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Analysis of Phytochemical Contents and Antibacterial Activity of an Endangered Tree (*Cynometra travancorica* Bedd.) of Western Ghats, India

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Abstract: Antibacterial activity of cold water and ethanolic crude extracts of leaves of *Cynometra travancorica* Bedd. was evaluated against Gram negative bacteria (*Escherichia coli, Pseudomonas fluorescens, Klebsiella pneumoniae* and *Enterobacter aerogenes*) and Gram positive bacteria (*Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus*, *Streptococcus mutans* and *Bacillus subtilis*). *Staphylococcus aureus* was the most sensitive strain of those tested with both the cold water and ethanolic extract of leaves of *Cynometra travancorica*. *Enterobacter aerogenes* showed no antibacterial sensitivity to the tested extracts.

Key words: Antibacterial Activity · Phytochemicals · *Cynometra travancorica* · Endangered Tree · Western Ghats

INTRODUCTION

The Southern Western Ghats or the Malabar region is one of the richest bio-geographic provinces of the Indian subcontinent. A good amount of biodiversity occurs here. Floristically, this region is one of the richest areas in the country harboring not less than 4950 species representing 30% of the flowering plants of India. It includes 1272 taxa endemic to Kerala and 483 among them are placed under threatened categories [1]. Some of these plants have been utilized as source of medicines and life supporting agents apart form food since time immemorial. Even today WHO estimated that up to 80% of people still rely mainly on traditional medicine. Its civilization is very ancient and the country as whole has long been known for its rich resources of medicinal plants. Physicians greatly depend on *Materia medica*, traditional knowledge and other ancient references. There are many plants that do not come to the light only because of their narrow distribution and high rate of extinction. Phytochemical analysis and antibacterial activities of wild plants were studied by Jain [2]. The antibacterial activity and phytochemicals of *Andrographis paniculata, Coleus aromaticus, Cinnamomum, Acaia nilotica, Samanea saman, Eucalyptus camaldulensis, Pterospermum diversifolium, Betula utis, Mallotus philippiensis, Physalis minima, Rauvolfia tetraphylla* and *Ocimum grattisimum* are also screened [3-13]. Reported works show that most of the researches are carried on medicinal plants which have wide distribution. *Cynometra* a Linnaean genus of approximately 75 species is widely distributed throughout the tropics. *Cynometra travancorica* Bedd. is an endangered tree (Endangered B1+2c ver 2.3) species and a member of Fabaceae [14]. The species is a tall tree 10-15 m height, leaflets 1 paired, unequal sided, very thin and creamy pink in color when young, Flowers are rosy white, ovary reddish and pods flat, semicircular, rugose, orange when dry. This species is locally known as *Koori* (in Malayalam) and timber is very useful and also grown as an avenue tree [15]. Many researchers were attempted to document the antimicrobial and phytochemical screening and estimation of the genus *Cynometra* [16, 17].

Narrow distribution and destruction of natural habitats make the plant less available for detailed phytochemical or pharmacological screening. A study conducted by M. S. Swaminathan Research Foundation to document the rare, endemic and threatened plants of

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Western Ghats shows that this plant is very rarely seen and narrowly distributed in evergreen and semi-evergreen forests of southern part of Western Ghats [15]. In this background, the present study aimed to screen the phytochemical contents and antibacterial properties of *C. travancorica* Bedd. leaf extracts against the selected clinically important pathogens.

MATERIALS AND METHODS

Plant material: The fresh leaves of *C. travancorica* Bedd. were collected from evergreen forests of Thamarassery (Wayanad) Ghat, Kozhikode district of Kerala. The identification was confirmed by using regional floras, monographs and comparison with voucher specimens deposited in herbaria of M. S. Swaminathan Research Foundation, Kalpetta, Kerala, India.

Culture and Maintenance of Bacteria: For the present study antibacterial assay: Gram negative bacteria; *Escherichia coli, Pseudomonas fluorescens, Enterobacter aerogen*s and *Klebsiella pneumoniae* and Gram positive bacteria; *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus*, *Streptococcus mutans* and *Bacillus subtilus* were used as test organisms. These microbes were grown in nutrient broth media and incubated at 37°C for 48 hrs. Each bacterial culture was further maintained on the same medium after every 48 hrs of transferring.

Preparation of Plant Extracts: Plant materials were surface sterilized and washed with clean sterile water and dried for 1 hr at 160°C. Three hundred grams of dried plant material was blended to fine powder and soaked in 150 ml of distilled water (cold water extract) and 95% ethanol (ethanolic extract) for 24 hrs. The slurry obtained was left in clean, sterile glass container and shaken vigorously to allow for proper extraction [18]. The slurry was filtered using a sterile muslin cloth. After which the extract obtained was air dried and stored at 4°C until required.

Analysis of Antibacterial Activity: The filter paper disc method was used for screening of the prepared cold water and ethanolic crude extracts for antibacterial activity [19]. Whatmann filter paper discs (6 mm in diameter) were sterilized by dry heat sterilization method at 140°C for 1 hr. Different dilutions of the plant extract were prepared in the order of 1, 2, 3, 4 and 5 mg/ml respectively. The filter paper discs were saturated with 10 µl of each concentration and known quantity of standard reference antibiotic (ampicilin) separately. The discs were then placed on the surface of the sterilized nutrient agar media that had been inoculated with test organisms and incubated at 37°C for 24 hrs. The zone of inhibition was considered as an indicator for the antibacterial activity. All the experiments were done in 5 replicates and mean values were computed [20, 21].

Activity Index of test microorganism was calculated by using the formula

$$\text{Activity Index (AI)} = \frac{\text{Inhibition area of the sample}}{\text{Inhibition area of the standard}}$$

Qualitative Phytochemical Analysis: The ethanolic leaf extracts were analyzed for the pholabatannins, tannins, sterols, lipids, glycosides, terpenoids, phenols, carbohydrates, anthraquinones, resins, reducing sugar, saponins, flavanoids, acidic compounds and alkaloids [22, 23].

RESULTS AND DISCUSSION

The present study carried out on *Cynometra travancorica* Bedd. revealed the presence of medicinally active compounds which possess antibacterial activity. The antibacterial activity of cold water and ethanolic extracts of leaves of *Cynometra travancorica* Bedd. was examined against 8 microorganisms and found to exhibit a promising antibacterial activity at 5 mg/ml against most of the studied Gram positive and Gram negative organisms.

Table 1 indicates the results of the antimicrobial activities of the cold water extract of leaves of *Cynometra travancorica* Bedd. with respect to the test organisms. The extract showed significant activity against eight test organisms. The data indicated that Gram-positive *Staphylococcus aureus* was the most sensitive strain of those tested with the cold water extract of leaves of *Cynometra travancorica*, with the strongest inhibition zone of 13.40 mm and the activity index of 0.837. The cold water extract also exhibited high antimicrobial to methicillin-resistant *Staphylococcus aureus* (MRSA) (IZ-11.6 mm and AI-0.725), *Streptococcus mutans* (IZ-10.8 mm and AI-0.432) and *Escherichia coli* (IZ-10 mm and AI-0.588). However, the cold water extract was found ineffective against *Enterobacter aerogenes*, where it showed no antimicrobial activity. However, the extract also showed moderate amount of antimicrobial activity against *Pseudomonas fluorescens, Klebsiella pneumoniae* and *Bacillus subtilis*. The inhibition zone observed for *Pseudomonas fluorescens* was 7mm. (AI-0.466), *Klebsiella pneumoniae* was 7mm (AI-0.350) and for *Bacillus subtilis* was found to be 7.8 mm (AI-0.520).

Table 1: Antibacterial activity of cold water extract of leaves of *Cynometra travancorica*

Test Microorganism	Inhibition zone of Standard Ampicilin in mm	Inhibition zone (IZ) in mm	Activity Index (AI)
Bacillus subtilis	15	7.8	0.520
Enterobacter aerogenes	22	0	0
Escherichia coli	17	10	0.588
Klebsiella pneumoniae	20	7	0.350
Methicillin-resistant *Staphylococcus aureus* (MRSA)	16	11.6	0.725
Pseudomonas fluorescens	15	7	0.460
Staphylococcus aureus	16	13.4	0.837
Streptococcus mutans	25	10.8	0.432

Table 2: Antibacterial activity of ethanolic extract of leaves of *Cynometra travancorica*

Test Microorganism	Inhibition zone of Standard Ampicilin in mm.	Inhibition zone (IZ) in mm	Activity Index (AI)
Bacillus subtilis	15	8.8	0.586
Enterobacter aerogenes	22	0	0
Escherichia coli	17	9.4	0.552
Klebsiella pneumoniae	20	9	0.450
Methicillin-resistant *Staphylococcus aureus* (MRSA)	16	9.2	0.575
Pseudomonas fluorescens	15	7.8	0.520
Staphylococcus aureus	16	10.2	0.637
Streptococcus mutans	25	8.6	0.344

Table 3: Phytochemical screening of ethanolic leaf extract of *Cynometra travancorica*

No	Phytochemical components	Status
1.	Acidic compounds	+
2.	Alkaloids	-
3.	Anrthraquinones	-
4.	Carbohydrates	+
5.	Flavanoids	+
6.	Glycosides	+
7.	Lipids/fats	+
8.	Phenols	+
9.	Pholabatannins	-
10.	Reducing sugars	+
11.	Resins	+
12.	Saponins	+
13.	Sterols	+
14.	Tannins	+
15.	Terpenoids	-

+ = presence; - =absence

Table 2 depicts the results of the antimicrobial activities of the crude ethanolic extract of leaves of *Cynometra travancorica* with respect to the selected test organisms. The extract showed noteworthy activity against 8 test organisms. The data indicated that Gram-positive *Staphylococcus aureus* was the most sensitive strain of those tested with the ethanolic extract of leaves of *Cynometra travancorica*, with the strongest inhibition zone of 10.2 mm and the activity index of 0.637. The ethanolic extract also exhibited high antimicrobial activity against *Escherichia coli* (IZ-9.4 mm and AI-0.552), methicillin-resistant *Staphylococcus aureus* (MRSA) (IZ-9.2 mm and AI-0.575), *Streptococcus mutans* (IZ-8.6 mm and AI-0.344) and *Bacillus subtilis* (IZ-8.8 AI-0.586). The ethanolic extract was found ineffective against *Enterobacter aerogenes*, where it showed no antimicrobial activity. However, the extract also showed moderate amount of antimicrobial activity against *Pseudomonas fluorescens* and *Klebsiella pneumoniae*. The inhibition zone observed for *Pseudomonas fluorescens* was 7.8 mm. (AI-0.520) and *Klebsiella pneumoniae* was 9 mm. (AI-0.450).

The ethanol extract was subjected to different qualitative phytochemical tests for detection of different biologically active chemical groups. The results are summarized in Table 3. In these screening process sterols, lipids/fats, glycosides, phenols, carbohydrates, tannins, resins, reducing sugars, saponins, flavanoids and acidic compounds gave positive results while pholabtannins, terpenoids, anthraquinones and alkaloids gave negative results. The antibacterial activity found in the plant extracts have been attributed to some of the secondary metabolites [24, 25]. The presences of phenolic compounds are thought to be toxic to microorganisms, inhibiting the enzymes which are essential for the growth of microorganisms [26].

Modern medicines depend heavily on antibiotics for bacterial infections. However, the high genetic adaptability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance [27]. Thus there has been a continuing search for new and more potent antibiotics. According to World Health Report on infectious diseases 2000, overcoming antibiotic resistance is the major issue of the WHO for the next millennium [28]. Hence, the last decade has witnessed an increase in the investigations on plants as a source of human disease management.

The present study was conducted to extend newer lead for enhanced and safer chemotherapeutic means of the plant origin from endangered taxa of Western Ghats because these RET plant species keeps unrevealed with its potential uses. From the work results, it is observed that the ethanolic and cold water crude extracts of leaf of *Cynometra travancorica* showed antibacterial activity against most of the tested organisms especially *Staphylococcus aureus*. This indicates the potential of a new drug that has medicinal value as well as commercial viability.

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