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IMPACT OF PARALIMNETIC MACROFLORA ON BACTERIAL POPULATIONS OF A TROPICAL FRESHWATER LAKE

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author MS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author KN managed and reviewed the literature searches and analyses. Both authors read and approved the final manuscript.

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ABSTRACT

Phytochemicals are playing some metabolic role and control development in living systems. They are also protective function in animals and are used as medicine especially, the steroidal alkaloids. The plant species *Acacia nilotica indica* is an indigenous plant in most of the South Indian freshwater lakes. The plant species provides enormous ecological and economical values. In the present study area Vellode Lake, *Acacia nilotica indica* is the most dominated plant species. Therefore, it is necessary to conduct phyto-chemical analysis of the plant species to find its chemical constitution and its impact on the ecosystem. Based on the standard methods of extraction, extracts of the leaves, bark and fruits are prepared and used for further qualitative and quantitative analysis. Parameters including pH, total phenols, alkaloids, saphonins, total flavonoids, total nitrogen, total phosphates, total potassium, calcium, magnesium and sodium of the plants parts were estimated. By the help of HPLC analysis presence of the major alkaloids like Dimethyl tryptamine, N- Methyl tryptamine, 5- Methoxy dimethyl tryptamine, Dimethyl tryptamine and flavonoids like 6,8 -bis C,B,D gluiopyranoside, (+) Catechin- 4,5 digallate, Melacacidin, (+) Catechin- 5,7 digallate, Quercetin 3-O-rutinoside, Melacacidin and 1,2 - Dimethyl benza antracene of the plant species were detected. Dominant bacterial species were isolated from the lake soil, water and bird droppings. The dominant bacterial bacteria species of the lake are *Escherichia coli, Staphylococcus sp., Bacillus sp., Pseudomonas sp., Streptococcai sp.* and *Clostridium sp.* Antibacterial tests were conducted with the plant extracts. The results were clearly indicated the great amount of antibacterial potential of *Acaci nilotica indica*. Thus, the present study demonstrate the natural efficiency of the lake ecosystem against the pathogenic bacterial population.

Keywords: *Acacia nilotica*; alkaloids; flavonoids; bacterial species; antibacterial activity.

1. INTRODUCTION

Phyto-chemicals are present in variety of plants utilized as important components of both human and animal diets. These include spices, fruits, herbs and vegetables. The usefulness of these plant materials medicinally is due to the presence of bioactive constituents such as alkaloids, tannins, flavonoids and phenolic compounds [1]. The role of plants in maintaining health of human, animals and ecosystems are well documented. Many of the indigenous species and their extracts are used in traditional medicine.

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Bioactive compounds from the plants exhibit physiological activity against bacteria and other microorganisms [2].

Alkaloids play some metabolic role and control development in living systems. They are also protective function in animals and are used as medicine especially the steroidal alkaloids. Tannins are known to inhibit pathogenic fungi. Saponins prevent disease invasion of plants by parasitic fungi, hence have some antifungal properties, they also have anti-fertility effects. Studies revealed that flavanoids apart from their antioxidant protective effects, inhibits the initiation, promotion and progression of tumors [3,4].

The plant species *Acacia nilotica indica* (Linn.) wild. Ex. Del. belongs to the leguminosae family and subfamily mimoseae. It consists of dried mature stem bark having moderate sized spiny, evergreen tree found throughout India. It is estimated that there are roughly 1380 species of *Acacia* worldwide. *Acacia nilotica indica* is belongs to subfamily mimoseae, family leguminosae, division magnoliate and phylum magnoliphyta. It commonly called gum arabic tree. It withstands extremes of temperature (-1 to 50°C). Trees are generally deciduous during the dry season, through riverine species can be almost evergreen [5].

A. nilotica is a plant 5 to 20 m high with a thick spherical crown, stems and branches usually sinister to black coloured, grey-pinkish slash, fissured bark, exuding a reddish low quality gum. The plant has straight, light, thin grey spines in axillary pairs, usually in 3 to 12 pairs, 5 to 7.5 cm long in young trees, mature trees commonly without throns. The leaves are bipinnate, with 3 to 6 pairs of pinnulae and 10 to 30 pairs of leaflets each, rachis with a gland at the bottom of the last pair of pinnuale. Flowers are globulous heads, with 1.2 - 1.5 cm in diameter of bright golden colour set-up either axillary or whorly on peduncles. Pods are strong constricted, white grey hairy thick [6].

Present study intended to find the role of *Acacia nilotica* on the indigenous bacterial populations. The ultimate objective of the study is to explore the importance of phytochemicals on the buffering natural environment quality and to create the awareness among the local community to preserve the ecological composition. The present was conducted at Vellode lake of Erode District, South India. The most part of the paralimnetic area is covered by Acacia plant species. The main water body almost holds water throughout the year. The lake serves as the habitat for wide range of avifaunal community and also act as major water source for the local community.

2. MATERIALS AND METHODS

Phyto-chemical analysis of the plant samples is extremely valuable in giving information about the nature of constituents found in each plant. It is necessary to correlate the nature of chemical constituents and its role on the ecosystem. In the present study area, *Acacia nilotica indica* is the most dominated plant species. Therefore, it is necessary to conduct phyto-chemical analysis of the plant species to find its chemical constitution and its impact on the ecosystem.

2.1 Collection of Plant Materials

The plant parts like bark, leaves, flowers and fruits of *Acacia nilotica indica* were collected from study area and authenticated by Botanical Survey of India, Coimbatore (certificate number No.: BSI/SRC/ 5/23 /2013- 14/tech/287).

2.2 Preparation of Samples for Analysis

The collected leaves and fruits were shadow dried and the barks were cut into small pieces and then shadow dried. After proper drying all the plant portions were powdered individually. All the powdered plant portions were stored at room temperature in separate air tight glass bottles.

2.3 Extraction of *Acacia nilotica indica* Plant Materials

The basic principle is to grind the plant materials finer to increase the surface area for extraction there by increasing the rate of extraction.

2.4 Choice of Solvents

Successful determination of biologically active compounds form plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. In the present analysis, water, acetone, alcohol and chloroform were used for the extraction of phyto-chemicals from *A. nilotica indica*.

2.5 Methods of Extractions

During the present phyto-chemical analysis the following methods were followed for the extractions of bioactive compounds from the plant portions of *A. nilotica indica*.

2.5.1 Plant tissue homogenization

Dried or wet fresh plant parts were grounded in a blender to fine particles. Then certain quantity of solvents were added, shaken vigorously for 5-10 minutes and after 24 hours stand, the extraction was collected and used for further analysis.

2.5.2 Maceration

In maceration, whole or coarsely powdered plant materials were kept in contact with a solvent in stoppered containers for a defined period with frequent agitation until soluble matter was dissolved.

2.5.3 Decoction

This method was used for the extraction of the water soluble and heat soluble constituents. By boiling the plant materials in water for 15 minutes, cooling and passing sufficient cold water through the filters required volume of sample was produced.

2.5.4 Serial exhaustive extraction

In this method, successive extractions were done with solvents of increasing polarity from a non-polar to a more polar solvent to ensure that wide polarity range of compound could be extracted.

2.6 Qualitative Analysis

Qualitative analysis was conducted to find the presence of phyto-chemical compounds like phenols, alkaloids, tannins, saphonins and flavonoids in extracts of *A. nilotica indica*.

2.7 Quantitative Analysis

The quantity of alkaloids, saponins, flavonoids, phenols, calcium, phosphates, chlorides, and sulphates were estimated.

2.8 HPLC Analysis

High performance liquid chromatography analysis was done for the conformation of the presence of the phyto-chemical compounds including alkaloids and flavonaoids.

2.9 Antimicrobial Analysis

2.9.1 Collection of samples

For the isolation of dominant indigenous bacterial species water, sediment and droppings were separately collected in well sterilized glass bottles. With proper labeling they transported to the laboratory and stored refrigerator at 4°C until further process.

2.9.2 Isolation of indigenous bacterial species

By serial dilution method, water, sediment and droppings were diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. All diluted samples were poured in separate petri-discs containing enough amount of nutrient agar medium (peptone: 5.0 g, beef extract: 3.0 g, NaCl: 5.0 g, Agar: 15.0 g and distilled water: 1000 ml).

All poured plates were inverted and kept in incubator for 24 hours at 35°C - 37°C. Based on the color and morphology grown colonies were counted with the assistance of colony counter. The top three dominant bacterial isolates from water, sediment and droppings were selected for the further identification tests. The isolates of water samples were named as WB1, WB2 and WB3. Similarly, isolates of sediment as named SM1, SM2 and SM3 and isolates of droppings as DM1, DM2 and DM3.

By continuous streaking method, the dominant three bacterial species of water, sediment and droppings were sub-cultured to obtain pure isolates for further identification tests.

2.9.3 Identification of bacterial isolates

Identification of the bacterial isolates up to genus level was done on the basis of morphological and biochemical tests of Bergey's manual [7].

2.9.4 Antibacterial activity

Isolated indigenous bacterial species were properly inoculated and used for the 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 *Acacia nilotica indica.* 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.

3. RESULTS

Qualitative, quantitative and antimicrobial analysis of bark, leaves and fruits of *Acacia nilotica indica* revealed interesting information.

3.1 Qualitative and Quantitative Analysis

Phyto-chemicals including alkaloids, glycosides, sapnonins, steroids, flavonoids, protein, terpenoids, tannins and phenols were present. By their positive results in qualitative analysis, extracts were undergone for the further quantitative analysis. The quantitative analysis for phyto-chemicals of the extracts from leaves, bark and fruits of *Acacia nilotica indica* revealed their phyto-chemical composition (Table 1).

Results of High Performance Liquid Chromatography (HPLC) analysis of the extracts for alkaloids and flavanoids are presented in Table 2.

3.2 Antibacterial Analysis

3.2.1 Isolation and identification of dominant indigenous bacteria

By counting the number of colonies of dominant three bacterial isolates in serial dilutions of the water, sediment and dropping samples suitable dilutions were selected for the further streaking. Bacterial colonies of dominant three bacterial isolates counted in serial dilutions of water, sediment and dropping samples. By continuous streaking, pure cultures were obtained for further identification tests.

Based on the morphological and biochemical tests, three dominant bacterial species were isolated from water (*Escherichia coli*, *Staphylococcus sp.* and *Bacillus sp.*), sediment (*Pseudomonas sp.*, *Bacillus sp.* and *Streptococci sp.*) and droppings (*Escherichia coli*, *Staphylococcus sp.* and *Clostridium sp*). Observations of morphological and biochemical characteristics were presented in Table 3.

3.2.2 Antibacterial tests

Antibacterial activity of leaves, bark and fruits extracts of *Acacia nilotica indica* against isolated dominant three bacterial groups from water, sediment and droppings were done by disc diffusion method. The inhibition zones were measured and presented in Table 4.

Table 1. Results of quantitative analysis of *Acacia nilotica indica*

S.N.	Parameters	Units	Composition		
			Leaves	Bark	Fruit
1	pH	-	5.86 ± 0.87	6.6 ± 0.53	6.35 ± 0.7
2	Total phenols	mg/g	12.2 ± 0.2	13.5 ± 1.1	7.5 ± 0.34
3	Alkaloids	mg/g	4.2 ± 1.2	8.7 ± 0.8	4.9 ± 0.83
4	Saphonins	%	0.156 ± 0.08	0.183 ± 0.1	0.026 ± 0.01
5	Total flavonoids	mg/g	9.7 ± 1.32	5.6 ± 2.1	3.8 ± 0.87
6	Total nitrogen	%	0.98 ± 0.61	2.1 ± 0.55	1.4 ± 0.92
7	Total phosphates	%	0.65 ± 0.04	0.59 ± 0.19	0.12 ± 0.01
8	Total potassium	%	0.96 ± 0.32	0.9 ± 0.4	0.74 ± 0.15
9	Calcium	%	0.63 ± 0.28	1.31 ± 0.71	0.48 ± 0.2
10	Magnesium	%	0.45 ± 0.09	0.63 ± 0.3	0.38 ± 0.14
11	Sodium	%	0.31 ± 0.08	0.3 ± 0.05	0.52 ± 0.2

Table 2. HPLC profile of *Acacia nilotica indica* extracts

Phyto-chemical	Extracts	Rf values	Time (Minutes)	Name of the detected chemical compound
Alkaloids	Leaves	10.2	16.9	Dimethyl tryptamine
		6.1	26.85	N- Methyl tryptamine
	Bark	1.2	4.83	5- Methoxy dimethyl tryptamine
	Fruit	6.1	13.8	Dimethyl tryptamine
Flavonoids	Leaves	0.34	24.9	6,8 -bis C,B,D gluiopyranoside
		0.71	38.2	(+) Catechin- 4,5 digallate
		0.27	48.1	Melacacidin
	Bark	0.69	27.3	(+) Catechin- 5,7 digallate
		0.17	42.3	Quercetin 3-O-rutinoside
	Fruit	0.26	8.4	Melacacidin
		0.76	34	1,2 - Dimethyl benza antracene

Table 3. Observations of morphological and biochemical characteristics of the bacterial isolates of water, sediment and bird droppings samples

S.N.	Tests	Water			Sediment			Droppings		
		WB1	**WB2**	**WB3**	**SM1**	**SM2**	**SM3**	**DM1**	**DM2**	**DM3**
1	Colony colour	Yellowish	Creamy	Grayish	Light yellow	Grayish	White mucoid	Yellowish	Creamy	Creamy white
2	Colony appearance	Rough	Raised & Smooth	Small round	Slightly raised	Small round	Rough	Rough	Raised & Smooth	Raised
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_2S test	-	-	-	-	-	-	-	-	+
12	Voges-Proskauer test	-	-	-	-	-	-	-	-	-
13	Nitrate reduction test	-	+	+	+	+	-	-	+	+
Suspected bacterial species		*E. coli*	*Staphylococcus sp.*	*Bacillus sp.*	*Pseudomonas sp.*	*Bacillus sp.*	*Streptococcai sp.*	*E. coli*	*Staphylococcus sp.*	*Clostridium sp.*

Table 4. Antibacterial activity of *Acacia nilotica indica* against indegenous bacteria (all values are mean ± standard deviation)

S. no.	Microorganisms	Inhibition Zone (mm)					
		Water extract			Ethanol extract		
		Leaves	Bark	Fruit	Leaves	Bark	Fruit
1	*Escherichia coli*	21 ± 5	26 ± 2	17 ± 5	24 ± 2	25 ± 5	17 ± 3
2	*Staphylococcus sp.*	16 ± 2	19 ± 4	19 ± 3	21 ± 3	22 ± 2	15 ± 1
3	*Bacillus sp.*	22 ± 3	25 ± 6	22 ± 3	23 ± 2	24 ± 4	22 ± 1
4	*Pseudomonas sp.*	14 ± 2	18 ± 3	9 ± 3	12 ± 1	16 ± 2	9 ± 2
5	*Streptococcai sp.*	12 ± 3	22 ± 1	15 ± 3	15 ± 3	25 ± 2	17 ± 2
6	*Clostridium sp.*	19 ± 2	18 ± 2	12 ± 5	19 ± 2	19 ± 3	15 ± 3

4. DISCUSSION

4.1 Phyto-chemical Composition of *Acacia nilotica indica*

A. nilotica indica occurs naturally and it is imperative in traditional, rural and agro pastoral systems. It is also an imperative multipurpose plant that has been used broadly for the treatment of various diseases. The phyto-chemicals contribute chemically to a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes. Previous study reported about role of *A. nilotica* in the recovery of waste lands on degraded lands. Because of the presence of variety of bioactive compounds in the plant can play a versatile role [6,7].

The present investigation revealed about high medicinal value of *A. nilotica* due to their phyto-chemical content and mineral content [8]. The chemical composition, mineral, profile and *in-situ* digestion kinetics of leaves of *A. nilotica* and estimated the amount of organic matter, fiber, lignin, ash, mineral and secondary metabolites in the leaf extracts of *A. nilotica* are also added the pharmacological values of the plant species [9]. Malviyo et al. [10] conducted a preliminary phyto-chemical investigation on *A. nilotica* and observed various phyto-chemical qualities of extracts of *A. nilotica* plant portions. The plant also consider as the multipurpose medicinal plant species [11].

4.2 Antimicrobial Activity of *Acacia nilotica indica*

An earlier study demonstrated the efficacy of *A. nilotica* extracts in treatment of gonorrhea, leucorrhoea, diarrhea, dysentery and wounds [12]. An investigation on phyto-chemical and antibacterial nature of bark extracts of *A. nilotica* and observed that the antimicrobial activity of ethanolic stem bark extract against *Salmonella viridis, Bacillus subtilis, Salmonella aureus, Eschericia coli* and *Salmonella sonnei* [13]. A study explained that antimicrobials destroy or inhibit the growth of microbes such as bacteria, viruses, fungi or protozoa and more than 50% of the reported antimicrobial substances are derived from plants [14].

Antibacterial and cytotoxic activities of *A. nilotica* extracts against *E. coli* and *Klebsiella* species and observed that killing capacity of the extracts against these bacterial species [15]. It was demonstrated that the effective tumor inhibition nature of extracts of *A. nilotica* in *in-vivo* models [16]. Vikrant [17] reviewed about ethanobotany, phytochemical and pharmacological profile of *A. nilotica* and concluded that *A. nilotica* is an important source of many therapeutically and pharmacologically active constituents. An investigation estimated the phyto-chemical components of *A. nilotica* and observed marked antibacterial activity against *Sterptococcus mutans* [18]. A researcher observed the antibacterial activity of *A. nilotica* against clinical isolates and reported that it is rich in phyto-chemicals and have potent antibacterial activity [19]. Similarly another study on antibacterial activity of methanolic extracts of *A. nilotica* against hospital isolates of Bangaluru also supports observations the present study [20].

Antibacterial tests were clearly expressed that both water and ethanol extracts of the bark of the plant is the major source to control the bacterial species except *Clostridium sp.* due to the obligate anaerobic nature of the bacterium. However, *Clostridium sp.* have the least population in the water, sediment and droppings samples of the lake. At the same time leaf extracts shows excellent controlling potential over the bacterial species. At this juncture, results revealed that the decomposition of the bark in lake ecosystem leads to the control of the bacterial populations of *Escherichia coli, Staphylococcus sp., Bacillus sp., Pseudomonas sp.,* and *Streptococcai sp.* Thus, the study proved that the microbial contamination of the lake ecosystem due to deposition of bird droppings and biotic decomposition is strongly buffered by the single most dominated plant species *Acaica nilotica indica.*

5. CONCLUSION

Due to its distinctive physicochemical and biological components, tropical lakes are considered as a highly sensitive and valuable ecosystem. The present study also add the values of biological components on the regulation of the natural process of the tropical freshwater lakes. The study explore the self-buffering potential of the lake ecosystem on bacterial population. The results revealed that the single dominant para-limnetic macro flora *Acacia nilotica indica* have highly efficient to check the blooming of population *Escherichia coli, Staphylococcus sp., Bacillus sp., Pseudomonas sp.,* and *Streptococcai sp.* This self-buffering potential of the lake is an example of the autonomic regulation of a tropical freshwater lake.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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