Plant Archives Vol. 21, Supplement 1, 2021 pp. 2005-2012 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.326

STUDY OF PHARMACOGNOSTICAL AND PHYTOCHEMICAL ANALYSIS OF FRUITS OF *MARTYNIA ANNUA* AND STUDY OF DIFFERENT FORMULATIONS OF ANTI-AGEING CREAM

Subhash Chandra*[1], Sarla Saklani[1], and Abhishek Mathur[2]
[1]Department of Pharmaceutical Chemistry, HNB Garhwal University, Srinagar, Garhwal, India; [2]Dept. of Research & Development (R&D), NCS Green Earth Pvt. Ltd., Nagpur, Maharashtra, India
***Author for correspondence:** subhashkothiyal@gmail.com

ABSTRACT

The medicinal plants are the resources of novel molecules and phytochemicals of wide pharmacological significance. Such novel agents in medicinal plants are significant as these play a key role in our body's vital needs. There is still a need to explore surplus biodiversity of medicinal plants and different novel agents that can be utilized pharmacologically. The present study was performed to prepare different formulations of anti-ageing cream using ethanolic extracts of fruits of *Martynia annua*. The cream formulations (F1-F6) prepared was having softness, whitening and antiseptic effects on the skin. These formulations can be safely used on the skin. The research work suggests that, the ethanolic extract of *Martynia annua* can be used to prepare an ideal face cream. The study finally concludes that, the composition of the extract and the base of the cream F5 and F6 were found to be more stable and safer in terms of all respect.

***Keywords*:** *Martynia annua*, fruits, ethanolic extracts, formulations, phytochemicals, novel agents, anti-ageing cream.

Introduction

Plants are said to be the repositories of natural molecules and phytochemicals. These phytochemicals are found to have significant pharmacological activities known to be effective against infections and diseases. In India, more than 20,000 medicinal plants have been recorded (Subhose *et al.*, 2005; Ballabh and Chaurasia, 2007; Dev, 1997). However traditional communities and local healers are using only 7,000-7,500 plants for curing different diseases (Perumal and Ignacimuthu, 1998a; 2000b; Kamboj, 2000). The medicinal plants are listed in various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy which 30 plant species for ailments (Rabe and Staden, 1997). Still there are many plants which are not explored yet. Even today, majorities of the medicines are prepared from the plant and animal products, minerals and metals etc. Major pharmaceutical industries depend on the plant products for the preparations of ayurvedic medicines. In the present context, the Ayurvedic system of medicine is widely accepted and practiced not only in the Indian Peninsula but also in the developed countries such as Europe, United States and Japan. Plant derived medicines have been the first line of defense in maintaining health and combating diseases (John, 1984). Biologically active compounds from natural sources have always been of great interest to scientists working on infectious diseases. Research to find out scientific evidence for claims of plants used for Indian Ayurvedic system of medicine has been intensified. Detailed research on the chemistry and pharmacology of products of plant origin are much essential and this may eventually lead to the discovery of medicine that can be used in the treatment of several diseases. Cosmetics are substances or products used to enhance or alter the appearance of the face or fragrance and texture of the body. There are many positive reasons for using herbal remedy such as it is made by pure herb extract, they provide nutrients and minerals. These don't have any side effect. The present investigation was carried out to develop the different formulations of anti-ageing cream using fruits of *Martynia annua* and study of their different parameters.

Materials and Methods

Collection and identification of plant material

The plant materials (fruits of *Martynia annua*) were collected from local areas of Srinagar Garhwal Uttarakhand, India during March 2019. The plant materials were identified by Dr. Painuli, Dept. of Botany, H.N.B. Garhwal University, Srinagar Garhwal, Uttarakhand, India.

Sampling of plant material

The fruits were dried at room temperature in the laboratory at 38°C for 10 days. After dried, the plant materials were ground to form fine powder. The powder was stored in air tight containers till further use.

Solvent extraction of the powdered plant material

The crushed powdered material of fruits of *Martynia annua* was passed through sieve no. 40 mesh, weighed and then used for extraction with petroleum ether, chloroform, ethyl acetate, ethanol and water in Soxhlet apparatus. Accurately weighed 500 g plant material was fed in a thimble and it was placed in a Soxhlet apparatus. Petroleum ether was taken in the round bottom flask and hot extraction was carried out for 24 h. The extract in the round bottom flask

was concentrated by distillation and the dry extract was weighed to get the petroleum ether soluble fraction. The left-over material was used for successive extraction with chloroform, ethyl acetate and ethanol separately. The final left-over material was extracted with water using decoction method. The resulting extracts were concentrated under reduced pressure using rotary vacuum evaporator to get the syrupy viscous masses. The viscous masses were transferred in porcelain dishes and dried. The amount of extract was weighed and stored in amber colored airtight container at 5-7°C. The extractive value (EV) (w/w) of plant materials in the solvents in which they were prepared were calculated.

Determination of extractive value

Extractive value is a measure of the content of the soluble extract of the plant materials by solvents. The extractive values of solvent extracts viz. petroleum ether, chloroform, ethyl acetate, ethanolic and water extracts were determined as per the modified method (Anonymous, 1989).

Determination of total ash value and total acid insoluble ash

The residue remaining after incineration is the ash content of drug, which simply represents inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it as a form of adulteration. Many a time, the crude drug are admixed with various mineral substances like silica, calcium oxalate, chalk powder. Ash value is a criterion to judge the identity or purity of crude drugs. Total ash usually consists of carbonates, phosphates, silica and silicates. Acid insoluble ash which is a part total ash insoluble in diluted hydrochloric acid is also recommended for natural drugs. Adhering dirt and sand may be determined by acid-insoluble ash contain. The methods for determination used were conventional.

Determination of total sugars and total starch

Estimation of total sugar in plant material was carried out according to spectrophotometric method (Bray and Thorpe, 1954).

Determination of total tannins

Estimation of tannin percentage in the plant material was carried out accordingly to the modified method (Wallis, 2002; Khandelwal, 2001).

Determination of total phenolics

Estimation of total phenolic content was performed by Folin-Ciocalteu reagent (Harborne, 1998)

Determination of total flavonoid

Total flavonoid content was estimated using the modified method (Anonymous, 1984; Ordon *et al,* 2006).

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

(I) Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) **Mayer's Test:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow-colored precipitate indicates the presence of alkaloids.

b) **Wagner's Test:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) **Dragendroff's Test:** Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) **Hager's Test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

(II) Detection of carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) **Molisch's Test:** Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

b) **Benedict's test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) **Fehling's Test:** Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

d) **Detection of glycosides:** Extracts were hydrolyzed with dilute HCl, and then subjected to test for glycosides.

(i) **Modified Bontrager's Test:** Extracts were treated with Ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

(ii) **Legal's Test:** Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

(III) Detection of saponins

a) **Froth Test:** Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) **Foam Test:** 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes, it indicates the presence of saponins.

(IV) Detection of phytosterols

a) **Salkowski's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

b) **Liebermann Burchard's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled.

Conc. sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

(V) Detection of phenols Ferric Chloride test: Extracts were treated with 3-4 drops of Ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

(VI) Detection of tannins Gelatin test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

(VII) Detection of flavonoids

a) Alkaline reagent test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

(VIII) Detection of proteins and amino acids

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

b) Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

Thin layer chromatographic studies

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7X6 cm (Merck). Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro liter by using capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system viz. (I) Hexane: Acetic acid (9:1); (II) solvent system, Hexane: Ethyl acetate: Acetic acid (5:4:1); (III) Hexane: Ethyl acetate: Acetic acid (4:4:2); (IV) Hexane: Ethyl acetate: Acetic acid (3:6:1); (V) Hexane: Ethyl acetate: Acetic acid (2:7:1) was used. After pre-saturation with mobile phase for 20 minutes for development were used. After the run plates were dried and sprayed with freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (Rf), values were calculated for different samples.

Antioxidant activity

Trolox standard and plant working solutions were prepared. A stock solution of concentration of 1 mg/ml in ethanol was firstly prepared for the plant extract and trolox. The working solutions of the following concentrations (1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80, 100 µg/ml) were prepared by serial dilution with ethanol from the stock solution.

(i) DPPH free radical scavenging activity

Spectrophotometric measurements were carried using DPPH solution and mixing of plant extracts within it in suitable volumes. The spectrophotometer was set zero using ethanol as a blank solution. The solutions were incubated in dark for 30 minutes at room temperature and the absorbance readings were recorded at 517 nm.

Percentage of inhibition of DPPH activity (%) = (A-B)/A ×100%

Where: A = optical density of the blank, B = optical density of the sample. The antioxidant half maximal inhibitory concentration (IC50) for the plant samples and the standard were calculated using Biodata Fit edition 1.02.

Formulation of herbal cream (O/W emulsion)

Oil in water (O/W) emulsion-based cream (semi-solid formulation) was formulated.

The process was distributed in two phases-

Phase 1 (Mixing of non-water-soluble components): The emulsifier (stearic acid) and other oil soluble components (cetyl alcohol, almond oil) were melted in a beaker by using a water bath on constant stirring.

Phase 2 (Mixing of water-soluble components): Methyl paraben, propyl paraben, triethanolamine, propylene glycol and concentrated aqueous extract of the fruits of *M. annua* with slight heating.

Phase 3 (Mixing of Phase 1 and Phase 2): Further, Phase 1 and Phase 2 were mixed with each other with constant stirring and heating at 75 °C with addition of perfume. Six different formulations (F1-F6) were prepared by using varying concentration of the aqueous extract, stearic acid and **liquid (Table 1).**

Table 1: Formulations of the cream

Ingredients	Category	F1	F2	F3	F4	F5	F6
Phase 1- Mixing of non-water-soluble components							
Stearic acid	Emulsifier	2.5	5	2	-	5	4
Steryl alcohol		4	6	5	4	6	5
Cetyl alcohol		7	1	6	7	-	2
Lanolin		10	7.5	7.5	10	5	5
Isopropyl mesritate		-	-	-	7	6.5	6
Almond oil		3	3	2.5	2.5	2	2
Phase 2- Mixing of water-soluble components							
EDTA		0.05	0.05	0.05	0.05	0.05	0.05
Polyethylene glycol		-	-	-	-	-	5
Propylene glycol	Moisturizer+ Binder	5	4.5	4	3	1	-
Methyl Paraben	Preservative	0.18	0.18	0.18	0.18	0.18	0.18
Triethanol amine		2.5	1	0.75	0.75	0.75	0.75
Glycerine	Moisturizer	7.5	8	10	8	7.5	5
Ethanolic extract	A. P. I.	3.5	3	1	1.5	2	2
Rose water		QS	QS	QS	QS	QS	QS
Water	Vehicle	QS	QS	QS	QS	QS	QS

Image 1: Images of the formulated cream as per Table 1.

Evaluation of Polyherbal Anti-aging Cream

(i) Determination of type of Emulsion (Dye Method)

A scarlet red dye was mixed with the cream. A drop of the cream was placed on a microscopic slide and examined under a microscope. If the disperse globules appear red and the continuous phase colorless, the cream is water-in-oil (w/o) type. The reverse condition occurs in oil-in-water (o/w) type cream i.e. the disperse globules appear colorless and the continuous phase red.

(ii) Organoleptic evaluation

The cream thus obtained was evaluated for its organoleptic properties like color, odor, and state. The appearance of the cream was judged by its color and roughness and graded.

(iii) Test for microbial growth in formulated cream samples

The formulated creams were inoculated on the plates of Muller Hinton agar media by the streak plate method and a control was prepared by omitting the cream. The plates were placed into the incubator and are incubated at 37 ^{0}C for 24 hours. After the incubation period, plates were taken out and check the microbial growth by comparing it with the control.

(iv) Stability studies

Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. To assess the drug and formulation stability, studies were done according to ICH guidelines. The stability studies were carried out as per ICH guidelines. The cream samples were filled in bottle and kept in humidity chamber maintained at 30 ± 2 °C/ 65 ± 5 % RH or 40 ± 2 °C / 75 ± 5 % RH for two months. At the end of studies, samples were analyzed for the physical properties and viscosity.

(v) pH of the formulated cream samples

The pH meter was calibrated using standard buffer solution. About 0.5 g of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured.

(vi) Homogeneity

The formulations were tested for the homogeneity by visual appearance and by touch.

(vii) Test for Emolliency

Emolliency, slipperiness and amount of residue left after the application of fixed amounts of formulated cream samples were checked.

(viii) Test for ease of Removal

The ease of removal of the cream applied was examined by washing the applied part with tap water.

(ix) Test for irritancy

Mark an area (1sq.cm) on the left-hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythematic, edema, was checked if any for regular intervals up to 24 hrs. and reported.

(x) Test for viscosity

Viscosity of the formulated samples was determined by Brookfield Viscometer at 100 rpm, using spindle no 7.

(xi) Smear test on skin surface

After application of formulated cream samples, the type of film or smear formed on the skin were checked.

(xii) Acid value

Taken 10 gm of substance dissolved in accurately weighed mixture; 50 ml mixture of equal volume of alcohol and solvent ether, the flask was connected to reflux condenser and slowly heated, until sample was dissolved completely. To this, 1 ml of phenolphthalein was added and titrated with 0.1N NaOH, until faint pink color gets appeared with slight shaking for 30 seconds.

Acid value = n*5.61/w n = the number of ml of NaOH required. w = the weight of substance.

(xiii) Saponification value

Taken 2 gm of substance refluxed with 25 ml of 0.5 N alcoholic KOH for 30 minutes, within the same, added 1 ml of phenolphthalein added followed with immediate titration using 0.5N HCL.

Saponification value = (b-a)*28.05/w The volume in ml of titrant = a The volume in ml of titrate = b The weight of substance in gm = w

(xiv) Spreadability test

Sample was applied between two glass slides and was compressed to uniform thickness by placing 100 g weight for 5 minutes. The time required to separate the two slides, i.e. the time in which the upper glass slide moved over the lower slide was taken as measure of spreading ability (18)

Spreadability =m*l/t

m = Weight tide to upper slide

l = length moved on the glass slide

t = time taken.

Results and Discussion

The extraction was done by the hot extraction method. Extractive value was measured by the content of the drug extracted in the solvents (water and methanol). Extractive values of the plant *Martynia annua* mature fruit are mentioned in Table 2. Physiochemical characters of the plant *M. annua* mature fruit was done by the conventional methods. The results of total ash value and acid insoluble ash is mentioned in Table 3. The percent sugar concentration is reported in Table 4. The percent starch content is reported in Table 5. The percent tannin content is reported in Table 6. The percent phenolic content is Table 7. The percent flavonoid content is reported in Table 7. The percent flavonoid content is reported in Table 8. The florescence study is reported in Table 9. The powder results are shown in Table 10. The phytochemical analysis is shown in Table 11. IC50 value of potent extract was determined is shown in Table 12. Metal analysis of fruit extract is shown in Table 13. The acid and saponification values determined are shown in Table 14. Microbial analysis of the formulations is shown in Table 15. The results of stability testing of formulations (F5; F6) are shown in Table 16. The pH of the cream was found to be in range of 5.6 to 6.8 which is good for skin pH. The herbal formulation was shown pH nearer to skin required i.e pH 6.3. The scarlet red dye is mixed with the cream, place a drop of the cream on a microscopic slide, covers it with a cover slip, and examines it under a microscope. The disperse globules appear in red color. The formulation was tested for the homogeneity by visual appearance and by touch, appearance and touch was good. When formulation was kept for long time, it was found that, no change in color of cream. Emollience, slipperiness and amount of residue left after the application of fixed amount of cream was found. After application of cream, the type of smear formed on the skin were non greasy. The cream applied on skin was easily removed by washing with tap water. The formulation shows no redness, edema, inflammation and irritation during irritancy studies. These formulations are safe to use for skin. The spreading ability test showed that formulation has good spreadable property. The DPPH radical scavenging activities of the ethanol extract of *Martynia annua* were evaluated. DPPH radicals react with suitable reducing agents which lose color stoichiometrically and the number of electrons consumed was measured spectrophotometrically at 517 nm. An ethanol fraction analyzed from a sample of *Martynia annua* exhibited considerable antioxidant activity with an IC_{50} value of 88.24 µg/mL while for gallic acid the IC_{50} was found to be 0.51 µg/ml. All the six formulations, passed the microbial limit test which included some parameters like total bacterial count and total fungal count. Pathogens like *E. coli*, *S. aureus*, *K. pneumoniae* and *B. cereus* were also found to be absent. Different types of preparations such as oil in water, several face creams correspondingly classified were from F1 to F6. The assessment of all formulations (F1 to F6) and the analysis of different parameters (pH, spread ability, stability and viscosity) was performed. The prepared face cream was O/W type emulsion, which can be easily washed with water. The studies suggested that, amongst the six formulations (F1 – F6), F5 and F6 displayed good spread ability, good consistency, homogeneity, appearance, pH of these formulations was found to be constant. There was no proof of a separation phase and ease of removal. The formulations F5 and F6 showed no redness or edema or erythema and irritation during irritancy studies.

Table 2: Extractive value of *Martynia annua* mature fruit

S. No	Parameter	Value (%)
1	Water soluble	6.5%
2	Ethanol soluble	29.3%

Table 3: Physico-chemical characters (Total ash content and Acid insoluble ash content) of *Martynia annua* mature fruit

S. No	Parameters	Value (%)
1	Total ash content	4.15
2	Acid insoluble ash content	0.7

Table 4: Percent sugar content in test solution of *Martynia annua* mature fruits

S. No	Plant sample	Total sugar content (%)
1	*M. annua* mature fruit	5.22

Table 5 : Percent starch content in test solution of *Martynia annua* mature fruits

S. No	Plant sample	Total starch content (%)
1	*M. annua* mature fruit	0.32

Table 6: Percent tannin content in test solution of *Martynia annua* fruits

S. No	Plant sample	Total tannin content (%)
1	*M. annua* mature fruit	0.5

Table 7: Percent phenolics content in test solution of *Martynia annua* fruits

S. No	Plant sample	Total phenolics content (%)
1	*M. annua* mature fruit	0.0426

Table 8: Percent flavonoid contents in test solution of *Martynia annua* fruits

S. No	Plant sample	Total flavonoid content (%)
1	*M. annua* mature fruit	0.0596

Table 9: Florescence study of powder of *Martynia annua* mature fruit

S.No	Chemical used	Color without addition of chemical in daylight	Color after addition of chemical in daylight	Color in short wavelength	Color in long wavelength
1	H_2SO_4	Black	Reddish	Bluish black	Purplish
2	50% H_2S0_4	Black	White	White	White
3	50% HNO_3	Black	Green	White	Light green
4	Toluene	Black	White	White	White
5	1N NaOH+H_2O	Black	Green	Black	Light green
6	NaOH+MeOH	Black	Greyish	White	White
7	Ferric Chloride	Black	Yellowish orange	Black	Green
8	1N HCL	Black	White	White	White

Table 10: Powder study of residue of *M. annua* mature fruit

S.No.	Chemical used	Color without addition of chemical in daylight	Color after addition of chemical in daylight	Color in short wavelength	Color in long wavelength
1	H_2SO_4	Black	Black	Black	Black
2	50% H_2S0_4	Black	Black	Black	Black
3	50% HNO_3	Black	Greenish Black	Black	Blackish green
4	Toluene	Black	Black	Black	Black
5	1N NaOH+H_2O	Black	Black	Black	Blackish green
6	NaOH+MeOH	Black	Black	Black	Black
7	Ferric Chloride	Black	Black	Black	Black
8	1N HCL	Black	Black	Black	Black

Table 11: Phytochemical analysis of extract of mature fruit of *Martynia annua*

Phytochemical constituents	Tests	*M. annua* fruit
Phenolic	Ferric chloride test	+
	Lead acetate test	+
Tannin	5% Ferric chloride	+
	5% NaOH	+
Sugar	Fehling's test	+
	Benedict's test	+
Saponin	Foam test	+
Flavonoid	Shinoda test	+
	Zinc chloride test	+

(+) present, (-) absent

Table 12: IC_{50} value of fruit extracts

S.No.	Sample name	IC_{50} value (mg/ml)
1	Quercetin	0.684
2	*M. annua* mature fruit	0.551

Table 13: Metals analysis of *M. annua* mature fruit extract

Metals	ppb
Cr	22475
Mn	9290.5
Fe	769000
Ni	19715
Cu	2602
Zn	12711
Pb	5285

Table 14: Test applied for acid value and saponification value

S. No.	Parameter	Formulation
1	Acid value	5.7
2	Saponification value	22.3

Table 15: Microbial analysis of mature fruit of *Martynia annua* (F1-F6)

Formulations	TBC (CFU/g)	TFC (CFU/g)	*E. coli*	*S. aureus*	*K. pneumonia*	*B. cereus*
F1	15×10^2	00	00	00	00	00
F2	4×10^2	00	00	00	00	00
F3	2×10^2	1×10^2	00	00	00	00
F4	12×10^2	3×10^2	00	00	00	00
F5	18×10^2	1×10^2	00	00	00	00
F6	26×10^2	00	00	00	00	00

TBC: Total bacterial count**; TFC: Total** fungal count**; Ab:** Absent

Table 16: Accelerated stability testing of formulations (F5, F6)

Days	Temperature	Formulation	Parameters						
			pH	A1	A2	A3	A4	A5	A6
0	Room Temperature	F5	6.58	**	NCC	**	E	NG	ES
		F6	6.67	**	NCC	**	E	NG	ES
	(40±1)°C	F5	6.58	**	NCC	**	E	NG	ES
		F6	6.67	**	NCC	**	E	NG	ES
10	Room Temperature	F5	6.51	**	NCC	**	E	NG	ES
		F6	6.37	**	NCC	**	E	NG	ES
	(40±1)°C	F5	6.42	**	NCC	**	E	NG	ES
		F6	6.40	**	NCC	**	E	NG	ES
20	Room Temperature	F5	6.74	**	NCC	**	E	NG	ES
		F6	6.81	**	NCC	**	E	NG	ES
	(40±1)°C	F5	6.61	**	NCC	**	E	NG	ES
		F6	6.83	**	NCC	**	E	NG	ES
30	Room Temperature	F5	6.76	**	NCC	**	E	NG	ES
		F6	6.83	**	NCC	**	E	NG	ES
	(40±1)°C	F5	6.65	**	NCC	**	E	NG	ES
		F6	6.85	**	NCC	**	E	NG	ES

Conclusion

The results of the study concluded that, *Martynia annua* is medicinally important plant, the ethanolic extract of rhizomes of *Martynia annua* was found to have wide range of pharmacological activities. The cream formulations (F1-F6) prepared was having softness, whitening and antiseptic effects on the skin. These formulations can be safely used on the skin. The research work suggests that, the ethanolic extract of *Martynia annua* can be used to prepare a face cream. The validation of cream was done and was found in the limits. The study finally concludes that, the composition of the extract and the base of the cream F5 and F6 are more stable and safer in terms of all respect.

References

Anonymous (1984). Official methods of Analysis of Association of Official Analytical Chemists (AOAC). *Virginia*, US.

Anonymous (1989). The Ayurvedic Pharmacopoeia of India". *Ministry of Health and Family Welfare*, Government of India, New Delhi, 17-18.

Ballabh, B. and Chaurasia, O.P. (2007). Traditional medicinal plants of cold desert Ladakh-Used in treatment of cold, cough and fever. *Journal of Ethnopharmacology*, 112(2): 341–349.

Bray, H.C. and Thorpe, W.V. (1954). Analysis of phenolic compounds of interests in metabolism. *Methods of Biochemical Analysis*, 1: 27–52.

Dev, S. (1997). Ethno-therapeutics and modern drug development: The potential of Ayurveda. *Current Science,* 73(11): 909-928.

Harborne, J.B. (1998). *Phytochemical methods-A guide to modern technique of plant analysis*, *3rd Edition*, Published by Chapman & Hall, London UK, Thomson Publishing, ISBN 0412-57260-5.

John, D. (1984). One hundred useful raw drugs of the Kani tribes of Trivandrum forest division, Kerala, India. *International Journal of Crude Drug Research*, 22(1): 17-39.

Kamboj, V.P (2000). Herbal medicine". *Current Science*, 78(1): 35-39.

Khandelwal, K.R. (2008). *Practical Pharmacognosy*, Nirali Prakashan Pune, 149-166. ISBN 978-81-85790-3-5

Ordonez, A.A.L. and Gomez, J.D. (2006). Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. *Food Chemistry*, 97(3): 452-458.

Rabe, T and Staden, J.V. (1997). Antibacterial activity of South African plants used for medicinal purposes. *Journal of Ethnopharmacology,* 56(1): 81–87.

Samy, R.P. and Ignacimuthu, S. (1998). Screening of 34 Indian medicinal plants for antibacterial properties. *Journal of Ethnopharmacology*, 62(2): 173-182.

Samy, R.P. and Ignacimuthu, S. (2000). Antibacterial activity of some folklore medicinal plants used by tribals in western Ghat of India. *Journal of Ethnopharmacology*, 69(1): 63-71.

Subhose, V. and Srinivas, P. (2005). Basic principles of pharmaceutical science in Ayurveda. *Bulletin of the Indian Institute of History of Medicine (Hyderabad),* 35(2); 83-92. PMID: 17333665.

Wallis, T.E. (2002). *Textbook of Pharmacognosy,* CBS Publishers & Distributors CBS- Delhi, *5th Edition*, P. No-68-583. ISBN 10: 8123908865