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Antimicrobial and antimalarial properties of medicinal plants used by the indigenous tribes of Andaman and Nicobar Islands, India

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ABSTRACT

In this study, methanol extracts of six medicinal plants (*Alstonia macrophylla*, *Claoxylon indicum*, *Dillenia andamanica*, *Jasminum syringifolium*, *Miliusia andamanica* and *Pedilanthus tithymaloides*) traditionally used by Nicobarese tribes of Andaman and Nicobar Islands were studied for antimicrobial and antimalarial activities as well as preliminary photochemical analysis. Plants were collected from Car Nicobar of Andaman and Nicobar Islands and the ethnobotanical data were gathered from traditional healers who inhabit the study area. The methanol extracts were obtained by cold percolation method and the antimicrobial activity was found using agar well diffusion method. Among the plants tested, *J. syringifolium*, *D. andamanica*, *C. indicum* were most active. The antimalarial activity was evaluated against *Plasmodium falciparum* chloroquine-sensitive MRC-2 isolate. The crude extract of *M. andamanica* showed excellent antimalarial activity followed by extracts of *P. tithymaloides*, *J. syringifolium* and *D. andamanica*. The chemical injury to erythrocytes was also carried out and it showed that, there were no morphological changes in erythrocytes by the methanol crude extracts. The *in vitro* antimicrobial and antimalarial activity might be due to the presence of alkaloids, flavonoids, triterpenes, sterols, tannins and saponins in the methanol extracts of tested plants.

1. Introduction

Traditional herbal medicines play a prominent role in the health care systems of many countries. In India, the natives implement various herbs for curing various ailments. The preparation and administration of drugs from these plants varies from place to place [16]. An Ethnomedicinal study offers immense opportunities to develop new drugs. Being closer to nature, traditional societies had gained knowledge regarding the use of wild flora and fauna, mostly unknown to the urbans, who are away from the natural ecosystem [17]. Though the art of herbal healing still exists through traditional folk healers, its knowledge seems to gradually perish [7].

Andaman and Nicobar Islands are a home to two distinct racial groups of primitive tribes' *viz*., Negretoes in the Andaman (Great Andamanese, Onges, Jarawas and Sentinelese) and Mongoloid in the Nicobar (Nicobarese and Shompens). More than 97% of the tribal population are Nicobarese, settled in different islands of Nicobar district. They belong to the mongoloid race of short-stature with stout and tough muscular body. The face bears a scanty beard with small thick lips and broad flattened nose [6]. The Nicobarese are coastal dwellers and enjoy the vicinity of exuberant and verdant tropical forests [18].

Tribal communities of Andaman and Nicobar Islands, especially the Nicobarese, depend on plant resources to prepare herbal medicines, for food, making household implements, as a sleeping mat and for fire. The former possess a great emporium of ethnobotanical wealth, as they are still isolated from the modern way of life and are still closer to nature. As a part of the ongoing studies, we documented their traditional treatment practices [2–4] and collected plants which were reported to be efficacious in the treatment of various diseases. In the present study, we generated the traditional knowledge among the Nicobarese of Nicobar group of islands [3] and six plant species were selected, which are regularly used in traditional medicines, to determine their antimicrobial and antimalarial activities as well as preliminary photochemical analysis.

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2. Materials and methods

2.1. Plant materials

Six plants were selected for evaluation on the basis of their ethnomedicinal histories. The names of these plants, their vernacular name (Nicobarese language), and the voucher numbers are presented in Table 1. Plant materials were collected in the Car Nicobar Island, Nicobar District and authenticated by the plant taxonomist at the Botanical Survey of India, Andaman and Nicobar Islands. Voucher specimens were deposited at the Regional Medical Research Centre (ICMR), Port Blair.

2.2. Extraction preparation

The leaves of the plants were separately dried under shade, pulverized by a mechanical grinder and passed through 40-mesh sieve, to obtain a fine powder. Hundred grams of coarsely powdered dry leaves were extracted by cold percolation method, by using 95% methanol as a solvent and keeping it for 72 h at room temperature [5]. The whole plant extract was collected in a conical flask, filtered, and the solvent was evaporated to dryness under reduced pressure in an evaporator (Eppendroff 5304) at 40–45 °C. Resulted residues were stored at 4 °C for further use.

2.3. Phytochemical analysis

The plant extracts were screened for the presence of different classes of secondary metabolites including alkaloids, flavonoids, triterpenes, sterols, tannins and saponins using previously described methods [8,9].

2.4. Antimicrobial activity

2.4.1. Microorganisms

The microorganisms used in this study included 10 bacterial and two fungal strains. These were *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 737), *Bacillus cereus* (MTCC 1272), *Staphylococcus epidermidis* (MTCC 3615), *Vibrio cholera* (MTCC 3906), *Proteus mirabilis* (MTCC 425), *Shigella flexneri* (MTCC 1457), *Salmonella enterica typhi* (MTCC 733), *Klebsiella pneumonia* (MTCC 129) and two fungal strains *Aspergillus niger* (MTCC 282), *Candida albicans* (MTCC 227), which were obtained from Microbial Type Culture Collection, IMTECH, Chandigarh, India.

2.4.2. Preparation of inoculums

Stock cultures were maintained at 4 °C on nutrient agar slants. Single colony of each microorganism from stock culture was transferred into sterile Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi. The bacterial cultures were incubated for 24 h at 37 °C and fungal cultures were incubated for 48 h at 25 °C.

2.4.3. Antimicrobial assay

The plant extracts were screened for antimicrobial activity using the agar well diffusion method [15]. The Muller-Hinton Agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petriplates and allowed to solidify. To this 0.1% inoculum suspension was swabbed uniformly, and allowed to dry for 5 min. Wells were dug in agar plates with the help of sterile metallic borer (diameter = 8 mm). 50 μl of various extracts were poured into the wells which were marked previously. Gentamycin and Nystatin were used as positive controls and pure dimethyl sulfoxide was used as negative control. The plates were allowed to diffuse for 15 min and later bacterial plates were incubated for 18 h at 37 °C and fungal plates were incubated for 48–72 h at 28 °C. At the end of incubation period, inhibition zone (IZ) formed around the disc were measured in millimeter, using a transparent scale. Each experiment was carried out in triplicates. The mean ± SD of the inhibition zone was taken for evaluating the antimicrobial activity of the plant extracts.

2.5. Antimalarial activity

2.5.1. Parasite cultivation

The antimalarial activity of plant extracts was assessed against chloroquine-sensitive *P. falciparum* (MRC-2) isolate obtained from the National Institute of Malaria Research (ICMR), New Delhi, India. *P. falciparum* are cultivated in human A Rh^+ red blood cells using RPMI 1640 medium (Sigma, India) supplemented with AB Rh^+ serum (10%), 5% sodium bicarbonate (Sigma, India) and 40 μg/ml of Gentamycin sulphate (Sigma, India) [19].

2.5.2. In vitro test for antimalarial activity

The *in vitro* activity of *P. falciparum* intraerythrocytic stage on crude plant extracts was evaluated by Schizont Maturation Inhibition (SMI) assay using *M-III method* [20]. Briefly, crude extracts were dissolved in DMSO and serially diluted with RPMI 1640 medium to reach 1 mg/mL before use. Serial double dilutions were made in 96-well microtiter plates (in triplicate) with concentration range of 1.56–100 μg/mL. The cultures, before testing were synchronized by treatment with 5% D-sorbitol with a parasitemia of 0.6–0.8%. Each well received 10 μL of parasite-infected erythrocytes, 5% haematocrit and 90 μL of different drug dilutions. Chloroquine and solvent controls contained similar concentrations of solvent, as that of test wells. The plates were incubated at 37 °C for 24 h. After confirmation of the presence of 10% mature schizonts in control wells (without drug), the blood from each well was harvested, and a thick film was prepared on a glass slide. The blood films were stained for 40 min with Giemsa stain at a dilution of 10% in double distilled water. Three independent optical-microscopy readings of the number of schizonts with three of more nuclei were carried out in 200 parasitized red blood cells for each dilution and duplicate. Growth inhibition was expressed as the percentage of schizonts in each concentration, compared with controls.

Table 1
Preliminary phytochemical analysis of the methanol extracts of ethnomedicinal plants used by Nicobarese.

Scientific name (voucher no.)	Local name	Alkaloids	Flavonoids	Triterpenoids	Steroids	Tannins	Saponins
A. macrophylla (ANH/CN/037)	Tachū rōi	+	+	−	−	+	+
C. indicum (ANH/CN/014)	Singënrő	+	−	+	−	+	−
D. andamanica (ANH/CN/081)	Kőiny	+	+	+	+	−	+
J. syringifolium (ANH/CN/018)	Panrāpő	+	+	+	+	+	−
M. andamanica (ANH/CN/038)	Topilei	+	+	−	−	+	+
P. tithymaloides (ANH/CN/055)	Thalaraf	+	−	+	+	−	−

'+' indicates *Present* and '−' indicates *Absent*.