STUDIES ON THE ANTIMICROBIAL ACTIVITY OF *TAMARINDUS INDICA* PLANT PART STEM AND THE PHYTOCHEMICAL ANALYSIS OF THEIR ALCOHOLIC AND AQUEOUS EXTRACT

[1]Pratibha Goyal, [1]Archana Srivastav and [2]Tejovathi Gudipati
[1]College of Life sciences CHRI Campus Gwalior
[2]VISM college, Turari, NH-75, Jhansi Road, Gwalior 475001

Abstract : Medicinal plants have enormous ability to synthesize wide variety of secondary metabolites with antimicrobial potential. *Tamarindus indica* plant belongs to leguminous family and its plant extract is rich source of antimicrobial agents. Qualitative analysis of alcoholic and aqueous extracts of *Tamarindus* stem revealed that the ethanol extract possessed alkaloids, flavonoids, tannins, saponins, carbohydrates, glycosides, protein and reducing sugars. while phytosterols and anthroquinones were absent. The methanol extract contained alkaloids, flavonoids, tannins, saponins, carbohydrates and reducing sugars ,while phytosterols, glycosides, protein were absence. Similarly phytochemical tests performed in stem aqueous extract indicated the presence of flavonoids, tannins, saponins, carbohydrates, reducing sugars and anthroquinones and absence of alkaloids, glycosides, protein and phytosterols. *In vitro* studies of antimicrobial property of *T. indica* stem ethanol, methanol and aqueous extracts against gram +ve bacterial strains *L. acidophilus, B. subtilis* and *S. aureus* and gram –ve bacterial strains *S. typhi* and *E. coli* were carriedout. The alcoholic and aqueous extracts showed the antimicrobial activity against all tested bacterial strains. Highest sensitivity was shown against *L. acidophilus* by aqueous extract and least against *S. aureus* by ethanolic extract.

Keywords: *Tamarindus indica*, Phytochemicals, Antimicrobial activity, Extracts, Bacterial strains

Introduction

Tamarindus indica L. (Tamarind) is a dicotyledonous tree plant belongs to family leguminosae and subfamily Caesalpiniaceae. In India *Tamarindus* is also known as Tetuli, Amli, Amali, Ambali, Ambli, Chinch, Chitz, Chinta, Imli, Nuli, Puli (Mishra *et al.*, 1997).

Tamarindus indica is rich in nutrients and is used as food ingredient. It contains high level of crude proteins, carbohydrates, vitamin A, iron and minerals such as potassium, phosphorus, calcium and magnesium (Morton *et al.*, 1958, Mohamed *et al.*, 1992, Yanez *et al.*, 1995). Extract of *T. indica* were found to have anti- meracidial and anti- cercaial activites (El *et al.*, 1990) and parts such as pulp, flower, leaves, bark, stem are reported to be used as a herbal medicine and traditional food (Nikkin *et al.*, 2003). Studies of Doughari, (2006), Ucheechukwu *et al.*, (2011) and Warda *et al.*, (2010) on antimicrobial activity of leaf, fruit pulp, stem bark extracts reported wide spectrum of activity of *Tamarindus.* Alfatimi *et al.*, (2007) reported that *T. indica* methanolic extract of flower possess strong *in vitro* antibacterial activity.

In the present study we report phytochemical profile and *in vitro* antimicrobial activity of *Tamarindus* stem ethanol, methanol and water extracts.

Methodology

Tamarindus indica stem was collected from different locations of Gwalior and initially washed thoroughly under running tap water. Later the water was blotted using filter paper and then dried completely at room

temperature. The dried stem was crushed into powder in mechanical grinder. Ethanolic and Methanolic (70%) and aqueous extracts were prepared using 5g of powder in soxhlet apparatus and then vacuum evaporated. Finally 0.025g dry extract was dissolved in 5 ml DMSO and used for antimicrobial studies. Presence of phytochemicals such as alkaloids, Saponins, Tannins, Flavonoids, carbohydrates, and sterols was analyzed using the methods given by Trease and Evans (1989); Harbone, (1998); Sofowora, (1993).

Test Microorganism

In vitro antimicrobial property of the stem extracts was analyzed against three gram + ve bacterial strains – *Lactobacillus acidophilus*(MTCC 10307), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 3160) and two gram –ve bacterial strains - *Salmonella typhi* (MTCC 3224), *Escherichia coli* (MTCC 1610).

Culture media

Different selective media- BHIagar medium (*Lactobacillus acidophilus*) EMBagar medium (*E.coli*) Manitol salt agar medium (*Staphylococcus aureus*), Nutrient agar medium(*Bacillus subtilis* and *Salmonella enteric sub typhimurium*), Candida agar (*Candida albicans*) and Potato dextrose agar (PDA) (*Aspergillus niger*) were used for the maintenance of microbial cultures and also for their growth in broth. While Muller Hinton Agar media was used for antimicrobial property evaluation studies.

Preparation of Standard inoculums

All the bacterial cultures were grown on specific media in broth and the turbidity of the culture was compared visually to that of 0.5 McFarland standards, which corresponds to the cell concentration of approximately 108cfu/ml (Baker et al., 1983). The bacterial culture turbidity was compared visually to the McFarland standards and equal turbidity culture broth was used for the antimicrobial studies.

Antibiotics

The antibiotics disc hexa G+7 with tibiotics are Amphicillin (Amp), Cephalothin (Cep), Clindamycin (Cd), Erythromycin (E), Oxacillin (Ox), Vancomycin (Va) was used as standard for comparison of the antibacterial property of the *Tamarindus* stem extracts.

Data collection and analysis

In vitro antimicrobial property of all the standard drugs and extracts was analyzed using Well Diffusion Method. The diameter of inhibition zone (IZ) around the well was recorded in millimeters (mm) and the level of sensitivity depending on the zone of inhibition size. . The data on zone of inhibitions was obtained from experiments was pooled and the mean and standard deviation was calculated.

Results and Discussion

Qualitative profiling of phytochemicals alkaloids, flavonids, tannins, saponins, carbohydrates, proteins, reducing sugar, phytosteroles, glycosides, anthroquinones was carried out in *Tamarindus indica* stem extracts and the data is presented in Table 1. The phytochemicals Alkaloids, Flavonoids, Tannins, Saponins, Carbohydrates, Glycosides, Protein and Anthroquinones were present in ethanol extract while Phytosterols and Reducing sugars were absent. in stem methanol extract Alkaloids, Flavonoids, Saponins, Carbohydrates, Reducing sugar and Anthroquinones were present and Tannins, Phytosterols, Glycosides and Protein were absent. While, aqueous extract possessed Flavonoids, Saponins, Carbohydrates and Alkaloids, Tannins, Protein, Phytosterols Glycosides, Reducing sugars and Anthroquinones were absent.

Table-1: Qualitative profile of phytochemicals in *Tamarindus indica* stem ethanolic, methanolic and aqueous extracts.

		Stem Extracts

S. No.	Phytochemical Name	Ethanol	Methanol	Aqueous
1	Alkaloids	+	+	-
2	Flavonoids	+	+	+
3	Tannins	+	+	+
4	Saponins	+	+	+
5	Carbohydrates	+	+	+
6	Phytosterols	-	-	-
7	Glycosides	+	-	-
8	Protein	+	-	-
9	Reducing sugars	+	+	+
10	Anthroquinones	-	-	+

Qualitative and quantitative analysis of various phytochemicals presence in different extracts of *Tamarindus* aerial parts (Chopra et al. 1958; Warda et al. 2007) including leaf, (Ugoh et al. 2013; Uchechukwu et al., 2011, Doughri , 2006; Gumgumjee et al. 2012), stem bark (Ugoh et al. 2013; Uchechukwu et al., 2011, Doughri , 2006), fruit (Ugoh et al. 2013; Uchechukwu et al., 2011; Isha and Miland, 2012), seed (Ara et al., 2009; Rasheed, 2014; Isha and Miland, 2012) and root have been reported earlier. Reports on phytochemical profile in *Tamarindus* stem extracts are very few. In our study we observed presence of reducing sugars, carbohydrates, saponins, Tannins and flavonoids all the stem solvent extracts while phytosterols are absent in all the extracts.

Table-2: *In vitro* Sensitivity (inhibition zone size in mm) of five bacterial cultures to standard antibiotics present in Hexa g+7 (Hi media) antibiotic comb.

Antibiotics	Concentration	Inhibition zone size (Diameter in mm ± SD)				
		L. acidophilus	*B. subtilis*	*S. aureus*	*S. typhi*	*E. coli*
Amp	10mcg	30.4±1.21	0±0.0	0±0.0	11±3.08	0±0.0
Cep	30mcg	24.6±1.86	26±3.11	9.2±0.37	14.4±0.68	14±0.55
CD	2mcg	21.4±0.93	16.8±1.39	0±0.0	7±0.32	10.4±0.51
E	15mcg	23.6±1.12	24.4±0.51	0±0.0	12.4±0.51	11.4±0.24
OX	1mcg	21.8±1.71	13±0.84	0±0.0	0±0.0	0±0.0
Va	30mcg	17±1.87	17±1.67	0±0.0	0±0.0	5.4±0.40

Table -3: *In vitro* antimicrobial activity of ethanol, methanol and aqueous extracts of *T. indica* stem against bacterial strains

Strains name	Inhibition zone (IZ) size (in mm ± SD)								
	Ethanol extract con. (µg)			Methanol extract con. (µg)			Aqueous extract con. (µg)		
	50	100	200	50	100	200	50	100	200

L. acidophilus	5.4±0.24	8.4±0.51	11.4±0.24	0±0.0	10.6±0.75	12.8±0.20	6.2±1.85	11.6±0.81	13.6±0.40
	+	++	++++	-	+++	++++	+	++++	++++
B. subtilis	0±0.0	0±0.0	13±0.84	0±0.0	0±0.0	10±0.32	5.2±0.20	5.2±1.32	8.4±0.40
	-	-	++++	-		+++	+	+	++
S. aureus	0±0.0	0±0.0	8±0.71	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
	-	-	++	-	-	-	-	-	-
S. typhi	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	6.0±0.71	0±0.0	9.4±0.89	10±0.71
	-	-	-	-	-	+	-	+++	+++
E. coli	0±0.0	0±0.0	9±0.71	8.6±0.89	9.4±0.89	11.8±0.45	0±0.0	0±0.0	9.8±0.84
	-	-	++	++	+++	++++	-	-	+++

***In vitro* sensitivity**

The data on *in vitro* sensitivity of tested bacterial strains and their inhibition zone (IZ) for standard antibiotics and *Tamarindus* stem extracts is presented in Tables 2 and 3.

L. acidophilus showed highest sensitivity (++++) towards ethanolic, methanolic and aqueous extracts with 11.4±0.24 to 13.6±0.40 mm IZ. While, it has shown highest sensitivity to all the standard antibiotics with IZ17.0±1.87 to 30.4±1.21mm IZ, which is significantly more than the effect of stem extracts at 200µg/ml concentration (Table2,3).

The *B. subtilis* also recorded growth inhibition with all the extracts. The alcoholic extracts at 200µg concentration recorded 13.4±0.84mm and 10.0±0.32mm IZ and aqueous extract recorded 8.4±0.4 IZ. *B. subtilis* recorded more sensitivity to standard antibiotics in hexa g+ (except for amp), whose IZ was higher than the *Tamarindus* stem extracts.

S. aureus has shown 9.2 ±0.3712mm against antibiotic Cep (30mcg) and resistance to all other antibiotics in the comb. While it recorded 8.0±0.71mm IZ for ethanol stem extract of *Tamarindus*, indicating its efficiency over antibiotics Amp, CD, E, OX and Va in inhibiting *S aureus*. (Table2,3).

The bacterial culture *S. typhi* demonstrated sensitivity to four (Amp, Cep, CD and E) antibiotics with 7.0 to 14.4mm size of zone of Inhibition (Table2). It also recorded sensitivity to methanol (200µg) and aqueous (100-200µg) extracts with 6.0 to 10.0±0.71mm inhibition zone which is greater than standard antibiotics CD, OX and Va (Table2,3)

The *in vitro* antimicrobial activity of extracts against culture of *E.coli* was observed with ethanol (200µg), methanol (100-200µg) and aqueous (200µg) extracts. The zone of inhibition was 8.6±0.89 mm to 11.8±0.45 mm (table2). The culture recorded sensitivity to standard drugs Cep, CD, E and Va, whose zone of inhibition was between 5.4±0.40mm to 14.0±0.55 mm size, indicate that the stem extract of *Tamarindus* was better than Amp, OX , Va CD and E (Table 2,3).

Study suggested that Tamarind stem extract show the phytochemicals and that perform antimicrobial activity against bacterial strains. Sensitivity of bacterial strains against six commercial antibiotics , Amp showed the higher IZ against *L. acidophilus* and *S. aureus* showed the IZ for Cep. For the bacterial strains stem extract of *Tamarindus* showed antimicrobial activity. Aqueous extract showed highest antimicrobial activity than ethanolic and methanolic extract of stem. Stem extract of *Tamarindus* was more effective for gram +ve bacterial strains than gram -ve bacterial strains. *L. acidophilus* was highly sensitive for all extract and *S. aureus* was sensitive for only ethanolic extract. *S. typhi* was resistant for ethanol extract and sensitive for methanolic and aqueous extract. *B. subtilis* and *E. coli* was showed inhibition zone for all extract of *Tamarindus indica* stem.

Acknowledgement
All authors are grateful to MPCOST, Bhopal, for providing partial facilities to carry out the work and the first author is thankful to College of life Sciences, CHRI, Gwalior and VISM group of colleges, Turari, NH-9, Gwalior for providing facilities.

References

1. Doughari JH, 2006. Antimicrobial Activity of *Tamarindus indica* Linn. *Tropical Journal of Pharmacology Research*. 5(2):597-603.
2. Al-Fatimi M, Wurster M, Schroder G, Lindequist U, 2007. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *Journal of Ethnopharmacology*. 111:657-666.
3. Baker CN and Thormsberg CH, 1983. Inoculum Standardization in Antimicrobial Susceptibility Tests: Evaluation of Overnight Age Culture. *J. Clin Microbial.*17: 140-457.
4. Harbone. J. B. 1998. Phytochemical methods. A Guide in Modern Techniques of plants Analysis. Chapman and Hall Ltd, London Pp 182-190.
5. Mishra RN, 1997. '*Tamarindus Indica* L: An Overview of Tree Improvement', Proceedings of National Symposium on *Tamarindus indica* L; Tirupathi. India (A.P.), organized by Forest Dept. of A.P., India.
6. Morton FJ, 1958.Tamarind (*Tamarindus indica* L.) its food, medicinal and Industrial uses. *Florida State Horticultural Society*. Pp288-294.
7. Sofawora, E.A., 1993. Meditional Plants and Traditional Medicines in Africa Chichester John Wiley &Sons, New York. Pp97-145.
8. Trease, G.E. and Evans, W.C., 1989. Textbook of Pharmacognosy (11th Ed). Balliere Trinadal Can. Macmillan Publishers, London. Pp239-241.
9. Warda S, Abdel G, Fathia M and Amel OB. Antibacterial activity of *Tamarindus indica* fruit and *Piper nigrum* seed. *Research Journal of Microbiology*. 2(11): 824-830.
10. Yanez E, Zacarias I, Aguayo M, Vasquez M and Guzman E, 1995. Nutritive value evaluation Rats of new cultivars of common beans (*Phaseolus vulgaris*) released on Chile. *Plant Foods Human Nutrition.* 47:301–307.