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REGULAR ARTICLE

PRELIMINARY SCREENING OF ANTIBACTERIAL COMPOUNDS FROM PALAR RIVER BASIN FLORA

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SUMMARY

Considering the significance of phytochemicals as antimicrobial agents, attempt was made in the present study, to categorize several rare plant species present in and around Palar river basin and to assess their antimicrobial activity. The densities of the green cover of the Palar river basin flora were assessed by the Google Earth software. Totally 28 plants were identified and classified into 17 families according to binomial classification system. Plant extracts were prepared from leaves of all collected plants by using methanol and chloroform. Thus, the crude methanol and chloroform extracts of 28 plant species were subjected to preliminary screening against 6 strains of human bacterial pathogen using the dick diffusion method at 500 µg/disc concentrations. The results indicated that 21 different plant species exhibited activity against one or more of the bacteria while four species, viz., *Ammania baccifera, Plectranthus* sp., *Vitex trifolia* and *Vitex negundo* showed activity against all test organisms. The plants containing bioactive metabolites demonstrated stronger anti-microbial properties stressing the need for further investigations using fractionated extracts and purified chemical components.

Keywords: Palar river basin, Antimicrobial activity, Crude extracts, Herbal medicine, Bacterial pathogen.
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1. Introduction

Despite emphasis being put in research of synthetic drugs, a certain interest in medicinal plants has been reborn, in part due to the fact that a lot of synthetic drugs are potentially toxic and are not free of side effects on the host [1]. This has urged microbiologists all over the world for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents [2, 3]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine [4]. Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant extracts as antimicrobials is very much in need. Antimicrobials of plant origin have enormous therapeutic potential and are effective in the treatment of infectious diseases while simultaneously mitigating

many of the side effects that are often associated with synthetic antimicrobials [5].

Medicinal plants are well-known natural sources for the treatment of various diseases since antiquity. About 20,000 plant species used for medicinal purposes as reported by World Health Organization (WHO) [6]. Furthermore, natural products, either pure compounds, or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity [7]. Antimicrobial agents from lower organisms and synthetic drugs sufficed in the treatment or control of various infectious diseases. Microbial drug resistance and increase in the opportunistic infections especially with ***acquired immune deficiency syndrome*** (AIDS) patients and individuals on immunosuppressive chemotherapy, many synthetic antifungal and antiviral drugs are of limited use due to toxicity. Hence need of searching more new natural products as a potential antimicrobial agent is in need for the society.

Large numbers of plants are utilized in various systems of medicine practiced in India and local health traditions for the treatment of human diseases since time immemorial. Most of these medicinal plants have been identified and their uses are well documented by different authors [8]. Particularly, plants withstand stress like drought, salinity and high temperatures exhibit more antimicrobial activity than plants in normal conditions [9]. With the introduction of a variety of antimicrobial compounds it became necessary to perform the antimicrobial susceptibility test described by Bauer *et al.,* [10] as a routine. As the medicinal use of various medicinal plants are increasing for the treatment against various diseases. Ascendancy of the human immunodeficiency virus (HIV) has spurred intensive investigation into the plant derivatives which may be effective, especially for use in underdeveloped nation with little access to expensive western medicines. Considering the significance of phytochemicals as antimicrobial agents, this study was designed to investigate several rare plant species present in and around Palar river basin for potential antibacterial activity by preliminary bioassay screening to categorize them.

2. Materials and Methods

Plant materials

The density of green cover on Palar river basin was assessed manually by using satellite image taken by EarthSat and by using Google-Earth software. Satellite image data revealed that only two locations within the Palar river bed at $12^0$48′01.12″ N $79^0$40′33.00″ E (Fig.1A) and $12^0$40′52.39″ N $79^0$56′55.58″ E (Fig.1B) found to be having considerable green cover. One more location at Narasamangalam hill station at $12^0$44′30.60 N $79^0$39′56.33″ E (Fig.1C) near Palar river basin was also selected based on satellite image analysis [11].

Totally 28 different plant species were collected among which 10 plant samples were from Palar river bed near Kanchipuram, 10 plant samples were from Palar river bed near Chengalpet and 8 plants were from Narasamangalam hills. The taxonomical classification was done with the help of taxonomists from Canter for Advance Studies in Botany, University of Madras. Plant extracts were prepared from leaves of all collected plants; shade dried at room temperature and was powdered by using mechanical grinder. Five grams of plant powder was then soaked in 100 ml of methanol and 100 ml of chloroform separately for 7 days under shaking (40 rpm) at room temperature. Then, both extracts were filtered through Whatman no. 1 filter paper. Prepared extracts were stored at 4°C in the dark for analysis [12].

Antibacterial efficacy

Test organisms

The antibacterial activities are carried out against six human pathogenic bacterial strains, *Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis*, *Escherichia coli, Salmonella typhi* and *Proteus mirabilis* which were obtained from Department of Microbiology, Christian medical college, Vellore, India.

Disk diffusion assay

The antimicrobial activity of the methanol and chloroform extracts was evaluated by the paper disc-agar diffusion method [13]. Test plates were prepared with Mueller-Hinton agar (Himedia) and inoculated on the surface with a cell suspension in sterile normal saline. In all cases, the concentration of the inoculum was adjusted to 1.5×10^8 CFU/ml. Test extracts (50mg) were dissolved in 1 ml of solvent used in extraction. Sterile paper discs (6.0mm) were aseptically impregnated with 10µl of the resulting solutions and then deposited on the surface of inoculated plates. After 24 h of incubation at 37°C, the activity was determined by the presence of clear zones of inhibition around the test extracts. Discs of tetracycline (30µg) were used as standard antibacterial control. All the assays were subjected to quality control procedures recommended by Clinical Laboratory Standard Institute [14].

3. Results and Discussions

Totally 28 plants were identified and classified into 17 families according to binomial classification system were shown in Table 1. Plant extracts were prepared from leaves of all collected plants, totally 28 methanolic extracts and 28 chloroform extracts were prepared from shade dried plant samples and their antibacterial activity was assessed. The results of the antibacterial screening are listed in Table 2. Out of the 28 samples, 21 plants showed antibacterial activity against at least one or more test organisms in which 10 plants namely, *Indigofera enneaphylla, Mollugo pentaphylla, Eupatorium odoratum, Ammania baccifera, Cleome pentaphylla, Mollugo cerviana*, *Plectranthus sp*., *Carissa carandas*, *Vitex trifolia* and *Vitex negundo* showed more than 15 mm zone of inhibition. Four of the plants, *Ammania baccifera, Plectranthus sp., Vitex trifolia* and *Vitex negundo* showed better activity against all test organisms. However seven plants namely, *Malvastrum coromandelianum, Xanthium indium*, *Ocimum bacillicum*, *Phyla nodiflora*, *Adhatoda zeylanica, Pseudarthria viscid* and *Ziziphus mauritiana* showed no activity against any of the test organisms.

Table 1. Collection of plant species from palar river basin and narasamangalam hill station

Region	Geographical coordinates	Plant species and family
Palar river bed near Kanchipuram	$12^0$48′01.12″ N $79^0$40′33.00″ E	*Indigofera enneaphylla* (Fabaceae), *Solanum xanthocarpum* (Solanaceae), *Mollugo pentaphylla* (Molluginoideae), *Lantana camara* (Verbenaceae), *Malvastrum coromandelianum* (Malvaceae), *Eupatorium odoratum*(Asteraceae), *Alysicarpus rugosus* (Fabaceae), *Xanthium indium*(Asteraceae), *Ocimum bacillicum* (Lamiaceae), *Portulaca oleracea*(Portulacaceae)
Palar river bed near Chengalpattu	$12^0$40′52.39″ N $79^0$56′55.58″ E	*Phyla nodiflora* (Verbenaceae), *Acalypha indica* (Euphorbiaceae), *Cardiospermum halicacabum*(Sapindaceae), *Adhatoda zeylanica* (Acanthaceae), *Ammania baccifera* (Lythraceae), *Cleome pentaphylla* (Capparaceae), *Capparis panamensis* (Capparaceae), *Capparis prisca* (Capparaceae), *Solanum surattense* (Solanaceae), *Bulbostylis barbata* (Cyperaceae)
Narasamangalam hill station near Palar river (Kanchipuram)	$12^0$44′30.60″ N $79^0$39′56.33″ E	*Citrullus lanatus* (Cucurbitaceae), *Mollugo cerviana* (Molluginoideae), *Plectranthus sp.* (Lamiaceae), *Carissa carandas* (Apocynaceae), *Pseudarthria viscid* (Fabaceae), *Ziziphus mauritiana* (Rhamnaceae), *Vitex trifolia* (Verbenaceae), *Vitex negundo* (Verbenaceae)

Table 2. In vitro screening for antibacterial activity of various plants extracts (500µg/disc)

Plant no	Plant species	Extracts	Zone of inhibition in mm[a]					
			SA	BC	EF	EC	ST	PM
1	*Indigofera enneaphylla*	M	8	12	15	16	-	-
		C	-	11	-	12	-	-
2	*Solanum xanthocarpum*	M	-	11	12	11	-	-
		C	-	-	-	10	-	-
3	*Mollugo pentaphylla*	M	13	15	10	-	15	9
		C	12	9	-	-	11	-
4	*Lantana camara*	M	14	13	12	-	13	12
		C	11	10	-	-	14	8
5	*Malvastrum coromandelianum*	M	-	-	-	-	-	-
		C	-	-	-	-	-	-
6	*Eupatorium odoratum*	M	11	15	12	-	11	10
		C	8	12	11	-	11	9
7	*Alysicarpus rugosus*	M	7	12	8	12	-	7
		C	-	12	7	13	-	-
8	*Xanthium indium*	M	-	-	-	-	-	-
		C	-	-	-	-	-	-
9	*Ocimum bacillicum*	M	-	-	-	-	-	-
		C	-	-	-	-	-	-
10	*Portulaca oleracea*	M	12	13	10	13	-	12
		C	14	13	7	12	-	-
11	*Phyla nodiflora*	M	-	-	-	-	-	-
		C	-	-	-	-	-	-
12	*Acalypha indica*	M	8	7	-	8	-	-
		C	7	11	-	11	-	-
13	*Cardiospermum halicacabum*	M	8	11	-	-	7	8
		C	7	11	-	-	8	9
14	*Adhatoda zeylanica*	M	-	-	-	-	-	-
		C	-	-	-	-	-	-
15	*Ammania baccifera*	M	22	19	8	13	20	11
		C	20	15	7	12	18	10
16	*Cleome pentaphylla*	M	-	13	8	-	11	8
		C	-	15	7	8	8	7
17	*Capparis panamensis*	M	-	11	-	-	12	-
		C	-	-	9	-	7	-
18	*Capparis prisca*	M	12	-	11	8	12	-
		C	11	-	10	7	-	9
19	*Solanum surattense*	M	12	-	-	-	9	-
		C	12	-	-	-	12	-
20	*Bulbostylis barbata*	M	11	-	-	-	-	-
		C	8	-	-	-	-	-
21	*Citrullus lanatus*	M	11	-	-	-	-	-
		C	11	-	-	-	-	-
22	*Mollugo cerviana*	M	-	11	7	-	12	-
		C	15	13	8	7	13	-
23	*Plectranthus sp.*	M	23	22	22	15	19	15
		C	20	18	19	16	17	12
24	*Carissa carandas*	M	-	15	7	9	15	-
		C	12	13	-	-	12	-
25	*Pseudarthria viscida*	M	-	-	-	-	-	-
		C	-	-	-	-	-	-

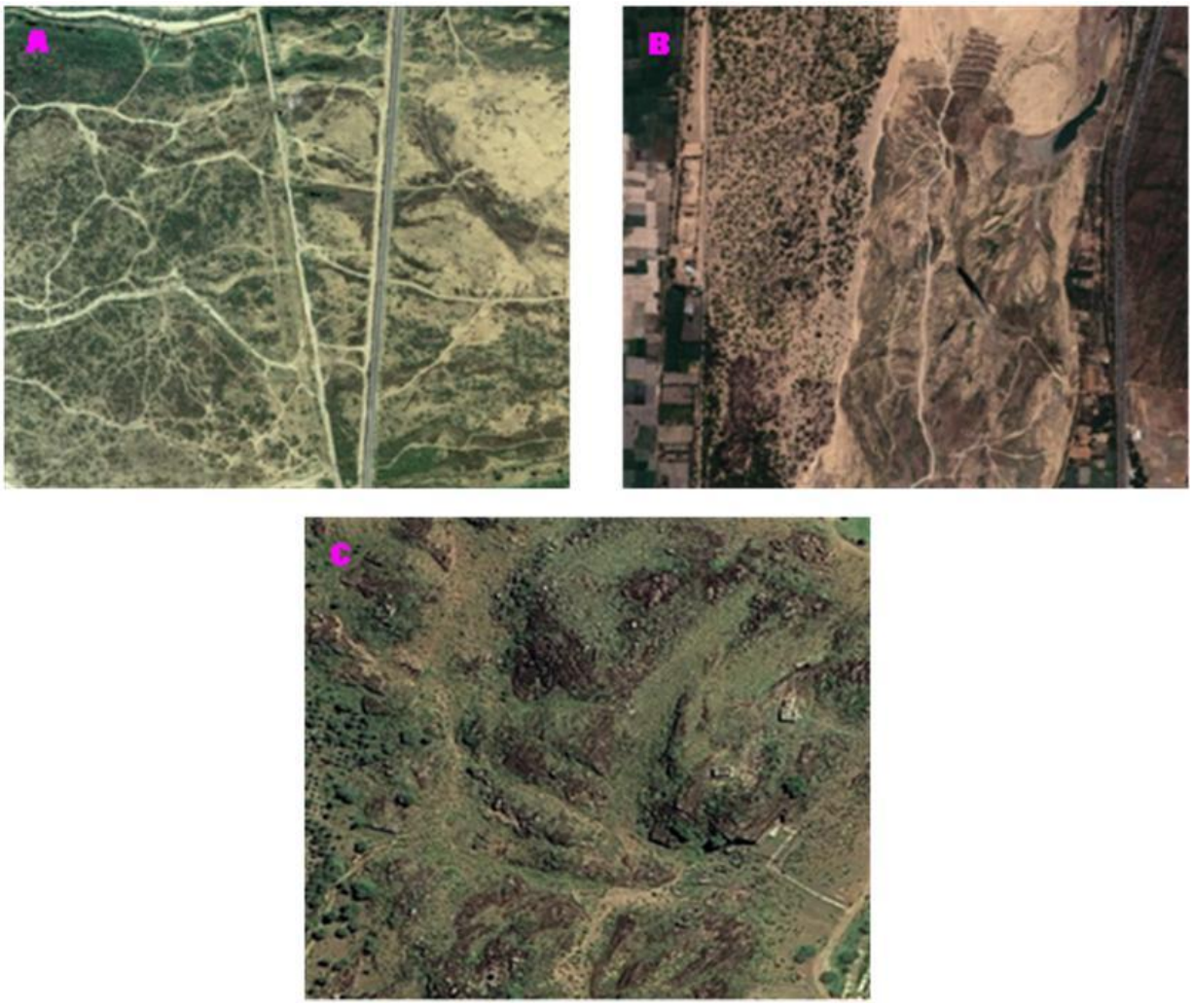
26	*Ziziphus mauritiana*	M	-	-	-	-	-	-
		C	-	-	-	-	-	-
27	*Vitex trifolia*	M	13	9	17	15	13	19
		C	13	12	8	12	15	11
28	*Vitex negundo*	M	12	13	15	10	12	15
		C	9	14	18	15	11	11
Methanol control			-	-	-	-	-	-
Chloroform control			-	-	-	-	-	-
Tetracycline[b]			20	24	32	27	25	18

EF: *Enterococcus faecalis*, SA: *Staphylococcus aureus,* BC: *Bacillus cereus,* EC: *Escherichia coli,* ST: *Salmonella typhi,* PM: *Proteus mirabilis,* (-) no inhibition, M: Methanol, C: Chloroform

[a]12 mm or less: resistant or no inhibition1, 13–17 mm: moderate inhibition, 18 mm or more: sensitive or maximum inhibition.

[b] The concentration of used standard drugs was 30µg/disc.

Figure 1. Image of zoom areas obtained from *google earth, A*. Palar river bed near Kanchipuram, B. Palar river bed near Chengalpattu, C. Narasamangalam hill station near Palar river (Kanchipuram)



Infectious disease of microbial origin, such as *Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis, Escherichia coli, Salmonella typhi* and *Proteus mirabilis* constitute the major cause of morbidity and/or mortality in developing countries like India. With the emergence of HIV, the oppurtunistic role of these microbes has even become worse as they facilitate the infection rate significantly in immune compromised host. The situation is further compounded by the lack of patient compliance to antibiotic regimen and by the exorbitant costs of the antibiotics. The preliminary results of the

present study, therefore, not only confirms the justifiable use of some of the plants against these micro-organisms in the traditional health care system but also reflects the hope for development and scientific validation of effective natural products from same or similar plants.

The results of this study support, to a certain degree, the traditional medicinal uses of the plants evaluated both for human and animal disease therapy [15] and reinforce the concept that the ethnobotanical approach [16] to screening plants as potential sources as successful bioactive substances. From this study we conclude that *Ammania baccifera, Plectranthus sp., Vitex trifolia* and *Vitex negundo* extracts were the most active antimicrobial plants. All of these species are perennial herbs widely distributed in palar river basin region. The fairly good degree of correlation of traditional therapeutic claims with the specific anti-microbial activity as observed in the present preliminary results warrant further investigation. Thus, activity guided fractionation of the constituents of the most promising plants as well as acute toxicity studies are already underway in our laboratory.

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