Asian Journal of Pharmaceutical Education and Research
Vol -10, Issue-2, April- June 2021
ISSN:2278 7496

RESEARCH ARTICLE Impact Factor: 7.014

FORMULATION OF POLYHERBAL FORMULATION FOR TREATMENT OF TOPICAL INFECTION AND ALOPECIA

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Received 17 Mar. 2021; Revised 21 Mar. 2021; Accepted 27 Mar. 2021, Available online 10 Apr. 2021.

Cite this article as: Dhakad R, Chouksey K, Ganju K. Formulation of Polyherbal Formulation for Treatment of Topical Infection and Alopecia. Asian Journal of Pharmaceutical Education and Research. 2021; 10(2): 27-38.

https://dx.doi.org/10.38164/AJPER/10.2.2021.27-38

ABSTRACT

Herb is a plant or part of a plant valued for its medicinal, aromatic, or savoury qualities. Herbs can be viewed as biosynthetic chemical laboratories, producing a number of chemical compounds. Herbal remedies or medicines consist of portions of plants or unpurified plant extracts containing several constituents, which often work together synergistically. Herbal formulations have more demanded in the market. The Hydroalcoholic extracts of *Allium cepa* L, *Withania somnifera*, *Spinacia oleracea* Linn possess almost all the phytochemicals. It could be seen from table no.7.2 that flavonoids and phenols were present in *Allium cepa* L, *Withania somnifera*, *Spinacia oleracea* Linn Hydroalcoholic extract. The total content of flavonoid (equivalent to quercetin) was 0.756 mg/100 mg, 0.639 mg/100 mg and 0.847mg/100mg in *Allium cepa* L, *Withania somnifer*, and *Spinacia oleracea* Linn respectively. The present investigation in this research work, the antifungal activity of polyherbal gel of *Allium cepa* L, *Withania somnifera* and *Spinacia oleracea* Linn were evaluated against Candida albicans pathogens used under present study. The polyherbal gel obtained from plant used to suitably dilute upto the concentrations of 100, 50 and 25 milligram per ml and applied on to the test organism using well diffusion method.

Keywords: *Allium cepa* L, *Withania somnifera*, *Spinacia oleracea* Linn, polyherbal gel, Topical infection

INTRODUCTION

Herbal medicines and their preparations have been widely used traditionally, for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects or dissatisfaction with the results of synthetic drugs. One of the characteristics of oriental herbal medicine preparations is that all the herbal medicines, either presenting as single herbs or as collections of herbs in composite formulae [1].

The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute mainly those traditional medicines which primarily use medicinal plant preparations for therapy [2]. These drugs are made from renewable resources of raw materials by eco-friendly processes and will bring economic prosperity to the masses growing these raw materials [3]. India is known as the “Emporium of Medicinal plants” due to availability of several thousands of medicinal plants in the different bioclimatic zones [4]. Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional systems of medicine. Attention is being focused on the investigation of efficacy of plant based drugs used in the traditional medicine because they are economy, have a little side effects and according to W.H.O, about 80% of the world population rely mainly on herbal remedies[5].

The World Health Organization has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today [6].

The uses of traditional medicines are widely spread and plants represent a large source of natural chemicals that might serve as leads for the development of the novel drugs [7]. Scientists have devised different ways of alienating the problem and one of the easy and cheapest options is herbal medicines. Herbs have been in use since long time to treat various diseases [8]. Almost one fourth of pharmaceutical drugs are derived from botanicals [9].

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, osteoarthritis, diabetes, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available (Kamboj, 2000). The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body [10].

Phenolic compounds possess potent antifungal, antiviral and antibacterial activity [11]. Many types of infections or diseases, including the dermal kind, are treated with a broad activity spectrum antibiotic. It may lead to the negative influence of antibiotics on natural microflora of the skin and lead to resistance of many bacterial strains [12]. Thus the activity of polyphenols has special significance in the case of strains resistant to antibiotics, e.g., *Staphylococcus aureus* resistant to methicillin, enterococci resistant to glycopeptide antibiotics and vancomycin, pneumococci resistant to β-lactam and macrolides,

and *Pseudomonas aeruginosa* with its defense mechanism against phagocytic activity of polymorphonuclear leucocytes [13,14]. Present investigation deals with The present scenario of infectious diseases shows that there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another important concern is the development of resistance to the antibiotic in clinical use. Hence, there is a pressing need to develop a natural formulation, which can act against the microorganisms causing skin diseases.

For many years, antibiotics have been used to treat topical microbial disease. However, antibiotic resistance has been increasing in prevalence within the dermatologic setting. The development of antibiotic resistance including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases. So our aim and objective to develop safe and effective polyherbal formulation for effective treatment of topical infection and alopecia.

MATERIAL AND METHODS

Material

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvent used in this study were of analytical grade. The pathogenic microbes used in the current study are obtained from Microbial Culture collection, National Centre Forcell Science, Pune, Maharashtra, India.

Methods

Collection of Plant material

Leaves of *Allium cepa* L, *Withania somnifera* and *Spinacia oleracea* Linn were collected from local area of Bhopal (M.P.) in the month of January, 2020.

Extraction of plant material

Dried powdered of leaves of *Allium cepa* L, *Withania somnifera* and *Spinacia oleracea* have been extracted with hydroalcoholic using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40^0C [15,16].

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical Screening

The chemical tests were performed for testing different chemical groups present in extracts.

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

b) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

5. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

6. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

7. Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes [17-19].

Estimation of total Phenolic and flavonoid Content

Total Phenolic content estimation

Principle: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method [20].

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25µg/ml was prepared in methanol.

Preparation of Extract: 10 mg of dried extracted dissolve in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenols.

Procedure: 2 ml of extract or 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 15min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoid content estimation

Principle: Determination of total flavonoids content was based on aluminium chloride method.

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol.

Preparation of extract: 10 mg of extract dissolved in 10 ml methanol and filter. Three (1mg/ml) of this extract was for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Formulation development of polyherbal gel

Method of preparation

Measured quantity of methyl paraben, glycerin, polyethylene glycol and hydroalcoholic extract of leaves of *Allium cepa* L, *Withania somnifera* and *Spinacia oleracea* 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 [21].

Table 1: Formulation of polyherbal gel

Ingredients (%)	PHG1	PHG2	PHG3	PHG4	PHG5	PHG6
Allium cepa L extract	1gm	1gm	1gm	1gm	1gm	1gm
Withania somnifera extract	1gm	1gm	1gm	1gm	1gm	1gm
Spinacia oleracea extract	1gm	1gm	1gm	1gm	1gm	1gm
Carbopol 940	0.5mg	0.75mg	1.0 gm	1.25 gm	1.5 gm	2.0 gm
Polyethylene Glycol	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
Methyl Paraben	0.08mg	0.08mg	0.08mg	0.08mg	0.08mg	0.08mg
Triethanolamine	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
Distilled Water (q.s)	100ml	100ml	100ml	100ml	100ml	100ml

Evaluation of polyherbal gel

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 Benoy [22].

Determination of Spreadability

Two glass slides of standard dimensions (6×2) were selected. The polyherbal 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 polyherbal gel formulation between the two slides was traced uniformly to form a thin layer. The weight was removed and the excess of the polyherbal 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 polyherbal gel formulation.

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)

m = weight tied to the upper slide (20 grams)

l= length of glass slide (6cms).

t = time taken is seconds.

Determination of pH

The pH of the polyherbal gels were 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. And constant reading was noted. The measurements of pH of each formulation were replicated two times [23].

Drug content

The drug content was determined by taking 1gm of gel in 10 ml volumetric flask diluted with methanol. 2 ml of stock solution 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 15min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [24].

Antifungal activity of polyherbal gel

The well diffusion method was used to determine the antibacterial activity of the polyherbal gel prepared from the *Allium cepa* L, *Withania somnifera* and *Spinacia oleracea* Linn using standard procedure. There were 3 concentration used which are 25, 50 and 100 mg/ml for antibiogram studies. The plates were incubated at 37°C for 24 hr. and then examined for clear zones of inhibition around the wells with particular concentration of drug.

RESULTS AND DISCUSSION

Percentage Yield of hydroalcoholic extract of *Allium cepa* L*, Withania somnifera* and *Spinacia oleracea* Linn was found to be12.36%, 8.47% and 11.66%. The Hydroalcoholic extracts of *Allium cepa* L, *Withania somnifera*, *Spinacia oleracea* Linn possess almost all the phytochemicals. It could be seen from table no.3 that flavonoids and phenols were present in *Allium cepa* L, *Withania somnifera*, *Spinacia oleracea* Linn Hydroalcoholic extract. The alkaloids were found to be absent in all three extracts. The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.042X-0.002, R^2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance. The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.06X+0.019, R^2= 0.999, where X is the quercetin equivalent (QE) and Y is the absorbance. The presence of phytochemicals (Phenols and Flavonoids) was quantitatively screened. Table No. 4 shows the overall flavonoid content of the *Allium cepa* L , *Withania somnifer, and Spinacia oleracea* Linn Hydroalcoholic extract. The total content of flavonoid (equivalent to quercetin) was 0.756 mg/100 mg, 0.639 mg/100 mg and 0.847mg/100mg in *Allium cepa* L, *Withania somnifer, and Spinacia oleracea* Linn respectively. In hydroalcoholic extract, the quantitative analysis of the total phenolic content showed that hydroalcoholic extract, quantitative analysis revealed total phenolic content (equivalent to gallic acid) in *Allium cepa* L. *Withania somnifer, and Spinacia oleracea* Linn was found to be of 1.074mg/100 mg and 0.985mg/100 mg and 1.155 mg/100 respectively. The phenolic content in *all the three plants* was found to be more as compare to flavonoid. It was observed that the freshly prepared formulations were off white to yellow in Dark brown. The Clogging was found absent in all formulations and having good homogeneity, texture was found in all formulations PHG1, PHG2, PHG3, PHG4, PHG5 and PHG6 respectively. Formulations were applied on the skin and then ease and extent of washing with water were checked manually. All the formulations exhibited good washability and left no traces over the skin on washing with water due to non-greasy properties. The all the prepared gel formulations were found Average extrudability and Good washability.

Spreadability of the formulations PHG1, PHG2, PHG3, PHG4, PHG5 and PHG6 were studied and found to in the range of 14.25±0.25, 13.15±0.36, 12.25±0.21, 13.36±0.45, 12.25±0.12 and 11.45±0.20 respectively. The Formulation PHG6 Showed the good Spreadability 11.45±0.20, among all formulation. It was found to be in the range of 6.8±0.1 to 7.2±0.1, the pH of prepared formulation was found to be similar to the skin pH 6.8. The Results of pH of all formulation were found to be near to skin pH so all

formulation considered as non-irritant. In the above formulations the viscosity of different sample of gel were determined and found that there is increase in viscosity. The formulation PHG6 has good viscosity. The viscosity of formulations PHG1, PHG2, PHG3, PHG4, PHG5 and PHG6 was found to be 3052±11, 2950±15, 2850±10, 3047±21, 2950±15 and 2875±23. The Phenolic Content of prepared formulations was found to be 75.6±0.2, 76.2±0.5, 74.3±0.3, 65.4±0.4, 68.8±0.5 and 82.5±0.4 percentage for formulations PHG1, PHG2, PHG3, PHG4, PHG5 and PHG6 respectively. The present investigation in this research work, the antifungal activity of polyherbal gel of *Allium cepa* L, *Withania somnifera* and *Spinacia oleracea* Linn were evaluated against *Candida albicans* pathogens used under present study. The polyherbal gel obtained from plant used to suitably dilute upto the concentrations of 100, 50 and 25 milligrams per ml and applied on to the test organism using well diffusion method.

Table 2: % Yield of hydroalcoholic extract

S. No.	Hydroalcoholic Extracts	% Yield (w/w)
1.	*Allium cepa* L	12.36
2.	*Withania somnifera*	8.47
3.	*Spinacia oleracea* Linn	11.66

Table 3: Result of Phytochemical screening of hydroalcoholic extracts

S. No.	Constituents	Hydroalcoholic extracts		
		***Allium cepa* L**	***Withania somnifera***	***Spinacia oleracea* Linn**
1.	Alkaloids	-ve	-ve	-ve
2.	Glycosides	+ve	-ve	+ve
3.	Flavonoid	+ve	+ve	+ve
4.	Diterpenes	-ve	-ve	-ve
5.	Phenol	+ve	+ve	+ve
7.	Carbohydrate	-ve	-ve	+ve
8.	Proteins	+ve	+ve	+ve
9.	Saponins	-ve	+ve	+ve

Table 4: Total Phenolic and Total flavonoid content

S. No.	Solvents→ Bioactive compound↓	Hydroalcoholic extracts		
		***Allium cepa* L**	***Withania somnifera***	***Spinacia oleracea* Linn**
1.	Total Phenol (Gallic acid equivalent (GAE) mg/100mg)	1.074	0.985	1.155
2.	Total flavonoid (Quercetin equivalent (QE) mg/100mg)	0.756	0.639	0.847

Table 5: Results of washability and extrudability

Formulation	Spreadability (gcm/sec)	pH	Viscosity (cps)	% Phenol content
PHG1	14.25±0.25	7.2±0.1	3052±11	75.6±0.2
PHG2	13.15±0.36	7.1±0.2	2950±15	76.2±0.5
PHG3	12.25±0.21	6.9±0.1	2850±10	74.3±0.3
PHG4	13.36±0.45	6.8±0.1	3047±21	65.4±0.4
PHG5	12.25±0.12	6.7±0.1	2950±15	68.8±0.5
PHG6	11.45±0.20	7.0±0.2	2875±23	82.5±0.4

***Average of three determinations (n=3)**

Table 6: Antifungal activity of standard and polyherbal gel formulation against *Candida albicans*

S. No.	Formulation	Zone of inhibition		
		100µg/ml	**50 µg/ml**	**25µg/ml**
1.	Fluconazole	18±0.5	15±0.47	13±0.86
		100mg/ml	**50 mg/ml**	**25mg/ml**
2.	Polyherbal gel	17±0.74	13±0.57	10±0.86

CONCLUSION

In conclusion our results indicated that PHG formulations developed from the combination of Hydroalcoholic extracts of *Allium cepa* L*, Withania somnifera* and *Spinacia oleracea* Linn showed the presences of phenols and flavonoid. The prepared gel possesses antimicrobial activity. The presence of phenols and flavonoid can be beneficial for the treatment of alopecia. Further studies with advanced therapeutic approaches like alopecia and clinical studies are underway.

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