Original Research

IN VITRO ASSESSMENT OF POLYHERBAL TOPICAL GEL FORMULATION OF ANTI-ACNE, ANTI-MICROBIAL AND ANTIOXIDANT ACTIVITY

***Priyanka Kantivan Goswami[1], Dr. Anubha Khale[1], Dr. Rashmi Srivastava[2]**

[1]H.K. College of Pharmacy, Oshiwara, Jogeshwari (W), Mumbai: 400102.
[2]Mumbai Education Trust's Institute of Pharmacy, Bandra (W), Mumbai: 400050.

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ABSTRACT

Acne vulgaris is one of the most common disease of the skin disorder characterize by non-inflammatory comedones or inflammatory papules, pustules and nodules. Although acne does not pose serious threat to general health, it is one of the most socially distressing conditions especially for adolescents.Acne therapy includes prolonged use of comedolytic agents, antibiotics and anti-inflammatory agents that are known to cause many side effects. Moreover, the widespread and long term use of antibiotics over the years has unfortunately led to emergence of resistant strains. To avoid side effects, traditional or herbal formulations are preferred. The anti-microbial, anti-acne and antioxidant activity of polyherbal formulation containing methanolic extracts of Neem leaves (*Azadirchataindica*, Family: Meliaceae), Turmeric (*Curcuma longa*, Family: Zingiberaceae), Coriander fruits (*Coriandrumsativum*, Umbelliferae) and Garlic (*Allium sativum*, Liliaceae) were determined. The antioxidant components analyzed were polyphenols, flavonoids and tannins. Antioxidant assays such as reducing power was carried out for formulation. Total Polyphenols, Flavonoids and Tannin were estimated. Higher absorbance indicated greater reducing capacity in 5% formulation. The antioxidant activities were correlated with the total phenolic content. Anti-microbial activity against gram negative microorganisms such as *Escherichia coli* was studied. The MIC is recorded as the lowest concentration of drug which showed clear fluid without turbidity. Minimum inhibitory concentration of *extract* ranged from 10 to 80 µg/ml. Formulation containing extracts, showed significant zone of inhibition for 0.5, 1, 2.5, 5% of which 5% showed maximum zone of inhibition of 19 mm as compared to marketed preparation. The present investigation revealed that gel formulation has potential antibacterial and antioxidant activity.

KEYWORDS: Acne, *Curcuma longa, Allium cepa,* diffusion method

Corresponding Author: Priyanka Kantivan Goswami
Email: priyanka8408@gmail.com
Mobile: +91-9833056028

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INTRODUCTION

Acne vulgaris is an extremely common disorder of skin (pilocebaceous unit) that affects virtually all individuals at least once during life. The incidence of acne peaks at teenage, but substantial numbers of men and women between 20-30 years of age are also affected by the disorder[1]. A changed keratinisation pattern in the hair follicle leads to blockage of sebum secretion. It is probable that hyper responsiveness to the stimulation of sebocytes and follicular keratinocytes by androgens leads to the hyperplasia of sebaceous glands and seborrhea that characterise acne. The enlarged follicular lumen attributable to inspissated keratin and lipid debris forms a closed comedone (whitehead). When the follicle has a portal of entry at the skin, the semisolid mass protrudes forming a plug, producing an open comedone (blackhead)[2]. Micro-organisms like Propionibacterium acne, Staphylococcus aureus and Staphylococcus epidermidis proliferate rapidly leading to the development of acne[3].

In spite of great advances of modern scientific medicine, traditional medicine is still the primary form of treating diseases of majority of people in developing countries including India; even among those to whom western medicine is available, the number of people using one form or another of complementary of alternative medicine is rapidly increasing. India has a rich heritage of traditional medicine and the traditional health care system has been flourishing in many countries. Most recently, there has been interest in other therapeutic lead compounds from an ancient system of therapy, which can be utilized for development of new drug. Over 50% of all modern drugs are of natural product origin and they play an important role in drug development programs of the pharmaceutical industry[4]. Hence a few medicinal plants are selected to evaluate anti-microbial, anti-acne and antioxidant activity in a herbal formulation viz. Turmeric (Curcuma longa, Family: Zingiberaceae), Neem (Azadirchataindica, Family: Meliaceae), Garlic (Allium sativum, Family: Liliacea) and Coriander (Coriandrumsativum, Family: Umbelliferae). The present study was undertaken to determine anti-microbial, anti-acne and antioxidant activity of a polyherbal formulation[5-8].

MATERIALS

Plant material

Dried Turmeric rhizome, Coriander fruits, Garlic buds and Neem leaves were procured from local market and air dried then stored in air tight container under refrigeration.

Chemical reagents and solvents

The chemicals used for the study were all of analytical grade. Methanol was used for extraction (maceration).

METHODS

Extraction of the sample

50 g of each material was soaked with 500 ml of methanol for 72 hours at room temperature with occasional shaking. Extract was then filtered with whatman filter paper number 1. The Filtrates was concentrated and stored at 4°C. For all experiments fresh extracts were used[9].

Preparation of hydrogel

The gel was composed of Carbopol 940- 2%, Propylene glycol(PEG) -30%, Propyl paraben -2.5%, Triethanolamine and distill water in a quantity sufficient to prepare 100gm of gel in case of blank gel .Water required for these formulations was divided into two parts. In one part the exact amount of extract was dissolved and in other part, Carbopol was soaked overnight and to this solution propylene glycol and propylparaben were added. Both these solutions were mixed in a beaker and pH 7.4 was adjusted with Triethanolamine. It is mixed properly to obtain the proper gel consistency. Formulation of 1% and 2 % extract ratio was prepared. Gel was placed in collapsible tubes[10].

Table 1: Poly herbal gel composition

Sr. No.	Ingredients	Gel I formula	Gel II formula
1	A. indica extract	1%	2%
2	C. longa extract	1%	2%
3	A. sativum extract	1%	2%
4	C. sativum extract	1%	2%
5	Carbopol 940	2% w/w	2% w/w
6	Propylene glycol (PEG)	30% w/w	30% w/w
7	Propyl paraben	2.5% w/v	2.5% w/v
8	Triethanolamine	q.s.	q.s.
9	Distill water	q.s.	q.s.

Formulation Optimization

Antioxidant estimation

Total phenolic compound analysis
Total polyphenol content was estimated using Folin-Ciocalteu (FC) assay which is widely used in routine analysis. A known amount of gel (10 mg/ml) was mixed with 1.0 ml of FC reagent and 0.8 ml of 2% Na_2CO_3 was added and the volume was made up to 10 ml using water- methanol (4:6) as diluting fluid. Absorbance was read at 740 nm after 30 min using spectrophotometer. Tannic acid (0 - 800 mg/L) was used to produce standard calibration curve. The total phenolic content was expressed in mg of Tannic acid equivalents (TAE) /100 g of sample.

Determination of total flavonoids
The total flavonoid content was determined using the Dowd method. A 5.0 ml of 2% aluminumtrichloride ($AlCl_3$) in methanol was mixed with the same volume of the gel solution (10 mg/ml). Absorption readings at 415 nm UV-VIS spectrophotometer were taken after 10 min against a blank sample consisting of extract solution with 5.0 ml methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin. Total flavonoid content is expressed as g of quercetin equivalents / 100 g of sample.

Total tannin estimation
Colorimetric estimation of tannins is based on the measurement of blue color formed by the reduction of phosphotungsto molybdic acid by tannin like compounds in alkaline solution. A known amount of gel was mixed with 5.0 ml of Folin- Denis reagent (FD) and Na_2CO_3 solution and made up to 100 ml, mixed well and absorbance was read at 760 nm after 30 min using spectrophotometer. Total tannin content as expressed as mg tannic acid equivalent /100 g of sample.

Reducing power
A spectrophotometric method was used for the measurement of reducing power. Different concentrations of extracts were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide (10 mg ml-1). The mixture was incubated at 50°C for 20 min, then rapidly cooled, mixed with 2.5 ml of 10% trichloroactic acid and centrifuged at 6500 rpm for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and then ferric chloride (0.5 ml, 0.1%) was added and allowed to stand for 10 min. The absorbance was read spectrophotometrically at 700 nm. The formulated gel is then compared with Ascorbic acid for antioxidant activity[11].

Anti-microbial and Anti-acne evaluation

Sample Preparations
Solutions of gels were prepared using 100 mg of gel in 10ml of dimethyl sulfoxide (DMSO). Similarly, Solution of marketed formulation was prepared. Vicco turmeric cream was (10mg/ml) was used as a standard.

Anti-microbial & Anti-acne Assay
The antibacterial activity of different formulations was determined by modified agar well diffusion method. In this method, nutrient agar plates were seeded with 0.2 ml of 24 h broth culture of *Staphylococcus aureus* and *Propioniobacterium acne*. The plates were allowed to dry. A sterile 8 mm borer was used to cut four wells of equidistance in each of plates; 0.5 ml of solutions of extract, marketed herbal formulation was introduced in to the wells at randomly. The plates were incubated at 37°C for 24 hours. The antibacterial activity was evaluated by measuring the diameter of zones of inhibition (in mm). The formulated gel is then compared with the marketed preparation for the activity[12].

RESULTS AND DISCUSSION

Polyherbal formulation (gel) and market preparation were tested for antioxidant effect by determining total phenol, flavonoids and total tannin content as well as by checking reducing power. And for anti-microbial and in vitro anti-acne activity, Poly herbal formulation (gel) and market preparation were tested against S. aureus and P. acne bacteria respectively. The formed gel was found to be having almost matching result with market preparation.

Table 2: Antioxidant evaluation

Determination	Formulation		Market preparation	
	Gel 1%	Gel 2%	Gel 1%	Gel 2%
Total polyphenols (mg/ 100ml)	67	98	96	101
Flavonoids (g/ 100g)	0.1	0.17	0.15	0.18
Total tannins (g/ 100g)	0.25	0.3	0.3	0.35

All values in the above table represent the mean ± SD (n = 4)

Reducing power: Higher absorbance indicated greater reducing capacity. It has been reported that the reducing power is associated with antioxidant activity. Hence, it is essential to determine the reducing power of phenolic constituents to explain the relationship between their antioxidant effect and their reducing power. Reducing power of different concentration was estimated. Highest reducing power was in 2% with R^2 = 0.5 and for marketed preparation R^2 = 0.6.

Table 3: Anti-microbial activity: Zone of Inhibition against strain (*S. aureus*)

Formulation (%)	Zone of Inhibition (mm)	Zone of Inhibition (mm) Market preparation
Gel with 1% Extract	12 mm	13 mm
Gel with 2 % Extract	13 mm	14 mm

Table 4: Anti-acne activity: Zone of Inhibition against strain (*P. acne*)

Formulation (%)	Zone of Inhibition (mm)	Zone of Inhibition (mm) Market preparation
Gel with 1% Extract	10 mm	10.5 mm
Gel with 2 % Extract	11 mm	10.5 mm

CONCLUSION:

In the present study, evaluation on poly herbal formulation for antioxidant, anti-microbial and anti-acne activity was carried out. Formulation containing 1% and 2% extract was prepared and evaluated. The phenolic compounds have capacity of reducing oxidative cellular damage caused by free radicals. The oxidative damage is very important effect of cellular free radicals which can leads to damage of cellular constituents. Their repair depends on presence of antioxidants. Zone of inhibition was used to study the anti-microbial and anti-acne effect. Thus this investigation gives valuable information about proposed work carried out.

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