



## DESIGN, DEVELOPMENT AND EVALUATION OF HERBAL CREAM CONTAINING LEAVES OF *MANGIFERA INDICA*

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### ABSTRACT

**Objective:** The present study was undertaken to design, develop and evaluate herbal cream containing leaves of *Mangifera indica* for anti-oxidant and anti-bacterial activity. **Methodology:** Leaves of *Mangifera indica* were collected, authenticated, shade dried and powdered. The powdered leaves were defatted with petroleum ether and ethanol extract prepared by hot extraction method. Preliminary phytochemical studies were performed and oil in water emulsion based cream was formulated with different compositions (F1 to F5) and evaluated. Then *in vitro* anti-oxidant studies using different anti-oxidant models like DPPH radical and nitric oxide radical scavenging activity and reducing power were carried out for the prepared formulations. Further all the prepared formulations were screened for *in vitro* anti-bacterial activity against two gram-positive bacterial strains i.e., *Staphylococcus aureus*, *Bacillus subtilis* and two gram-negative bacterial strains *Proteus vulgaris* and *Escherichia coli* using cup-plate method. **Results & Conclusion:** The observations of the study showed that all the prepared formulations possess all the characteristics of typical creams and also proved to have anti-oxidant activity and anti-bacterial activity. Furthermore, the formulation (F5) was found to possess good anti-oxidant and anti-bacterial activity and have scope to develop as a cost effective herbal cream for anti-aging treatment.

### INTRODUCTION

World is endowed with a rich wealth of medicinal plants. These plants have always been the principle form of medicine throughout the world, as people strive to stay healthy in the face of chronic stress and pollution, and to treat illness with medicines that work in concert with the body's own defense.<sup>[1]</sup> Apart from therapeutic use in traditional medicine, medicinal plants are used in skin care and beautifying products. They are not only used for beautification purpose but also in the treatment of different skin ailments. These products improve the functioning and texture of the skin by

Boosting collagen growth by eradicating harmful effects of free radicals. Now-a-days the usefulness of herbs in the cosmetics production has been extensively increased in personal care system and there is a great demand for the herbal cosmetics.<sup>[2]</sup> The basic idea of skin care cosmetic lies deep in the Rigveda, Yajurveda, Ayurveda, Unani and Homeopathic system of medicine. These are the products in which herbs are used in crude or extract form. These herbs have varieties of properties like anti-oxidant, anti-inflammatory, anti-septic, emollient, anti-seborrhatic, anti-kerolytic activity and anti-bacterial etc.<sup>[3]</sup> In this context there was a

wide interest in the herbal cosmetic industry in the development of herbal formulations with anti-oxidant principles for preventing aging and skin disorders. There are numerous herbs available naturally having different uses in cosmetic preparations for skin care, hair care and as anti-oxidants. The tribal people of chittoor district, in Andhra Pradesh use *Mangifera indica* to treat ailments where free radicals involved.<sup>[4]</sup> Further ethnobotanical data and in ayurvedic literature mentioned that Genus *Mangifera* is rich in anti-oxidant principles and anti-bacterial properties. Considering the vast potentiality of plants as sources of anti-oxidants and increased day to day demand for herbal cosmetics the present study was undertaken to formulate a herbal cream using the leaves of *Mangifera indica*.

#### **METHODOLOGY:**

##### **Collection of plant material:**

Leaves of *Mangifera indica* were collected from Karakampalli village near Penumur in Chittoor district and authenticated by Dr. K. Madhav chetty, Botanist, Department of Botany, SV University, Tirupati and Voucher specimen [N0: 0643] was deposited in SVU Botany department.

**Preparation of extract:** Leaves of *Mangifera indica* were shade dried and coarsely powdered. 100 gm of leaf powder was taken and defatted with petroleum ether (60-80°C) and ethanol extract was prepared by maceration followed by hot extraction method.

**Preliminary phytochemical screening:** Ethanol extract was subjected to preliminary phytochemical studies according to standard methods of Harborne *et al.*, 1973.<sup>[5]</sup>

**Formulation of herbal creams:** Oil in water (O/W) emulsion-based cream (semisolid formulation) was formulated. The emulsifier (stearic acid) and oil soluble component (Cetylalcohol), were dissolved in the oil phase (liquid paraffin) and heated to 75°C. The preservative (Methyl paraben), Emollient (Propylene glycol), ethanol extract of *Mangifera indica* (EEMI) were dissolved in the aqueous phase and heated to 75°C. After heating, the aqueous phase was added in portions to the oil phase with

continuous stirring until cooling of emulsifier took place. The formula for the cream was followed as mentioned in Table 1.

**Evaluation of Cream:** The standard procedure was followed to evaluate all the parameters in the prepared formulations.<sup>[6]</sup>

**Organoleptic evaluation:** The obtained formulations were evaluated for its organoleptic properties like colour, odour, and state. The appearance of the cream was judged by its color and roughness and graded.

**Determination of type of emulsion:** The scarlet dye method was adopted to determine the type of emulsion.

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

**After feel, Type of Smear and Removal:** Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked. After application of cream, the type of film or smear formed on the skin was checked. The ease of removal of the cream applied was examined by washing the applied part with tap water.

**Rheological studies:** The formulated cream was found to be non-newtonian. A fixed quantity 10gms of cream was taken in a 10 ml beaker. Kept it in intact for 1 hr. The beaker was inclined to one side and observed whether the cream is liquefied or not. Beaker is shaken to and fro for continuous 5 min and checked whether consistency was changed or not. The beaker was again tilted and checked for pourability of the cream.

**Viscosity and pH:** The viscosity determinations were carried out using a Brookfield Viscometer (DV II+ Pro model) using spindle number S-64 at a 20 rpm at a temperature of 25°C. The determinations were carried out in triplicate and the average of three readings was recorded. The pH was determined using pH meter.<sup>[7]</sup>

**Acid value and Saponification value:** Acid value and saponification values are determined for the prepared formulations as per the standard methods mentioned in Singh *et al.*, 2011.<sup>[6]</sup>

**Stability studies:** The stability studies were carried out as per ICH guidelines. The formulations were kept at room and elevated temperature and observed on 0<sup>th</sup>, 5<sup>th</sup>, 15<sup>th</sup>, 20<sup>th</sup>, 25<sup>th</sup> and 30<sup>th</sup> day at 8<sup>o</sup>C, 25<sup>o</sup>C. At the end of studies, samples were analyzed for the physical properties.<sup>[8]</sup>

**In vitro anti-oxidant studies:** The anti-oxidant profile of prepared herbal cream with ethanol extract of leaves of *Mangifera indica* has been evaluated. The percentage inhibition activity was recorded in graded response. Further anti-oxidant activity was studied by comparing with marketed herbal anti-aging cream which acts as standard. The anti-oxidant activity was studied by adopting standard procedures in various methods namely DPPH radical scavenging activity, Nitric oxide radical scavenging activity and reducing power assay.<sup>[9-12]</sup>

**Anti-bacterial activity:** All the prepared formulations F1, F2, F3, F4 and F5 and the standard at concentrations 100 and 150mg/ml were screened for *in vitro* anti-bacterial activity against two gram-positive bacterial strains i.e., *Staphylococcus aureus*, *Bacillus subtilis* and two gram-negative bacterial strains i.e., *Proteus vulgaris* and *Escherichia coli* using cup-plate method in nutrient agar media by measuring the zone of inhibition. Streptomycin at a concentration of 10mg/ml was used as positive control and tested for anti-bacterial activity.<sup>[13]</sup>

## RESULTS:

**Preliminary phytochemical screening:** Preliminary phytochemical studies of ethanol extract revealed the presence of various bioactive phytoconstituents namely flavonoids, steroids, terpenoids and glycosides.

### Evaluation of Cream

**Organoleptic evaluation:** The prepared formulations F1, F2, F3, F4 and F5 found to be creamish-green (F1) to light green (F5) in colour with characteristic odour and smooth texture (Figure-1).

**Type of emulsion:** All the formulations F1 to F5 found to be of the O/W type emulsion by dye test.

**Homogeneity:** All formulations F1 to F5 produced a uniform distribution of extracts

in cream which was confirmed by visual appearance and touch (Table-2).

**After feel, Type of Smear and Removal:** Emolliency and slipperiness after the application of fixed amount of cream was found good, the cream produced non-greasy film on the skin surface and was easily removed by washing with tap water.

**Rheological studies:** Rheological behavior of prepared formulations was studied and confirmed that the cream had pseudo plastic flow behavior.

**Viscosity and pH:** Viscosity of prepared formulations was in the range of 1410 to 19380 cps and the pH of the formulations were found to be between 6.19 to 6.74 which is good for skin pH and are represented in Table 2 .

**Acid value and Saponification value:** The acid value and saponification value of all formulations are presented in Table 2 and showed satisfactory values.

**Stability studies:** Upon subjecting the formulations for stability studies it was found that there was no change in properties of cream like pH, homogeneity, appearance etc. and the cream was stable (Table-3).

**In vitro anti-oxidant studies:** The results of anti-oxidant studies revealed that all the prepared formulations exhibited appreciable anti-oxidant properties and they were represented in Table 4.

**Anti-bacterial activity:** The formulated creams showed significant anti-bacterial activity and F5 found to have comparatively more anti-bacterial activity against all the bacterial strains. The anti-bacterial activity represented in terms of zone of inhibition was tabulated in Table 5.

## DISCUSSION:

In present days, the knowledge and experience of usage of herbs are blend with advanced cosmetic technology to develop safe and elegant beauty products which has wide range of people acceptability. With the science of Ayurveda, several herbs are used in the preparations of herbal cosmeceuticals which not only beautified the skin but acted as a shield against any kind of external effects on the body.<sup>[14]</sup>

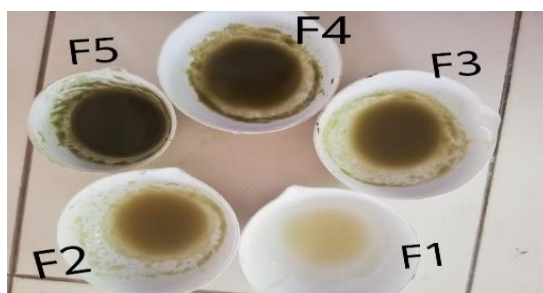


Figure 1: Prepared Herbal cream Formulations

Table -1: Composition of designed herbal creams

Ingredients	F1	F2	F3	F4	F5
Ethanol extract of <i>Mangifera indica</i>	1gm	2gm	3gm	4gm	5gm
Cetylalcohol	0.6ml	0.6ml	0.6ml	0.6ml	0.6ml
Stearic acid	0.6ml	0.6ml	0.6ml	0.6ml	0.6ml
Liquid paraffin	0.66ml	0.66ml	0.66ml	0.66ml	0.66ml
Methyl paraben	0.05gm	0.05gm	0.05gm	0.05gm	0.05gm
Propylene glycol	3ml	3ml	3ml	3ml	3ml
Water	Q.S	Q.S	Q.S	Q.S	Q.S

Table-2: pH, Acid and saponification values and Viscosity of Formulations

S.NO	FORMULATION	pH	ACID VALUE	SAPONIFICATION VALUE	VISCOSITY (cp)
1	F1	6.19	61.7	67.30	1410
2	F2	6.74	66.759	77.13	2380
3	F3	6.50	67.88	110.79	8260
4	F4	6.47	70.96	75.73	15000
5	F5	6.35	105.465	127.62	19380
6	FS	6.55	22.44	308.55	11400

Table-3: Stability studies of Formulation- F5

S.No	Parameter	Day 0		Day 30		Day 60		Day 90	
		8°C	25°C	8°C	25°C	8°C	25°C	8°C	25°C
1	Temperature	8°C	25°C	8°C	25°C	8°C	25°C	8°C	25°C
2	pH	6.12	6.35	6.14	6.35	6.14	6.38	6.14	6.38
3	Homogenicity	Good	Good	Good	Good	Good	Good	Good	Good
4.	Colour	Pale Green	Pale Green	Pale Green	Pale Green	Pale Green	Pale Green	Pale Green	Pale Green
5.	Apprearence	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid
5	Spreadability	Good	Good	Good	Good	Good	Good	Good	Good
6	After feel	Emollient	Emollient	Emollient	Emollient	Emollient	Emollient	Emollient	Emollient
7	Ease of removal	Easy	Easy	Easy	Easy	Easy	Easy	Easy	Easy
8	Type of Smear	Non greasy	Non greasy	Non greasy	Non greasy	Non greasy	Non greasy	Non greasy	Non greasy

**Table- 4: In vitro anti-oxidant studies of formulations**

S.No	Formulation	DPPH	Scavenging of nitric oxide	Reducing power
1	F1	1.6%	23.29%	20.76%
2	F2	3.4%	24.8%	25.42%
3	F3	3.49%	28.96%	29.11%
4	F4	7.08%	36.67%	31.9%
5	F5	8.2%	39.76%	38.42%
6	FS	18.2%	45.75%	55.44%

**Table-5: Anti-microbial activity of formulations**

Formulation	Zone of Inhibition for the microorganisms (cm)							
	<i>Staphylococcus Aureus</i>		<i>Proteus vulgaris</i>		<i>Bacillus subtilis</i>		<i>Escherichia coli</i>	
	100 mg/ml	150 mg/ml	100 mg/ml	150 mg/ml	100 mg/ml	150 mg/ml	100 mg/ml	150 mg/ml
<b>F1</b>	0.4	0.5	0.5	0.6	0.5	0.6	0	0.5
<b>F2</b>	0.7	0.8	0.7	0.9	0.8	0.8	1.0	1.0
<b>F3</b>	0.8	0.9	0.8	1.0	0.8	1.0	1.0	1.0
<b>F4</b>	0.7	1.0	0.8	1.0	0.9	1.4	1.0	1.0
<b>F5</b>	1.2	1.5	0.9	1.5	1.0	1.5	1.4	1.5
<b>FS</b>	-	-	-	-	-	-	0.6	0.7
<b>BLANK</b>	-	-	-	-	-	-	-	-
<b>STANDARD</b>	2.5	3	2	2.5	2	2.5	2.5	3.0

There is growing scientific evidence that plants possess vast and complex active phytochemicals which are able for skin smoothing, restore, heal and protect the skin from various effects including aging.<sup>[15]</sup> The use of anti-oxidants for a particular topical formulation appears to be an interesting approach to protect skin against oxidative stress caused by different extrinsic agents.<sup>[16]</sup> Many plants such as *Centella asiatica* oil, *Spilanthes acmella* oil, *Zingiber officinale*, *Nardostachys jatamansi*, and *Punica granatum* which are rich in anti-oxidant principles proved to have beneficial effects in protection of skin.<sup>[17]</sup> Hence in our current study for the formulation of herbal cream, leaves of *Mangifera indica* which was proved to be rich in anti-oxidant principles like flavanoids, saponins, terpenoids and other phenolic compounds

Were selected. Upon preliminary phytochemical screening of ethanol extract of leaves of *Mangifera indica* it was found that the extract is rich in anti-oxidant principles such as flavanoids and other phenolic compounds which supported the earlier studies.<sup>[18]</sup> In present study five formulations (F1, F2, F3, F4 and F5) were designed in which the concentration of ethanol extract is varied. In formulation cetylalcohol and stearic acid were used as emulsifiers. The Cetylalcohol was preferred since apart from emulsification it also acts as an emollient and thickening agent while Stearic acid aids as a surfactant. As a moisturizing agent is an essential ingredient in formulation of anti-aging herbal creams, in present formulation development, liquid paraffin and propylene glycol were used. The liquid paraffin not only helps in moisturizing but also acts as lubricant and

emollient and thus helps skin to feel more comfortable and less itchy. Methyl paraben is one of the most preferred preservative used in cosmeceuticals and hence methyl paraben was used as a preservative agent. The prepared formulations were evaluated for various organoleptic and physical parameters and the results revealed that they are acceptable in appearance and found to be satisfactory with typical characteristics of pharmaceutical creams. All formulations were found to be homogeneous, good in spreadability, and ease in removal. Further the formulations were found to be non-irritant and the pH's of them were close to the skin pH. Viscosity is an important parameter in studying the rheological properties of creams,<sup>[19]</sup> our formulations found to be pseudoplastic in behavior and the results were found to be comparable with marketed formulation. Acid value and saponification value are important parameters of oils and creams. The prepared formulations found to have normal acid and saponification values as that reported in various herbal creams reported by Sekar *et al.*, 2017 and Mandeep *et al.*, 2011;<sup>[20]</sup> and the values found to be nearer to that of standard. Moreover, stability studies performed for the formulations showed that all the prepared formulations were stable. Thus, all the evaluation parameters showed that all properties are within the acceptable range and in harmony with previous reports of herbal anti-aging creams.<sup>[17,21]</sup> Prepared herbal formulations F1 to F5 were evaluated for anti-oxidant activity by performing DPPH, nitric oxide scavenging activity and reducing power assay. The anti-oxidant activity raised gradually from F1 to F5 which may be due to the increase in the concentration of extract. These results were comparable with the marketed anti-aging formulation which is used as standard. In line with previous reports on herbal anti-age creams, our herbal cream also showed good anti-oxidant activity which supports it can be used to protect the skin from oxidative stress which leads to aging effect.<sup>[22]</sup>

Further anti-bacterial activity was evaluated for all the formulations and it was found that they are potent inhibitors of the

growth of tested microorganisms. When compared with the standard marketed formulation the prepared formulations exhibited more zone of inhibition against tested bacterial strains at both lower and higher concentrations. Among all the formulations F5 showed maximum zone of inhibition and proved to be more effective in preventing bacterial growth. The anti-bacterial activity of the cream may help to protect the skin from extrinsic microbes which damages the epidermal layers and thereby to pre-aging effect. Based on all the parameters of the study among the prepared formulations F5 found to more effective since apart from all characteristics of typical creams it also proved to have more anti-oxidant activity and anti-bacterial activity. In comparison with the marketed formulation the prepared formulation F5 was advantageous in the aspect that the formulation exhibit both anti-oxidant and anti-bacterial activities. It may be more effective in preventing the skin from damaging as it scavenges free radicals and also protects the layers of the skin from bacterial effects.

#### CONCLUSION:

The present study resulted in formulation of herbal cream using the ethanol extract of leaves of *Mangifera indica*. The study also depicts the anti-oxidant, anti-bacterial activity of the formulations. Furthermore, the formulation (F5) was found to possess good anti-oxidant and anti-bacterial activity and have scope to develop as a cost effective herbal cream for anti-aging treatment.

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