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ORIGINAL RESEARCH

Formulation development of herbal anti acne gel

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Abstract

Antibiotics have been used to treat acne vulgaris. However, antibiotic resistance has been increasing in prevalence within the dermatologic setting. The development of antibiotic resistance includes the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. It is a very good attempt to establish the herbal gel containing hydro-alcoholic extract of Rhizome of *Curcuma longa, Curcuma Caesia* and *Curcuma Amada*. This study revealed that the developed single herbal formulation F3 was comparatively better than other formulation.

Keywords: Herbal, Anti-Acne, *Curcuma longa, Curcuma Caesia, Curcuma Amada*

Introduction

Herbal drugs

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 [1]. 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 [2]. 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. 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. India is known as the "Emporium of Medicinal plants" due to availability of several thousands of medicinal plants in the different bioclimatic zones [3, 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.

Herbal medicine

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care [8]. 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 [9-11]. 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.

In the early 19th century, when chemical analysis first became available, scientists began to extract and modify the active ingredients from plants.

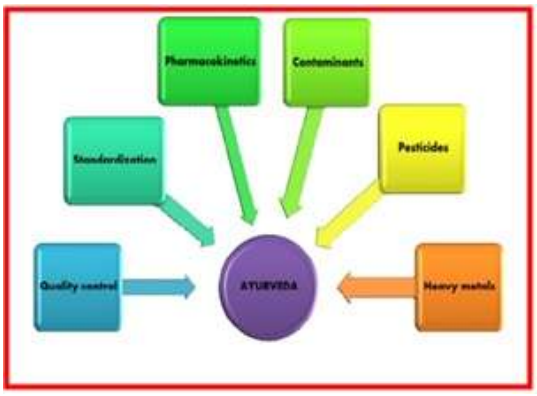

Figure 1: Challenges in herbal formulation

History of herbal medicine

Plants had been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medical systems (such as Siddha, Ayurveda, Unani and TCM) in which herbal therapies were used [12-15]. The consumption of plant-based medicines and other botanicals in the West has increased manifold in recent years. About two centuries ago, our medicinal practices were largely dominated by plant-based medicines. However, the medicinal use of herbs went into a rapid decline in the West when more predictable synthetic drugs were made commonly available. In contrast, many developing nations continued to benefit from the rich knowledge of medical herbalism. For example, Siddha & Ayurveda medicines in India, Kampo Medicine in Japan, traditional Chinese medicine (TCM), and Unani medicine in the Middle East and South Asia are still used by a large majority of people.

Importance of herbal medicine

The points of thought are why common people divert to use the Ayurvedic, Chinese and other herbal medicines? Though it is used all over the world, in India, its use is much more because of their easy accessibility, no expert consultation required, are considered safe to use and also because primary health care services fall short of peoples' need both in qualitative and quantitative terms. We should make all these easily marketed ayurvedic, and other herbal medicines FDA approved and increase public awareness about pros and cons of their uses. The common belief that anything natural is safe is not correct. Herbal Medicines are readily available in the market from health food stores without

prescriptions and are widely used in India, China, USA and all over the world. According to recent survey the majority of people who use herbal medicines do not inform their physicians about their consumptions that can cause abnormal test results and confusion in proper diagnosis. However, natural medicines seem to be barely able to provide convincing alternatives to conventional western medicine for global health-care.

Acne vulgaris is an extremely common skin disorder that affects areas containing the largest oil glands, including the face, back, and trunk. *Propionibacterium acnes* (P. acnes), an anaerobic pathogen, plays an important role in the pathogenesis of acne. It is implicated in the development of inflammatory acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils. Although not a serious threat to general health, acne is one of the most socially distressing skin conditions, especially for adolescents, who must deal with a disfiguring disease that erupts just when sexual maturity makes them most sensitive about their appearance.

Experimental

Plant Profile

1. Curcuma longa Rhizomes

Common Names

Kunyit, Curcuma, Haldi,

Classfication

Kingdom: Plantae
(unranked): Angiosperms
(unranked): Commelinids
Order: Zingiberales
Family: Zingiberaceae
Genus: Curcuma

Figure 2: *Curcuma longa* Rhizomes

Turmeric contains protein (63%), fat (5.1%), minerals (3.5%), carbohydrates (6.94%) moisture (13.1%) and essential oil (0.8%). The steam distillate of turmeric contains curcuminoids.

In the last few decades, efforts have been made to isolate curcuminoids from different sources. They have been shown to be scavengers of free radicals and reactive oxygen species (ROS), such as hydroxyl radicals.

Parts used

Rhizomes, Leave, Root & Stem

Uses

The active compound curcumin is believed to have a wide range of biological

effects including anti-inflammatory, antioxidant, antitumour, antibacterial, and antiviral activities, which indicate potential in clinical medicine.

2. Curcuma Caesia Rhizomes

Common Names

Curcuma Kuchoor, Kali Haldi, Krishna kedar, Yaingang Amuba,

Classfication

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Commelinids
Order:	Zingiberales
Family:	Zingiberaceae
Genus:	Curcuma

Figure 3: *Curcuma caesia* Rhizomes

Curcuma caesia, black turmeric or black zedoary is a perennial herb with bluish-black rhizome, native to North-East and Central India. Black turmeric is also sparsely found in the Papi Hills of East Godavari, West Godavari, and the Khammam districts of Andhra Pradesh. The rhizome of black turmeric has a high economic importance owing to its putative medicinal properties. In west Bengal, the rhizome of the plant is used in Kali Puja, and hence the plant is called Kali haldi. The cultivation and harvest practices are similar to that of common turmeric which is used in recipes. In the fields, the rhizomes are washed thoroughly and are placed in a wide mouthed cauldron.

Parts used

Rhizomes, Leave, Root & Stem

Uses

The rhizomes are used as a rub efficient to rub the body after taking a Turkish bath. It is used in the fresh state-turmeric. The powder of rhizomes is used by tribal women as a face-pack during their engagement and marriage period

3. *Curcuma Amada* Rhizomes

Common Names

Amiyaa haldi, Mango ginger, maamidi, Temu mangga, Ambiya haladi, amragandha,

Classfication

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Commelinids
Order:	Zingiberales
Family:	Zingiberacea
Genus:	Curcuma

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Figure 4: *Curcuma Amada* Rhizomes

Curcuma amada (mango ginger) is a plant of the ginger family Zingiberaceae and is closely related to turmeric. The rhizomes are very similar to ginger but have a raw mango taste. Mango ginger (*Curcuma amada*) *is* a spice of high usage in pickles, sauce, culinary formulations and traditional/folk systems of medicine for therapeutic actions in Asian countries.

Parts used

Rhizomes, Leave, Root & Stem

Uses

Curcuma amada used as various type of ayurvetic preparation like Kandu, Vrana, Kasa, Svasa, The rhizomes are used externally in the form of paste as an application for bruises and skin diseases and combined with other medicines it is useful in improving quality of blood.

Methodology

Crude drugs are derived from natural sources like plants, animals and minerals. It is important that they should be properly identified and characterized for their physical and chemical characteristics and their quality should be enforced.

Plant material collection

Rhizome of *Curcuma longa, Curcuma Caesia* and *Curcuma Amada* were collected from local area of Bhopal (M.P.) in the month of March, 2018.

Extraction of plant material

Dried powdered Rhizome of *Curcuma longa, Curcuma Caesia* and *Curcuma Amada* has been extracted with hydroalcoholic using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40^0C.

Determination of percentage yield

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

Percentage yield = (Weight of Extract/ Weight of powder drug taken) x 100

Phytochemical Screening

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

Test for alkaloids: To the extract dilute hydrochloric acid was added. Then it was boiled and filtered.

i. *Mayer's test*

To 2-3 ml of filtrate, few drops of the Mayer's reagent were added. Formation of cream precipitate indicated the presence of alkaloids.

ii. *Dragendorff's test*

To 2-3 ml of filtrate, few drops of the Dragendorff's reagent were added. Formation of orange brown precipitate indicated the presence of alkaloids.

iii. *Hager's test*

To 2-3 ml of filtrate, few drops of Hager's reagent were added. Formation of yellow precipitate indicated the presence of alkaloids.

Test for carbohydrates

i. *Molisch's test* **(**General test)

In a test tube containing 2 ml of extract, 2 drops of freshly prepared 10 per cent alcoholic solution of α- naphthol was added. Then it was shaken and 2 ml of Conc. sulphuric acid was added from sides of the test tube. So the violet ring was formed at the junction of two liquids, indicated the presence of carbohydrates.

ii. *Fehling's test* (Reducing sugars)

To 2 ml of extract, equal volume of mixture of equal parts of Fehling's solution A and B were added and boiled for few minutes in boiling water bath. Formation of red or brick red coloured precipitate indicated the presence of reducing sugars.

Test for flavonoids

i. *Ferric-chloride test:*

Test solution with few drops of ferric chloride solution shows intense green colour.

ii. *Alkaline reagent test:* To 2 ml of test solution add 2 ml alkali, gives yellow color, which disappears on addition of dil. HCl it disappears, which indicates presence of flavonoids.

Test for proteins

i. Biuret's test (General test)

To 1 ml of test extract, 4% of sodium hydroxide solution and few drops of 1% copper sulphate solution were added. Formation of a violet red colour indicated the presence of proteins

Test for tannins

i. Lead acetate test

Extract solutions were treated with 5% lead acetate solution. Formation of white precipitate indicated the presence of hydrolysable tannins

iii. Gelatin test

3 ml of test solution when treated with gelatine solution (3ml) gave white precipitate.

Table 1: Composition of the the herbal formulation

Ingredients (%)	F1	F2	F3	F4	F5	F6
Curcuma longa extract (mg)	1000	1000	1000	1000	1000	1000
Curcuma Caesia extract (mg)	1000	1000	1000	1000	1000	1000
Curcuma Amada extract (mg)	1000	1000	1000	1000	1000	1000
Carbopol 940 (mg)	250	500	750	250	500	750
Polyethylene Glycol	0.2ml	0.2ml	0.2ml	0.4ml	0.4ml	0.4ml
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 herbal gel

Appearance and consistency

The physical appearance was visually checked for the texture of herbal gel formulations.

Washability

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

Extrudability determination of formulations

The herbal 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.

Determination of Spreadability

An important criterion for anti-acne gels is that it must possess good spreadability. Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application to skin. The therapeutic efficacy of a formulation also depends on its spreading value

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

Anti-acne activity

Pathogenic microbes used

The pathogenic microbes used in the current study are three bacteria obtained from Microbial Culture collection, National Centre for cell science, Pune, Maharashtra, India.

Media preparation (broth and agar media)

Agar - 1.5 gm
Beef extract - 0.3 gm
Peptone - 0.5 gm
Sodium chloride - 0.55 gm
Distilled water - to make 100 ml

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

Sterilization of culture media

The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch^2 (121°C) for 15 minutes.

Preparation of plates

After sterilization, the nutrient agar 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 °C overnight to check the sterility of plates. The plates were dried at 50 °C for 30 minutes before use.

Revival of the bacterial cultures

The Bacterial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques the lyophilized cultures are inoculated in sterile nutrient broth broth than incubated for 24 hours at 37°C. After incubation the growth is observed in the form of turbidity.

Antibiogram studies

Broth cultures of the pure culture isolates of those test microorganisms which are sensitive towards the 100 mg/ml concentration of phyto extract used in present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37 °C for 24-48 hours. The well diffusion method was used to determine the antibacterial activity of the extracts prepared from the Rhizome of *Curcuma longa, Curcuma Caesia* and *Curcuma Amada* using standard procedure. There were 3 concentration used which are 25, 50 and 100 mg/ml for antibiogram studies.

Results and Discussion

Percentage Yield

The crude extracts so obtained after the maceration process, each extracts were further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. 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.

Table 2: % Yield of hydroalcoholic extract

S. No.	Species	% Yield (w/w)
1	*Curcuma longa*	4.9%
2	*Curcuma Caesia*	5.5%
3	*Curcuma Amada*	6.7%

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Phytochemical screening of extract

A small portion of the dried extracts were subjected to the phytochemical test using Kokate (1994) methods to test for alkaloids, glycosides, tannins, saponins, flavonoids and steroids separately for extracts of all samples. Small amount of each extract is 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 3.

Table 3: Phytochemical screening of hydroalcoholic extracts

S. no	Constituents	*Curcuma longa*	*Curcuma Caesia*	*Curcuma Amada*
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

Total Phenolic and flavanoid content estimation

Total Phenolic content estimation (TPC)

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, R^2= 0.998,** where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Total flavonoids content estimation (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **Y=0.040X + 0.009, R^2=0.999**, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 4: Evaluation of herbal gel

Formulation	Colour	Washability	Extrudability	Texture
F1	Brown	Good	Good	Smooth
F2	Brown	Good	Good	Smooth
F3	Brown	Good	Good	Smooth
F4	Brown	Poor	Good	Smooth
F5	Brown	Poor	Good	Smooth
F6	Brown	Poor	Good	Smooth

Antibiogram studies

The formulation F3 was suitably diluted upto the concentrations of 100 mg/ml, 50 mg/ml and 25mg/ml microgram per ml and applied on to the test organism using well diffusion method.

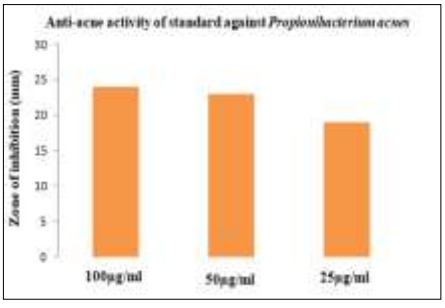

Figure 5: Anti-acne activity of standard against *Propionibacterium acnes*

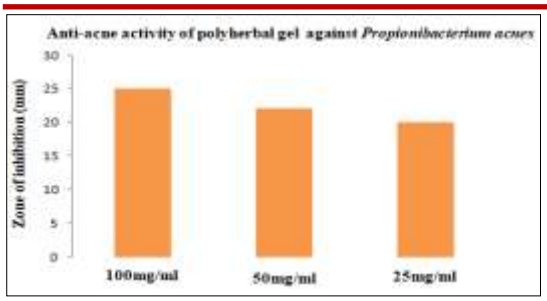

Figure 6: Anti-acne activity of F3 against *Propionibacterium acnes*

CONCLUSION

Antibiotics have been used to treat acne vulgaris. 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. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. It is a very good attempt to establish the herbal gel containing hydro-alcoholic extract of Rhizome of *Curcuma longa, Curcuma Caesia* and *Curcuma Amada*. This study revealed that the developed single herbal formulation F3 was comparatively better than other formulation.

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