UPI Journal of Pharmaceutical Medical, and Health Sciences
Content Available at www.uniquepubinternational.com ISSN: **2581-4532**

Open Access **Research Article**

Evaluation of antimicrobial activity of hydro-alcoholic extract of *thunbergia fragrans*

Rupesh Kumar M, Aishwarya B.M*, Hrishikesh Balu, Ranjith S, Chandan K, Bharathi D.R, Syed Sagheer Ahmed
Department of Pharmacology, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B.G. Nagara, Karnataka

Article History

Received: 16-06-2021
Revised: 28-06-2021
Accepted: 25-07-2022

Keywords

Robodoc, Robota, Programmable Universal machine, prostatectomy surgery, Knee arthroplasty.

***Corresponding Author**
Aishwarya B.M

DOI: https://doi.org/10.37022/jpmhs.v5i3.79

Abstract

Hydroalcoholic extract of *Thunbergia fragrans* was investigated for phytochemical as well as Anti-microbial screening. Phytochemical evaluation of the plant extract revealed the presence of Flavonoids, Phenols, Tannins, and other constituents. The extract was prepared by maceration technique, the solvent was used in ratio of 70:30 [ethanol: water]. The obtained hydro alcoholic extract was tested against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus.* Whereas the extract showed no significant antimicrobial effects even though *Thunbergia Fragrans* is believed to be used to treat open wounds in Indian siddha medicine, further studies are required to find out the full potential of the plant.

Introduction

Microbiology is emerging as the key biological science. The models for molecular biology study are provided by microorganisms. This research at the molecular level has provided, and continues to provide, the answers to numerous fundamental questions in genetics, metabolism, and cell forms and functions. The interactions between species in mixed populations can also be studied using model systems provided by microorganisms. Three interrelated aspects, including the microbe, host resistance, and therapy, affect how an infection develops. The most crucial of them is the interaction between the host and the pathogenic microorganisms, or more specifically, the equilibrium between the pathogens' virulence and the host's resistance to the pathogens. The role of antimicrobial agents, although often decisive, is mainly to shift the balance in favor of the host, giving the host time to metabolize its resistance mechanisms. Some bacterial species are inherently resistant to some antibiotic classes, either because they lack the required receptor or because the antibiotic cannot pass through their cell wall. Bacteria can develop resistance in a number of ways. The most typical method of resistance is when bacteria develop an enzyme that breaks down antibiotics. An important factor in the spread of resistance is the transfer of genetic material from one microorganism to another, even from a non-pathogen to a pathogen. There is widespread antibiotic resistance among harmful microorganisms [1].

Antibiotics resistant in the bacteria spread at three levels:

I. By transfer of bacteria between people.
II. By bacteria exchanging resistance genes (usually on plasmids).
III Using transposons, resistance genes may be transferred across genetic components of bacteria.

Transposons is possible for some stretched DNA to be moved (transposed) from one plasmid to another as well as from plasmids to chromosomes or the other way around.This is because integration of these segments of DNA, which are called transposons.

Between 1938 and 1952, a period of 15 years, the yearly death rate from infectious illnesses decreased by an average of 8.2 percent per year, which was the largest rate

of drop in infectious disease mortality in the USA. Pneumonia, influenza, and TB were the infectious illnesses that were mostly responsible for this drastic reduction. These declines coincided with the introduction of sulphonamides in 1935, penicillin in 1941, and streptomycin in 1943 into clinical practice. In addition to streptomycin, several other combination drugs, including para-amino salicylic acid in 1944 and isoniazid in 1952, were also introduced for the treatment of tuberculosis. Antimicrobials are crucial for the management of infectious disorders, as this association amply demonstrates. Recent data also demonstrates our success in combating infectious illnesses [2].

Antimicrobial drugs have greatest contribution to therapeutics. They are one of the few treatments available. Antibiotics are chemicals created by microbes that, at extremely low concentrations, stop the development of or actually kill other germs.If an antibiotic works against both Gram-positive and Gram-negative bacteria, it is considered to have a limited range of action. Antimicrobial drugs can be classified in many ways according to their chemical structure, mechanism of action, types of organisms, spectrum of activity, type of action, source of origin. Antibiotics are bactericidal or bacteriostatic. Bacteriostatic antibiotics prevent bacterial cells from growing and multiplying but do not really kill the pathogens. Instead, they allow the host to destroy the germs. Bactericidal antibiotics kill and sometimes lyse the cells. They are administered in accordance with their type and dosage concentration. Bacteriostatic medications include Tetracycline, Chloramphenicol, Clindamycin, Sulphonamides, Trimethoprim, and Macrolides, whereas bactericidal medications include Penicillin, Cephalosporin, Cephamycin, Aminoglycosides, Glycopeptides, Polymyxin, Bacitracin, Monobactams, and Carbapenems.

Since the dawn of human civilization, the plant world has been the finest natural supply of pharmaceuticals and medicines.In the preparation of antibiotic, many pharmaceutical industries process and utilize plants and plant parts as raw material to produce plant derived drugs. But most of the information of plant use as medicinal purposes is seeded in the rural people and indigenous community. Although Bangladesh is a tiny nation, it is abundant in natural medicine.More than five thousand vascular plant species are present throughout the forests, hills, plains, crop fields, marshy lands and home gardens, 750 species, the most of which having some degree of antibacterial qualities, have reportedly been employed in traditional medicine for the country's millions of residents80 percent of people worldwide rely on herbal remedies for their basic medical requirements, according to the WHO.[3]

Herbal medicines may be employed as a source of direct therapeutic agents [4], as well as an alternative method for finding a replacement for currently used medications and validating the safety and efficacy of conventional and traditional medicinal plants [5].

Plants have an almost infinite potential to produce aromatic compounds, most of which are secondary metabolites, of which at least In all, 12,000 have been found, which is less than 10% of the total. These substances often serve as chemicals that defend plants from herbivores, insects, and microorganisms. Additionally, some of which could be implicated in flavor and smell. The world's pharmaceutical market is dominated by natural products and derivatives, especially antibiotics, which account for over half of all pharmaceuticals in use. Higher plants make up around a quarter of the total. Consequently, medicinal plants are thought to represent a significant source of novel chemical compounds with therapeutic potential [6].

Traditional medicines rely significantly on plants, and humans relied greatly on the healing effects of medicinal plants long before chemical medications were invented. Some people regard these plants as valuable because they were created with the intention of providing man with food, medicinal treatment, and other benefits [7]. Herbal remedies Both emerging and industrialized nations engage in considerable research, and in recent years, the need for plant-based medications has increased [8].

Natural medicine Both emerging and developed nations engage in substantial research, and in recent years, there has been an increase in the demand for medications made from plants.

Antimicrobial drugs used for therapeutic purposes have virtually always been accompanied by the emergence of antimicrobial resistance. Shigellae and other gram-negative bacilli quickly developed sulfonamide resistance after the first therapeutic use of sulfonamides in the late 1930s and early 1940s. Likewise, almost all types of *Staphylococcus aureus* were sensitive when penicillin was first used in medicine. However, significant issues started to emerge in many important medical facilities in less than ten years. These issues arose as a result of *S. aureus* acquiring b-lacta-mase genes, making it penicillin resistant.

Gram-negative bacilli that were resistant to penicillin and cephalosporins as well as broad-spectrum antibiotics

(such as tetracyclines, chloramphenicol, and aminoglycosides) gained significant notoriety in the decades that followed. Serratia marcescens, Enterobacter cloacae, and multidrug-resistant strains of nonfermenters like Pseudomonas aeruginosa are only a few examples of the multidrug-resistant Enterobacteriaceae that have posed significant issues, especially for very ill patients in intensive care units [9].

To treat infections brought on by Gram-positive bacteria including Staphylococcus aureus, Streptococcus pneumoniae, and Streptococcus pyogenes, macrolides are among the most often prescribed families of clinically significant antibiotics.

The widespread proliferation of resistance strains has been a natural result of the massive usage of these medicines. Macrolides can induce the expression of several of the resistance-determining factors. Erm methyltransferases, which precisely methylate a specific nucleotide inside the macrolide binding region, are of special interest. The ribosome stall inside the translated regulatory open reading frame before to the Erm cistron is a necessary component of the Erm induction process, which is also thought to be strongly connected to the general mode of macrolide action on protein synthesis. However, the specifics of the Erm induction process remain unknown [10].

The bacterium Escherichia coli is amazing and diversified. This generally benign commensal simply needs to accumulate a set of mobile genetic components to transform into a highly adapted pathogen that may infect the urinary tract, circulation, and central nervous system in addition to causing gastroenteritis. These illnesses have a staggering global toll, affecting hundreds of millions of people each year [11].

People in rural areas of Asia, Africa, and Latin America frequently use herbal medicine, a chemically defined component of a herbal mixture, for basic health care [12]. Herbal medicines may be employed as a source of direct therapeutic agents [13], as well as an alternative method for finding a replacement for currently used medications and validating the safety and efficacy of conventional and traditional medicinal plants [14].

The genus *Thunbergia*, which contains over 100 species of annuals, perennials, and shrubs, includes the species *Thunbergia fragrans roxb*. This versatile genus has several twining climbers as well as some shrubby varieties. The majority of the plants in this genus are decorative, but some also have therapeutic uses [15 16].

A blooming plant with flavonoids, tannins, and phenols is *T. fragrans.* Those phytoconstituents are crucial for the neuroprotective effect [17]. *Thunbergia grandiflora*, a member of the same genus, has neuroprotective properties. An old-world tropical genus called *Thunbergia* has a variety of decorative and therapeutic purposes. According to reports, the plants of the *Thunbergia* genus include a number of intriguing iridoid glycosides, Researchers have discovered a subclass of iridoid (mono terpenoid) compounds from the plants *T. alata, T. grandiflora, T. fragrans, T. mysorensis*, and *T. laurifolia* that include a glycoside moiety, which is typically found at the C-1 position [18].

In Indian Siddha Medicine *T. fragrans* is used as antipyretic agent, the tender twigs of the plant were made to paste and applied all over the body against fever, the leaf juice of *T. fragrans* poured in the nose and the paste of the leaf was used to treat giddiness, fresh root juice was used as eye drops, leaf paste was used on open wounds, and sometimes the leaf paste was applied on forehead to treat headache [19].

Geographical distribution [20]

It is native to Andaman Islands and Asian continent.

Plant description [21]

Taxonomy.

PLANT PROFILE

Kingdom – Plantae
Sub kingdom – Tracheobionta
Super division – spermatophyta
Division – Magnoliophyta
Class – Magnoliosida
Sub class – Asteridae
Order – Scrophulariales
Family – Acanthaceae
Genus – Thunbergia
Species – fragrans

Synonym: *Thunbergia volubilis Pers*, *Flemingia grandiflora Roxb*. ex Rottler.

Common name: White lady, white thunbergia, sweet clock-vine, white clock-vine.

Fig :1. Leaves of *Thunbergia fragrans*

Chemical constituents

T. fragrans Roxb, is known for its wide range of medicinal properties, including its chemical constituents of Palmitic acid, Cis-9-Hexadecenal, and Campesterol that possess anticancer activity [22]. It also includes flavonoids, tannins and other common constituents that are present in plants, but *Thunbergia* species contain iridoid glycosides that has unexplored medicinal properties [23].

Methodology

Plant material

The plant was collected *Thunbergia fragrans* Plants from B. G Nagara, Mandya district. Plant leaves was washed, shade dried, subjected for size reduction into coarse powder and stored in airtight container at room temperature for further usage. The plant leaf was authenticated by S. Noorunnisa Begum, Curator, FRLHT.

Fig. 2 Fresh *Thunbergia fragrans* leaves

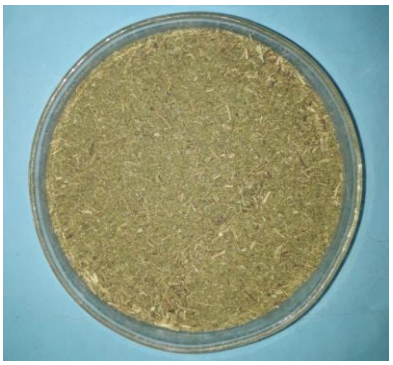

Fig. 3 *Thunbergia fragrans* powder

Preparation of plant extract

The coarsely powdered leaves of *Thunbergia fragrans* was be extracted by maceration using hydro alcohol (70:30). The extract was filtered via a cotton plug, whatman filter paper (no. 1), and rotary evaporator before being concentrated.

Fig. 4. Filtration **Fig.5. Rotary evaporation**

Qualitative chemical tests for crude extracts

Wet chemical tests are one method for determining the chemical composition, precise identification, polarity, etc. of the components in the crude extract. Because a colour reaction or precipitate is an outcome of a particular chemical, typically a class of compounds. These tests can be helpful for researching chemical compounds and assessing how well an extraction procedure is working. Chemical qualitative analysis was performed on the petroleum ether, ether, ethyl acetate, methanol, ethanol, and aqueous extracts. The extracts were subjected to a number of assays, with the results recorded, for substances such as steroids, terpenoids, flavones, anthraquinones, sugars, glycosides, alkaloids, quinones, phenols, tannins, and saponins.

1.Test for steroids

Liebermann Burchard test:

A few drops of concentrated sulphuric acid were put around the edges of the test tube after dissolving 1 mg of the test material in a few drops of chloroform, 3 ml of acetic anhydride, 3 ml of glacial acetic acid, and 3 ml of acetic anhydride were added, warmed and cooled under the faucet. When two layers meet, a reddish-brown ring forms, and the top layer turns blue green, which indicates the presence of steroids.

2. Test for terpenoids

Noller's test:

The substance was warmed with tin and thionyl chloride. A pink coloration indicates the presence of triterpenoids.

3. Test for flavones

a. Shinoda's test: To the substance, a few magnesium turnings and a few drops of concentrated HCL were added and boiled for five minutes. A red coloration indicates the presence of flavones.

b. To the substance in alcohol, 10 percent NOAH solution or ammonia was added. A dark yellow color indicates the presence of flavones.

4. Test for anthraquinones:

Borntrager's test: The substance was macerated with ether and after filtration; aqueous ammonia or caustic soda was added. A pink, red or violet colour in the aqueous layer indicates the presence of anthraquinones.

5. Test for glycosides:

The substance was mixed with a little anthrone on a watch glass. One drop of concentrated sulphuric acid was added, made into a paste and warmed gently over a water bath. A dark green coloration indicates the presence of glycosides.

6. Test for sugars:

The substance was mixed with Fehling's I and II solutions. A red coloration indicates the presence of sugars.

7. Test for alkaloids:

a. To the test substance, a few drops of acetic acid and Dragendorff's reagent were added and shaken well. An orange red precipitate indicates the presence of alkaloids.

b. The substance was mixed with little amount of dilute hydrochloric acid and Mayer's reagent. A white or cream precipitate indicates the presence of alkaloids.

8. Test for quinones:

To the test substance, sodium hydroxide was added. A blue green or red colour indicates the presence of quinones.

9. Test for phenols:

To the substance, a few drops of alcohol and ferric chloride solution were added. A bluish green or red color indicates the presence of phenol.

10. Test for tannins:

The substance was mixed with basic lead acetate solution. A white precipitate indicates the presence of tannins.

11. Test for saponins:

The substance was shaken with water. A copious lather formation indicates the presence of saponins.

12. Test for proteins and free amino acids:

a. The substance was treated with sulphosalicylic acid solution. A white precipitate indicates the presence of proteins.

b. The substance was mixed with Millon's reagent and heated on a water bath. A red precipitate indicated the presence of proteins.

Screening of Antimicrobial activity

Agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. The agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 mL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested [24].

Results

Phytochemical constituents present in extracts of *Thunbergia fragrans*leaves.

Details of qualitative phytochemical tests:

SL.No.	Chemical test	Hydroalcoholic extract
1.	Terpenoids	Absent
2.	Flavonoids	Present
3.	Steroids	Absent
4.	Anthraquinone	Absent
5.	Glycosides	Absent
6.	Sugars	Present
7.	Alkaloids	Absent
8.	Quinones	Absent
9.	Phenols	Present
10.	Tannins	Present
11.	Saponins	Absent
12.	Proteins & free amino acids	Present

The present investigation involves hydroalcoholic extraction of *TunbergiaFragrans* commonly found in this area. The hydroalcoholic extracts were used to determine their antibacterial activity using pathogenic Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli as test bacteria. The results obtained are presented as follows:

Fig 6. Standard against against Pseudomonas aurogenosa

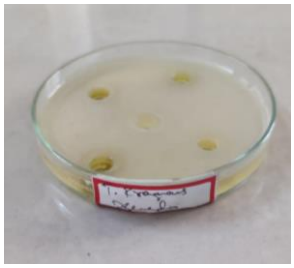

Fig 7*T.Fragrans* extract against Pseudomonas aurogenosa

Fig 8. Standard against E Coli

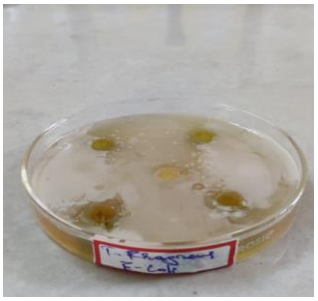

Fig 9.*T. Fragrans* extract against E Coli

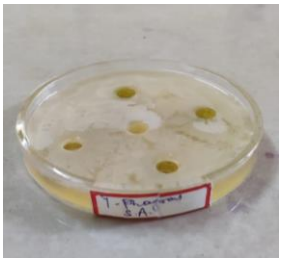

Fig 10. Standard againstStaphylococcus aureus

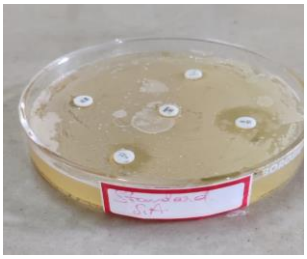

Fig 11.*T.Fragrans* extract against Staphylococus aureus

Discussion

The present investigation was carried out to determine the antimicrobial activity of *Tunbergia Fragrans* commonly found in rural parts of India. These plants components and derivatives are known to be used in the treatment of open wounds, by traditional practitioners. Microorganisms are important component of health-related illnesses or goodness as they affected the skin and many internal organs in men and all animals. It is known that normal micro biota synthesizes many nutrients essential for health.For the determination of antimicrobial activity of plants *Tunbergia Fragrans* hydroalcoholic extracts with different concentration were attempted using established procedures.

Extraction of plant phytochemicals

Water soluble polysaccharides, polypeptide, including fabatine and lectins of plant origin act as an inhibitor of microbial parthogens (Zhang and Lewis 1997). The majority of the plant components that have been found to be effective against infections are aromatic or saturated organic compounds. They are more efficiently extracted using organic solvents such as methanol ethanol etc. In the present investigation ethanol and water were used in ratio of 70:30 as solvents for hydroalcoholic extraction of phytochemicals.

Antimicrobial activity of *T. Fragrans* leaf extract:

T. Fragrans popularly known as Indrapushpa is considered to have very important strong wound healing activity. In the present study, its hydroalcoholic extracts did not possess significant antimicrobial activity against E. coli, Staphylococcus aureus and Pseudomonas aeruginosa.

Conclusion

The present study was aimed to determine the antimicrobial effect of *T. Fragrans.* The *T. Fragrans* was extracted using Maceration process. The antimicrobial activity was performed by Agar well diffusion method against Staphylococcus aureus, E. coli and Pseudomonas aeruginosa. The obtained results were not significant hence, further study is needed to evaluate pharmacological potential of *T. Fragrans* leaf extract.

Reference

1. Awasthi, A. K.; Bisht, G. S.; Anroop B.; Invitro antimicrobial activity of Swertia chirata. Indian Journal of Natural Products. PP. 27 – 28.
2. Aminov R. History of antimicrobial drug discovery: Major classes and health impact. Biochemical pharmacology. 2017 Jun 1;133:4-19.
3. Pasha and Uddin, 2013, Yusuf et al., 1994, 2009; Ghani, 1998, Balandrin et al., 1985,WHO, IUCN & WWF, 1993.
4. European Medicines Agency (2011) Guideline on quality of herbal medicinal products/traditional herbal medicinal products: EMA/ CPMP/QWP/2819/00 Rev.2. pp113http://www.ema.europa.eu/docs/en_GB/ document_library/Scientific_guideline/2011/09/ WC500113209.pdf.
5. Roy VK, Chenkual L, Gurusubramanian G (2015) Protection of testis through antioxidant action of Mallotusroxburghianus in alloxan induced diabetic rat model. J Ethnopharmacol, 176: 268–280.
6. Ertuk, O., Kati H., Yayli, N. and Demirbag, Z.Antimicrobial properties of Silene mutifida (Adams) Rohrb. Plant extract. Turk J. Biol. 2006, 30(1):17-21.
7. OM FR, Kumar R, Tamizh Mani T. A REVIEW OF HEPATOPROTECTIVE NATURAL PRODUCTS.
8. Ahmed SS, Prakash KC, Tabassum S, Salma N, Devi KH. Pharmacognostical and Pharmacological Review on Tridax procumbens Linn. Research Journal of Pharmacology and Pharmacodynamics. 2019;11(1):11-6.
9. Aminov R. History of antimicrobial drug discovery: Major classes and health impact. Biochemical pharmacology. 2017 Jun 1;133:4-19.]
10. Gaynor M, Mankin AS. Macrolide antibiotics: binding site, mechanism of action, resistance. Current topics in medicinal chemistry. 2003 May 1;3(9):949-60.
11. İşcan G, Kirîmer N, Kürkcüoğlu M, Başer HC, Demirci F. Antimicrobial screening of Mentha

piperita essential oils. Journal of agricultural and food chemistry. 2002 Jul 3;50(14):3943-6.

12. Croxen MA, Finlay BB. Molecular mechanisms of Escherichia coli pathogenicity. Nature Reviews Microbiology. 2010 Jan;8(1):26-38.
13. European Medicines Agency (2011) Guideline on quality of herbal medicinal products/traditional herbal medicinal products: EMA/ CPMP/QWP/2819/00 Rev.2. pp113http://www.ema.europa.eu/docs/en_GB/ document_library/Scientific_guideline/2011/09/ WC500113209.pdf.
14. World Health Organization (2013) WHO traditional medicine strategy 2014–2023. World Health Organization, WHO Press, Geneva, Switzerland.
15. Lakshmi S. Pharmacognostic, Phytochemical and Pharmacological Evaluation of *Thunbergia fragrans* Roxb. (*Acanthaceae*) (Doctoral dissertation, College of Pharmacy, Madurai Medical College, Madurai).
16. Sultana K, Chatterjee S, Roy A, Chandra I. An Overview on Ethnopharmacological and Phytochemical properties of *Thunbergia sp*. Med Aromat Plants. 2015;4(217):2167-0412.
17. Gutierrez-Merino C, Lopez-Sanchez C, Lagoa R, K Samhan-Arias A, Bueno C, Garcia-Martinez V. Neuroprotective actions of flavonoids. Current medicinal chemistry. 2011 Mar 1;18(8):1195-212.
18. Charles A, Ramani VA. Chemosystematics of genus *Thunbergia* (a-mini review). International Journal of Scientific Research and Modern Education-IJSRME. 2016; 25:5-11.
19. Quattrocchi, Umberto (3 May 2012). CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology (5 Volume Set).ISBN 9781420080445.
20. POWO (2022). "Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://www.plantsoftheworldonline.org/ Retrieved 27 January 2022.
21. Chandrasekaran C, K R. Molecular docking studies of potential anticancer agents from Thunbergia Fragrans against Colorectal cancer mutant genes through in silico study. IJAM [Internet]. 2021 Dec. 31 [cited 2022 Feb. 15];12(4):792-5.
22. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis. 2016 Apr 1;6(2):71-9.