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ANTIMICROBIAL ACTIVITY OF DIFFERENT VARIETIES OF *TERMINALIA CATAPPA* LEAVES

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ABSTRACT: *Terminalia catappa* Linn. belongs to the family Combretraceae found in tropical regions of Asia, Africa and Australia, which is commonly called as Indian almond, sea-almond, tropical almond, wild badam. The fruit is useful in bronchitis and bowels. Juice of leaves is used in the preparation of the ointment for scabies, leprosy, cutaneous diseases and also as analgesic. The root bark is used in dysentery and diarrhoea. The stem bark cures fevers. The oil yielded from kernels is used commercially as a substituted for almond oil. According to the literature the different parts of the plants is used in many therapies including, antibacterial anticlastogenic, antioxidant, antifungal, antiulcer and molluscidal activity, management of sickle cell aneamia, anticancer, anti HIV reverse transcriptase, hepato-protective, anti-inflammatory, antidiabetic. In this study the methanol extracts of two varieties of *Terminalia catappa* (yellow and red variety) leaf was screened for antifungal like *Candida albicans* and *Aspergillus niger* and antibacterial like *Eschiershea coli*, *Staphylococcus aureus, Citrobacter species, Enterococcus faecalis, Pseudomonas aerogenosa* and *Bacillus cereus* was evaluated. The variation in the intensity of inhibitory activity of this microorganism between two varieties was observed.

INTRODUCTION: Herbal medicines have been known to man for centuries, the therapeutic efficacy of many indigenous plants for several disorders discovered by well known scientist. Antimicrobial properties of medicinal plants are being increased reported from different parts of the world, the plant extract or their active constituents used as fold medicines in traditional therapies of 80% of the world's population.

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The harmful microorganism can be controlled with the drugs and these results in the emergence of multiple drug resistant bacteria and it has created an alarming clinical situation in the treatment of infections, in effort to expand the spectrum of antibacterial agents from natural resources.

Medicinal plants contain active chemical constituent in various parts of plants like root, leaf and stem. Plant extract has active ingredients [1]. The combination of plant extract and antibiotics could be useful in fighting emerging drug - resistance microorganisms. *Terminalia catappa* Linn. is an ornamental tropical tree belonging to the family Combretaceae is native to Southeast Asia in the tropical regions of Asia, Africa and Australia. It is known by the common names Bengal almond,

Country almond, false kamani, Indian almond, Malabar almond, and about 35m (115 ft) tall, 9 m in width of its symmetrical canopy [2] and horizontal branches. The greenish yellow leaves are clustered in axillary spikes, small and inconspicuous. It usually commences flowering within 2 - 3 years of out planting but this may vary with site and genotype. It is a large deciduous stately tree with a characteristic pagoda shape [3]. It is cultivated in Nigeria solely as a shade tree and for its fruits and seeds as well as medicinal uses [4]. The several reports revealed the identification, characterization and standardization of *Terminalia catappa* as medicinally important plant [5, 6].

Plant with red leaves are used to expel worms applied to rheumatism where as ordinary leaves are mixed with the oil and rubbed on to the breast to relieve mammary pain, secondary metabolites which are useful for the growth and development of plant [7]. The pharmacological significance of the plant is an updated review is for the traditional medicinal uses which play a major chemical constitutes reported on this tree and plant was sensitive to pollution control in Nature [8].

Many scientists have contributed and studied phytochemicals [9] play an important role in the treatment of different types of diseases and disorders and are still used in both traditional and modern medicine. Phenolic compounds are known as powerful chain breaking antioxidants [10]. Juices from leaves were used as an ointment for scabies, leprosy and other skin infections [11]. The plant showed a rich source of bioactive compounds and justifying their traditional use to treat any disease and disorder is postulated. The leaves have antioxidant property anticancer used to cure liver diseases [12] anti HIV reverse transcriptase and management of sickle cell anemia [13], hepato-protective, anti-inflammatory [14] antidiabetic [15, 16] fish pathogen [17].

The leaves found to possess to fight against the infectious microbes which might be due to the phytochemicals identified in the leaf thus acting as a potent antimicrobial agent [18, 19, 20, 21, 22]. Methanol extract showed significant antifungal activity against most susceptible mold [23, 24]. *Terminalia catappa* as a source of naturally derived anti-microbial formulations to herbal medicines against bacterial infection [25] and in pharma industry. In present study the methanol extracts of two varieties of *Terminalia catappa* (yellow and red variety) leaf was screened for antimicrobial activities.

MATERIALS AND METHODS:

Collection of Material: The leaves of *Terminalia catappa* were collected in the reference of two varieties that is the yellow and red variety from Bangalore city. The two yellow varieties are called as T1 and T2. The two red varieties were called as T3 and T4.

Preparation of Plant Extract:

Extraction: The leaves of *Terminalia catappa* were air dried and then the sample was powdered in a homogenizer 10 g was used for methanol extraction by Soxhlet method. The solvent was then evaporated to dryness under reduced pressure in Roto evaporator and the extracted compound left was used for the antimicrobial assay.

Preliminary Phytochemical Assay: The methanolic extract of different varieties of *Terminalia catappa* leaves were subjected to preliminary phyto-chemical assay to detect the presence of different bioactive compounds like proteins, carbohydrates, glycosides, flavanoids, tannins and phenol [26, 27].

Quantitative Analysis of Phytochemicals:

Determination of Total Phenol: The total phenol concentration in methanolic leaf extracts were determined by using Folin-Ciocalteu method [28]. To 1 ml of leaf extract, 0.5 ml of 0.2N Folin- Ciocalteu reagent and 1.5 ml of 0.7 M sodium carbonate solution was added and adjusted to 10ml with distilled water. After incubation in dark at 25 ºC for 1 h, the absorbency of reaction mixture was measured at 725 nm. Catechol was used as a standard to produce the calibration curve.

Determination of Total Flavonoids: The total flavonoid content of leaf extract was measured using aluminium chloride method [29]. One ml of leaf extract was mixed with 0.5 ml of 1.2% aluminium chloride and 0.5 ml of 120 mM potassium acetate. The volume was then increased to 10 ml with distilled water. The mixture was incubated for 30 min at room temperature. The absorbance was measured at 415 nm. Rutin was used as standard for the calibration curve.

Test Microorganisms and Growth Media: The following microorganisms such as *Eschiershea coli, Pseudomonas aerogenosa, Bacillus cereus, Staphylococcus aureus, Staphylococcus mutans, Enterococcus* and fungal strains like *Candida albicans, A. niger, Enterococcus, Klebsiella, Citrobacter* were chosen based on their clinical and pharmacological importance.

The bacterial and fungal stock strains were incubated for 24 h at 37 ºC on nutrient agar and potato dextrose agar medium (PDA), stock cultures were maintained at 4 ºC. All bacterial strains were procured in lyophilized form from the Institute of Microbial Technology (IMTECH), Chandigarh.

Antimicrobial Activity:

Determination of Zone of Inhibition: *In-vitro* antibacterial and antifungal activities were examined for methanol extract against six pathogenic bacteria (Gram positive and Gram negative) and two pathogenic fungal were investigated by well diffusion method. Each purified extracts were dissolved in methanol for the determination of zone of inhibition and standard antibiotic ampicillin (10 mg/ml) used for comparison of the results.

All the extracts (T1, T2, T3, T4) were screened with different concentration of plant extract (50, 100, 150, 200 µg/ml) against the *Eschiershea coli, Pseudomonas aerogenosa, Bacillus cereus, S. aureus, Staphylococcus mutans, Enterococcus, Klebsiella pneuomonia, Citrobacter* and fungal strains like *Candida albicans, Aspergillus niger*. The zones of growth inhibition around the discs were measured after 18 to 24 h in incubation at 37ºC and 28 ºC for bacterial and fungal respectively. For each extract three replicate trials were conducted against each organism. The diameters of the inhibition zones were measured in mm.

RESULTS AND DISCUSSION: According to the result of qualitative analysis of chemical constitutes of different varieties in *Terminalia catappa* showed the presence of amino acid, protein, carbohydrates, glycosides, alkaloids (+) and saponin, tannin, flavanoid showed very good result (++) shown in **Table 1**.

The quantitative analysis with a focus on flavanoids, and phenol from the methanol extract of different varieties of the sample in **Table 2**. The phenol content was determined by Folin-ciocalteau reagent. The maximum concentration of phenol was observed T3-195 µg/ml, T4-185 µg/ml, T2-180 µg/ml minimum concentration was observed in-T1-178 µg/ml. The flavanoid content was evaluated by aluminium chloride method highest amount of flavanoid content was obtained in sample T1-0.89 µg/ml, T4-0.86 µg/ml, T3-0.75 µg/ml and low in sample T2-0.72 µg/ml.

TABLE 1: QUALITATIVE ANALYSIS OF LEAF EXTRACT

Qualitative Test	Methanol			
	T1	T2	T3	T4
Protein	+	+	+	+
Alkaloids	+	+	+	+
Saponins	++	++	++	++
Carbohydrates	+	+	++	+
Glycosides	+	+	++	+
Tannin	++	++	++	++
Flavanoid	++	++	++	++
Amino acid	+	+	++	+

TABLE 2: QUANTITATIVE ANALYSIS OF PHENOL AND FLAVANOID

Sample	Amount of flavanoid	Amount of phenol
T1	990µg/ml	178 µg/ml
T2	585 µg/ml	180 µg/ml
T3	587 µg/ml	195 µg/ml
T4	950 µg/ml	185 µg/ml

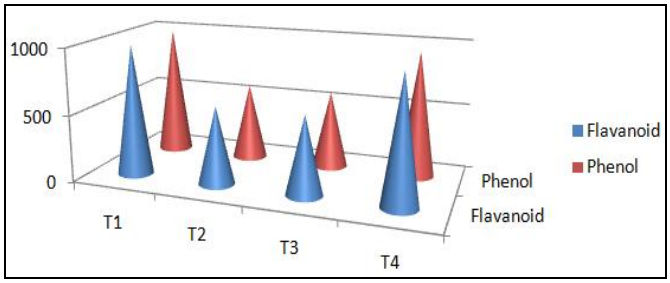

FIG. 1: REPRESENTING THE QUANTITATIVE ESTIMATION OF PHENOL AND FLAVANOID

Antimicrobial activity of methanol extract of *Terminalia catappa* leaf variety was studied with selected G+ve and G-ve pathogens such as *Bacillus cereus, Staphylococcus aureus, K. pneumonea, Eschiershea coli, Citrobacter sp., Pseudomonas aerogenosa* and fungus such as *Candida albicans* and *Aspergillus niger* was performed in well diffusion method.

Result revealed that the methanol extract of *Terminalia catappa* leaves was found to be effective against standard ampicillin (40 mm), G +ve such as *Staphylococcus aureus* with the zone of inhibition is 40 mm in T1 and T2, 30 mm in T3,

20 mm in T4. The G+ve *Bacillus cereus* showed the zone of inhibition 22 mm in T2 and T3, no zone of inhibition in T1 and T4.

An antifungal activity of G+ve like *Candida albicans* showed 12 mm in T1, 26 mm in T2, 07 mm in T4, no zone of inhibition in T3. *Aspergillus niger* with the zone of inhibition as 20 mm in T2,18 mm in T3, 10 mm in T4, no zone of inhibition in T1. G–ve bacteria like *Eschiershea coli* showed 40 mm in T1, T2 and 32 mm in T3, 20 mm in T4. *Pseudomonas aeurogenosa* has 20 mm in T1 and 22mm in T2 and 19 mm in T3, 11mm in T4. *Citrobacter sp* showed 16 mm in T1, 12 mm in T2, and 08 in T3, 12 mm in T4. *Klebsiella pneumonia* showed the zone of inhibition as 18 mm in T1, T2 and T3 is 10 mm, 12 mm in T4 respectively. The positive control, ampicillin has showed the highest level of 40 mm when compared to sample taken.

According to the **Table 3**, variety T2 showed significant antibacterial and antifungal activities in all pathogens tested in different concentrations. The G+ve bacteria *Staphylococcus aureus*, G-ve bacteria *Eschiershea coli* was significantly inhibited in methanolic extract of all varieties with the standard in accordance with earlier report [30] and its bioactive compounds can be used as chemo preventive agents to treat bacterial diseases without risk assessment [31].

TABLE 3: ANTIMICROBIAL ACTIVITY

S no.	Samples (Methanol)	*Eschiershea coli*	*Pseudomonas aeurogenosa*	*Bacillus cereus*	*Staphylococcus aureus*	*Citrobacter enterococcus*	*Klebsiella pneumonia*	*Candida albicans*	*Aspergillus niger*
1	T_1	40 200mg/ml	20 200mg/ml	-------	40 200mg/ml	16 200mg/ml	18 50mg/ml	12 50mg/ml	---
2	T_2	40 200mg/ml	22 150mg/ml	22 150mg/ml	40 200mg/ml	12 100mg/ml	18 100mg/ml	26 100mg/ml	20 150mg/ml
3	T_3	32 150mg/ml	19 150mg/ml	22 150mg/ml	30 200mg/ml	8 100mg/ml	10 150mg/ml	---	18 150mg/ml
4	T_4	20 200mg/ml	11 100mg/ml	----	20 200mg/ml	12 100mg/mls	12 200mg/ml	07 100mg/ml	10 100mg/ml
5	Standard ampicillin antibacterial	40 200mg/ml	40 200mg/ml	40 200mg/ml	40 200mg/ml	40 200mg/ml	40 200mg/ml	40 200mg/ml	40 200mg/ml

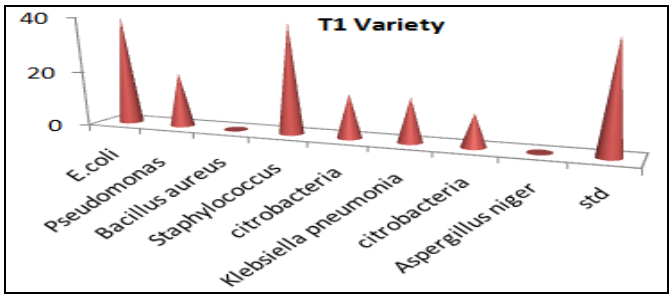

FIG. 2: T1 VARIETY

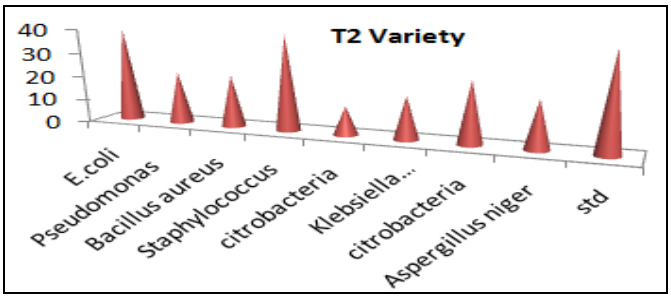

FIG. 3: T2 VARIETY

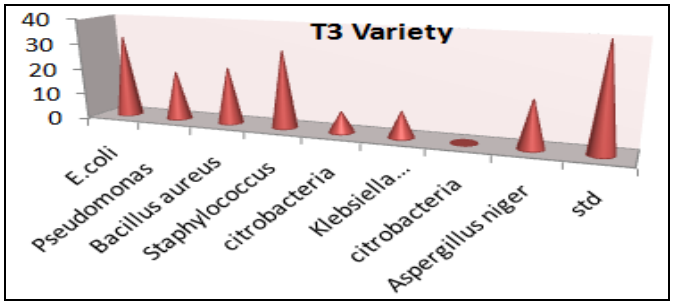

FIG. 4: T3 VARIETY

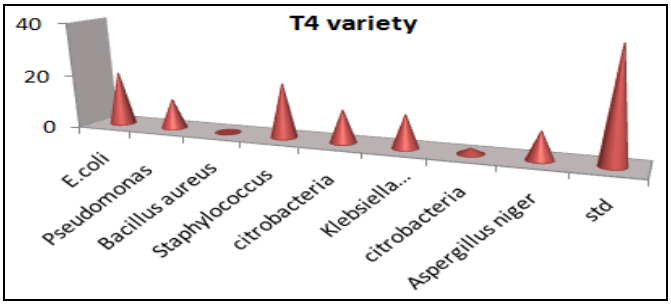

FIG. 5: T4 VARIETY

CONCLUSION: In the current investigation, the methanol extracts of different varieties of *Terminalia catappa* leaves showed high antibacterial activity, exactly to standard whereas antifungal activity was half to the standard. Therefore the antibacterial activity of four different varieties is equally important and can be used for clinically isolated microorganisms as compared with the standard.

The present study justified that the different varieties of *Terminalia catappa* leaves can be used in the traditional system to treat various infectious diseases caused by microbes especially by two microorganisms like *staphylococcus aureus* (a Gram -positive bacteria), *Eschiershea coli* (Gram - negative bacteria) were more susceptible and for the further investigations in the potential discovery of new natural bioactive compounds.

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CONFLICT OF INTEREST: Nil

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