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Total Phenolic Content, Antioxidant and Antimicrobial Activity of *Talinum cuneifolium* (Vahl.) from Dibrugarh, North East India

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ABSTRACT

In present study we evaluated the phytochemical constituents, antioxidant and antimicrobial activity of the plant *Talinum cuneifolium*. The total phenol and flavonoid content and anti-oxidant activity of the extracts were spectrophotometrically determined; catechol, quercetin and ascorbic acid were taken as standard in case of total phenol and flavonoid content and anti-oxidant activity respectively. Antimicrobial activity done by Agar well diffusion method. This study confirms the presence of tannins, phlobatannin, flavonoids, glycosides, cardiac glycosides, saponins, carotenoids, alkaloids, phenol and reducing sugars and absence of terpenoids, steroids and free anthraquinone in the sample. Both the total phenol and flavonoid content, DPPH and ABTS radical scavenging activity were recorded highest in methanol extract. Hexane extract showed the good inhibition (12mm) against *E. coli* followed by *P. vulgaris* (10mm) and *B. cereus* (10mm).

Keywords: *Phytochemical constituents, antioxidant and antimicrobial activity.*

Introduction

India is one of the largest producers of medicinal herbs. More than 30% of the entire plant species of India are used for medicinal purposes [1]. Due to the current global trends of shifting to obtain drugs from plant sources, attention has been given to the medicinal value of herbal remedies [2]. The medicinal value of these plants lies in the bioactive phytochemical constituents. These natural compounds formed the base of modern drugs [3-7]. Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, phenolic compounds, steroids, saponins, tannins, terpenoids [8, 9]. Dubey *et al* [10] mentioned that the complete phytochemical investigations of medicinal plants of India should be carried out to determine these secondary metabolites. Compared with synthetic drugs, drugs derived from plants are frequently considered to be less toxic with fewer side effects [11].

Talinum cuneifolium Linn. (Protulacaceae) is locally used as medicinal plants in Assam. Leaves and roots are used for treatment of diabetes, mouth ulcer, as aphrodisiac, in cough, gastritis, pulmonary tuberculosis, diarrhoea and stomachic [12-14]. The plant is rich in vitamin A and mineral content [15] and endowed with wide range of pharmacological activities [16].The preliminary phytochemical analysis of this plant was carried out by various researchers from various parts of India [7, 14, 17, 18]. The present study focused on the total phenolic content, anti-oxidant and antimicrobial activity of the species from Dibrugarh, N.E. India.

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Materials and Methods

Sample collection

Plant was collected from Dibrugarh, Assam, N.E. India. The plant was botanically authenticated. A voucher specimen

(DUL.Sc.468) of the plant has been deposited to the herbarium of the Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam. The material was shade dried and grounded to fine powder using electric grinder.

Sample extraction

Samples were macerated with four solvents viz. water, methanol, acetone and hexane for 48 hours and filtered through Whatman No 1 filter paper. The filtrate was evaporated at 50°C (within the lowest boiling point of the selected solvents, 56.2°C for acetone) until a semi dried powder/sticky mass of crude extract and fractions were obtained. The crude extract and fractions were dissolved in Dimethyl sulphoxide (DMSO) as neutral solvent to make final concentration for biochemical analysis.

Experimental

Following methods were used for the phytochemical analysis, antioxidant & antimicrobial activity of the plant- Phytochemical analysis, Total phenol content (TPC) and Total flavonoid content (TFC), antioxidant acivity.

The qualitative phytochemical analysis was performed following the standard laboratory methods described by Edeoga *et al* [3]; Aja *et al* [19] and Ajayi *et al* [20]. Quantitative estimation of TPC was done by the method described by Malik and Singh [21] and TFC by the method described by Mervat and Hanan [22]. Antioxidant activity study was performed using DPPH and ABTS radical scavenging method as described by Anti-Stanojevic *et al* [23] and Re *et al* [24] respectively.

Antimicrobial activity study

The antimicrobial test was carried out by agar well diffusion method described by Nair et al. [25] using 6mm borer in triplicate. The activity was determined by measuring the diameter of zone of inhibition (ZOI) exhibited by the extract.

Table 1: Qualitative phytochemical analysis of *T. cuneifolium*

Phytochemicals		Presence /absence
Tannin		+
Phlobatannin		+
Flavonoids		+
Terpenoids		-
Steroids		-
Glycosides		+
Cardiac glycosides		+
Alkaloids	Dragendroff reagent	+
	Mayer's reagent	+
	Picric acid	+
Saponins		+
Carotenoids		+
Reducing suger		+
Phenol		+
Free anthraquinone		-

+ indicates presence of constituents and – indicate absence of constituents

Selected strains for antimicrobial study

Five Gram-Positive bacterial strains viz, *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 8750), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermis* (MTCC 3615) and *Proteus vulgaris* (MTCC 744); two Gram-Negative strains viz, *Escherichia coli* (MTCC 443), *Enterococcus faecalis* (MTCC 439) and two fungal strains viz- *Candida albicans* (MTCC 3017) and *Penicillium chrysogenum* (MTCC 947) were used in the study. Strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial strains were maintained on nutrient agar slants and fungal strains on PDA slants and stored in freeze. Strains were regularly sub-cultured using nutrient broth for bacterial strains and PDB for fungal strains.

Standard antibiotics

Standard antibiotics viz, Chloramphenicol (C) 30 mcg, Clotrimazole (CC) 10 mcg were taken for bacterial and fungal strains for comparison of ZOI with the solvent extracts.

Results and Discussion

Qualitative phytochemical analysis of the plant is presented in Table 1. The study confirms the presence of tannins, phlobatannin, flavonoids, glycosides, cardiac glycosides, saponins, carotenoids, alkaloids, phenol and reducing sugars and absence of terpenoids, steroids and free anthraquinone in the sample. Janapati et al. [18] from Kadapa showed the presence of alkaloids, tannins, flavonoids in the present sample which also supports our results but presence of steroids does not support our results. Savithramma *et al* [7] from Tirupathi, showed the presence of flavonoids, glycosides, saponins, and steroid but they could not detect alkaloids, phenols, tannins, terpenoid; in this case absence of terpenoid corroborate our result but presence of steroids does not do so. These variation in phytochemicals in the present study and earlier study may be due to the habitat differences of the plant which play important role in production of secondary metabolites. Table 2 shows the TPC and TFC of different solvent extract of *T. cuneifolium*. Here methanol extract showed highest amount of TPC and TFC than other solvent extract. Table 3 presents the DPPH and ABTS radical scavenging activity of the plant. All the solvent extract showed good amount of antioxidant activity, though the values were less than ascorbic acid. Here also methanol extract showed the highest result. Table 4 shows the anti-microbial activity of the plant; hexane extract of leaves showed maximum inhibitory effect on the growth of *E.coli* (12mm) followed *by B. cereus* (10mm) and *P vulgaris* (10mm). Savithramma *et al* [16] reported that methanolic extract of leaves showed maximum effect on the growth of *Proteus* (25.8 mm) followed by *Bacillus* (24.62 mm) and *E. coli* (19.42 mm). The present study does not show antifungal activity against *C. albicans* and *P. crysogenum*; on the other hand Savithramma *et al* [16] recorded pronounced activity against *C. albicans*. This difference of the present and earlier study may be due to the

climatic, edhapic and various other environmental factors of the study area. Antibacterial activity obtained were found to be encouraging as compared to that of standard antibiotics though the standard antibiotics showed larger inhibitory effect than the different solvent extract of *T. cuneifolium*.

The present study revealed that *T. cuneifolium* is a good source of natural antioxidant which might be helpful in preventing the progress of various oxidative stresses and may be a good antimicrobial agent against certain diseases caused by *E. coli, S. epidermis, B. cereus*. It is observed from our study that TPC, TFC, antioxidant and antimicrobial activity of plant varies among different solvent extract. The results of the present study deviated from the earliar studies perhaps due to method of extraction, concentration of the extract and habitat condition of the plant. Further study incorporating mere parameters would be needed in this aspect.

Table 2: TPC and TFC of different solvent extracts of *T. cuneifolium*.

Samples	Total phenol (mg catechol equivalent/g dry material)				Total flavonoid (mg quercetin equivalent/g dry material)			
	Ethanol extract	Methanol extract	Petroleum ether extract	Water extract	Ethanol extract	Methanol extract	Petroleum ether extract	Water extract
T. cuneifolium	1.31± 0.00	1.62± 0.00	1.27± 0.01	1.10± 0.01	0.87± 0.03	1.13± 0.03	1.10± 0.01	0.87± 0.10

**Values tabulated are average of triplicate.*

Table 3: Antioxidant activity of different solvent extracts of *T. cuneifolium*

Sample	Antioxidant activity (% inhibition in mg/ml)							
	DPPH radical scavenging activity				ABTS radical scavenging activity			
	Water extract (500µl)	Methanol extract (500µl)	Acetone extract (500µl)	Hexane extract (500µl)	Water extract (500µl)	Methanol extract (500µl)	Acetone extract (500µl)	Hexane extract (500µl)
num eifolium	59.29± 0.33	62.16± 0.13	60.41± 0.41	30.00± 0.09	69.86± 0.43	76.33± 0.34	70.66± 0.00	47.93± 0.39
orbic acid	90.28±0.03				83.00±0.00			

**Values tabulated are average of triplicate.*

Table 4: Antibacterial and Antifungal activity of different solvent extracts of *T. cuneifolium*

Microbial strains	Antibacterial activity							Antifungal activity	
Extracts(60µl)	*B. subtilis*	*B. cereus*	*S. aureus*	*S. epidermis*	*E. coli*	*E. facaelis*	*P. vulgaris*	*C. albicans*	*P. crysogenum*
Water extract	-	-	-	-	-	-	-	-	-
Methanol extract	-	8	-	8	8	8	8	-	-
Acetone extract	-	8	8	8	8	8	8	-	-
Hexane extract	8	10	8	8	12	-	10	-	-
Chloramphenicol(C) 30mcg	15	-	-	30	-	8	-	-	-
Clotrimazole (CC) 10mcg	20	10	14	20	8	-	26	11	32

ZOI including 5mm well diameter.

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