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Original Article

VARIABLE RESPONSE OF THREE MORPHOTYPES OF TECOMELLA UNDULATA (SM.) SEEM TOWARDS HUMAN PATHOGENIC BACTERIA

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ABSTRACT

Objective: Medicinal Tree *Tecomella undulata* [Sm.] Seem belonging to family Bignoniaceae has three distinct morphotypes. Present study was undertaken to assess antimicrobial potential of the three morpho types of *Tecomella undulata* against human pathogenic bacteria to select suitable morphotype for medicinal use.

Methods: Disc diffusion method was used to test and compare the antimicrobial activity exhibited by 13 populations belonging to three morphotypes of this tree against five human pathogenic bacteria i. e. *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Results: Methanol and acetone extract of bark of the tree showed considerable activity against all the five bacteria tested. Differences were observed in the activity not only between the morphotypes but also within the morphotypes against the bacteria tested. Out of the three morpho types yellow morphotypes showed the highest antimicrobial action against all the five bacteria which were comparable to standard antibiotic Chloramphenicol. The trend in antibacterial activity observed was yellow>red>orange in acetone extract and yellow>orange>red in methanol extract towards all microbes except against bacillus bacteria where it was yellow>orange>red [acetone] and orange>yellow>red [methanol].

Conclusion: The experimental results of the present study revealed that *Tecomella undulata* plants show great intraspecific variability in their antibacterial potential. Hence there is need to evaluate germplasm to select superior genotype for medicinal, multiplication, conservation or plantation purpose.

Keywords: *Tecomella undulata,* Antimicrobial activity, Disc diffusion method, Activity index, Conservation, Human pathogenic bacteria.

INTRODUCTION

Present decade has seen the revival of traditional Indian medicines based upon natural sources, mainly plants that are more effective and at the same time less toxic to humans. Crude extracts of plants and plant products have been traditionally used for the treatment of infectious diseases [1-3]. Search of substances with antimicrobial activity has lead to screening of the number of plants [4-8]. This has led to exponential market and public demand for the plants with curative properties and simultaneously increased the exploitation of these plants for preparing herbal formulation either individually or in combination. Many a time selection of the herbal plants is made without actually knowing the amount, quality or effectiveness of active principle in it as they vary with the genotype of the plant. This effect of genotype is more pronounced in cross pollinated plants. In biological world lot of things change due to existing inter and intraspecific genetic variability.

Sometimes environment also plays a major role in expression of a character as result of genotypic and environmental interactions. In nutshell we can say that for selection of particular plant or its genotype for use one must first be assured of relative bio potential and suitability of that genotype for that purpose otherwise any effort made in haste not only will be waste of time, labour and money but also can lead to great extinction risk to many plants and obviously the loss of genetic diversity. One such tree of medicinal importance is *Tecomella undulata* [Sm.] Seem [Vern Rohida or Marwar Teak]. The tree has three distinct morphotypes based on flower colour i. e. red, orange and yellow [9-11].

It is a threatened tree species distributed in Rajasthan, Maharashtra, Gujarat, Haryana and Punjab regions of India and Sind and Waziristan regions of Pakistan [12-13]. The plant is known as a cure for syphilis, eczema gonorrhoea, leucoderma, treatment of enlarged spleen and liver diseases [14]. Bark has mild relaxant, cardiotonic and chloretic activities [15-16]. Many herbal formulations of the plant are available in the market such as Rohitakarista, Rohitakyadi churna, Livonil Syrup, Mediliv-DS, Exol, Gynicare, Culiv, Livosan, U Liv, Kamalahar etc [17-18] and most of them are liver tonics. Tree is also known to have antibacterial activity [19-21, 12], Anti-cancer potential [22], immuno-modulatory activity [23] and anti HIV agents [24]. The present study deals with testing of antimicrobial activity of the leaves and bark of the three morphotypes of the species to assess variability in response of their extracts towards selected pathogenic bacteria. For this purpose 13 populations of the tree collected from various sites from Punjab were used. Present study clearly points to the need for assessing genetic variability of medicinal plants before using it for medicinal purpose or plantation of the same for the said purpose.

MATERIALS AND METHODS

Extract preparation

Leaves and bark of the trees were collected, washed thoroughly and dried in shade. Dried material was crushed to powder and stored in air tight jars till further use. Three types of extracts were prepared for testing i.e. Aqueous, acetone and methanol. Five grams of the powdered material were soaked in 100 mL of the different solvents [48 hrs in acetone and methanol and 24 hrs in distilled water]. The extracts were filtered and dried. Dried extracts were weighed and dissolved in Dimethylsulphoxide [DMSO] to make final concentration of 100 mg/mL of the extract and stored in refrigerator till further used.

Bacterial cultures

Antimicrobial activity was tested against five bacteria i. e. *Bacillus subtilis, Staphylococcus aureus* [both gram positive], *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [all the three gram negative procured from IMTECH, Chandigarh]. Bacteria were sub cultured in 13mg/L nutrient broth [Himedia] at 37^{o}C before use. Sterilized petriplates were filled with 30 mL of agar [Himedia] containing growth medium and then inoculated with 0.5 mL of inoculum [inoculum size 10^{4} cells/mL].

Collection of material

Fresh leaves and bark were collected from trees of *T. undulata* growing at 13 different locations in seven districts of Punjab, India belonging to three morphotypes: Yellow [2], red [4] and Orange [7].

Experiment

Disc diffusion method as proposed by Baurer *et al.* [25] was used to test the antimicrobial potential of the leaves and bark of the tree. DMSO was used as negative control and Chloramphenicol at the concentration of 2mg/mL was used as positive control. Every disc [7 mm diameter] was impregnated with 20 µl of the test solution. All experiments were conducted in triplicate. Inhibition zone was noted in mm after 24 hours of incubation at 37° C from inoculated petriplates**.** Activity index of the extract against each bacterium was calculated as per Singh *et al*. [26] by comparing the zone of inhibition by extract with that of Chloramphenicol.

RESULTS AND DISCUSSION

To compare the antimicrobial activity of three morphotypes of the species, samples were collected from plants growing at thirteen locations in Punjab [Table1].

The test results showed that the acetone and methanol extract of the bark of the tree showed the antibacterial activity while aqueous extract of the bark showed no such activity. Similar observation has been made by Parekh and Chanda [4-5] and Thanawalla and Jolly [27]. Further when the test was carried out by using the methanol and acetone extract of selected accessions of various morphotypes, variability was seen in the zone of inhibition, not only between the morphotypes but also within the morphotypes against the tested organism. Activity index of each extract was also calculated and then average and range of activity index was calculated for each morphotype with standard deviation [Table 2 and Fig 1].

Table 1: Collection sites of various populations of Tecomella undulata from Punjab

Morphotype	Locality and altitude	Population
Yellow flowered	Barnala: Maur Maksudan, 228m	Y-BR1
	Sangrur: Sangrur-Dhanaula road, 236m	Y-SN1
Red flowered	Muktsar: Dhani Gurjant Singh, Sotha road, Doda, 184m	R-MK1
	Fazilka: Amarpura to khandwalla road, 177m	R-FZ1
	Mansa: Phulwal Dogra, 212m	R-MN
	Patiala: Punjabi University campus, 249m	R-PT1
Orange flowered	Barnala: Badbar Bir, 228m	O-BR2
	Fazilka: Amarpura Fields, 177m	O-FZ2
	Muktsar: Abul khurana, 184m	O-MK2
	Faridkot: Maharaja Bir, 195m	O-FR1
	Bathinda: Raiya, 201m	O-BT1
	Mansa: Tahlian-field, 212m	O-MN2
	Patiala: Punjabi University campus, 249m	O-PT2

Table 2: Activity index of methanol extract (ME) and acetone extract (AE) of bark of plants of three morphotypes in Tecomella undulata

Bacteria		Yellow		Red				Orange						
		Y-BR1	Y-SN1	R-MK1	R-FZ1	R-MN1	R-PT1	O-BR2	O-FZ2	O-MK2	O-FR1	O-BT1	O-MN2	OPT-2
P. aeruginosa	ME	0.73	0.59	0.55	0.68	0.64	0.5	0.5	0.64	0.59	0.5	0.64	0.73	0.55
	AE	0.73	0.64	0.55	0.68	0.59	0.59	0.41	0.73	0.55	0.5	0.64	0.77	0.5
E. coli	ME	1.05	0.7	0.6	0.65	0.7	0.55	0.77	0.85	0.55	0.65	0.75	0.95	0.65
	AE	0.8	0.85	0.8	0.65	0.8	0.75	0.85	0.75	0.65	0.65	0.65	0.85	0.75
S. aureus	ME	0.77	0.68	0.64	0.68	0.73	0.55	0.64	0.73	0.64	0.64	0.73	0.77	0.77
	AE	0.64	0.82	0.77	0.64	0.77	0.68	0.73	0.73	0.59	0.59	0.59	0.77	0.68
B. subtilis	ME	0.5	0.35	0.0	0.42	0.42	0.35	0.35	0.42	0.42	0.46	0.5	0.58	0.42
	AE	0.54	0.42	0.42	0.46	0.46	0.42	0.38	0.5	0.46	0.42	0.46	0.65	0.42
K. pneumoniae	ME	0.84	0.63	0.0	0.63	0.68	0.53	0.58	0.68	0.63	0.58	0.63	0.84	0.63
	AE	0.79	0.89	0.79	0.68	0.84	0.74	0.79	0.74	0.68	0.58	0.63	0.94	0.68
Average [AI]	ME	0.78±0.2	0.59±0.14	0.36±0.33	0.61±0.11	0.63±0.12	0.5±0.08	0.57±0.16	0.66±0.16	0.57±0.09	0.57±0.08	0.65±0.1	0.77±0.14	0.60±0.13
	AE	0.7± 0.11	0.72±0.2	0.67±0.17	0.62±0.09	0.69±0.16	0.64±0.14	0.63±0.22	0.69±0.11	0.59±0.09	0.55±0.09	0.59±0.08	0.8± 0.11	0.61±.0.14

In plants of yellow morphotype the range of average activity index against all test organism was between 0.59± 0.14 to 0.78± 0.2 [methanol extract] and between 0.70± 0.11 to 0.72± 0.2 [acetone extract]. The maximum AI value was against *E. coli* followed by *K. pneumoniae* and *S. aureus* [Fig 1].

The methanol extract of the bark of accession collected from Barnala [Y-BR1] showed better antimicrobial activity [Table 2]. Further, the performance of acetone extract of bark was better than methanol extract in yellow morphotype except in *E. coli* where methanol extract showed more activity and in *S. aureus* where both acetone and methanol extract were equally effective.

The bark extract of four plants of red flowered morphotype was evaluated for antimicrobial action. The study showed the overall AI values in the range of 0.36±0.33 to 0.63± 0.12 in methanol extract and 0.62± 0.09 to 0.069± 0.16 in acetone extract. All the collections of red morphotype behaved in almost similar fashion against tested organism except for the methanol extract of the bark of plant collected from Muktsar [R-MK1] that showed no activity against

Bacillus subtilis and *Klebsiella pneumoniae* [Table 2]. In red flowered morphotype, acetone extract of the bark of collected accessions was more efficient than methanol extract against all the tested organisms. Study of antimicrobial activity in the orange morphotype revealed the greatest intra-morphotypic variation with range of average activity index between 0.57± 0.08 to 0.77± 0.14 [methanol extract] and 0.55± 0.09 to 0.8± 0.11 [acetone extract]. Among the collected orange morphotypes the performance of a plant collected from Mansa area [O-MN2] was quite appreciable with highest average activity index of 0.77± 0.14 in methanol extract and 0.8±0.11 in acetone extract [Table 2].

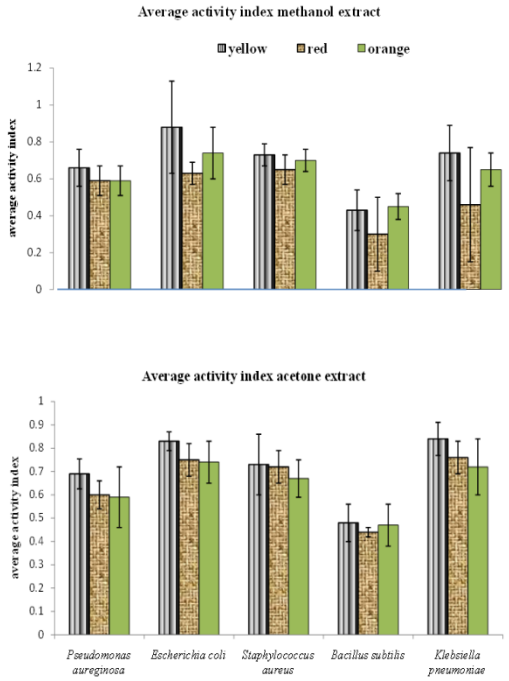

Fig. 1: Average activity index of bark extract of Tecomella undulata against each bacterium

Like the AI, performance of acetone and methanol extract of accessions of orange flowered morphotype also showed lot of variation. The efficiency of the two types of extracts was similar against *P. aeruginosa* and *E. coli.* However, against *B. subtilis* and *K. pneumoniae* acetone extract showed more activity than methanol extract while methanol extract performed better than acetone extract against *S. aureus*.

Overall comparison of the three morphotypes was done by comparing the average activity index against each bacteria strain [Fig1]. Out of the three morphotypes the bark of the yellow morphotypes showed highest antimicrobial activity against all the five bacteria which were comparable to standard antibiotic Chloramphenicol. The trend observed in average antibacterial activity among three morphotypes was yellow>red>orange in the acetone extract and yellow>orange>red in the methanol extract except against *B. subtilis* bacteria where the trend was yellow>orange>red [acetone] and orange>yellow>red [methanol]. On an individual basis one orange flowered tree growing at Mansa [O-MN2] exhibited maximum antimicrobial activity followed by yellow flowered plant at Barnala [Y-BR1] [Table 2]. Thanawala and Jolly [27] observed that *T. undulata* stem extract showed different response toward various organisms. There was no antimicrobial activity against *P. aeruginosa* [both extracts], *B. subtilis* [alcoholic extract], *E. coli* [acetone extract] and *S. aureus* [acetone extract]. Parekh and Chanda [4] observed *T. undulata* was more active against gram positive bacteria as compared to gram negative bacteria. Present study results revealed that both alcoholic [methanol] and acetone extracts of the 13 accessions collected were capable of inhibiting the growth of all the above mentioned bacteria except for methanol extract of one accession [R-MK1] which was not effective against *B. subtilis* and *K. pneumoniae*.

The leaf extracts [aqueous, acetone and methanol] did not show any antibacterial activity against any of the five bacteria used [*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*].

However, recently Kapoor and Bansal [28] and Sharma *et al.* [29] reported the antimicrobial potential of alcoholic extracts of leaves of *T. undulata.* Presently, none of the thirteen collections of three morphotypes of the species showed antibacterial action of leaf extract.

CONCLUSION

It is worth to mention here that the experimental results of the present study revealed that *Tecomella undulata* plants show great intraspecific variability in their antibacterial potential which may be attributed to genotype or environment or both. Variation observed in the antibacterial potential of the three morphotype could be considered as an important factor for herbal formulation as effectiveness of the herbal medicine may be influenced by the type of genotype and morphotype used for its preparation. Further, it suggests that there is need for testing and selection of superior genotype of this plant before recommending the same for medicinal purpose, multiplication, mass plantation or conservation.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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