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INVESTIGATION ON EFFECTIVENESS OF CARBOPOL BASED GEL AS ANTI-ACNE PREPARATION CONTAINING *GLYCORHIZAGLABRA* ROOT EXTRACTS

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ABSTRACT

Skin diseases are one of the common problems sometimes caused by microbial infections where most fequently due to *Staphylococcus aureus*, *Streptococcus pyogenes*, and coryneform bacteria. In case of skin diseases topical lotions, shampoos, ointments or antifungal drugs are used in general. The present study was aimed at investigation on anti-acne potential of carbopol based gels containing *Glycorhizaglabra*root extracts *in vitro* against two common skin pathogens *Staphylococcus aureus* and *Propionibacterium acnes*. The caropole based gel was prepared with the using ethanolic extract of *G. glabra*defatted root powder. The antimicrobial activity of extract and gels were performed against *Staphylococcus aureus* (MTCC-737) and *Propionibacterium acnes* (MTCC-1951) by well diffusion method shows an inhibitory potential at lowest concentration of 0.25 µg/20µl in each well individual extract with a zone size of 7 mm diameter against *S. aureus* whereas as zone size of 12 mm diameter against *P. acnes* as low as at 1.0 µg/20µl concentration of extract per well. The hydroethanolic extract was reported to contain alkaloids, flavonoids, tannins, saponins and glycosides.The prepared gel containing *G. glabra*extractwas clear golden yellow and homogenous appearance with a pH of 7.2, 41.25 µg/ml of TPC having spreadability range of 70.84 sq. cm also posses inhibitory potential against the same test microbes.Due to the availability of active phytodrug constituents that impart pharmacological activity could be further investigated in order to commercialize such therapeutic preparations for topical use in skin cure or improvement.

K**eywords:** Skin diseases, Anti-Acne gel, Carbopol-940, *Glycyrrhizaglabra*.

INTRODUCTION

Skin diseases are caused by viruses, rickettsiae, bacteria, fungi, and parasites. Skin infections may be either primary or secondary. Primary infections have characteristic morphologies and courses, are initiated by single organisms, and usually occur in normal skin. They are most frequently caused by *Staphylococcus aureus*, *Streptococcus pyogenes*, and coryneform bacteria. Cellulitis, impetigo, boils and folliculitis are the most common bacterial skin infections seen by the family physician [1, 2].

In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases. Moreover, Indian folk medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds, inflammation, skin infections, leprosy, diarrhoea, scabies, venereal disease, ulcers, snake bite[3].

In case of skin diseases topical lotions, shampoos, ointments or antifungal drugs available to treat skin diseases mostly do not respond or have the tendency to relapse or reoccur and cause many side effects. Plant derived antimicrobial substances could be an effective option for development of formulations against skin disease compared to the highly effective synthetic drug with lot of side effects and unaffordable cost sometimes [4].

One of the important medicinal plant *Glycyrrhizaglabra, Linn* belongs to family Laguminsae is a hardy perennial shrub and is also known as licorice or sweetwood [5]. The root of licorice have been reported to posses analgesic, expectorant, wound-healing, antiseptic, antiallergic, antidotal, cholagogic, antimutagenic, hypotensive, hepatoprotective, hypolipidemic, antidiuretic, diaphoretic and tonic properties [6]. This plant is widely used as sweetening additives in food industries and is it also reported to have anti-bacterial, anti-fungal and antiviral potentials and has been used in treatment of respiratory tracts, lungs stomach, and kidneys, disorders [7]. Thus present study was aimed at investigation on anti-acne potential of carbopol based gels containing *Glycorhizaglabra*root extracts *in vitro* against two common skin pathogens *Staphylococcus aureus* and *Propionibacterium acnes*.

MATERIALS AND METHODS

Sample Collection and Processing

The roots of *Glycorhizaglabra*were collected from local market of Bhopal and were authenticated by botanical literatures, images and web resources. The collected roots were washed thoroughly with tap water, cleaned and dried at room temperature followed by grinding them into fine powder. These finely grounded rootpowder were subjected to phytochemical extraction.

Phytochemical Extraction and Analysis

The fine powdered roots of *G. glabra*were first defatted with petroleum ether at ambient temperature for 24 hours and then the defatted merc was further subjected to phytochemical extraction using soxhlet extractor in 50% ethanol as solvent. The menstrum so obtained after extraction was allowed to concentrate in a boiling water bath to evaporate the solvent. The percentage yield of extraction and preliminary phytochemical analysis was done according to the methods described by Harborne, (1983); Khandelwal, (2005); and Tenguria *et al*., (2014) [8, 9, 10].

Antimicrobial activity of Extract

The antimicrobial activity was performed using well diffusion method against the two bacterial species *Staphylococcus aureus* (MTCC-737) and *Propionibacterium acnes* (MTCC-1951). The bacterial cultures

were cultured in nutrient broth separately for 24-48 hours at 37 ^{o}C to increase their number of cells in substantial quantity. With the help of sterile swab each bacterium was seeded on to the nutrient agar plates and allowed to dry for about 5 minutes this was followed by preparing wells of 6 mm diameter using sterile well punch. A stock of phytochemical extract of 100 mg per ml concentration was serially diluted upto 5 dilutions and poured 20 µl of each concentration into the wells of each culture plate. The culture plates were incubated for 24 hours at 37 ^{o}C after which the zone of inhibitions were recorded using zone scale (HiMedia). The experiment was repeated twice [11, 12].

Preparation of Carbopol Based Gel

Stock of phytochemical extract was prepared in sterile distilled water with a concentration of 100 mg/ml. In present study the Carbopol gel were prepared based on the investigation of Mohammad Haneefa KP., *et al*., (2016); Muhammad Ubaid*et al*., (2016); and Krongrawa, *et al*., (2018) with suitable modifications [13, 14, 15]. The composition of the gel base used in present work is depicted in table 1. The stockconcentration of 100 mg per ml crud phytochemical extract was prepared in sterile distilled water homogenized and degased in ultrasonic bath which was used to be added in gel to make varied final concentrations of extract in per gram of gel.

Table 1: Composition of Cabopol -940 gel base

S.N.	Composition	Amount
1	Water	100 ml
2	Carbopol -940	1 gm
3	Methyl paraben	200 mg
4	Glycerol	1 ml
5	Phyto extract stock	As required

Analysis of Prepared Gel

Appearance and Homogeneity

The prepared gels were visually evaluated for colour, physical appearance and homogeneity.

pH measurement

Using pH meter (Electronic India), the pH of prepared gel was evaluated by completely dipping the glass electrode of calibrated pH meter measurement of the gel was carried out using a digital pH meter by [16].

Spreadability

Approx. on gram of herbal gel formulation was placed on the center of 12×12 inch glass plate and sandwiched by another glass plate of same dimension and 1kg of weight was placed over this system for 10 minutes. The diameter of circular spreading of the gel in the plate was taken area of circle was determined using following formulae:

$$Area\ of\ spreading = \pi r2$$

Estimation of TPC gel formulation

One gram of prepared gel was dissolved in 10 ml of ethanol and filtered with the use of 0.22 micron dissociable syringe filter (Moxcare). This filtrate after suitable dilution was then subjected to estimation of total polyphenolic content using the Folin-Ciocalteu method with suitable modification [17, 10]. The filtrate was oxidized with Folin-Ciocalteu reagent, and the reaction was neutralized with pinch of sodium carbonate. The absorbance of the resulting blue colour was measured at 650 nm after 60 min. Using Gallic acid as standard total phenolic content (standard curve was prepared using concentrations 0-50 µg/ml) was expressed as µg GA equivalent/ml of extract.

In vitro antimicrobial potential of gel

Similar to the antimicrobial activity of extracts the inhibitory potential of herbal gel in the form of zone of inhibition against the *S. aureus* and *P. acnes* was done by putting approx. 20 mg of gel in single well on pre-inoculated culture plates where zone of inhibition was read after incubation.

RESULTS & DISCUSSION

Preliminary Phytochemical analysis of Extract

The ethanolic extracts of *G. glabra* when extracted with 60% ethanol in soxhlet extractor is reported to have the presence of alkaloids, tannins, flavonoids, glycosides and saponins when tested as per the method described by Harborne, (1983) and Tenguria *et al*., (2013) [8,12]. The extract was only devoid for the present of terpenoidal groups. The phytochemical groups that were found positive in the extract are generally polyphenols and flavonoids that are responsible for most of the pharmacological properties of any plant derived drug. The results of phyto analysis are summarized table 2.

Table 2: Results of Preliminary Phytochemical analysis of *G. glabra* root extract

S.N.	Phytoconstituents	Observations
1.	Alkaloids	+Ve
2.	Flavonoids	+Ve
3.	Tannins	+Ve
4.	Terpenoids	–Ve
5.	Saponins	+Ve
6.	Glycosides	+Ve

Antimicrobial Activity of Extract

In present investigation the results for *in vitro* antimicrobial potential of ethanolic extract of *G. glabra* roots against the two common bacterial species *S. aureus* and *P.acnes*using sensitivity assay are depicted in table 3. The extracts are effective at lowest concentration of 0.25 µg/20µl of extract used for *S.aureus*in the well while 1 µg/20µl for *P.acnes* forms a zone of inhibition of size 7mm and 12 mm respectively.At such a low concentration describes the potentially of plant extract against the test microorganisms.

Table 3: Results of antimicrobial sensitivity of ethanolic *G. glabra* root extract against the *S. aureus*and *P. acnes*

S.N.	Extract Concentration in µg/20µl	Zone of inhibition in mm	
		Against *S.aureus*	Against *P.acnes*
1	2	16	18
2	1	13	12
3	0.5	10	0
4	0.25	7	0
5	0.125	0	0

Analysis of Gel

It was observed that the appearance of gel was clear homogenized with golden yellow colour with a pH 7.2 even after 30 days of preparation. The results of gel analysis are summarized in table 4. Polyphenols are aromatic compounds either developed from shikimic acid or polyacetyl metabolism. Anthocyanins, coumarins, lignins, flavonoids, tannins, quinones, acids and phenols generally are the families of different polyphenols [18, 19]. One of the phenol tannins acts as free radical scavengers, anti-inflammatory and anti-microbial in case of skin diseases and infections [20, 21]. In present investigation when 100 mg/ml

concentration of *G. glabra* extract was used to add into the gel, the total polyphenolic content within the gel was reported as 41.25 µg/ml of gel compare to standard plot with phenol equivalent to gallic acid (table 5 and figure 1). Also the gel in a volume of 20 mg in was inhibitive against both *S. aureus* and *P. acnes* bacterial. This much availability of TPC in gel convinces the beneficial therapeutic prosperities of any formulation.

Table 4: Evaluation of Topical Gel formulation

S.N.	Parameter	Observations
1.	Colour	Golden yellow
2.	Appearance	Clear and homogenous
3.	pH	7.2
4.	Spreadablility	70.84 sq. cm.
5.	TPC in Gel	41.25 µg/ml
6.	*In vitro* antimicrobial potential of gel	Positive on both bacteria

Table 5: Gallic acid as standard concentration vs absorbance at 765 nm to plot standard curve for estimation in samples using Folin-Ceucaltues Method

S.N.	Concentration (µg/ml)	Absorbance (λ)
1	10	0.237
2	20	0.592
3	30	0.870
4	40	1.159
5	50	1.532

Instrument Used: Single beam visible range digital microprocessed spectrophotometer from Electronic India model EI-2305.

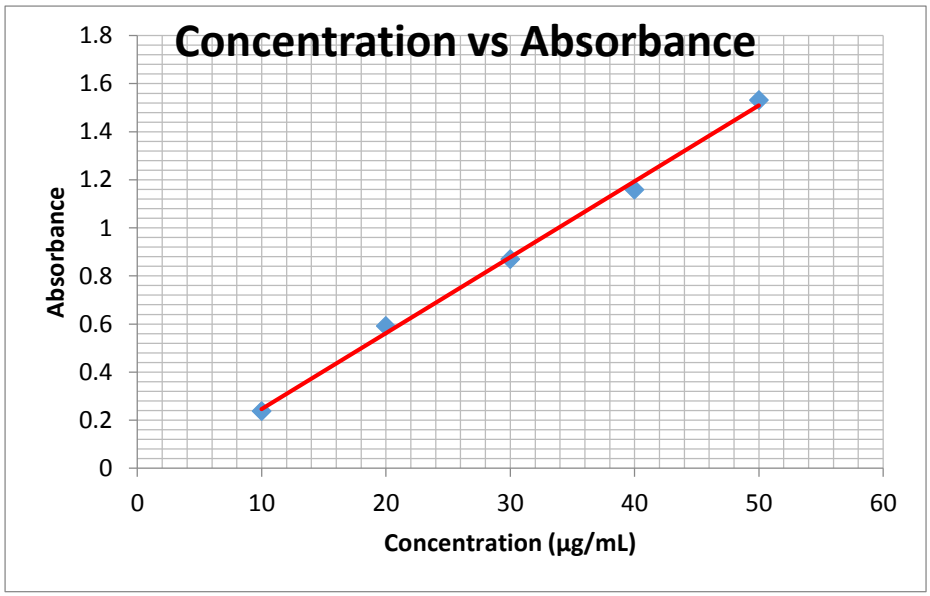

Figure 1: Standard Plot for know concentration of *Gallic Acid Standard*. The Graph is obtained from Excel 2010 linear regression function

Conclusions

The results of antimicrobial activity of *G. glabra* extracts as well as carbopol based gel containing *G. glabra* extracts against *S. aureus* and *P. acnes* suggest the utility of such preparations in therapeutic use against the skin diseases and acne problems. The physicochemical properties of prepared gels are also satisfying under lab tests. The availability of phytochemical, flavonoids, polyphenols and other active phytodrug constituents responsible for pharmacological activity could be further investigated in order to commercialize such therapeutic preparations for topic use in skin cure or improvement.

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