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Antibacterial and antifungal activities of *Ruellia tuberosa (l.)* leaf and stem extract

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Abstract

This research work was carried out with an objective to examine the antibacterial and antifungal potentials of leaves of *Ruellia tuberosa*. The purpose of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on selected bacterial and fungal strains. In the present study, the microbial activity of extracts of leaf and stem of *Ruellia tuberosa* was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar disc diffusion method. The antibacterial and antifungal activities of leaf and stem extracts (50, 100, 150 µg/ml) of *Ruellia tuberosa* were tested against two Gram-positive—*Staphylococcus aureus,* Gram-negative—*Escherichia coli* human pathogenic bacteria; and fungal strains *Candida albicans*. Zone of inhibition of extracts were compared with that of standard drug tetracycline for antibacterial activity and amphotericin B for antifungal activity. The results showed that the significant inhibition of the bacterial growth was shown against the tested organisms. Henceforth, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

Keywords: *Staphylococcus aureus, Escherichia coli, Candida albicans*, antibacterial activity and antifungal activity.

1 Introduction

Medicinal plants are due to the presence of bioactive Plants have been a major source of therapeutic agents since time immemorial. The increasing acceptance of traditional herbal systems of medicine, like Ayurveda, within India and outside has resulted in the revival of ancient traditions of medicine. Medicinal plants and their derivatives are thus looked upon not only as a source of affordable healthcare but also as an important commodity item of international trade and commerce. In India, medicinal plant sector has traditionally occupied an important position in the socio-economic, cultural and spiritual area of rural and tribal lives. (Allice Kurian and M. Asha Sankar,2007).

Medicinal plants are abundant source of antimicrobial molecules. A wide range of medicinal plants extracts are used to treat several infections as they have potential antimicrobial activity. Some of these bioactive molecules are screened and traded in market as raw material for many herbal industries (Renisheya *et al.,* 2011). Experts turned their concentration back towards obtaining advantages from medicinal plants after observing more side effects of synthetic drugs compared to their benefits (Bushra et al., 2012). It is estimated that about 35,000 to 70,000 plants species are used as medicinal plants out of 422127 reported worldwide plant species (Bibi *et al.,* 2011).

Ruellia tuberosa (L.) is an erect, sub erect or diffuse perennial herb up to 60-70 cm tall and belongs to the family *Acanthaceae*, a native of Central America introduced into Indian gardens as ornament and widely distributed in South East Asia including Thailand and Laos. It is used medicinally in West Indies, Central America, Guiana and Peru. Ruellia tuberosa (L.) is commonly known as "Cracker plant" (Pandey 2005, Medicinal plants of the Guiana's and Chothani *et al.* 2010). In Siddha system of medicine, leaves are given with liquid copal as remedy for gonorrhea and ear diseases (Suseela and Prema 2007), used in stomach cancer (Reddy et al. 1991). Dried and ground roots in dose of two ounces cause abortion and also used in sore eyes (Kirtikar and Bashu 1935).

The objective of this study was to explore the antibacterial and antifungal activity of *Ruellia tuberosa* (L.) leaf and stem to determine the scientific basis for its use in folk medicine to treat microbial pathogen and other infectious diseases.

2 Experimental Sections

2.1. Collection and plant material

The leaves and stem of Ruellia tuberosa were collected from nearby villages of Tirupattur district, Tamil Nadu and the leaves are washed with water and dried carefully in the absence of sunlight to remove the water molecules present in the leaves. The dried leaves and stem are made into fine powder using blender. Then the fine powders are stored properly in an airtight container for future purpose.

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2.2. Extraction

About 40g of the fine powder of the leaves and stems of *Ruellia tuberosa* are taken in a thimble which is placed in a Soxhlet extractor for the purpose of extraction of phytochemicals present in the leaves. The extraction is carried out using ethanol. The extracts obtained are collected separately and the solvents are evaporated using vacuum distillation and dried. The dried samples are stored in an airtight container for further analysis.

2.3. Antimicrobial activity

The antimicrobial test is an important technique used in pharmacology to study the efficacy and potency of antimicrobial agents from herbal extracts against microorganisms. The pathogenic microorganism chosen for the antimicrobial activities are *Staphylococcus aureus, Escherichia coli* and *Candida albicans.*

2.4. Disc Diffusion Method.

Disc diffusion method was used for antimicrobial activity. A stock solution of extract was prepared by dissolving 0.1 g of extract with 100 mL of their respective solvents (distilled water and absolute ethanol) to produce a final concentration of 100 mg/mL. The stock solution was then diluted to concentrations of, 50,100 and 150 mg/mL of extract. 20 μL of each dilution was impregnated into sterile, blank discs 6 mm in diameter. 5 μL of extract was spotted alternately on both sides of the discs and allowed to dry before the next 5 μL was spotted to ensure precise impregnation. Distilled water and ethanol loaded discs were used as negative controls for aqueous and ethanol extracts, respectively. All discs were fully dried before the application on bacterial lawn. The positive controls used were tetracycline antibiotic discs all strains. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the discs. The assay was repeated trice. Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the leaf extract.

3. Results and Discussion

3.1. Antimicrobial activity

The antimicrobial activity was performed by disc diffusion method. The aqueous and ethanol extract from *Ruellia tuberosa* exhibited significant antimicrobial activity towards all the three microorganisms and the corresponding zone of inhibition values are given in the Table 1,2 and 3.

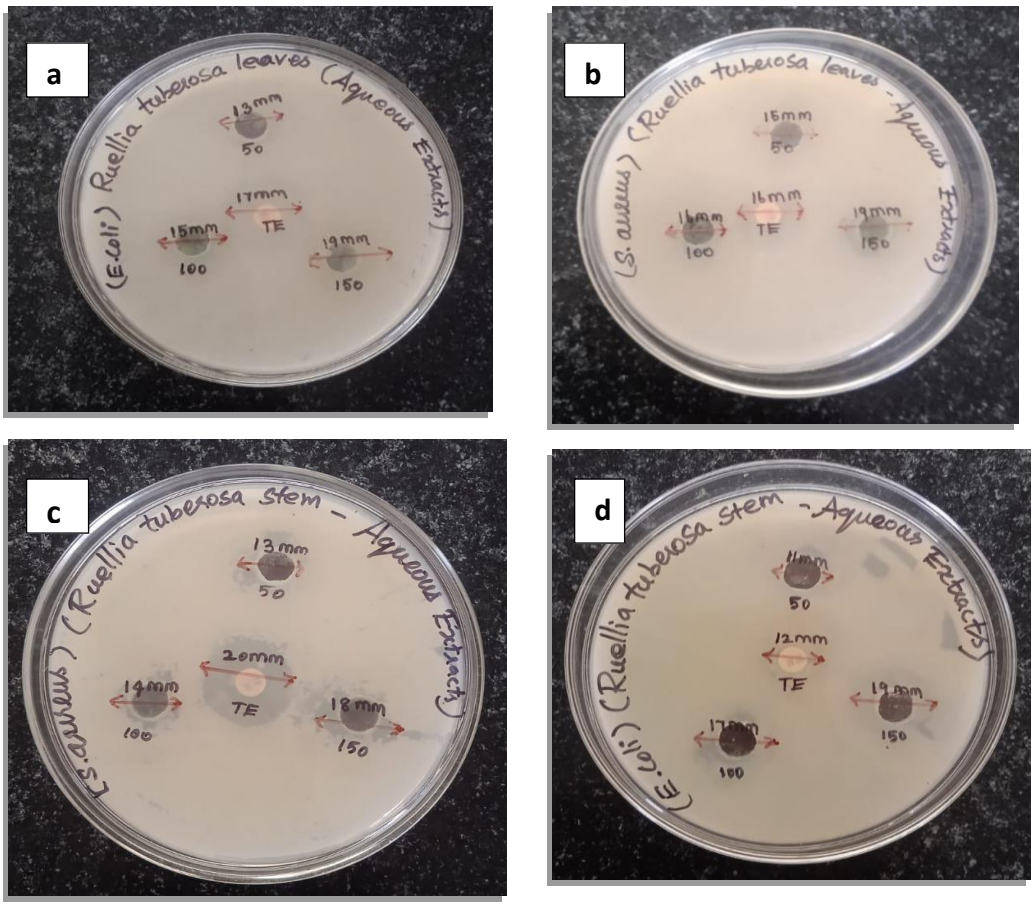

Fig 1. (a,b,c & d) antibacterial activity of *ruellia tuberosa* (50,100,150µg concentration) in leaf and stem aqueous extract with standard drug tetracycline (100 µg) against Escherichia coli and Staphylococcus aureus.

Table: 1 Antibacterial activity of *Ruellia tuberosa* in aqueous extract

S.No.	Name of the Microorganism	ZONE OF INHIBITION IN mm							
		Aqueous Leaf extract				Aqueous Stem extract			
		Standard (100µg)	50	100	150	Standard (100µg)	50	100	150
1	***E- coli***	17	13	15	19	12	11	17	19
2	***Staphylococcus aureus***	16	15	16	19	20	13	14	18

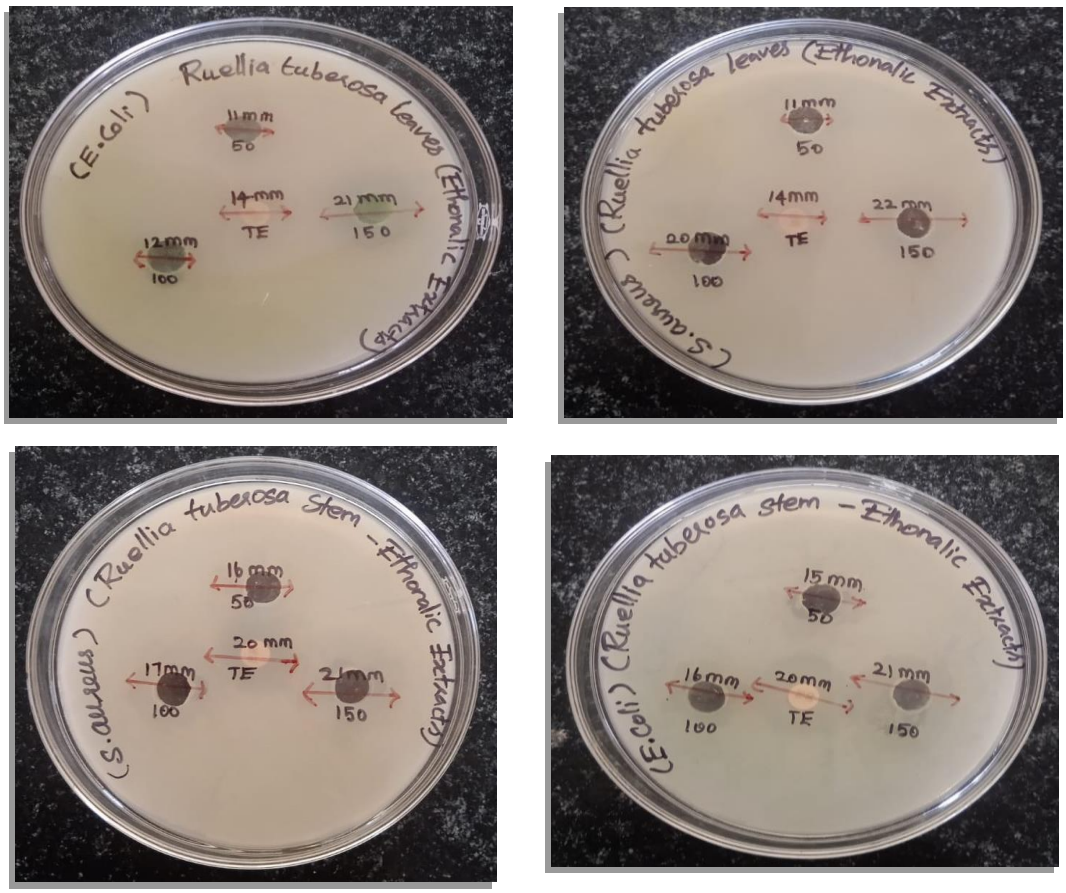

Fig 2. (a,b,c & d) Antibacterial activity of *Ruellia tuberosa* (50,100,150µg concentration) in leaf and stem ethanolic extract with standard drug tetracycline (100 µg) against Escherichia coli and Staphylococcus aureus.

Table: 2 Antibacterial activity of *Ruellia tuberosa* in ethanol extract

S.No	Name of the Microorganism	ZONE OF INHIBITION IN mm							
		Ethanol Leaf extract				Ethanol Stem extract			
		Standard (100µg)	50	100	150	Standard (100µg)	50	100	150
1	*E- coli*	14	11	12	21	20	15	16	21
2	*Staphylococcus aureus*	14	11	20	22	20	16	17	21

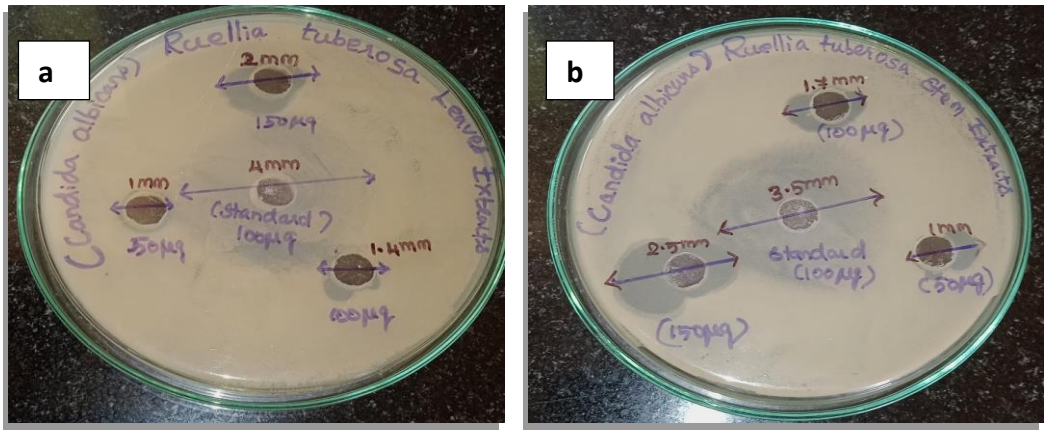

Figure:3 (a&b) Antifungal activity of *Ruellia tuberosa* (50,100,150µg concentration) in leaf and stem ethanolic extract with standard drug amphotericin b (100 µg) against *Candida albicans* .

Table: 3 Antifungal activity of *Ruellia tuberosa* in ethanol extract

S.No.	ZONE OF INHIBITION IN mm							
	Name of the Microorganism	Ethanol Leaf extract				Ethanol Stem extract			
		Standard (100µg)	50	100	150	Standard (100µg)	50	100	150
1	***Candida albicans***	4	2	1	1.4	3.5	1.7	2.5	1.0

Antibacterial activity of *Ruellia tuberosa* (50,100,150µg concentration) in leaf and stem aqueous and ethanolic extract with standard drug tetracycline (100 µg) against escherichia coli and staphylococcus aureus shown in figure 1 and 2. Antifungal activity of *Ruellia tuberosa* (50,100,150µg concentration) in leaf and stem ethanolic extract with standard drug amphotericin b (100 µg) against *candida albicans* figure 3. E- coli was found to be maximum towards ethanol extract with inhibitory zone of 19mm and minimum activity towards ethyl acetate observed with 12mm. Staphylococcus aureus was found to be maximum towards ethanol extract with inhibitory zone of 13mm and also minimum activity towards methanol observed with 10mm. The ethnaol and aqueous extract of *Ruellia tuberosa* leaves and stem show significant antimicrobial activity towards all the three microorganisms.

4. Conclusion

The results of this study exhibited that *Ruellia tuberosa* leaf and stem extracts have the most significant applications against human pathogens, including those that cause enteric infections. The results of various concentrations that the leaves and stem have some measurable inhibitory action against Staphylococcus aureus *Escherichia coli* and *Candida albicans.* The current results will form the basis for selection of plant species for further examination in the potential discovery of new natural bioactive compounds. Additional studies which aimed at the isolation and structure elucidation of antimicrobial active constituents from the plant have been initiated.

Conflict of Interest: Nil

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