FORMULATION AND EVALUTION OF POLY HERBAL EXTRACT GEL FOR ANTI FUNGAL ACTIVITY

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Abstract

Research in complementary therapies and alternative medicines is increasing and formulating therapeutic medicines based on these findings has become the need of the hour. The present research aims to formulate and evaluate a polyherbal gel containing ***Acalypha indica*** and Plectranthus amboenicus extract. The formulation was designed by using alcoholic as well as aqueous extract of Achyranthes aspera and Argryeia nervosa in varied concentrations. The extracts were subjected to various physicochemical evaluations. The gel was prepared by using a suitable gelling agent- Carbopol. The polyherbal formulation was evaluated for its antifungal activity and it was found that the formulation have elicited promising antifungal activity which was comparable with that of standard drug. However the activity was found to be due to the synergestic blend of herbal drugs. The polyherbal formulation exhibits a promising topical gel for fungal infections of the skin. Thus the present study demonstrates an immense scope for development of such polyherbal formulations and also explores the vast potential to further carry out research by exploiting synergistic effect in herbal extracts

Keywords: Acalypha indica and Plectranthus amboenicus extract, Antifungal activity, Gel

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Introduction

Acalypha indica is one of weed plants that contain important medicinal values for human health applications. It can be found commonly in India, Sri Lanka Thailand and Pakistan. The extracts of various parts of the plant, leaves, roots and stem parts are used for medicinal purposes to treat various diseases such as the eye infections, respiratory problems, rheumatism, and skin problems and to decrease blood sugar level. Different extraction methods are used for obtaining active components from *Acalypha indica*. Generally, Soxhlet extraction has a high efficiency and accuracy but the thermal stress might degrade target photochemical components. Herbal medicines have been playing a vital role in treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. This type of treatment, also known as conventional treatment, was the main source of medical treatment during this time [1]. However, civilization has changed and with it has come the introduction of more advanced techniques and methods, leading the next generations to tend to choose modern treatment over conventional treatments. The information related to conventional treatments are gradually vanishing since the previous generations are getting older and dying without successors. This knowledge is passed on to the next generation, through experiments and observation and oral teaching [2]. Therefore, it is crucial to have proper documentation from the extant practitioners since conventional treatments are an alternative path to treating various types of human diseases [3]. Traditional or conventional medicinal practices based on natural plants have been recognized by the World Health Organization (WHO, 2002) as reliable medicinal sources for therapeutic activities. The medicinal plants are available around backyards, settlements, spreading along roadsides, and house compounds Medicinal plants are recognized as potential source of bioactive compounds. More than 80%of modern drugs are derived directly from sources of plants and microbes. Natural products derived from medicinal plants have wide range of pharmacological significance. Bioactive compounds as they contain therapeutic and their complex nature will able to interact with mammalian cell targets. Phytochemicals naturally isolated from the medicinal plants (MAPs) are used specifically in drug industries. However, these Phytochemicals' have certain limitations of low absorption, high toxicity, and other side effects, bioavailability and efficacy. Irrespective of the advantages of synthetic, combinatorial chemistry and molecular modelling, they remain an important source for new drugs discovery Aim of the current research work is to formulation and evaluation of poly herbal extract gel for anti-fungal activity

Objective

To formulate and evaluate poly herbal extract gel containing Sodium CMC, Carbopol and HPMC. To study the effect of different polymer on the release of plant extract from the formulation. To study effect of physicochemical parameters of the formulation like, pH Measurement, Homogeneity, Viscosity, Spreadability etc.

Need for the study:

Topical application of the drug at the affected site offers potential advantages of delivering the drug directly to the site of action23. Skin injuries or local infections can best be treated by application of products, which form transparent water vapour and air permeable film over the skin surfaces from which the drug releases continuously to the site24. The gels are becoming more popular due to use of application and better percutaneous absorption than other semi-solid preparations Hence, a study on formulation of anti-fungal activity of poly herbal extract gels with different polymers and permeation enhancers at various concentrations is selected.

Plan of Work

Selection of Plants and Excipients

Procurement of Plants And Excipients

Experimental Work

Compatibility Testing of Plant with Excipients

Preparation of gels

Evaluation of gels

Physical appearance

pH of formulations

Spreadability

Viscosity

Stability Studies

Anti-fungal activity

MATERIALS AND METHODS:

Formulation & evaluation of gels

Preparation of HPMC gel: The gel based on HPMC system was prepared as follows:

Accurately weighed amount of HPMC was placed in known amount of distilled water, DMSO and Propylene glycol. After complete dispersion, the polymer solution was kept in dark for 24 hours for complete swelling. Accurately weighed amount of poly herbal extract was dissolved in a specified quantity of suitable solvent.The poly herbal extract was added slowly to the aqueous dispersion of polymer with the help of high speed stirrer (500 rpm) taking precaution that air did not entrap. Finally, the remaining ingredients were added to obtain a homogeneous dispersion of gel. The formed gel was then neutralized by sufficient quantity of triethanolamine. Final volume was adjusted by Distilled water.

Preparation of Carbopol gel:

The gel based on Carbopol system was prepared as follows:

Accurately weighed amount of Carbopol was placed in known amount of distilled water, DMSO and Propylene glycol After complete dispersion, the polymer solution was kept in dark for 24 hours for complete swelling. Accurately weighed amount of poly herbal extract was dissolved in a specified quantity of suitable solvent.

The drug solution was added slowly to the aqueous dispersion of polymer with the help of high speed stirrer (500 rpm) taking precaution that air did not entrap.

Finally, the remaining ingredients were added to obtain a homogeneousdispersion of gel.

The formed gel was then neutralized by sufficient quantity of triethanolamine. Final volume was adjusted by Distilled water.

Preparation of Na CMC gel:

The gel based on Sodium CMC system was prepared as follows:

Accurately weighed amount of Sodium CMC was placed in known amount of distilled water, DMSO and Propylene glycol After complete dispersion, the polymer solution was kept in dark for 24 hours for complete swelling. Accurately weighed amount of poly herbal extract was dissolved in a specified quantity of suitable solvent.

Table 1.formulation and evaluation of a polyherbal extract gel

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9
DRUG	1g	1g	1g	1g	1g	1g	1g	1g	1g
CARBOPOL	0.5 g	1 g	1.5 g	-	-	-	-	-	-
HPMC	-	-	-	0.5 g	1 g	1.5 g	-	-	-
NaCMC	-	-	-	-			0.5 g	1 g	1.5 g
OLEIC ACID	100mg	100m	100mg	100mg	100mg	100mg	100mg	100mg	100mg
DMSO	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
PG	1.5ml	1.5ml	1.5ml	1.5ml	1.5ml	1.5ml	1.5ml	1.5ml	1.5ml
TEA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
PROPYL PARABEN	10mg	10mg	10mg	10mg	10mg	10mg	-	-	-
DISTILLEDWATER	Upto10g	Upto10g	Upto10g	Upto10g	Upto10g	Upto10g	Upto10g	Upto10g	Upto10g

1. The drug solution was added slowly to the aqueous dispersion of polymer with the help of high speed stirrer (500 rpm) taking precaution that air did not entrap.
2. Finally, the remaining ingredients were added to obtain a homogeneousdispersion of gel.
3. The formed gel was then neutralized by sufficient quantity of triethanolamine.
4. Final volume was adjusted by Distilled water.

EVALUATION OF POLY HERBAL EXTRACT GELS:

Clarity

The clarity of various formulations was determined by visual inspection under black and white background and it was graded as follows; turbid: +, clear: ++, very clear (glassy): +++.

pH :

2.5 grams of gel was accurately weighed and dispersed in 25 ml of distilled water. The pH of dispersion was measured by using digital pH meter (Systronics µ pH system 362).

Homogeneity: All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container for their appearance and presence of any aggregate.

Viscosity:

Brookfield viscometer was used to determine viscosity. The sufficient quantity of ointments, gels and creams were filled in wide mouth jar separately. The height of the ointments were filled in the jar should sufficiently allow to dip the spindle. The RPM of the spindle was adjusted to 2.5 RPM. The viscosities of the formulations were recorded.

Spreadability:

Spreadability of the formulations was determined by an apparatus suggested by Mutimer et al, which was suitably modified in the laboratory and used for the study. It consists of a wooden block which was provided by a pulley at one end. A rectangular ground glass plate was fixed on the block. An excess of gels (about 2g) under study was placed on this ground plate. The gel was then sandwiched between this plate and another glass plate having the dimensions of the ground plate and provided with the hook. A 300g weight was placed on the tope of two plates for five minutes to expel air and to provide a uniform film of the gel between the plates. Excess of the gel was scrapped off from the edges. The top plate was then subject to a pull of 30g. With the help of a string attached to the hook and the time (in sec) required by the top plate to cover a distance of 10cms be noted. A shorter the time interval indicates better Spreadability.

The Spreadability was determined by special apparatus and it was calculatedusing the formula

$S = ML/T$

Where:

S = Spreadability

M = Weight tide to upper slide

L = Length moved on the glass slide

T = Time taken to separate the slide completely from each other

Stability Study:

Stability testing of substance, drug and drug product begins as a part of drug discovery and synthesis or development-preformulation efforts and ends only with the demise of the compound or commercial product. FDA and ICH specifies the guidelines for stability testing of new drug products, as a technical requirement for the registration ofpharmaceuticals for human use.

The ICH Tripartite Guidelines have established that long term stability testing should be done at 25ºC/60% RH; stress testing should be done at 40ºC/75% RH for 6 months. If significant change occurs at these stress conditions, then the formulation should be tested at an intermediate condition i.e. 30ºC/75%RH. The following table shows different temperatures and period of stability testing.

Table: 2. Storage Condition for stability study`

Conditions	Temperature	Duration
Freezer Conditions	-20º to –10ºC	-
Refrigerator	2ºC to 8ºC	-
Controlled room temperature	15ºC to 30ºC	Till expiry date

Microbial and Anti Fungal Activity:

Formulation and evaluation of a poly herbal extract gel for antifungal activity involves several steps. Here's a general outline of the process:

Collection and identification of polyherbal:

Identify a suitable source of polyherbal, which could be a specific plant, marine organism, or any other natural material known for its antifungal properties.

Extraction of polyherbal: Extract the active compounds from the polyherbal using a suitable solvent or extraction method. This may involve grinding, maceration, or other extraction techniques to obtain a concentrated polyherbal extract.

Gel formulation: Develop a gel formulation to incorporate the polyherbal extract. The choice of

gel base can vary depending on the desired characteristics such as viscosity, stability, and compatibility with the extract. Common gel bases include carbomers, cellulose derivatives, and natural polymers like alginate or gelatin.

Optimization of gel formulation: Conduct preliminary experiments to optimize the gel formulation by adjusting the concentration of the polyhedra extract, viscosity modifiers, gelling agents, and other excipients. The aim is to achieve a stable gel with suitable rheological properties for easy application.

Antifungal assay: Perform antifungal assays to evaluate the efficacy of the polyhedra gel extract. This can be done using various methods such as agar diffusion, broth dilution, or microdilution techniques. Use different fungal strains to assess the broad- spectrum activity of the gel extract.

Measurement of antifungal activity: Quantify the antifungal activity of the gel extract by measuring parameters such as minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). MIC is the lowest concentration of the extract that inhibits fungal growth, while MFC determines the concentration that kills the fungus.

Comparison with standard antifungal agents: Compare the antifungal activity of the polyhedra gel extract with commercially available antifungal agents, such as fluconazole or amphotericin B. This provides a reference point for evaluating the effectiveness of the extract.

Stability and safety assessment: Evaluate the stability of the polyhedra gel extract under different storage conditions, such as temperature and light exposure. Additionally, assess the safety of the gel formulation through skin irritation tests and cytotoxicity assays.

Statistical analysis: Perform statistical analysis to determine the significance of the results obtained. This helps establish the reliability of the antifungal activity exhibited by the polyhedra gel extract.

Further studies: If the polyhedra gel extract shows promising antifungal activity, additional studies such as in vivo evaluations, time-kill kinetics, and formulation optimization can be conducted to enhance its effectiveness and potential for commercialization.

It is important to note that the specific protocols and methodologies used in each step may vary depending on the nature of the polyhedra extract, gel formulation, and available r**e**sorceses**"**

RESULTS & DISCUSSION

Table 3.evaluation of poly herbal extract gel

FORMULATIONS	APPEARANCE	CLARITY	HOMOGENEITY
F1	Opaque,off white	++	Good
F2	Opaque,off white	++	Good
F3	Translucent, yellowish	++	Good
F4	Translucent, yellowish	++	Good
F5	Translucent, yellowish	++	Good
F6	Translucent, yellowish	++	Good
F7	Opaque, slightyellowish	++	Good
F8	Opaque, slightyellowish	++	Good
F9	Opaque, slight yellowish	++	*Good*

Physical Appearance:

The physical appearance of the formulations was checked and compared visually and by physical observation. The results are given in Table 3. The carbopol formulations are found to be opaque, off white, HPMC and Na CMC formulations are found to be translucent, yellowish and Methyl cellulose formulations are found to be opaque, slightyellowish.

Table 4. pH of the Formulations:

S.no:	Formulations	pH		
		Trial I	Trial II	Average
1	F1	7.4	7.3	7.35
2	F2	7.6	7.6	7.6
3	F3	6.7	6.5	6.6
4	F4	7	7.1	7.05
5	F5	7.5	7.3	7.4
6	F6	7.8	7.6	7.7
7	F7	7.7	7.3	7.5
8	F8	7.8	7.4	7.6
9	F9	7.5	7.6	6.6

pH of formulations:
The pH of the formulations was determined by using the pH meter and observations are shown in the Table 4. The pH of the formulations was found to be amenable for application on the skin, because skin pH lies in this range.

Table 5. Spreadability of the formulations

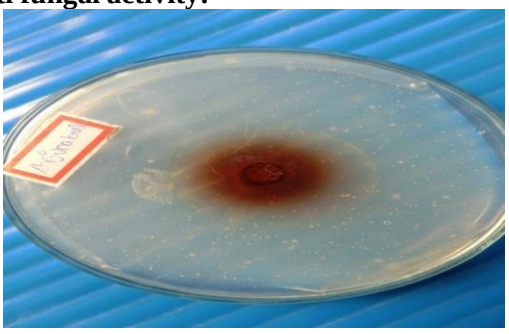
S.No:	Formulations	Spreadability (gm/sec)
1	F1	13.60
2	F2	13.95
3	F3	15.25
4	F4	15.70
5	F5	16.15
6	F6	16.50
7	F7	14.75
8	F8	15
9	F9	13.75

Spreadability of the formulations: The results of the spreadability were shown in Table 5. The gels showed better spreadability. Out of the eight gel formulations F1&F2 showed the better spreadability among all the formulations

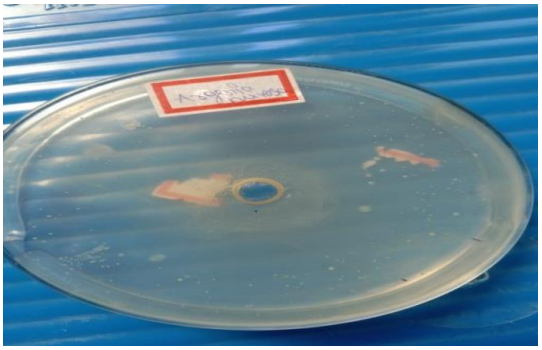
Table 6. Viscosity Determination:

S.no:	Formulations	Viscosity(cps)		
		Trial I	Trial II	Average
1	F1	2450	2440	2445
2	F2	4700	4750	4725
3	F3	7630	7600	7615
4	F4	15000	15020	15010
5	F5	9010	9030	9020
6	F6	18200	18220	18210
7	F7	5450	5420	5435
8	F8	10880	10850	10865
9	F9	10909	10911	10910

Anti fungal activity:



Arjuna Bark



Argyria Nervosa

Mixed Gel

Summary

Utility of gel based drug delivery systems are being employed in recent past for therapeutic effectiveness of topical applied drugs. Topical route for poly herbal gel was selected up to avoid GIT irritation and to maximize the drug concentration at the site ofaction.

In the present study an attempts were made to formulate and evaluate topical gel of poly herbal gel. In our preliminary study the standardization of poly herbal gel was carried out for purity and identity. The preformulation studies include identification, pH, solubility were carried out. All the gels were evaluated for their appearance, pH, viscosity, spredability, Visually Carbopol gels were Opaque, off white The pH range of Carbopol gels, HPMC gels, NaCMC gels were found to be suitable for topical application. The plant extract of formulated. The viscocity measurement was done for selected gels using Brookfield viscometer at room temperature.

The order of spreadibility was Carbopol > HPMC > NaCMC. Among all gel formulations the drug release was greater in Carbopol934. The pH of gel was found to be in range of 7.4 to 7.6. The drug release mechanism from all the formulated gels was found to be predominantly, the stability studies of formulated gels were carried out at room temperature. Result showed no significant variation with respect to drug content, pH, viscosity, spreadability.

Conclusion

The data obtained from the study of formulation and evaluations of Poly Herbal Extract gels, the following conclusion were made The drug content, pH, and spreadability was found within acceptable range. The viscosity of gel formulation was increased as concentration of gelling agent increased. Based on the results the gels which are prepared with carbapol was selected as optimized formulations, because of their better spreadability and as the concentration of carbapol increased spreadability also increases upto certain concentration, so finally F2 formulation was considered as an optimized formulation**.** The results of anti fungal activity, the gels showed better antifungal activity. on Comparsion of anti fungal activity of individual extracts with poly herbal extract gel ,gel shows almost equal and better anti fungal activity.

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