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Original Research Article

FORMULATION AND CHARACTERIZATION OF POLYHERBAL GEL FOR EFFECTIVE FUNGAL TREATMENT

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ABSTRACT

This work aims at creating a polyherbal gel from *Butea monosperma*, *Helicteres isora* and *Eclipta prostrata* that can be used to treat a fungal infection. The hydroalcoholic extract of *Helicteres isora* had revealed the presence of diterpenes, phenolics, saponins, flavonoids and alkaloids. Phytochemical screening of extract of *Eclipta prostrata* showed the present of tannins, proteins, phenolics, carbohydrate, flavonoids and glycosides. Total phenol content was found to be 0.52 mg/100mg, 0.63 mg/100mg and 0.77 mg/100mg in *Butea monosperma*, *Helicteres isora* and *Eclipta prostrata* respectively. Total flavonoids content was found to be 0.87mg/100mg, 0.75mg/100mg and 0.96mg/100mg in *Butea monosperma*, *Helicteres isora* and *Eclipta prostrata* respectively. All the formulated polyherbal gels exhibited a light brown color, with no clogging, good homogeneity and smooth texture. All formulations (PHG1 to PHG6) exhibited good washability while extrudability of the polyherbal gels varied. The results of spreadability PHG4 showed the highest spreadability value (33.4 ± 4 gcm/sec), indicating that it can easily spread over a larger area on the skin upon application. PHG1, PHG4, and PHG6 showed pH values of 6.9 ± 0.2, 6.8 ± 0.2, and 7.1 ± 0.1, respectively, which are also close to neutral. The viscocity of formualtions of 10 ± 0.47 mm. As the concentration of the gel increased to 20µg/ml, the zone of inhibition also increased to 15 ± 0.57 mm. Results of *In-vitro* drug release study of polyherbal gel ranged from 2074±24 cps to 2896±27. Further, PHG5 exhibited the highest flavonoids content with a percentage of 82.5 ± 0.6%. At 10 µg/ml concentration, PHG5 showed a zone of inhibition formulation (PHG5) indicated that about 98.12% drug was released in 120minutes. Thus, the formulated PHG5 exhibited all ideal characteristics & can be used to cure fungal infection.

Keywords: Fungal infections, Medicinal plants, *Butea monosperma*, *Helicteres isora*, *Eclipta prostrata*, Polyherbal gel

INTRODUCTION

The fungi are everywhere around us, but not all of them cause disease. Fungi can infect the body when they are breathed in, come into touch with the skin, cut, wound, or injected into the skin. People with weakened immune systems are more likely to experience it. Over one billion people have fungal infections annually throughout the world. In 2017, an estimated 1.6 million fatalities were recorded. The number has been rising, and in 2020 there will likely be 1.7 million deaths.According to the Global Action Fund for Fungal Infections, yearly incidences of fungal asthma exceed 10 million, while long-term pulmonary

aspergillosis affects about 3 million people (Romani., 2004; Brown *et al*., 2012).

Only 300 of the approximately 100,000 fungal species are known to infect animals or people. A small number of fungus, such as dermatophytes, live on the skin, hair, and nails of both humans and animals and are highly contagious (Candida spp.). The majority of human fungal infections go untreated and unreported. The most typical fungal infections in people are caused by dermatophytic fungi. Isolation of fungi from blood cultures or sterile sites, isolation of the same fungus from the source wound, radiographic proof, the absence of other infections, and an improvement in the patient's clinical condition after the start of antifungal therapy are all requirements for establishing an IFI (Mukherjee *et al*., 2005; Hay, 2006).

The introduction of strains that are resistant to the currently employed antifungal medicines complicates the treatment of fungal infection. The number of antifungal medicines now in use is limited, and many of them are both harmful and expensive. Since fungal infections frequently recur, controlling an opportunistic illness becomes more difficult. In order to broaden the range of actions and battle strains displaying resistance to the current antifungals, these circumstances necessitate the creation of novel antifungal drugs (Meis and Verweij, 2001; Kyle and Dahl, 2004).

There is already evidence of the usage of herbal medicine. They are presently in high demand not because they are cheaper but because they are more widely accepted culturally, have less adverse effects, and work better with the human body. The old medical systems are significantly reliant on by a large portion of the rural communities. The number of people turning to alternative treatments instead of conventional medications has increased dramatically in recent years (Solanki, 2011; Bussmann and Glenn, 2011).

The Indian Subcontinent and Southeast Asia are home to *B. monosperma*, a plant belonging to the Fabaceae family. Rural and tribal people typically utilize it to treat a variety of ailments. Extract, juice, infusion, powder, and gum are all forms of using plant materials. The leaves have aphrodisiac, diuretic, depurative, and astringent properties. Flowers are used to treat a wide range of illnesses, including enlargement of the spleen, eye disease, epilepsy, leprosy, chronic fever, leucorrhoea, liver diseases, antifertility activity, and gout, among others. They also have antifungal, anti-inflammatory, and anti-leucorrhoeal properties. When the seed is mashed with lemon juice and applied to the skin, it has good anthelmintic properties and works as a rubefacient. (Akram *et al*., 2011; Surin and Ananthaswamy, 2011).

Helecteres isora Linn. (Sterculiaceae), often known as the Indian screw tree and also known by the names Marodphali, Marorphali, Avartani, and Enthani, is a highly regarded medicinal plant in South-East Asia. The presence of cucurbitacin, flavones, triterpenoids, phytosterols, saponin, sugars, and flavonins. The roots and barks have anti-inflammatory, antibacterial, and antispasmodic qualities that can be used for snakebites, hypoglycemia, and expectorants (Pandey *et al*., 2021; Gaikwad and Dhasade, 2019).

Eclipta prostata is also referred to as Bhringraj locally and is also referred to as False Daisy or Ink Plant in English. It is widely dispersed in Asia, Africa, and South America's tropical and subtropical climates. For the treatment of skin issues, gastrointestinal issues, respiratory issues, such as asthma, and other symptoms including fever, hair loss and hair whitening, cuts and wounds, spleen enlargement, etc., this herb is widely utilized throughout India. In Nepal, the leaves and shoots are used to cure and prevent wound infection. It is utilized in Ayurveda for its rejuvenating and anti-aging qualities (Timalsina and Devkota, 2021; Feng *et al*., 2019).

Additionally, topical antifungal gels are used to treat and manage fungal infections. To shield and moisturize the skin, the formulation could incorporate a moisture barrier. Antifungal gels are applied both as a palliative treatment for fungal infections that have already developed and as a preventative measure when a fungal infection is a possibility (Chen *et al*., 2015). Thus, the goal of this effort is to combine the therapeutic qualities of all three plants and create a polyherbal gel that can be used to treat a fungal infection).

MATERIALS & METHODS

Selection and Collection of plant material

The plants have been selected on its availability and folk use of the plant. Every parts of the plant like bark, leaves, flowers, roots, fruits and seeds may contain active secondary metabolites. *Butea monosperma*, *Helicteres isora* and *Eclipta prostrata* were collected from ruler area of Bhopal (M.P.) in the month of February, 2022.

Defatting & extraction

52 gram of *Butea monosperma,* 45 gram of *Helicteres isora* and 49 gram of *Eclipta prostrata* shade dried plant material were coarsely powdered and subjected to extraction with petroleum ether in a maceration method. The extraction was continued till the defatting of the material had taken place. Defatted plant materials of *Butea monosperma*, *Helicteres isora* and *Eclipta prostrata* were exhaustively extracted with hydroalcoholic solvent (Ethanol: Aqueous: 70:30v/v) by maceration method.

Determination of percentage yield

The percentage yield was calculated by dividing weight of extract of by weight of powder drug taken multiplied by 100.

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods (Khandelwal, 2005; Kokate, 1994).

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Parkhe and Bharti, 2019).

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm (Parkhe and Bharti, 2019).

Formulation development of polyherbal gel

Measured quantity of methyl paraben, glycerin, polyethylene glycol and hydroalcoholic extract of *Butea monosperma*, *Helicteres isora* and *Eclipta prostrata* were dissolved in about 35 ml of water in beaker and were stirred at high speed using mechanical stirrer (or sonicator). Then Carbopol 940 was added slowly to the beaker containing above liquid while stirring. Neutralized the solution by slowly adding triethanolamine solution with constant stirring until the gel is formed.

Table 1: Formulation of polyherbal gel

Ingredients	PHG 1	PHG 2	PHG 3	PHG 4	PHG 5	PHG 6
Butea monosperma extract	1gm	1gm	1gm	1gm	1gm	1gm
Helicteres isora extract	1gm	1gm	1gm	1gm	1gm	1gm
Eclipta prostrata extract	1gm	1gm	1gm	1gm	1gm	1gm
Carbopol 940	0.25 mg	0.5m g	0.75 mg	1.0 gm	1.25 gm	1.5 gm
Polyethylene glycol	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
Methyl paraben	0.08 mg	0.08 mg	0.08 mg	0.08 mg	0.08 mg	0.08 mg
Triethanola mine	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
Distilled Water (q.s)	100m l	100m l	100m l	100m l	100m l	100m l

Evaluation of polyherbal gel

Appearance and consistency

The physical appearance was visually checked for the texture of polyherbal gel formulations for color, odor and texture (Yamini and Onesimus, 2013).

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually and observed.

Extrudability determination of formulations

The polyherbal gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked (Bhaskar *et al.*, 2009).

Spreadability

Two glass slides of standard dimensions (6×2) were selected. The gel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms along the slide. 100 grams of weight was placed up on the upper slide so that the gel formulation between the two slides was traced uniformly to form a thin layer.

The weight was removed and the excess of the gel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cms and separate away from lower slide under the direction of the weight was noted. The

experiment was repeated and the average of 6 such determinations was calculated for each gel formulation.

Determination of pH

The pH of the gels was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation until constant reading obtained.

Flavonoids content

The drug content was determined by taking 1gm of gel in 10 ml volumetric flask diluted with methanol. 3 ml of stock solution was mixed with 1 ml of 2% $AlCl_3$ solution. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 420 nm using a spectrophotometer.

In vitro Antifungal activity

In vitro antifungal activity was performed on isolated colonies of *C. albicans*. The Agar well diffusion technique was used for determining the zone of inhibition and minimum inhibitory concentrations (MIC). The strains of *C. albicans* were inoculated in prefabricated agar plate. Plates were dried and 4 wells were made with the help of 6 mm agar well cutter. 0.5mg/ml, 0.25 mg/ml & 0.125mg/ml of prepared polyherbal gel solution was loaded in all the respective wells. The agar plates were kept undisturbed to allow the passive diffusion of polyherbal gel solution into the agar culture medium. Then the plates were incubated at 37°C for 24 hours. The zone of inhibition was calculated in mm (Bauer *et al*., 1966).

***In vitro* drug diffusion study**

The *in-vitro* diffusion study is carried by using franz diffusion cell. Egg membrane is taken as semi permeable membrane for diffusion. The Franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm^2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32 ± 0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell. During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength 282nm of drug.

RESULTS AND DISCUSSION

The percentage yield of *Butea monosperma, Helicteres isora* and *Eclipta prostrata* were found 7.5%, 6.8% and 8.2% respectively. The phytochemical screening of the *Butea monosperma* extract revealed the presence of several bioactive compounds, including flavonoids, tannins, phenolic compounds and diterpenes. These compounds are known to possess various pharmacological properties, which might contribute to the medicinal value of *Butea monosperma*. The hydroalcoholic extract of *Helicteres isora* had revealed the presence of diterpenes, phenolics, Saponins, flavonoids and alkaloids. Phytochemical screening of extract of *Eclipta prostrata* showed the present of tannins, proteins, phenolics, carbohydrate, flavonoids and glycosides.

Total phenol content was found to be 0.52 mg/100mg, 0.63 mg/100mg and 0.77 mg/100mg in *Butea monosperma*, *Helicteres isora* and *Eclipta prostrata* respectively. Total flavonoids content was found to be 0.87mg/100mg, 0.75mg/100mg and 0.96mg/100mg in *Butea monosperma*, *Helicteres isora* and *Eclipta prostrata* respectively.

All the formulated polyherbal gels exhibited a light brown color. The uniformity in color suggests that the herbal ingredients used in the formulations contributed to a consistent appearance.The data shows that clogging was absent in all the formulations (marked as "Absent"). This indicates that the polyherbal gels had appropriate consistency, preventing any blockage or obstruction in the dispensing system. The polyherbal gels showed good homogeneity (marked as "Good"). This suggests that the ingredients were well mixed and uniformly distributed throughout the gel matrix. The texture of all the polyherbal gels was reported as smooth. A smooth texture ensures that the gel is easily spreadable and readily absorbed into the skin upon application

All formulations (PHG1 to PHG6) exhibited good washability. Good washability implies that the gel can be easily removed from the skin surface with water or a mild cleansing agent, leaving no residue behind. The extrudability of the polyherbal gels varied, with some formulations demonstrating average extrudability and others exhibiting good extrudability.

The results of spreadability PHG4 showed the highest spreadability value (33.4± 4gcm/sec), indicating that it can easily spread over a larger area on the skin upon application. PHG1, PHG4, and PHG6 showed pH values of 6.9 ± 0.2, 6.8 ± 0.2, and 7.1 ± 0.1, respectively, which are also close to neutral. These formulations are likely to be well-tolerated by the skin and may offer good skin compatibility. Overall, the pH values of the polyherbal gel formulations appear to be within an acceptable range for topical formulations. The viscocity of formualtions ranged from 2074±24 cps to 2896±27.

Further, PHG5 exhibited the highest flavonoids content with a percentage of 82.5 ± 0.6%. This indicates that PHG5 has the highest concentration of flavonoids among the formulations.

The results of the antifungal activity evaluation reveal the ability of the optimized polyherbal gel formulation (PHG5) to inhibit the growth of *Candida albicans* at different concentrations. At 10 µg/ml concentration, PHG5 showed a zone of inhibition of 10 ± 0.47 mm. As the concentration of the gel increased to 20µg/ml, the zone of inhibition also increased to 15 ± 0.57 mm. This indicates that the polyherbal gel formulation has a dose-dependent antifungal effect against *Candida albicans*.

Results of *In-vitro* drug release study of polyherbal gel formulation (PHG5) indicated that about 98.12% drug was released in 120minutes. By analyzing the drug release profile and comparing it to the desired release characteristics, it is possible to optimize the formulation and tailor the drug release for the intended therapeutic application.

Table 2: Results of percentage yield of extract of *Butea monosperma*, *Helicteres isora* and *Eclipta prostrata*

Hydroalcoholic extracts	Percentage yield (w/w)
Butea monosperma	7.5%
Helicteres isora	6.8%
Eclipta prostrata	8.2%

Table 3: Result of phytochemical screening of extract of *Butea monosperma*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	A) Wagner's Test:	-Ve
	B) Hager's Test:	-Ve
2.	Glycosides	
	A) Legal's Test:	-Ve
3.	Flavonoids	
	A) Lead acetate Test:	-Ve
	B) Alkaline Reagent Test:	+Ve
4.	Saponins	
	A) Froth Test:	-Ve
5.	Phenol	
	A) Ferric Chloride Test:	+Ve
	B) FC reagent test:	+Ve
6.	Proteins	
	A) Xanthoproteic Test:	-Ve
7.	Carbohydrate	
	A) Fehling's Test:	-Ve
	B) Benedict test:	-Ve
8.	Diterpenes	
	A) Copper acetate Test:	+Ve
9.	Sterols	-Ve
10.	Tannins	
	A) Gelatin test:	+Ve

Table 4: Result of phytochemical screening of extract of *Helicteres isora*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 +Ve -Ve
2.	Glycosides A) Legal's Test:	 -Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 +Ve +Ve
4.	Saponins A) Froth Test:	 +Ve
5.	Phenolics C) Ferric Chloride Test: D) FC reagent test	 +Ve -Ve
6.	Proteins A) Xanthoproteic Test:	 -Ve
7.	Carbohydrate C) Fehling's Test: D) Benedict test	 -Ve -Ve
8.	Diterpenes A) Copper acetate Test:	 +Ve
9.	Sterols	-Ve
10.	Tannins A) Gelatin test	 -Ve

Table 5: Result of phytochemical screening of extract of *Eclipta prostrata*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 -Ve -Ve
2.	Glycosides A) Legal's Test:	 +Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 -Ve +Ve
4.	Saponins A) Froth Test:	 -Ve
5.	Phenolics E) Ferric Chloride Test: F) FC reagent test	 -Ve +Ve
6.	Proteins A) Xanthoproteic Test:	 -Ve
7.	Carbohydrate	

	E) Fehling's Test: F) Benedict test	-Ve +Ve
8.	Diterpenes A) Copper acetate Test:	 -Ve
9.	Sterols	-Ve
10.	Tannins A) Gelatin test	 +Ve

Table 6: Estimation of total phenolic and flavonoids content of *Butea monosperma, Helicteres isora* and *Eclipta prostrata*

S. No.	Hydroalcoholic extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	*Butea monosperma*	0.52	0.87
2.	*Helicteres isora*	0.63	0.75
3.	*Eclipta prostrata*	0.77	0.96

Table 7: Results of physical appearance

Formulation	Colour	Clogging	Homogeneity	Texture
PHG1	Light Brown	Absent	Good	Smooth
PHG2	Light Brown	Absent	Good	Smooth
PHG3	Light Brown	Absent	Good	Smooth
PHG4	Light Brown	Absent	Good	Smooth
PHG5	Light Brown	Absent	Good	Smooth
PHG6	Light Brown	Absent	Good	Smooth

Table 8: Results of washability and Extrudability

Formulation	Washability	Extrudability
PHG1	Good	Average
PHG2	Good	Average
PHG3	Good	Average
PHG4	Good	Good
PHG5	Good	Good
PHG6	Good	Average

Table 9: Results of pH and spreadability

Formulation	Determination of pH	Spreadability (gcm/sec)
PHG1	6.9±0.2	26.7±4
PHG2	6.5±0.1	30.8±2
PHG3	7.4±0.2	27.6±1
PHG4	6.8±0.2	33.4±4
PHG5	7.2±0.2	15.4±3
PHG6	7.1±0.1	20.9±2

Table 11: Results of flavonoids content and Viscosity

Formulation	Flavonoids content (%)	Viscosity (cps)
PHG1	77.2±0.2	2351±12
PHG2	80.9±0.3	2536±20
PHG3	73.4±0.4	2074±24
PHG4	75.6±0.3	2296±19
PHG5	**82.5±0.6**	2763±28
PHG6	74.5±0.4	2896±27

Table 13: Antifungal activity of polyherbal gel formulation (PHG5) against *Candida albicans*

S. No.	Microbes	Zone of inhibition (mm)		
		0.5mg/ml	0.25 mg/ml	0.125mg/ml
1.	*Candida albicans*	8±0.74	11±0.5	13±0.86

Table 14: Results of *In-vitro* drug release study of polyherbal gel formulation (PHG5)

S. No.	Time (Min.)	Percentage drug release
1.	15	32.25
2.	30	46.65
3.	60	58.98
4.	90	69.98
5.	120	98.12

CONCLUSION

Everyone has experienced the discomfort of a fungus infection at some point. Numerous different substances and combinations have been employed for antifungal action over the years. Additionally, the majority of earlier antifungal formulations contain components that are not necessarily environmentally friendly and are therefore not profitable. Some of the earlier antifungal formulations also include ingredients that are bad for the skin in the long run. More people are turning to natural herbs while making antifungal products.

From this study it can be concluded that *Butea monosperma*, *Helicteres isora* and *Eclipta prostrata* extracts can act synergistically when combined in gel form and could be used for combating fungal infections. However, these assertions call for additional research, the identification of each component's active phytoconstituents, and an examination of their potential as antifungal agents.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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