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Afr. J. Biomed. Res. Vol. 27(4s) (December 2024); 10194-10198

Research Article

Herbal Cream Formulation And Antioxidant Evaluation Of Flueggea Leucopyrus

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Abstract:

In developing countries like India, the commercial demand for herbal products is increasing continuously as natural products are safe with fewer side effects and are economical compared to synthetic medicines. The research work relates to the herbal topical composition comprising of the herbal extract of Flueggea Leucopyrus by topical drug delivery. Flueggea leucopyrus plant is useful for the treatment of scrotal swelling, swollen testicles lead to enlargement of testicles and is used in treatment of skin swelling and puffiness of tissue. Furthermore, Flueggea leucopyrus plant extract is effective for the treatment of stomach discomfort, hepatomegaly and increased portal venous pressure. The present invention covers the process for the preparation of the herbal cream formulation and its biological evaluation for antioxidant activity.

Keywords: Herbal creams, topical cream, Flueggea Leucopyrus, Antioxidant

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Received 21/09/2024, Acceptance 28/09/2024

DOI: <https://doi.org/10.53555/AJBR.v27i4S.2065>

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Introduction:

Herbal medicines are commonly known as phytomedicine. Herbal medicines utilize the different plant parts covering seeds, roots, leaves, bark, or flowers useful for curing various disease conditions. Herbalism deals with the traditional use of medicines outside the range of conventional medical use. Herbalism has gained popularity due to advancements in analysis, quality control and clinical operations, showing the importance of herbal medicinal products in the treatment of various diseases¹⁻⁴.

The usage of herbal plants for the treatment and prevention of various disease conditions has been

reported long back before history. Ancient Egyptian and Chinese papyrus written scripts disclose ancient uses of herbal plants, around 3,000 BC before. According to literature, African and Native American cultures used herbal medicine in their healing rituals, rest of the culture developed traditional treatment procedures including Ayurveda and panchakarma. Many countries adopted traditional Chinese medicine as herbal therapies & procedures. It was found in the literature that beginning of the 19th century when analysis of chemical substances became available first time to the world, researchers started extracting and altering the active ingredients from plants. After a few years, researchers started developing new models of plant compounds, and,

later, herbal medicine started to turn down in favor of synthetic drugs⁵⁻⁷. Ayurvedic treatment methods give more importance to the bonding between plants and humans throughout the progress of human culture. Currently, increase in the demand for herbal medicines due to being free from toxicity and side effects as compared to allopathic medicines⁴. According to WHO (World Health Organization), herbal medicinal products are finished labeled medicinal products that cover active medicinal ingredients, aerial or underground parts of the plant or other plant material or combinations⁵. It has been observed that an increase in demand & usage of herbal medicinal products worldwide in the previous few years down the line. In 2006, the global market reached up to \$5.6 billion; whereas it was growing at an average annual growth rate of 1.7%, it is expected to rise above \$6.1 billion in 2011⁸⁻¹⁰.

Flueggea leucopyrus is a genus of thorny shrubs belonging to the family Euphorbiaceae, and found as two species in Sri Lanka. *Flueggea leucopyrus* Wild is naturally found with a large bush, long straggling thorny branches, and small globose white berries. *F. leucopyrus* is found in Sri Lanka, India and Burma⁷. *Flueggea leucopyrus* is becoming popular now a days because it becomes the active ingredient in several 20 traditional therapeutic systems. *Flueggea leucopyrus* is useful in curing lung carcinoma, gastric pain, hemorrhoids, leiomyomas, fibromas, scrotal swelling, swollen testicles lead to enlargement of testicles and is used in treatment of skin swelling and puffiness of tissue. As reported in the literature, the whole plant is used to cure foot melanoma, bloating abdomen, hepatomegaly or liver enlargement and increased portal venous pressure. The bark of the stem is used for the treatment of dental pain. It is reported in the literature that leaves of *Flueggea leucopyrus* are effective for abdominal discomfort, and hemorrhoids. The roots of plants are effective in the treatment of swollen testicles leading to enlargement of testicles and skin swelling and puffiness of tissue. The entire plant of *Flueggea leucopyrus* is very effective in treating swelling of testicles and in the cure of swelling or puffiness of the tissue. There are literature reports that abdominal lumps, liver hypertrophy and portal hypertension are cured by *Flueggea leucopyrus* plant extract¹¹⁻¹⁴.

Creams are topical formulations containing homogenous, semisolid, or viscous preparations. It exhibits liquid consistency and is deliberately for external use. Creams are formulated for therapeutic,

protective, or prophylactic applications. Creams comprise either solutions or dispersions of medicinal agents in a suitable base to get semisolid consistency. Oil in water type of cream base is preferred because of certain advantages like ease of application, smooth texture and aesthetic appearance. Herbal creams have advantages over allopathic creams such as free from harmful chemicals & reagents, and having fewer skin diseases and side effects. Herbal creams contain crude or extract forms of medicinal herbs¹⁵⁻¹⁶. The herbal topical compositions have been suitable for handling skin properties for a longer duration and their effects are widely accepted by developing countries. *Flueggea leucopyrus* plant extract which was utilized in the present investigation exhibits applications in Indian traditional medicine systems to treat various skin diseases¹⁷.

Materials and Methods:

The entire plant of *Flueggea leucopyrus* was collected from different regions in Sri Lanka like Wijerama Hambantota, Kohuwela in the winter session of November 2023. The plant was identified and authenticated at Alarsin Ayurvedic Pharmaceuticals, Mumbai. All the chemicals were pharmaceutical grade. L-Ascorbic Acid is procured from sigma Aldrich.

Preparation of Flueggea leucopyrus aqueous extract:

The leaves of *Flueggea leucopyrus* were first collected and then washed thoroughly with distilled water, followed by air dried for 4 weeks. The dried leaves were powdered in the mixture. 200gm powder of *Flueggea leucopyrus* and distilled water 400 ml were refluxed for 16 hours. The brownish-red solution which was obtained, was filtered properly with filter paper. The filtered solution was gently heated at 80°C to 100°C for 48 hours. The filtrate in which a clear solution or clear extract of *Flueggea leucopyrus* was used to prepare the cream product. Aqueous extracts of plant materials were evaluated for various quality control tests such as Na⁺ content, K⁺ content, conductivity, and pH¹⁸⁻²⁰.

Preparation of Formulation:

An aqueous extract of *Flueggea leucopyrus* having a concentration of 2mg/ml was incorporated in all formulations (F1-F7). Along with active ingredients, the cream consists of Cetyl alcohol (Stabilizer), Isopropyl myristate (thickener), triethanolamine (emulsifier), Tween 80 (surfactant), Glycerine (alkaline agent), Methylparaben (preservative) and water as the vehicle as shown in Table 1.

Table 1. The formulations of herb extract cream formulations

Sr. No.	Ingredients	Conc. in mg						
		F1	F2	F3	F4	F5	F6	F7
1	Extract of Fluggea Leucopyrus	2	2	2	2	2	2	2
2	Cetyl alcohol	6	8	10	12	14	16	18
3	Isopropyl myristate	1	1	1	3	5	7	9
4	Triethanolamine	0.2	0.4	0.6	0.8	0.10	0.12	0.14
5	Tween80	1.3	2.3	2.9	3.9	4.7	5.7	6.7
6	Methyl paraben	0.04	0.04	0.04	0.04	0.04	0.04	0.04
7	Glycerine	2.1	2.3	2.5	2.7	2.9	3.1	3.3
8	Distilled water	qs	qs	qs	qs	qs	qs	qs
9	Lavender oil	qs	qs	qs	qs	qs	qs	qs

Formulations F1-F7 creams were prepared in the laboratory using beaker method. Triethanolamine & Isopropyl myristate were mixed and added in Cetyl alcohol to form oil phase. Glycerine was added in oil phase with continuous stirring. Extract of Fluggea Leucopyrus, methyl paraben, tween 80 and Lavender oil were added to form water phase; Mixing the oil phase and water phase to form final cream formulation.

Physicochemical properties of formulations:

Organoleptic properties: Organoleptic properties of the cream formulations F1-F7 were studied by evaluating colour, odour, and texture of cream.

Centrifugation test: A centrifugation test was performed at 2000 rpm for 30 min, followed by appearance and phase separation conducted by macroscopic examination.

pH measurement: The pH measurement was conducted using a pH meter at room temperature ($30 \pm 2^\circ\text{C}$)

Viscosity and flow measurement: Brookfield rheometer (model LVDV-III Ultra) was utilized to evaluate the viscosity and rheological properties of the F1-F7 prepared formulations at room temperature. The spindle No. 6 at 10 rpm. was used for the experiment.

Spreadability: The spreadability experiment was conducted by keeping two sets of glass slides of standard dimension.

Later, a topical cream formulation was placed on the side. A certain load weight was placed on the upper slide so that the cream between the two slides was evenly distributed to form a thin layer. After that load was removed, excess formulation adhering to the slides was scrapped off. The upper slide was allowed to slip off freely by the force of the load tied to it. The time taken by the upper slide to slip off was recorded.

Washability: A 1gm of herbal formulation was applied on the right hand of the volunteer and washed with distilled water to perform the washability test.

Antioxidant test: An antioxidant study of seven formulated creams was conducted in the Laboratory by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method. 96-well plate and a microplate reader (NS-100 Nano scan) were utilized in DPPH assay method. The concentrations of 20, 40, and 100 $\mu\text{L/mL}$ were prepared for cream formulations. Furthermore, the samples weighed 1g were mixed with 1 mL of distilled, vortexed, and sonicated for 15 min to get a clear solution. In contrast, L-ascorbic acid (0.1-40 $\mu\text{g/mL}$ diluted in water) was utilized as the positive control for antioxidant testing.

The DPPH solution with a concentration of 6×10^{-5} M was prepared in absolute ethanol and the samples were mixed with the DPPH solution. The mixtures were stored in the dark at room temperature for 20 minutes and measured spectrophotometrically at 517 nm. The scavenging activity of formulations was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \frac{1 - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

Antioxidant activity data was mean $\pm$ standard deviation (SD). Statistical comparisons were made using Student's t-test performed by SPSS statistics 20.

Results and Discussion:

Preparation of Fluggea Leucopyrus Herbal extract: F. Leucopyrus extract yielded 42.64% yield. The aqueous extract was observed pale yellowish-gray colour with a pleasant odour.

Antioxidant activity of Fluggea Leucopyrus Herbal extract: Screening of antioxidant activity of F. Leucopyrus Herbal extract was performed using the DPPH technique. The EC_{50} value of F. Leucopyrus herb extract was observed as 2.512 mg/mL whereas, the positive control, ascorbic acid was marked EC_{50} value as 2.714 $\mu\text{g/mL}$. We can conclude that less of substances are contained in the extract.

Physicochemical properties of formulations:

Organoleptic properties: Organoleptic properties of the seven formulations were evaluated for the color,

odour, and texture of the cream. It was observed that all formulations exhibit a pale yellowish-gray colour with a pleasant odour, smooth texture & semisolid state.

Table 2: Physical evaluation of Formulations F1-F7

Sr.no	Parameters	F1	F2	F3	F4	F5	F6	F7
1	Colour	Yellowish Grey	Yellowish Grey	Yellowish Grey	Yellowish Grey	Yellowish Grey	Yellowish Grey	Yellowish Grey
2	Odour	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
3	Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
4	State	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid

Centrifugation test: Centrifugation test was performed at 2,000 rpm speed for 30 min, all seven formulations F1-F7 remained homogeneous, therefore, prepared formulations were stable under experimental conditions. In the centrifugation test, the distinct separation of two phases in the emulsion influences several characteristics, particularly, the solubility of active ingredients. It is

conceded that the creams are formulations with high water content. In the prepared creams, percentages of water content started from 46.20 to 56.20 %w/w.

pH measurement: The pH values of the *F. Leucopyrus* herbal extract F1-F7 formulations were considered in the range of skin pH values.

Table 3: pH values of cream formulations (F1-F7)

Parameter	F1	F2	F3	F4	F5	F6	F7
pH value	5.70 ± 0.00	5.72 ± 0.00	5.74 ± 0.00	5.72 ± 0.00	5.72 ± 0.00	5.72 ± 0.00	5.74 ± 0.00

Viscosity and rheological measurement: It is recognized that when the shear rates increase the viscosity of the cream formulation tends to decrease. At speeds of 45, 60, and 90 rpm, there was no statistical difference in the viscosity between different formulations. Brookfield viscometer with spindle No.6

at 10 rpm at a temperature of 37°C ± 0.5°C was utilized to determine the viscosity of F1-F7 cream formulations was determined by Brookfield viscometer with spindle No.6 at 10 rpm at a temperature of 37°C ± 0.5°C. The viscosity values of cream formulations F1-F7 are depicted in Table 4.

Table 4: Viscosity of cream formulations

Formulation	Viscosity [centipoise]
F1	11,140
F2	9,250
F3	10,340
F4	14,140
F5	15,200
F6	5,450
F7	3,510

Antioxidant activity of *F. Leucopyrus* herbal extract cream formulations:

In Table 5, it showed that the radical scavenging activity in terms of the percentage of reduction of initial radical absorbance by the cream formulations at different concentrations (20, 40, 100 µL/mL)

EC50 of Ascorbic acid as a positive control was detected at 2.588 µg/mL. One blank formulation without any *F.*

Leucopyrus herbal extract was detected with devoid of antioxidant activity.

F. Leucopyrus herbal extract containing seven formulations F1-F7 expressed the existence of antioxidant activity as shown in Table 5. According to the results of antioxidant activity, it was concluded that the antioxidant properties were solely caused by *F. Leucopyrus* herbal extract which was added to the cream base.

**Table 5: % Scavenging activity of F1-F7 cream formulations
(mean $\pm$ SD, n=5 where n is a number of replications)**

Concentration μ L/mL	% Scavenging activity						
	F1	F2	F3	F4	F5	F6	F7
20	24.08 $\pm$ 0.88	24.10 $\pm$ 0.52	24.11 $\pm$ 0.22	24.12 $\pm$ 0.46	24.14 $\pm$ 0.30	24.15 $\pm$ 0.28	24.18 $\pm$ 0.42
40	48.28 $\pm$ 0.13	48.24 $\pm$ 0.28	48.25 $\pm$ 0.1	48.28 $\pm$ 0.13	48.30 $\pm$ 0.13	48.32 $\pm$ 0.13	48.34 $\pm$ 0.13
100	82.20 $\pm$ 0.53	82.21 $\pm$ 0.36	82.22 $\pm$ 0.72	82.24 $\pm$ 0.18	82.25 $\pm$ 0.28	82.26 $\pm$ 0.42	82.28 $\pm$ 0.55

Conclusion: It was concluded based on the result demonstrated that *F. Leucopyrus* herbal extract was loaded into cream formulations containing Cetyl alcohol & Triethanolamine. All seven formulations F1 to F7 exhibit acceptable value of physicochemical Properties. All seven cream formulations F1-F7 possess sufficiently good antioxidant activity, which can be regarded as an efficient and economical herbal cream product.

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