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

Kumar GS*, Jayveera KN1, Ashok Kumar CK2, Vrushabendra Swamy BM3, Sanjay Umachigi P4, and Kishore Kumar DV5.

*Department of Pharmacognosy, 3Department of Pharmaceutics, 4Department of Pharmacology, 5Department of Pharmaceutical Chemistry, Rural College of Pharmacy, D.S.Road, Devanahalli- 562110, Bangalore Rural Dist, Karnataka, (India).
1Department of Chemistry, Jawaharlal Nehru Technological University College of Engineering, Ananthpur- 02, Andhra Pradesh, (India).
2Department of Pharmacognosy, Sree Vidyanikethan College of Pharmacy, A.Rangamepeta, Tirupathi, Chitoo Dist. Andhra Pradesh, (India).

*Corresponding author:
KUMAR.G.S
Assistant Professor,
Department of Pharmacognosy,
Rural College of Pharmacy,
D.S.Road, Devanahalli- 562110,
Bangalore Rural Dist, Karnataka, (India).
pharmacysk@rediffmail.com
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), Cocculus 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 °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 ± 10°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 is introduced into the jar which changes the colour from white to blue in absence of anaerobiosis. Staphylococcus epidermidis was incubated in tryptic soy broth (TSB) for 24 h at 37 °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°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°C 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°C for 48 h. For *Staphylococcus epidermidis* the test tubes were incubated at 37 ± 1°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) Nepheloturbidometer. 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)
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*, *Symlocos racemosa*, *Mucuna pruriens* and *Jasminum grandiflora* showed strong inhibitory effects. Interestingly, *Berberis aristata* and *Symlocos 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. *Symlocos racemosa*, showed the potent antimicrobial effect. The MIC values against both organisms were equal (0.044 mg/ml) and the MBC values were 0.044 and 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.

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Susceptibility of bacteria to medicinal plant extracts*a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Propionibacterium acnes</td>
</tr>
<tr>
<td></td>
<td>MIC (mg/ml)</td>
</tr>
<tr>
<td><strong>Quercus infectoria</strong></td>
<td>0.052</td>
</tr>
<tr>
<td><strong>Berberis aristata</strong></td>
<td>0.656</td>
</tr>
<tr>
<td><strong>Symplocos racemosa</strong></td>
<td>0.044</td>
</tr>
<tr>
<td><strong>Mucuna pruriens</strong></td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Couroupita guianensis</strong></td>
<td>0.675</td>
</tr>
<tr>
<td><strong>Hibiscus syriacus</strong></td>
<td>&gt;5</td>
</tr>
<tr>
<td><strong>Coccus mucifera</strong></td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Jasminum grandiflora</strong></td>
<td>0.885</td>
</tr>
<tr>
<td><strong>Verbena officinalis</strong></td>
<td>1.50</td>
</tr>
<tr>
<td><strong>Vitex trifolia</strong></td>
<td>5</td>
</tr>
<tr>
<td><strong>Ammania baccifera</strong></td>
<td>&gt;5</td>
</tr>
<tr>
<td><strong>Tectona grandis</strong></td>
<td>5</td>
</tr>
<tr>
<td><strong>Clindamycin</strong></td>
<td>56</td>
</tr>
</tbody>
</table>

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 dragendorff’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 *Symlocos 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 *Symlocos racemosa* may act in the same mechanism to inhibit *Propionibacterium acnes* and *Staphylococcus epidermidis*. Therefore, the active component of the *Symlocos racemosa* extract could be of interest for further development as an alternative treatment for acne.

References:


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

