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Studies on antimicrobial efficacy of medicinal tuberous shrub *Talinum cuneifolium*

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Abstract

Talinum cuneifolium (Vahl.) Willd. an erect shrub with subterranian tuber (Portulacaceae) is endowed with wide range of pharmacological activities. The antimicrobial efficacy of the plant is evaluated against three bacteria and two fungal species by disc diffusion method. Preliminary phytochemical screening was carried out among hexane, ethylacetate, methanolic extracts of leaf and root tuber for different potent chemicals. The leaf methanolic extract of *T. cuneifolium* showed maximum effect on the growth of *Proteus* (25.8 mm) followed by *Bacillus* (24.62 mm) and *E. coli* (19.42 mm). The tuberous methanolic extract of *T. cuneifolium* showed maximum effect on growth of *Proteus* (28.15 mm) followed by *Bacillus* (26.88 mm) and *E. coli* (24.51 mm). The Gram-positive bacterial strains (*Bacillus)* were more susceptible to the extractions of *T. cuneifolium* as compared to Gram-negative bacteria (*E.coli, Pseudomonas aeruginosa, Proteus vulgaris and Klebsiella pneumoneia)*. The antifungal activity of selected plant leaf and root tubers exhibit pronounced activity against *Candida albicans* rather than *Aspergillus niger*. These studies showed that the methanolic extracts of *T. cuneifolium* plant parts were certainly much better and powerful. This may be due to the better solubility of the action components in organic solvent.

Key words

Antimicrobial efficacy, *Talinum cuneifolium*, Root tuber, Medicinal plant

Introduction

Medicinal plants have a strong linkage with human health. Indian system of Medicine like Ayurveda, Yunani and Sidha depend on medicinal plants for herbal drugs. Many chemicals are currently employed for the control of fungal and bacterial diseases. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Harborne and Baxter, 1995). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important bio active compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga *et al.,* 2005). The antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena, 1999). Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests and identification of natural products in plants with antimicrobial activity represents a potentially useful area for development of chemotherapeutic agents (Suffredini *et al.,* 2004). The phytochemical research based on ethnopharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Kloucek *et al.,* 2005). The identification of natural products in plants with antimicrobial activity represents a potentially useful area for development of chemotherapeutic agents and helps in explaining the use of some plant materials in traditional medicine (Shanmugavadivu *et al.,* 2008)

Talinum cuneifolium (Vahl.) Willd., an erect shrub with subterranean tuber belongs to the family Portulacaceae. It is used as a green leafy vegetable due to its rich Vitamin A and mineral content (Anon, 2004). Five to ten leaves are eaten daily in the morning to control blood sugar level in the diabetic patients (Savithramma, 2003). The tuberous roots are used for cough, gastritis, diarrhoea and pulmonary tuberculosis (Madhavachetty *et al.,* 2008).

In Ethiopia the leaves are applied medicinally against eye diseases and the root against cough and gonorrhoea (Saradvathi *et al*., 2009). Validation is necessary for isolation of active compounds for further use in the preparation of medicine. Hence, the present study is aimed to screen for phytochemicals and antimicrobial activity of leaf and root tuber of *Talinum cuneifolium* extracted in different solvents.

Materials and Methods

Plant material and preparation of extracts: Fresh healthy leaves and root tubers of *T. cuneifolium* collected from the Botanical garden of Botany Department, Sri Venkateswara University, Tirupati, Andhra Pradesh, India, were cleaned under running tap water and cut into small pieces with knife and dried under shade condition. The dried

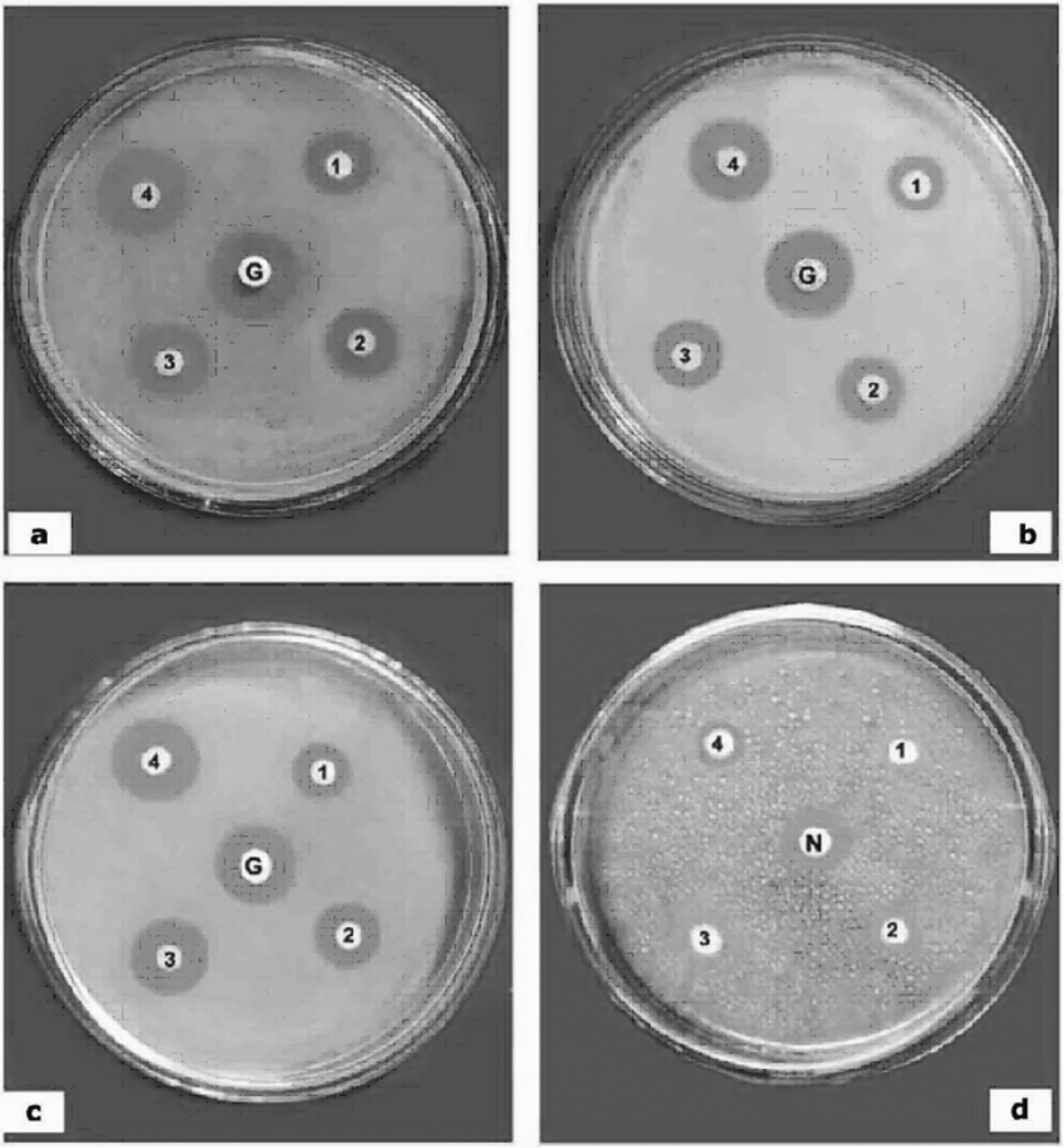

Fig. 1: Antimicrobial activity of *Talinum cuneifolium* leaf (methanolic extract) (a) *Escherichia coli,* (b) *Bacillus subtilis,* (c) *Proteus vulgaris,* (d) *Candida albicans* (1) 500 μg disc^{-1}, (2) 500 μg disc^{-1}, (3) 2000 μg disc^{-1}, (4) 2500 μg disc^{-1} (G- Gentamycin - 10 μg disc^{-1}, for Bacteria/N - "Nystatin - 10 μg disc^{-1} for fungi used as standard

plant materials were ground into fine powder and stored in screw cap bottles until further use. The extraction of the plant material (leaf and root tubers) was prepared according to Harborne (1998). Fine powder (100 g) was taken and soxhlated for 6-8 hr using soxhlet apparatus with solvents, based on the increasing polarity using hexane solvent initially followed by ethyl acetate and methanol to get their soluble parts. The components were separated in to the solvents based on their polarity. The extract was subjected to rotary evaporator at 40°C to remove the excess solvent from the extract. The extracts were dissolved in 10% dimethyl sulfoxide (DMSO) and these solutions were preserved at 4°C until further use.

Phytochemical screening: The successive crude extracts from leaves and tubers of *T. cuneifolium viz.* hexane, ethylacetate and

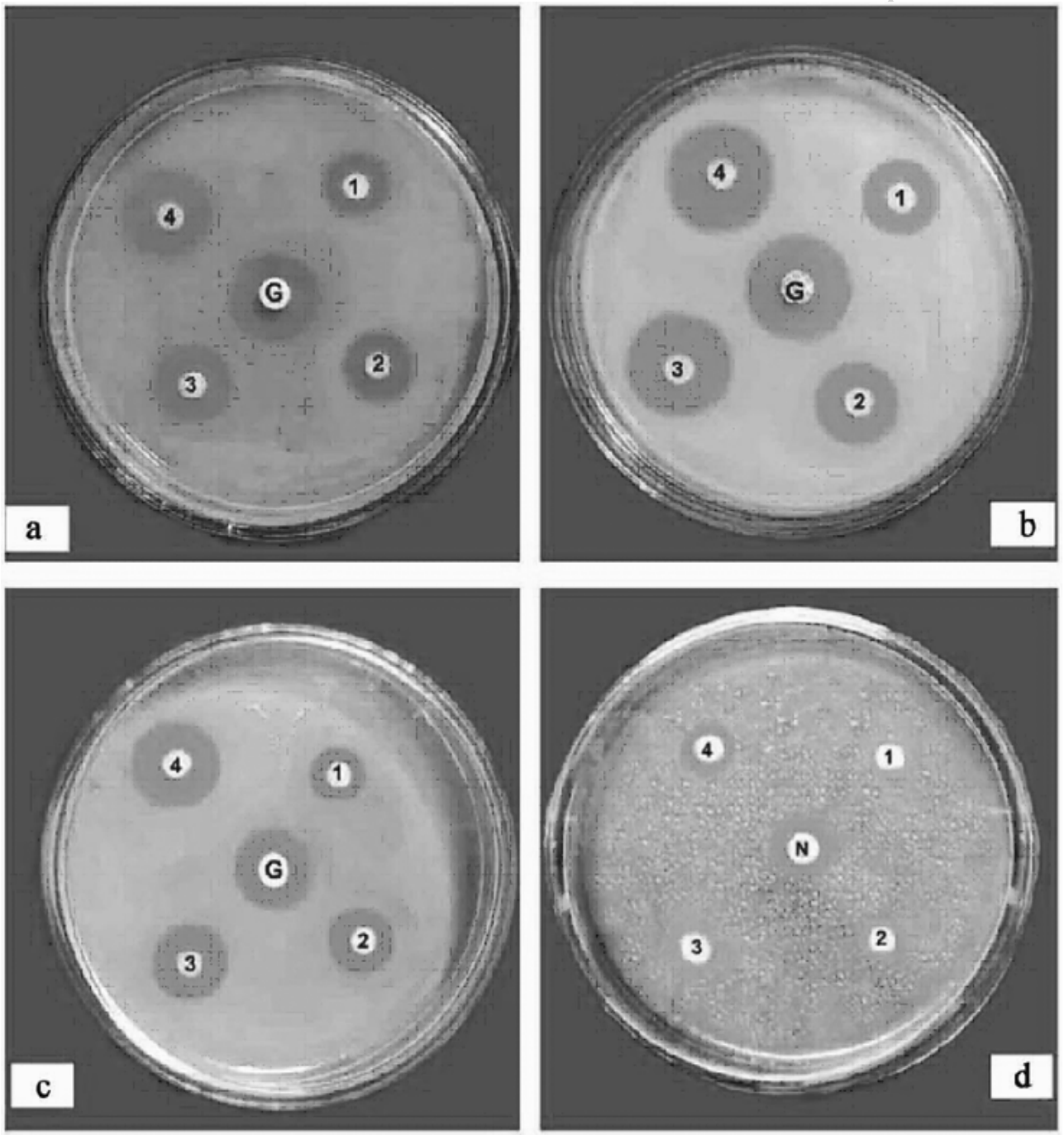

Fig. 2: Antimicrobial activity of *Talinum cuneifolium* tuber (methanolic extract) (a) *Escherichia coli*, (b) *Bacillus subtilis*, (c) *Proteus vulgaris*, (d) *Candida albicans* (1) 500 µg disc^{-1}, (2) 500 µg disc^{-1}, (3) 2000 µg disc^{-1}, (4) 2500 µg disc^{-1} (G- Gentamycin - 10 µg disc^{-1}, for Bacteria/N - "Nystatin - 10 µg disc^{-1} for fungi used as standard

Table 1: Screening of secondary metabolites from tuber and leaf extracts of *Talinum cuneifolium*

Tests for secondary metabolites		*T. cuneifolium* (Tuber)			*T. cuneifolium* (Leaf)		
		H	EA	M	H	EA	M
Flavonoids	$FeCl_3$ test	+	+	+	–	–	+
	Shinoda's test	+	+	+	–	–	+
	Zinc HCl reduction test	+	+	+	–	–	+
	Lead acetate	+	+	+	–	–	+
Glycosides	Kellar kilani test	–	+	+	+	–	+
Saponins		–	–	+	–	–	+
Steroids	Slakowski	–	+	+	+	+	+
	Libermann burchard	–	+	+	+	+	+
Reducing sugars	Benedicts test	–	–	+	–	–	–

+ = Presence, – = Absence, H = Hexane, EA = Ethyl acetate, M = Methanol

Table 2: Antibacterial activity of tuber and leaf methanolic extracts of *Talinum cuneifolium*

Concentration of extract (μg)	Bacterial inhibition zone		
	E. coli	*B. subtilis*	*P. vulgaris*
		(A) Tuber methanolic extract	
500	16.86 ± 0.27	18.11 ± 0.27	16.43 ± 0.19
1000	18.25 ± 0.24	20.08 ± 0.18	19.15 ± 0.36
2000	22.74 ± 0.16	23.07 ± 0.25	23.04 ± 0.28
2500	24.51 ± 0.36	26.88 ± 0.14	28.15 ± 0.19
		(B) Leaf methanolic extract	
500	10.43 ± 0.31	12.5 ± 0.14	12.56 0.35
1000	12.58 ± 0.21	14.76 ± 0.15	15.71 ± 0.18
2000	16.7 ± 0.35	18.28 ± 0.12	18.22 ± 0.26
2500	19.42 ± 0.16	24.62 ± 0.28	25.8 ± 0.29

Values are mean of triplicates ± SE

methanol have been screened for secondary metabolites following the standard procedures adopted by the earlier workers (Harborne, 1998).

Anti microbial activity: The successive methanolic extracts from leaves and tubers of *T. cuneifolium* were screened for antibacterial and antifungal activity by employing paper disc diffusion method (Jairaj *et al.,* 1999). Whatman No.1 filter paper discs of 6 mm diameter were prepared and sterilized in autoclave in a clean air tight and dry petriplate. Stock solutions of desired concentrations (0.5, 1.0, 2.0 and 2.5 mg) were applied to each sterilized filter paper disc with the help of micro pipette. Later the discs were dried on the laminar air flow. These completely dried discs were used for antimicrobial studies. The antimicrobial standards such as Nystatin (10 μg $disc^{-1}$) for fungi and Gentamycin (10 μg $disc^{-1}$) for bacteria were procured from Hi-media and used as standard.

Tested organisms: The microbial cultures of *Escherichia coli, Bacillus subtilis, Proteus vulgaris, Candida albicans* and *Aspergillus niger* were procured from the Department of Microbiology, Sri Venkateswara University and Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati. Nutrient agar media (NA) for bacterial cultures and potato dextrose agar media (PDA) for fungal cultures were used. Inoculum was prepared by transferring a loopful of stock culture to a 250 ml Erlenmeyer flask containing 80 ml of NA broth for bacteria and PDA broth for fungal cultures.

Preparation of petriplates: The nutrient agar media and potato dextrose agar media were sterilized by autoclaving at 121°C for 15 min. The petriplates and pipettes plugged with cotton and were sterilized in an oven at 150°C for 1 hr. About 25 ml of molten agar medium was poured in each sterilized petriplate (diameter 10 cm) under aseptic conditions. About 0.5ml of inoculum broth of different strains of bacteria and fungi were added to the respective petriplates. The contents of petriplates were mixed thoroughly by rotary motion. The medium containing inoculum were allowed to solidify at room temperature. A sterile filter paper disc (6 mm) containing different concentrations of plant extract was placed at the centre of the petriplate. These were incubated at 37°C for 24 hr for bacteria. Fungal growth was examined for 48 hr (Hernandez *et al.,* 1999). The results were expressed in terms of the diameter of the inhibition zone (Saeed and Tariq, 2007).

Results and Discussion

Secondary metabolites from tuber and leaf extracts of *Talinum cuneifolium* are shown in Table 1. The tuber showed maximum

Table 3 : Antifungal activity of tuber and leaf methanolic extracts of *Talinum cuneifolium*

Concentration of extract (μg)	Fungal inhibition zone	
	Candida albicans	*Aspergillus niger*
	(A) Tuber methanolic extract	
500	10.49 ± 0.2	–
1000	14.6 ± 0.25	–
2000	16.1 ± 0.37	–
2500	19.03 ± 0.12	–
	(B) Leaf methanolic extract	
500	8.08 ± 0.24	–
1000	10.49 ± 0.06	–
2000	14.52 ± 0.16	–
2500	16.21 ± 0.45	5.64 ± 0.15

Values are the mean of triplicates ± SE, - = No inhibition

number of compounds in the methanolic extract. Both methanolic and ethyl acetate extracts were rich in flavonoids, steroids and glycosides and differs in saponins and reducing sugars, whereas the hexane extract showed positive results only for flavonoids. The leaf methanolic and hexane extracts were rich in flavonoids steroids and glycosides. The ethyl acetate extract given positive results only for steroids. Alkaloids, phenols, lignin, tannins, terpenoids, quinones, fixed oil and volatile oils were absent in leaf and tuber extracts of *T. cuneifolium*. Edeoga *et al.* (2005) screened number of medicinal plants for phytochemical constituents. Based on the results obtained it concluded that majority of the chemical constituents were dissolved in the methanol solvent. Hence, in the present study screening of antimicrobial activity was carried out using methanolic extract of tuber and leaf of the selected plant.

Methanolic leaf extract of *T. cuneifolium* showed maximum effect on the growth of *Proteus* (25.8 mm) followed by *Bacillus* (24.62 mm) and *E. coli* (19.42 mm) at the concentration of 2500 μg disc^{-1} (Table 2, Fig. 1 a-c). Leaf methanolic extract (2000 μg) of *Sebastiania chamaelea* proved maximum activity on Gram-positive and negative bacteria (Shanthisree *et al.,* 2010). The methanolic leaf extracts of *Artemisia nilagirica* showed high inhibition at the minimal concentration for most of the clinical pathogens in comparison to other extracts, but hexane leaf extract exhibits maximum inhibitory activity against all the phytopathogens in comparison to other extracts (Ahameethunisa and Hopper, 2010). The highest activity of *Basilicum polystachyon* leaf extract was observed against *B. subtilis*, *E. coli* was least inhibited followed by *P. aeruginosa* and *S. aureaus* (Chakraborty *et al.,* 2007). *Tectona grandis* leaf extracts showed activity on *Mycobacterium tuberculosis* at the concentration of 200 mg ml^{-1} onwards (Purushotham *et al.,* 2010). The methanolic extract of root tuber showed a maximum effect on the growth of *Proteus* (28.15 mm) followed by *Bacillus* (26.88 mm) and *E. coli* (24.51 mm) at a higher concentration of 2500 μg disc^{-1} (Table 2, Fig. 2 a-c). The tuber extracts of the *Gloriosa superba* showed antimicrobial activity against all the Gram-negative bacteria (Hemaiswarya *et al.,* 2009).

Methanolic extract of both leaf and tubers of the *T. cuneifolium* exhibit pronounced activity against *Candida albicans* rather than *A. niger*. Methanolic tuber extract displayed the maximum zone of inhibition (19.03 mm) (Table 3, Fig. 2d) A hundred percent inhibition of *A. niger* was observed with tuber extract of *Gloriosa superba* during the first 24 hr of incubation whereas, a significant reduction was noted on the next 24 hr of incubation (Hemaiswarya *et al.,* 2009). The roots extract of *Clitoria ternatea* widely varied across the species of *Klebsiella* and *A. niger* (Shanmugavadivu, 2008). While the methanolic leaf extract exhibited maximum zone of inhibition (16.21 mm) against *Candida albicans* (Table 3, Fig.1 d). The growth of inhibition of *A. niger* (5 mm) was found to be negligible with methanolic leaf extract at higher concentrations. According to Prasanna *et al.* (2007) fraction two of the methanolic leaf extract of *Croton sparsifloras* showed better antibacterial and antifungal activity than other fractions on *A. nigher*, *A. flavus* and *Candida albicans*. The leaf extract of *Basilicum polystachyon* showed highest antifungal activity against *A. niger* (Chakraborty *et al.,* 2007).

Tribes of various localities have been using the *T. cuneifolium* as a medicinal plant to treat different ailments (Savithramma, 2003). It has been suggested through preliminary phytochemicals analysis that the leaf and root tuber having number of potential chemical compounds, moreover screening of antimicrobial activity reveals that the plant may be useful to inhibit bacterial and fungal infections.

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