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STUDIES ON PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF *TERMINALIA TOMENTOSA* WIGHT & ARN.

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ABSTRACT: The present work aimed to screen the phytochemical constituents and antibacterial activity in leaves of different extracts of *Terminalia tomentosa* (Combretaceae). Phytochemical analysis was performed to check the presence of flavonoids, alkaloids, phenolic compounds, steroids, saponins, tannins, anthocyanins, lignins, and glycosides. The antibacterial activity of aqueous, ethanol and methanol extracts of *T. tomentosa* was studied against gram-positive bacteria and gram-negative bacteria, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*. Alkaloids, flavonoids, phenolics, tannins, lignins, steroids, saponins, and glycosides were present in all the three extracts, whereas anthocyanidins were barred. Among the three fractions, ethanol has possessed the highest zone of inhibition on *E. coli* (19.8 mm), and the minimum inhibition zone was observed in aqueous against *K. pneumonia* (7.8 mm). In comparison with the extracts and counting with the antibiotics, ciprofloxacin (19-31 mm) has possessed the highest zone of inhibition. The obtained results of leaf extract are worthy for further studies to discover the unrevealed part of it and to prove its efficacy as an alternate for antibacterial infections.

INTRODUCTION: *Terminalia tomentosa* Wight & Arn (Combretaceae) is a large deciduous tree, grows in moist deep heavy clayey soil. It is commonly known as 'Asan' and casually called 'Crocodile bark tree' due to its characteristic bark pattern. It is one of the most exploited plants which finds wide usage in tasar, pharmaceutical, tanning and timber industry [8]. It is a primary host plant for the larvae of tropical tasar silkworm *Anthreae mylitta* D, reared for commercial production of cocoon.

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The leaves are hard, sparsely hairy on the above and on the lower side of the midrib with one-two stalked glands near the base and densely covered with soft pubescence beneath when fully grown. Plants and their parts have its own defense mechanism, which backing it to grow and to behave in a condition when external bioactive agents barrage on it.

This defense mechanism comprises of a number of bioactive constituents which show great efficacy against many-sided pathogenic diseases. Nowadays, the traditional healing system is inclining around the world by utilizing herbal remedies, an important resource for modern drug discovery. Plants have been and still are the major, rich, and diverse source of a variety of phytochemicals, including potent anti-microbial

molecules. Interestingly and apparently, from way back, plant extracts contain copious phytoconstituents; therefore, using plant extracts may pose some adversity counter to the advancement of bacterial resistance as compared to the single molecular drug [12]. One of the most paramount medicinal species in genus Terminalia, widely used in traditional medicine is *Terminalia tomentosa*, which exhibits a number of medicinal activities is might be due to the presence of a large number of different types of phytoconstituents.

The leaf of tree possesses diverse health benefits and has been used as traditional medicine for household remedy against various human ailments since antiquity. This drives the need to curtain for narrative bioactive compounds as plant-based drugs are biodegradable and safe. The present work involved in phytochemical screening and anti-bacterial activity of leaf extract of *T. tomentosa*.

MATERIALS AND METHODS:

Sample Collection: Leaves were collected from three year old plants of *Terminalia tomentosa* maintained at Department of Sericulture, Sri Padmavati Mahila Visvavidyalayam, Tirupati. The samples were amassed by excluding too tender and over matured leaves for each treatment with three replications. Antibacterial activity conducted with aqueous, ethanol and methanol leaf extracts of *T. tomentosa*. *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*, were two gram-positive and one gram-negative bacterium obtained from the Department of Microbiology, Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, Andhra Pradesh.

Authentication of Plant Material: The collected plant material specimen No: AKV-01 was identified and authenticated by Professor N. Yasodamma, Department of Botany, Sri Venkateswara University, Tirupati as *Terminalia tomentosa* (Roxb) Wight & Arn. (Combretaceae) and compared with standard herbarium SVUTY, Department of Botany, S.V. University, Tirupati with specimen voucher No: 735.

Preparation of Leaf Extract for Phyto-constituents: Leaves of *T. tomentosa* were shade dried and pulverized to a coarse powder and sequentially extracted with aqueous, alcohol and methanol by using standard procedures. 5 gms of leaf powder was taken into 250 ml of each different solvents and were soaked for 48 h.

Finally, these extracts were filtered and used for preliminary screening. Phytochemical analysis was conducted for the recognition of bioactive constituents in three different extracts of the leaf sample as per the Harborne [2] standard procedure.

Determination of Anti-bacterial Assay: Streptomycin, ciprofloxacin, and penicillin were the standard drugs as positive controls used for this assay. Bacterial cultures were collected in nutrient broth agar medium by agar well diffusion method.

These wells were loaded with sample extract and control of about 100 µl. The plates were incubated at 37 ºC for 24 h and the inhibition zone was measured in diameter (mm) as they were produced around each well by the plant extracts and antibiotics.

TABLE 1: PHYTOCHEMICAL ANALYSIS IN DIFFERENT EXTRACTS OF LEAF OF *TERMINALIA TOMENTOSA*

S. no.	Secondary Metabolites	Name of the test	Leaf extractions		
			Aqueous	Ethanol	Methanol
1	Alkaloids	Mayer's test	++	++	++
		Wagner's test	++	++	++
2	Flavonoids	Shinoda's test	++	+	+
		Ferric chloride test	++	++	+
3	Phenolic compounds	Phenols test	++	++	++
		Ellagic acid test	++	++	++
4	Steroids	Salkowski test	-	+	+
		Libermann's test	-	+	+
5	Saponins	Saponins test	++	-	-
6	Tannins	Gelatin test	++	+	+
		Ferric chloride test	+	+	+
7	Anthocyanidins	Anthocyanin test	-	-	-
8	Lignins	Lignin's test	++	++	++
		Labat test	++	++	++
9	Glycosides	Kilerkillani test	+	+	+

RESULTS AND DISCUSSION: For screening of phytochemicals, three different extracts were used and all these exhibited a decent amount of the secondary metabolites, but out of these, aqueous was showed high array results. In aqueous extract alkaloids, flavonoids, phenols, saponins, tannins, and lignin showed high array concentration except in glycosides and barred in steroids. Alcohol and methanol extract resulted in high alkaloids, phenolics, and lignins, while on the contrary, steroids, saponins, tannins, and glycosides were exhibited moderately. Alkaloids, flavonoids, phenolics, tannins, lignins and glycosides were present in all the three extracts as shown in **Table 1**. Alkaloids and phenols array high concentration in all the extracts; flavonoids are high in aqueous and alcohol but moderate in methanol. Saponins were observed only in aqueous, which possessed high concentration but barred in other two solvents.

Tannins were also exhibited high in aqueous and moderately in alcohol and methanol. Glycosides were observed moderately in all the extracts whereas anthocyanidins were barred in all the three extracts

Anti-bacterial Activity of *Terminalia tomentosa* Leaf: The Aqueous, ethanol, and methanol fractions of the antibacterial activity showed results at a concentration of 100 µl/well against all bacteria. The zone of inhibition was measured in mm and results were presented in **Table 2**.

TABLE: 2 ANTI-BACTERIAL ACTIVITY OF AQUEOUS, ETHANOL AND METHANOL FRACTIONS AGAINST TESTED MICROORGANISMS

S. no.	Microorganism	Zone of inhibition (mm)					
		Extract fractions			Control		
		Aqueous	Ethanol	Methanol	Ciprofloxacin	Streptomycin	Penicillin
1	*Staphylococcus aureus*	9.16 ± 0.16	17.5 ± 0.28	14.13 ± 0.18	31.33 ± 0.35	22.3 ± 0.18	16.33 ± 0.33
2	*Escherichia coli*	7.93 ± 0.06	19.8± 0.17	15.76 ± 0.14	30.83 ± 0.44	21.8 ± 0.11	15.93 ± 0.06
3	*Klebsiella pneumonia*	7.8 ± 0.11	13.83 ± 0.12	12.76 ± 0.14	19.0 ± 0.11	16.33 ± 0.33	13.26 ± 0.37

All values are represented as mean ± SD (n = 3)

The three fractions of the leaf extracts have inhibited the growth of both gram-positive and gram-negative bacteria, which is *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*. Ethanol fraction (13-19 mm) exhibited high antibacterial activity followed by methanol (12-15 mm) which is moderate and low in aqueous (7-9 mm).

Bearing on antibiotics, ciprofloxacin (19-31 mm) exhibited the highest inhibition zone, which was followed by streptomycin (16-22 mm) and then penicillin (13-16 mm).

In comparison with the extracts and with the antibiotics, ciprofloxacin has possessed highest zone of inhibition.

Among the three fractions, ethanol has possessed the highest zone of inhibition in *E. coli* (19.8 mm) followed by methanol in *E. coli* (15.76 mm) and aqueous in *S. aureus* (9.16 mm). In **Table 2**, ciprofloxacin exhibited the highest zone of inhibition against *S. aureus* (31.33 mm) followed by *E. coli* (30.83 mm). Minimum inhibition zone was observed in aqueous against *K. pneumonia* (7.8 mm) followed by *E. coli* (7.93 mm) in aqueous.

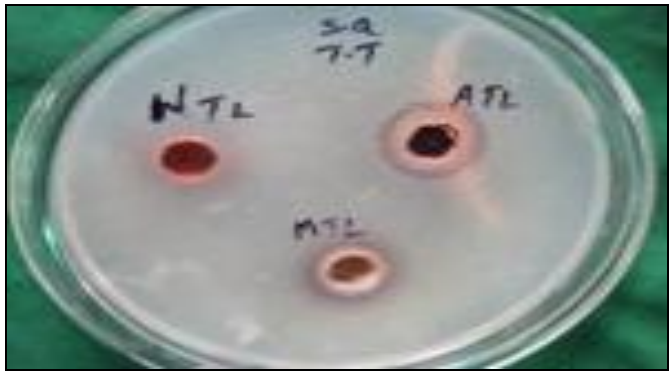

FIG. 1: ANTI-BACTERIAL ACTIVITY OF AQUEOUS, ETHANOL AND METHANOL EXTRACT AGAINST *S. AUREUS* IN LEAF OF *T. TOMENTOSA*

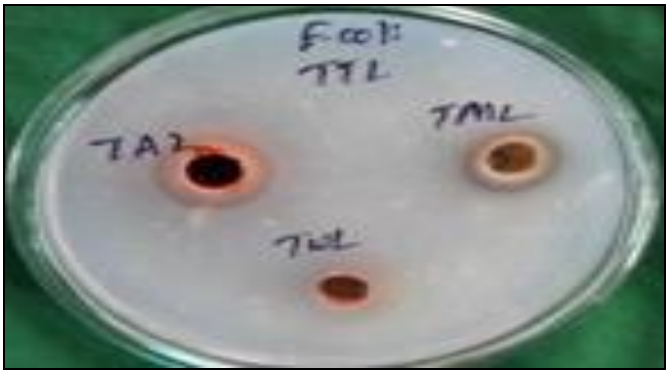

FIG. 2: ANTI-BACTERIAL ACTIVITY OF AQUEOUS, ETHANOL AND METHANOL EXTRACT AGAINST *E. COLI* IN LEAF OF *T. TOMENTOSA*

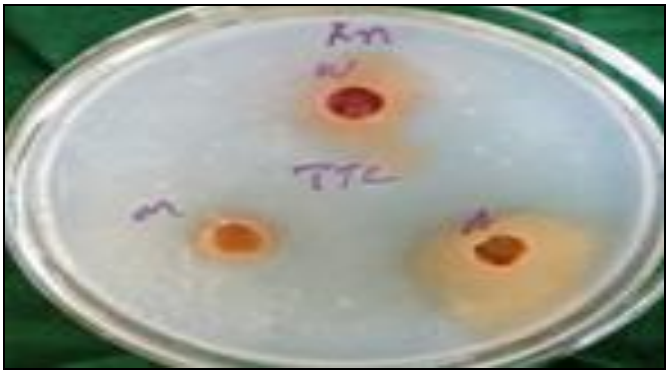

FIG. 3: ANTI-BACTERIAL ACTIVITY OF AQUEOUS, ETHANOL AND METHANOL EXTRACT AGAINST *K. PNEUMONIA* IN LEAF OF *T. TOMENTOSA*

From the above results, different leaf extracts of *T. tomentosa* revealed the presence of alkaloids, flavonoids, phenols, steroids, saponins, tannins, liginins and glycosides. The phytoconstituents of *T. tomentosa*, such as alkaloids, flavonoids, phenols, lignins, and tannins, are the vital factors for the efficient action of the species of tomentosa. Some findings have also observed that flavonoids, glycosides, saponins, phenols and tannins showed positive results in aqueous and methanolic extracts of leaves of *T. tomentosa* [15]. Studies on ethanolic extract of *T. catappa* contain phenolics and flavonoids.

Ethanolic leaf extract of *T. catappa* significantly inhibit the migration of cell and invasion capacities of squamous cell carcinoma (SCC4) cells [1]. Camelina leaves showed highest alkaloids with methanol extract followed by ethanol [11]. Ethanol and acetone extracts were the most effective in inhibiting the growth of the microorganisms compared to methanol and water extracts in leaf and fruit extracts of *T. ferdinandiana* [13]. From the methanolic extracts of crude powder of leaf of *G. latifolia*, *T. tomentosa* and *L. parviflora* showed the presence of flavonoids, phenols, saponins, tannins and glycosides [9].

Alkaloids, an important class of secondary metabolites having numerous physiological effects on humans and other animals, for plants, they work as a defense line against various pathogens and herbivorous [8]. These are heterocyclic nitrogen compounds that can interact with the bacterial cyto-plasmic membrane, intercalate with DNA and inhibit efflux pumps [4, 5]. The antimicrobial action of alkaloids could be through intercalation with cell wall and DNA constituents. Flavonoids are eminently liable for the color and aroma of the plant. These are of a particular interest, owing to their preventive role counter to several human ailments, stemming from their wide spectrum of biological activities, including anti-inflammatory, anti-oxidant, anti-viral, anticancer and neuro-protective activities. Plant phenols are a group of natural products, and a major component of any plant organism with variable structure acts as a strong defense mechanism against pathogens and plays crucial role in growth mechanisms of plant, such as pigmentation, reproduction, *etc.*, inside the plant and also well known for their beneficial effects on health possess significant antimicrobial and antioxidant activities. Different crude extracts having polyphenol contents such as *T. bellerica*, *T. cattappa*, *T. myriocarpa* and other Terminalia species [16].

In genus Terminalia, tannins content in most of the species was more than 1% with the highest value (>12%) [3]. Tannins are polyphenols, in addition to its use in the leather processing industry, it has shown potential antiviral, antibacterial, and anti-parasitic effects. It inhibits bacterial growth by binding to bacterial enzymes and interfering with phos-phorylation, and sometimes forming complexes with transition metal ions, which are essential for bacterial growth.

It is reported that the presence of saponins in plants are responsible for the toxic and stimulating activities observed in Chinese and Japanese medicinal herbs. The heightened consumer demand for natural products makes steroids, triterpenoids, and saponins promising compounds for industrial applications.

Glycoside compounds, containing carbohydrate and non-carbohydrate residue (moiety) in the same molecule stimulates cardiac muscle contractions and is used therapeutically.

Lignins are the most important secondary metabolite and second most biopolymer. It has components in the global carbon cycle; the resistance of lignin to microbial degradation enhances its persistence in soils. In addition, lignin itself can also be used as a resource for the field of energy or the pharmaceutical industry.

Phytochemicals act on multihued biochemical targets of the bacterial cell and are usually responsible for the antibacterial properties of the plant [19].

The antimicrobial activity of bioactive constituents from plants is due to their ability to reach a site of action in the microbial cell death which is Electron Transport Chain (ETC) and ATPases, embedded in the plasma membrane and mitochondria [7]. Terminalia species possess a number of antibacterial compounds [14]. The antibacterial activity of leaf of ethanol extracts showed better activity towards gram-negative bacteria than gram-positive.

The zone of inhibition in methanolic extract of *Terminalia catappa* showed maximum (20 mm) antibacterial activity against *S. aureus* and minimum (11 mm) against *E. coli* when compared with *Anacardium occidentale* and both extracts were able to inhibit six bacterial strains [10]. The aqueous and methanolic extracts of the leaves of *Terminalia catappa* show different degrees of activity against *S. aureus*, *E. coli* and *K. pneumoniae* [6]. The methanolic extract is significantly more efficient than the aqueous extract in inhibiting the microbial strains [6]. In *Terminalia chebula* Retz. methanol (11-17 mm) extract showed that growth of bacteria inhibited at 200 µl concentration and also similar results were obtained with ethanol extract [18]. The study of traditional human uses of plants is recognized as an effective way to discover future medicines. Medicinal plants mean for therapeutic agents in conjunction with essential raw materials used in the manufacture of traditional and modern medicine [17].

Terminalia tomentosa is a customary traditional medicine is being used by folk tribals and rurals since antient times. Research finding stated that the leaves are useful for fast wound healing, to treat liver problems, to promote sexual health, to relieve headache, stomach ache, as purgative and also applied in sprain to ease swelling and pain [9].

CONCLUSION: The present study was carried out to screen the phytoconstituents and to determine the antibacterial activity of the leaves of *T. tomentosa*. Crude extracts of this species have been revealed the presence of eight bioactive constituents and antibacterial activity, which is proved to be effective against *E. coli*, *S. aureus* and followed by *K. pneumoniae*. From the above results, the leaf extract is worthy of further studies to discover the unrevealed part of it and to prove its efficacy as an alternate for antibacterial infections.

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