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Research Article

In Vitro Sun Protection Factor Determination of Selected Medicinal Plants and Formulation of Sunscreen Cream

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ABSTRACT

Introduction: Plants, due to presence of secondary metabolites like flavonoids, phenols, have been used for various skin care purposes. The ethanolic extracts of different parts of various plants were screened for phytochemicals. Total phenols, total flavonoids, *in vitro* Sun Protection Factor (SPF) were determined and sunscreen cream was formulated and evaluated from the most potent plant extract.

Method: Extraction was done by double maceration. The total phenol content was determined colorimetrically using Folin-Ciocalteu reagent and flavonoid content by aluminium chloride colorimetric method. *In vitro* SPF was determined by spectrophotometric analysis using Mansuer equation. Formulation was done by incorporating oil phase into aqueous phase.

Results: Phytochemical analysis suggested the presence of various phytoconstituents. Among nine plants, the maximum total phenolic and flavonoid content was of *Magnifera indica* extract. Four formulations were prepared containing 0%, 1%, 2.5% and 5% of extract in a cream base. The evaluation of all the formulations has been done by analysis of different parameters such as pH, acid value, saponification value, *in vitro* SPF, stability for 21 days. The pH of the formulation was within the range of 5.01-6.22. Acid value was within the range of 5.98-14.21. The saponification value of cream was between 23.27-33.25. The formulations containing 5% plant extract showed the highest SPF value. The stability study of the formulation with 5% plant extract showed homogeneity under 4°C and room temperature but non-homogeneity under higher temperature i.e. 40°C.

Conclusion: This will be a better, cheaper and safer alternative to harmful chemical sunscreens which are abundantly available in the market.

Keywords: Flavonoids, *In vitro* sun protection factor, Medicinal plants, Phenols, Sunscreen cream

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INTRODUCTION

Before the introduction of orthodox drugs, different plant extracts have been used for the treatment of several diseases. Due to the presence of secondary metabolites, plants poses antifungal, antimicrobial, anticancer, antidiabetic and other pharmacological activities (Larbie CO, *et al*., 2019). Similarly, natural ingredients have been traditionally used for skin care purposes. Source of natural ingredients can be herbs, flowers, fruits, roots and leaves (Rebeiro AS, *et al*., 2015). Natural ingredients are gaining more popularity as consumers seek more natural ingredients in their personal care product because they are less hypo allergic compared to synthetic cosmeceutical ingredients and consumer need not to worry about skin irritations. Cosmeceutical preparations with natural ingredients supply the skin with nutrients like vitamin A, vitamin C, vitamin E, phenolic compounds, flavonoids and terpenoids which act as antioxidant, anti-inflammatory, antiaging and anti-melanogenic effect on skin and enhance the skin health (Lohani A *et al*., 2018).

Exposure to Ultraviolet (UV) radiation triggers the rapid generation and accumulation of Reactive Oxygen Species (ROS) in the skin. When there is imbalance between ROS and antioxidants, they can also take part in the pathological process known as oxidative stress and leads to cell damage and aging (Petruk G *et al*., 2018). UV radiation consists of three components, UVA, UVB and UVC. Among these components UVA and UVB reach the earth surface in sufficient amount to damage the skin whereas UVC is almost completely absorbed by the ozone layer (Yang Y and Li S, 2015). UVB is responsible for erythema and sunburn. In contrast to UVB, UVA is more efficient in inducing immediate and delayed pigment darkening and delayed tanning. UVA induces several adverse effects including immunosuppression, photoaging, ocular damage and skin cancer. UV radiations also have beneficial effect as it increases synthesis of vitamin D in skin. There are a lot of different types of sunscreen products (oils, sticks, gels, creams, lotions) which absorb UV rays and prevents them from penetrating the skin (Korac RR and Kambholja KM, 2011).

Different class of antioxidants such as flavonoids, polyphenols, carotenoids and vitamins show protectant activity against UV radiation. Different bioactive compounds responsible for skin protection against UV radiation are quercetin, resveratrol, lycopene, beta-carotene, vitamin C and vitamin E (Petruk G *et al*., 2018). Phenolic compounds act as antioxidant and protect leaf from photodamage. Different physical sunblock such as zinc oxide, titanium dioxide are also used. Besides that, the skin's natural sun blockers are proteins, lipids and nucleotides (Korac RR and Kambholja KM, 2011).

Sun protection factor is defined as the UV energy required to produce a Minimal Erythema Dose (MED) on protected skin divided by the UV energy required producing a minimal erythema dose on unprotected skin. MED is defined as the dose of UV irradiation sufficient to produce a minimal erythema on unprotected skin. The higher the SPF, more effective is the product in preventing sunburn. Photo protection afforded by sunscreen against solar radiation exposure can be determined by photo testing in human volunteer (Dutra EA, *et al*., 2004).

Topical cream formulations are considered to be more acceptable to patients as they can be effortlessly applied on various part of the skin without imparting the greasy feel. Creams are more potent and occlusive for long period of time as compared to lotions. Topical creams are prepared by the fusion process wherein the oil phase and the water phase components are separately heated and mixed with continuous agitation (Mendonsa NS, *et al*., 2019). Creams and ointments containing plant extract are being formulated due to rapid expansion of demand for herbal cosmetics (El-Gied AA, *et al*., 2015). Plants or their parts used in herbal cosmetic preparation should have various properties like antioxidant, anti-inflammatory, antiseptic, emollient and antibacterial (Aswal A, *et al*., 2013) (*Table 1*).

Table 1: List of selected plants and parts used for the study

S.No	Scientific name	Common name	Part used
1	*Allium cepa L.*	Onion	Bulb
2	*Allium sativum L.*	Garlic	Leaves
3	*Cinnamomum tamala*	Bay leaf	Leaves
4	*Ficus religiosa L.*	Banyan	Bark
5	*Magnifera indica L.*	Mango	Bark
6	*Mimosa pudica L.*	Touch me not plant	Roots
7	*Ocimum tenuiflorum L.*	Holy basil	Leaves
8	*Rubus ellipticus Sm.*	Himalayan raspberry	Leaves
9	*Solanum tuberosum L.*	Potato	Peels

MATERIALS AND METHODS

Plant collection and identification

All the plants were collected from Rupatal, Deurali, and Khudi of Kaski district at an altitude ranging from 800-1200 m. The herbaria of collected plants sample were prepared. Then it was identified by comparing the morphological characteristics and was confirmed by taxonomist at National Herbarium and Vegetation laboratories, Godawari, Lalitpur, Nepal. All the plant samples were made clean from mud, dirt, or lichens present and then cut into pieces with the help of plant cutter and knives and the left for shade drying until dried completely before extraction started.

Extraction of plants

Bark, leaves, roots, bulb and peels of the selected plants were separated, dried in shade at room temperature and grinded to coarse powder using blender. The 100 gram dried powder of each plant was extracted with 800 ml ethanol for 24 hours. Then the residue obtained from the initial extraction was again extracted with 800 ml ethanol for 24 hours. All the extract was concentrated to dryness, under reduced pressure and controlled temperature, using rotary evaporator.

Phytochemical screening

The phytochemical analysis was performed for testing the different chemical groups present in the ethanolic extract of various plants. The presence of phytochemical like alkaloids, flavonoids and phenols was evaluated according to methods described by Zhang J, *et al*., 2011 with some modifications.

Reagents used for phytochemical screening

Mayer's reagent: About 1.358 gram of $HgCl_2$ was dissolved in 60 ml of water and it was mixed with a solution of 5 g KI in 10 ml water and volume was adjusted upto 100 ml by water.

Wagner's reagent: Potassium iodide of about 16.6 gram of was dissolved in 100 ml of distilled water and few crystals of iodine were added to the solution and stirred properly.

Ferric chloride solution: Fifteen grams of ferric chloride hexahydrate was dissolved in 100 ml of distilled water.

Lead acetate solution: Ten grams of lead acetate was dissolved in 100 ml of carbondioxide free water.

Sodium hydroxide solution: Twenty grams of NaOH was dissolved in 100 ml of distilled water.

Phytochemical tests

Alkaloids test: Each of the sample extracts were dissolved individually in dilute hydrochloric acid and filtered. Then the filtrates were treated with Mayer's reagent and Wagner's reagent to test for the presence of alkaloids.

Phenolic test: All extracts were treated with 5 ml $FeCl_3$ solution.

Flavonoids test: In alkaline test, all extracts were treated with 5 ml of sodium hydroxide and observed. In lead acetate test, all extracts were treated with 5 ml of lead acetate solution and observed.

Determination of total phenolic content

The total phenolic content of plant extract was determined using a spectrophotometric method according to Zhang J, *et al*., 2011 with slight modifications.

Preparation of gallic acid solutions and test samples

Gallic acid was taken as standard phenolic compound. Different concentrations of gallic acid (7.82125 µg/ml, 15.625 µg/ml, 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml) were prepared. From dried extract of plants 1 mg/ml concentration was prepared as a test sample.

Preparation of 10% sodium carbonate solution: Ten grams of sodium carbonate was dissolved in distilled water and diluted up to 50 ml to make 10% of solution (Indian Pharmacopoeia).

Determination of total phenolic content

About 1 ml of test sample was added to 1 ml of 2 N Folin reagent followed by the addition of 5 ml of distilled water and left for five minutes. Then, 1 ml of 10% $NaCO_3$ was added and incubated at room temperature for 1 hour. Finally, the absorbance of the reaction mixture was measured at 760 nm against blank ethanol. The total phenol content was expressed as µg gallic acid equivalents (GAE)/mg of extract, using the calibration curve of gallic acid (*Figure 1*) (7.8125-1000 µg/ml) standards. All the determinations were carried in triplicate.

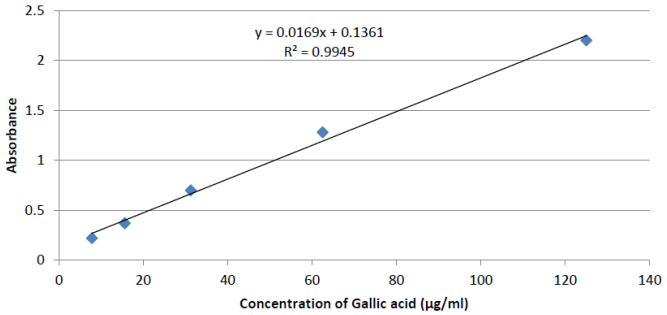

Figure 1: Calibration curve of gallic acid

Determination of total flavonoid content

The content of flavonoids in plant extracts was determined using a spectrophotometric method according to Zhishen J, *et al*., 1999 with slight modifications.

Preparation of quercetin and test samples

Quercetin was taken as standard flavonoid compound. Different concentrations of quercetin (7.8125 µg/ml, 15.625 µg/ ml, 31.25 µg/ ml, 62.5 µg/ ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml) were prepared. From the stock solution 1 mg/ml concentration of extract were prepared as test samples.

Preparation of aluminium chloride (10%): Ten grams of aluminium chloride was dissolved in 100 ml of water to prepare 10% of aluminium chloride solution.

Determination of total flavonoid content

Total flavonoid content was determined by Aluminium chloride colorimetric method. 1 ml of each extract solution (1 µg/ml) was mixed with 4 ml of distilled water. Then, 0.3 ml of 5% sodium nitrate was added. Af-

ter 5 minutes, 0.3 ml of 10% aluminium chloride was added and allowed to stand for 6 minutes. Then, 2 ml of 1 M sodium hydroxide was added and absorbance was measured at 510 nm using UV spectrophotometer. The calibration curve was prepared using quercetin as the standard (*Figure 2*). Total flavonoid content was calculated from the calibration curve and results were expressed as µg quercetin equivalent per gram dry extract weight.

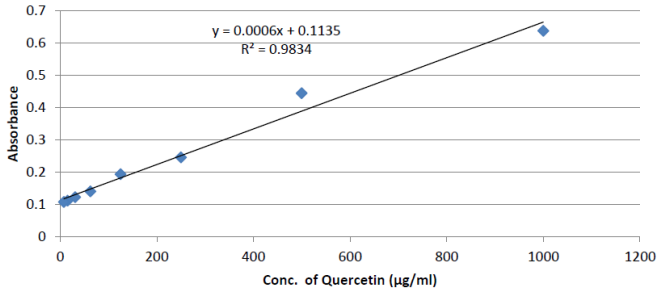

Figure 2: Calibration curve of quercetin

Determination of sun protection factor

In vitro SPF was determined using spectrophotometric analysis. For the determination of SPF, 1% w/v solution was prepared in ethanol and from this 0.01% w/v concentration was prepared. The absorbance of the sample solutions were taken by UV-visible spectrophotometer in the range of 290-320 nm, in every 5 nm interval using ethanol as a blank. Three readings were taken at every interval. The SPF value was calculated by following equation-

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

Where,

CF=Correlation factor

EE=Erythemogenic effect of radiation with wavelength

Abs=Spectrophotometric absorbance values of wavelength

The values of EE(λ) × I(λ) is constant at a fixed wavelength (3).

Formulation of cream

For the formulation of cream in laboratory method described by Farboud ES, *et al*., 2011 with slight modifications was used. Required amount of both aqueous phase and lipid phase were

heated separately at 70 ± 2°C. The aqueous phase was added to the lipid phase, with continuous stirring, using magnetic stirrer until congealed. Four formulations, having concentrations of 0%, 1%, 2.5% and 5% plant extract, were prepared. All the formulations, containing certain level of plant extracts, were evaluated for different parameters. The formulation components, used during formulation of cream, are listed below (*Table 2*).

Evaluation of pH of cream

The pH meter was calibrated using standard buffer solution. pH of 0.5 g was measured by digital pH meter (Aswal A, *et al*., 2013).

Appearance

The appearances of the creams were evaluated visually for their color and homogeneity (Joshi P, *et al*., 2019).

Evaluation of SPF of cream

Sunscreen samples were diluted using ethanol, final concentration of cream having 1 µg/ml, and analyzed by UV-Visible spectrophotometry ranging from 290 nm to 320 nm, at an interval of 5 nm.

Evaluation of acid value

Acid values of creams were determined according to method described by Aswal A, *et al*., 2013. Four grams of cream was dissolved in 20 ml mixture of equal volume of ether and ethanol and heated until sample dissolved. Five ml of this mixture was taken in a conical flask and titrated with 0.1 N NaOH until fairly pink color persisted for 30 seconds. 1 ml of phenolphthalein was used as indicator.

$$\text{Acid value} = \frac{n}{w} \times 5.61$$

where,

n=No.of ml of NaOH required

w=The weight of substance (Aswal A, *et al*., 2013).

Evaluation of saponification value

Saponification values of creams were determined according to method by Aswal A, *et al*., 2013. 2 g of cream was refluxed with 25 ml of 0.5 N of alcoholic KOH, for 30 minutes, and the sample was titrated with 0.5 N HCl, phenolphthalein as an indicator. Then, saponification value was calculated from following formula.

$$\text{Saponification value} = \frac{(b-a)}{w} \times 28.5$$

Where,

a=Volume of titrant (in ml)

b=Volume of titrate (in ml)

w=Weight of substance (in grams) (Aswal A, *et al*., 2013).

Stability testing

Formulated creams were stored at three different temperatures, 4°C, room temperature (21 ± 5°C) and 40 ± 2°C, and the stability study were carried out at 0th, 7th, 14th, and 21st day (Smaoui S, *et al*., 2019).

RESULTS

Extraction yield value

Obtained yield values of each ethanolic extracts were expressed as extract yield percentage (*Table 3*).

Phytochemical screening

Phytochemical screening of various plant extract confirmed absence or the presence of alkaloids, flavonoids and phenols. The results are as follows (*Table 4*).

Table 2: List of components used during the formulation of the cream

Ingredients	Amount				Uses
	Cream base	1% extract	2.50% extract	5% extract	
Stearic acid	5 g	4.95 g	4.87 g	4.75 g	Emollient and co-emulsifier
Cetostearyl alcohol	3.75 g	3.713 g	3.66 g	3.56 g	Emulsifier

Lanolin	5 g	4.95 g	4.88 g	4.85 g	Emollient
Triethanolamine	0.62 ml	0.61 ml	0.604 ml	0.58 ml	PH adjuster
Glycerol	5 ml	4.95 ml	4.88 ml	4.75 ml	Humectant
Propyl parabean	0.04 g	0.039 g	0.039 g	0.038 g	Preservatives
Distilled water	80.64 ml	79.83 ml	78.624 ml	76.58 ml	Solvent
Magnifera indica extract	0 g	1 g	2.5 g	5 g	Active ingredient

Table 3: Yield value of the extract of selected plants

S.No	Plants	Amount of crude drug(g)	Dry extract (g)	Yield value (%)
1	*O. tenuiflorum*	100	16.88	16.88
2	*F. religiosa*	100	8.9	8.9
3	*C. tamala*	100	8.54	8.54
4	*M. pudica*	100	7.52	7.52
5	*M. indica*	100	14.57	14.57
6	*R.ellipticus*	100	10.11	10.11
7	*S. tuberosum*	100	6.54	6.54
8	*A. cepa*	100	15.53	15.53
9	*A. sativum*	100	3.2	3.2

Table 4: Phytochemical screening of selected plants

Phytochemicals	Tests	A_1X_1	A_1G_1	A_1C_1	A_1M_1	A_1R_1	A_1T_1	A_1P_1	A_1B_1	A_1Z_1	Results
Alkaloids	Mayer's test	+	+	-	-	+	-	-	-	-	Yellowish white precipitate
	Wagner's test	-	-	-	-	-	-	-	+	+	Yellow cream precipitate
Flavonoids	Alkaline reagent test	+	+	+	+	+	+	+	+	+	Intense yellow color withalkali and colorless with acids
	Lead acetate test	+	+	+	+	+	+	+	+	+	Formation of yellow precipitate
Phenols	Ferric chloride test	+	+	+	+	+	+	+	+	+	Bluish black precipitate
	Lead acetate test	+	+	+	+	+	+	+	+	+	Yellow color isformed

Total phenol content

The quantitative determination of total phenolic content was carried out using Folin-Ciocalteu reagent in terms of gallic acid equivalent (GAE). The calibration curve of gallic acid. *Table 5* shows total phenolic content expressed as µg gallic acid equivalent per milligram dry extract weight.

Table 5: Total phenolic content of selected plant samples

S.No	Plants	Total phenolic content (µg GAE/ mg dry wt. of extract)
1	*Allium cepa*	22.22 ± 0.46
2	*Allium sativum*	16.48 ± 0.65
3	*Cinnamomum tamala*	11.35 ± 0.44
4	*Ficus religiosa*	124.24 ± 0.70
5	*Magnifera indica*	182.09 ± 0.3
6	*Mimosa pudica*	70 ± 0.73
7	*Ocimum tenuiflorum*	167.87 ± 0.30
8	*Rubus ellipticus*	51.59 ± 0.53
9	*Solanum tuberosum*	10.68 ± 0.70
Note: Data were expressed in mean ± SD (n=3)		

Total flavonoids content

Quantitative determination of total flavonoid was performed by precipitating with aluminium chloride in an alkalinized medium. The calibration curve with quercetin as the standard is shown in figure while *Table 6* shows the total flavonoid content of plant extracts.

Table 6: Total flavonoid content of selected plant samples

S.No	Plants	Total flavonoid content (µg QE/mg dry wt. of extract)
1	*Allium cepa*	143.44 ± 10
2	*Allium sativum*	766 ± 24.20
3	*Cinnamomum tamala*	712.85 ± 9.92
4	*Ficus religiosa*	788.34 ± 6.26
5	*Magnifera indica*	2949.49 ± 8.97
6	*Mimosa pudica*	402 ± 4.48
7	*Ocimum tenuiflorum*	514.33 ± 13.38
8	*Rubus ellipticus*	1569.36 ± 9.15

DISCUSSION

In our study, nine different plants were collected from Deurali, Rupatal, Rajakochautara and Bijaypur of Kaski district. Ethnobotanical information revealed that the selected plants in this study are traditionally used for various medicinal purposes and possess different pharmacological properties. The thesis work included phytochemical screening, determination of total phenol and flavonoid content, *in vitro* SPF of selected nine plants, formulation and evaluation of sunscreen cream from most potent plant extract. Phytochemical screening was performed for different phytochemical constituents such as alkaloids, phenols and flavonoids. Ethanolic extract of all the plants showed the presence of phenols and flavonoids. According to Mustapha AA, *et al*., 2014, *M. indica* showed the presence of phenols, flavonoids and alkaloids which supports this research work. According to Makhija IK, *et al*., 2010, *Ficus religiosa* showed the presence of flavonoids which supports this research work.

M. indica showed highest amount of phenolic content. Total phenolic content of *M. indica* was found to be in the range of 63.89 to 116.80 mg GAE/g dry weight of extract in 80% methanol (Sultana B, *et al*., 2012), but in this research work total phenolic content of this plant was found to be 182 mg GAE/g dry weight of extract in ethanol. This variation may be due to variation in solvent. The plant having high phenolic content showed the highest SPF value (Ebrahimzadeh MA, *et al*., 2014), which supports our study. Flavonoids do not seem to be simply detectable by any method therefore $AlCl_3$ was used as a complexing reagent. The method is based on the formation of a stable complex between $AlCl_3$ keto and hydroxyl group of flavones and flavonoids (Hassan SM, *et al*., 2013). *M. indica* showed the highest flavonoid content. Total phenolic content of the *M. indica* was found to be 925.55 CE/g dry weight of extract in 80% methanol (Sultana *et al*., 2012). In this research, total flavonoids content of ethanolic extract of *M. indica* bark was found to be 2949 mg QE/g dry weight of extract. This may be due to variation in solvent and may be due to variation in comparison with standard where catechin was used in previous work and quercetin in this work. The plant having high flavonoid content showed the highest SPF value (Costa SC, *et al*., 2015). From above discussion we can conclude that there may be correlation between phenolic flavonoid compounds and SPF value. Flavonoids and phenols have been reported as an important functional compound in plants which play a vital role in management of inflammation due to solar radiation (Imam S, *et al*., 2015).

There is growing demand for herbal cosmetics in the world (Aswal A, *et al*., 2013). Therefore, an herbal sunscreen cream containing ethanolic extract of M. *indica* was prepared. Four formulations were prepared and evaluated for various parameters such as pH, acid value, saponification value, *in vitro* SPF and stored at three different temperatures for their stability testing (*Table 7*). The measurement of SPF is an ultimate way to determine the effectiveness of the sunscreen formulation. The higher the SPF the more protection the sunscreen offers against UV light. Sunscreen is used to aid the body's defense mechanism to protect against harmful UV-radiation from the Sun (Lohani A, *et al*., 2018). Here, in this research work, the formulation containing 5% plant extract showed the highest SPF value. Therefore, this formulation may protect skin against UV-radiation. Acid value is the mass of KOH in mg that is required to neutralize one gram of chemical substance. Higher the acid value, it will cause irritation to skin after application (Fatima S, *et al*., 2017; Swarnlata S, *et al*., 2011). Saponification value number represents the number of mg of KOH required to saponify 1g of fat. Saponification value influences pH and stability of cream. Higher saponification value means higher amount of free fatty acid which are prone to hydrolysis and can cause rancidification (Saraf S, *et al*., 2011). In our research work it is found that the saponification value is decreasing on increasing the concentration of plant extract.

This study revealed that formulations I,II and III were found to be stable at all storage conditions except the formulation IV, which was unstable and resulted in breakdown of emulsion, at 40 ± 2°C from the 7th day. The formulation IV, which is kept at 40 ± 2°C, was found to be unstable i.e. liquefaction started at increased temperature along with increase in concentration of extract. Monitoring the pH of cream is necessary for determining the stability of pharmaceutical and cosmeceuticals (*Table 8*). Any changes in the pH of the product *indica*te the possible interaction which may provide an idea on the quality of the product (Smaoui S, *et al*., 2012). The pH of the human skin normally ranges from 4.5 to 6. Due to frequent washing and use of soap, the acidity of the skin is lost.

Table 7: SPF value of selected plant samples

EE*I		Absorbance								
		A_1X_1	A_1G_1	A_1C_1	A_1M_1	A_1R_1	A_1T_1	A_1P_1	A_1B_1	A_1Z_1
290	0.015	0.172	0.206	0.396	0.207	0.917	0.547	0.481	0.182	0.439
295	0.081	0.161	0.187	0.334	0.176	0.89	0.505	0.385	0.173	0.431
300	0.287	0.129	0.171	0.307	0.149	0.87	0.465	0.28	0.168	0.431
305	0.327	0.143	0.158	0.295	0.135	0.847	0.428	0.237	0.161	0.437
310	0.186	0.142	0.146	0.292	0.124	0.819	0.394	0.208	0.16	0.446
315	0.037	0.135	0.135	0.294	0.112	0.776	0.362	0.191	0.159	0.448
320	0.018	0.127	0.123	0.297	0.1	0.707	0.331	0.181	0.158	0.443
SPF	-	1.39 ± 0.3	1.6 ± 0.13	3.03 ± 0.16	1.39 ± 0.08	8.44 ± 0.35	4.33 ± 0.43	2.55 ± 0.01	1.64 ± 0.06	4.38 ± 0.13

Note: EE-Erythemal Effect spectrum; I-Solar intensity Spectrum and the data was expressed in mean ± SD (n=3).

Table 8: Stability results of different creams at 3 different temperatures

Temperature	Cream	pH				Appearance (Color, homogeneity)			
		0th day	7th day	14th day	21st day	0th day	7th day	14th day	21th day
4°C	Base	-	6.12	6.13	6.14	White, homogenous	White, homogenous	White, homogenous	white, homogenous
	1% Extract	-	5.57	6.09	6.11	Light brown, homogenous	Light brown, homogenous	Light brown, homogenous	Light brown, homogenous
	2.5% Extract	-	5.46	5.79	5.86	Light brown, homogenous	Light brown, homogenous	Light brown, homogenous	Light brown, homogenous
	5% Extract		5.04	5.38	5.38	Dark brown, homogenous	Dark brown, homogenous	Dark brown, homogenous	Dark brown, homogenous
Room temperature	Base	6.16	6.22	6.3	6.55	White, homogenous	White, homogenous	White, homogenous	white, homogenous
	1% Extract	5.15	5.48	5.68	5.8	Light brown, homogenous	Light brown, homogenous	Light brown, homogenous	Light brown, homogenous
	2.5% Extract	5.3	5.43	5.63	5.69	Light brown, homogenous	Light brown, homogenous	Light brown, homogenous	Light brown, homogenous
	5% Extract	5.03	5.13	5.16	5.3	Dark brown, homogenous	Dark brown, homogenous	Dark brown, homogenous	Dark brown, homogenous
40°C	Base	-	5.37	5.42	5.88	White, homogenous	White, homogenous	White, homogenous	white, homogenous
	1% Extract	-	5.01	5.1	5.26	Light brown, homogenous	Light brown, homogenous	Light brown, homogenous	Light brown, homogenous
	2.5% Extract	-	5.01	5.09	5.2	Light brown, homogenous	Light brown, homogenous	Light brown, homogenous	Light brown, homogenous
	5% Extract	-	5.04	5.23	5.29	Dark brown, non-homogenous	Dark brown, non-homogenous	Dark brown, non-homogenous	Dark brown, non-homogenous

Note: All the results were expressed in mean ± SD

Therefore, moisturizer has an acidic range which is used to normalize the skin. Acceptable pH range of the moisturizer should be between 5-8. The pH of the cream was found to be in the range 5.01-6.76. The result revealed that the pH of the cream was within the range (Saraf S, *et al*., 2011). The formulations have almost constant pH throughout the study. The appearance of the cream was not changed. In this study sunscreen cream containing 1% extract showed acid value and saponification value 8.6 ± 0.41 and 30.4 ± 0.83 respectively. In a previous research, antiaging facial cream containing 1% curcumin extract was formulated, which showed acid value and saponification value of 5.7 and 25.7 respectively (Panda S, *et al*., 2018). This difference in acid value and saponification value may be due to ingredients used in cream and chemical constituents present in extract (*Table 9*). According to Akter S, *et al*. 2013, on varying the concentration of stearic acid and cetyl alcohol, saponification value also varies. Saponification value goes on decreasing on increasing the concentration of those constituents. In this study, the saponification value goes on decreasing on increasing the concentration of extract.

Table 9: Acid value, saponification value and SPF of the cream stored at room temperature

Cream	Acid value	Saponification value	SPF
Base	10.86 ± 0.32	33.25 ± 0.822	0.64 ± 0.011
1% extract	8.60 ± 0.41	30.4 ± 0.83	1.26 ± 0.05
2.5% extract	14.21 ± 0.33	24.7 ± 0.91	2.35 ± 0.03
5% extract	5.98 ± 0.647	23.27 ± 1.645	7.19 ± 0.12

According to Pratama G, *et al*., 2019, the cream having high amount of extract was very good for counteracting UV rays because it has high SPF value when compared to other formulations. Also, in this study, the formulation containing 5% extract showed the highest SPF i.e. 7.19 ± 0.12.

CONCLUSION

The study provided reasonable data to conclude that *Magnifera indica* has highest phenolic and flavonoid value which may have resulted in the highest SPF value among the nine medicinal plants taken during the study. The sunscreen cream, which is o/w type emulsion, containing different concentration yields good physical characteristics formulation. Method used for the evaluation of sunscreen cream, used in this work, is simple, fast, economical and easy to use.

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