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Original Research Article

DEVELOPMENT AND CHARACTERIZATION OF ANTI-ACNE GEL CONTAINING EXTRACT OF *PIPER BETLE* L.

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ABSTRACT

Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various diseases. Acne is an inflammatory skin disease that occurs due to blockages in polysebase and inflammation that are caused by bacteria. Topical and systemic antibiotics are always used for treatment of acne, but the gradual resistance to antibiotics can affect the success rate of acne cure. *Piper betle* L. extract showed that has antibacterial activity against acne- causing bacteria. In the present study anti-acne gels were prepared using polymer carbopol 940 along with the hydroalcoholic extracts of plants *P. betle* and evaluated for their physicochemical properties, like pH, washability, extrudability, spreadability and viscosity. The extract and formulations were tested for the anti-acne activity by well diffusion method against *Propionibacterium acnes* to find the minimum inhibitory concentration (MIC). Results showed that the gels were non-irritant, stable and posses anti-acne activity. The efficacy of gel when tested with an extract was almost double to that of extract. This suggests that *P. betle* has potential against acne causing bacteria and hence they can be used in topical anti-acne preparations and may address the antibiotic resistance of the bacteria.

Key words: *Propionibacterium acnes, piper betle* L, Carbopol 940, Anti-acne activity, Well diffusion method, Physicochemical properties.

INTRODUCTION:

Acne vulgaris, a chronic inflammatory disease of skin, could possibly have affected almost everyone at various points in their lives (Webster, 1996). The inflammatory acne lesion, a crucial event of the disease often results in scarring and permanent mark (Layden,1995; Cunliffe, 1998; Farrar and Ingram, 2004; Williams et al., 2012). There is a wide range of individual clinical expression with males tending to have more severe forms, the incidence is similar in males and females until mid-20s; thereafter acne is more prevalent in females, but the severity and frequency are markedly decreased (Sams and Lynch, 1996). Four main pathogenetic factors of acne include hyperproliferation of follicular epithelial cells, excess sebum production and colonization of *Propionibacterium acnes* and inflammation (Layden,1995; Cunliffe, 1998). *P. acnes* have been denoted as a predominant bacterium of acne due to its unique immunomodulatory

effect which mainly induces the inflammatory process (Bojar and Holland, 2004; Dessionioti and Katsambas. 2010). Skin macrophages were directly induced by *P. acnes* heat-shock protein to produce several pro-inflammatory cytokines including interleukin-6 (IL-6) and neutrophils chemoattractants; interleukin-8 (IL-8), mainly stimulates neutrophils migration leading to acne lesion and pus formation (Farrar and Ingram, 2004; Bojar and Holland, 2004; Dessionioti and Katsambas. 2010; Kim et al., 2002). Neutrophils subsequently generate oxygen free radicals for killing the bacteria. However, excessive production of the free radicals, stimulated by *P. acnes*, leads to the leakage of the free radicals within extracellular space, which destroys follicular epithelium and accelerates progression of the inflammatory responses (Akamatsu and Horio, 1998). Therefore, *P.acnes* has been recognized as one of the main targets for acne treatment (Webster, 1996). Nowadays, the attempts to find an alternative treatment for acne from natural resources have been considerably expanded every single year due to the antibiotic resistance of *P. acnes* and skin side effects, which might be occurred through the usage of conventional topical medicines (Webster, 1996; Chomnawang et al., 2005; Eady et al., 2003; Tsai et al., 2010). Ayurveda, the Indian system of medicine, has been an integral part of Indian culture and materia medica. From the rich Indian biodiversity, it has identified various plants/herbs that have been associated with a number of potential therapeutic efficacies (Chatterjee and Pakrashi, 1995). *Piper betle* L. (Piperaceae) leaves have a strong pungent aromatic flavour and is widely used as masticatory in Asia. The leaves are credited with many properties (digestive, stimulant). Medicinally the leaves are useful in catarrhal and pulmonary affections (The Wealth of India, 1969). The phenolic constituent allyl pyro catechol from the leaves showed activity against obligate oral anaerobes responsible for halitosis (Ramji et al., 2002). The leaf extract has significant stimulatory influence on pancreatic lipase activity in experimental rats (Prabhu et al., 1995). The leaf extract inhibits radiation induced lipid peroxidation. The extract also increased the activity of superoxide dismutase activity in a dose dependent manner, indicating elevation of antioxidant status in Swiss albino mice (Choudhury and Kale, 2002). *P. betle* leaves also afforded a significant hepatoprotective effect and improved the tissue antioxidant status by increasing the levels of non-enzymatic antioxidants (reduced glutathione, vitamin C and vitamin E) and the activities of free radical-detoxifying enzymes in liver and kidney of ethanol-treated rats [Saravanan et al., 2002). *P. betle* leaf extract inhibited platelet aggregation via both antioxidative effects and effects on thromboxane B2 (TXB2) and prostaglandin-D2 (PGD2) production. Piperbetol, methylpiperbetol, piperol A and piperol B, isolated from *P. betle* are effective platelet activating factor (PAF) receptor antagonists in vitro (Zeng et al., 1997). There are many literatures reporting the ethno-medicinal values of *P. betle*, but there is little scientific proof for further using this plant commercially or in a more effective form. Therefore, an attempt was made to evaluate the anti-acne activity of *P. betle* extracts against *P. acnes.*

MATERIAL AND METHOD

Plant materials

The leaves of plant of *P. betle* were collected from local shop of Bhopal (M.P), India in the months of December 2018. Plant material (leaves) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvent used in this study were of analytical grade. The pathogenic microbes used in the current study were obtained from Microbial Culture collection, National Centre Forcell Science, Pune, Maharashtra, India.

Extraction

Dried powdered leaves of *P. betle* has been extracted with hydroalcoholic solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C and stored in an air tight container free from any contamination until it was used. Finally, the percentage yields were calculated of the dried extracts.

Qualitative phytochemical analysis of plant extract

The *P. betle* extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Kokate and Khandelwal (Kokate, 2011; Khandelwal, 2005). The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavanoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Total flavonoids content estimation

The total flavonoid content was determined using the method of Olufunmiso *et al* (Olufunmiso and Afolayan, 2011). 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extracts or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

Formulating anti-acne gel

Measured quantity of methyl paraben, glycerine, polyethylene glycol and hydroalcoholic extract of *P. betle* were dissolved in about 35 ml of water in beaker and were stirred at high speed using mechanical stirrer (or sonicator). Then carbopol 940 was added slowly to the beaker containing above liquid while stirring. Neutralized the solution by slowly adding triethanolamine solution with constant stirring until the gel is formed. All the samples were allowed to equilibrate for 24 hours at room temperature prior to performing rheological measurements (Table 1).

Table 1 Formulation of polyherbal gel

Ingredients (%)	HG1
Piper betle extract	1gm
Carbopol 940	2.0gm
Polyethylene Glycol	0.2ml
Methyl Paraben	0.08mg
Triethanolamine	1.0ml
Distilled Water (q.s)	100ml

Evaluation of prepared gels

The Psycho Rheological characteristics were studied for topical gel formulations like colour, clogging, homogeneity and texture etc. Consistency or hardness of gel was measured by Penetrometer. Three containers were filled carefully and completely with formulation, without forming air bubbles and stored at 25±0.5^{0}C for 24 hrs. Test samples were placed on Penetrometer and position of spindle was adjusted as such that, its tip just touches the surface of sample. Penetrating object was released for 5 sec. Depth of penetration was measured. Same was repeated with remaining formulation. Extrudability study was performed by gel formulations were filled into aluminum collapsible tubes. The tubes were pressed by applying weight to extrude the material. Weight was measured which required to extrude the gel from collapsible tubes. An important criterion for gel 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. A special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time taken to slip a movable slide from another fixed slide placed in a frame with formulation under the application of a certain load. Lesser the time taken for the separation of two slides, better the spreadability. The experiment was repeated and the average of 6 such determinations was calculated for each gel formulation.

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

Where, S=Spreadability (gcm/sec)

m = weight tied to the upper slide (20 g)

l= length of glass slide (6 cm).

t = time taken is seconds.

pH of gel was determined by digital pH meter. Ten gram of gel was taken and the electrode was then dipped in to gel solution for 30 min until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated three times. The viscosity of the prepared gel was determined using Brookfield digital viscometer. The viscosity was measured using spindle no. 6 at 10 rpm at ambient room temperature 25-30^{0}C. The sufficient quantity of gel was filled in appropriate wide mouth container. Wide mouth container use to allow spindle of the Viscometer inside of the container. Viscosity value was noted down after stable of reading. Samples of the gel were allowed to settle over 30 min at the constant room temperature before the measurements. The stability of the gels was tested using freeze thaw cycling method. The gels were subjected to a temperature of 4°C for 7 days, 25°C for 7 days and then at 40°C for 7 days. The gels were exposed to the ambient room temperature after each step and noted for synerisis, viscosity, and pH changes (Barry, 1983; Jain et al., 2007; Lachman et al., 1986; Schoch, 1968).

In-vitro anti acne activity

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 37ºC overnight to check the sterility of plates. The plates were dried at 50ºC for 30 minutes before use.

Revival of the bacterial and fungal 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 than incubated for 24 hours at 37ºC. After incubation the growth is observed in the form of turbidity. These broth cultures were further inoculated on to the agar plates with loop full of bacteria and further incubated for next 24 hours at 37ºC to obtain the pure culture and stored as stocks that are to be used in further research work.

Antibiogram Studies

The well diffusion method was used to determine the antibacterial activity of the herbal gel prepared from the leaves of *P. betle using* standard procedure (Bauer et al., 1966). There were 3 concentration used which are 25, 50 and 100 mg/ml for each formulation in antibiogram studies. Its essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted overnight broth cultures should never be used as an inoculum. The plates were incubated at 37ºC for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

Results and discussion

The crude extracts so obtained after the maceration extraction process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. The % yield of hydroalcoholic extracts of *P. betle* was obtained 4.5 w/w. Phytochemical analysis of hydroalcoholic extracts of plants showed the presence of flavonoid, protein, carbohydrate and diterpines while, alkaloids, glycosides and oils and fats were reported to be absent Table 2. The total flavonoid content of the extracts of was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of Hydroalcoholic extract of *P. betle* showed the content values of 1.352. Results are provided in Table 3 and Fig. 1. From the Psycho Rheological characteristics studies of optimized formulation showed that all of them have light brown colour, No clogging, good homogeneity and smooth texture Table 4.

Table 2 Result of Phytochemical screening of hydroalcoholic extracts

S. No.	Constituents	*Piper betle*
1.	Alkaloids	-ve
2.	Phenol	-ve
3.	Flavonoids	+ve
4.	Diterpenes	-ve
5.	Proteins	+ve
6.	Saponins	+ve
7.	Carbohydrate	+ve

Table 3 Total flavonoid content of _Piper betle_

S. No.	Extracts	Total flavonoid (QE) (mg/100mg)
1.	**Hydroalcoholic**	1.352

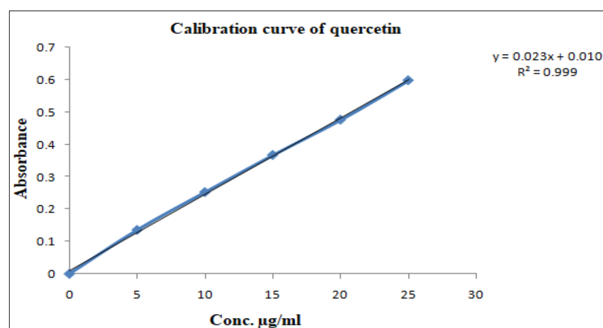

Fig. 1 Graph of Estimation of Total flavonoid content

Table 4 Results of Psycho rheological characteristics

Formulation	Colour	Clogging	Homogeneity	Texture
HG1	Light Brown	Absent	Good	Smooth

The results of washability, extrudability, spreadability, pH, viscosity were given in table 5. Extrudability study was performed by gel formulations were filled into aluminium collapsible tubes, the formulation has average extrudability. The skin irritation test performed showed no signs of sensitivity, erythema and edema. So the prepared formulations were considered to be non-irritant. The efficacy of the anti-acne gels from herbal extracts is shown in Table 6. The anti-acne gels could inhibit the growth of the microorganisms that inhabit acnes and the herbal gel exhibited comparatively more efficacy to extract.

Table 5 Results of washability, extrudability, spreadability, pH, Viscosity

Formulation	Washability	Spreadability (gcm/sec)	pH	Extrudability	Viscosity
HG1	Good	4.5±0.12	7.05±0.14	Average	3317±18

Table 6 Anti-acne activity of extract and herbal gel formulation against *Propionibacterium acnes*

S. No.	Formulation	Zone of inhibition		
		100mg/ml	**50 mg/ml**	**25mg/ml**
1.	Extract	14±0.5	11±0.74	10±0.57
2.	Herbal gel	25±0.94	23±0.5	21±0.5

Conclusion

The present study was aimed to developed herbal gels for anti acne treatment using hydroalcoholic extracts of *P. betle a*n aqueous based carbopol gel system and evaluated for their physicochemical properties, like pH, spreadability, viscosity and microbial assay. The anti acne activities of the mentioned gel were more than extract, this needs to be fully clarified by further assay methods and using additional concentrations of extracts. Further

phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its anti acne activity and to explore the existence of synergism if any, among the compounds.

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