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Research Article

Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria

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Abstract

Propionibacterium acnes and *Staphylococcus epidermidis* have been recognized as pus-forming bacteria triggering an inflammation in acne. The present study was conducted to evaluate antimicrobial activities of Indian medicinal plants against these etiologic agents of acne vulgaris. Ethanolic extracts of *Hemidesmus indicus* (roots), *Eclipta alba* (fruits), *Coscinium fenestratum* (stems), *Curcubito pepo* (seeds), *Tephrosia purpurea* (roots), *Mentha piperita* (leaves), *Pongamia pinnata* (seeds), *Symplocos racemosa* (barks), *Euphorbia hirta* (roots), *Tinospora cordyfolia* (roots), *Thespesia populnea* (roots), and *Jasminum officinale* (flowers) were tested for antimicrobial activities by disc diffusion and broth dilution methods. The results from the disc diffusion method showed that 07 medicinal plants could inhibit the growth of Propionibacterium acnes. Among those *Hemidesmus indicus, Coscinium fenestratum, Tephrosia purpurea, Euphorbia hirta, Symplocos racemosa, Curcubito pepo* and *Eclipta alba* had strong inhibitory effects. Based on a broth dilution method, the *Coscinium fenestratum* extract had the greatest antimicrobial effect. The MIC values were the same (0.049 mg/ml) for both bacterial species and the MBC values were 0.049 and 0.165 mg/ml against Propionibacterium acnes and Staphylococcus epidermidis, respectively. In bioautography assay, the *Coscinium fenestratum* extract produced strong inhibition zones against Propionibacterium acnes. Phytochemical screening of *Coscinium fenestratum* revealed the presence of alkaloid which could be responsible for activity. Taken together, our data indicated that *Coscinium fenestratum* had a strong inhibitory effect on *Propionibacterium acnes* and *Staphylococcus epidermidis*.

Keywords: Acne; *Propionibacterium acnes; Staphylococcus epidermidis;* Antimicrobial activity

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INTRODUCTION

Acne vulgaris is a most common skin disorder of Pilosebaceous unit. That affects areas containing the largest oil glands, including the face, back, and trunk[1]. It is generally characterized by formation of seborrhea, comedone, inflammatory lesions and presence of bacteria *Propionibacterium acnes, Staphylococcus epidermidis* and *Malassezia furfur* in the follicular canal and sebum production [2]. Propionibacterium acnes have been described as an obligate anaerobic organism. It is implicated in the development of inflammatory acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils. On the contrary, Staphylococcus epidermidis, an aerobic organism, usually involves in superficial infections within the sebaceous unit[3]. These factors provide a potential target for treatment. *Propionibacterium acnes* and *Staphylococcus epidermidis* are the target sites of antiacne drugs[4, 5]. Long term use of antibiotics against acne is outdated because of exacerbated antibiotic resistance[6, 7]. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases. In the present study, 12 medicinal plants, which have been traditionally used as antimicrobial and anti-inflammatory agents were examined for antimicrobial activities against microorganisms frequently involved in acne inflammation, Propionibacterium acnes and Staphylococcus epidermidis.

MATERIALS AND METHODS

Plant material

The 12 plant materials used in this study were collected from various locations in India. Authentication of the plant materials was done by comparison with plant specimens located at Bangalore. Herbarium and Botanical Section of Regional Research Institute (Ayurveda), Jaynagar, Bangalore. The specimens were deposited at Department of Pharmacognosy, Rural College of Pharmacy, Devanahalli, Bangalore Rural District, Karnataka, India.

Microorganisms and media

The test organisms used in this study were as followed: *Propionibacterium acnes* (MTCC 1951) and Staphylococcus epidermidis (MTCC 931). These bacteria were obtained
from the Microbial Type Culture Collection and Gene Bank, Chandigarh, India All media were purchased from Himedia.

Preparation of plant extracts

Dried parts of the plants were made into coarse powder. 400 g *Hemidesmus indicus*(roots, 24.2% w/w), *Eclipta alba* (fruits, 13.1% w/w), *Coscinium fenestratum* (stems, 20.4% w/w), *Curcubito pepo* (seeds, 17.9% w/w), *Tephrosia purpurea* (roots, 6.9% w/w), *Mentha piperita* (leaves, 14.3% w/w), *Pongamia pinnata* (seeds, 23.1% w/w), *Symplocos racemosa* (barks, 19.5% w/w), *Euphorbia hirta* (roots, 17.4% w/w), *Tinospora cordyfolia* (roots, 18.4% w/w), *Thespesia populnea* (roots, 17.6% w/w), and *Jasminum officinale* (flowers, 12.5% w/w) were macerated in ethanol. The macerate was filtered after seven consecutive days, filtrate was dried under reduced pressure and finally under vaccum desicator.

Antimicrobial susceptibility testing

Disc diffusion method

This experiment was performed by the method of Hayes and Markovic[8] with some modifications. Propionibacterium acnes was incubated in brain heart infusion medium (BHI) with 1% glucose for 48 h under anaerobic conditions and adjusted to yield approximately 1.0×10^8 CFU/ml. Aliquots of molten BHI with glucose agar were used as the agar base. A prepared inoculum was added to the molten agar, mixed, poured over the surface of the agar base and left to solidity. A sterile paper disc was impregnated with test material (100 mg/ml).and placed on the agar. Clindamycin (10 µg/ml) was used as the standard. The plates were then incubated at 37^0C for 48 h under anaerobic

TABLE 1: Antimicrobial Activity of Medicinal Plant Extracts

Plant extracts	Susceptibility of bacteria to medicinal plant extracts Zone of inhibition (mm)[a]*	
	Propionibacterium acnes	***Staphylococcus epidermidis***
Hemidesmus indicus	13	14
Eclipta alba	12	10
Coscinium fenestratum	15	16
Curcubito pepo	12	14
Tephrosia purpurea	12	13
Mentha piperita	08	12
Pongamia pinnata	08	09
Symplocos racemosa	14	14
Euphorbia hirta	13	12
Tinospora cordyfolia	07	06
Thespesia populnea	05	05
Jasminum officinale	06	07
Clindamycin	19	20

[a]*Concentration of the extract used: 100 mg / ml, Clindamycin: 100 μg / ml*
**Mean of triplicate measurements*

conditions in an anaerobic jar (Hi-Media) with gas pack and indicator strip and the jar was kept in an incubator for 48 h at 37 ± 1^{0}C. Gas packs containing citric acid, sodium carbonate and sodium borohydride were used to maintain and check the anaerobiosis, where citric acid releases carbon dioxide and sodium borohydride releases hydrogen when they come in contact with oxygen. An indicator strip of methylene blue, when introduced into the jar, changes in colour from white to blue in the absence of anaerobiosis. Staphylococcus epidermidis was incubated in tryptic soy broth (TSB) for 24 h at 37^{0}C and adjusted to yield approximately 1.0×10^8 CFU/ml. The procedures were the same as mentioned above except the plates were incubated at 37^{0}C for 24 h under aerobic conditions. All disc diffusion tests were performed in three separate experiments and antibacterial activity was expressed as the mean of inhibition diameters (mm) (see Table 1).

Determination of minimum inhibitory and bactericidal concentrations

The minimal inhibitory concentration (MIC) values were determined by broth dilution assay[9, 10,11]. The cultures were prepared at 24 h and 48 h broth cultures of *Staphylococcus epidermidis* and *Propionibacterium acnes*, respectively. The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms.

3ml of the Nutrient yeast glucose broth (NYG) for *Propionibacterium acnes*, and Nutrient broth for *Staphylococcus epidermidis*, in 10 ml glass screw cap test tube was sterilised by autoclaving at 121^{0}C for 15min. The medium was cooled and inoculated with 50 μl of the bacterial suspension containing 1 x 10^8 cells/ml. 1 ml of the plant extracts (100mg/ml) was added to corresponding test tubes under anaerobic condition. 3 ml of NYG broth inoculated with 50μl

of organisms was taken as positive control. The test tubes were then kept in anaerobic jar (Hi-Media) with gas pack and indicator strip and the jar was kept in incubator for 48 h at 37 ± 1^0C. Gas packs containing citric acid, sodium carbonate and sodium borohydride were used to maintain and check the anaerobiosis, where citric acid releases carbon dioxide and sodium borohydride releases hydrogen when they come in contact with oxygen. An indicator strip of methylene blue, when introduced into the jar, changes in colour from white to blue in the absence of anaerobiosis. For *Staphylococcus epidermidis*, the test tubes were incubated at 37 ± 1^0C for 24 h aerobically and growth of *Propionibacterium acnes* and *Staphylococcus epidermidis* was measured as a function of turbidity at 660 nm using (Systronics 131) Nephaloturbidometer. The MIC and MBC values of 12 medicinal plant extracts against *Propionibacterium acnes* and *Staphylococcus epidermidis* were determined. The results are shown in Table 2 as average values from three separate experiments per medicinal plants and indicate the susceptibility of bacteria to the medicinal plant extracts.

Phytochemical screening[12]

The ethanolic extract was subjected to preliminary phytochemical testing for the detection of major chemical groups. The details of the tests are as follows:

1. For phenols: The ethanolic extract was spotted on a filter paper. A drop of phoshomolybdic acid reagent was added to the spot and was exposed to ammonia
Vapours (Blue coloration of the spot indicates the presence of phenols).
2. Braemer's test for tannins: To a 2–3 ml of methanolic extract, 10% alcoholic ferric chloride solution was added. (Dark blue or greenish grey coloration of the solution indicate the presence of tannins in the drug).
3. Liebermann-Burchardt test for steroids and terpenoids: To 1 ml of methanolic extract of drug, 1 ml of chloroform, 2–3 ml of acetic anhydride and 1 to 2 drops of concentrated sulfuric acid were added. (Dark green coloration of the solution indicate the presence of Steroids and dark pink or red coloration of the solution indicate the presence of terpenoids).
4. Alkaloids: A drop of methanolic extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Dragendorff's reagent. (Orange coloration of the spot indicates the presence of alkaloids).
5. Bornträger's test for anthraquinones: About 50 mg of methanolic extract was heated with 10% ferric chloride solution and 1 ml of concentrated hydrochloric acid. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract
was further extracted with strong ammonia. (Pink or deep red coloration of aqueous layer indicate the presence of anthraquinones).
6. Shinoda test for flavonoids: To 2–3 ml of methanolic extract, a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid were added. (Pink red or red coloration of the solution indicate the presence of flavonoids in the drug).

Bioautography

Bioautography was performed with bacterial cultures exhibiting high sensitivity to the extracts. Developed TLC plates were carefully dried for complete removal of solvent,
overlaid with agar containing an aliquot of an overnight culture and incubated at 37 ◦C. The plates were run in duplicate; one set was used as the reference chromatogram and the other was used for bioautography.

RESULTS

In the present study, 12 medicinal plant extracts were examined for antimicrobial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis*. The results showed that 07 extracts could effectively inhibit the growth of *Propionibacterium acnes*. Among these, ethanolic extracts of *Hemidesmus indicus, Eclipta alba*, *Coscinium fenestratum, Curcubito pepo, Symplocos racemosa, Euphorbia hirta* and *Tephrosia purpurea* showed strong inhibitory effects Table 1) Interestingly, *Coscinium fenestratum*, *Hemidesmus indicus*, and *Symplocos racemosa* extracts showed promising antibacterial activities against both Propionibacterium *acnes* and *Staphylococcus epidermidis.* The remaining 5 plant extracts had

Table 2: The MIC and MBC values of 12 medicinal plant extracts against *Propionibacterium acnes* and *Staphylococcus epidermidis*. The results are shown as mean of triplicate measurements

Plant extracts	Susceptibility of bacteria to medicinal plant extracts[a]			
	Propionibacterium acnes		*Staphylococcus epidermidis*	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Hemidesmus indicus	0.051	2.5	1.25	>4
Eclipta alba	0.665	>5	0.312	>5
Coscinium fenestratum	0.049	0.049	0.049	0.165
Curcubito pepo	1.25	1.25	2.5	5
Tephrosia purpurea	0.675	1.25	2.5	>5
Mentha piperita	>5	>5	>5	>5
Pongamia pinnata	2.5	>5	2.5	>5
Symplocos racemosa	0.685	1.35	0.685	>4
Euphorbia hirta	1.55	1.95	2.5	5
Tinospora cordyfolia	5	5	5	>5
Thespesia populnea	>5	>5	>5	>5
Jasminum officinale	5	>5	>5	>5
Clindamycin*	78	85	76	72

[a]*The results indicate of average of 3 separate experiments*

**Clindamycin- All values are in µg/ml*

no detectable activity against *Staphylococcus epidermidis*. Subsequent experiments were conducted to determine inhibitory concentrations of all selected plant extracts. *Coscinium fenestratum* showed the potent antimicrobial effect. The MIC values against both organisms were equal (0.049 mg/ml) and the MBC values were 0.049 and 0.165 mg/ml against *Propionibacterium acnes* and *Staphylococcus epidermidis*, respectively (Table 2). Further, the plant extracts was subjected to preliminary Phytochemical screening for the presence and absence of different chemical groups (Table 3).

DISCUSSION

The similar values of MIC and MBC obtained from this plant against Propionibacterium acnes suggest that the ethanolic extract of *Coscinium*

Table 3: Preliminary phytochemical screening of 12 medicinal plant extracts

Plant extracts	Phenols	Tannins	Steroids	Alkaloids	Glycosides	Flavonoids	Terpenoids
Hemidesmus indicus	+++	++	--	--	+	+++	++
Eclipta alba	++	+++	--	--	--	+++	+
Coscinium fenestratum	++	--	--	+++	--	+++	+
Curcubito pepo	+	++	++	--	+	+	+
Tephrosia purpurea	+	--	+	--	++	+++	--
Mentha piperita	+	--	++	--	--	--	+++
Pongamia pinnata	--	--	++	++	--	--	++
Symplocos racemosa	--	++	++	+++	++	--	--
Euphorbia hirta	++	++	--	--	+	+++	--
Tinospora cordyfolia	+	--	+	+++	--	++	+
Thespesia populnea	--	--	+	+++	+	--	--
Jasminum officinale	++	--	--	--	--	+++	+++

– = absent; + = Trace; ++ = moderate, +++ = Abundant

fenestratum could possibly act as a bactericidal agent to this microorganism. In addition, the *Hemidesmus indicus* extract also showed good antimicrobial effects against *Propionibacterium acnes* with a MIC of 0.051 mg/ml but a high concentration was required to kill both *Propionibacterium acnes* and *Staphylococcus epidermidis* as compared to the ethanolic extract of *Coscinium fenestratum*. *Symplocos racemosa*, showed outstanding antimicrobial properties against *Propionibacterium acnes* based on the disc diffusion assay, each had a MIC value of 0.685 mg/ml and a MBC of 1.35 mg/ml for *Propionibacterium acnes.* The plant extracts were further analyzed by phytochemical screening for detection of phytoconstituents. The assay for bioautography demonstrated strong inhibition zones of *Coscinium fenestratum* extract against the growth of *Propionibacterium acnes*. The clear zones were located in separate places on the TLC plate, suggesting that more than one compound possessed an antimicrobial effect. There were no inhibition zones presented above the bands of the other plant extracts covered with Propionibacterium acnes. This implied that the strongest effect of the *Coscinium fenestratum* extract was against *Propionibacterium acnes.* Phytochemical screening of *Coscinium fenestratum* extract showed positive results for the presence of alkaloids. Alkaloid and its derivatives have activities against *Staphylococcus aureus* and methicillin-resistant *S. aureus*[13]. The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmane[14] is attributed to their ability to intercalate with DNA[15]. It is possible that berberine an alkaloid present in *Coscinium fenestratum* may act in the same mechanism to inhibit *Propionibacterium acnes* and

Staphylococcus epidermidis. Therefore, the active component of the *Coscinium fenestratum* extract could be of interest for further development as an alternative treatment for acne.

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