Research Article

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ANTIACNE EFFECT OF POLYHERBAL GEL FORMULATION IN MALE SPRAGUE-DAWLEY RATS

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ABSTRACT

Acne vulgaris is the commonest skin disorder to affect humans, characterized by both non-inflammatory (comedones) and inflammatory lesions (papules, pustules, and nodulocystic lesions). Topical gel formulation has been developed containing *Barleria prionitis, Butea monosperma, Casuarina equisetifolia, Dalbergia sissoo* and *Lagenaria siceraria*. In vivo antiacne activity was performed for the formulations using male sprague dawley rats. Physical observations were carried out at regular intervals on the rat ear and progressive fading of comedo was found on treated animals. Test group showed significant improvement on comedo reduction of rat pinna observed photographically. Test group showed a significant improvement compared to standard. The present study scientifically evaluates the antiacne potential of the herbs as a single entity as well as in combination using in vivo methods. The results obtained will help in identification and isolation of bioactive constituents for treating the root causes of acne without side effects.

Keywords: Acne vulgaris, comedo, *Propionibacterium acnes*, Antibacterial assay, in-vivo

INTRODUCTION

Acne vulgaris is the commonest skin disorder to affect humans, characterized by both non-inflammatory (comedones) and inflammatory lesions (papules, pustules, and nodulocystic lesions). It is a disease of the pilosebaceous follicle with comedones resulting from the hyper cornification of the keratinocytes of the duct wall and usually preceding inflammatory lesions.[1] Of particular interest in the pathophysiology of inflammatory acne is the role of the normal skin commensal bacterium *Propionibacterium acnes*. Although not a requirement for comedogenesis, a number of observations have suggested that *P. acnes* is implicated in the pathogenesis of inflammatory acne. The density of *P. acnes* increases markedly during puberty coinciding with the onset of the disease.[2]Treatments that reduce *P. acnes* numbers lead to clinical improvement of acne and finally, the emergence of antibiotic-resistant *P. acnes* strains are linked to the failure of antibiotic treatment[3,4]

Many synthetic drugs like Benzoyl peroxide, antibiotics, anti-androgens are used to treat this disorder but these drugs also exhibit several side effects like dryness of skin, dermatitis, bleaching cloth, darkening of skin and recurrence after withdrawal.[5]

In literature review it was found that *Barleria prionitis, Butea monosperma, Casuarina equisetifolia, Dalbergia sissoo* and *Lagenaria siceraria* were used for most of the skin ailments including acne.[6] So, taking into consideration the literature review and the presence of different chemical constituents like alkaloids, flavonoids, tannins, steroids and saponins, an attempt is made to formulate a polyherbal topical gel in order to develop a safer, effective and cheaper remedy for healing acne. The in-vivo activity was done on the rat ear model. Histological parameters like hyperkeratosis, comedo formation, dilated sebaceous gland, leucocyte infiltration and acanthosis were studied.

MATERIALS AND METHODS

Collection of plant material

The plant materials were collected from the Ahmednagar District and authenticated at Botanical Survey of India, Pune (Voucher specimen number SATBAP 1, SATBUM 2, SATCAE 3, SATLAS 4 AND SATDAS 5, vide letter No.BSI/WRC/Tech/2010.) (Table 1)

Table 1: Plant materials used

Plant	Family	Part used
Barleria prionitis	Acanthaceae	Leaves
Butea monosperma	Papilionaceae	Leaves
Casuarina equisetifolia	Casuarinaceae	Bark
Dalbergia sissoo	Papilionaceae	Bark
Lagenaria siceraria	Cucurbitaceae	Fruit

Extraction

Dried powder of the plant parts were extracted by continuous hot extraction (soxhlet) method using ethanol (95%). The extract obtained was concentrated and then subjected to phytochemical screening.[7]

Formulation of gel

Required quantity of Carbomer 940 was soaked in sufficient amount of distilled water for 2 to 3 hrs. (Phase I). The extracts were dissolved in small amount of propylene glycol. Propyl paraben and methyl paraben were later added as preservatives (Phase II). Phase I and II were mixed and adjusted to a pH of 6.8-7.4 by drop wise addition of triethanolamine. The remaining quantity

of distilled water was then added to make up the final 100gm weight. The formulation was stirred with mechanical stirrer to homogenize the formulation. (Table 2)[8]

Table 2: Formulation of gel

Ingredient	Concentration
Carbopol 940	2.0
Propylene glycol	10
Propyl paraben	0.08
Methyl paraben	0.2
Triethanolamine	q.s
B.prionitis	1.2
B.monosperma	1.0
C.equisetifolia	0.3
D.sisso	0.7
L.siceraria	1.0
Distilled water	q.s

In-vivo Antiacne activity

To conduct the animal studies, an approval from the institutional animal ethical committee was obtained (Letter No. ACP/IAEC/11-12/25-10). Groups of 5 adult male Sprague-Dawley rats weighing 180-220 gm were shaved in the interscapular area. Twenty four hours later, the test preparation, control and standard were applied locally to the shaved area. The treatment was continued for three weeks. Controls received glycerin. Clindamycin was used as a standard. The animals were sacrificed 24 h after the last dose administration. Pieces of skin from the interscapular region were excised and processed for evaluation by electron microscopy. Intradermal injection of killed *P.acnes* into the rat ear was used to induce chronic acne like inflammation characterized by edema, cell infiltration and formation of comedones.[9] The histological parameters observed before and after application of the formulation were hyperkeratosis, comedo formation, dilated sebaceous gland, leucocytic infiltration, acanthosis and necrosis of epithelium.

HPTLC Chemoprofiling of the extracts and the formulation

The HPTLC chemoprofiling of the extracts was done to confirm for the presence of the chemical constituents in the extract as well as in the formulation. About 100 mg of dry powdered herbal extract was mixed with little quantity of methanol by sonication and then the volume was made up to 10 ml with methanol. Pre-coated silica gel $G_{60}F_{254}$aluminium plates were used. 10µl of the individual ethanolic extracts and the extract of the formulation was applied by using HPTLC applicator. Toluene: Ethyl acetate: Formic acid (5:4:1) was used as the mobile phase. The TLC plates were developed in a developing chamber to a sufficient distance. The detection of spots on TLC plates was carried out by using the spray reagents as 5 % Ferric Chloride & Dragendroff's reagent[10].

RESULT AND DISCUSSION

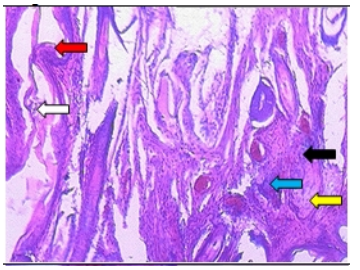
The in vitro antiacne potential of the plant materials against *Propionibacterium acnes* and *Staphylococcus epidermidis* have already been proven in our previous paper[11]. The plant extracts showed the presence of tannins, steroids, saponins and flavonoid compounds (Table 3). These extracts in a polyherbal formulation showed a significant reduction in the comedones without necrosis as compared with the standard clindamycin. (Table 4) .

Table 3: Phytochemical screening of the extracts

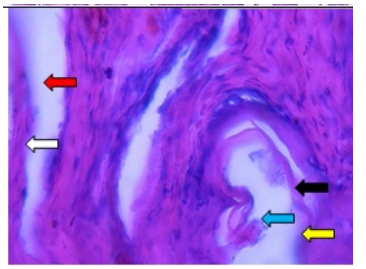
Phytochemical	Test	*Barleria prionitis*	*Butea monosperma*	*Casuarina equisetifolia*	*Dalbergia sisso*	*Lagenaria siceraria*
Carbohydrates	Molish test	+	+	+	+	+
Proteins	Biuret test Ninhydrin test	-	+	-	-	+
Alkaloids	Dragendroff's test Mayer's test	+	+	-	-	+
Tannins	$FeCl_3$ test Lead acetate test	+	+	+	+	-
Flavonoids	Shinoda test	+	+	+	+	-
Steroids	Liebermann Burchard test	-	-	-	+	+
Saponins	Foam test	+	+	-	-	+
Fixed oils & Fats	Saponification test	-	-	-	-	-
Mucilage	Ruthenium red test	-	-	-	-	-

Table 4: In vivo antiacne activity of the formulation

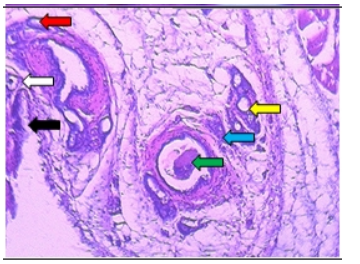
Group	Control	Standard	Formulation
Hyperkeratosis	++++	++	++
Comedo formation	+++	+	+
Dilated sebaceous gland	++++	+	+
Leucocytic infiltration	+++	+	+
Acanthosis	+++	+	+
Necrosis of epithelium	+++	++	+



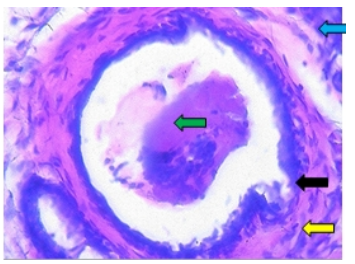
A Control group: H&E stain 100X



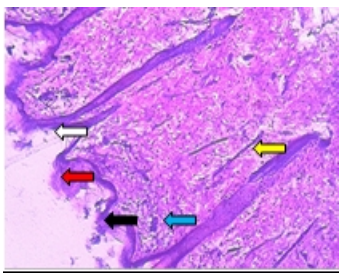
B Control group: H&E stain 400X



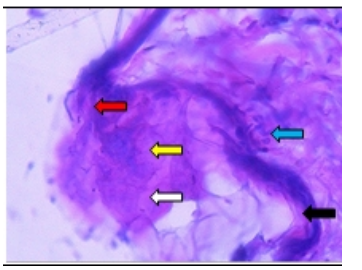
C Control group: H&E stain 100X



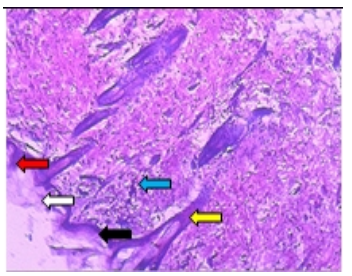
D Control group: H&E stain 400X



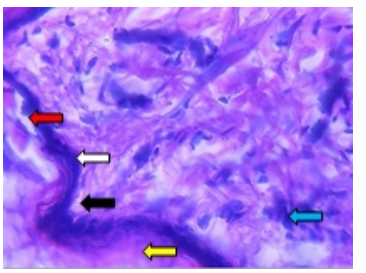
E Standard group: H&E stain 100X



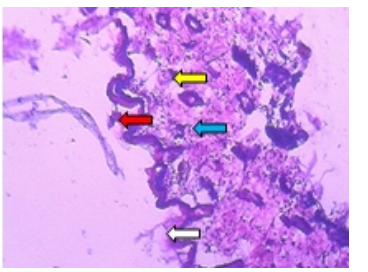
F Standard group: H&E stain 400X



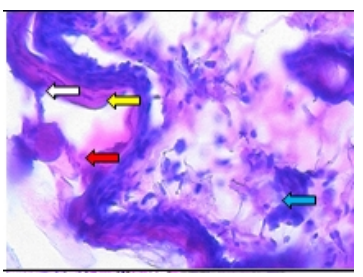
G Standard group: H&E stain 100X



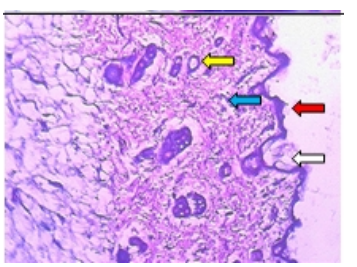
H Standard group: H&E stain 400X



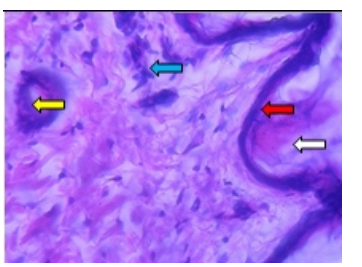
I Test group: H&E stain 100X



J Test group: H&E stain 400X

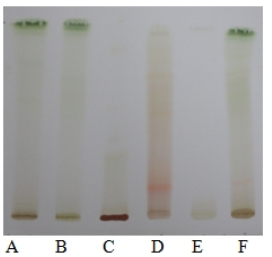


K Test group: H&E stain 100X

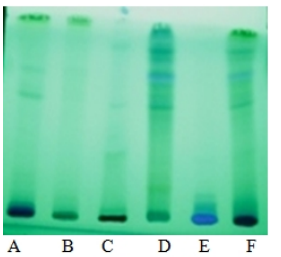


L Test group: H&E stain 400X

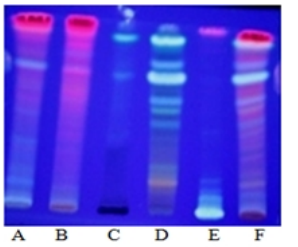
Figure 1: Antiacne activity of the control group, standard and the formulation
Hyperkeratosis (white arrow), dilated sebaceous gland (yellow arrow), necrosis (black arrow), acanthosis (red arrow), cellular infiltration (blue arrow), comedo formation (green arrow)



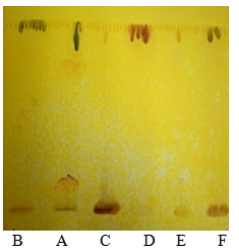
A: TLC for Tannins (Visible region)



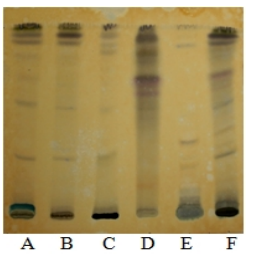
B: TLC for Tannins (UV 254 nm)



C: TLC for Tannins (UV 366 nm)



D: TLC for Alkaloids
Spraying reagent (Dragendorff's reagent)



E: TLC for Alkaloids
Spraying reagent (Ferric chloride)

Figure 2: HPTLC Chemoprofiling of the extracts and the optimized formulation
A=*Barleria prionitis*; B=*Butea monosperma*; C=*Casuarina equisetifolia*; D=*Dalbergia sisoo*; E=*Langeria sisararia*; F=Formulation

The histograms of the activity are shown in Figure 1. HPTLC chemoprofiling showed the presence of tannins in the extracts and the formulation as shown in Figure 2.

CONCLUSION

The antiacne activity of the extracts can be assumed due to the presence of chemical constituents like tannins, flavonoids, steroids, saponins and alkaloids. Tannins may have a similar type of activity like Benzoyl peroxide since Benzoyl peroxide releases the oxygen from the carboxyl group and this oxygen creates an aerobic oxidizing environment for the anaerobic bacteria which is toxic. Tannins have also shown potential free radical scavenging, antiviral, antibacterial and antiparasitic effects.[12] .The better antiacne potential of the formulations can be attributed to a synergistic effect shown by all the extracts. Hereby it is concluded that the herbal plant materials show a promising antiacne potential as compared to the synthetic medicine. The future work includes isolation of active phytoconstituents, its formulation and evaluation for its antiacne potential.

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