ANTIBACTERIAL SCREENING OF SELECTED INDIAN MEDICINAL PLANTS AGAINST ACNE-INDUCING BACTERIA

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Summary

The present study was conducted to evaluate antibacterial activities of Indian medicinal plants against *Propionibacterium acnes* and *Staphylococcus epidermidis* which have been recognized as pus-forming bacteria triggering an inflammation in acne which are etiologic agents of acne vulgaris. Ethanolic extracts of *Ammania baccifera, Hibiscus syriacus, Quercus infectoria, Berberis aristata, Couroupita guianensis, Tectona grandis, Verbena officinalis, Symplocos racemosa, Mucuna pruriens, Vitex trifolia, Coccus mucifera,* and *Jasminum grandiflora* 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 *Ammania baccifera, Hibiscus syriacus, Quercus infectoria, Berberis aristata, Couroupita guianensis, Symplocos racemosa,* and *Mucuna pruriens* had strong inhibitory effects. Based on a broth dilution method, the ethanolic extract of *Symplocos racemosa* had the greatest antimicrobial effect. The MIC values were the same (0.044 mg/ml) for both bacterial species and the MBC values were 0.044 and 0.156 mg/ml against *Propionibacterium acnes* and *Staphylococcus epidermidis*, respectively. In bioautography assay, the *Symplocos racemosa* extract produced strong inhibition zones against *Propionibacterium acnes*. Antimicrobial activity from fractions of column chromatography revealed one of the active compounds in *Symplocos racemosa* is an alkaloid harmine, which could be responsible for activity. Further a HPTLC method was followed to quantify harmine in *Symplocos racemosa.* Precoated Silica gel G plates were used as stationary phase and toluene: ethyl acetate: methanol (60:20:20) was used as mobile phase. Detection and quantification were performed by densitometry at λ 324 nm.

Keywords: Acne, Plant extracts, MIC, Harmine, *Propionibacterium acnes*, *Staphylococcus epidermidis.* HPTLC,

Introduction

Acne vulgaris is a chronic inflammatory disorder in adolescents consists of the pilosebaceous follicles, characterized by comedones, papules, pustules, cysts, nodules and often scars, mainly of face, neck, back and trunk (1). The microorganisms involved include *Propionibacterium acnes* and *Staphylococcus epidermidis*. *Propionibacterium acnes* (2) 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 (Rcp/Pcog/Herb/10-21/2006) 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.

High performance thin layer chromatography (HPTLC)

Plate: Precoated silica gel 60F $_{254}$ HPTLC plate (E. Merck) (0.2 mm thickness). Spotter: CAMAG Linomat 5, Developing chamber: CAMAG glass twin trough chamber. Scanner: CAMAG TLC scanner 3 and WINCATS 4.0 integration software.

Preparation of plant extracts

Dried parts of the plants were made into coarse powder. 300 g *Verbena officinalis* (roots, 20.2% w/w), *Quercus infectoria* (fruits, 19.1% w/w), *Berberis aristata* (roots, 25.4% w/w), *Coccus mucifera* (seeds, 19.9% w/w), *Couroupita guianensis* (roots, 16.9% w/w), *Jasminum grandiflora* (flowers, 17.3% w/w), *Mucuna pruriens* (seeds, 20.1% w/w), *Symplocos racemosa* (barks, 19.5% w/w), *Tectona grandis* (roots, 17.7% w/w), , *Hibiscus syriacus* (roots, 18.4% w/w),

Ammania baccifera (roots, 15.6% w/w), and *Vitex trifolia* (roots, 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 vacuum desiccator.

Antimicrobial susceptibility testing

Disc diffusion method

This experiment was performed by the method of (8) (9) 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 an agar base. A prepared inoculum was added to molten agar, mixed and poured over the surface of the agar base and left to solidity. A sterile paper disc was impregnated with test material (100 mg/m), Clindamycin as standard 10 µg/ml) and the disc was placed on the agar. Plates were then incubated at 37 $^{\circ}$C for 48 h under anaerobic conditions in anaerobic jar (Hi-Media) with gas pack and indicator strip and the jar was kept in incubator for 48 h at 37 $\pm$ 1^0c. Gas packs containing citric acid, sodium carbonate and sodium borohydride were used to maintain and check the anaerobiasis, where citric acid releases carbon dioxide and sodium borohydride releases hydrogen when they come in contact with oxygen. An indicator strip of methylene blue is introduced into the jar which changes the colour from white to blue in absence of anaerobiasis. *Staphylococcus epidermidis* was incubated in tryptic soy broth (TSB) for 24 h at 37 $^{\circ}$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 $^{\circ}$C for 24 h under aerobic conditions. All disc diffusion tests were performed in three separate experiments and the antibacterial activity was expressed as the mean of inhibition diameters (mm).

Determination of MIC and MBC

The minimal inhibitory concentration (MIC) values were determined by broth dilution assay (10) (11) (12). 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. 3 ml 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 sterilized by autoclaving at 121^0c for 15 minutes. 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 (100 mg/ml) was added to corresponding test tubes under anaerobic condition. 3 ml of NYG broth inoculated with 50 µl of organisms was taken as positive control were placed in anaerobic condition at 37 ± 1^0 c for 48 h. For *Staphylococcus epidermidis* the test tubes were incubated at 37 ± 1^0 c for 24 h aerobically and growth of *Propionibacterium acnes* and *Staphylococcus epidermidis* was measured as 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*. The results are shown Table 1 as average values from three separate experiments Medicinal plants Susceptibility of bacteria to medicinal plant extracts *Propionibacterium acnes* *Staphylococcus epidermidis* MIC (mg/ml) MBC (mg/ml) MIC (mg/ml) MBC (mg/ml)

Phytochemical chemical screening

The ethanolic extract was subjected to preliminary phytochemical testing (13) for the detection of major chemical groups like phenols, alkaloids, cardiac glycosides, tannins, terpenoids, steroids and flavanoids.

HPTLC analysis (14)

Preparation of standard solution of harmine:

A stock solution of harmine was prepared by dissolving 10 mg of accurately weighed harmine in methanol and making up the volume to 100 ml with methanol. From this stock solution standard solutions of 100 to 500ng/µL were prepared by transferring aliquots (1 to 6 mL) of stock solution to 10 ml volumetric flasks and adjusting the volume with methanol.

Preparation of sample solutions:

Accurately weighed 1 g amount of powder of stem bark of *S. racemosa* was extracted for 6 hrs with 1 ml of Ammonium hydroxide and 10 ml of methanol and made up to 10 ml methanol in a volumetric flask.

Estimation of harmine from *S. racemosa* stem bark:

A 10 µl volume of sample solution was applied in triplicate on a precoated silica gel G60 HPTLC plate (E. Merck) with the CAMAG Linomat V Sample spotter. The plate was developed and scanned. The peak areas were recorded. The amount of harmine present in the sample was calculated using the calibration curve for harmine.

Bioautography

Bioautography (15) 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 *Quercus infectoria, Berberis aristata, Couroupita guianensis, Symplocos racemosa, Mucuna pruriens* and *Jasminum grandiflora* showed strong inhibitory effects Interestingly, *Berberis aristata* and *Symplocos racemosa* extracts showed promising antibacterial activities against both *Propionibacterium acnes* and *Staphylococcus epidermidis.* The remaining 5 plant extracts had no detectable activity against *Staphylococcus epidermidis*. Subsequent experiments were conducted to determine inhibitory concentrations of all selected plant extracts. *Symplocos racemosa,* showed the potent antimicrobial effect. The MIC values against both organisms were equal (0.044 mg/ml) and the MBC values were 0.044and 0.156 mg/ml against *Propionibacterium acnes* and *Staphylococcus epidermidis*, respectively (Table 1). Further, the plant extracts was subjected to preliminary Phytochemical screening for the presence and absence of different chemical groups

HPTLC

Of the various solvent systems tried that containing toluene-ethyl acetate-methanol (60:20:20 v/v) was found to be the most suitable one. In this system, harmine was resolved (R_f = 0.35) (Figure 1a) in the presence of other compounds in the sample extract (Figure 2). The identity of the bands of harmine in the sample extracts was confirmed by overlaying their UV absorption spectra with those of the standard harmine using a CAMAG TLC Scanner 3.

Table 1: The MIC and MBC values of 12 medicinal plant extracts. [a] The results indicate of average of 3 separate experiments *Clindamycin- All values are in µg/ml.

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)**
Quercus infectoria	0.052	2.5	1.50	>4
Berberis aristata	0.656	1.25	0.322	>5
Symplocos racemosa	0.044	0.044	0.044	0.154
Mucuna pruriens	1.5	1.25	2.0	5
Couroupita guianensis	0.675	1.85	2.0	>5
Hibiscus syriacus	>5	>5	>5	>5
Coccus mucifera	2.5	>5	2.5	>5
Jasminum grandiflora	0.885	1.35	0.980	>4
Verbena officinalis	1.50	2.0	2.5	5
Vitex trifolia	5	5	5	>5
Ammania baccifera	>5	>5	>5	>5
Tectona grandis	5	>5	>5	>5
Clinda-mycin*	56	66	55	69

The purity of harmine band in the sample extracts was confirmed by comparing the absorption spectra at start, middle, and end position of the bands. Further band of harmine were detected by spraying with dragendroff's reagent, after which the compounds appeared as violet bands.

The harmine content of *S. racemosa* was estimated by the above validated HPTLC method. The amount of harmine was found to be 0.268 (w/w). The described method allows the reliable quantification of harmine with good resolution from other constituents of *Symplocos racemosa.*

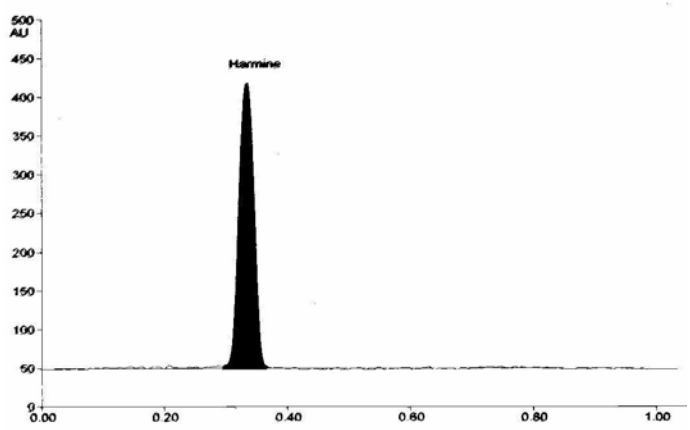

Fig. 1a - Standard Harmine peak

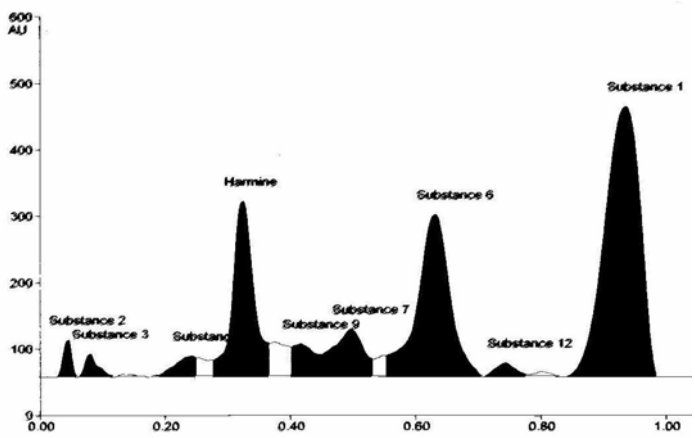

Fig. 2 – Sample extract Harmine peak

Discussion

The same value of MIC and MBC obtained from this plant against Propionibacterium acnes suggested that ethanolic extract of *Symplocos racemosa* could possibly act as a bactericidal agent to this microorganism. In addition, the *Quercus infectoria* extract also showed good antimicrobial effects against *Propionibacterium acnes* with a MIC of 0.052 mg/ml but a high concentration was required to kill both *Propionibacterium acnes* and *Staphylococcus epidermidis* as compared to the ethanolic extract of *Symplocos racemosa, Berberis aristata,* showed outstanding antimicrobial properties against *Propionibacterium acnes* based on the disc diffusion assay, each had a MIC value of 0.656 mg/ml and a MBC of 1.25 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 *Symplocos racemosa* 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 was no inhibition zones presented above the bands of the other plant extracts covered with *Propionibacterium acnes*. Phytochemical screening of *Symplocos racemosa* extract showed positive results for the presence of alkaloids. The amount of harmine was found to be 0.268 (w/w). The described method allows the reliable quantification of harmine with good resolution from other constituents of *S. racemosa.* This implied that the strongest effect of the *Symplocos racemosa* extract was against *Propionibacterium acnes.* Alkaloid and its derivatives have activities against *Staphylococcus aureus* and methicillin-resistant *S.* aureus (16). The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmine (17) is attributed to their ability to intercalate with DNA (18).

It is possible that berberine an alkaloid present in *Symplocos racemosa* may act in the same mechanism to inhibit *Propionibacterium acne*s and *Staphylococcus epidermidis*. Therefore, the active component of the *Symplocos racemosa* extract could be of interest for further development as an alternative treatment for acne.

References:

1. Leyden JJ..Current issues in antimicrobial therapy for the treatment of acne. J Eur Dermatol Venereol. 2001;15(3):51-55.

2. Leyden JJ. Therapy for Acne vulgaris. The New Eng J Med. 1997;156–1162.

3. Burkhart CG, Burkhart CN, and Lehmann PF. Acne: a review of immunologic and microbiologic factors. J Post Grad Med. 1999; 75:328–331.

4. Leyden JJ. The evolving role of Propionibacterium acnes in acne. Semin Cutan Med Surg. 2001; 20:139-143.

5. Lalla JK, Nanndedkar SY, Paranjappe MH and Talreja NB. Clinical trials of ayurvedic formulations in treatment of acne vulgaris. J Ethnopharmacol. 2001;18: 99-102.

6. Degroot HE and Friedlander SF. Update Acne. Curr Opin Pediatr. 1998;10: 381-386.

7. Swanson IK Antibiotic resistance of *Propionibacterium acnes* in Acne vulgaris. Dermatol Nurs. 2003;5:359–361

8. Hayes AJ, and Markovic B. Toxicity of Australian essential oil *Backhousia citriodora* (Lemon myrtle). Part 1. Antimicrobial activity and in vitro cytotoxicity. Food Chem Toxicol. 2002; 40: 535– 543.

9. Isao Kubo, Hisae Muroi and Aya Kubo. Naturally occurring antiacne agents. J Nat Prod. 1994;57(1): 9-1 7.

10. Kumar GS and Salma K. Antiacne activity of few natural products. Ind J Nat Prod. 2004;30(4):7-9.

11. Sahin F, Karaman I, Gulluce M, Ogutcu H, Sengul M, Adiguzel A, and Ozturk Kotan R. Evaluation of antimicrobial activities of *Satureja hortensis*. J Ethnopharmacol. 2003;87:61–65.

12. Kumar G S, Vrushabendra Swamy BM, Ashok Kumar CK, Jayveera KN, Sanjay Umachigi P, Dhanapal R, and Sreedhar C. Phytochemical and antiacne activity of *Hemidesmus indicus* R.Br. var. indicus. Adv Pharmacol Toxicol. 2007; 8(1): 75-78.

13. Ravishankara MN, Neeta S, Harish P, and Rajani M. Evaluation of antioxidant properties of root bark of Hemidesmus indicus R. Br. (Anantmul) Phytomed. 2002;9: 153–160

14. Kumar GS, Vrushabendra Swamy .BM, Ashoka Babu VL, Sreedhar C, Sreenivasa Rao T, and Tarakaram K. HPTLC estimation of harmine from the stem bark of *Symplocos racemosa* Roxb. Asian J Chem. 2006;18(4):2851-55.

15. Kumar GS, Jayaveera KN, Ashok Kumar CK, Umachigi Sanjay P, Vrushabendra Swamy BM, and Kishore Kumar DV.

Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. Trop J Pharm Res. 2007; 6(2):717-723

16. Valsaraj R, Pushpangadan P, and Smitt UW. Antimicrobial screening of selected medicinal plants from India. J Ethnopharmacol. 1997;58: 75-83.

17. Hopp KH, Cunningham LV, Bromel MC, Schermeister LJ, and Wahba KSK. *In vitro* antitrypanosomal activity of certain alkaloids against *Trypanosoma lewisi.* Lloydia. 1976; 39:375–377.

18. Phillipson JD and Neill MJO. New leads to the treatment of protozoal infections based on natural product molecules. Acta Pharm Nord. 1987;1:131–144.