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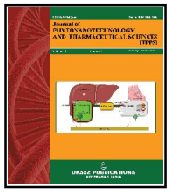

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Antimicrobial polyherbal cream and serum

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Abstract

The topical infectious with antimicrobial resistances compels for herbal drug discovery and development. Thus, this study aimed to formulate and evaluate herbal creams and serums from ethanolic extracts of papaya leaf, moringa leaf, pashanbhed, orange peel, and neem leaf for topical therapies. Formulated herbal creams and serums with ethanolic extracts of papaya leaf, moringa leaf, orange peel, pashanbedh and neem leaf of 1% w/v, respectively, were prepared. Physicochemical properties and *in vitro* antimicrobial activities of the cream and serums were evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. All the cream and serum formulations prepared showed acceptable pH, globule size, viscosity, and organoleptic properties. They were also non-irritant to the skin, and for up to three weeks. There was no microbiological growth, all the formulations were found to be stable at room temperature. The results of the cream showed significant inhibitory zones against *E. coli* and *S. aureus*. Findings from the cream and serum formulations suggest that polyherbal formulations could be used as antimicrobial skin care products that promote natural and holistic skincare. Antimicrobial results from the current study provide an evidence-based overview of polyherbal cosmetic formulations and lay the basis for potential future dermatological and cosmetic beneficial use.

1. Introduction

Plants have always been the main source for active cosmetic ingredients, having proven health beneficial effects on human, such as antiaging, antioxidant, anti-inflammatory, UV protective, anticancer, antiwrinkle, skin soothing, whitening, moisturizing, *etc*. (Georgiev *et al.,* 2018). Extracts from herbal, aromatic and/or medicinal plants have been widely used as effective active ingredients in cosmeceuticals or nutricosmetics, especially in products for topical application and skincare formulations. However, over the past decade, there has been an increasing interest to plant cell culture - derived active cosmetic ingredients. These are "new generation" of high-quality natural products, produced by the modern plan biotechnology methods, which usually showed stronger activities than the plant extracts obtained by classical methods (Georgiev *et al.,* 2018). In this article, the advantages, and the current progress in plant cell culture technology to produce active cosmetic ingredients have been summarized and discussed in detail within a presented case study for calendula stem cell product development (Barbulova *et al.,* 2015).

As per the Ayurvedic pharmacy, any polyherbal formulations restrain two or more than two herbs. The idea of polyherbal is peculiar to Ayurveda even though it is tricky to explain in terms of modern parameters. The Ayurvedic, literature Sarang Dhar Samhita tinted the idea of polyherbal to attain greater therapeutic efficacy. Herbal formulation has been used all around the earth due to its medicinal and therapeutic application. It is also recognized as polyherbal therapy or herb-herb combination (Karole *et al.,* 2019). The active phytochemical constituents of individual plants are inadequate to attain the desirable therapeutic effects. When polyherbal and herbo-mineral formulations combine multiple herbs in a meticulous ratio, it will give an enhanced therapeutic effect and decrease toxicity. The active constituents used from individual plants are inadequate to provide attractive pharmacological action. There is evidence that crude plant extracts often have greater potency rather than isolated constituents. In traditional medicine, whole plants or mixtures of plants are used rather than isolated compounds (Sharma *et al.,* 2022). Herbal creams are products that are used to improve moisturizing, nourishing, whitening, and treating various diseases (Gade *et al.,* 2015).

Serum offers quick absorption and the capacity to penetrate deep layers of the skin, as well as a non-oily finish and a deep formula with a high concentration of active ingredients (Yeskar *et al.,* 2023; Kalayan *et al.,* 2023; Singh *et al.,* 2020).

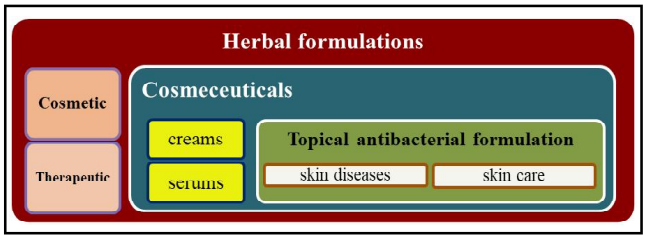

Figure 1: Different types of herbal formulations.

Herbs like papaya leaf demonstrate antioxidant, anti-inflammatory, and antimicrobial properties that may help improve the performance of the formulations (Singh *et al.,* 2020; Gajalakshmi *et al.,* 2004). Moringa leaf, which is packed with vitamins and has anti-inflammatory, antibacterial, and skin protecting qualities, may also have advantages for skin stabilization and protection. Wound healing

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and antimicrobial effects may be improved with neem leaf powder. As neem leaf powder has typical antibacterial, antifungal, and wound healing abilities (Gajalakshmi *et al.,* 2004; Maizuwo *et al.,* 2017), it will synergistically act and enhance the antimicrobial and wound healing activities in the formulations. Orange peel powder has antimicrobial, anti-inflammatory and antioxidant properties that protect, and rejuvenate the skin (Maizuwo *et al.,* 2017; Nazir *et al.,* 2022). Pashanbhed powder possesses diuretic, anti-inflammatory and antimicrobial properties that are used in the formulation and will have a similar action (Nazir Anum *et al.,* 2022; Sulaiman *et al.,* 2022). Novel polyherbal cream and polyherbal serum formulated with the ethanolic extract of use papaya leaf powder, moringa leaf powder, neem leaf powder, orange peel powder, and pashanbhed powder could be a potential therapeutic approach for skincare and wound healing. These botanicals have a vast traditional medicinal system for curing several ailments. However, scientific research must be done to improve the formulation, and therapeutic dose and make it safer to handle.

There is a need for independent clinical trials to confirm the efficacy of polyherbal combinations on wound healing and skincare. Moreover, quality control methods need to be established to help in the production of quality products. Overall, the formulation of polyherbal cream and polyherbal serum incorporating these botanical ingredients holds great promise in offering synergistic therapeutic effects, minimal side effects, and natural alternatives for skincare and wound healing. Continued research and development in this field will contribute to the advancement of herbal medicine and provide valuable options for individuals seeking safe and effective skincare solutions.

2. Materials and Methods

2.1 Procurement of raw material and preparation of herbal extract

The papaya leaf powder, neem leaf powder, orange peel powder, moringa leaf powder and pashanbhed powder were procured from the market. Each of these powders (2 g) is separately extracted using a maceration process with 20 ml of ethanol, dried at room temperature, and stored in a desiccator to obtain the dried ethanolic extract of each drug (Sulaiman *et al.,* 2022).

2.2 Formulation development

For the making of base cream and serum, to ensure the desired consistency, stability, and efficacy of the final product, a selection of ingredients is essential. The base cream formulation consists of both aqueous and oil phases, each containing specific ingredients tailored to their functions and properties listed in Table 1. The preparation of the oil phase involves dissolving stearic acid, cetyl alcohol, and liquid paraffin together, forming a homogeneous mixture. This mixture is then placed in a beaker within a water bath set to 75^{0}C. Subsequently, the aqueous phase, containing glycerine, methylparaben, and triethanolamine, is heated separately within the same water bath. Once both phases reach the desired temperature, the aqueous phase is added gradually to the oil phase with continuous stirring. This process allows for the formation of a stable emulsion as the two phases combine (Cubliffe, 1989; Goulden *et al.,*1997; Payal *et al.,*2020).

The formulation of the serum base, as per Table 2, is prepared by mixing the oil phase components for 10 min to ensure uniformity. Simultaneously, the aqueous phase is prepared by mixing flax seeds gel, glycerine, and a small amount of distilled water uniformly. The oil phase is then added dropwise to the aqueous phase under continuous stirring to create an oil-in-water-based emulsion (Maria *et al.,*2016; Gillbro and Olsson, 2011).

Once the base was ready the different formulations of cream and serum were prepared by blending the base with the extracts in Tables 3 and 4 to prepare different cream formulations like C1, C2, C3, and C4 and different serum formulations like S1, S2, S3, and S4 which were further evaluated for their physicochemical properties as well as antibacterial activity stability and other tests.

Table 1: Formulation of base cream

Aqueous phase

Ingredients	Quantity	Role
Glycerine	5 g	Humectant
Methylparaben	0.05 g	Preservative
Tri ethanol amine	0.05 g	Emulsifier, pH adjuster
Distilled water	Up to 100 ml	Aqueous phase

Oil phase

Ingredients	Quantity	Role
Stearic acid	10 g	Stabilizer
Cethyl alcohol	4 g	Emulsifier, thickener
Liquid paraffin	4 g	Emollient

Table 2: Formulation of serum base

Aqueous phase

Ingredients	Quantity	Role
Flax seeds gel	50 ml	Humectant, thickner

Oil phase

Ingredients	Quantity	Role
Almond oil	0.1 ml	Emollient
Tween	1 ml	Emulsifier
Span	2 ml	Emulsifier
Methyl paraben	0.01 g	Preservative

Table 3: Polyherbal cream formulations

	C-1	C-2	C-3	C-4
Papaya extract	0.1 g	0.1 g	-	-
Orange peel	0.1 g	0.1 g	-	-
Moringa	0.1 g	-	0.1 g	-
Neem	0.1 g	-	0.1 g	-
Pashanbhed	0.1 g	-	-	0.1 g
Base serum	9.5 g	9.8 g	9.8 g	-
Total quantity	10 g	10 g	10 g	10 g

Table 4: Polyherbal serum formulations

	S-1	S-2	S-3	S-4
Papaya extract	0.1 g	0.1 g	-	-
Orange peel	0.1 g	0.1 g	-	-
Moringa	0.1 g	-	0.1 g	-
Neem	0.1 g	-	0.1 g	-
Pashanbhed	0.1 g	-	-	0.1 g
Base serum	19.5 ml	19.8 ml	19.8 ml	19.9 ml
Total quantity	20ml	20 ml	20 ml	20 l

2.3 Physicochemical characterization of formulations

Formulation evaluation tests for herbal emulsion typically involve assessing various aspects of the emulsion's physical and chemical properties to ensure its quality, stability, and effectiveness (Drallos and thaman, 2006; Leveque and Agache; Wikipedia contributor: Olive oil; Srivastava *et al.,* 2014; Moneruzzaman *et al.,* 2015; Nadkarni, 1993).

The commonly performed physiochemical tests are appearance, odour, pH, and spreadability, consistency, homogeneity, globule size of herbal cream/ serum formulations have been performed as per the previous published research work (Estanqueiro *et al.,* 2014).

2.3.1 Rheological properties

The viscosity of the formulations was determined by a Brook-filed viscometer. The viscosity measurements were done using Brook-**filed DV-a!+Pro**.Viscometer using spindle no-64. The developed formulation was poured into the adaptor of the viscometer and angular velocity increased gradually from 100 to 200 rpm.

2.3.2 Microbiological testing

This method ensures the prepared formulations are free from harmful microorganisms. Here, the formulations are spread to the solidified nutrient agar in a sterile petriplate and incubated for 24 h at 45 h.

2.3.3 Skin irritation testing

Conduct patch tests on human volunteers to evaluate the cream's potential for skin irritation or sensitization. Mark an area on the left-hand dorsal surface the creams and serums were applied to the specified area and time was noted. Irritancy, erythema, and oedema were checked at regular intervals up to 24 h and reported.

2.3.4 Antibacterial activity

The well diffusion method was employed for determining the zone of inhibition of the prepared formulations against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus* microbes.

3. Results

The drugs prepared buy extraction for the formulation of cream and serum are collected in petri plates delivered nearly 5%w/w of dried papaya leaf extract (i) moringa leaf extract (ii) neem leaf extract (iii) and (iv) pashanbhed extract. These extracts were used for the preparation of four cream formulations and four serums, *viz.,* C1, C2, C3, C4, S1, S2, S3, and S4. The eight formulations showed physiochemical properties as described in Table 5. All the cream and serum formulations prepared showed acceptable organoleptic properties, pH and globule size, and viscosity, and were found non-irritant to the skin. There was no microbial growth observed in all the formulations up to three weeks as per Table 7, hence showing good stability at room temperature.

The zone of inhibition for antimicrobial activity for the formulations are reported in Table 8 the zone of inhibition measured in millimetres indicates the extent to which the formulations inhibit the growth of various microbial strains.

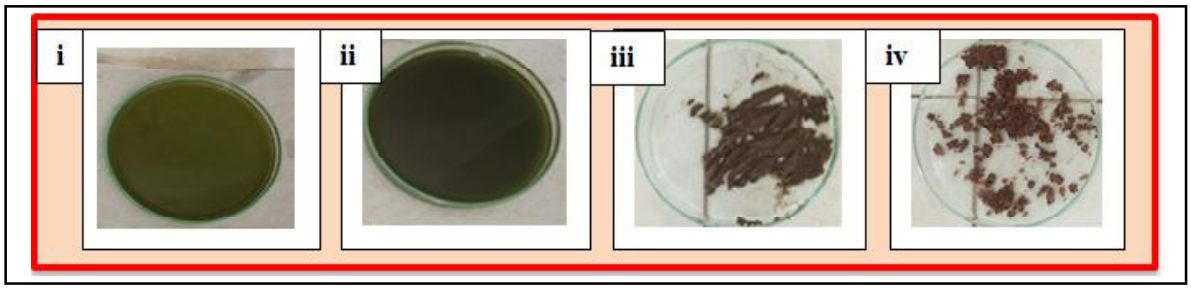

Figure 1: Dried ethanolic extracts of (i) papaya leaf (ii) moringa leaf (iii) neem leaf (iv) pashanbhed.

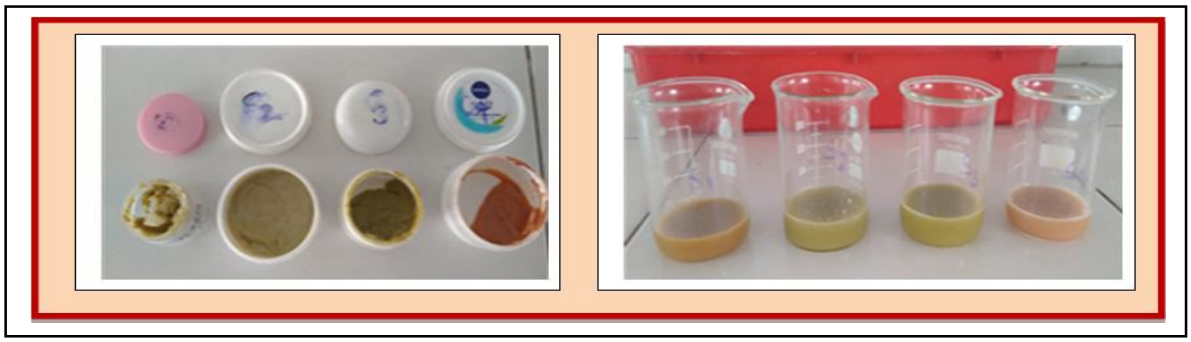

Figure 2: Prepared creams C1,C2,C3 and C4 and serums S1, S2, S3 and S4.

Table 5: Physiochemical evaluation of prepared formulations

	Cream				Serum			
	C-1	C-2	C-3	C-4	S-1	S-1	S-3	S-4
Colour	Green	Creamish green	Light green	Peach	Greenish brown	Green	Light green	Peach
Odour	Pleasant smell	Pleasant smell	Pungent smell	Pleasant smell	Pleasant smell	Pleasant smell	Pleasant smell	Pleasant smell
Texture	Smooth, thick, easily absorbed	Smooth, thick, easily absorbed	Smooth, thick,easily absorbed	Smooth, thick, easily absorbed	Easily absorbed thick oily.	Easily absorbed thick oily.	Easily absorbed thick oily.	Easily absorbed thick oily.
Irritation test	No redness, irritation, edema.	No redness, irritation, edema.	No redness, irritation, edema.	No redness, irritation, edema.	No redness, irritation, edema.	No redness, irritation, edema.	No redness, irritation, edema.	No redness, irritation, edema.
Emulsion	o/w	o/w	o/w	o/w	o/w	o/w	o/w	o/w
pH	6.5	6	6-7	6	8	7.5	6.5	6
Globule size (µm)	0.1 - 2.01	0.1 - 2.1	0.4 - 3.6	0.1 - 1.1	0.001 - 0.2	0.001- 1.11	0.06 -0.1	0.003 -0.9

Table 6: Rheological properties of base cream

Rpm	Temperature	Cp	%t
20	33.0^{0}C	3359	11.5
30	33.1^{0}C	3439	17.2
50	33.2^{0}C	2256	18.6
60	33.2^{0}C	2280	22.5
100	34.7^{0}C	1488	24.8

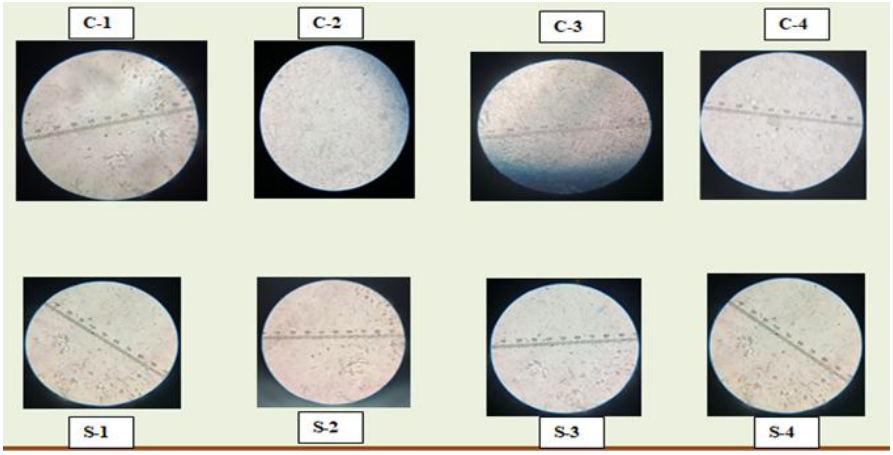

Figure 3: Globule size determination of the prepared formulations.

Table 7: Observations of microbial test preformed on the prepared formulations

Formulation	Microbial growth		
	1st Week	2nd Week	3rd Week
C1	None	Not observed	Not observed
C2	None	Not observed	Not observed
C3	None	Not observed	Not observed
C4	None	Not observed	Not observed
S1	None	Not observed	Not observed
S2	None	Not observed	Not observed
S3	None	Not observed	Not observed
S4	None	Not observed	Not observed

Table 8: Antimicrobial activity of the formulations (Zone of inhibition in mm)

	Zone of inhibition (mm) of the 1%w/v cream					Zone of inhibition (mm) of the 1%w/v serums				
Microbes	**C_1**	**C_2**	**C_3**	**C_4**	**C_5**	**S_1**	**S_2**	**S_3**	**S_4**	**S_5**
E. coli	15	-	-	-	20	-	-	-	7	-
Ps. aeruginosa	-	-	-	-	-	-	-	9	7	-
Bacillus subtilis	11	11	-	7	15	7	-	-	-	-
S. aureus	11	-	11	13	15	7	9	-	11	-

4. Discussion

The increasing demand for antimicrobial tropical formulations due to propelling resistant microbial strains there is a huge opportunity to establish herbal formulations showing synergistic activity. Since the cream and serum are easy to apply, cost-effective and have good stability are preferred for preparing various antimicrobial topical formulations. In this study, the antimicrobial activity highlights the potential of polyherbal cosmetic formulations in combating pathogenic microorganisms. Both the creams and serums demonstrated varying degrees of effectiveness against the tested microbes. The creams showed promising results with significant inhibition zones observed against *E. coli* and *Staphylococcus aureus* these findings suggest that Poly herbal formulations have the potential to serve as effective skin care products laying the foundation for further studies and potential applications in skin care and dermatology.

5. Conclusion

Human skin needs to be protected from microbes causing skin infections and diseases. Herbal drugs are the potential focus for drug discovery and development in this area. This study focuses on the preparation and evaluation of formulated herbal cream and serum from ethanolic extracts of the above-mentioned drugs indicating the capability and potentiality of the plant towards the discovery of the antibacterial agent that could be used in the treatment of skin infections caused by bacteria used in our study. The ethanolic extracts of papaya, moringa, neem, orange peel, and pashanbhed have been studied for their antioxidant, anti-inflammatory, and antimicrobial activities, which can contribute to the overall efficacy of the formulations. The usefulness of creams was significantly pronounced due to the synergistic effect of bioactive compounds, which are predominantly flavonoids, terpenes, tannins and terpenoids. Therefore, this study has substantiated the ability of drug extract to serve as a natural resource from which antimicrobial creams or serums could potentially be discovered and applied to combat skin infections caused by microbes. The formulation of herbal cream and serum incorporating these botanical ingredients holds great promise in offering synergistic therapeutic effects, minimal side effects, and natural alternatives for skincare applications.

Conflict of interest

The authors declare no conflict of interest relevant to this article.

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