



ANTI-MICROBIAL ACTIVITY OF *TECOMARIA CAPENSIS* LEAVES EXTRACT

Saini N.K.^{1*}, Singhal M.¹, Srivastava B.¹, Sachdeva K.¹, Singh C.

School of Pharmaceutical Sciences, Jaipur National University, Jagatpura, Jaipur-302025, India.

*Corresponding author's E-mail: pharmaniraj@hotmail.com

Accepted on: 25-01-2011; Finalized on: 04-03-2011.

ABSTRACT

The aim of the present study was to evaluate the potential anti-microbial activity of *Tecomaria capensis* leaves extract (TCLE) using in-vitro anti-bacterial and anti-fungal models. In antibacterial method, Test TCLE used at different concentration 2, 4, 6 and 8 mg/ml and STD Ciprofloxacin 5mcg. TCLE showed more potency compare to STD ciprofloxacin at 8 mg/ml concentration. In antifungal method, TCLE used at different concentration 2, 6 and 8 mg/ml and compared with positive control and negative control, it showed growth inhibition of fungus is increasing with increase in concentration. These results show that TCLE presents significant anti-microbial activity.

Keywords: *Tecomaria capensis*, anti-microbial, anti-bacterial, anti-fungal.

INTRODUCTION

Despite of tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. There impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance.¹ During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics² has lead to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages.³ Current research on natural molecule and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses.⁴ *Tecomaria capensis* (family: Bignoniaceae) also known as Cape-honeysuckle⁵ is a fast growing, scrambling shrub which may grow up to 2-3m high and spread more than 2.5m. *Tecomaria capensis* is an evergreen plant in warm climate areas but loses its leaves in colder areas. It has pinnately compound leaves that have oval leaflets with blunt teeth. Flowering time for this shrub is very erratic and often it flowers all year round. Flowers are orange in color. Plant is used as a traditional medicine to relieve pain and sleeplessness.⁶ Dried powdered bark infusions are taken for sleeplessness⁷, reported to induce sleep.⁸ It included in the list of African plants evaluated for *in vitro* antiplasmodial activity against *Plasmodium falciparum*.⁹

MATERIALS AND METHODS

Plant material

The leaves of *Tecomaria capensis* were collected from Jaipur, Rajasthan, India and a voucher specimen (RUBL 20847) for this plant material was preserved in the herbarium of Department of Botany, Rajasthan University, Jaipur. The leaves, dried in shade were

powdered and subjected to soxhlet with methanol for 72 hr. The extract collected was evaporated (yield 26.66% w/w), and stored in a vacuum desiccator. The preliminary phytochemical investigations (table 1) with the methanolic extract revealed the presence of flavonoids, flavones, phenolic compound, tannins, volatile oil, fixed oil, steroids, saponins, glycosides.

Drugs and chemical

The following drugs namely Ciprofloxacin and chemicals methanol, DMSO, PDA, NA were used during the experimental study.

Micro-organisms

Bacteria: *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC29213), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (ATCC6633) and *Enterococcus faecalis* (MTCC439).

Fungus: Clinically isolates of *Alternaria alternata* and *fusarium solani* fungi.

In-vitro antibacterial activity (Well-diffusion method)

The determination of the inhibitory effect of the TCLE on test bacteria was carried out by agar well diffusion method.¹⁰ Bacterial cultures were grown at 37°C for 24 h in Nutrient Broth. The culture suspensions were adjusted by comparing against 0.5 McFarland standard 2-3×10⁸ CFU. Petri dishes with 10 ml of Nutrient Agar were prepared, previously inoculated with 100 µL of the culture suspension. The wells (7.0 mm) were made and the TCLE and reference (ciprofloxacin, 5mcg) which is dissolved in DMSO was added to wells (100 µL) and same volume (100 µL) of DMSO was used as a control. The inoculated plates were incubated for 24 h. After incubation, the diameter of the inhibition zone was measured with calipers. The measurements were done basically from the edge of the zone to the edge of the well.



In-vitro antifungal susceptibility

The procedure for the PDA method.¹¹ PDA powder was dissolved in distilled water to a final concentration of 39g/liter and then sterilized at 121°C for 15 min. The sterilized PDA solution was placed in a water bath, and the temperature was cooled to and maintained at 55 to 60°C. The antifungal agent stock solutions were mixed with the PDA solution to produce a series of different final concentrations as 2%, 6% and 8%. Drug-free agar containing only 1% DMSO was used as a positive control and without inoculation one plate used as negative control. The mixtures of antifungal agent and PDA solutions were poured directly into the plates. After the plates were cooled to room temperature, freshly made fungal suspension (5×10^3 to 2×10^4 /ml) was inoculated onto the agar plate. The plates were incubated aerobically at 35°C until the 7 days and then measure the growth of the fungi on the plate.

RESULTS

Table 1: Preliminary phytochemical investigations with the methanolic extract

TEST	OBSERVATION
Alkaloids	-
Carbohydrates	-
Flavonoids	+
Flavones	+
Phenolic compound	+
Tannins	+
Coumarins	+
Volatile oils	+
Fixed oil and fats	+
Steroids	+
Proteins	-
Saponins	+
Glycosides	+

In-vitro antibacterial activity (Well-diffusion method)

Five bacteria were tested for their sensitivity to TCLE, antibacterial potency was determined by the well diffusion method. Table 2 presents diameters of inhibition zones (clear zones around wells) exerted by the

various concentrations of TCLE and standard ciprofloxacin. Concentration of TCLE 8mg/ml shown more potency then compare to standard Ciprofloxacin.

In-vitro antifungal susceptibility

Two fungi were tested for their sensitivity to TCLE, antibacterial potency was determined by the antifungal susceptibility method. Table 3 presents diameters of growth zone of fungi exerted by the various concentrations of TCLE. We have seen that increase in concentration of extract inversely proportional to increase in zone of growth. We compared the result with positive control.

DISCUSSION

Medicinal plants have been used for ages in the treatment of diseases.¹² In recent years, herbal medicines have increasingly been using to treat infections difficult to manage.¹³ Plants are known to produce certain chemicals which are naturally toxic to bacteria, and a large body of literature has validated the antimicrobial activity of plant extracts.^{14,15}

Plant contains phenolic compounds. Most of the studies on the mechanism of phenolic compounds focused on their effects on cellular membranes. Actually, phenolics not only attacked cell wall and cell membrane, thereby destroying its permeability and releasing of intracellular constituents (ribose, Na glutamate, etc.) but also interfered with membrane function e.g. electron transport, nutrient uptake, protein and nucleic acid synthesis and enzyme activity. That was, active compounds might have several invasive targets which could lead to inhibit the bacteria. Furthermore, leakage of intracellular material was a general phenomenon induced by many antibacterial substances, the level of leakage from *Escherichia coli* and *Staphylococcus aureus* observed was in correlation with the concentration of phenolics present.^{16,17}

TCLE showed very good antibacterial activity against *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC29213), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (ATCC6633) and *Enterococcus faecalis* (MTCC439) and antifungal activity against *Alternaria alternata* and *fusarium solani*. So, TCLE can be considered as a good antimicrobial agent.

Table 2: Anti-bacterial Activity of TCLE by compare with ciprofloxacin as a standard drug

S. No	Bacteria	Zone of inhibition (In mm)				
		TCLE 2mg/ml	TCLE 4mg/ml	TCLE 6mg/ml	TCLE 8mg/ml	STD (Ciprofloxacin) 5mcg
1.	<i>Escherichia coli</i>	16±1.63	20±0.82	36±1.22	38±0.82	35±1.22
2.	<i>Staphylococcus aureus</i>	14±0.82	18±1.22	35±0.41	38±0.82	30±0.82
3.	<i>pseudomonas aeruginosa</i>	12±0.82	16±1.63	34±1.22	35±0.82	30±1.63
4.	<i>Bacillus cereus</i>	16±1.63	20±0.82	35±0.41	36±0.41	30±1.63
5.	<i>Enterococcus faecalis</i>	15±0.41	21±1.63	34±0.82	37±0.82	29±0.41

Presented data given in Mean±S.D., n =3



Figure 1: Antibacterial activity of TCLE using ciprofloxacin as a standard

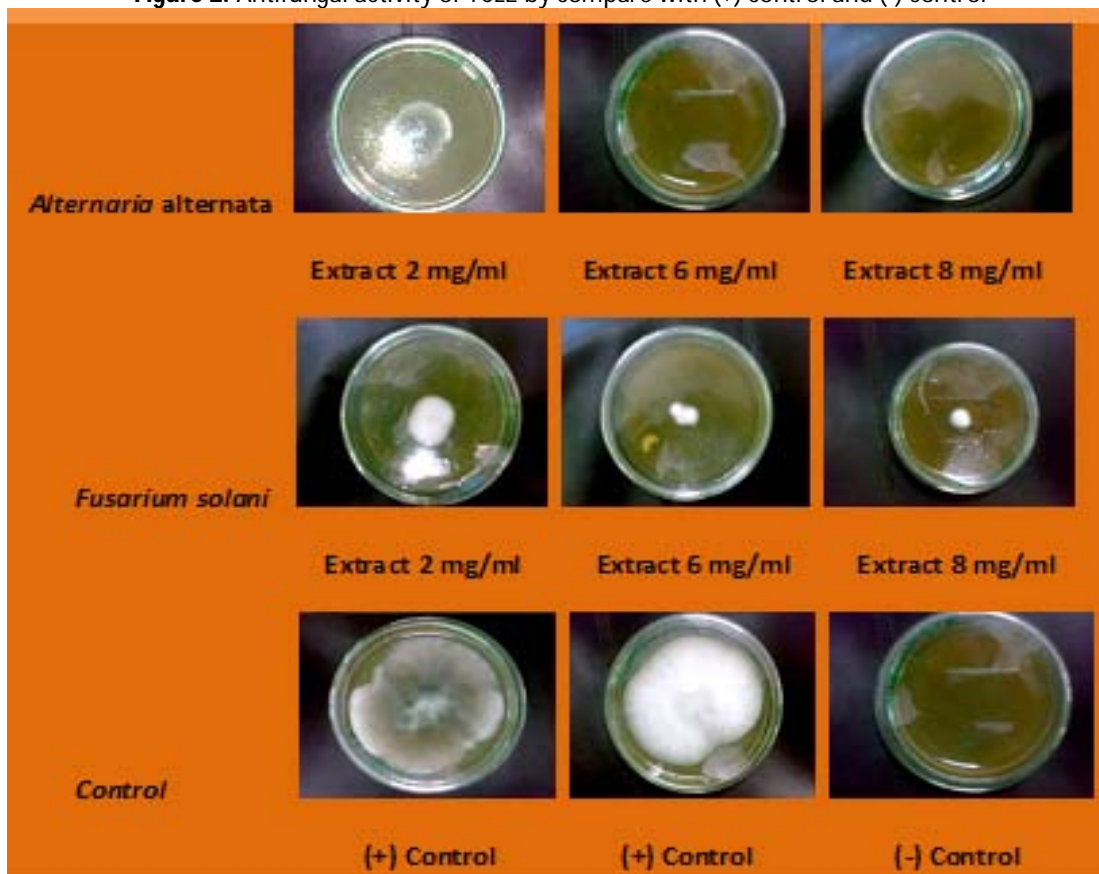


Table 3: Anti-fungal activity of TCLE by compare with positive control

S. No.	Fungi	Zone of growth (In mm)			
		Positive Control	TCLE 2mg/ml	TCLE 6mg/ml	TCLE 8mg/ml
1.	<i>Alternaria alternata</i>	81.17±0.94	37.5±1.22	No growth	No growth
2.	<i>Fusarium solani</i>	81.83±0.62	31.5±0.82	16.5±1.22	9±1.63

Presented data given in Mean±S.D, n =3

Figure 2: Antifungal activity of TCLE by compare with (+) control and (-) control



CONCLUSION

The methanolic extract of the *Tecomaria capensis* (TCLE) showed antimicrobial activity against both Gram negative and Gram positive bacteria and some species of fungus. According to the results of this study, the TCLE may be suggested as a new potential source of Broad spectrum natural antimicrobial.

REFERENCES

1. Zampini IC, Cuello S, Alberto MR, Ordonez RM, Almeida RD, Solorzano E, Isla MI, Antimicrobial activity of selected plant species from the Argentine puna against sensitive and multiresistant bacteria. *Journal of Ethnopharmacology*, 2009, 124: 499-505.
2. Okemo PO, Bais HP, Vivanco JM, *In vitro* activities of *Maesa lanceolata* extracts against fungal plant pathogens. *Fitoterapia*, 2003, 74: 312-316.



3. Bouamama H, Noel T, Villard J, Benharref A, Jana M, Antimicrobial activities of the leaf extract of two Moroccan *Cistus* L species. *Journal of Ethnopharmacology*, 2006, 104: 104-107.
4. Arora DS, Kaur GJ, Antibacterial activity of some Indian medicinal plants. *Journal of Natural Medicine*, 2007, 61:313-317.
5. USDA, ARS, National Genetic Resources Program, *Germplasm Resources Information Network - (GRIN)* [Online Database], National Germplasm Resources Laboratory, Beltsville, Maryland. URL: <http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?7183> (10 December 2010).
6. South African National Biodiversity Institute's (SANBI) Integrated Biodiversity System, information URL:<http://www.plantzafrica.com/planttuv/tecomar.cap.htm> (10 December 2010).
7. Roberts, M., Indigenous Healing Plants. Halfway House. Southern Book Publishers, 1990.
8. Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg, 1996.
9. P. Pillaya, V.J. Maharaj, P.J. Smith, Investigating South African plants as a source of new antimalarial drugs, *Journal of Ethnopharmacology*, 2008, 119 438–454.
10. Kalembe, D., Kunicka, A., Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry*, 2003, 10, 813–829.
11. National Committee for Clinical Laboratory Standards, Reference method for broth dilution antifungal susceptibility testing of yeasts. Proposed standard M27-P. National Committee for Clinical Laboratory Standards, Villanova, 1992, Pa.
12. Penalver P, Huerta B, Borge C, Astorga R, Romero R, Perea A. *APMIS* 2005;113:1.
13. Lin RD, Chin YP, Lee MH. *Phytother Res* 2005;19:612.
14. Basile A, Giordano S, Lopez-Saez JA, Cobiainchi RC. *Phytochemistry* 1999;52:1479.
15. Pesewu GA, Cutler RR, Humber DP. *J Ethnopharmacol* 2008;116:102.
16. Denyer, S.P., Hugo, W.B. (Eds.), *Mechanisms of Action of Chemical Biocides; their Study and Exploitation*, The Society for Applied Bacteriology, Technical Series No 27. Oxford: Oxford Blackwell Scientific Publication, 1991, pp. 171–188.
17. Tassou, C., Koutsoumanis, K., Nychas, G.-J.E., Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. *Food Research International* 2000, 33, 273–280.

About Corresponding Author: Mr. Neeraj Kumar Saini



Mr. Neeraj graduated from Rajasthan University of Health Science's (RUHS), Jaipur and going to complete post graduation from Jaipur National University (JNU), Jaipur. In post graduation level his specialization is in Pharmacology.