Antibacterial potentials of selected species of *Thunbergia* Retz. (Acanthaceae) - An inquiry

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Thunbergia is a genus of flowering plants that belongs to the family Acanthaceae and includes around 100 species distributed in the tropical region. Though many studies have been carried out in identifying various active biochemical compounds in different species of *Thunbergia*, a comparative analysis of the four species *vizT.alata, T.erecta, T.grandiflora* var blue and white and *T.fragrans* is a novel attempt in terms of its antimicrobial activities as well as phytochemical screening. From the present study it is clear that *T.erecta T. elata* and *T.grandiflora* contains rich quantity of secondary metabolities in it. The least value for flavonoids phenols and alkaloids were exhibited by *T.fragrans*. The antibacterial activity of the species reveals that *T.grandiflora* and *T.fragrans* possess high antimicrobial activities. The zones of inhibition in all the species were higher than that of the streptomycin standard against *Streptococcus aureus*. The presence of active phytochemical compounds and exhibition of antibacterial activities in these species corresponds to its pharmaceutical applications.

IndexTerms - Anti-bacterial activity, *Thunbergia alata, T.erecta, T.fragrance, T.grandiflora*

I. INTRODUCTION

Acanthaceae is a taxon of dicotyledonous flowering plants, containing approximately 250 genera and nearly 4000 species. *Thunbergia* is a genus of flowering plant of Acanthaceae family, native to tropical regions of Africa, Madagascar, Australia and South Asia. *Thunbergia* species includes around 100 species of annuals and perennials.There are many twinning climbers, as well as some shrubby types of plants in this variable genus.Most of the plants of this genus have ornamental value but rarely some of them have medicinal applications (Sultana et al., 2015). Some of the common *Thunbergia* species are *Thunbergia alata, Thunbergia annua,Thunbergia coccinea, T. cordata, T. erecta T. elegans* etc. Although genus *Thunbergia* was generally reported to contain variety of compounds e.g. flavonoids, iridoid glycosides but very few phytochemical studies have been reported from the leaves of the *Thunbergia* species.*Thunbergia alata*shows antibacterial activities in chloroform extract against *Pseudomonas aeruginosa* and significant antibacterial activity in higher concentration of ethanolic leaf extract against *Salmonella typhi*(Jenifer et al). Methanolic crude extract of *Thunbergia grandiflora* leaves against some Gram positive and Gram-negative bacteria also shows significant antibacterial activity (Chowdhury et al 2012). Methanolic extract of flower of *Thunbergia grandiflora* showes antibacterial activity against *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus cereus, Proteus mirabilis* and *Streptococcus pyogenes* due to the presence of phenols, alkaloids and flavonoids in them (Jeeva et al 2011).Aqueous extract of *Thunbergia laurifolia* exhibites inhibitory and antitumor or antiproliferative effects on MCF-7 breast cancer cells of human using multiple cellular and molecular approaches (Jetawattana et al 2015).Purification, identification and characterization of unexplored bioactive compounds showing pharmacological activities of these species will be the area of future work. Production of bioactive compounds from these plant species can be increased since they possess pharmacological activities like antibacterial, antifungal, anti-diabetic, antipyretic, anti-inflammatory, anti-helmentic, antioxidant and hepatoprotective which may be advantageous for pharmaceutical industry to explore effective drugs from different species of the *Thunbergia* plant.

MATERIALS AND METHODS

Carbohydrate content was measured spectrophotometrically (Hedge& Hofreiter, 1962) using anthrone method. Glucose was used as the standard. Reducing sugars were determined based on Miller, 1972.Starch content was estimated spectrophotometrically using anthrone reagent (Hedge &Hofreiter,1962. Protein determination was carried out based on Lowry *et al*., 1951. Determination of amino acid was carried out following the protocol of Moore & Stein, 1948.Total phenol content was detected spectrophotometrically based on Malick, & Singh, 1980**.** The tannin content was determined by Vanillin Hydrochloride method. Flavanoid content was determined spectrophotometrically. Alkaloid, Saponin, terpenoid and oxalate content were determined spectrophotometrically. Vitamin C content was determined by iodine titration. Ascorbic acid was used as the standard. Iron content was calculated colorometrically. Anti-bacterial activity was determined by agar well diffusion method.

RESULTS AND DISCUSSIONS

The amount of carbohydrate present in all the five species under study shows that *T. fragrance* is a good source of carbohydrate (21.05±0.21) and the least concentration is exhibited by *T.grandiflora* blue (17.72±1.07). There was only a minute difference between the carbohydrate concentrations of all the five species.The starch content was only half the amount of carbohydrate present in the sample. There was only a marginal difference in the total starch content among the species with the highest starch content exhibited by *Thumbergia erecta* (10.41±0.97) and the least content was shown by *T.grandiflora* white (8.14±1.11). The reducing sugar content was highest in *T.fragrans* with a value of 9.87±0.67 the least content of the same was exhibited by *T.grandiflora* blue (6.87±0.56).There was only a marginal difference between the three species (*T.erecta, T.grandiflora* and *T.alata*). The total protein content was highest in *T.erecta*(8.94±0.45) and the minimal concentration was exhibited by *T.fragrans*(4.78±0.67). The other three species showed only marginal variations. The aminoacid value was almost similar to that of the protein. The total amino acid content was highest in *T.grandiflora* blue with 4.89±0.45mg and the minimal concentration was exhibited by *T.fragrans* (2.24±0.11). The other three species showed only marginal variations. The total vitamin C content was highest in *T.grandiflora* blue (2.65±0.67) and the minimal concentration was exhibited by *T.fragrans*(1.24±0.48). The other three species showed only marginal variations. The total iron content was highest in *T.grandiflora* blue with 1.79±0.56mg and the minimal concentration was exhibited by T.fragrance0.94±0.04mg. The other three species showed only marginal variations (Table 1). From this study it can be concluded that all the species under study were good source of carbohydrates and proteins.The total tannin content was highest in *T.grandiflora* white (0.785±0.06) and the minimal concentration was exhibited by *T.fragrans* (0.027±0.05). The other three species showed only marginal variations. The total flavonoid content was highest in *T.erecta* (0.789±0.05) and lowest in *T.grandiflora* blue (0.456±0.07). The total phenolic content was highest in *T.fragrans* (0.45±0.07) and the minimal concentration was exhibited by *T.grandiflora* blue (0.14±0.08). The other three species showed only marginal variations. The total alkaloid content was highest in *T.grandiflora* white (0.985±0.04) and lowest in *T.fragrans* (0.458±0.06). The other three species showed only marginal variations. The total saponin content was highest in *T.grandiflora* blue and lowest was exhibited by *T.grandiflora* .The saponin content and terpenoid content was highest in *T.erecta* and lowest in *T.alata*. The oxalate content was highest in *T.alata* (0.49±0.07) and lowest in *T.erecta* (0.19±0.09). The presence of flavonoids phenols and alkaloids suggests the pharmaceutical applications of the species. Pharmaceutical and ethano-pharmacological aspects of *T.laurifolia* have been carried by Chan et al in 2011 which points to the pharmacological applications of other species. Previous studies in *T grandiflora* suggests the content of flavonoids and tannins in it which would correspond to its antimicrobial activities (Ibrahim et al 2017). Studies also report the members of the genus as good antioxidant.

Anti-bacterial activity

The anti-bacterial activities of *Thunbergia* species viz *T.alata, T.erecta, T.grandiflora* blue, *T.grandiflora* white and *T.fragrans* against *Staphylococcus aureus* was carried out. Streptomycin was used as standard and the zone of inhibition was 20mm. In *Thunbergia erecta* the zone of inhibition was maximum at 100µl concentration (Table 3). The zone of inhibition was much greater than that of standarad (30mm). At 50µl concentration also the zone of inhibition was 27mm which was also greater than that of the standard. Hence it can be concluded that the methanolic leaf extract of *Thunbergia erecta* possess antibacterial activity (Table 4). The zone of inhibition of the methanolic leaf extract of *T.grandiflora* var blue has antibacterial activity in minimum concentration of 25µl, greater than that of the streptomycin standard. The maximum zone of inhibition was exhibited at 100 µl with 36mm. The zone of inhibition is 16 mm greater compared to the standard that corresponds to its efficient antibacterial activity (Table 5). The antimicrobial activity of *T.grandiflora* var. white is comparatively lesser than other species. The zone of inhibition at 50µl and 100µl is 25mm and 27mm respectively. There is only a difference of 7mm in the zone of inhibition from the standard to the maximum concentration of the plant extract. The antibacterial activities exhibited by *T.fragrans* reveals that it exhibits high antibacterial activity whereas in *T.alata* the antibacterial activity is comparatively low. The zone of inhibition was much higher than the standard in *T.fragrans* while in *T.alata* the zone of inhibition at maximum concentration showed only a marginal difference of 6mm. According to the present study the least anti-bacterial activity is exhibited by *T.elata*(Table 6 &7).The result obtained here suggests that the *Thunbergia* species under study exhibit antibacterial property against *S. aureus*. Previous reports suggest that in T.*laurifolia* also exhibits excellent antibacterial and antifungal activities against various strains of bacteria and fungi (Junsi et al 2015). The results obtained here are also supported by the previous studies carried out by Gulzar et al. (2015).

Table 1: Table showing the quantitative analysis of biochemical compounds in different species of *Thunbergia*

Sl no	Species	Carbohydrate	Starch	Reducing sugar	Protein	Amino Acid	Vitamin C	Iron
1	*Thunbergia erecta*	20.91±1.35	10.41 ±0.97	8.14±0.22	8.94±0.45	4.14± 1.32	1.98±1.09	1.47± 0.09
2	*Thunbergia grandiflora blue*	17.72±1.07	8.93± 0.54	6.87±0.56	7.98±0.22	4.89± 0.45	2.65±0.67	1.79± 0.56
3	*Thunbergia grandiflora white*	18.74±1.53	8.14± 1.11	7.14±1.09	5.89±0.22	2.98± 0.59	2.64±0.56	1.49± 0.09
4	*Thunbergia fragrans*	21.05±0.21	9.77± 0.87	9.87±0.67	4.78±0.67	2.24± 0.11	1.24±0.48	0.94± 0.04
5	*Thunbergia alata*	19.22±1.11	9.11± 0.57	8.91±0.54	7.25±0.95	2.98± 1.17	1.97±1.09	0.99± 0.05

Table 2: Table showing the quantitative analysis of secondary metabolites in *Thunbergia* species

Sl no	Species	Tannin	Flavonoid	Phenolics	Alkaloids	Saponins	Terpenoids	OXALATE
1	*Thunbergia erecta*	0.098± 0.09	0.789±0.05	0.15±0.09	0.910±0.06	0.241±0.11	0.43±0.07	0.19±0.09
2	*Thunbergia grandiflora blue*	0.051±0.07	0.456±0.07	0.14±0.08	0.789±0.07	0.981±0.09	0.33±0.04	0.27±0.004
3	*Thunbergia grandiflora white*	0.785± 0.06	0.756±0.12	0.19±0.05	0.985±0.04	0.478±0.11	0.28±0.03	0.44±0.09
4	*Thunbergia fragrans*	0.027± 0.05	0.478±0.05	0.45±0.07	0.458±0.06	0.547±0.11	0.36±0.04	0.46±0.006
5	*Thunbergia alata*	0.741± 0.04	0.531±0.16	0.17±0.04	0.125±0.08	0.258±0.08	0.18±0.06	0.49±0.07

Table 3: Table showing antimicrobial activity of *T.erecta*

Sample	Concentration (µl)	Zone of inhibition (mm)
Streptomycin	20	20
THUNBERGIA ERECTA	25	16
	50	27

	100	30

Table 4: Table showing antimicrobial activity of *T.grandiflora var.* blue

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Sample	Concentration (µl)	Zone of inhibition (mm)
Streptomycin	20	20
THUNBERGIA GRANDIFLORA BLUE	25	27
	50	30
	100	36

Table 5: Table showing antimicrobial activity of *T.grandiflora var.* white

Sample	Concentration (µl)	Zone of inhibition (mm)
Streptomycin	20	20
THUNBERGIA GRANDIFLORA WHITE	25	18
	50	25
	100	27

Table 6: Table showing antimicrobial activity of *T.fragrance*

Sample	Concentration (µl)	Zone of inhibition (mm)
Streptomycin	20	20
THUNBERGIA FRAGRANS	25	23
	50	28
	100	31

Table 7: Table showing antimicrobial activity of *T.elata*

Sample	Concentration (µl)	Zone of inhibition (mm)
Streptomycin	20	20
THUNBERGIA ALATA	25	23
	50	25
	100	27

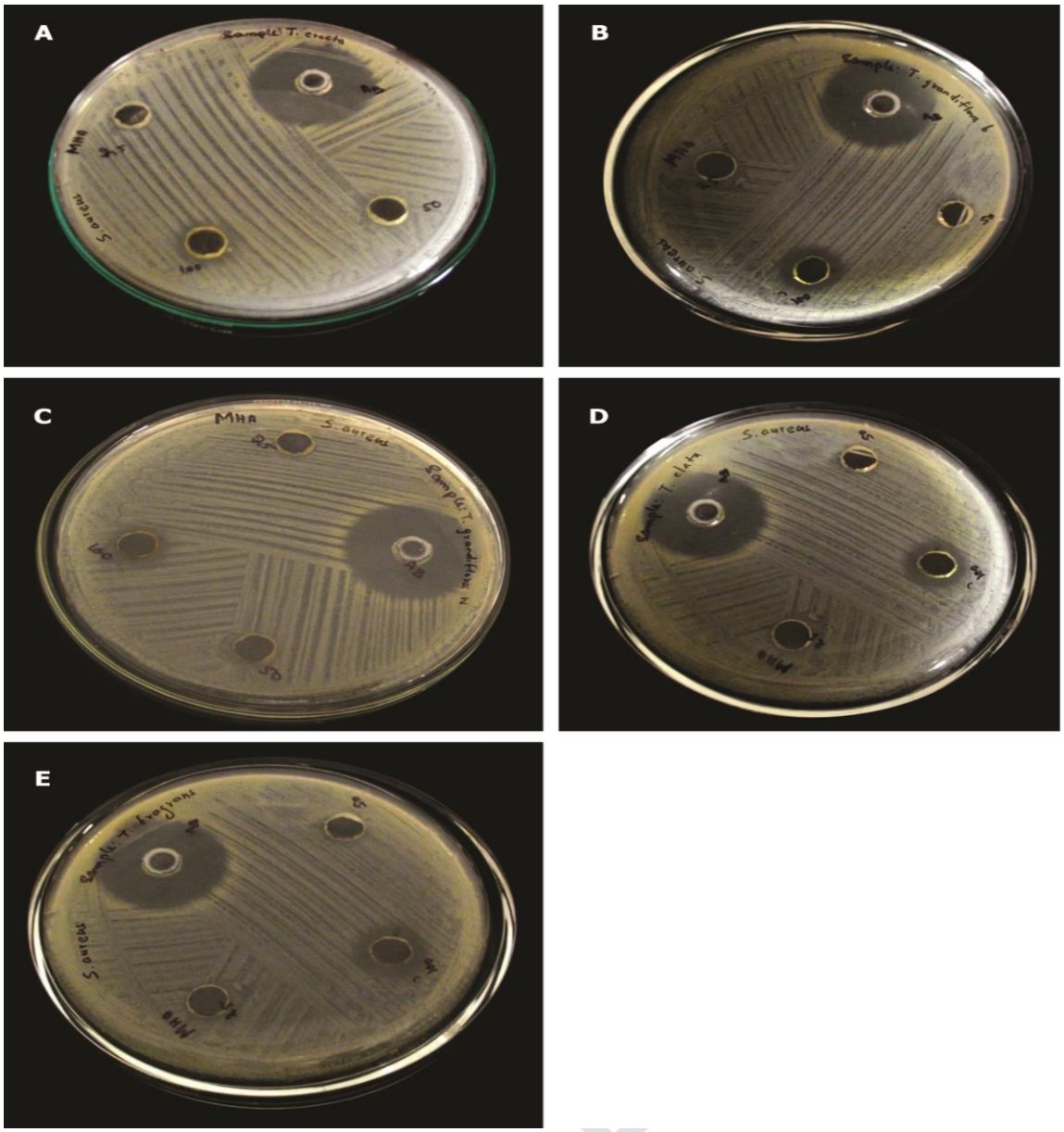

Plate 1: Antibacterial activity of the selected plants against *Staphylococcus aureus*
A. Antibacterial activity of *Thunbergia erecta* against *S. aureus*
B. Antibacterial activity of *Thunbergia grandiflora* (blue) against *S. aureus*
C. Antibacterial activity of *Thunbergia grandiflora* (white) against *S. aureus*
D. Antibacterial activity of *Thunbergia alata* against *S. aureus*
E. Antibacterial activity of *Thunbergia fragrans* against *S. aureus*

CONCLUSION

The study provides an insight into the antibacterial potentials of the selected taxa against the screened strains of microbes. Further, the phytochemical constituents like flavonoids, phenolics, terpinoids etc have also been screened. The pharmacognostic potential of these species could be exploited subsequently in comparison with other commonly used drug plants of the family.

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