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Formulation Development, Evaluation and Anti-fungal Activity of Poly-Herbal Cream

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Abstract

The present research has been undertaken with the aim to formulate and evaluate the herbal cream containing ethanolic flower extract of *Blumea lacera* (Burm.f.) DC., *Leucas cephalotes* Roxb. and *Peristrophe bicalyculata* (L) Merr. The poly herbal cream was prepared using ethanolic flower extract in varied concentrations and was evaluated using physiological measurements. The polyherbal cream was prepared by using bees wax, paraffin wax, olive oil, soya powder, tocopherol, glycerine, rose water, peppermint oil and sandalwood oil and required amount of distilled water. Then skin pH (6.8-7) was maintained by drop wise addition of tri-ethanolamine. The physiochemical parameters of formulations (pH, viscosity, spreadability etc.) were determined. The results showed that formulation PHC-III have maximum drug content and drug release. Further PHC-III was screened for anti-fungal activity against *Candida albicans, Cryptococcus neoformans* and *Aspergillus flavus*. The zone of inhibition was recorded and anti-fungal activities were determined and results were compared with standard marketed herbal cream.

Key words: Polyherbal Cream, Plant Extract, Anti-fungal activity

Introduction

The use of medicinal plants as raw materials in the production of new drugs is ever increasing because of their potentials in combating the problem of drug resistance in micro-organisms. Demand for medicinal plants is increasing in both developing and developed countries. Research on medicinal plants is one of the leading areas of research globally . [1-2]

Blumea lacera is an annual herbaceous plant belonging to the Asteraceae family. It has a wide variety of compounds that have great potential for medicinal use. Numerous beneficial phytochemical components are present in it, such as lupeol and lupeol acetate, hentriacontane, ß-sitosterol, acetates, alpha-amyrin, and stigmasterol. Numerous pharmacological characteristics, including astringent, hepatoprotective, anthelmintic, diuretic, antipyretic, anti-inflammatory, antimicrobial, cytotoxic, anxiolytic, antiviral, analgesic, hypothermic, anti-bacterial, anti-leukemic, and tranquillizing effects, are displayed by these phytochemicals. [3] *Leucas cephalotes* (Family: Lamiaceae) also known as 'Dronapushpi' in Sanskrit, is an edible rainy season weed. In Ayurveda, it has been recommended for inflammation, psoriasis, scabies, chronic skin eruptions, edema, diaphoresis, chronic malaria, asthma, eye diseases, jaundice, paralysis and obstinate urinary troubles. The plant possess labellenic acid and beta-Sitosterol, stigmasterol, flavones pillion, gonzalitosin, ricin, cosmosin, apigenin, anisofolin A and Luteolin as a major phytochemicals. [4] *Peristrophe bicalyculata,* a member of the Acanthaceae family, can grow to a height of 60 to 180 cm and is widespread in Africa, Afghanistan, and India. It is frequently referred to as kakajangha in Sanskrit and kali aghedi in Hindi. The herb is used to cure ear and eye infections, bone fractures, sprains, fevers, colds, coughs, and snake poison. It also has antibacterial (tuberculostatic) properties. The dried aerial components' chemical makeup revealed 35-hydroxynonatriacontanal and 14-methyl-tritriacont-14-en-15-ol. [5]

During past few years plant derived extracts and their isolated phytochemicals are gaining importance and are also a new emerging area of research. In last two decades anti-fungal effects in the category of anti-microbial is of great interest. The present study was designed to evaluate formulate and evaluate polyherbal cream prepared from extract of these plants.

Material and Methods

Selection and Collection of Plant Material

Three plants i.e., *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers, *Leucas cephalotes* Roxb. (Gumma) Flowers and *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers were collected from Vindhya Region on Madhya Pradesh and was selected f for the present study.

Authentication of Plant/Plant Material

The plant parts viz., BLF= *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers, LCF= *Leucas cephalotes* Roxb. (Gumma) Flowers and PBF= *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers were collected and identified morphologically, microscopically and compared with standard pharmacopoeial monograph. The sample of drug was also identified & authenticated by Dr. S. N. Dwivedi, Retd. Prof. and Head, Department of Botany, Janta PG College, A.P.S. University, Rewa, (M.P.) Voucher Specimen J/Bot/BLF-019; LCF-020; PBF-021 was allotted.

Successive extraction of selected herbs [6]

Sample were shattered and screened with 40 mesh. The shade dried coarsely powdered plant matertial (250 gms) were loaded in Soxhlet apparatus and was extracted with petroleum ether (60-62°C), Chloroform, ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined.

Formulation of Polyherbal Cream

Bees wax is first melted at 60 to 70 degrees Celsius, followed by the addition of paraffin wax, soya powder, olive oil, and tocopherol. This creates a water-in-oil emulsion cream. Aqueous phase and extract were added, and they were heated to 50°C. Glycerine and rose water were then added, and the mixture was cooled to 40°C. For homogeneous dispersion, the two phases were

continually combined. After being slowly cooled, peppermint and sandalwood oils were added for scent. [7]

Preparation of Oil Phase

In accordance with tables 1, the appropriate amounts of beeswax, paraffin wax, and olive oil were taken and melted at 70°C in a porcelain dish.

Preparation of Aqueous Phase

Glycerine, soy powder, water and the ethanolic extract were added to another clean dish and heated to 70°C.

Addition of Aqueous Phase to Oil Phase

At room temperature, the aqueous phase was continuously stirred into the oil phase. The last ingredients were rose water, peppermint oil, and sandalwood oil. The mixture was then transferred into an appropriate container.

Table 1: Composition of Polyherbal Cream containing Ethanolic Flower Extract

Ingredients	Control (Placebo) (CP)	Ethanolic Flower Extract		
		PHC-I	PHC-II	PHC-III
Bees Wax (gm)	5	5	5	5
Paraffin wax (gm)	2	2	2	2
Olive Oil (ml)	10	10	10	10
Soya Powder (gm)	1.5	1.5	1.5	1.5
Tocopherol (ml)	0.5	0.5	0.5	0.5
Extract (%) EEBLF EELCF EEPBF	-	2.5	5	10
Glycerine (ml)	2.5	2.5	2.5	2.5
Rose water(ml)	2	2	2	2
Peppermint oil	1	1	1	1
Sandalwood oil	0.5	0.5	0.5	0.5
Aqueous Phase (ml)	qs	qs	qs	qs

Evaluation Parameters of Herbal Cream [7]

Physical Evaluation

Clarity and transparency of the polyherbal cream were assessed visually as part of its physical test. The samples were examined against a white background in the light.

Determination of pH

The pH metre was calibrated initially, and a reading of zero was noted. The samples were placed in a beaker after being diluted with water to produce a suspension, and measurements were obtained using a calibrated electrode. Three average readings were taken after repeating the technique.

Determination of Viscosity

Utilising spindle no. 01 at 20 rpm and temperatures between 4 and 37 degrees Celsius, the viscosity of the herbal cream was measured using a Brookfield viscometer. A beaker was filled with 15 ml of samples from each batch, and the spindle was then submerged in the formulation.

The reading was taken at different temperatures both before and after rotation. The reading was three times recorded.

Determination of Homogeneity

By visually inspecting each batch of manufactured herbal cream, homogeneity was verified and the existence of any aggregates in the formulation was assessed.

Determination of Spreadibility

All of the prepared herbal cream's spreadability was evaluated. The glass slide with the recipes on it was topped with another glass slide that was empty and contained no gel. The formula was set up so that it was sandwiched between two slides. The slides were found to be occupied at a distance of 7.5 cm. The herbal cream was pressed into a thin, even coating and placed between two slides. The herbal cream was freed from its weight. It was removed the extra herbal cream seen on the slides. The two slides were fastened together, and 20 0.5 g of weight were connected to the upper glass slide. The time it took to cover the 7.5 cm separation distance was recorded as both slides being separated due to weight. The mean time was calculated after the three readings were obtained. Spreadability was determined as follows:

S=mxl/t: Where, l: length of the slide (7.5 cm), m: the weight which is tied to slides, and t: time taken in second.

Determination of Wetness

Applying the prepared herbal cream to the skin's surface helped evaluate how moist it was. The quantity of cream extracted from each batch was enough to cover a 10 mm2 area of skin surface.

Determination of type of Smear

The produced herbal cream was applied to the skin's surface, and the type of film or smear generated on the skin was noted. The amount of cream collected from each batch was enough to cover a 10 mm2 area of skin surface.

Determination of Emolliency

We evaluated the herbal cream's emolliency, slipperiness, and amount of residue that remained after application. The quantity of cream taken from each batch was enough to cover a surface area of the skin 10 mm2 in size.

Determination of type of Emulsion

Dilution Test

Depending on the type of emulsion, either o/w or w/o, the created herbal cream was diluted with oil or water, and the results were recorded.

Dye Solubility Test

The resulting herbal cream was combined with an amaranth-based water soluble dye and examined under a microscope. The collected results were interpreted.

Determination of Drug Content

The herbal cream's composition was calculated using a UV-Visible spectrophotometer. The dosage was roughly 1g of the formulation in 50 ml of volumetric flask. The right amount of methanol was added to the solution. Whatman filter paper Grade 1 was used to filter the mixture after it had been shaken. The filterate was further diluted to a volume of 10 ml with solvent before being estimated at the appropriate wavelength. The standard curve created at the maximum wavelength, or 510 nm, was used to assess the amount of medication present in the extract.

***In Vitro* Drug Release**

The herbal cream was put into the semi-permeable dialysis membrane bag (7 cm long), which had been produced. Ten dialysis bags were suspended in 50ml of a 1: ethanol: water mixture with a temperature of 37°C plus or minus 0.5 °C. At predetermined intervals (2, 4, 6 and 8 hours), about 1ml of sample was removed from the membrane and a fresh equivalent volume was concurrently reintroduced. The samples were taken out, diluted, and subjected to UV Visible spectrophotometer analysis at the appropriate maximum times, i.e., 2, 4, 6, and 8 hours, for the polyherbal cream containing extract. The experiment was carried out three times, and the readings were used to compute the total amount of drug release.

Anti-fungal of Polyherbal Cream [8-10]

Fungal strain

Fungal strain i.e., *Candida albicans, Cryptococcus neoformans* and *Aspergillus flavus* were used for the present investigation. The innoculum of strains were transferred to the recultured before staring the lab work.

Screening of Anti-fungal activity (Disc diffusion method)

Preparation of Disc

Disc of whatsmann filter paper of one quarter inch in diameter was prepared and the same was sterilized using autoclave.

Preparation of samples entrapped disc

The accurately weighed polyherbal cream were dissolved in ethanol of different stock solutions (10, 20, 30, 40, 50 µg/ml**)** solutions were prepared. All the dilution prepared was applied to whatsmann filter paper disc using a micropipette. The disc were then dried and sterilized.

Preparation of culture plate

The sabouraund's agar and mueller Hinton agar media were prepared by dissolving media in 1000 ml of distilled water and sterilized by autoclave at 121^{o}C for 1 hour. The media were cooled and poured in sterilized petri plate to solidified at room temperature.

Evaluation of Zone of inhibition

The re-cultured fungal strains were used for antifungal evaluation. The strains were streak on the Mueller Hinton media and the drug entrapped patches were placed. For negative control disc of distilled water and for positive control marketed formulation (Clotrimazole cream) (10 µg) were used. The petri plates were kept in incubator for 24 hrs. After 24 hrs the petri-plates were checked for zone of inhibition. The zone of inhibition diameter was recorded with the help of zone reader scale. The zone of inhibition was calculated by subtracting diameter of sample or standard or control by diameter of disc. The more the zone of inhibition the more will be antifungal activity.

Results and Discussion

No any defects were noted in any of the formulated batches of polyherbal cream. The formulated polyherbal cream was evaluated as per standard methods and the results were mentioned in table 2. The drug content of polyherbal creams using ethanolic flower extract of *Blumea lacera* (Burm.f.) DC., *Leucas cephalotes* Roxb. and *Peristrophe bicalyculata* (L) Merr. and various excipient ratios was assessed. According to the findings (tables 2, when compared to all formulated polyherbal cream, the drug content and drug release was found to be at its highest in PHF-III

Table 3: Evaluation parameters of Polyherbal Cream containing Ethanolic Flower Extract

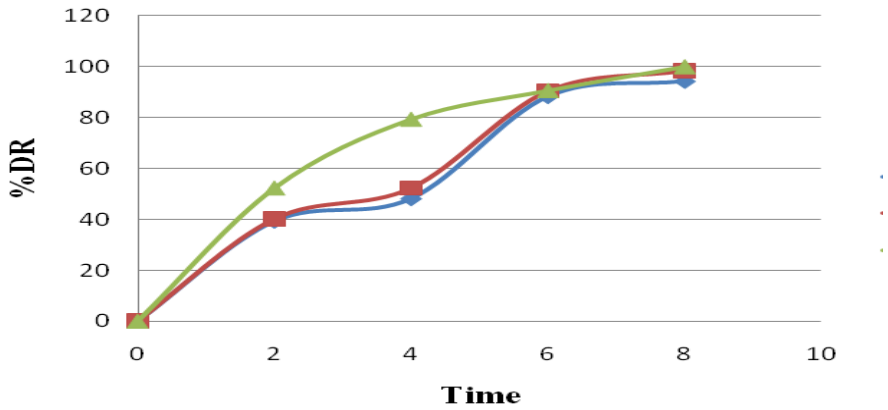
S/No.	Parameters	CP	PHC-I	PHC-II	PHC-III

1.	Appearance	Clear	Clear	Clear	Clear
2.	pH	6.7	6.9	7.0	7.0
3.	Viscosity(cp)	3844	4412	4434	4626
4.	Homogeneity	H	H	H	H
5.	Spreadibility (gcm/sec)	48.39	50.28	56.36	58.12
6.	Wetness	++	++	+++	+++
7.	Type of Smear	NG	NG	NG	NG
8.	Emolliency	NRL	NRL	NRL	NRL
9.	Type of Emulsion	o/w	o/w	o/w	o/w
10.	Drug Content	-	80.24	88.40	95.38

Note: H=Homogeneous, +=Good, ++=Better, +++=Best, G=Greasy, NG= Non-greasy, NRL=No residue left, o/w=oil in water

Table 3: *In-Vitro* Drug Release of Polyherbal Cream containing Ethanolic Flower Extract

Time (Hrs)	% Drug Release		
	PHC-I	PHC-II	PHC-III
0	0	0	0
2	39.23	40.11	52.25
4	48.15	52.20	79.27
6	87.22	89.20	90.54
8	95.29	97.23	98. 85



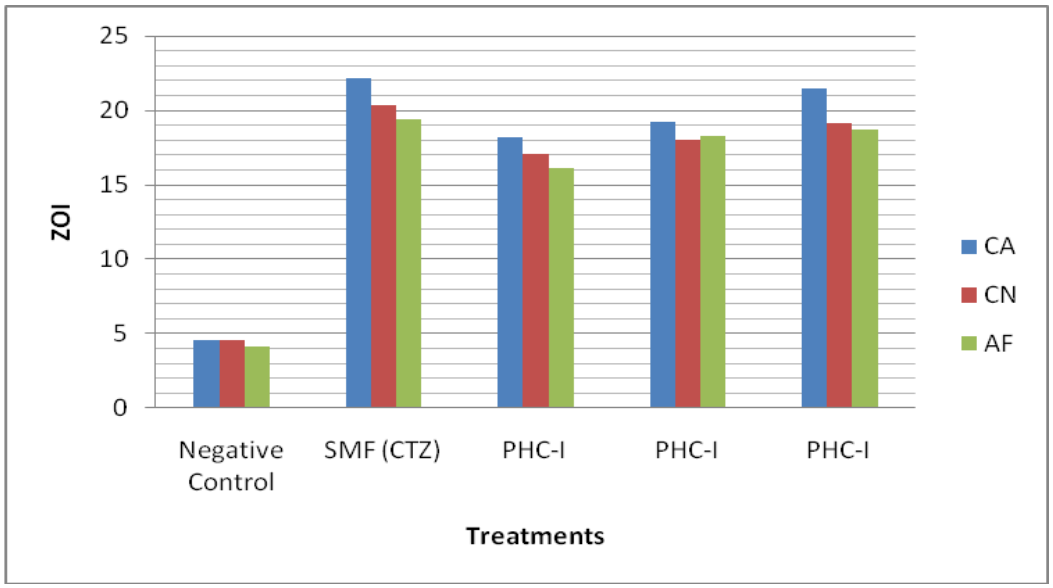
Graph 5.10: % Drug release of polyherbal cream

Anti-fungal activity of polyherbal cream containing flowers extract of *Blumea lacera* (Burm.f.) DC., *Leucas cephalotes* Roxb. and *Peristrophe bicalyculata* (L) Merr. were evaluated. The zone of inhibition of PHC-I, PHC-II & PHC-III on *Candida albicans, Cryptococcus neoformans* and *Aspergillus flavus* were presented in table 3. Results indicate that PHC-III have significant anti-fungal activity when compared with standard marketed formulation (Clotrimazole cream).

Table 4: Anti-fungal activity of Polyherbal Cream containing Ethanolic Flower Extract

S/No.	Treatments	Zone of Inhibition (mm)		
		CA	CN	AF
1.	Negative Control	4.61±0.11	4.54±0.07	4.12±0.10
2.	SMF (CTZ)	22.14±0.16**	20.38±0.11**	19.38±0.04**
3.	PHC-I	18.21±0.21*	17.11±0.03*	16.16±0.06*
4.	PHC-I	19.20±0.01**	18.02±0.08**	18.27±0.12**
5.	PHC-I	21.43±0.11**	19.11±0.11**	18.72±0.07**

Note: All values are expressed as Mean (X) ±SEM, (n=3). One way ANOVA followed by student test, values are statistically significance *P<0.001, **P<0.01 when compared with control and standard.



Graph 2: Anti-fungal activity of Polyherbal Cream

Conclusion

Natural and herbal-based medications have the ability to heal a variety of fungal-caused skin problems, including many indigenous people who depend on the nation's medicinal plants for treatment. Based on traditional knowledge that adopts a holistic and systemic approach, several scientists have concentrated on different parts of specific medicinal plants utilising different approaches to cure skin-related disorders. In the future, a variety of fascinating medicinal plants that are used in many nations—either alone or in combination with manufactured medications—may be helpful for treating skin disorders and perhaps even serving as sources of maintaining overall health. The phytoconstituents in the powerful plants demonstrated biological action.

The polyherbal cream containing ethanolic flower extract of *Blumea lacera* (Burm.f.) DC., *Leucas cephalotes* Roxb. and *Peristrophe bicalyculata* (L) Merr. Were formulated and evaluated and the results found were satisfactory and upto the marks. Out of three formulated polyherbal

cream, PHC-III were most prominent. Further anti-fungal activity using three fungal strains—*Candida albicans, Cryptococcus neoformans,* and *Aspergillus flavus*—were tested of polyherbal cream and standard marketed formulation (SMF-Clotriamazole (CTZ). When compared to a typical medication, the results show that PHC-III have strong anti-fungal activity. To produce new phyto-formulations, more research is necessary to identify the molecules causing the biological activity that has been seen.

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