

**ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL PROFILING
OF ROOTS OF *TODDALIA ASIATICA* (L.) LAM.****Mutyala Naidu L^{*1}, Satyasri B¹, Nookanna Dora SVVS², Krishna Rao M³ &
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ABSTRACT:

Antibiotic toxicity and multi drug resistant pathogens are the two greatest challenges being faced by today's medical world. These days, there is a greater search for plant extracts with strong antimicrobial properties. The aim of the present study is an attempt to explore the antimicrobial activity of hexane, chloroform, methanol, ethanol and aqueous extracts of roots of *Toddalia asiatica* by agar well diffusion method. The results observed that methanol extract exhibited promising result against fungal species *Saccharomyces cerevisiae*, *Aspergillus niger* and *Candida albicans* while *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris* were found to be effective only at higher concentrations, whereas *Klebsiella pneumoniae* and *Streptomyces griseus* were resistant. From the MIC values, it was observed that methanol extract showed high value against *Saccharomyces cerevisiae*, which was 500 µg/ml, while ethanol extract showed MIC values of 250 µg/ml against *Candida albicans*. Preliminary phytochemical screening revealed the presence of carbohydrates, cardiacglycosides, terpenoids, alkaloids, flavonoids, phenols, tannins, amino acids, quinines and saponins. The findings showed that

these spices have a significant concentration of secondary metabolites, which give them strong antimicrobial properties and make them useful for both therapeutic and biopreservation purposes.

KEYWORDS: *Toddalia asiatica*, Phytochemicals, Extracts, Biological activity, Microorganisms.

1. INTRODUCTION

A medical plant is any species in the kingdom of plants whose parts—flowers, leaves, roots, stems, fruits, or seeds—are used either directly or in a preparation to treat a disease or ailment. Understanding how medicinal plants can help cure illnesses is a great way to protect the biological and cultural variety of many ethnic groups. Antimicrobial, anticancer, antiviral, antioxidant and enzyme inhibitory, anti-aging, anti-inflammatory, antihypertensive, neuroprotective, and anticoagulant effects are just a few of the many advantageous qualities associated with medicinal plants (Ali et al., 2019; Lesellier et al., 2021). Throughout the world, medicinal plants are extremely valuable, both on their own and in conjunction with conventional medicine. Some diseases and conditions, including diabetes, sweating and bleeding, menstrual cycle regulation, and the reduction of severe bleeding, stomach pain, inflammation, and toothache, are treated with medicinal plants as traditional herbal remedies (Lari et al., 2020; Shafaghat, 2009). PEOs have been extracted from specific medical plants, and medicinal plants have recently emerged as significant raw materials for essential oil extraction (Jugreet and Mahomoodally, 2020; Zhang and Yao, 2019). The increased interest in medicinal plant cures is because, primarily plants as medicines are safe, less rigorous and more affordable than synthetic drugs. Thus, there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Owk & Lagudu, 2018).

In South Asia and Africa, *Toddalia asiatica* has a long history that dates back to traditional tribal medicine. A "biting vine" that promotes digestion and relieves respiratory congestion is mentioned in passing in early Ayurvedic manuscripts from the first millennium CE. Although it's fascinating to note that Linnaeus only added the genus name in 1753 during the early botanical investigations, Ayurvedic compendia such as the Charaka Samhita classified *Toddalia* under stimulating herbs (Deepana) during the medieval age. Indian hill tribes in Karnataka made root decoctions to ease monthly discomfort and stomach cramps, while coastal communities in Tanzania and Kenya valued the juice of crushed leaves for topical

treatments of arthritis and skin infections. Its reputation changed over time. British botanists noted *T. asiatica* strong scent and bitter taste in their medicinal manuals during the 1800s colonial botanical era. Missionary physicians in Africa experimented with leaf extracts to treat malaria fevers by the early 20th century; this practice is now mostly out of use. The herb is still used by traditional healers to treat rheumatic pain in Tamil Nadu and toothaches in some parts of Ethiopia. Reputable ethnobotanical surveys from the 1980s onward highlight its function as an expectorant and digestive aid, despite some claims of mystical "anti-venom" qualities. The traditional legacy of this thorny vine, which is never to be undervalued and offers a spicy taste of Ayurvedic wisdom, has endured despite narrowing usage patterns. Its efficacy on diseases proved the future usefulness of different species of *T. asiatica*. Therefore, the aim of the present study is an attempt to explore the antimicrobial activity of hexane, chloroform, methanol and aqueous extracts of roots of *T. asiatica* by agar well diffusion method.

2. MATERIAL AND METHODS

2.1. Collection, identification and extraction

Toddalia asiatica roots were gathered from the Kambalakonda woodland area in Visakhapatnam, Andhra Pradesh, India. Prof. M. Venkaiah of the Department of Botany at Andhra University in Visakhapatnam, India, identified the collected plant. The stem bark sample was collected and allowed to dry completely in the shadows. It was then packed in a Soxhlet device after being ground into a powder in a mixture grinder. Hexane, chloroform, and methanol were used in a sequential extraction process (Aniel Kumar et al., 2015). A rotary evaporator was used to remove the solvents at reduced pressure at 40 °C in order to concentrate the filtrates. After being labeled, the concentrated crude extracts were kept at 4 °C. Simultaneously, the aqueous extract of the stem bark was prepared by adding boiled water to the powdered in a beaker on water bath, with occasional stirring for 4 hr. The aqueous extract was then filtered and reduced under pressure.

2.2. Microorganisms used

The Institute of Microbial Technology (IMTECH) in Chandigarh, India, provided the strains listed below. *Bacillus subtilis* MTCC B2274, *Enterococcus faecalis* MTCC B3159, *Escherichia coli* MTCC B1560, *Klebsiella pneumoniae* MTCC B4030, *Micrococcus luteus* MTCC B1538, *Pseudomonas aeruginosa* MTCC B2297, *Proteus vulgaris* MTCC B7299, *Staphylococcus aureus* MTCC B3160, *Streptococcus pneumoniae* MTCC B2672, and three

fungal strains—*Aspergillus niger* MTCC F4325, *Candida albicans* MTCC F7315, and *Saccharomyces cerevisiae* MTCC F2567.

2.3. Antimicrobial screening

After sub culturing the lyophilized culture, the working stock culture concentration was determined to be 10^{-6} CFU/ml. A predetermined amount of nutrient agar was made and aseptically plated. The antimicrobial susceptibility test for crude extracts and DMSO (negative control) was conducted using the agar well diffusion technique, whereas the antimicrobial susceptibility test for standard antibiotic discs was conducted using the agar disc diffusion method. The known concentrations of 50 mg/ml, 75 mg/ml, and 100 mg/ml were obtained by dissolving the extracts in DMSO. Tetracycline discs (10 µg/disc) were used to compare the activity. An antibiotic zone reading scale (HiAntibiotic ZoneScale-c) was used to measure and tabulate the zone of inhibition following a 24-hour incubation period at 37 °C. For the antifungal activity, the same method as for bacteria was adopted of nutrient agar, Saboraud dextrose agar was used. The inoculated medium was incubated at 25 °C for two days for the *C. albicans*, *S. cerevisiae* and three days for *A. niger* (Kumar et al., 2014). About 500 µg of nystatin was dissolved in 1 ml of sterile de ionized water. About 10 µl of 0.5 mg/ml nystatin (equivalent to 5 µg dose).

The minimum inhibition concentration (MIC) experiment was performed on the extracts that showed inhibitory zones using serial two-fold dilution (Kumar et al., 2010). An initial concentration of 2000 µg/ml was obtained by dissolving 0.6 g of each extract in 300 ml of sterile nutrient broth. The stock was then serially diluted twice to achieve concentrations of 1000, 500, 250, 125, 62.5, 31.2, and 15.6 µg/ml. After adding one milliliter of standardized inoculums of each test organism to each extract nutrient broth mixture, the mixture was incubated at 37 degrees Celsius. The extracts' minimum inhibitory concentration (MIC) was defined as the lowest concentration that prevented growth. For two (*C. albicans*, *S. cerevisiae*) to three (*A. niger*) days, the infected media was incubated at 25 °C for the fungi.

2.4. Statistical analysis

Each experimental data from triplicates was subjected to one way ANOVA using Minitab version 15. A significant level of $p < 0.01$ was used for all statistical analyses.

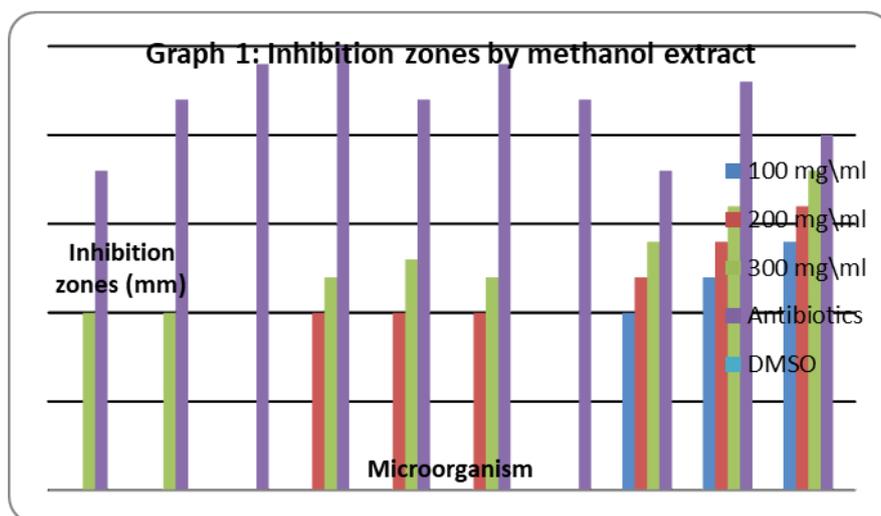
3. RESULTS AND DISCUSSION

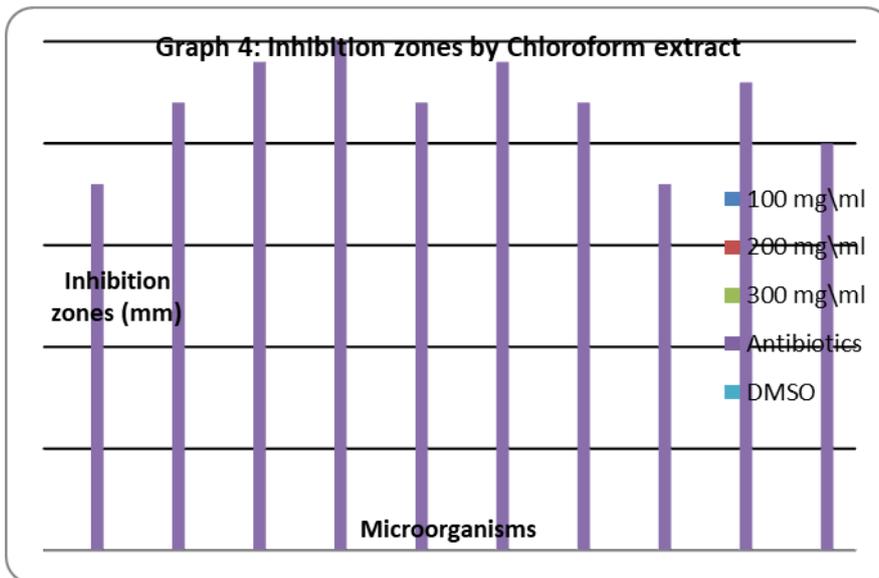
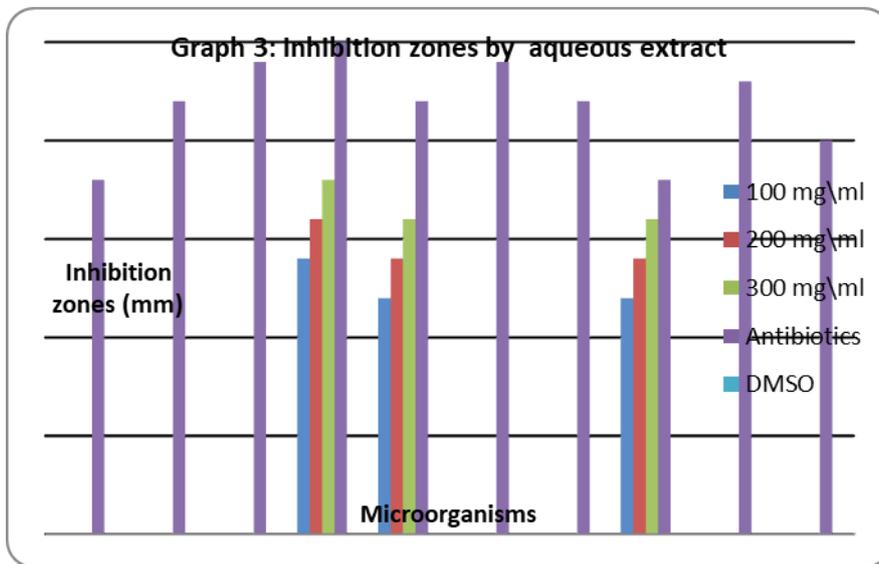
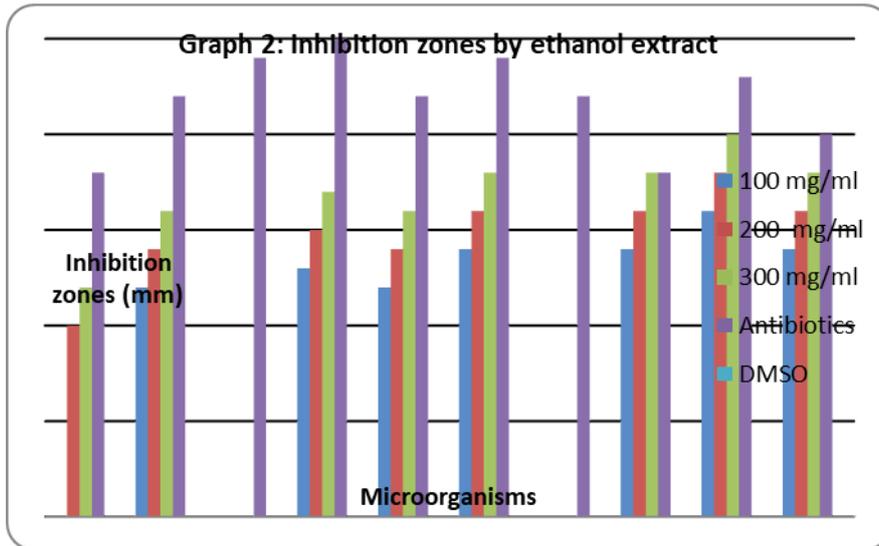
The different solvent extracts obtained from the roots of *T. asiatica* exhibited moderate antimicrobial activity. Among these, methanol, ethanol and aqueous extracts exhibited

antimicrobial activity on some microorganisms. The chloroform and hexane extracts were inactive against all tested microorganisms (Graph 1-5). From the zone of inhibition, it was observed that methanol extract exhibited promising result against fungal species *Saccharomyces cerevisiae*, *Aspergillus niger* and *Candida albicans* while *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris* were found to be effective only at higher concentrations, whereas *Klebsiella pneumoniae* and *Streptomyces griseus* were resistant. Ethanol extract was active against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* *Staphylococcus aureus*, *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae* while *Klebsiella pneumoniae* and *Streptomyces griseus* did not show any inhibition zones. Aqueous extracts showed activity mainly against *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Aspergillus niger*.

The type of solvent employed in the extraction process has a significant impact on the subsequent isolation of biological components from plant material. Although water is the main solvent used by traditional healers and practitioners, the current study found that methanol extracts of these plants were unquestionably superior and more potent than other extracts. This could be because the active ingredients are more soluble in the organic solvent. De Boer et al (2005) similarly observed comparable findings.

From the MIC values, it was observed that methanol extract showed high value against *Saccharomyces cerevisiae*, which was 500 µg/ml, while ethanol extract showed MIC values of 250 µg/ml against *Candida albicans*. Aqueous extract showed high MIC value of 500 µg/ml against *Pseudomonas aeruginosa*. (Table 1). Narrow spectrum of antimicrobial activity was exhibited against all tested microorganisms except ethanol extract against *Aspergillus niger*.





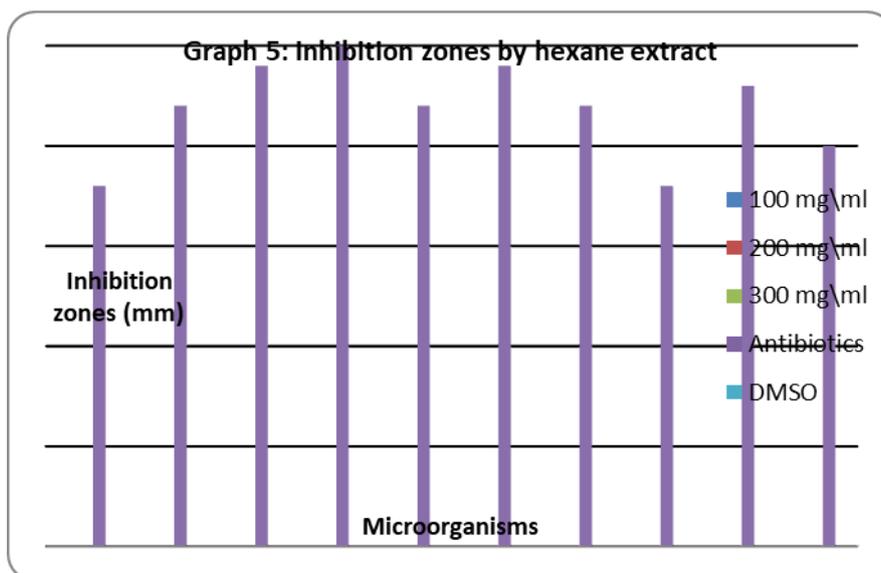


Table 1: MIC values of roots of *T. asiatica*.

Microorganisms	Methanol (µg/ml)	Ethanol (µg/ml)	Water (µg/ml)	Chloroform (µg/ml)	Hexane (µg/ml)
<i>B. subtilis</i>	>1000	>1000	–	–	–
<i>E. coli</i>	>1000	1000	–	–	–
<i>K. pneumoniae</i>	–	–	–	–	–
<i>P. aureginosa</i>	>1000	500	500	–	–
<i>P. vulgaris</i>	>1000	1000	1000	–	–
<i>S. aureus</i>	>1000	500	–	–	–
<i>S. greseus</i>	–	–	–	–	–
<i>A. niger</i>	1000	500	1000	–	–
<i>C. albicans</i>	1000	250	–	–	–
<i>S. cerevisiae</i>	500	500	–	–	–

The minimum inhibitory concentration (MIC) of an antibiotic that, following an overnight incubation period, will prevent a microbe from growing visibly is known as the MIC. It depends on numerous parameters, and comparing MIC values from one author to another is challenging due to non-standardized methodologies. It is helpful, therefore, as a useful indicator of primary action against a particular pathogenic microbe. In this investigation, the minimum inhibitory concentration was employed to quantitatively evaluate the in vitro antibacterial properties of crude solvent extracts made from *T. asiatica* root, which are used in traditional medicine to treat a variety of illnesses. Every extract that was tested showed a different level of inhibitory activity on common strains of fungus and Gram-positive and Gram-negative human pathogenic bacteria. Gram positive and Gram negative bacteria have MIC values ranging from 62.5 to 1000 µg/ml, while fungi have MIC values ranging from 250 to 1000 µg/ml. The antibacterial activity of plant extracts is not categorized using any

particular cutoff values as a reference or standard. Crude extracts were classified as having good activity in this study if their MIC value was less than 500 µg/ml, and as having considerable activity of pharmacological interest if their MIC value was less than 100 µg/ml (Owk & Lagudu 2016). Since little amounts of the extracts that are below their toxicity limit can be applied without causing negative effects, a lower MIC value suggested that the compound was very effective as an antibacterial agent *E. hirta* also observed similar findings (Kader et al., 2013).

When tested against pathogenic microbes, some of the extracts showed no inhibitory zones. Negative results, however, do not indicate that the plant is inert or that there are no bioactive components. The crude extracts may include an active chemical or compounds in inadequate amounts to demonstrate activity at the dose levels used. Taylor et al. (2001) also noted similar findings in traditional medicinal herbs. Therefore, only with high dosages can lack of activity be demonstrated. Mutyala & Krishna came to similar conclusions (2025). Alternatively, other components may have antagonistic effects or counteract the beneficial effects of the bioactive molecules if the active principle is present in sufficient amounts. Some of the chemical components present in these plants may have undergone conformational changes as a result of the drying process. Extracts that have no antibacterial action might be effective against other bacterial species that weren't examined in this study.

The preliminary phytochemical screening of *T. asiatica* root revealed the presence of phytoconstituents such as carbohydrates, cardiacglycosides, terpenoids, alkaloids, flavonoids, phenols, tannins, amino acids, quinines and saponins. The micromolecules play an important role in plant species defense against herbivores, pathogenic microorganism, to combat environmental stress, fire, etc. These plant components have been used as drugs for millennia. Therefore, the screening for phytochemicals serves as the initial step in predicting the types of potentially bioactive compounds. (Chew *et al.*, 2004). Alkaloids, saponins, anthraquinones, glycosides, phenolics, terpenoids and flavonoids have been documented in this study. These principles have been known for many years to exhibit biological activity, such as effects on the central nervous system, antimicrobial, antitumor and antihelminthic activity.

Higher plants are known to contain antibacterial compounds (Srinivasan, 2001). Since medications derived from plants have significantly improved human health, plants have served as a source of inspiration for new therapeutic molecules. As with the Unani and

Ayurvedic medical systems, phytomedicine can be utilized to treat illnesses or serve as the foundation for the creation of new medications (Didry et al., 1998). Reports of medicinal plants' antimicrobial qualities are growing from all over the world. According to estimates from the World Health Organization, 80% of the world's population uses plant extracts or their active ingredients in traditional medicines.

4. CONCLUSIONS

The phytochemical profile revealed the therapeutic potential of crude drugs and also supports the folk claims. The findings showed that these spices have a significant concentration of secondary metabolites, which give them strong antimicrobial properties and make them useful for both therapeutic and biopreservation purposes. Further, the purification and characterization of active principles is being carried out in the laboratory to understand the molecular basis of pharmacological property of the crude drugs. The information gathered from this study may be utilized to create natural bioactive substances that enhance human health.

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