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Evaluation of antibacterial activity of leaf extracts of *Mansoa alliacea* (Lam.) A. H. Gentry, *Tecomaria capensis* (Thunb.) Spach and *Tecoma stans* (L.) Juss. Ex H. B. & K.

Sundas Iltaf*[1], Zaheer-Ud-Din Khan[1], Rizwana Rafique[1], Anjum Parveen[2]

[1]*Department of Botany, GC University, Lahore, Pakistan*

[2]*Center for Plant Conservation, University of Karachi, Pakistan*

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Abstract

The current investigation was carried out to evaluate the antibacterial potential of different extracts (*n*-hexane, chloroform, ethanol and aqueous) of the leaves of *Mansoa alliacea*, *Tecomaria capensis* and *Tecoma stans*, belonging to family Bignoniaceae, using agar well diffusion method against 2 Gram-positive bacteria, i.e. *Bacillus subtilis, Staphylococcus aureus* and 2 Gram-negative bacteria, i.e. *Escherichia coli* and *Pseudomonas aeruginosa*. The diameter of inhibition zone of the leaf extracts were compared with those of different standards like ampicillin (10µg), amikacin (30µg) and the significant inhibition of the growth of bacteria was shown by leaf extracts against the test microbes, e.g. in *Tecomaria capensis*, 45.36mm and 42.33mm by *n*-hexane extract against *S. aureus* and by chloroform extract against *E. coli*, respectively. Highest percentage extraction yield was exhibited by the aqueous extract of leaves, i.e. 12.52, 15.48 and 17.98 for *Mansoa alliacea*, *Tecoma stans* and *Tecomaria capensis*, respectively. The ranges of Minimum Inhibitory Concentration (MIC) were 10-2.5mg/mL for *Mansoa alliacea* and *Tecoma stans*, while it was 10-1.25mg/mL for *Tecomaria capensis*. It suggested that these plants can be used as bioactive natural products to inhibit the growth of pathogenic bacteria.

***Corresponding Author:** Sundas Iltaf ✉ sundas2006@yahoo.com

Introduction

Plants are an important component of biodiversity, representing more than 500,000 species. The drug derivatives from plants show a vital role in the prevention and cure of various diseases, so they are necessary for the survival of mankind as well as animals (Battu *et al.*, 2011). Many plants are being used medicinally as having antimicrobial compounds, but the search of more plants for better and safer antimicrobial drugs production is still continuous (Ray and Majumdar, 1976). The source of almost fifty percent of medicines available in the market is natural plant material. As many of the useful plant ingredients cannot be synthetically prepared, so there is an increasing demand of herbal medicine over synthetic ones (Dey *et al.*, 2012). Screening of the plants for discovery of antibacterial and antifungal compounds used in well-known medicines is the first step towards the development of antimicrobial agents (Paz *et al.*, 1995). In the treatment of infections, an alarming clinical situation has occurred due to the emergence of multiple drug-resistant bacteria. A number of new antibiotics have been produced by the pharmacological industries for those bacteria that have developed great resistance against the antibiotics, especially against the synthetic drugs being utilized as therapeutic agents (Towers *et al.*, 2001).

The Bignoniaceae is a plant family consisting of 120 genera and 800 species (Nasir, 1979). Plants of interest, i.e. *Mansoa alliacea* (Lam.) A.H. Gentry, *Tecomaria capensis* (Thunb.) Spach and *Tecoma stans* (L.) Juss. ex Kunth and belong to family Bignoniaceae commonly called as trumpet creeper or trumpet vine family, *Bignonia* family, *Jacaranda* family or *Catalpa* family. Literature survey reveals that the Bignoniaceae family is a huge reservoir of a variety of secondary metabolites like, alkaloids, anthralene derivatives, carbohydrates, coumarines, flavonoids, glycosides, iridoids, quinines, terpenes, tannins, steroids, saponins, etc. (Choudhury *et al.*, 2011). The secondary metabolites have antimicrobial ability as revealed by Dahanukar *et al.*, 2000.

Tecoma stans also known as yellow bell or yellow trumpet brush is native of tropical South America and is cultivated on wide range in tropical and subtropical areas, commonly in gardens. It is predominantly a fast growing small tree or erect shrub of approximately 6-10 m in height. It is extensively used for the treatment of urinary disorders and diabetes in Central America and Mexico (Shapiro and Gong, 2002) and also for feeding goats and cattle (Susano-Hernández, 1981).

Phytochemical investigation of *Tecoma stans* showed the presence of alkaloids, anthraquinones, flavonoids, phenols, saponins, steroids and tannins, which shows that this plant can be the source of good antimicrobial and antioxidant agents (Govindappa *et al.*, 2011). *Tecomaria capensis* (Thunb.) Spach, an ornamental plant with orange flowers, native of South Africa also known as Cape-honey suckle commonly found in tropical regions is deciduous and evergreen; scrambler to small tree with a round crown. Alkaloids, saponins, flavanoids, carbohydrates and anthraquinone glycosides were reported as important constituents present in *Tecoma capensis* by Begum *et al.*, 2015. *Mansoa alliacea* (Lam.) A.H. Gentry is commonly named as garlic vine, because its crushed leaves give odor just like garlic. It's an ornamental plant with attractive pink flowers and native of Brazil and Peru. Quantitative analysis showed highest amount of phenols in leaf and flavonoid contents in roots. This plant has importance for treatment of fever, cold, cough and upper respiratory condition (Patel *et al.*, 2013). Antibacterial activity of alcoholic extracts of *Boerhaavia diffusa, Eclipta alba, Cassia auriculata, C. Lantana,* and *Tinospora cardiofolia*. was checked by Girish and Satish, 2008. Antimicrobial activity of 39 plants belonging to 22 different families of Tanzania from Buna district was carried out by Maregesi *et al.*, 2008.

The objective of the study was the evaluation of antimicrobial potential of leaf extracts (*n*-hexane, chloroform, ethanol and aqueous) of *Mansoa alliacea, Tecoma stans and Tecomaria capensis* against clinically isolated microbes.

Materials and methods

The plants, i.e. *Mansoa alliacea* (Lam.) A. H. Gentry, *Tecomaria capensis* (Thunb.) Spach and *Tecoma stans* (L.) Juss. Ex H.B. & K. were collected from Botanic Garden GC University, Lahore. They were deposited as voucher specimen in Dr. Sultan Ahmad Herbarium, Department of Botany, GC University Lahore, after identification with Flora of Pakistan (Nasir, 1979).

Preparation of the extracts

Fresh and healthy leaves of the plants were air dried at room temperature and crushed to powder form in a mechanical grinder. The dried leaf powder (80g) of each plant was extracted by maceration, i.e. soaking in 150 ml of *n*-hexane for eight days. After filtration the residue was air dried and then subjected to further fractions by successive solvent extractions with chloroform, ethanol and water by soaking in each solvent for eight days with repeated agitation. Ultimately all the extracts procured were desiccated and concentrated using rotary evaporator under reduced pressure, lyophilized and stored at 4°C until further use. The following formula was used to calculate the percentage extraction yield of the plants:

$$\text{Extraction yield (\%)} = \frac{\text{Wt. of the extract}}{\text{Wt. of the initial plant sample taken}} \times 100$$

Antimicrobial activity

The clinical and pharmacological important bacterial strains.i.e. *Bacillus subtilis* (ATCC 15029), *Escherichia coli* (ATCC 14962), *Staphylococcus aureus* (ATCC 14923) and *Pseudomonas aeruginosa* (ATCC 14971) were maintained on nutrient agar medium for 24 hrs and then used as test microbes. The zone of inhibition was estimated using agar well diffusion method following Jorgensen and Turnidge, 2007. During this technique positive control (antibiotic discs) and negative controls (blank solvents *n*-hexane, chloroform, ethanol and distilled water) were used. The inoculum (1.5×10^8 CFU/mL adjusted to 0.5 McFarland turbidity standards) was spread homogeneously on the sterilized solidified medium in the Petri plates.

After making wells, 1ml of stock solution (20mg/mL) of each plant extract was transferred to wells in the Petri dishes and incubated at 37°C for 24 hrs. The diameter of inhibition zone was measured in mm by means of verniercalliper and recorded in Table 2-5. Evaluation of Minimum Inhibitory Concentration (MIC) was carried out by Jorgensen and Turnidge, 2007 using serial dilution method by preparing different concentrations (10, 5, 2.5, 1.25, 0.625mg/mL) of each plant extract. Two ml of each dilution was poured in Petri plate containing 18mL of sterile nutrient agar medium. After solidification inoculum of bacterial suspension was spread homogeneously on the surface of media in prepared Petri plates. These plates were incubated at 37°C for 24 hours. After incubation, Petri plates were analyzed for the presence (+) or absence (-) of microbial proliferation. The least concentration that inhibited the bacterial growth was considered as MIC. Nutrient agar medium with bacteria were taken as positive control while nutrient agar medium with no bacterial suspension as negative control.

Antibacterial analysis was carried out in triplicates and the significant value of the analysis was determined statistically by applying DMRT at 5% significance level using Cost at 6.4 version (Steel *et al.*, 1997).

Results and discussion

The percentage extraction yield of *Mansoa alliacea* range from 3.00-12.52, the potential of the percentage yield extract in different solvents was aqueous > ethanol > Chloroform >*n*-hexane. *Tecoma stans* showed 1.76-15.48 % extraction yield with the potential for different solvents, i.e. aqueous > ethanol >*n*-hexane > chloroform, while 1.12-17.98 % extraction yield was exhibited by *Tecomaria capensis* in different solvents as aqueous > ethanol >*n*-hexane > chloroform. The crude extracts provided the clue that the % extraction yield is directly proportional to the polarity index of the solvents (Tadeg *et al.*, 2005), however the chloroform extracts of *Tecoma stans* and *Tecomaria capensis* were exception in the regard.

The inhibition zone exhibited by the leaf extracts of *Mansoa alliacea* ranged from11.60-25.10mm. The maximum activity, i.e. 25.10 was exhibited by *n*-hexane extract against *P. aeruginosa* also supported by the results of a study conducted by Guilhon *et al*., 2012. Ethanol extract showed good potential, i.e.22.40mm zone of inhibition against *P. aeruginosa* among the all bacteria. Chloroform and aqueous extracts exposed less activity against the test microbes (Table 2), The zone of inhibition of *Tecoma stans* ranged from 11.10-26.80mm, the maximum by *n*-hexane extract against *E. coli*, i.e. 26.80mm. Ethanol extract also showed maximum activity, i.e. 24.30 but against *P. aeruginosa*. Similarly maximum zone of inhibition was shown by the methanolic extracts of roots of *Tecoma stans* against *P. aeruginosa* by Ramesh *et al*., 2009. The chloroform extract exhibited 11.10-17.13mm, while the aqueous extract 13.67-20.87mm against all the bacteria tested. Overall higher antibacterial activity of all leaf extracts was shown against *P. aeruginosa* (Table 3). The zone of inhibition of all the extracts of *Tecomaria capensis* ranged between 12.13-45.36mm against the tested bacteria, e.g. maximum by *n*-hexane extract against *S. aureus*, i.e. 45.36 mm. Chloroform extract showed best activity against *E. coli* with the inhibition zone of 42.33mm, while ethanol and aqueous extracts showed intermediate response against all the tested bacteria (Table 4). In comparison to Standard Antibiotics, *n*-hexane and Chloroform extracts of *Tecomaria capensis* showed remarkable inhibition of *S. aureus* and *E. coli*, respectively (Table 1 & 4).

Table 1. Inhibition zone (mm) of the standard antibiotic discs against bacterial strains.

Antibiotic standard disc	Conc. (µg)	Zone of Inhibition (mm)			
		B. subtilis	*E. coli*	*P. aeruginosa*	*S. aureus*
Amikacin	30	18±1.3	13±0.3	14±0.8	17±0.5
Ampicillin	10	11±1.5	12±2.5	11±0.5	22±3.9

Table 2. Zone of inhibition of the extracts of *M. alliacea* against tested bacteria.

Plant species	Extracts	Inhibition zone (mm)			
		B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
M. alliacea	*n*-Hexane	12.43±0.48^{c}	11.67±0.33^{d}	16.47±0.54^{de}	25.10±0.35^{a}
	Chloroform	11.60±1.05^{c}	13.43±0.41^{c}	17.27±0.42^{cd}	13.87±0.34^{f}
	Ethanol	17.57±0.90^{b}	14.07±0.41^{c}	19.13±0.32^{c}	22.40±0.40^{b}
	Aqueous	15.50±0.61^{b}	16.10±0.35^{b}	21.40±0.47^{b}	17.63±0.80^{c}

*Different superscripted alphabets in the same column indicate significant differences ($p < 0.05$) between means.

Table 3. Inhibition zone of the extracts of *Tecoma stans* against tested microbes.

Plant species	Extracts	Inhibition zone (mm)			
		B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
Tecoma stans	*n*-Hexane	11.10±0.38^{d}	12.20±0.17^{cd}	26.80±0.74^{a}	25.37±0.43^{c}
	Chloroform	12.70±0.31^{cd}	11.10±0.31^{d}	15.60±0.36^{c}	17.13±0.54^{c}
	Ethanol	22.10±0.26^{a}	13.60±0.45^{bc}	19.20±0.52^{b}	24.30±0.57^{a}
	Aqueous	16.27±0.47^{b}	13.67±0.74^{bc}	15.370±0.62^{c}	20.87±1.16^{b}

Table 4. Inhibition zone of the extracts of *Tecomaria capensis* against tested microbes.

Plant species	Extracts	Inhibition zone (mm)			
		B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
Tecomaria capensis	*n*-Hexane	14.07±0.44^{e}	45.36±1.43^{a}	20.93±0.68^{b}	25.10±0.21^{b}
	Chloroform	13.23±0.52^{e}	15.20±0.26^{c}	42.33±1.55^{a}	15.83±0.35^{cd}
	Ethanol	21.20±0.47^{c}	12.13±0.34^{d}	17.97±0.57^{c}	25.27±0.55^{b}
	Aqueous	17.53±0.88^{d}	21.33±0.52^{b}	16.06±0.49^{c}	13.30±0.61^{d}

Table 5. Minimum inhibitory concentration of different extracts of *Mansoa alliacea*, *Tecomaria capensis* and *Tecoma stans* against bacterial pathogens.

Bacterial isolates	Conc. (mg/mL)	Plants											
		Mansoa alliacea				*Tecoma stans*				*Tecomaria capensis*			
		Hex	Chl	Eth	Aq	Hex	Chl	Eth	Aq	Hex	Chl	Eth	Aq
B. subtilis	10	-	-	-	-	-	-	-	-	-	-	-	-
	5	+	+	-	-	+	+	-	+	+	+	-	-
	2.5	+	+	+	+	+	+	+	+	+	+	+	+
	1.25	+	+	+	+	+	+	+	+	+	+	+	+
	0.625	+	+	+	+	+	+	+	+	+	+	+	+
S. aureus	10	-	-	-	-	-	-	-	-	-	-	-	-
	5	+	-	-	-	+	+	+	+	-	+	+	-
	2.5	+	+	+	+	+	+	+	+	-	+	+	+
	1.25	+	+	+	+	+	+	+	+	-	+	+	+
	0.625	+	+	+	+	+	+	+	+	+	+	+	+
E. coli	10	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	+	-	+	-	-	-	-
	2.5	+	+	+	-	-	+	+	+	+	-	+	+
	1.25	+	+	+	+	+	+	+	+	+	-	+	+
	0.625	+	+	+	+	+	+	+	+	+	+	+	+
P. aeruginosa	10	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	+	-	+
	2.5	-	+	-	+	-	+	-	+	-	+	-	+
	1.25	+	+	+	+	+	+	+	+	+	+	+	+
	0.625	+	+	+	+	+	+	+	+	+	+	+	+

*Key: - = Absence of bacterial growth, + = Presence of bacterial growth.

*Macerates: Hex = *n*-hexane, Chl = Chloroform, Eth = Ethanol, Aq =Aqueous

On the whole *n*-hexane extract of *Tecomaria capensis* showed best activity against *S. aureus* (Fig. 1), similar to the results obtained by Ugbabe *et al.* (2010),while ethanol extract of *Tecoma stans* exhibited maximum zone of inhibition against *B. subtilis* (Fig. 2). Similarly the chloroform extract of *Tecomaria capensis* showed maximum activity against *E. coli* (Fig. 3) Almost the same antibacterial activity, having 25.10-25.37mm zone of inhibition was exhibited by *n*-hexane extract of all the three plants against *P. aeruginosa* (Fig. 4). The results showed that the leaf extracts of the plants were found more active against Gram positive bacteria as compared to the Gram negative bacteria. This observation was supported by different studies carried out by Torres *et al.*, 2013 during the evaluation of *invitro* antimicrobial activity of twenty climber species belonging to family Bignoniaceae and antimicrobial potential of some species of Bignoniaceae by Rasadah and Houghton, 1998.

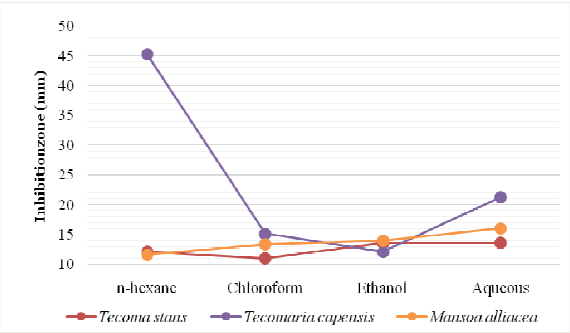

Fig. 1. Comparative inhibition zones of the leaf extracts of the plants against *Staphylococcus aureus*.

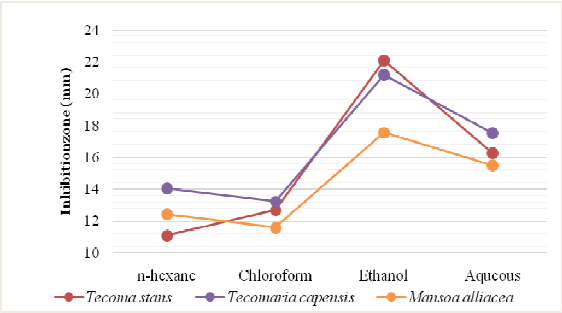

Fig. 2. Comparative inhibition zone of the leaf extracts of the plants against *Bacillus subtilis*.

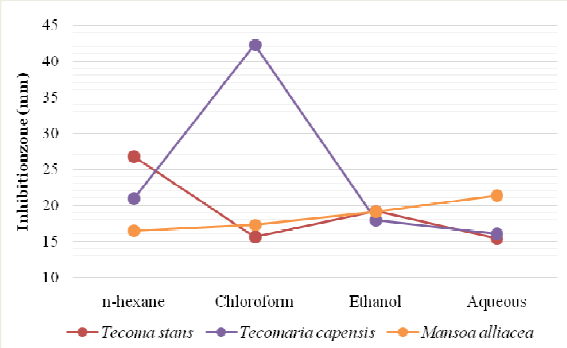

Fig. 3. Comparative inhibition zone of the leaf extracts of the plants against *Escherichia coli*.

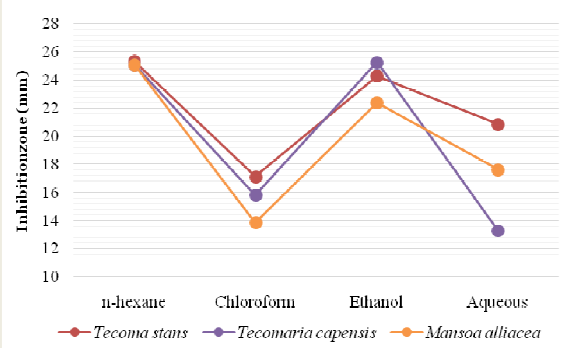

Fig. 4. Comparative inhibition zone shown by the leaf extracts of the plants against *Pseudomonas aeruginosa*.

The Minimum Inhibitory Concentration (MIC) verified the antimicrobial potential of *Mansoa alliacea* (Lam.) A.H. Gentry, *Tecoma stans* (L.) Juss. Ex H.B. & K. and *Tecomaria capensis* (Thunb.) Spach. The ranges of MIC were 10-2.5mg/mL for *Mansoa alliacea* and *Tecoma stans*, while it was 10-1.25mg/mL for *Tecomaria capensis* (Table 5).

The best MIC (1.25mg/mL) was exhibited by *n*-hexane and chloroform extract of *Tecoamria capensis* against *S. aureus* and *E. coli* and promising MIC potential (2.5mg/mL) was exhibited by *n*-hexane and ethanol extracts of the tested plants against *P. aeruginosa* as also supported by the MIC determined by the hexane extract of *Mansoa difficilis* (Guilhon *et al.*, 2012), aqueous extract of *M. alliacea* against *E. coli*, *n*-hexane extract of *Tecomastans* against *E. coli*. MIC less than 5mg/mL was defined as strong actitivty (Bussmann *et al.*, 2010). The results showed that *S. aureus* and *E. coli* were susceptible to *n*-hexane and chloroform extracts of *Tecomaria capensis*, while *P. aeruginosa* was susceptible to the *n*-hexane and ethanol extracts *M. alliacea* and *Tecoma stans* as compared to the other bacterial strains as its MIC was 2.5mg/mL for the tested plants. The MIC potential of *Mansoa alliacea* and *Tecoma stans* showed that these plants were active against Gram-negative as compared to the Gram-positive bacteria which is similar to the investigations carried out on *Sauromatum venosum* Ait by Ajaib *et al.*, 2011. The MIC results of the extract for the investigation of antibacterial potential were in accordance with the results obtained from the evaluation of zone of inhibition of ethanol extracts. The MIC results obtained in this study were higher than the results reported by Bissmann *et al.*, 2010, while they were in the range of the concentrations observer by Peswu*et al.*, 2008 or lower than the concentration range reported by Kloucek *et al.*, 2007. The maximum zone of inhibition was shown by the *n-hexane* and chloroform extract of *Tecomari acapensis* against *S. aureus* and *E. coli*, i.e. 45mm and 42mm. The results showed that the extracts having maximum zone of inhibition show MIC at less concentrations.

Conclusion

The current study showed that the extracts from leaves of *Mansoa alliacea*, *Tecomaria capensis* and *Tecoma stans* belonging to Bignoniaceae had strong antibacterial activities. The findings suggest that these plants can be explored for the preparation of antibacterial drugs for pharmaceutical industries, as

all the extracts showed significant activity against the four strains of bacteria. These antibacterial activities could be due to the presence of secondary metabolites such as alkaloids, anthraquinone, flavonoids, saponins, steroids and tannins etc. which are antibacterial in their mode of action.

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