



Formulation and Evaluation of Polyherbal Gels for Dermatological Disorders

Vaibhav Agarwal¹, Ashutosh Badola^{1*}

¹Department of Pharmaceutics, School of Pharmaceutical Sciences, SGRR University
Patel Nagar-248001, Dehradun, Uttarakhand, India,

*Corresponding authors: Dr. Ashutosh Badola

E-mail: ashutoshbadolampharma@gmail.com

ABSTRACT

The present study was designed to formulate and evaluate polyherbal gel containing extracts of *Berberis aquifolium*, *Curcuma longa*, *Echinacea angustifolia*, and *Thuja occidentalis* for the treatment of dermatological disorders. The prepared formulations were evaluated for appearance and homogeneity, pH, viscosity and rheological studies, spreadability, skin irritation test (patch test), and washability. The formulations were also screened for antimicrobial activity by disc plate method against *B. cereus*, *E. gergoviae*, *S. flexneri*, *S. aureus*, *B. subtilis*, *A. niger*, *S. epidermidis*, and *E. coli*, which are representative types of Gram-positive and Gram-negative organisms. The results of the study revealed that all formulations under study viz. 17 mm, 18 mm, 18mm, 24 mm, and 21 mm showed better zones of inhibition as compared with the control. However, formulation F2 exhibited maximum activity against the selected strains which may be attributed to its greater amount of herbal extracts compared to formulations F1, F3, F4, F5 and F6. The polyherbal gel formulations were observed to possess antimicrobial action. The effective activity may be attributed to the synergistic action of the plant constituents present in the formulation. Based on our research, it could be concluded that these formulations possess antimicrobial activity and can be used safely on human skin.

Keywords: *T. occidentalis*, *C. longa*, Carbopol 940, polyherbal gel and acne.

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INTRODUCTION

More than 80% of the world's population still greatly depends on traditional medicines for the treatment of various skin diseases. Traditional medicines play an important role in health services around the globe. Medicinal plants represent a rich source of potent and powerful drugs. The treatment of human and animal diseases depends mainly on natural products derived from plants, animals, microorganisms, and minerals [1]. There are large numbers of medicinal plants which are widely used in the treatment of skin diseases and are also known to possess antimicrobial activity [2]. Plants comprise primary and secondary metabolites, which show and play various therapeutic functions. The primary and secondary metabolites are used as pressure and deterrents. Its miles are referred to as plant herbal products [3]. Dermatological disorders include common skin rashes to severe skin infections, which occur due to a range of things, such as infections, heat, allergens, system disorders, and medications. The foremost common skin disorders are eczema, psoriasis, acne, and rosacea. Topical application of gels at pathological sites offers great advantages in the faster release of a drug directly to the site of action as compared to creams and ointments [4, 5].

B. aquifolium (Berberidaceae) is an evergreen shrub growing 1-3 m (3-10 ft) [6] and has dense clusters of yellow flowers in early spring, followed by dark bluish-black berries [7, 8]. *T. occidentalis* is rich in Vitamin C. Due to the presence of the neurotoxic compound thujone, its internal use can be harmful if used for prolonged periods or while pregnant. It is commercially used for rustic fencing and posts, lumber, poles, shingles, and in the construction of log cabins [9]. It is the preferred wood for structural elements, such as the ribs and planking of birchbark canoes and the planking of wooden canoes. The essential oil within the plant has been used for cleansers, disinfectants, hair preparations, insecticides, linings, room sprays, and soft soaps. Others have used the twigs to make teas to relieve constipation and headaches [10]. In the 19th century, *T. occidentalis* extract was in common use as an externally applied tincture or ointment for the treatment of warts, ringworm, and thrush [11].

E. angustifolia is a perennial herb, with spindle-shaped taproots and moderately to densely hairy [12]. The medicinal properties of *E. angustifolia* are sometimes used in veterinary practice. A weak antibiotic substance, the main root metabolite echinacoside (q.v.) is a marker constituent for *E. angustifolia* and *E. pallid* [13]. *Curcuma longa* (turmeric, haldi) belonging to the ginger family Zingiber-aceae [14] has a wide range of pharmacological effects like anti-HIV, antiseptic, anti-inflammatory, antibacterial, antioxidant, anti-fungal, antiviral, antitumor, and antimicrobial activities. Curcumin being the main constituent of *C. longa* is responsible for its beneficial activities. Curcumin displays anticancer, antidiabetic, and anti-inflammatory activities [15]. Cyclooxygenase (COX-2) has a vital role in the initiation of colon cancer. The HT-29 colon cancer cells treated with different concentrations of curcumin decreased the expression of COX-2. Curcumin aids in the prevention of colon cancer and breast cancer cell lines (MCF-7) were assessed through SRB and MTT assays for cytotoxicity and cell viability, respectively which exhibited augmented caspase 3/9 activity and initiation of apoptosis indicating downregulation of miR-21 and the expression of miR-21 in MCF-7 cells by upregulation of PTEN/Akt signaling pathway [16].



Figure 1:- Plants of *B. aquifolium*, *C. longa*, *E. angustifolia* and *T. occidentalis*.

MATERIAL AND METHODS

Plant material

The fresh part of plants of *B. aquifolium*, *C. longa*, *E. angustifolia*, and *T. occidentalis* were collected from adjoining areas of the “Himalaya Drug Company Saharanpur” in the month of February 2020. The plant was authenticated by the ‘Botanical Survey of India’, Dehradun Uttarakhand India.

Chemicals

All the chemicals and reagents used for the experimental work included sodium hydroxide, methanol, ethyl alcohol, hydrochloric acid, sulphuric acid, Carbopol 940, propylene glycol-400, ethanol, methylparaben, propylparaben, triethanolamine, and EDTA used in this study were of analytical grade and purchased from Merck (India). The solid media and broth used for microbial culture were procured from Hi-Media Pvt. Limited, Bombay, India.

Preparation of plant Extract

The plant material was separated into its selected parts: leaf, rhizome, and leaf air-dried and ground to a moderately fine powder and Soxhlet extracted with increasing polarity solvents (Petroleum ether, chloroform, methanolic, and water). Each extract was evaporated to dryness under reduced pressure using a rotary evaporator. The coarse powder of leaf, rhizome, and leaf was subjected to successive hot continuous extraction with various solvents each time before extracting with the next solvent. The powdered material will be air dried (weight of crude extract 500gm). The various concentrated extracts were stored in air-tight containers for further studies [17].

Phytochemical analysis

The qualitative phytochemical properties of the dried powdered sample were determined using standard methods. The extracts obtained as above were subjected to qualitative tests for the identification of various plant constituents. In addition, 50 g of air-dried or fresh plant material was also subjected to hydrodistillation to detect the presence of different contents. The plant material was subjected to preliminary phytochemical screening for the detection of various plant constituents as per the methods described [18].

Antimicrobial activity of the polyherbal gel

The antimicrobial activity testing was performed by relating the diameter of zones of inhibition (in mm), which indicates the effectiveness of an antimicrobial agent [19]. The polyherbal gel of *B. aquifolium*, *C.*

longa, *E. angustifolia*, and *T. occidentalis* was observed for its antimicrobial properties toward dermatological causing organisms, such as *Staphylococcus aureus* (MTCC-6538P), *Escherichia coli* (MTCC-8739), and *Candida albicans* (MTCC-18804). Its activity was also compared with standards, such as Erythromycin (10 mg/ml). It is believed that the antimicrobial property might be due to the presence of strong flavonoids in the extract and the weak antioxidant nature of the gel, which in turn increases the shelf life of the product from photodegradation and oxidative degradation. The antimicrobial property of the gel might be due to the high percentage content of flavonoids, which makes the preparation highly effective against the studied microorganisms.

Additionally, the antioxidant property that protects epidermal cells from UVA-induced damage is mainly due to the presence of orthophosphoric acid in the gel, which protects the skin from conditions of extreme pH modification [20].

Formulation of Polyherbal Gel

The polyherbal gel containing *B. aquifolium*, *C. longa*, *E. angustifolia*, and *T. occidentalis* was incorporated into the optimized 2% carbopol gel base. Different concentrations of ethanolic extract of *B. aquifolium*, *C. longa*, *E. angustifolia*, and *T. occidentalis*, including 1, 1.5, and 2%, were also incorporated into the gel base. The *B. aquifolium*, *C. longa*, *E. angustifolia*, and *T. occidentalis* concentrations were kept constant [5 mL] in all gel bases. The formulations of the designed polyherbal gel are presented in Table 5. The formulated gel was checked visually for color, appearance, and homogeneity; the results are listed in Table 6 and Figure 7, which indicate the absence of aggregates in F1 and F2, and the presence of slight aggregates in F3 (2% *Vigna radiata* extract). This indicates a problem of in homogeneity in F3. [21].

pH determination

A good topical preparation should have a pH that is acceptable for the skin, ranging from 4.2 to 6.5. Gels that are too alkaline will result in scaly skin. On the other side, if the pH is too acidic, it will irritate the skin. The pH of the formulation was 5.7–5.9. The pH of the prepared gel showed its compatibility with the skin. Even though the ideal pH range is below 5.0, the addition of stabilizers contributes to this pH range, making it suitable for topical application and penetration [22].

Determination of viscosity

Rheological characteristics of gels vary and show reversible deformation, similar to that experienced by elastic materials, rather than flowing at low shear stresses. They flow like liquids when a specific shear stress is exceeded, which is known as the yield value or yield stress. In general, the consistency of gel compositions is reflected in their viscosity. Non-Newtonian flow (shear thinning) shows how the viscosity of gels reduces with an increasing shear rate; this behavior is desired because of its low flow resistance when used under high shear conditions. The rheological property helps in determining consistency and influences the diffusion rate of a drug from a gel. By maintaining the viscosity below 15,000 cps, the advantages of more attractive cosmetic characteristics and ease of accurate application over the skin through better flow and spreadability can be achieved.

Additionally, this low viscosity is an indication of the viscoelastic behavior of the gel upon applied stress, which makes it easier to flow from the container to the applied area and suck back to the container upon the release of stress [23]. The results are tabulated in Table 7. It can be observed that all formulations have low viscosity, which indicates promising applicability to skin administration.

Spreadability

Manufactured gels must have good spreadability and satisfy the ideal quality for topical application since the spreadability of the gel aids in the uniform application of the gel to the skin. In addition, it is thought that this is a key element in patient adherence to therapy. Spreadability denotes the area and the extent to which a gel readily spreads upon topical application [24]. The spreadability of different gel formulations was studied. The formulation F2 produced better spreadability than the other formulations. The results of the three physical parameters are presented in Table 7. To have good permeation across the skin, the gel should have ideal properties and stability over a long period. From the results obtained for the physical parameters, such as pH, viscosity, and spreadability, it can be seen that the formulation F2 is ideal; thus, it was chosen for further characterization, such as texture analysis [25].

Extrudability

This mechanical property plays a vital role in the selection, packing, and removal of a gel from its container. To assess how easily topical preparations, such as ointments, creams, and gels, can be removed and applied, extrudability must be quantified. The consistency of a product can change over the course of its shelf life, and product developers can analyze these changes and modify formulations accordingly. This allows producers to evaluate the compatibility of packaging materials and their design. The rheological property also influences the spreadability, firmness, and in vivo performance of a product upon its application to the skin [26]. The results corresponding to extrudability are shown in Table 9 and Figure S2 (supporting

information). The results indicate that the gel requires 3.401 kg of force to extrude through the outlet. Therefore, the formulated gel has ideal extrudability.

RESULTS AND DISCUSSION

Table 1. Organoleptic characteristics and the extractive value of these plants.

S. No	Plants	Color	Odor	Methanol extract (%)	Water extract (%)	Ash Value (%)	Acid soluble extract (%)	Foreign matter (%)
1.	<i>Berberis aquifolium</i>	Bright and deep color	Reminiscent of roses	5.00	11.0	8.60	0.50	1.70
2.	<i>Curcuma longa</i>	Deep yellowish-brown color	Mild aromatic	5.50	10.5	9.15	0.80	1.35
3.	<i>Echinacea angustifolia</i>	Dark green	Faint and aromatic	6.00	10.8	10.1	0.70	1.50
4.	<i>Thuja occidentalis</i>	Dull Green	Camphoraceous	6.20	9.60	9.50	0.75	1.80

Table 2. Phytochemical screening of *B. aquifolium*, *C. longa*, *E. angustifolia*, and *T. occidentalis* plants.

Test	BA Extract	CL Extract	EA Extract	TO Extract
Carbohydrates/ glycosides	(-)	(-)	(+)	(-)
(1) Molish test	(+)	(-)	(+)	(-)
(2) Fehling test	(+)	(-)	(+)	(-)
(3) Benedict test				
Alkaloid				
(1) Mayer's test	(-)	(+)	(+)	(-)
(2) Dragondroff test	(-)	(+)	(-)	(-)
Flavonoids				
(1) Shinoda/pew	(+)	(+)	(+)	(+)
(2) Ammonia	(+)	(-)	(-)	(-)
Saponins	(+)	(+)	(+)	(-)
Tannins				
(1) Pyrogallol & catechol	(+)	(+)	(+)	(+)
(2) Gallic acid	(+)	(+)	(+)	(+)
Unsaturated sterol/triterpenes				
(1) Liebermann Burchard test	(+)	(+)	(+)	(+)
(2) Salkowiskis test	(+)	(+)	(+)	(+)
Phenolics compound	(+)	(+)	(+)	(+)
(1) Ferric chloride				
Protein and amino acid	(+)	(+)	(+)	(+)
(1) Xanthoprotien				

Table 3. Antioxidant activities (mm AAE/100G FW) in these plants.

Methanol plant extract	ABTS Assay	FRAB Assay	DPPH Assay
<i>Berberis aquifolium</i>	12.46 ± 0.10	12.13 ± 0.03	40.51 ± 0.15
<i>Curcuma longa</i>	13.27 ± 0.03	12.69 ± 0.18	39.02 ± 0.58
<i>Echinacea angustifolia</i>	15.40 ± 0.25	14.23 ± 0.05	31.10 ± 0.20
<i>Thuja occidentalis</i>	19.40 ± 0.05	15.23 ± 0.10	37.10 ± 0.20

Table 4. Formulation of polyherbal gel (100 gm).

S. No	Ingredients	F1	F2	F3	F4	F5	F6
1.	BA Extract	1%	1%	1%	1%	1%	1%
2.	CL Extract	1%	1%	1%	1%	1%	1%
3.	EA Extract	1%	1%	1%	1%	1%	1%
4.	TA Extract	1%	1%	1%	1%	1%	1%
5.	Carbopol 940	1%	1.5%	2%	-----	-----	----
6.	HPMC K100 M	----	-----	----	1%	1.5%	2%
7.	Methyl Paraben	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
8.	Propyl Paraben	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
9.	Triethanolamine	2%	2%	2%	2%	2%	2%
10.	Propylene glycol	2%	2%	2%	2%	2%	2%
11.	Distilled water	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.

Table 5. Measurement of pH, viscosity, and spreadability.

Formulation Code	Appearance	pH	Viscosity (50 rpm)	Spreadability (gm-cm/sec)	Extradibility amount (%)	Thermo-Stability and self life for 1 Months
F1	Mustard orange	6.7	4321	3.1±0.2576	81.95	Semisolid
F2	Yellow orange	6.9	4764	3.4±0.2545	86.70	Semisolid
F3	light yellow	6.5	4356	2.6±0.2554	82.20	Semisolid
F4	Copper orange	6.5	4656	3.0±0.2566	85.10	Semisolid
F5	light Copper	6.8	4210	3.2±0.1234	80.10	Semisolid
F6	Dark copper	6.9	4825	2.8±0.2654	84.40	Semisolid

Table 6. Antibacterial activity of ten bacterial strains against BA, CL, EA, and TA plant fruit extract.

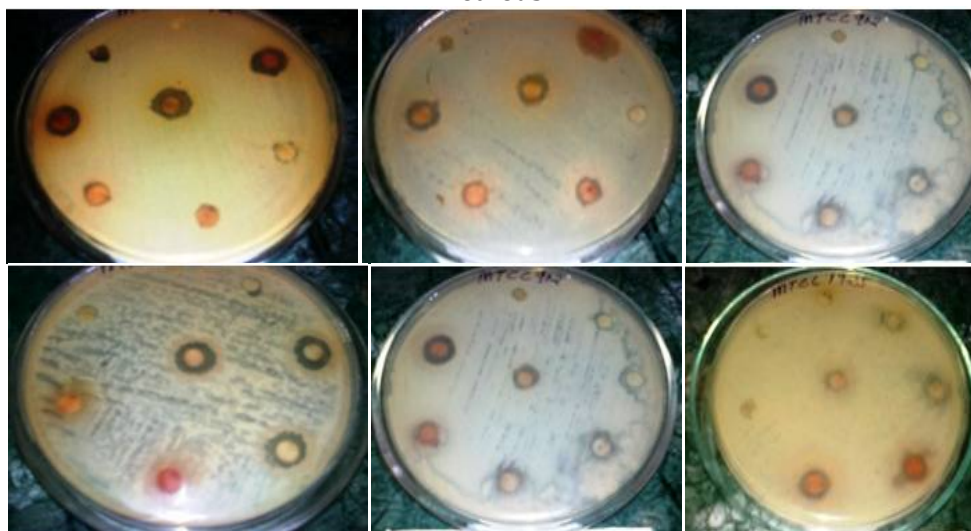
Bacterial Name		Erythromycin	BA Methanol Extract	CL Methanol Extract	EA Methanol Extract	TO Methanol Extract
Genus /Species/Subspe.	MTCC (Code)	10 Mg/ml	50 mg/ml	50 mg/ml	50 mg/ml	50 mg/ml
Bacillus cereus	1272	12	16	17	13	14
Escherichia coli	729	14	16	17	13	14
Enterobacter gergoviae	621	13	17	18	14	15
Klebsiella pneumonia	432	11	16	17	13	14
Salmonella entericatypim	98	10	17	19	14	16
Shigella flexneri	1457	10	17	18	14	15
Staphylococcus aureus	902	11	22	24	19	20
Staphylococcus epidermidis	435	10	21	22	18	19
Streptococcus pyogenes	1925	12	19	20	16	17
Escherichia coli	443	13	19	21	16	18

Table 7. Antifungal activity of three fungal strains against BA, CL, EA, and TA plant extract.

Fungal Name		Ketoconazole	BA Methanol Extract	CL Methanol Extract	EA Methanol Extract	TO Methanol Extract
Genus /Species/Subspe.	MTCC (Code)	10 mg/ml	50 mg/ml	50 mg/ml	50 mg/ml	50 mg/ml
Candida albicans	3017	10	13	14	10	11
Aspergillus flavus	2798	8	12	13	9	10
Aspergillus parasiticus	2796	9	12	13	9	11

Note: - Disc size, 5 mm, Inhibitory zone size ± 1 mm, mm means (millimeters) and - indicate (NIZ) No inhibitory zone.

Figure 2:-Antibacterial and antifungal activity of BA, CL, EA, and TO extracts against disc diffusion methods.



Plants are considered to be a vital source of potentially useful constituents for the development of new therapeutic agents, as most of them are safe with less or no side effect(s). Topical application of gels at pathological sites offers great advantages in the faster release of a drug directly to the site of action as compared to creams and ointments. Considering the results of the study, it was concluded that the prepared polyherbal formulations F1 to F6, which comprised of methanolic extract of *B. aquifolium*, *C. longa*, *E. angustifolia*, and *T. occidentalis* in a concentration of 50 mg/ml respectively produced no skin irritation after performing a gel test of 24 h. Also, physical analysis and stability studies of the prepared polyherbal gel proved potency and efficacy. Thus, these formulations can be used safely on human skin. The effective activity exhibited by the polyherbal formulations may be attributed to the synergistic action of the plant constituents present in the formulation. The high amount of plant extracts (0.5 %) increased the antimicrobial activity of the formulation. In the present scenario, gels have been used as a vehicle for drug delivery to the body. Plants with specific medicinal properties can be used in this dosage form as active ingredients in order to provide additional value.

CONCLUSION

The development of polyherbal formulations has drawn increasing attention due to their historical roots, economic viability, and patient compliance. The preliminary assessment and antimicrobial study of *B. aquifolium*, *C. longa*, *E. angustifolia*, and *T. occidentalis* demonstrated a strong antimicrobial effect of the extract against skin infection. This investigation revealed antimicrobial and antifungal activity against selected bacterial and fungal strains. Gels, semisolid preparations, can give controlled release, stable and improved absorption, which increases medicinal drugs' bioavailability. The polyherbal gel formulated in this study indicates that it might be a good gel for topical application. The qualitative phytochemical analysis test showed the presence of carbohydrates and glycosides, alkaloids, flavonoids, saponins, tannins, unsaturated triterpenoids and sterols, resin.

Further studies to characterize the pharmacokinetics of the polyherbal gel and to establish its safety, stability, and dermatological activity will be investigated using various new formulations at varying strengths and dosage forms, as well as with different plant extracts.

Disclosure of interest

The authors declare that they have no competing interests.

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