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## STUDIES ON ANTIMICROBIAL ACTIVITY OF *ANOGEISSUS LATIFOLIA* (ROXB. EX DC.) AND *TERMINALIA TOMENTOSA* WIGHT & ARN.

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### Abstract:

The global burden of bacterial infections is very high and has been exacerbated by increasing resistance to multiple antibiotics. Antibiotic resistance leads to failed treatment of infections, which can ultimately lead to death. To overcome antibiotic resistance, it is necessary to identify new antibacterial agents. In this study, two medicinal important plants namely *Anogeissus latifolia* (Roxb. ex DC.) Wall. Ex Guillem. & Perrplants from and *Terminalia tomentosa* Wight & Arn. The present investigation was aimed to focus on the antibacterial activity of two selected plants of *Anogeissus latifolia* and *Terminalia tomentosa* in aqueous, ethanolic and Chloroform extracts. Different concentrations of Chloroform, ethanol and aqueous extracts were used for antibacterial activity. Maximum zone of inhibition was observed in 30% w/v Ethanolic extract than other extracts. Different extracts of *A. latifolia* and *T. tomentosa* has the medicinally useful secondary metabolites and also act as antibacterial agent on various bacterial strains. The findings of this research suggest that the extracts of both plants can be a source of natural antibacterial agents with pivotal applications in pharmaceutical companies to control pathogenic bacteria causing severe illness in humans

**Key Words:** multiple antibiotic resistances; human pathogens; antibacterial activity; ethanolic, Chloroform extracts *Anogeissus latifolia* and *Terminalia tomentosa*

### Introduction:

Medicinal plants have long been used to treat diseases (Mohanta *et. al.*, 2012, Mohanta *et.al.* 2014). Plants are commonly used as sources of new pharmaceuticals due to the presence of promising therapeutic compounds. Infectious diseases play a significant role in the deaths of millions of people worldwide, in part due to the mutagenic nature of the bacterial genome. Moreover, the exchange and uptake of plasmids among bacteria results in the development of multiple antibiotic resistant strains. Antimicrobials from different plants

have enormous therapeutic potential and lesser side effects than synthetic antibiotics (Verma, and Singh (2008). Dubey *et.al.* 2004). accordingly, it is desirable and essential to develop an effective, safe and natural product to control multiple drug resistance (MDR) pathogens. Medicinal plants contain active principles generated by various natural metabolic processes and each plant species has its own metabolome that governs the presence of chemical components or bioactive molecules (Cown, (1999).

India is one of the richest countries in the world with regards to the genetic resource of medicinal plants (Parekh and Chanda (2008). The country has a wide range of topography and climate, which influences its vegetation and floristic composition. Worldwide searches for antimicrobial agents continued to focus on lower plants, fungi and bacteria (Fabry *et.al.*, 1998). There are many approaches that can be used to select plants of potential therapeutic interest (Vlietinck and Vanden Berghe (1991). Compounds can be identified through random, ethno- (including Ethnobotanical, Ethnomedical and Ethnopharmacological) and ecological searches (Fabricant and Farnsworth (2001). The random collection of plant samples from certain habitats with high species diversity (for example tropical rain forests) can be very useful for identification of novel chemical entities. However, this method is time consuming and labor intensive (Vuorela, P *et. al.*, 2004). This kind of sampling is most likely to be used in industry to evaluate the industrial approach and most likely to be used for evaluating plants for bioactive compounds.

*Anogeissus latifolia* one of the important medicinal plant has since Ayurveda in cardiac disorder. The plant is useful in UTI infections, skin diseases, liver complaints, fever, epileptic fits etc. The plant is rich in pharmacologically active phenolic phytoconstituent-ellagic acid. It possess healing potential, microbicidal activities, antiulcer potential, hypolipidemic activities and hepatoprotective potential. *Anogeissus latifolia* (DC.) is medium sized deciduous tree belonging to the family combretaceae and it is commonly known as gahtti. It attains height of about 30-40 feet. Leaves are opposite or sub-opposite. Bark is smooth with grey-white colour and exfoliating in irregular thin scales. Flowers sessile, in dense heads. Fruit small, compressed, winged with beak, seed ovoid. Tree flowers and fruits in the month of Sept-March.

*Terminalia* is a genus of large trees of the flowering plant family Combretaceae, comprising around 100 species distributed in tropical regions of the world. *Terminalia tomentosa* (syn. *Terminalia alata* Heyne ex Roth, *Terminalia elliptica* (Roxb.) Wight & Arn is a species of *Terminalia* native to southern and south east Asia in India, Nepal, Bangladesh, Myanmar, Thailand, Laos, Cambodia and Vietnam. It is a prominent part of both dry and moist deciduous forests in southern India up to 1000 m. *Terminalia tomentosa* {Roxb.} Wight & Arn has been traditionally the stem bark of the plant is used as cardiac stimulant and in treatment of atonic diarrhea & callous ulcer. (Chetty and Sivaji Rao (2008).

Several studies have provided evidence that the antimicrobial compounds isolated from different solvent extracts never provided the expected final output based on the activity of crude extracts and fractions (Eloff, *et. al.*, 2008). This is probably because different plant metabolites often work in combination with other compounds to regulate microbial infections and may therefore not be effective alone (Lewis, and Ausubel 2006.) Therefore, in the present study, an aqueous extract was used in the preliminary screening (agar diffusion method). It is believed that methanol could efficiently penetrate the cell membranes, permitting the extraction of high amounts of endocellular components in contrast to low polarity solvents such as chloroform

and petroleum ether which can only extract extracellular material. Methanol primarily dissolves polar constituents together with medium and low polarity compounds extracted by cosolubilization. Therefore, the present investigation was conducted to evaluate both the aqueous and methanolic (80%) extracts of two different plants belonging to Combretaceae of *Anogeissus latifolia* and *Terminalia tomentosa* a wide range of plants based on random sampling. The result presented herein will be useful to further search of novel plants with antibacterial properties.

### **Material and Methods:**

Leaves of *Anogeissus latifolia* and Bark of *Terminalia tomentosa* were collected. These were washed in tap water, air dried and ground to fine powder and stored in airtight bottles. The specimens were identified by a taxonomist. The healthy leaves and bark were dried at low temperature without allowing the growth of any type of fungi, or bacteria. These extracts were powdered separately using a mortar and pestle then passed through a 40–60 mm mesh size sieve to obtain uniform powdered samples. Preparation of Plant Extracts A total of 100 g of each powdered sample was dissolved in 200 mL of sterile distilled water and 80% Ethanol separately in wide mouth bottles. The aqueous samples were then steamed with distilled water for 30 minutes, after which they were stored overnight. Next, the suspensions were filtered separately (Whatman No. 40 paper) and used to investigate the antimicrobial properties. The methanol extracts were dried in a rotary evaporator at 50 °C and stored in a refrigerator until further analysis.

### **Test microorganisms and microbial culture:**

Four bacterial strains & two fungal strains were used in this study, two gram-positive *Staphylococcus aureus* (MTCC 98) and *Bacillus cereus* (MTCC 430) and two gram-negative (*Escherichia coli* (MTCC 1687) & *Pseudomonas aeruginosa* (MTCC 1688), *Yeast Candida albicans* (MTCC 227) & Mould *Aspergillus niger* (MTCC 281). The bacterial strains were cultivated at 37°C and maintained on nutrient agar slant at 4°C & Fungal strain were cultivated at 25°C and maintained on sabouraud dextrose agar slant at 4°C.

### **Preparation of antimicrobial disc:**

Sterile discs were procured from HI media and used for the preparation of antimicrobial disc. The extracts of the medicinal plants were incorporated to the sterile disc. Each sterile disc was incorporated individually with the volume equivalent to 50 mg/ml dose of the extracts using a calibrated micropipette. Precautions were taken to prevent the overflow of the solvent from the outer surface of the disc. To ascertain this, the discs applied in small quantities and the discs were allowed for air drying followed by another dose of the extract.

### **Assay of Antibacterial activity:**

The cultures were smeared on the sterile, air-dried nutrient agar plates and sabouraud dextrose agar plates using sterile cotton swab. Sterile discs loaded with known quantity of antibacterial compounds were placed on the surface of agar petriplates with the help of flame sterilized forceps. Control discs were placed in the agar plates incorporating at the solvents only. Then the bacterial petriplates were incubated at 37°C for 24 hrs and fungal petriplates were incubated at 25°C for 48- 72 hrs. The zone of inhibition was observed and measured with the help of a Vernier calipe.

## Results and discussion:

No inhibitions were observed with the ethanol and chloroform extracts of both *A. latifolia* and *T. tomentosa* samples. The water extracts of the plants possess antibacterial properties (Willey, (2006). In the antibacterial studies on the water extracts of both *A. latifolia* showed sensitive inhibition in 50 mg/ml dose against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. *T. tomentosa* showed sensitive inhibition against *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*, no inhibition against *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*.

Thus plants extracts are promising natural antibacterial agents with pivotal roles in pharmaceutical industries for regulating the pathogenic microbes. As a medicinal plant, *A. latifolia* are as since Ayurveda in cardiac disorder. The plant is useful in UTI infections, skin diseases, liver complaints, fever, epileptic fits etc. *T. tomentosa* plant extract are using chest pain (angina). Some research shows that taking *Terminalia* by mouth with conventional medications improves symptoms in people experiencing chest pain after a heart attack. Other research shows that taking *Terminalia* by mouth improves symptoms and reduces the need for chest pain medication in people with long-term chest pain. In this present study, we tested the antimicrobial activity of *A. latifolia* leaf extract and *T. tomentosa* bark extract (with three different solvents, i.e., Water, ethanol and Chloroform) against six pathogenic strain as well as *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

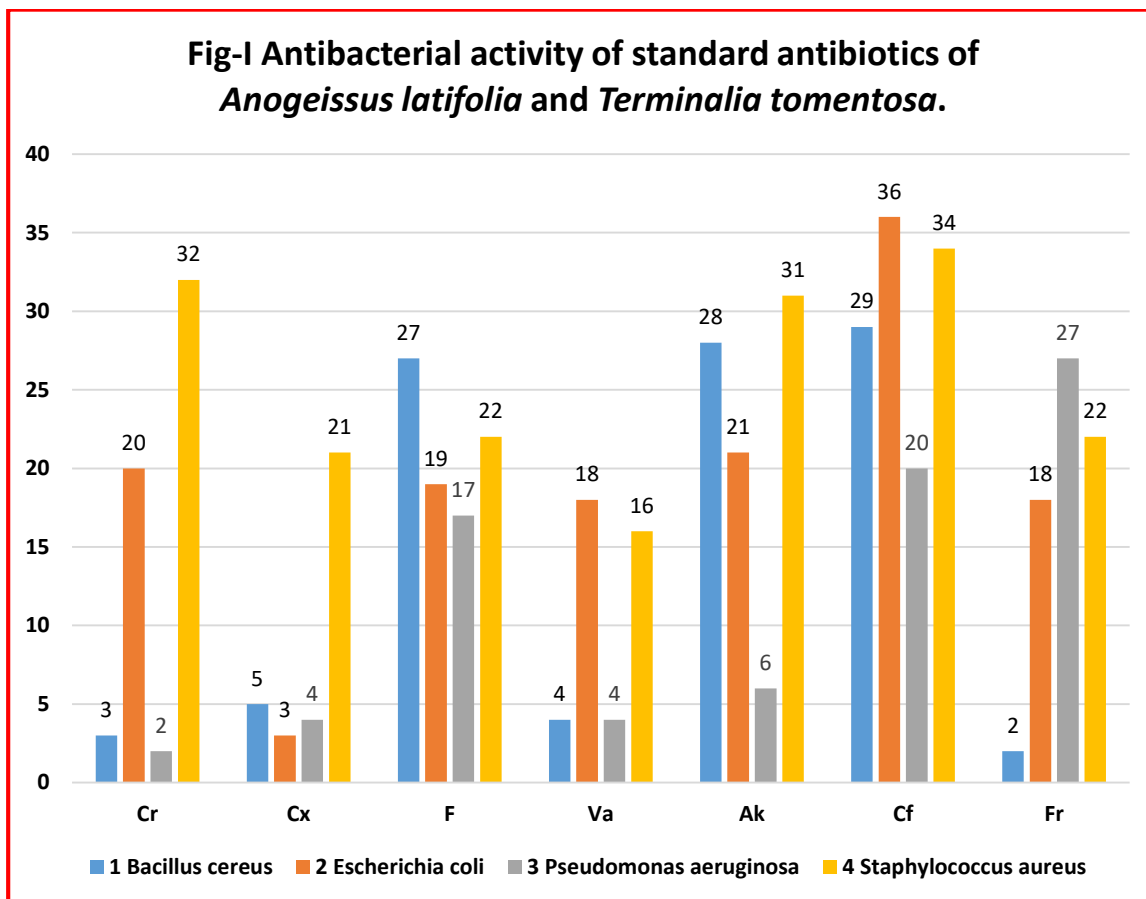
**Table 1. Antibacterial activity of standard antibiotics of *Anogeissus latifolia* and *Terminalia tomentosa*.**

Sl. No	Name of microorganisms	Zone of inhibition (diameter) in mm							
		Cr	Cx	F	Va	Ak	Cf	Fr	
1	<i>Bacillus cereus</i>	03	05	27	04	28	29	02	
2	<i>Escherichia coli</i>	20	03	19	18	21	36	18	
3	<i>Pseudomonas aeruginosa</i>	02	04	17	04	06	20	27	
4	<i>Staphylococcus aureus</i>	32	21	22	16	31	34	22	

Cr = Cephaloridine, Ak = Amikacin, Cx = Cloxacilin, Cf = Ciprofloxacin, F = Framycetin, Fr = Furozolidone, Va = Vancomycin

**Table 2. Antibacterial activity of *Anogeissus latifolia* extracts from Leaves**

Sl. No	Name of microorganisms	Zone of inhibition (diameter) in mm		
		Water	Ethanol	Chloroform
1	<i>Aspergillus niger</i>	7 mm	No zone	No zone
2	<i>Candida albicans</i>	12 mm	No zone	No zone
3	<i>Bacillus cereus</i>	8 mm	No zone	No zone
4	<i>Escherichia coli</i>	8 mm	No zone	No zone
5	<i>Pseudomonas aeruginosa</i>	No zone	No zone	No zone
6	<i>Staphylococcus aureus</i>	14 mm	8 mm	9 mm



**Table 3. Antibacterial activity of *Terminalia tomentosa*. Extracts from Bark**

Sl. No	Name of microorganisms	Zone of inhibition (diameter) in mm		
		Water	Ethanol	Chloroform
1	<i>Aspergillus niger</i>	9 mm	No zone	No zone
2	<i>Candida albicans</i>	No zone	No zone	No zone
3	<i>Bacillus cereus</i>	10 mm	No zone	No zone
4	<i>Escherichia coli</i>	11 mm	No zone	No zone
5	<i>Pseudomonas aeruginosa</i>	No zone	No zone	No zone
6	<i>Staphylococcus aureus</i>	14 mm	8 mm	9 mm

### Conclusions:

In Conclusion, this study highlights the antibacterial activity of *A. latifolia* and *T. tomentosa* which are worthy of further investigation for their phytochemical analysis. Our results support the use of these plants as traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial and antifungal properties that can be used as effective antimicrobial agents in the field of biomedical science.

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