.0±0.0	7.3±0.5	7.1±0.3	6.0±2.4	41.1±8.9
HS8	7.8±2.7	7.7±0.9	5.8±1.6	7.7±0.5	7.0±0.0	8.0±0.0	44.0±5.7
HS9	5.8±1.6	7.1±0.6	6.4±0.7	7.0±0.5	6.8±0.6	7.4±0.5	40.5±4.5
HS10	7.0±2.8	6.6±2.0	7.5±0.9	7.3±0.7	7.2±0.6	7.0±1.2	42.6±8.2
HS11	3.4±2.7	4.1±3.2	3.5±1.8	5.2±2.0	5.3±1.5	5.8±2.8	27.3±14.0
HS12	8.6±1.3	7.5±0.9	5.7±0.7	7.0±0.0	6.7±0.5	7.8±0.4	43.3±3.8
HS13	4.6±2.3	4.6±2.6	5.3±2.2	6.4±0.5	6.3±0.9	7.1±0.9	34.3±9.4
HS14	5.0±3.7	3.7±2.9	7.6±1.0	7.1±0.3	7.1±0.9	6.7±1.2	37.2±10.0
P value	0.003	0.0001	< 0.0001	< 0.0001	0.0001	0.0017	------

All the values are represented as Mean ± SD (n=10), p value found to be significant for all by ANOVA.

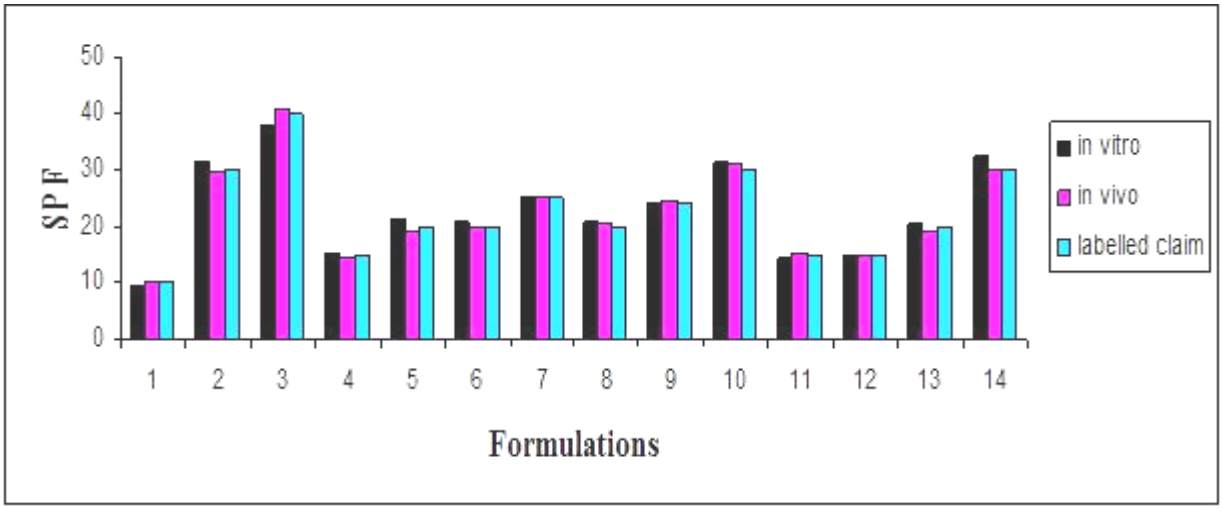

Figure 3: SPF determined by In-vitro, In-vivo method and claimed SPF.

ANOVA (63). Best known scale used in consumer testing is the 9 point hedonic scales (64–69) that has been used for present study [9 = liked extremely, 5 = neither like nor dislike, 1 = disliked extremely].

STATISTICAL SECTION

Statistical analysis was carried out by using STATS (70) software and results were expressed as mean S.D. Physicochemical, safety, efficacy and psychometric parameters were statistically analysed at 95% confidence level in the column. Statistical result of psychometric evaluation was further tested by ANOVA [One way analysis].

RESULTS

Colour of tested sunscreens was found to be white, cream, whitish-pink and orangish-white, pH of sunscreens was found to be in range of 6.09±0.01 to 8.30±0.03 and type of emulsions exist by tested sunscreens is either o/w or w/o. Key chemical parameters include non volatile matter [9.93±0.2% - 41.02±0.5%], volatile matter [58.98±0.5% - 90.07±0.2%], saponification value [6.01±0.2 - 224.40±0.5], acid value [17.27±0.5% - 1.56±0.6%], ester value [4.44±0.4 - 221.48±0.1], ash value [0.01±1 gm - 0.51±1 gm], and fatty content [15.92±0.6 gm - 12.21±0.7 gm] were found to be in controlled range for all tested sunscreens [Table 2].

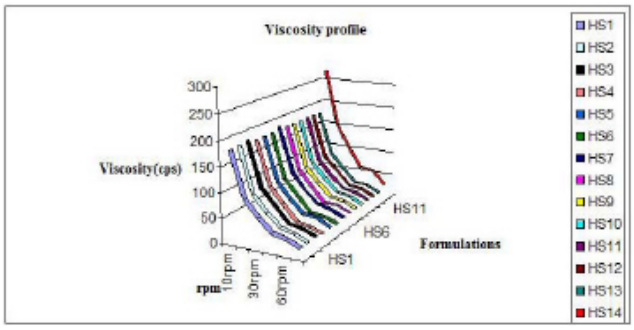

Figure 1: Viscosity profile of herbal sunscreens.

During the storage and handling of cosmetic formulations, spreadability, layer thickness and viscosity are the prime parameters which affect the formulation's acceptability. Spreadability and layer thickness were found to be in the range of 97±0.6% - 98±1.0% and 28.99±1.55 µm - 32.25±1.00 µm for formulations [Table 1]. As the speed of rotation has increased viscosity of tested sample decreased, this behaviour of all formulations [Table 3, Figure 1] revealed the pseudoplastic behaviour of products. Formulations with a pseudoplastic flow produce a coherent protective film covering the skin surface and this activity is important for adherence on the skin especially for higher SPF product (48–49).

Table 2: Physicochemical evaluation parameters

Sunscreens	Colour	Type of emulsion	pH	NVM (%)	VM (%)	SV	AV	Ester value	Ash value (gm)	FC (gm)	S (%)	LT(µm)
HS1	white	w//o	7.03±0.01	14.91±0.5	85.09±0.5	66.22±0.5	6.73±0.3	59.49±0.2	0.06±1	15.8±0.9	98±0.1	32.21±1.68
HS2	whitish-pink	o/w	7.64±0.01	31.44±0.1	68.56±0.1	207.57±0.3	6.95±0.5	200.61±0.2	0.01±3	13.23±0.8	97±1.0	31.39±1.00
HS3	cream	w/o	7.23±0.03	15.26±0.6	84.74±0.6	56.10±0.4	11.44±0.5	44.66±0.1	0.04±2	12.29±0.5	97±0.9	29.99±1.99
HS4	white	o/w	6.30±0.02	24.82±0.6	75.18±0.6	224.40±0.5	2.91±0.6	221.48±0.1	0.02±2	12.21±0.7	98±0.8	29.29±1.92
HS5	orangish-white	o/w	6.20±0.01	41.02±0.5	58.98±0.5	6.01±0.2	1.56±0.6	4.44±0.4	0.01±4	15.45±0.6	97 ±0.7	32.25±1.00
HS6	white	o/w	7.76±0.02	9.93±0.2	90.07±0.2	25.24±0.5	9.42±0.5	15.82±0.0	0.05±1	14.67±0.9	97±0.8	32.01±1.76
HS7	orangish-white	w/o	6.90±0.03	29.03±0.3	70.97±0.3	81.34±0.1	10.32±0.5	71.02±0.4	0.01±2	15.92±0.6	97±0.9	29.90±1.65
HS8	orangish-cream	o/w	6.58±0.02	24.41±0.3	75.59±0.3	30.85±0.4	6.28±0.6	24.57±0.2	0.01±4	13.99±0.8	96±0.9	31.75±1.20
HS9	white	w/o	6.09±0.01	18.60±0.5	81.40±0.5	101.12±0.4	10.15±0.2	90.96±0.2	0.01±1	14.8±0.6	98 ±1.0	29.21±2.00
HS10	orangish-cream	w/o	8.30±0.03	19.98±0.3	80.02±0.3	220.13±0.1	5.64±0.1	214.48±0.0	0.08±2	15.22±0.6	98±0.7	28.99±1.55
HS11	whitish-cream	o/w	6.98±0.03	16.01±0.2	83.99±0.2	50.49±0.2	8.75±0.3	41.73±0.1	0.51±1	13.30±0.8	97±1.0	31.49±1.00
HS12	white	o/w	7.11±0.01	10.66±0.2	89.34±0.2	164.09±0.3	6.28±0.4	157.80±0.1	0.01±4	12.78±0.5	97±0.6	29.89±1.08
HS13	white	o/w	7.21±0.02	23.69±0.6	76.31±0.6	120.61±0.2	6.17±0.5	114.44±0.3	0.01±3	12.61±0.7	98±0.9	31.29±1.92
HS14	creamish-white	w/o	7.46±0.02	28.43±0.5	71.57±0.5	129.03±0.5	17.27±0.5	111.75±0.0	0.03±2	14.23±0.6	98±0.8	31.58±1.66

All the values are represented as Mean ± SD (n=3), p < 0.001in the column, (S) Spreadability, (LT) Layerthickness, (AV) Acid value, (SV) Saponification value, (VM) volatile mater
(NVM) Non volatile matter, (FC) Fatty content.

Table 3: Safety evaluation parameters

Sunscreens	Microbial examination				Stability		
	Microbial Count (CFU/gm)	Pseudomonas aeruginosa	Staphylococcus aureus	Canidida albicans	Centrifugation 13500 (rpm)	Freeze Thaw	Erythema score
HS1	32±2	A	A	A	+	-	0
HS2	32±3	A	A	A	-	+	0
HS3	34±1	A	A	A	--	+	0
HS4	32±3	A	A	A	-	+	0
HS5	31±2	A	A	A	+	+	0
HS6	31±1	A	A	A	+	+	0
HS7	31±1	A	A	A	--	+	0
HS8	32±2	A	A	A	---	+	0
HS9	34±2	A	A	A	--	--	0
HS10	31±2	A	A	A	+	-	0
HS11	31±3	A	A	A	-	+	0
HS12	33±1	A	A	A	--	-	0
HS13	32±3	A	A	A	+	+	0
HS14	32±2	A	A	A	+	+	0

(CFU) colony forming unit, (rpm) rotation per minute,(A) absence,(+) stable,(-) unstable,(0) no redness/no irritation

Table 4:Viscosity profile of herbal sunscreens

Sunscreens	Viscosity(cps)					
	10rpm	20rpm	30rpm	50rpm	60rpm	100rpm
HS1	180.6	90.3	60.2	36.1	30.1	18.1
HS2	183.0	91.4	60.9	36.5	30.5	30.5
HS3	186.0	95.3	66.1	34.2	29.9	19.2
HS4	180.0	90.0	60.0	28.3	23.5	15.9
HS5	180.0	90.0	60.0	36.0	33.0	18.0
HS6	181.0	89.2	58.4	28.7	25.4	16.1
HS7	187.0	95.1	62.3	37.1	30.5	18.4
HS8	182.0	91.5	60.6	30.45	29.99	18.0
HS9	181.0	90.0	61.5	34.5	30.0	18.0
HS10	182.0	91.0	62.0	36.0	32.0	18.0
HS11	182.4	90.6	60.7	36.2	30.8	18.5
HS12	180.5	90.0	62.0	35.0	31.6	18.0
HS13	180.0	91.6	60.1	33.8	30.2	18.5
HS14	270.5	150.6	100.2	60.5	54.9	28.4

Centipoise (cps)

Table 6: List of photostable herbal sunscreens

UVB	UVB & UVA 2	UVB & UVA (1 + 2)
HS1 to HS 14	HS11	HS1 to HS5, HS7 to HS 8, HS10, HS12 to HS14

Microbial examination [Table 3] revealed that all tested herbal sunscreens are free from *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Canidida albicans*, it is the desired property of all cosmetic formulation (51). Microbial count of all formulations was found to be in range of 31±1–34±2 CFU/gm indicating the acceptable range (51) resulting safety of the products. During patch analysis on human volunteers, erythema score of 0 was observed in all formulation by visual observation according to COLIPA and BIS guidelines.

Stability results, tested by centrifugation and freeze thaw method were shown in Table 3. Phase separation at 13500 rpm was observed in HS2, HS3, HS4, HS7, HS9 and HS11, this shows the unstability of these formulation at high stress conditions [stroke]. Water was separated from HS1, HS9, and HS10 and oil oozed out from HS11 and HS12 during freeze thaw study.

During photostability evaluation after 30 min of UV_{nat} exposure HS6 and HS9 were found to unstable under UVA 1 and UVA 2 range as [AUCI <0.80] and stable under UVB range [AUCI >0.80]. HS11 showed photostability in UVA 2 and UVB range. HS1, 3, 4, 7, 8, 12 and 13 were found to be photostable after UV_{nat}; in the UVA range the AUCI was between 0.80 and 0.89 after 120 min and between 0.84 and 0.90 in the UVB range. HS 2, 5, 10 and 14 were found to be photostable after UV_{nat}; in the UVA range the AUCI was between 0.89 and 0.98 after 120 min and between 0.91 and 0.92 in the UVB range. Figure 2a–c shows the change in absorption spectra of HS6, 9 and 11 before and after the UV_{nat} exposure.

SPF values of all formulations [HS1-HS14] were determined using *In vitro* and *In-vivo* method [Table 7]. All values of table 6 are graphically represented in Figure 3. SPF of sunscreen determined by *In-vitro* UV method and by *In-vivo* subjective method shows standard deviation [2.6 to 1.0] and [1.7 to 1.0] respectively to the labelled claim SPF.

The statistical result of psychometric analysis by STAT software is represented in table 7. p value of all the five attributes [Appearance, fragrance feel, softness, smoothness and after affect] by ANOVA were found to be in the range of 0.003 to 0.0001[extremely significant]. Overall rating of tested formulation was calculated by adding all values of five attributes. The higher ranking goes to formulation HS8 [44.0±5.7, Table 8]. The comparative analysis of all datas indicates that all parameters remain in close proximity for each sunscreen.

DISCUSSION

Physicochemical parameters are important to collect the information regarding product rheological behaviour, stability and skin compatibility. Result of their analysis justified the compatibility of tested herbal sunscreens with all type of skins. Non volatile matter, saponifcation value, acid value, fatty content, spreadability and layer thickness value confirmed the good cosmetological property of all tested sunscreens. All formulations hold the pseudoplastic flow that is desirable property needed by all creams and lotion for the considerable stability. All sunscreens were found to be free from microbiological contamination and the irritancy test evaluation of all formulations [HS1–HS14] indicates no irritation [no redness] on skin, this verified the safety labelled claim.

During stability study phase separation was observed in HS1 to HS4, HS7, HS9 to HS12 revealing that these formulations could not bear the major changes of environments during travelling as well product transport. These datas gave an idea that above products require more attention and protection from major changes of temperatures and environmental strokes.

The photounstable sunscreens start to degrade rather rapidly when exposed to the sun. It is general thinking that commercial sunscreens give, good UVA and UVB protection. However, the photostability of the sunscreen in the UVA range is not always adequate. Most sunscreens offer good protection against UVB while the UVA photostability of some products decreases substantially during UV exposure. Results of our study also confirmed the above statement by showing stability of all formulations in UVB range [Table 6] and showing instability of three sunscreens in UVA range. The change observed in absorption spectra of HS6, 9 and 11 after 30, 90 and 120 min of UV natural exposure proved that these products are not stable in entire UVA range and minute changes were observed in absorption spectra in UVB range confirming the photostability of tested products in UVB range [Figure 2a–c]. Rarely any manufactures mention about the photostability of sunscreens which is an important criteria effecting the sun protection factor.

Protecting effect of herbal sunscreens had been confirmed by subjective evaluation. All sunscreens found to be give sun protection factor as per claimed on their labels [98 ± 5%]. So both the *In-vitro* and *In-vivo* method are suitable and reliable method to find the SPF of herbal sunscreens.

SPF determination by *In vivo* method on human subject verified that herbal extracts of plant that are incorporated as ingredients in these tested sunscreens are effectively produce the protection from solar radiation. Possible mechanism to get protection from UV solar radiation, associated with tested sunscreens is due to the presence of photo shielding flavanoids, which quench the production of free radicals in the skin. Herbs like aloe vera, cucumber, basil, wheat germ, constituents have polyphenolic structure which absorb the solar radiation and protect the skin from harmful sunrays. HS8 after psychometric evaluation found to be highly preferred by consumers, due to its acceptable and pleasant fragnance, softness, smoothness and after effect.

Results of our study revealed that 100% of selected herbal sunscreens are photostable in the UVB range, and 71% of them are stable in both UVA and UVB range. Subjective study by *in vivo* SPF determination revealed that 98% of the sunscreens effectively provide protection to the skin from sunburns. Over all data obtained after quality evaluation study substantiate that all products are safe and efficacious.

CONCLUSION

The present study is a building step towards the development of quality control methods for herbal products. Research backed by evidenced shall generate confidence for their continued use. This is bound to serve as the interests of both the industry and the community. Investigation by subjective study shows the effectiveness of herbal sunscreens for protecting skins from UVA and UVB rays and photostability study shows that out of 14 sunscreens three of the sunscreens were degraded in the absorption region in the UVA range therefore photostability should be marked on herbal sunscreen product. Study reveals that UV-V spectroscopy is the rapid, acceptable and reproducible method for the evaluation of herbal sunscreen. Compilation of all quality parameters by using various standards methods from different branches of allied sciences can assist the regulatory authorities, scientific organizations and manufacturers in developing uniform standards for herbal sunscreens.

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