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DEVELOPMENT AND EVALUATION OF A HERBAL COSMETIC CREAM FROM THE STANDARDIZED EXTRACT OF *OCIMUM BASILICUM* LEAVES

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Keywords:

Ocimum basilicum, HPTLC, Formulation, Anti-microbial activity, Anti-oxidant activity

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ABSTRACT: Objective: The present study aimed to develop and evaluate herbal cosmetic cream by incorporating standardized Ocimum basilicum leaf extracts to exert its anti-oxidant potential as anti-wrinkle properties. **Methodology:** The hydro-alcoholic and aqueous extracts were prepared and standardized through high-performance thin-layer chromatography studies using the mobile phase ethyl acetate - acetic acid- formic acid- water (100:11:11:26 v/v/v/v) along with the standard rutin. Then, the anti-oxidant potential of the extracts were analysed through *in-vitro* DPPH free radical scavenging assay. Further, the formulations were developed using hydro-alcoholic and aqueous extracts (F_1 to F_6). The formulations were analysed for stability, physical characterization, HPTLC studies and anti-bacterial properties. **Results:** The studies resulted the spot at Rf 0.40 and the calibration showed the linear relationship with the r^2 value 0.99716 in the concentration ranging from (400-1500) ng per spot. The anti-oxidant activity resulted in a percentage inhibition of 84.62% for hydro-alcoholic extract and 71.23% for aqueous extract, comparable with the standard ascorbic acid, which was 94.02%. The cream was characterized by its bloom strength, extrusion, firmness and spreadability. The HPTLC studies of the formulation revealed that the quantity of rutin present was found to be 0.041±0.002 ng/gm. The formulation was shown to have activity against both gram-positive and gram negative bacteria. Hence, the developed formulation can be further subjected to *in-vivo* analysis to explore *Ocimum basilicum* as a novel formulation in the market.

INTRODUCTION: *Ocimum basilicum (*Lamiaceae) is an erect herb popularly known as sweet basil. It was distributed throughout India. This plant is grown for its ornamental purpose and also exerts its excellent culinary properties. The leaves are greenish with aromatic odour due to presence of numerous oil globules. The leaves of *Ocimum basilicum* are used to treat various ailments such as nausea, dysentery and flatulence.

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The volatile oil obtained from the leaves exerts its antibaterial and larvicidal properties [1]. Several biological activities have been proven for *Ocimum basilicum* leaves in the treatment of various disorders. The monoterpene, sesquiterpenes, and flavonoids in the hexane and dichloromethane extracts of *Ocimum basilicum* leaves were proven with antioxidant properties.

The amino fatty alcohols, lignins, coumarins, and sesquiterpene lactones were found to be in the methanolic extracts of *Ocimum basilicum,* and the extracts were shown to control hypertension in low doses [2]. The essential oil obtained from *Ocimum basilicum* was found to have the potential to inhibit the fungal biofilm of *Candida albicans* [3, 4].

MATERIALS AND METHODS:

Authentication of Plant Material: The plant materials proposed for the study were collected from the medicinal garden at PSG College of Pharmacy, Coimbatore, Tamil Nadu, India and authenticated by Scientist G, Botanical Survey of India, Coimbatore. Authentication no: BSI/SRC/5/23/2016/Tech/2028.

Process of Extraction: The aerial part of *Ocimum basilicum* was shade dried and powdered coarsely. The processes of extraction with each solvent (aqueous, hydro alcohol) was repeated three times by using cold maceration method and the resultant extracts were combined together, the solvents were distilled off using rotary evaporator. The dried extracts were weighed, and their required quantity was dissolved in appropriate solvents for further investigation. [5].

Anti-oxidant Activity of the Extract: The purpose of this study was to evaluate the antioxidant activity of hydro-alcoholic and aqueous extracts of *Ocimum basilicum* proposed for anti-wrinkle activity using the DPPH free radical scavenging assay. The percentage of antioxidant activity (AA %) of each extract was assessed by DPPH free radical scavenging assay. The DPPH free radical scavenging activity was measured according to the methodology described [6].

Formulation of Herbal Cosmetic Cream: By incorporating *Ocimum basilicum* extracts, Oil in water (O/W) emulsion-based cream (semisolid formulation) was formulated [7]. The formulation's ingredients were mixed in various proportions, creams were formulated and named from F_1 to F_6, respectively, which are tabulated in **Table 1.**

TABLE 1: FORMULATION OF ANTI-WRINKLE CREAM FROM HERBAL EXTRACTS

Ingredients	F_1	F_2	F_3	F_4	F_5	F_6
Extract	5% HAE*	5% HAE	10% HAE*	10% HADE**	10% AE***	10% ADE****
Stearic Acid	6%	4%	4%	4%	4%	4%
Cetyl Alcohol	4%	3%	2%	2%	2%	2%
Glycerol	6%	3%	4%	4%	4%	4%
Moisturizer	5%	3%	4%	4%	4%	4%
Methyl Paraben	0.03%	0.02%	0.02%	0.02%	0.02%	0.02%
Propyl Paraben	0.02%	0.03%	0.02%	0.02%	0.02%	0.02%
Ethylene Diamine Tetra Acetate	0.02%	0.01%	0.01%	0.01%	0.01%	0.01%
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s
Flavouring Agent	q.s	q.s	q.s	q.s	q.s	q.s
Water	q.s	q.s	q.s	q.s	q.s	q.s

*HAE - Hydro-Alcoholic Extract. **HADE - Hydro-Alcoholic Decolourized Extract. ***AE - Aqueous Extract, ****ADE - Aqueous Decolourized Extract.

Stability Testing: Stability studies are an essential component of pharmaceutical development that allows the evaluation of active pharmaceutical ingredient (API) stability or drug product stability under the influence of a variety of environmental factors such as temperature, humidity and light [7]. Stability was tested in the prepared formulations and conducted for six samples for 30 days at room temperature. The formulations F1-F6 were kept at room temperature for 30 days and regularly observed on pH, colour, odour, appearance and homogeneity.

Physical Characterization of the Formulation:

Dilution Test: The formulated product was mixed with 10 ml of distilled water. The status of phase separation was identified and recorded.

Dye Test: The scarlet red dye was mixed with the formulation. A drop of the sample was placed on a microscopic slide, covered with a cover slip, and examined under a microscope.

Texture Analysis: Texture profile analysis was performed using Texture Analyzer (stable micro systems TA.XT plus). Firmness, consistency, adhesiveness, stringiness, spreadability and bloom strength were performed using Texture analyzer [8].

HPTLC Evaluation of Herbal Formulation:

Instrumentation: The HPTLC system (Camag, Muttenz, Switzerland) consists of TLC scanner III with Wincats 3 software (version 1.4.10), Linomat 5 applicator fitted with 100 µL syringe and

connected to a nitrogen cylinder, a twin trough chamber (20×10 cm) [9].

Sample Preparation:

Preparation of Standard Stock Solution: Standard rutin was dissolved in methanol (2mg/10ml).

Preparation of Extract Stock Solution: One gram of the *Ocimum basilicum* Extract was dissolved in 10ml of methanol and centrifuged to remove the insoluble particles.

Chromatography and HPTLC Scanning:

- Samples were applied to the plates, 5-6mm wide and 10mm from the bottom on Si 60F $_{254}$ TLC plates using Linomat V applicator.
- Chromatogram was developed by using the principle of ascending chromatography.
- Ethyl acetate-glacial acetic acid-formic acid-water (100: 11: 11:26) in a twin trough chamber.
- After the development, the plates were dried and detected using UV chamber.
- The plates were then scanned using the Wincats software (ver 1.4.10) [10].

Anti-microbial Evaluation of Herbal Formulation:

Media: Mueller Hinton agar media.

Organism used: Staphylococcus aureus and Escherichia coli strains were collected from the biotechnology laboratory of PSG College of Pharmacy, Coimbatore, Tamil Nadu-641004.

Procedure:

- The molten Mueller Hinton agar was brought to the temperature of 45-50°C and the standardized inoculum was added to the molten media and mixed well.
- The molten media was added to the petri dish and kept to get solidified.
- After solidification of the inoculated media, to the each plate, holes of 7 mm in diameter were made at equal distance using sterile cork borer [11].
- Measuring 20-30µl of Hydro-alcoholic extract, aqueous extract, formulation and standard amikacin of concentration 1000µg/ml were dissolved in sterile water for injection. It was added to each hole on the medium and opened for 5-15 minutes in the laminar air flow chamber.
- After complete diffusion of the test substance to the media, the petri plate was closed and kept for incubation at 37°C for 18-24 hours at incubator.
- After 24 hours, the plates were taken and checked for zone of inhibition.
- If the zone of inhibition was seen, the diameter of the zones were measured in millimetre scale and compared with the standard zone produced in standard drug (Amikacin 1000µg/ml) [12].

RESULTS AND DISCUSSION:

Standardization of Extracts through HPTLC Method: The developed HPTLC method showed a good correlation in the concentration range of 400-1500 ng/spot with respect to peak area, gave a sharp, thin and well defined peak at Rf=0.40 and resulted with the concentration of 0.126±0.002ng/g and 0.094±0.002ng/g of hydro-alcoholic **Fig. 1** and aqueous **Fig. 2.** extracts respectively.

TABLE 2: QUANTITATIVE ESTIMATION OF RUTIN IN DIFFERENT EXTRACTS

S. no.	Particulars	Report	
		Hydro-alcoholic extract	Aqueous extract
1	Solvent system	Ethyl acetate-formic acid-glacial acetic acid-water(100:11:11:26)	
2	Standard Rf (RUTIN)	0.40	0.40
3	Linearity range (ng)	400-1500	400-1500
4	Limit of detection (ng)	200	100
5	Regression (r^2) Area	0.99716	0.99894
6	Standard deviation Area	4.49%	3.10%
7	Quantity of rutin present (ng/g).	0.126±0.002	0.094±0.002

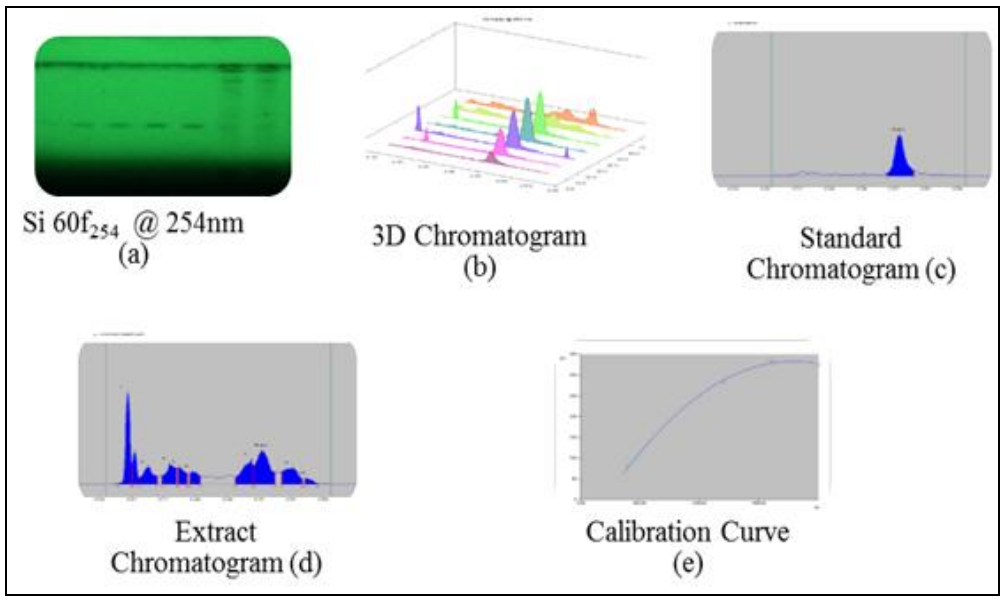

FIG. 1: CHROMATOGRAM FOR HYDRO-ALCOHOLIC EXTRACTS

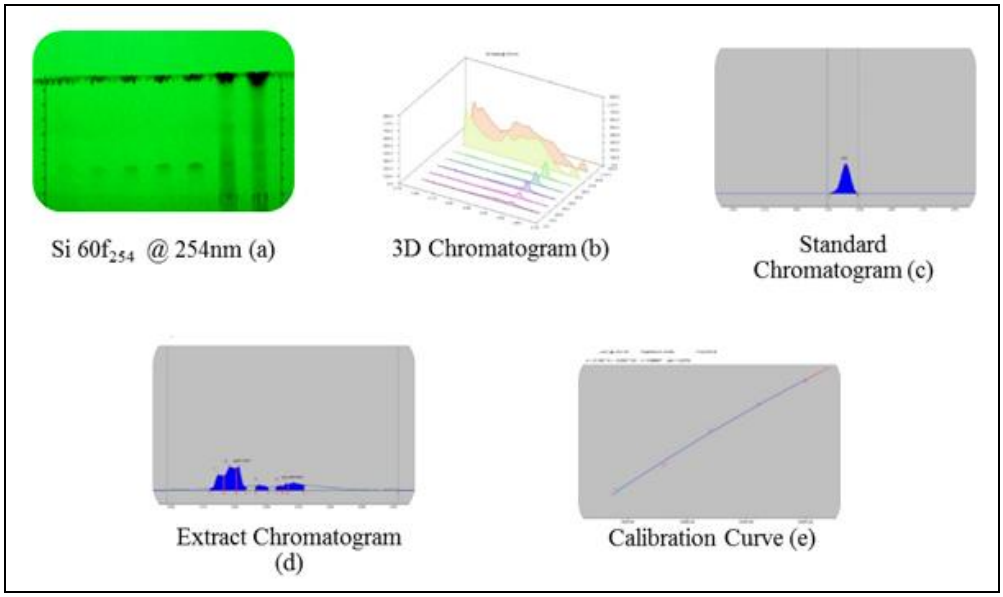

FIG. 2: CHROMATOGRAM FOR AQUEOUS EXTRACT

Anti-oxidant Studies of the Extracts: The *Ocimum basilicum* leaf extracts shows scavenging activity on DPPH radical tabulated in **Table 3**. The *Ocimum basilicum* extracts and the positive control (Ascorbic acid) displayed concentration dependent inhibitory activities on DPPH free radical.

The inhibitory effect of the hydro-alcoholic extract (84.62%) is higher than the effect of aqueous extract (71.23%) both were comparable to the inhibitory effect of the standard ascorbic acid. Since hydro-alcoholic extract have more anti-oxidant activity than aqueous extract, hydro-alcoholic extract was the most promising extract of *Ocimum basilicum* leaves to revert the wrinkles present in the face.

TABLE 3: ANTI-OXIDANT STUDIES

Extracts	Average of 3 values (%)
Hydro-alcoholic extract	84.62
Aqueous extract	71.23
Ascorbic acid	94.02

Stability Studies of the Formulation: The formulated creams were evaluated on stability testing parameters such as pH, colour, odour, appearance; homogenecity and washability were reported on **Table 4**. The work was done for the period of 30 days under normal room temperature and humidity. At the end of 30 days, all the cream fails the stability testing as there were a change in colour, fluctuation in pH and poor homogenecity, wash ability and appearance except the cream F_6

which passes the test as there was no any changes in the stability parameter of the cream. So, the passed cream F_6 was considered as the stable cream and preceded with further evaluation and rest of the creams F_1.F_5 were discarded.

TABLE 4: STABILITY STUDIES

Para Meters	Days	F_1	F_2	F_3	F_4	F_5	F_6
	0	6.35	6.42	5.83	5.32	5.71	5.62
	15	5.88	6.18	5.74	5.41	5.24	5.57
pH	30	5.14	4.85	5.64	5.46	5.82	5.56
	0	green	Green	green	pale green	dark green	pale yellow
	15	pale green	pale green	pale green	pale green	dark green	pale yellow
Colour	30	colourless	Colourless	colourless	light green	pale green	pale yellow
Odour	0	***	***	***	***	***	***
	15	**	**	***	***	***	***
	30	*	*	**	***	**	***
Appearance	0	***	***	***	***	***	***
	15	*	*	**	***	**	***
	30	*	*	**	**	**	***
Homogenecity	0	***	***	***	***	***	***
	15	**	**	***	***	***	***
	30	*	*	**	***	**	***
Washability	0	***	***	***	***	***	***
	15	*	*	***	***	***	***
	30	*	*	**	***	**	***

*** - excellent ** - good * - poor.

Physical Characterization of the Selected Formulation: The physical measurements were performed to characterize the cream and to study the effect of the emulsifying agent on the quality of the final product. It doesn't form any phase separation on dilution test; on dye test, the disperse globules appear red and the background colourless. So, the dilution test and dye test confirmed the formulated cream was the O/W cream. The assessment of firmness, consistency, adhesiveness, and stringiness of the cream was performed. The samples were analyzed in triplicate, the average values were calculated, and images were given in Fig. 3 and tabulated in **Table 5.**

TABLE 5: PHYSICAL PARAMETERS

S. no.	Parameters	F_6
1	Dilution test	O/W cream
2	Dye test	O/W cream
3	Viscosity	159082 Pa.S
4	Bloom strength	54.895 g
5	Extrusion	2399. 163 g
6	Firmness	1571.851 g
7	Spreadability	1669.611 g.sec

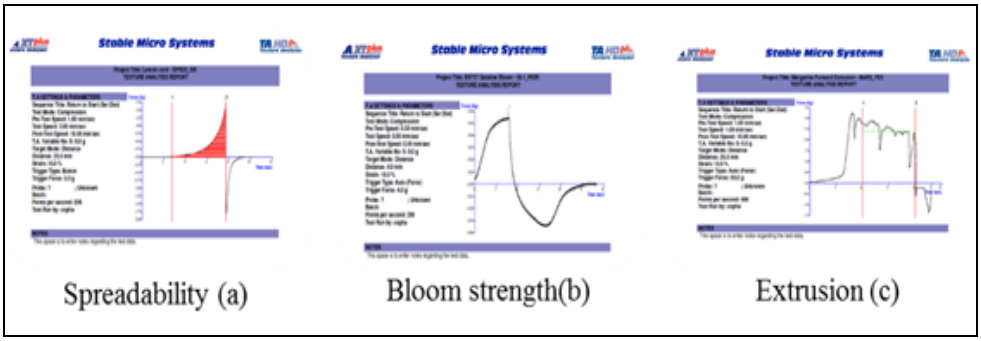

FIG. 3: TEXTURE ANALYSIS REPORT

Analysis of Rutin Content in the Formulation using HPTLC Method: According to chromatography in a thin layer of silica gel, spots in the formulation has similar colour to the standard

of rutin, and the retention factors (Rf) is 0.40 **Fig. 4**, which are also the same as the standards. The quantity of the rutin present in the formulation was found to be 0.041±0.002ng/gm of formulation, which is tabulated in **Table 6**. This indicates that the rutin content was retained in the formulation and there was a certain decrease in the flavonoid rutin quantity.

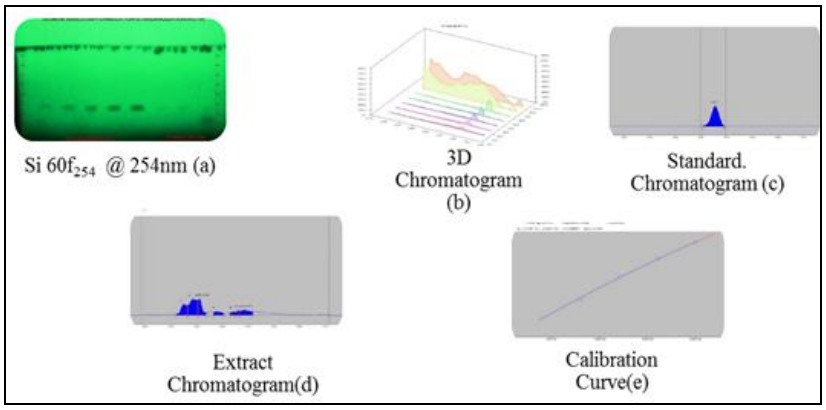

FIG. 4: CHROMATOGRAM FOR FORMULATION

TABLE 6: ESTIMATION OF RUTIN CONTENT IN FORMULATION CONTAINING *OCIMUM BASILICUM* EXTRACTS

S. no.	Particulars	Report
		Formulation
1	Solvent system	Ethyl acetate-formic acid-glacial acetic acid-water(100:11:11:26)
2	Standard Rf (RUTIN)	0.40
3	Linearity range (ng)	400-1500
4	Limit of detection (ng)	100
5	Regression	0.99894
6	Standard deviation	3.10%
7	Quantity of rutin present(ng/g)	0.041±0.002

Anti-bacterial Studies: Results of the anti-bacterial screening tests are given in **Table 7.** As can be clearly seen in the above picture **Fig. 5**, we can easily conclude that the Hydro-alcoholic, aqueous extract and formulation of *Ocimum basilicum* Linn exhibited inhibitory activity against Gram +Ve (*Staphylococcus aureus*) and Gram –Ve (*Escherichia coli*) organism. Compared with standard amikacin, the hydro-alcoholic extract have a wide range of anti-bacterial activity followed by aqueous extract and formulation. Upon testing the samples using Staphylococcus aureus and Escherichia coli, the formulated cream can be used to treat pimples and acne.

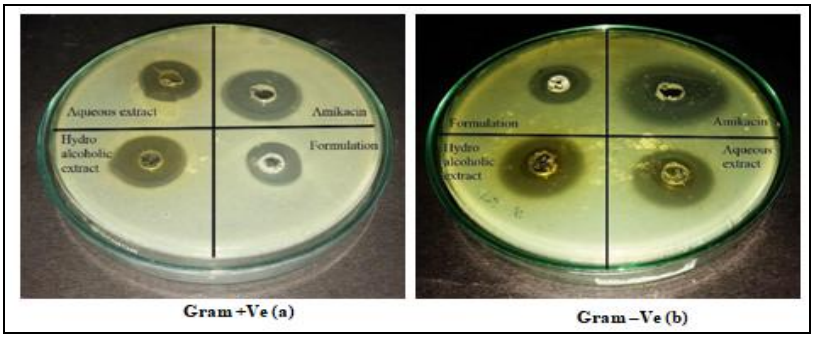

FIG. 5: ANTI-BACTERIAL ACTIVITY

TABLE 7: ANTI-BACTERIAL STUDIES

Substance	Zone of Inhibition (mm) (Average of three)	
	Staphylococcus aureus	***Escherichia coli***
Standard drug (Amikacin)	25	26
Aqueous extract	12	21
Hydro-alcoholic extract	22	19
Formulation	17	15

CONCLUSION: Despite the wide range of alternatives available in the market, the interest for anti-wrinkle natural products was increasing globally. The medicinal plants available in our traditional medicine present a great potential in developing new anti-wrinkle products considering different mechanism of actions such as antioxidant effects, stimulation of collagen synthesis and improvement of the skin elastic properties. Besides, natural ingredients are well tolerated in humans and the incidence of side effects to herbal extracts is extremely rare. The standardized extracts were incorporated in an O/W cream base in these formulations for the effective anti-oxidant activity and anti-wrinkle effect. In summary of the work, analysis of rutin content in raw material as well as in finished formulation shows the retention of active content after processing into formulation. This formulation may prosper and become effective in exerting anti-wrinkle properties. The mentioned therapeutic efficacy might be due to the mechanism of the scavenging of destructive free radicals of the constituents present in the *Ocimum basilicum* leaves used in the cream. This study revealed that the extracts' flavonoidal content (rutin) may be responsible for the anti-wrinkle activity through anti-oxidant mechanism. However, evidence-based studies on their anti-wrinkle effects are still in demand. Further, the assessment of clinical efficacy for the developed formulation should be continued in future.

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CONFLICT OF INTEREST: The authors declare that there are no conflict of interest

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