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Phytochemical Screening and Antimicrobial Activity of Leaf Extract of *Wrightia tomentosa*

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Abstract

Phytochemical screening and antimicrobial activities of solvent extracts of leaf of Wrighitia tomentosa (Roxb.) Roem and Schult. have been studied for the presence of secondary metabolites and to find out their activity against microbes. The results revealed that the aqueous, chloroform, ethyl acetate, petroleum ether and methanolic leaf extracts of W. tomentosa contain alkaloids, ellagic acids, iridoids, methylene dioxy compounds, steroids, tannins and triterpenoids. The antimicrobial activity of these leaf extracts were tested against gram positive and gram negative bacteria through well diffusion method. Among all the extracts of leaf the highest sensitivity was recorded for methanolic, ethyl acetate and chloroform extracts. None of the aqueous extract exhibited any antimicrobial activity. The results provided evidence that the species W. tomentosa can be used as a potential source of antimicrobial agent.

Keywords: Phyotochemical screening, antimicrobial activity, leaf extract, zone of inhibition.

Introduction

Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind[1]. Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment inhibiting bacterial or fungal growth[2,3]. About 30% of drugs used worldwide are based on natural products[4]. Emergence of multiple drug resistant strains of microorganisms due to indiscriminate use of antibiotics to treat infectious diseases has generated a renewed interest in herbal medicine. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectre of untreatable bacterial infections and adds urgency to the search for new infection combating strategies and new effective therapeutic agents[5].

The medicinally important secondary metabolites produced by plants offer a new source of novel drugs that can act against many diseases and microbes. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants[6]. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic resistant strains[7].

One among such plants possessing a number of biologically active compounds is *Wrightia tomentosa* (Roxb.) Roem and Schult. (Apocynaceae) an endangered medicinal tree with many medicinal uses. *W. tomentosa* is widely used to treat stomach ache, tooth ache, fever, hemorrhage, arthritis and snake bite[8]. The purpose of the present study was to investigate the presence of phytochemicals and antimicrobial properties of *W. tomentosa.* In this research article we report the results of antimicrobial activity of leaf extracts on bacterial pathogens in order to orient future investigations towards the finding of new, potent and safe antimicrobial compounds. The antimicrobial activities of plant extract can be determined by various methods such as disc diffusion, agar well diffusion and twofold serial dilution techniques[9]. The agar well diffusion technique for screening of the antimicrobial activity of medicinal plant is normally considered[10].

Our present investigation envisages the collection, identification, extraction and antimicrobial evaluation of *W. tomentosa.*

Material and Methods

Collection and Preparation of plant material: Fresh leaves of *W. tomentosa* were collected from Kakatiya Arboretum, Department of Forest Research and Development, Warangal (AP), India. The collected plant material was washed thoroughly under running tap water followed by distilled water to remove surface contaminants and was shade dried at room temperature for two weeks. The dried plant material was ground into fine powder using an electric blender and stored in air tight containers at ambient pressure until further use.

Extraction of the plant material: The powdered plant material (250g) was placed in Soxhlet apparatus. Then materials were extracted with different solvents such as petroleum ether,

chloroform, ethyl acetate methanol (MeOH) and water at 72 cycles (8 h per day for 9 days) and when the solvent was drained colourless, the extraction process was stopped. The extracts were filtered using Whatman No.1 filter paper and then concentrated in vaccum at 40ºC using a rotatory evaporator. A semisolid mass is obtained for analytical study.

Phytochemical screening: The phytochemical tests were performed to find out the presence of biologically active chemical constituents such as alkaloids, ellagic acid, iridoids, lignans, methylene dioxy compounds, steroids, tannins and triterpenoids by following the procedures given below.

Alkaloids: For testing the presence of alkaloids, a small quantity of the methanolic extract of the plant was taken into a test tube followed by the addition of 1% HCl and tested with Dragendorff's and also with Mayer's reagents. When a precipitate forms or when the solution turns turbid, the reaction was considered as positive for the presence of alkaloids.

Ellagic acid: For the detection of ellagic acid, the methanolic extract was treated with a few drops of each of 5% acetic acid and 5% sodium nitrate solution. The extract turns yellow olive brown, niger brown or deep chocolate, depending upon the amount of ellagic acid present.

Iridoids: 1ml of Trion-Hill reagent was added to the concentrated methanolic leaf extract. It was then heated for a few minutes. The presence of iridoids was inferred when the solution turned to blue-green or red.

Lignans: Using Badouni test, the presence or absence of lignans was determined. In this process, 2% furfuraldehyde was added to 2ml of methanolic extract when this was acidified with HCl, the development of red colour was conceived as positive reaction for the presence of lignans.

Methylene-dioxy compounds: Gallic acid was added to the methanolic extract of leaf acidified with few drops of concentrated H_2SO_4. The development of blue green colour was the indication for the presence of methylene dioxy compounds.

Steroids: Salkowski test was used to detect the presence or absence of steroids. Conc.H_2SO_4 and chloroform were added to the methanolic leaf extract in a test tube. When the wine red colour developed, the reaction was considered as positive and the presence of steroids was inferred.

Tannins: The presence of tannins was detected by adding 1% potassium dichromate solution to equal volume of concentrated methanolic extract of leaf, the development of generous precipitate is considered to be positive for the presence of tannins.

Triterpenoids: Libermann-Burchard[11,12] test was adopted to know the presence of triterpenoids and steroids. Conc.H_2SO_4 was added to the mixture of methanolic leaf extract and acetic anhydride. Development of bluegreen colour was the indicative for the presence of triterpenoids.

Antimicrobial activity: The antimicrobial activity was carried out by agar well diffusion method. For this, the different solvent extracts of leaf of *W. tomentosa* have been tested against gram positive and gram negative bacteria to find out the antibacterial activity. A nutrient broth was prepared (beef extract-3gm/L; Peptone-5gm/L; NaCl-5gm/L; Agar-16gm/L) and then sterilized in an autoclave at 15lb pressure for 15 minutes. The sterilized medium was poured into petridishes. The solidified plates were bored with 6mm diameter sterilized cork borer. The plates with wells were used for the antibacterial studies.

Various solvent extracts of leaf of *W. tomentosa* were subjected to individual microbiological tests to ascertain their antimicrobial activity against six species of microorganisms, *Bacillus cereus, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae and Salmonella typhimurium* for their antimicrobial activity. The evaluation of antimicrobial activity of the extracts was done by observing the clear zone around disks and measuring the diameter of the zone of inhibition (ZI) exhibited by the extracts around disks in mm by sliding digital vernier caliper.

Well diffusion method: Antibacterial activity of various solvent extracts of leaf of *W. tomentosa* was tested using agar well diffusion method[13,14]. The bacterial strains (*Streptococcus* sp.*, B.cereus, A. hydrophila V. cholerae* and MRSA33) were inoculated in the nutrient broth under aspectic condition and incubated at 37ºC for 18 hours for antimicrobial analysis of Root extracts of *Pseudarthria viscida* Wight and Arn and *Desmodium gangeticum* (Linn) DC[15]. Wells were made on the agar surface with 6 mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37ºC±2ºC for 24 hours for bacterial activity. The plates were observed for the zone clearance around the wells. Gentamycin was also used as standard antibiotics. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

Results and Discussion

The phytochemical constituents and the percentage yield of extracts prepared from leaf extracts of *W. tometosa* are shown in tables 1-2. The methanolic leaf extract screened for the presence of various biologically active constituents were responded positive for the alkaloids, ellagic acid, iridioids, lignans, methylene dioxy compounds, steroids, tannins and triterpenoids. Zaki *et al*[16] have also reported to possess unsaturated sterols, terpenes, flavonoids, phenolic acids and alkaloids. Similarly phytochemical constituents were also observed from different solvent extracts of leaf and bark extracts of *W. tometosa*[17].

During the present investigations the highest yield was found in petroleum ether, methanol and aqueous extracts of leaf followed

by ethylacetate and chloroform (table-2). The difference in the yield of extraction products may be dependent on the polarity difference of solvents used for extraction, solubility of various ingredients, and type of extraction method used[18].

The antimicrobial potential of leaf extracts was tested against three gram positive and three gram negative bacterial pathogens viz., *B. cereus, S. aureus, B. subtilitis* and *E. coli, K. pneumonia, S. typhimurium* respectively (table-3). The ZI was highest in chloroform extracts against *E. coli*, followed by *S. typhimurium, B. subtilitis, S. aureus, K. pneumoniae* and *B. cereus*. Ethyl acetate extract showed highest ZI against *S. aureus*, followed by *B. subtilitis*, *E. coli, S. typhimurium, S. aureus* and *B. subtilis*, aqueous extract has recorded lowest ZI against *S. aureus* followed by *K. pneumonia*, and *S. typhimurium* and showed zero against *B. cereus, B. subtilis, E. coli.* The gram positive bacteria were more sensitive to methanol (*S. aureus*) and chloroform (*B. subtilitis*) extracts. The higher sensitivity of gram-positive bacteria could be attributed to their outer peptidoglycan layer which is not an effective permeability barrier[19]. *Cinnamomum zeylanicum* essential oil is mainly composed in cinnamaldehyde, cinnamyl acetate and cinnamyl benzoate. This composition can justify the significant antimicrobial activities observed[20]. The secondary metabolites detected in different solvent extracts could be responsible for the observed antimicrobial activity. These metabolites have been shown to be responsible for various therapeutic activities of medicinal plants[21].

Conclusion

In conclusion, the present investigation of phytochemical screening and antimicrobial activity of leaf extracts of *W. tomentosa* reports that the various solvent extracts of leaf contains biologically active secondary metabolites which showed the antimicrobial activity against bacterial pathogens of both gram positive and gram negative. Thus it can be employed as an antibacterial agent against many bacterial diseases. Further investigations on other medicinal activities are in progress.

Table-1
Showing various secondary metabolites present in the methanolic leaf extract of *W.tomentosa*

Leaf extract	Alkaloids	Ellagic acid	Iridoids	Lignans	Methylene dioxy compounds	Steroids	Tannins	Triterpenoids
W.tomentoa	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Table-2
Showing the percentage yield of different solvent extracts of leaf of *W.tomentosa*

Plant extract	Weight of raw material (gm)	Weight of the extract (gm)	Percentage of yield
Petroleum ether	250	20	8.0
Methanol	250	20	8.0
Ethyl acetate	250	18	7.2
Chloroform	250	15	6.0
Aqueous	250	20	8.0

Table-3
Data showing antibacterial activity of leaf extracts of *W.tomentosa* by agar well diffusion method

Name of the solvent	Test microorganism and zone of inhibition (in mm) +ve Bacteria			Test microorganism and zone of inhibition (in mm) –ve Bactria		
Leaf Extract	***Bacillus cereus***	***Staphylococcus aureus***	***Bacillus subtilis***	***Escherichia coli***	***Klebsiella pneumoniae***	***Salmonella typhimurium***
Chloroform	8	9	11	15	9	13
Pet-ether	4	2	3	4	9	7
Ethyl Acetate	3	8	12	10	3	10
Methanol	5	14	9	9	13	10
aqueous	-	3	-	-	2	2
Gentamycin (Control)	18	18	19	20	20	20

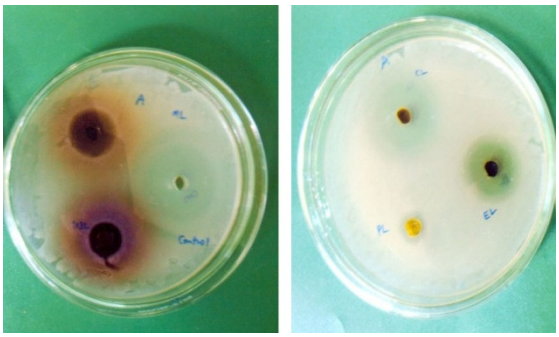

Figure-1
Showing the zone of inhibition of *Bacillus cereus* against various solvent extracts of leaf of *W. tomentosa*

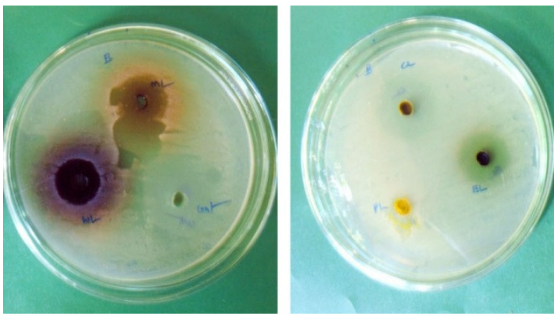

Figure-2
Showing the zone of inhibition of *Staphylococcus aureus* against various solvent extracts of leaf of *W. tomentosa*

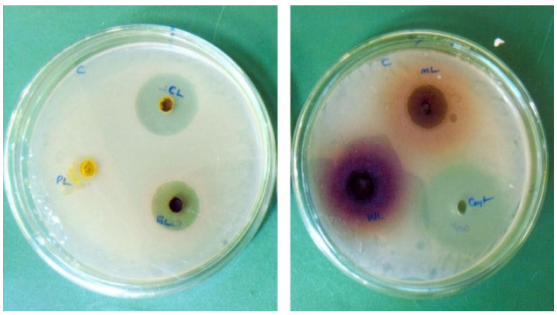

Figure-3
Showing the zone of inhibition of *Bacillus subtilis* against various solvent extracts of leaf of *W. tomentosa*

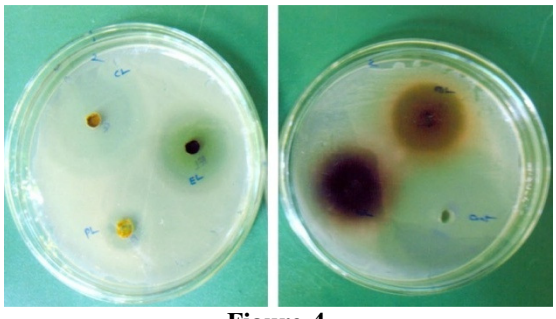

Figure-4
Showing the zone of inhibition of *Escherichia coli* against various solvent extracts of leaf of *W. tomentosa*

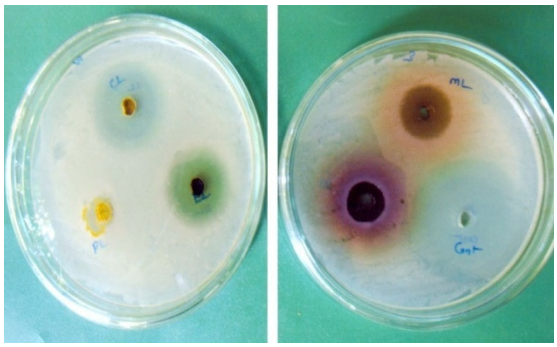

Figure-5
Showing the zone of inhibition of *Klebsiella pneumoniae* against various solvent extracts of leaf of *W. tomentosa*

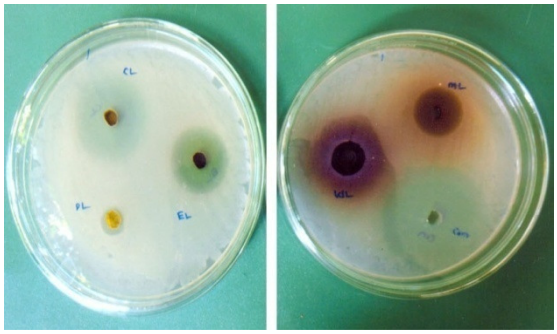

Figure-6
Showing the zone of inhibition of *Salmonella typhimurium* against various solvent extracts of leaf of *W. tomentosa*

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References

1. Kaewseejan N., Puangpronpitag D. and Nakornriab M., Evaluation of phytochemical composition and antibacterial property of *Gynura procumbens* extract, *Asian J. of Plant Sciences,* **11(2)**, 77-82 **(2012)**
2. Chopra R.N., Nayer S.L. and Chopra I.C., Glossary of Indian Medicinal Plants, 3rd Edn. New Delhi, *Council of Scientific and Industrial Research,* 7-246 **(1992)**
3. Bruneton J., Pharmacognosy, Phytochemistry, Medicinal plants, France: Lavoisiler Publishing Co., 265-380 **(1995)**
4. Subhash J.B. and Vaishana K., Comparison of three plant tissue culture media for efficient micropropagation of an important tropical medicinal plant, *Gynura procumbans* (Lour) Merr., *American-Eurasian J. Agricult. Environ. Sci.,* **8,** 474-481 **(2010)**
5. Parekh J. and Chanda S., *In vitro* Antimicrobial activity of some Indian folklore medicinal plants, *Journal of Cell and Tissue research,* **6(1)**, 577 **(2006)**

6. Duraipandiyan V., Ayyanar M. and Ignacimuthu S., Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamilnadu, India. BMC Complimentary and alternative medicine, 635 **(2006)**

7. Kordali S., Kotan R., Mavi A., Cakir A., Ala A. and Yildrim A., Determinatiobn of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus*, and of the antifungal and antibacterial activities of turkis *Artemisia absintthium, A.dracunculus, Artemisia sanctonicum* and *Artemisia spicigera* essential oils, *J Agric food chem*., **53,** 9452-9458 **(2005)**

8. Srinivas P., Shyamsundarachary R., Samatha T. and Ramaswamy N., Plant regeneration from immature zygotic embryo culture of *Wrightia tomentosa* – an endangered medicinally important forest tree species, *J. of Theor. And Exp. Biol.,* **8 (3and 4),** 105-111 **(2012)**

9. Khan Z.K., *In vitro* and *in vivo* screening techniques for antimicrobial and antifungal activity of medicinal plants, In: International workshop on Medicinal plants, their Biodiversity, screening and Evaluation. Lucknow, India: CDRI **(1997)**

10. Pepeljnjak S., Stanic G. and Potocki P., Antimicrobial activity of the ethanolic extract of *Satureja montana* spp *Montana, Acta Pharmaceutica*, **19**, 65-69 **(1999)**

11. Liebermann N.C., Uber das Oxychinoterpen. *Ber*. **18**, 1803 **(1885)**

12. Burchard H., Beitraegezur Kenntnis des Cholesterins, *Chem. Zentralbl*., **61**, 25 **(1890)**

13. Bauer A.W., Kirby W.M., Sherris J.C. and Truck M., Antibiotic susceptibility testing by standardized single disc method, *Am. J. Clin. Pathol.,* **44,** 493-496 **(1996)**

14. Desta B., Ethiopian traditional herbal drugs part II. Antimicrobial activity of 63 medicinal plants, *J. Ethnopharmacol*., **100,** 168-175 **(2005)**

15. Illath Sujina and Subban Ravi, *In vitro* antimicrobial and cytotoxic avtivity of methanolic extract of *Osbeckia wynaadensis*, *ISCA Journal of Biological Sciences*, **1(4)**, 33-38 **(2012)**

16. Zaki A.Y., El-Tohamy S.F., Abd El-Fattah S.M., *Egypt.J.Pharm.Sci.,* **22**, 105 **(1981)**

17. Nagarajan K., Majumder A. and Ghosh L.K., Comparative antimicrobial evaluation studies of the extracts and isolates of leaves and bark of *Wrightia tomentosa, Ancient Science of Life,* **26(1and2),** 1-7 **(2006)**

18. Mahour K., Mishra A., Kumar A. and Vihan V.S., Preliminary pharmacognostical and phytochemical investigation on *Feronia elephantum* corr. Fruit. *J. Pharm. Res.,* **1**, 45-48 **(2008)**

19. Hawaze S., Deti H. and Suleman S., *In vitro* antimicrobial activity and phytochemical screening of Clematis Species Indigenous to Ethiopia, *Indian Journal of Pharmaceutical Sciences,* **74(1),** 29-35 **(2012)**

20. Yehouenou B., Sessou P., Houinsou R. de L., Noudogbessi J.P., Alitonou G.A., Toukourou F. and Sohounhloue D., Chemical composition and Antimicrobial activities of *Cinnamomum zeylanicum* Blume dry Leaves essential oil against Food-borne Pathogens and Adulterated Microorganisms, *I. Res. J. Biological Sci.,* **1(6),** 18-25 **(2012)**

21. Tijjani M.B., Bello I.A., Aliyu T., Olurishe S.M., Habila J.D. and Balogun E.O., Phytochemical and antibacterial studies of root extract of *Cochlospermum tinctorium* A. rich. (Cochlospermaceae), *Res. J. Med. Plant*, **3**, 16-22 (**2009**)