Research Article

www.ijrap.net (ISSN:2229–3566)

FORMULATION AND EVALUATION OF POLYHERBAL ANTI-ACNE GEL

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Received on: 09/07/20 Accepted on: 25/08/20

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DOI: 10.7897/2277-4343.1105151

ABSTRACT

The main aim of the research was to formulate and evaluate polyherbal anti-acne gel. Four herbal extracts i.e., leaves of *Azadirachta indica,* leaves of *Carica papaya,* rhizomes of *Curcuma longa* and fruits of *Vitis vinifera* were used for the formulation of anti-acne gel. Total eight formulations were prepared by using these four herbal extracts and two polymers as gelling agents; out of which one is natural (*Aloe vera*) and the other is synthetic (Carbopol 934). The prepared gels were evaluated for physical appearance, pH, viscosity, and Spreadability. Anti-bacterial activity for eight formulations was carried out and compared with two commercial formulations by using three bacterial strains like *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Enterococcus species*. The study was performed for eight days with the help of agar well diffusion method. Diameters of zone of inhibition were recorded. The prepared polyherbal anti-acne gel showed good pH, viscosity and Spreadability. F6, prepared using 1% of the four herbal extracts using Carbopol 934 as a gelling base demonstrated strong antibacterial activity against all three bacterial species, across all formulations. Therefore, prepared polyherbal gel (F6) could be effective in acne treatment.

Keywords: *Azadirachta indica,* Anti-acne, Anti-bacterial activity, *Staphylococcus aureus,* Polyherbal, Spreadability.

INTRODUCTION

Acne vulgaris is a skin condition that affects almost all persons at least once throughout their lifespan and its frequency is closely linked to sebum development, which is itself caused by swollen sebaceous glands following androgenic stimulants. Multiple physiological factors were found in the pathogenesis of acne, including follicular hyper proliferation and increased sebum production, followed by follicle blockage and colonization of various microorganisms, such as *Propionibacterium acnes*[1].

Antibiotics are the most effective treatment, but long-term support cannot be provided. Research from preclinical initiation to clinical presentation of active lesions also suggests the crucial function of cellular inflammatory events in acne lesion production at each point. As a predominantly sebaceous hyper proliferative follicular condition, the focus thus changed from acne to that of an infectious skin disorder[2].

The most potent acne therapies function by stimulating the sebaceous gland and reducing sebum production. Sometimes, physicians use a hybrid medication approach to cure acne utilizing topical medications such as antibacterial, antibiotics and retinoids before prescribing oral antibiotics, hormone therapy, or oral isotretinoin. Light and laser devices have been effective, too. The suggested solution is to formulate effective care protocols based on the predominant causes and general clinical extent of acne lesions[3].

For many years, hormones and chemotherapeutic agents have been extensively used for treating acne. However, those drugs are most concerned with severe side effects and drug resistance. Consequently, herbal remedies and photodynamic therapy with high antibacterial efficacy and without side effects have been widely studied as an approach to inactivating various gram-positive bacteria, such as *P. acne*[3].

Herbs are natural and pose fewer side effects, thus most people turn to herbal medicine. Most of the herbal ingredient-based cosmetic items are daily appearing in the market. In this backdrop, the present study was designed for the formulation and evaluation of polyherbal anti-acne gel using the extract of *Azadirachta indica* leaves, *Carica papaya* leaves*, Curcuma longa* rhizomes and *Vitis vinifera* fruits.

MATERIALS AND METHODS

Fresh leaves of *Azadirachta indica* and *Carica papaya*, rhizomes of *Curcuma longa* and fruits of *Vitis vinifera* were collected from Foundation for Revitalization of Local Health Traditions (FRLHT), Bangalore. Various excipients for the preparation of anti-acne like Carbopol, methyl paraben, propyl paraben, methanol, ethanol, triethanolamine, and propylene glycol were collected from Karnataka Fine Chem., Bangalore. The medium used for growth Muller-Hinton agar was collected from HiMedia-50, Mumbai. 10 per cent ammonia, Hydrochloric acid, chloride, ether, chloroform, anhydrous iron (III), H_2SO_4 condensed acetic acid solution, Dragendroff's reagent, Mayer reagent (Potassium mercury iodide solution), sodium hydroxide concentrated in 1N, potassium permanganate and physiologically safe sodium de-mineralized water were the various chemicals and reagents used in this project.

Extraction of Herbs

Azadirachta indica leaves were washed and dried for 15 days under the shade and then powdered. 50 g of the powdered leaf was taken in a beaker with 250 mL of methanol and stored at room temperature for 48 hours. The mixture was filtered using Whatman filter paper and the filtrate was vaporized in an oven at a temperature of 60°C for 40 hours to dryness[4]. Afterwards, the sample was stored at 4°C for further analysis. Similarly, *Carica*

papaya leaves' powder and *Curcuma longa* rhizomes' powder were extracted by using maceration method of extraction[5,6]. *Vitis vinifera* fruits were washed under running tap water. Then juice was prepared from fresh grapes by steam extraction (60 min, 75–85°C), sieved (270 mesh) and stored at 18°C until use using carrier agent i.e., Maltodextrin (15%) which was added to juice with continuous stirring until complete dissolution. Prepared mixture was kept for spray drying[7]. Thus, the powder extract of *Vitis vinifera* was obtained.

Phytochemical screening

The hydroalcoholic extracts of *Azadirachta indica, Carica papaya, Curcuma longa* and *Vitis vinifera* were subjected to preliminary qualitative phytochemical investigations as per the procedures described by Shubham S, *et al*[8].

Preparation of Aloe vera gel base

Leaves were washed with running tap water and cut into pieces transversely. Then the thick epidermis was separated with a peeler, and spoon removes the pulp. The pulp collected is properly minced and then homogenized to obtain uniform consistency in a mixer. Then, about 20 ml 0.1 N NaOH was added to make it alkaline. 1% Carbopol was taken in 30-40 ml of water and kept at room temperature for overnight. In alkaline aloe, this was added and then homogenized for 1 hour with mechanical stirrer. Then it was added with continuous stirring 2-3 drops of triethanolamine[9].

Preparation of Carbopol gel base

Carbopol gel was prepared by using 2% of Carbopol 940 P polymer.

Formulation and evaluation of anti-acne gel

Total 8 formulas were produced using 4 separate proportions of both natural (*Aloe vera*) and synthetic (Carbopol) gel base extracts.0.5%, 1%, 1.5% and 2% of *Azadirachta indica* (leaves), *Carica papaya* (leaves), *Curcuma longa* (rhizomes) and *Vitis vinifera* (fruit) extracts had been incorporated into prepared gel bases[10]. Formulation chart is given in Table 1.

Evaluation parameters

Spreadability

Two glass slides of standard dimension (7.5 cm) were used to measure the diffusability of all formulations. Around 1 gm of gel was put between two glass sheets and 20 g of weight was attached to the upper glass plate. The collection had been kept upright throughout. For the top of the diaphragm the gap of 7.5 cm was taken and separated by weight from the bottom of the diaphragm. The propagation rate of the following formula was calculated[10].

$$S = m \times l/t$$

S = Spreadability coefficient, **M** = Weight tied to upper plate.
l = Length of glass plate, **t** = Time taken in sec.

pH and Viscosity

Viscosity of the gel was determined by using Brookfield viscometer (DV-II + Pro EXTRA). T shape spindle no F96 was fixed to viscometer and it was immersed in the 25 g of gel. The viscometer was operated at different rpm and viscosity was noted in cps at the room temperature. While the pH measurement of gels was done using digital pH meter (pH Meter 115, Bangalore)[11].

Anti-bacterial activity

The anti-bacterial activity of 8 herbal formulations (F1-F8), marketed herbal anti-acne cream (HCA) and marketed gel (ADP) was evaluated against *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA) and *Enterococcus species* (ES), adopting agar well diffusion method.

Preparation of agar plates

Medium was prepared and held for 20 minutes to autoclave. After cooling the medium was poured into Petri plates and kept at room temperature for cooling. Total 12 plates of agar had been prepared.

Inoculation of agar plates

The surface of one or two colonies of micro-organisms was touched by a sterile cotton swab and streaked over the surface of agar plates. This cycle was repeated three times; the plate was rotated approximately 60 degrees each time to ensure the inoculum was distributed evenly. The plates were then placed in the incubator for 24 hours at 37°C.

Agar well diffusion assay

The wells were created by stinging holes with a sterile borer into the MH inoculated agar plate. Each well was 5 mm in diameter and a sterile needle was used to extract the cut from the agar. Weighed and inserted a desired quantity of 8 formulations, as well as 2 standards into each well. Then prepared agar plates were kept for 8 days in the incubator at 37°C and inhibition zone diameters were observed and compared with the help of the ruler each day[12].

RESULT

Mainly four herbs i.e., leaves of *Azadirachta indica, Carica papaya*, rhizomes of *Curcuma longa* and fruit juice of *Vitis vinifera* were selected for the present study. *Azadirachta indica* leaves had been selected due to its antibacterial, anti-inflammatory, antifungal as well as anti-oxidant activity. *Carica papaya* leaves were selected due to its anti-acne activity because no work had been done so far related to acne activity. Similarly, rhizomes of *Curcuma longa* and fruit juice of *Vitis vinifera* had been selected due to its antimicrobial and anti-oxidant activity respectively.

Extraction of the leaves of *Azadirachta indica* and *Carica papaya,* rhizomes of *Curcuma longa* and fruits of *Vitis vinifera* was carried out. The obtained extracts were stored and used for further formulation and evaluation. The leaves of *Azadirachta indica* and *Carica papaya*, *Curcuma longa* rhizomes and *Vitis vinifera* fruits were collected. The extracts obtained were stored and then used for further formulation and assessment.

Phytochemical Tests

Phytochemical tests were performed to determine the presence of various constituents. Inference of phytochemical tests of different herbal drugs like *Azadirachta indica, Carica papaya, Curcuma longa* and *Vitis vinifera* are given in the Table 2. All the extracts possess flavonoids which might be the possible reason for anti-oxidant activity that consequences in anti-acne activity.

Table 1: Formulation chart

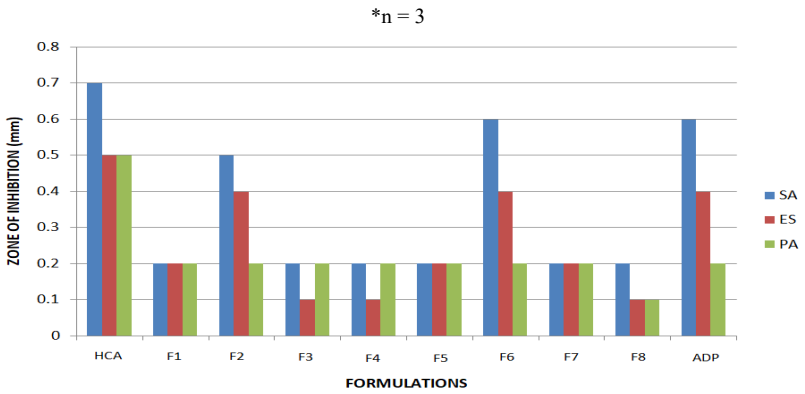
Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Leaf extract of *Azadirachta indica*	0.5%	1%	1.5%	2%	0.5%	1%	1.5%	2%
Leaf extract of *Carica papaya*	0.5%	1%	1.5%	2%	0.5%	1%	1.5%	2%
Rhizome extract of *Curcuma longa*	0.5%	1%	1.5%	2%	0.5%	1%	1.5%	2%
Fruit extract of *Vitis vinifera*	0.5%	1%	1.5%	2%	0.5%	1%	1.5%	2%
Methyl paraben	0.3 g	0.3 g	0.3 g	0.3 g	0.3g	0.3 g	0.3 g	0.3 g
Propyl paraben	0.03g	0.03 g	0.03 g	0.03 g	0.03 g	0.03 g	0.03 g	0.03 g
Triethanolamine	2 ml	2 ml	2ml	2 ml	2 ml	2 ml	2 ml	2 ml
Propylene glycol	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Carbopol 934 up to	-	-	-	-	25 g	25 g	25 g	25 g
Aloe vera up to	25 g	25 g	25 g	25 g	-	-	-	-

Table 2: Phytochemical Tests

S. No.	Component	Physicochemical test	Inference			
			Extract of *Azadirachta indica* leaves	Extract of *Curcuma longa* Rhizomes	Extract of *Carica papaya* leaves	Extract of *Vitis vinifera* juice
1	Alkaloids	Meyer's test	+ve	-ve	+ve	+ve
2	Carbohydrates	Molisch's test	-ve	-ve	+ve	+ve
		Fehling's test	+ve	-ve	+ve	+ve
3	Proteins and amino acids	Million's test	+ve	+ve	-ve	+ve
		Biuret test	-ve	-ve	-ve	-ve
4	Phytosteroids	Liebermann-Burchard's	+ve	-ve	-ve	+ve
5	Phenolic compounds and flavonoids	Ferric chloride	+ve	+ve	+ve	+ve
		Lead acetate	+ve	+ve	+ve	+ve

Table 3: Evaluation parameters of F1 to F8

Formulation	pH (Mean ± SD) *	Spreadability (g cm/s) (Mean ± SD) *	Viscosity (Pascal. s) (Mean ± SD) *
F1	8.13 ± 0.58	11.35 ± 1.26	74.84 ± 1.23
F2	7.55 ± 0.10	7.15 ± 0.34	71.20 ± 0.86
F3	5.67 ± 0.16	9.05 ± 0.86	64.86 ± 1.32
F4	8.34 ± 0.33	12.42 ± 2.27	55.76 ± 0.23
F5	7.77 ± 0.18	6.83 ± 0.31	79.21 ± 0.65
F6	7.84 ± 0.20	9.47 ± 1.19	67.45 ± 0.05
F7	7.65 ± 0.09	10.95 ± 1.91	65.87 ± 0.46
F8	8.69 ± 0.28	9.24 ± 0.84	58.43 ± 0.34



SA: *Staphylococcus aureus*; ES: *Enterococcus species*; PA: *Pseudomonas aeruginosa*

Figure 1: Anti-bacterial studies of polyherbal anti-acne formulations

Preparation of herbal gel

The gelling agent had been chosen based on the polymer's gelling ability. Two polymers had been selected i.e., one natural polymer and one synthetic polymer to allow a direct distinction. *Aloe vera* and Carbopol were selected merely because of the high gelling property. Since the viscosity of *Aloe vera* and Carbopol was the highest among other concentrations of polymers, 1 percent concentration was selected as the appropriate one for topical application.

Propylene glycol was used as a humectant and as the chosen medium, it was used for preservatives (methyl paraben and propyl paraben) and volume was provided by purified water. Since *Aloe vera* is naturally acidic, 0.1N NaOH was used to make it alkaline. Triethanolamine was being used as a pH adjuster for Carbopol and *Aloe vera*.

Evaluation of gel

Total eight formulations were prepared using four different herbs with different concentration and evaluated for pH, Spreadability and viscosity. Results are given in Table 3.

The pH range for an ideal gel for topical use is 4.5-7. Hence, pH of all the F1 to F8 formulations was found to be 8.13, 7.55, 5.67, 8.34, 7.77, 7.84, 7.65 and 8.69 respectively.

The Spreadability of semi-solid formulations, i.e. the ability of a gel or cream to permed uniformly on the skin, plays a crucial role in the daily dosage of a medicated formulation and in the effectiveness of a topical substance being applied to the skin.

Spreadability in the range of 9-15 is considered to have good Spreadability. All eight formulations showed good Spreadability.

Viscosity was determined by using T-bar spindle (96F) at 50 rpm. The viscosity of F1 to F8 was found to be 74.84 ± 1.23, 71.20 ± 0.86, 64.86 ± 1.32, 55.76 ± 0.23, 79.21 ± 0.65, 67.45 ± 0.05, 65.87 ± 0.46 and 58.43 ± 0.34 Pascal's respectively. Carbopol gel exhibited higher viscosity owing to higher swellability compared to *Aloe vera*.

Anti-bacterial study

Anti-bacterial activity was performed for eight formulations against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus species.* F6 had shown comparable anti-bacterial potency using *Enterococcus species* whereas exhibited superior potential in *Staphylococcus aureus* when compared to marketed formulations. Among all the formulations, F6 (1% of extract of all herbs *i.e., Azadirachta indica, Carica papaya, Curcuma longa* and *Vitis vinifera*) has shown comparable zone of inhibition to that of Adapalene gel (ADP) and Himalaya Clarina anti-acne cream (HCA). Therefore, 1% of extracts of all herbs in combination with Carbopol had shown the superior antibacterial activity in comparison with standard (ADP) as well as other formulations. Diameters of zone of inhibition were recorded. The zone of inhibition is given in the Figure 1.

CONCLUSION

Anti-acne polyherbal gel was prepared with combination of different herbal extracts i.e., leaves of *Azadirachta indica, Carica papaya*, rhizomes of *Curcuma longa* and fruit juice of *Vitis vinifera*. Extracts of different herbs was obtained with maceration as well as spray drying method. Total eight formulations were prepared with different concentration of extracts i.e., 0.5%, 1%, 1.5% and 2% of extracts of all herbs. In this work an attempt was made to prepare polyherbal gel with natural and synthetic gelling agents. Aloe was used as a natural gelling agent while Carbopol was used as a synthetic gelling agent. Viscosity was achieved by employing the 1% of *Aloe vera* and Carbopol 934. Results indicate a comparable antibacterial activity of polyherbal formulation to that of commercial formulation. Therefore, this could be a promising formulation for the treatment of acne.

ACKNOWLEDGEMENT

The authors are thankful to Acharya and BM Reddy college of Pharmacy, Karnataka, Bangalore for providing the necessary facilities and GKVK campus, Bangalore for providing spray drying facility.

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Cite this article as:

Sneha Bharti and Harini Chowdary Vadlamudi. Formulation and evaluation of Polyherbal anti-acne gel. Int. J. Res. Ayurveda Pharm. 2020;11(5):110-113 http://dx.doi.org/10.7897/2277-4343.1105151

Source of support: Nil, Conflict of interest: None Declared