



Research Article

Antibacterial efficacy of *Fabaceae* plants of a tropical lake of South India

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ABSTRACT

Obtaining the plant drugs for the production of commercial products is the recent research scenario. But still, the role of a plant species in its ecosystem is not much explored. The present study intended to find antibacterial activity of *Fabaceae* plant species in a tropical freshwater lake against isolated indigenous bacterial species. Results revealed that the *Fabaceae* plants *Abrus precatorius*, *Acacia nilotica*, *Cajanas cajan*, *Cassia auriculata*, *Clitoria ternatia*, *Crotalaria vergosa*, *Crotia ternatia*, *Mimosa pudica*, *Pongamia pinnata*, *Sesbania grandiflora*, *Tephrosia purpuria* and *Vigna mungo* of Vellode lake have potential antibacterial activity against indigenous bacterial isolates from soil and water samples.

Key words: *Fabaceae* plants; Indigenous bacteria; Antibacterial efficacy; Tropical Lake

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INTRODUCTION

India is proud to be rich in biodiversity and possess about 8 % of estimated biodiversity of the world with around 12600 species.¹ There are about 400 families of the plants in the world which 315 are represented in India.² According to World Health Organization (WHO), plants are best sources to obtain variety of drugs to treat various diseases.³ Plants are widely used in human therapy, veterinary, agriculture, scientific research and countless other areas.⁴ Diversity of ethno-medicinal plants and their usage in various local communities have been studied by various researchers.⁵⁻⁸ Similarly, antimicrobial nature of plants is also studied by various researchers.⁹⁻¹¹ However, potential antimicrobial nature of the plants in an ecosystem against their indigenous microbes is not very much focused. By finding the efficiency of a plant in an ecosystem might explore the significance of the plant. These observations may also give clue to know the level of utilization or recovery of plants from its own ecosystem. The prime objective of the present study is to record the role of highly eco viable *Fabaceae* plants over the much variable bacterial population of a tropical freshwater lake. The present study was carried out in the Vellode Lake of Erode district, South India. It lies between 11⁰⁸'04.04" N and 77⁰³⁷'52.42" E with the elevation of 764 ft. High summer temperature and heavy monsoon rainfall of the lake express its typical tropical climatic nature.

MATERIALS AND METHODS

Collection and identification of plants: In the present study, whole or plant portions (leaves, flowers and fruits) of *Fabaceae* plant species were collected and preserved by pressing and drying method. All collected plant species were identified by the assistance of Botanical Survey of India (BSI) Southern Zone, Coimbatore. The authentication numbers of *Abrus precatorius*, *Acacia nilotica*, *Cajanas cajan*, *Cassia auriculata*, *Clitoria ternatia*, *Crotalaria vergosa*, *Crotia ternatia*, *Mimosa pudica*, *Pongamia pinnata*, *Sesbania grandiflora*, *Tephrosia purpuria* and *Vigna mungo* are BSI/SRC15/23/2013-14/tech/287-98.

Extraction from plants: The collected leaves, flowers, bark and fruits of a species were shadow dried and powdered. The powdered plant portions of all the plant species were stored at room temperature in separate labeled air tight glass bottles. With proper homogenization, maceration, decoction and serial exhaustive extraction methods concentrated extracts with all bioactive compounds were obtained. Quantitative analysis was conducted to determine the phytochemical composition of the plants.

Estimation of phytochemicals: The quantity of alkaloids, saponins and total phenols were determined by using Harborne (2001) methods.¹² Okeke and Elekwa (2003) method was used for tannin determination.¹³ The mineral elements such as

calcium, phosphorus, magnesium, potassium and nitrogen were determined using wet digestion extraction methods as described by Owjuwala (1998),¹⁴ Andrew (1999)¹⁵ and Nivozamsky et al., (2007).¹⁶

Collection of water and sediment samples: For the isolation of indigenous bacterial species, water and sediment samples were collected in separate, sterilized container and with proper labelling, they were transported to the laboratory and stored in refrigerator at 4°C until further process. Basic parameters including pH, total nitrogen, total phosphates, total potassium, calcium and magnesium were analyzed.

Isolation and identification of bacterial species: By serial dilution method, water and soil samples were diluted to 10⁻¹, 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵ concentrations. All diluted samples were poured in separate petri-dishes containing enough amount of nutrient agar medium (peptone: 5.0g, beef extract: 3.0g, NaCl: 5.0g, Agar: 15.0g and distilled water: 1000ml).

All poured plates were inverted and kept in incubator for 24 hours at 35°C - 37°C. Based on the color and morphology, the grown colonies were counted with the assistance of a colony counter. The top three dominant bacterial isolates from water and soil were selected for further identification tests. The isolates of water samples were named as WB1, WB2 and WB3. Similarly, isolates of soil as named SB1, SB2 and SB3. By continuous streaking method, the dominant three bacterial species of water and soil were sub-cultured to obtain pure isolates for further identification tests.

Identification of the bacterial isolates up to genus level was done on the basis of morphological and biochemical tests of Bergey's Manual.¹⁷

Antibacterial activity: Isolated indigenous bacterial species were properly inoculated and used for the assessment of antimicrobial activity. The antibacterial tests were done by following the disc diffusion method. 10 µl of test microorganisms were seeded into respective petri plates with agar medium. The paper discs (5mm in diameter) were impregnated in solidified medium after dipping in extracts of *Fabaceae* plants. After 24 hours of incubation period, the inhibition zones of the discs were measured. By comparing with inhibition zone of control, the inhibition zones of extracts were calculated.

RESULTS

***Fabaceae* plants species:** In the present investigation totally, 11 *Fabaceae* plants including 3 trees (*Acacia nilotica*, *Pongamia pinnata* and *Sesbania grandiflora*), 2 shrubs (*Cajanas cajan* and *Cassia auriculata*), 2 herbs (*Tephrosia purpuria* and *Vigna mungo*), 4 climbing herbs (*Abrus precatorius*, *Clitoria ternatia*, *Crotalaria vercosa* and *Mimosa pudica*) were selected (table 1).

Table 1: *Fabaceae* family plants of Vellode lake

S. N.	Species Name	Common Name	Vernacular Name
1	<i>Abrus precatorius</i>	Crab's eye	Gundumani
2	<i>Acacia nilotica</i>	Gum Arabic tree	Karuvallam

3	<i>Cajanas cajan</i>	Pigeon Pea	Dhurai
4	<i>Cassia auriculata</i>	Tanner's cassia	Aavarai
5	<i>Clitoria ternatia</i>	Butterfly pea	Sangu poo
6	<i>Crotalaria vercosa</i>	Rattle pods	Vellai Avari
7	<i>Mimosa pudica</i>	Shy plant	Tottachinughi
8	<i>Pongamia pinnata</i>	Indian beech	Poovarasan
9	<i>Sesbania grandiflora</i>	Hummingbird tree	Agathi
10	<i>Tephrosia purpuria</i>	Wild indigo	Kozlungi
11	<i>Vigna mungo</i>	Black gram	Ullundhu

Quantification of phytochemicals: The phytochemicals including alkaloids, flavonoids, tannins, saponins, and phenols were estimated and presented in Fig.1. Mineral elements such as phosphorus, calcium, magnesium, potassium and nitrogen were determined and their compositions are represented in Fig 2.

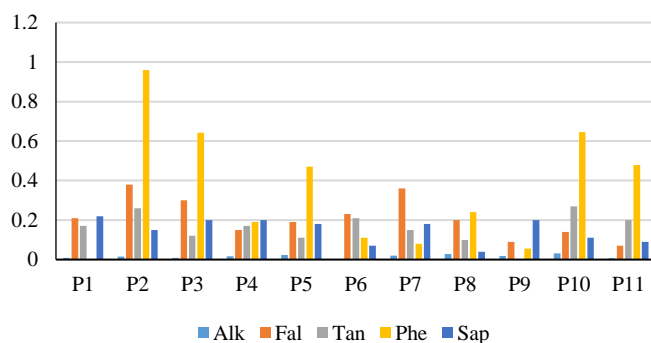


Figure 1: Quantitative estimation of phytochemical contents of *Fabaceae* plants

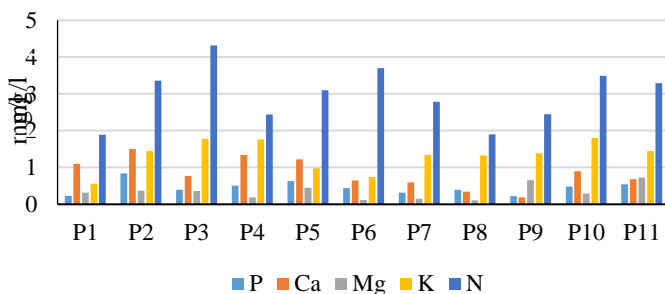


Figure 2: Quantitative estimation of mineral elements of *Fabaceae* plants

Physico-chemical analysis of water and soil samples: Water and soil samples collected from Vellode lake for the isolation bacterial species were subjected to physicochemical analysis. Parameters including pH, total nitrogen total phosphorus, total potassium, calcium, magnesium and chlorides were estimated for both water and soil samples. C/N ratio and N/P ratio were calculated for soil samples only. Physicochemical characteristics of the water and soil samples of the lake were represented in Table 2.

Isolation of Bacterial species: From the collected water and soil samples of the Vellode lake, dominant three bacterial isolates of pour plates were taken for further subculture. From those subcultures required amount of the slant cultures were

obtained and utilized for further biochemical tests for the identification of the isolates (Table 3).

From the water samples WB1 (*E. coli*), WB2 (*Staphylococcus sp.*) and WB3 (*Bacillus sp.*), from soil samples SB1 (*Pseudomonas sp.*), SB2 (*Bacillus sp.*) and SB3 (*Streptococcai sp.*) were isolated. *Bacillus sp.* of both water and soil samples were similar in morphology and in other biochemical tests.

Antibacterial Activity: Antibacterial activity of the 11 *Fabaceae* plants of Vellode lake against the indigenous bacterial isolates of water and soil samples i.e *Escherichia coli*, *staphylococcus sp.*, *Bacillus sp.*, *Pseudomonas sp.* and *Streptococcai sp.* were observed in the form of zone of inhibition (mm) by the disc diffusion method in petri plates. The results obtained in the study were represented in Fig. 3.

Table 2: Physicochemical analysis of water and soil samples of Vellode Lake

S.N.	Parameters	Water	Soil
1	pH	6.82 ± 0.3	8.03 ± 1.2
2	Total Nitrogen	11.45 ± 2.2 mg/l	0.192 mg/kg
3	Total Phosphates	0.13 ± 0.35 mg/l	0.04 ± 0.01%
4	Total Potassium	34.46 ± 1.7 mg/l	192 ± 23 %
5	C: N ratio	NA	12.74 ± 2.1 %
6	N: P ratio	NA	4.29 ± 1.8 %
7	Calcium	29 ± 1.76 mg/l	46 ± 4 mg/kg
8	Magnesium	7.89 ± 0.91 mg/l	16.2 ± 0.9mg/kg
9	Chlorides	72.7 ± 1.38 mg/l	40.8 ± 3 mg/kg

Table 3: Observation of biochemical tests for identification of bacterial isolates of water and soil samples of Vellode Lake

S. N.	Tests	Water			Soil		
		WB1	WB2	WB3	SB1	SB2	SB3
1	Colony colour	Yellowish	Creamy	Grayish	Light yellow	Grayish	White mucoid
2	Colony appearance	Rough	Raised & Smooth	Small round	Slightly raised	Small round	Rough
3	Motility test	+	-	+	+	+	-
4	Gram staining	-	+	+	-	+	+
5	Catalase test	+	+	+	+	+	-
6	Oxidase test	-	-	+	+	+	-
7	Indole test	+	-	-	-	-	-
8	Citrate test	+	+	-	+	-	+
9	Methylred test	+	-	+	-	+	-
10	Urease test	-	+	-	-	-	-
11	H ₂ S test	-	-	-	-	-	-
12	Voges-Proskauer test	-	-	-	-	-	-
13	Nitrate reduction test	-	+	+	+	+	-
Suspected bacterial species		<i>E. coli</i>	<i>Staphylo coccus sp.</i>	<i>Bacillus sp.</i>	<i>Pseudo monas sp.</i>	<i>Bacillus sp.</i>	<i>Strepto coccai sp.</i>

Note: + indicates positive result; - indicates negative result

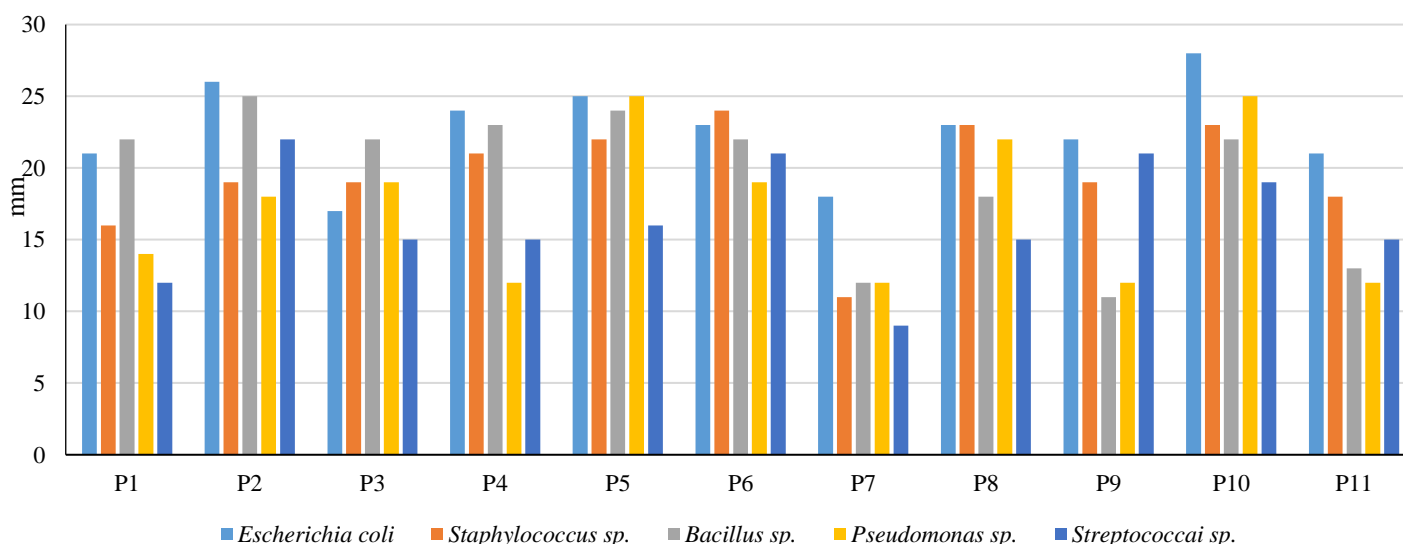


Fig. 3: Antibacterial activity (zone of inhibition in mm) of *Fabaceae* plants against bacterial isolates from water and soil samples of Vellode Lake

DISCUSSION

Even though the climatic and soil quality favors, the diversity of the *Fabaceae* plants were very much limited because of the strong competition from the invasive plants.¹⁸ Quantification of phyto-components among the *Fabaceae* plants indicates variable nature of phytochemical composition of family of plants. These composition also indicates the uniqueness among the closely related plants of a family.¹⁹ However, it is suggested that further investigation on the phytochemical composition can widen the differentiations among the plants of this family.

Physicochemical analysis of water and soil samples suggested that the conditions of the lake are very much suitable for the wide range of microbial (bacterial, algal and fungal) populations. Especially, pH, total nitrogen of water samples and C:N ratio, N:P ratio of the soil samples revealed the availability of different kinds of micro climates for varied kind of microbial populations. However, competitive nature of similar niche groups and temporal variations might give the dominance to some bacterial species.^{20,21}

Antibacterial test results demonstrated the high efficacy of the all the plants against *E.coli* and least efficacy with *Streptococcal sp.* where, the plants have highly diverse range of inhibition rate against the remaining isolates. In fact, each plants species showed different magnitude inhibition with every isolates. Thus, the present investigation showed the highly varied range of characteristics among the plants of a family which indicates the importance of a particular species in its own habit. It is strongly recommended that the immediate actions to remove the invasive plants esp. *Prosopis juliflora* and *Parthenium sp.* of this lake ecosystem, due to their strong competition behavior (in seed dispersal, light, water and nutrition) with native *Fabaceae* and other family plants also²². Because, in an ecosystem there will be no compensation for loss of a species or loss of its abundance.

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