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Formulation, development and evaluation of polyherbal gel for topical infection

Deepak Soni[1], Mukesh Kumar Patel[1], Ashish Manigauha[1], Arun Pandey[2*]

[1]*Mittal Institute of Pharmacy, Bhopal-462038, MP (India)

[2]*Mittal Group of Institutions, Bhopal-462038, MP (India)

Abstract

Objective: The purpose of this research was to formulate polyherbal gel, a combination of *Acorus calamus*, *Annona squamosa* and *Chenopodium album* and to evaluate anti-acne activity against *Propionibacterium acnes*.

Methods: Topical gel formulations were prepared using carbopol 940 as a gelling agent. Test parameters for topical gel include organoleptic, extrudability, spreadability and pH. The anti-acne activity test of *Acorus calamus*, *Annona squamosa* and *Chenopodium album* using agar well diffusion method was carried out.

Results: The results of this study showed that *Acorus calamus*, *Annona squamosa* and *Chenopodium album* extracts had anti-acne activity. Anti-acne activity combination of *Acorus calamus*, *Annona squamosa* and *Chenopodium album* extract is synergistic. The results of preliminary phytochemical investigation showed the presence of carbohydrates, cardiac glycosides, phenols and flavonoids. Quantitative analysis of total phenolic and flavonoid content compounds showed high content of phenolic compounds and flavonoids. Other parameters such as spreadability, pH, viscosity and extrudability were found to be well within the limits.

Conclusion: Hydroalcoholic extracts of *Acorus calamus*, *Annona squamosa* and *Chenopodium album* can be formulated as hydrogels with satisfactory physicochemical parameters for anti-acne activity.

Keywords: *Acorus calamus*, *Annona squamosa*, *Chenopodium album*, Polyherbal gel, Anti-acne activity, *Propionibacterium acnes*

Introduction

The Gram-positive bacterium *Propionibacterium acnes* (*P. acnes*) are considered as a skin commensal, which preferentially resides in sebaceous follicles. The bacterium's presence on the human skin is suggested to be beneficial, for instance due to the ability to lower the skin pH by acidic fermentation products, thereby protecting the follicles against colonization by harmful pathogens [1]. However, several lines of evidence indicate that, under certain conditions, *P. acnes* can act as an opportunistic pathogen [1, 2]. Fungal infection of the skin is now a day's one of the common dermatological problem. There are wide choices for treatment from solid dosage to semisolid dosage form and to liquid dosage formulation. Among the topical formulation, gels have widely accepted in both cosmetics and pharmaceuticals. Within the major group of semisolid preparations, the use of gels has expanded both cosmetics and pharmaceutical preparations [3]. Polyherbal are the formulations containing two or more than two herbs are called polyherbal formulations. The popularity of polyherbal formulation is due to its high effectiveness towards a number of diseases.

Traditional medicine, since ages have been an important source of potentially useful new compounds to develop chemotherapeutic agents and nature is contributing to an impressive number from which numbers of modern drugs have been isolated [4]. *Acorus calamus* is mentioned in Ayurveda and belongs to the genus *Acorus L.* of the family Acoraceae and is widely distributed temperate to sub temperate regions. It is commonly used in traditional medicinal systems of Asian and European countries to treat appetite loss, diarrhoea, digestive disorders, bronchitis indigestion, chest pain, nervous disorders [5]. *Annona squamosa* belongs to Annonaceae family is a deciduous plant used in traditional medicines. Studies on *Annona squamosa* have reported antibacterial, antidiabetic, antitumor, anti-malarial, anthelmintic and anti-genotoxic potential [6]. *Chenopodium album* (L.) of the family Chenopodiaceae belongs to the genus Chenopodium. Medicinally, this plant has been used to treat various symptoms attributable to nutritional deficiencies. The leaves of *C. album* are being used in traditional medicines. It has been found to have antipruritic and antinociceptic [7], sperm immobilizing agent [8], cryptomeridiol and 8-alphaacetoxycyryptomeridiol as growth promoting activity.

Thus the aim of the study was to develop a topical polyherbal formulation consisting of *Acorus calamus*, *Annona squamosa* and *Chenopodium album* extract and to evaluate anti-acne activity against *Propionibacterium acnes*. The objective was to evaluate the formulation for its pharmaceutical as well as pharmacological properties.

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Materials and methods

Materials

All reagents and chemicals used were of analytical reagent grade.

Methods

Plant material

Rhizome of *Acorus calamus*, seeds of *Annona squamosa* and leaves of *Chenopodium album* was collected from local area of Bhopal (M.P.) in the month of February, 2018 and authenticated from Department of Botany, Safia College Bhopal (Voucher No. 280/bot/saf/11) by the Head of Department of botany Dr. Zia Ul Hasan Professor of Safia College of Science Bhopal.

Extraction

Dried powdered rhizome of *Acorus calamus*, seeds of *Annona squamosa* and leaves of *Chenopodium album* has been extracted with hydroalcoholic solvent using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40 °C.

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

Phytochemical screening of the extracts was carried out according to the standard procedures of Trease [9]. The hydroalcoholic extracts were subjected to preliminary phytochemical screening to identify the various phyto-constituents present in them i.e. alkaloids, terpinoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins and tannins.

TLC (Thin Layer Chromatography) profile

For the separation of different phytochemical compounds in the hydroalcoholic extract of *Acorus calamus*, *Annona squamosa* and *Chenopodium album*, the extract was spotted manually using a capillary tube on precoated silica gel G TLC plates (15x5 cm with 3 mm thickness). The spotted plates were put into a solvent system to detect the suitable mobile phase as per the method of Wagner et al., [10, 11]. After the separation of phytochemical constituents, the spraying reagents such as Dragendorff reagent, 10% ethanolic sulphuric acid, 10% sulphuric acid, 5% ferric chloride, Kedde reagent, vanillin phosphoric acid reagent and vanillin sulphuric acid reagent were used to identify the respective compounds. The colour of the spots were noted and Rf values were calculated by using the following formula: Retention time (Rf) = Distance travelled by the solute/Distance travelled by the solvent

Quantitative phytochemical analysis

Total phenols determination

The amount of total phenolic contents of extract of *Acorus calamus*, *Annona squamosa* and *Chenopodium album* was determined by the spectrophotometric method of Kim et al., [12] with slight modification. A diluted hydroalcoholic extract (1 ml) or gallic acid standard phenolic compound was added to a 25 ml volumetric flask, containing 9 ml of distilled water. 1 ml of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was mixed in to the test sample. The solution was diluted to 25 ml distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23 °C, after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The Total phenolic content was expressed as milligrams of Gallic acid equivalents (GAE) per gram of dried sample.

Total flavonoid determination

The total flavonoid assay was conducted according to Katasani Damodar [13]. Total flavonoid content was determined by using aluminium chloride colorimetric method. Hydroalcoholic extract of *Acorus calamus*, *Annona squamosa* and *Chenopodium album* (0.5 ml) was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 510 nm using UV-Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 0 to 150 µg/ml in methanol. The total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dried sample.

Preparation of gel containing extract

1 g of carbopol 940 was dispersed in 50 ml of distilled water. It was kept aside to swell, which was further stirred to form a gel. Required quantity of methyl paraben was dissolved in distilled water with the aid of heat on water bath. Solution was cooled and propylene glycol was added to it. Further required quantity of hydroalcoholic extract of *Acorus calamus*, *Annona squamosa* and *Chenopodium album* at different concentration was mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. All the ingredients were mixed properly and with continuous stirring. Triethanolamine was added drop wise to the formulation for the adjustment of skin pH (6.8-7) and also to obtain a gel at required consistency. The same method was followed for the preparation of control sample. The method describes above and the formulae were tabulated in Table 1.

Arun Pandey, E-mail:- arun3177@gmail.com

Table 1: Formulation of polyherbal gel

Ingredients (%)	F1	F 2	F3	F4	F5	F6
Acorus calamus extract (mg)	500	500	500	500	500	500
Annona squamosa extract (mg)	500	500	500	500	500	500
Chenopodium album extract (mg)	500	500	500	500	500	500
Carbopol 940	0.25 mg	0.5 mg	0.75 mg	1.0 gm	1.25 gm	1.5 gm
Polyethylene glycol	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Methyl paraben	0.08 mg	0.08 mg	0.08 mg	0.08 mg	0.08 mg	0.08 mg
Triethanolamine	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Distilled water (q.s)	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

Evaluation of gel

Following parameters were used for the evaluation of gel

Homogeneity

All developed gels were tested for visual homogeneity inspection after the gels have been set in the container. They were tested for their appearance.

pH

The pH values of 1 gel were measured by a pH meter (Lab Electronics Ltd.)

Extrudability study

After the gels were set in the container, formulations were filled in the collapsible tubes. The extrudability of formulation was determined in terms of weight in grams required to extrude a 0.5 cm ribbon of gel in 10 sec.

Viscosity

The viscosity of the gel formulations was determined using Brookfield viscometer with spindle no. 4 at 10 rpm.

Spreadibility

The spreadibility of the gel formulations was determined at 24 h after permeation, by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm × 20 cm) after one min.

Anti-acne activity

Propionibacterium acnes were obtained from Microbial Culture Collection, National Centre for Cell Science, Pune, Maharashtra, India.

Media preparation (broth and agar media)

Compositions of broth and agar media are mention in table 2 & table 3

Table 2: Composition of nutrient agar media

Composition	Weight
Agar	1.5 gms
Beef extract	0.3 gms
Peptone	0.5 gms
Sodium chloride	0.55 gms
Distilled water	to make 100 ml

Table 3: Composition of potato dextrose agar media

Composition	Weight
Potato infusion	20 gms
Dextrose	2 gms
Agar	1.5 gms
Distilled water	to make 100 ml

Method of preparation

This agar medium was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients were transferred to flask containing required quantity of distilled water and heated to dissolve the medium completely.

Sterilization culture media

The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch2 (121°C) for 15 min.

Preparation of plates

After sterilization, the media in flask was immediately poured (20 ml/ plate) into sterile petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37 ºC overnight to check the sterility of plates. The plates were dried at 50 ºC for 30 min before use.

Revival of the microbial cultures

The microbial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques the lyophilized cultures are inoculated in sterile nutrient and potato dextrose broth than incubated for 24 hrs at 37 ºC. After

Arun Pandey, E-mail:- arun3177@gmail.com

incubation the growth was observed in the form of turbidity. These broth cultures were further inoculated on to the nutrient and potato dextrose agar plates with loop full of microbes and further incubated for next 24 hrs at 37 °C to obtain the pure culture and stored as stocks that are to be used in further research work.

Antimicrobial sensitivity

The antimicrobial sensitivity test was employed on to *Propionibacterium acnes* used under present study with polyherbal gel obtained from hydroalcoholic extracts of rhizome of *Acorus calamus*, seeds of *Annona squamosa* and leaves of *Chenopodium album*. For this experiment 6 mm diameter wells, stock of 100 mg/ml of extract separately applied on it. A nutrient and potato dextrose agar plate was seeded with particular microbes with the help of spread plate technique prior and left for 5 min then incubated for 24 hrs at 37 °C. After incubation, plates were observed to see the sensitivity of polyherbal gel towards test bacteriums at particular concentration in the form zone of inhibition.

Antibiogram studies

Broth cultures of the pure culture isolates of those test microorganisms which are sensitive towards the polyherbal gel used in present study were prepared by transferring a loop of culture into sterile nutrient and potato dextrose broth and incubated at 37°C for 24-48 hrs. A loop full was taken from these broths and seeded onto sterile nutrient and potato dextrose agar plates through sterile cotton swab to develop diffused heavy lawn culture.

The well diffusion method was used to determine the antimicrobial activity of the polyherbal gel obtained from hydroalcoholic extracts of rhizome of *Acorus calamus*, seeds of *Annona squamosa* and leaves of *Chenopodium album* using standard procedure [14]. There were 3 concentration used which are 25, 50 and 100 mg/ml for polyherbal gel in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37 ºC for 24 hrs and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

Results and discussion

To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from hydroalcoholic as solvent is depicted in the table 4.

Table 4: Percentage yield of hydroalcoholic extract

S. No.	Solvent	% Yield (w/w)
1	*Acorus calamus*	7.56%
2	*Annona squamosa*	1.80%
3	*Chenopodium album*	2.16%

Table 5: Result of phytochemical screening of hydroalcoholic extracts

S. No.	Constituents	*Acorus calamus*	*Annona squamosa*	*Chenopodium album*
1.	Alkaloids	-ve	-ve	+ve
2.	Glycosides	-ve	-ve	-ve
3.	Flavonoids	+ve	+ve	-ve
4.	Diterpenes	+ve	+ve	+ve
5.	Phenolics	+ve	+ve	+ve
6.	Amino acids	-ve	-ve	-ve
7.	Carbohydrate	-ve	+ve	+ve
8.	Proteins	-ve	-ve	-ve
9.	Saponins	+ve	-ve	+ve
10.	Oils and fats	-ve	-ve	-ve

+ve= Positive; -ve=Negative

Table 6: TLC of extracts

Hydroalcoholic extracts	Toluene: Ethyl acetate: Formic acid (5:4:1) Quercetin (Rf value)	Toluene: Ethyl acetate: Formic acid (7:5:1) Gallic acid (Rf value)
Acorus calamus	0.65	0.478
Annona squamosa	0.649	0.47
Chenopodium album	0.651	0.477

Arun Pandey, E-mail:- arun3177@gmail.com

A small portion of the dried extracts were subjected to the phytochemical test to test for alkaloids, glycosides, tannins, saponins, flavonoids and steroids separately for extracts of all samples. Small amount of each extract was suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the table 5.

Results of comparative thin layer chromatography of hydroalcoholic extract confirmed the presence of quercetin as flavonoids and gallic acid as phenol compound in the extract. The developed TLC methods will help the manufacturer for quality control and standardization of herbal formulations, such finger printing is useful in differentiating the species from the adulterant and act as biochemical markers for this medicinally important plant species in the Pharma industries and plant systematic studies. The data presented here could be helpful in standardizing extracts of these plants.

Phenols and flavonoids seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. The content of total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.011X+0.011, R2= 0.998, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100 mg) using the equation based on the calibration curve: Y=0.040X + 0.009, R2=0.999, where X is the quercetin equivalent (QE) and Y is the absorbance.

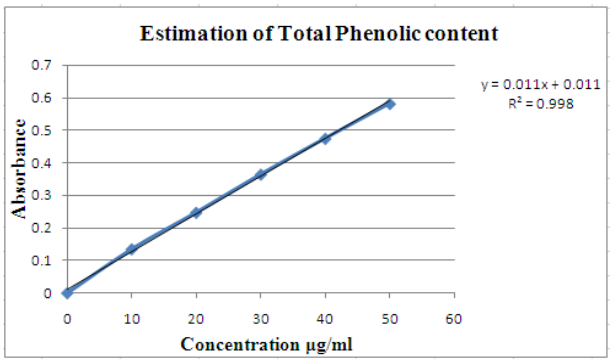

Figure 1: Estimation of total phenolic content

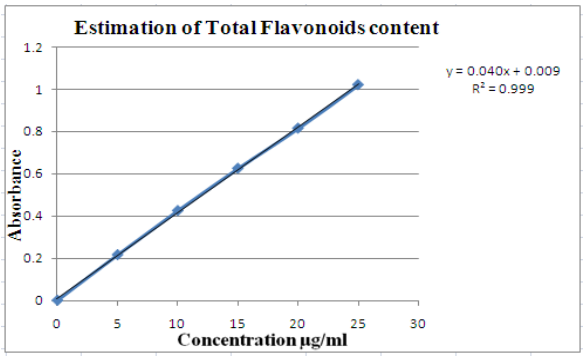

Figure 2: Estimation of total flavonoids content

Table 7: Calibration curve of gallic acid

S. No.	Concentration	Absorbance
0	0	0
1	10	0.135
2	20	0.247
3	30	0.364
4	40	0.474
5	50	0.581
6	Extract	0.148

Table 8: Calibration curve of quercetin

S. No.	Concentration	Absorbance
0	0	0
1	5	0.216
2	10	0.425
3	15	0.625
4	20	0.815
5	25	1.021
6	Extract	0.752

Results of evaluation of formulation of polyherbal gel are depicted in the table 10. In the above formulations of gels, it has been noted that all of them has clear colour, No clogging, good homogeneity and smooth texture. Results of washability and extrudability of polyherbal gel are depicted in the table 11. Results of spreadibility of polyherbal gel are depicted in

Arun Pandey, E-mail:- arun3177@gmail.com

the table 12. The formulation F5 has good viscocity. Results of phenol content of polyherbal gel are depicted in the table 12. In all above formulations of gel the spreadibility of F5 was found good. Results of determination of pH of polyherbal gel are depicted in the table 12. Results of determination of viscosity of polyherbal gel are depicted in the table 12.

The anti-acne activity of polyherbal gel of hydroalcoholic extracts of rhizome of *Acorus calamus*, seeds of *Annona squamosa* and leaves of *Chenopodium album* was evaluated against *Propionibacterium acnes* pathogens and results of the experiment are being concluded in the table 13.

Table 9: Total phenolic and total flavonoids content

S. No.	Solvents⟶ Bioactive compound↓	Hydroalcoholic extracts		
		Acorus calamus	***Annona squamosa***	***Chenopodium album***
1.	Total phenol (Gallic acid equivalent (GAE) mg/100mg)	1.17	1.2	2.06
2.	Total flavanoid (Quercetin equivalent (QE) mg/100mg)	0.45	1.85	0.10

Table 10: Results of physical characteristics

Formulation	Colour	Clogging	Homogeneity	Texture
F1	Brown	Absent	Good	Smooth
F2	Brown	Absent	Good	Smooth
F3	Brown	Absent	Good	Smooth
F4	Brown	Absent	Good	Smooth
F5	Brown	Absent	Good	Smooth
F6	Brown	Absent	Good	Smooth

Table 11: Results of washability and extrudability

Formulation	Washability	Extrudability
F1	Good	Average
F2	Good	Average
F3	Good	Average
F4	Good	Average
F5	Good	Average
F6	Good	Average

Table 12: Determination of spreadability, pH, viscocity and % phenolic content

Formulations	Spreadability (gcm/sec)	pH	Viscosity (cps)	% Phenolic content
F1	14.56±0.45	6.75±0.32	2565±12	74.45±0.45
F2	16.56±0.32	6.32±0.35	2872±35	79.12±0.65
F3	13.25±0.46	6.87±0.65	3012±47	75.65±0.58
F4	13.10±0.58	6.89±0.45	3125±45	78.98±0.65
F5	12.25±0.36	7.02±0.58	3256±32	84.65±0.45
F6	14.65±0.25	6.85±0.45	3314±14	74.12±0.48

*n=3±SD

Table 13: Anti-acne activity of standard and polyherbal gel formulation against *Propionibacterium acnes*

S. No.	Standard/Formulation	Zone of inhibition		
		100µg/ml	**50 µg/ml**	**25µg/ml**
1.	Clindamycin (STD)	33±0.45	32±0.76	22±0.45
1.	Polyherbal gel	34±0.69	30±0.58	25±0.45

The use of traditional medicine is increasing day by day due to the high cost of the allopathic medicines and their potential side effects [15, 16]. The results of the present study also supplement the folkloric usage of the studied plants which

Arun Pandey, E-mail:- arun3177@gmail.com

possess several known and unknown bioactive compounds with bio-activity. By isolating and identifying these bioactive compounds new drugs can be formulated to treat various diseases and disorders. TLC analysis revealed the presence of different types of phytochemicals based on the number of spots. The compounds present in the mixture are to be screened and purified for qualitative estimation.

Conclusion

Medicinal plants are very important to human beings in preserving our health. The use of antibiotics to control diseases produces adverse toxicity to the host organs, tissues and cells. The toxicity produced by the antimicrobial agents can be prevented by using herbs. In conclusion, the of polyherbal gel of hydroalcoholic extracts of rhizome of *Acorus calamus*, seeds of *Annona squamosa* and leaves of *Chenopodium album* offer potential anti-acne property against bacterial strains. The plant merits further investigation to isolate its active constituents and to establish the activity in animal models. Further studies should be undertaken to elucidate the exact mechanism of action of antimicrobial effect to identify the active ingredients which can be used in drug development program.

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Conflict of Interest: None declared

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Arun Pandey, E-mail:- arun3177@gmail.com