

IN VITRO EVALUATION OF ANTIMICROBIAL PROPERTY OF TAMARINDUS INDICA LEAF ALCOHOLIC AND AQUEOUS EXTRACTS AGAINST SEVEN BACTERIAL AND FIVE FUNGAL STRAINS

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Abstract: In the present investigation an attempt has been made to study the antimicrobial effect of *T. indica* leaf extract against three gram +ve, four gram -ve and five fungal strains. This was compared to the standard antibacterial and antifungal drugs. The combi hexa g+7 (Himedia) containing six commercial antibiotics – Ampicillin (Amp), Cephalothin (Cep), Clindamycin (Cd), Erythromycin (E), Oxacillin (Ox), Vancomycin (Va) was used to study for bacterial strains and Strp (Streptomycin): Flu (Fluconazole): Itra (Itraconazole): Keto (Ketoconazole): Metro (Metroconazole) for fungal cultures. Ethanolic extract did not show any inhibition zone for all tested microorganisms. Methanolic extract has shown inhibitory effect against all the tested microorganisms except *Candida albicans*, *Candida glabrata*. While aqueous extract of leaf showed inhibition zone only against *S. aureus* and *E. coli*.

Key Words: *Tamarindus indica*, leaf extract, Antimicrobial, inhibition zone, Antibiotics

Introduction

All through history irrespective of culture, plants have been first and most important source of medicine (Stockwell, 1988; Thomson, 1978). The WHO estimated that 80% of the population of developing countries rely on traditional medicines mostly plant drugs for their primary health care needs. Modern pharmacopoeia shows at least 25% drugs derived from plants and many others are synthetic analogues built on prototype compounds isolated from plants (Bruneton et al., 1995). *Tamarindus indica* is a leguminous medicinal tree plant. In India Tamarind is also known as Tetuli, Amli, Amali, Ambali, Ambli, Chinch, Chitz, Chinta, Imli, Nuli, Puli (Mishra et al., 1997). The leaves are reported to contain proteins, fat, fiber and some vitamins like thiamine, riboflavin, niacin, ascorbic acid and β -carotene and some metabolites such as flavonoids and polyphenols (El-Siddig et al., 2006; Chitra, 1999). *Tamarindus* leaves have an extensive ethnobotanical use due to their antimicrobial and antiseptic effects (Khare et al., 1995; Melendez et al., 2007; Lans et al., 2007; Shankar et al., 2005). *Tamarindus* has

been used traditionally for curing stomach disorders, general body pain, jaundice, and also used as blood tonic and skin cleaner (Fabiya et al., 1993; Atawodi et al., 2002). Limited work has been reported on the antimicrobial property of *Tamarindus indica* (Doughari, 2006; Bhadoriya et al., 2011; Khanzada et al., 2008; Shital et al., 2010; Naznin and Monirul, 2009; Shanker et al., 2005; Uchechukwu et al., 2011; Ugoh et al., 2013) against limited microorganisms.

Material And Methods

The present study on antimicrobial property of alcoholic and aqueous extracts of *T. indica* - leaf was analyzed. The leaves were collected in bulk from different regions of Gwalior, M.P in 2012-2013 and 2013-2014 in different seasons.

Extraction procedure

The antimicrobial property of *T. indica* leaf was analyzed using three solvent extracts i.e. Ethanol (70%), methanol (70%) and Aqueous. All the solvent extracts were prepared using Soxhlet apparatus. About 5g of finely grounded powder of the samples was Soxhlet extracted in 200ml of solvent and the concentrated extract collected was vacuum dried.

About 0.025g of each sample was dissolved completely in 5ml DMSO and used immediately for anti microbial studies. The extracts were used at 50µg, 100µg and 200µg concentrations for the present work.

Tamarindus indica leaf was evaluated against seven bacterial strains (3 gram +ve bacteria)- Lactobacillus acidophilus (MTCC no 10307), Bacillus subtilis (MTCC no 441), Staphylococcus aureus (MTCC no 3160), (four gram -ve bacteria)- Escheria coli (MTCC no 1610), Salmonella typhi (MTCC no. 3224), P. aeruginosa (MTCC no. 6458), P. mirabilis (MTCC no. 425). All bacterial cultures were maintained on Nutrient agar medium. Five fungal strains- clinically isolated strain of Candida sp. (From the pus sample of a patient at Department of Microbiology CLS, CHRI, Gwalior) and the type cultures - Candida albicans(MTCC 3017), Candida glabrata(MTCC3019), Candida krusei(MTCC9215), and Aspergillus niger(MTCC 478) were used for present work. All the fungal cultures were maintained on Potato Dextrose Agar medium.

In vitro sensitivity test was used to analyze antimicrobial property. This was done on MHA (Muller-Hinton agar) medium. All microbial culture suspension were prepared according to McFarland standard.

Each extract was used at 50, 100 and 200µg concentrations. The inhibition effect was compared to standard drugs.

Each experiment was done in duplicate and each experiment was repeated twice. The data on inhibition zone (IZ) was pooled and mean and standard error was calculated. According to the size of IZ diameter (in mm), the sensitivity of each microorganism was grouped.

Results

Sensitivity of bacterial strains against six commercial antibiotics

The comb hexa g+7 (Himedia) containing six commercial antibiotics - Amphicillin (Amp), Cephalothin (Cep), Clindamycin (Cd),

Erythromycin (E), Oxacillin (Ox), Vancomycin (Va) was used in this study. The data on the mean size of the inhibition zone (IZ) with standard error and the level of sensitivity of each bacterial strain against each antibiotic is given in the Table 1.

The results presented in the Table 1 clearly shows that L. acidophilus was very highly (++++) sensitive to all the antibiotics tested. The diameter of IZ varied from 17.0±1.87mm to 30.4±1.21mm. The IZ size against amp, Cep, CD, E, OX and VA was 30.4±1.21mm, 24.6±1.86, 21.4±0.93, 23.6±1.12, 21.8±1.71 and 17±1.87mm respectively (Table 1) Similarly B. subtilis also recorded very high sensitivity (++++) against all the antibiotics used except the ampicillin (Table 1). The least IZ was recorded as 13.0±0.84mm against OX and maximum inhibition zone of 26±3.11mm recorded against Cep. The inhibition zone against CD, E and VA was 16.8±1.39mm, 24.4±0.51 and 17±1.67mm respectively (Table 1). P. aeruginosa and P. mirabilis recorded sensitivity for only two antibiotics. The IZ was 7.5±0.50mm against CD and maximum inhibition zone of 9.0±1.00mm recorded against Cep. Similarly P. mirabilis also recorded 8.5±0.50mm IZ against Cep and maximum inhibition zone of 9.5±0.50mm recorded against E (Table 1).

Sensitivity of fungal strains against five selected antidrugs

Fungal cultures C. albicans and C. glabrata showed high to very high sensitivity against streptomycin and Fluconazole. C. albicans recorded growth inhibition up to 10.2±3.80 mm and 18.2±0.58 mm and C. glabrata recorded growth inhibition up to 12.4±0.51mm and 11.0±0.50mm against Streptomycin and Fluconazole respectively. C. albicans and C. glabrata were also inhibited by drug Metroconazole and they demonstrated 6.4±0.51 mm and 10.4±0.81 mm of inhibition zone respectively against this drug. For the rest of the drugs these fungal cultures recorded resistance. C. krusei was sensitive to only

itraconazole and showed 9.20 ± 0.37 mm (+) zone of inhibition. Similarly, *Candida* sp. was resistant for all the drugs except for ketocanazole. It recorded 6.8 ± 0.37 mm zone of inhibition. While *C. glabrata* has demonstrated resistance against ketocazole and Itracozole. But it was sensitive to Streptomycin (12.4 ± 1.14 mm), Fluconazole (11.0 ± 1.58 mm) and metroconazole (10.4 ± 1.82 mm) (Table 2). The fungal culture *A. niger* demonstrated high sensitivity to Fluconazole (16.2 mm IZ) and minimum sensitivity to Metroconazole (5.4 ± 0.24 mm IZ). But *A. niger* was resistant to rest of the drugs tested (Table 2).

In vitro studies on antimicrobial property of *T. indica* plant leaves

Tamarindus indica is an evergreen tree and its leaves were used in herbal medicine and also in traditional Indian food. In the present study, the antimicrobial property of leaf was studied using three solvents - ethanolic, methanolic and aqueous extracts. The data on zone of inhibition (mean in mm \pm SE) was calculated and given in Table 3, 4 and 5.

The Table 3, representing the data of *T. indica* leaf ethanol extract results clearly shows that ethanolic extract has completely failed to inhibit the growth of any of the bacterial strains tested. It has also failed to inhibit the fungal growth too.

The methanolic extract results, given in the Table 4, have shown relatively high antimicrobial activity against the *L. acidophilus*, *B. subtilis*, *E. coli* and *S. aureus*. At low concentration ($50 \mu\text{g}$) methanolic leaf extract has resulted minimum (+) inhibition against *L. acidophilus* with IZ 5 ± 0.0 mm. At 100 and $200 \mu\text{g}$ concentrations this bacterial culture exhibited high (+++) to very high (++++) sensitivity with 9.4 ± 0.60 mm and 11.8 ± 0.37 mm zone of inhibition respectively (Table 4).

B. subtilis demonstrated minimum (+) to high (+++) sensitivity to methanolic extract at 50 and $100 \mu\text{g}$ of methanolic extract. Size of the IZ was

5.0 mm to 9.4 ± 0.81 mm (Table 4). *E. coli* culture at $100 \mu\text{g}$ and $200 \mu\text{g}$ concentrations has shown medium (++) to high (+++) sensitivity and the IZ was recorded as 7.2 ± 0.20 mm and 9.4 ± 0.60 mm respectively. Similarly, the methanolic leaf extract has demonstrated high (+++) to very high (++++) antimicrobial effect against *S. aureus* at $100 \mu\text{g}$ and $200 \mu\text{g}$ concentrations with 10.0 ± 0.55 mm ($20 \mu\text{l}$) and 11.0 ± 0.32 mm ($200 \mu\text{g}$) IZ. *S. typhi* did not show any inhibition effect (Table 4). *P. mirabilis* show sensitivity at $200 \mu\text{g}$ concentration and IZ was with error 6.5 ± 0.71

Leaf methanolic extract has no antimicrobial property against fungal cultures *C. albicans* and *A. niger*. Strains *C. krusei* and *Candida* sp. have shown inhibition at $100 \mu\text{g}$ and $200 \mu\text{g}$ concentration with 7.50 ± 0.50 , 11 ± 1.00 and 5.5 ± 0.50 , 9 ± 1.00 mm IZ respectively (Table 4).

The Table 5 gives result of *Tamarindus* leaf aqueous extract show limited antimicrobial property. It has failed to inhibit the growth of *L. acidophilus*, *B. subtilis*, *S. typhi* *C. albicans* or *A. niger* at all concentrations ($50 \mu\text{g}$ $100 \mu\text{g}$ and $200 \mu\text{g}$) tested (Table 5). It has completely failed to inhibit any of the fungal strains tested. Leaf aqueous extract inhibited only *E. coli* growth up to 10.0 mm at both $100 \mu\text{g}$ and $200 \mu\text{g}$ concentration and up to 5.0 ± 1.26 mm size for *S. aureus* culture at $200 \mu\text{g}$ concentration (Table 5).

Discussion

Medicinal plants are rich source of antimicrobial agents. This may be attributed to their ability to synthesize limitless number of phytochemicals. Measuring microbial growth and their inhibition by plant crude extracts or purified compounds by well diffusion method against different types of pathogenic microorganism and analyzing the zone of inhibition around the well, against each pathogenic microorganism, is the most common and primary step for the antimicrobial activity studies (Fooks and Gibson, 2002; Savadogo et al., 2004). The antimicrobial property of *Tamarindus* plant part extracts to various gram +ve, gram -ve bacterial and fungal cultures were reported earlier by Melendez et al., 2006,

Doughari et al., 2006; Ucheechukwu et al., 2011. Ugoh et al., 2013; Shital et al., 2010, Julio et al.,; Dipali et al., 2010; Shaymaa et al., 2014).

Isu (2005), Doughari (2006) and Aram et al., (2014) reported the antimicrobial property of Tamarindus aqueous extracts on E. coli. Julio et al. used leaf, stem bark and fruit, water and ethanolic extracts and demonstrated that Tamarindus has broad spectrum antimicrobial property. Doughari, 2006 evaluated the antimicrobial property of stem bark and leaf, acetone, water and ethanol extracts against 13 microorganism. The studies reveal that S. paratyphi and B. subtilis showed the lowest and S. aureus showed the highest MIC and MBC and leaf extracts generally showed lower activity compared to bark extract. He also reported no mycotic activity of T. indica extract against A. flavus, A. fumigatus, A. niger and C. albicans.

Leaf aqueous extract at 10mg/ml concentration recorded promising antimicrobial activity against E. coli, S. aureus, S. paratyphi, B. subtilis, A. niger and C. albicans (Aram et al., 2014). Dipali et al., 2010 reported low antifungal activity of pulp extract against A. niger. While Julio et al., 2008 recorded that C. albicans are resistant to all Tamarindus aqueous and hydro alcoholic extracts. In the present study, leaf extracts used at 50-200µg concentration, we have seen no antimicrobial activity of ethanolic leaf extract against all tested 12 microorganism. S. typhi and P. aurengosa are resistant to leaf methanolic extract. Similarly methanolic extract has no inhibitory effect on Candida sp., C. glabrata and A. niger. The aqueous has only inhibitory activity against E. coli and S. aureus.

Conclusion

The Tamarindus leaf has demonstrated antimicrobial property against various microorganisms tested. It has shown broad antimicrobial activity against E. coli, S. aureus, L. acidophilus, B. subtilis, P. mirabilis, C. kursei and Candida sp. at 50-

200 µg concentrations. The concentration increase may probably inhibit rest of the microbial growth. Further screening of leaf extract for phytochemicals and their qualitative and quantitative analysis may through light on the probable compounds that can be used in drug development.

References

1. Aram Abuzied, Mawadda Adam, Rashid Eltayeb Abdalla, Abu baker Osman Uro, Amel Ali Suleiman, (2014). The Antimicrobial effect of Aqueous extract of Tamarind (*Tamarindus indica*) Leaves. *Journal of Biomedical and Pharmaceutical Research*. Vol. 3, 141-146.
2. Atawodi, S.E., Ameh, D.A. and Ibrahim, S. (2002). Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. *Journal of Ethnopharmacology*, 79(2): 279-282.
3. Bruneton, J.; Hatton, C.K. (1995) *Pharmacognosy, translator Paris Lavoisier publisher.*, 30-35.
4. Chitra Arya. (1999) Post Infectious Changes in Flavonoids and Phenolic Acids by Fungal Pathogens in *Tamarindus indica* L. *Res J Chem Environ.* 3:305-17.
5. Dipali Y. Jadhav, Akshaya K. Sahoo, Jai S. Ghosh, Rahul C. Ranveer and Aruna M. Mali, (2010). Phytochemical Detection and in vitro Evaluation of Tamarind fruit Pulp for potential Antimicrobial Activity;; 5(3): 68-72.
6. Doughari JH, (2006). Antimicrobial Activity of *Tamarindus indica* Linn. *Trop. J. Pharm. Res.* 5(2):597-603.
7. El-Siddig, Gunasena, Prasad, Pushpakumara, Ramana, Vijayanand, Williams. (2006). *Tamarind, Tamarindus indica*. Southampton, UK: Southampton Centre for Underutilised Crops.
8. Fabiyi, J.P., Kela, S.L., Tal, K.M. and Istifanus, W.A (1993). Traditional therapy in the State of Bauchi-Nigeria. *Journal of Medicinal Plants*, 38(2): 193-195.

9. Fooks, L.J. and G.R. Gibson (2002). *In vitro* investigations of the effect of probiotics and prebiotics on selected human intestinal pathogens. *FEMS Microbiol. Ecol.*, 39:67-75.
10. Isu, N.R., (2005). Antibacterial effect of *Aframomum meleguata*. *Nig. J. Nat. Prod. Med.*, 9: 22-25.
11. Julio Cesar Escalona- Arranz, Renato Peres-Roses, Imilci Urdaneta- Laffita, Miladis Isabel Camaacho- Pozo, Jesus Rodriguez- Amado and Irina Licea- Jimenez, (2010). Antimicrobial activity of extract from *Tamarindus indica* L. leaves *Pharmacogn mag.* 6(23): 242-247.
12. Khanzada SK, Shaikh W, Sofia S, Kazi TG, Usmanghani K, Kabir A, Sheerazi TH (2008). Chemical constituents of *Tamarindus indica* L. Medicinal plant in Sindii. *Pak. J. Bot.*; 40: 2553-2559.
13. Khare CP , (2004). *Ayurvedic and other traditional usage*, Botany. 2nd ed. New Delhi: Springer Verlay;. *Encyclopedia of Indian medicinal plant- rational Western therapy*.
14. Lans, C. (2007). Comparison of plants used for skin and stomach problems in Trinidad and Tobago with asian ethnomedicine. *J Ethnobiol Ethnomed.*;3:102-9.
15. Melendez PA, Carriles VA. (2006). Antibacterial properties of tropical plants from Puerto Rico *Phytomedicine*; 13:272-6.
16. Melendez PA, Carriles VA (2006). Antibacterial properties of tropical plants from Puerto Rico *Phytomedicine*; 13:272-6.
17. Mishra RN. (1997). Tirupathi. India (A.P.). Jun 27-28, 'Tamarindus Indica L: An Overview of Tree Improvement', *Proceedings of National Symposium on Tamarindus indica L*; 1997 organized by Forest Dept. of A.P., India.
18. Naznin Ara and MD. Monirul Islam, (2009). Phytochemical screening and *In vitro* antimicrobial activity of *Tamarindus indica* seeds ethanolic extract. *Pakistan Journal of Pharmacology* Vol. 26. No. 1. Pp. 19-23.
19. Santosh Singh Bhadoriya, Aditya Ganeshpurkar, Jitendra Narwaria, Gopal Rai and Alok pal jain, (2011). *Tamarindus indica*: Extent of explored potential. *Pharmacogn Rev.*, 5(9): 73-81.
20. Savadogo, A., C.A.T. Quattara, I.H.N. Bassole and A.S.Tarore, (2004). Antimicrobial activity of lactic acid bacteria isolated from Burkina Faso fermented milk. *Pak. J. Nutr.*, 3:174-179.
21. Shankar EM, Subhadra N, Usha AR (2005). The effect of methanolic extract of *Tamarindus indica* Linn.on the growth of clinical isolates of *Burkholderia pseudomallei*. *Indian J Med Res.*122:525-8.
22. Shankar EM, Subhadra N, Usha AR (2005). The effect of methanolic extract of *Tamarindus indica* Linn.on the growth of clinical isolates of *Burkholderia pseudomallei*. *Indian J Med Res.* ;122:525-8.
23. Shaymaa Fouad Rasheed, (2014). Antibacterial activity of *Tamarindus indica* seeds extract and study the effect of extract on adherence and Biofilm production of some bacteria. *International journal of Biological and Pharmaceutical Research*; 5(1):42-47.
24. Shital S. Waghmare, Dipali Y. Jadhav, Jai S. Ghose and Akshay K. Sahoo, (2010). Characterization of some Antimicrobial substances from Seed coat of *Tamarindus indica* Linn. *British Journal of pharmacology and Toxicology* 1(1): 29-32.
25. Stockwell, C. (1988). *Nature's Pharmacy*. CenturyHutchinson Ltd., London, UK. Pp 27-32.
26. Thomson, W.A.R. (1978). *Medicines from the Earth*. McGraw-Hill Book Co. Maidenhead, UK. Pp 632-640.
27. Uchechukwu U. Nwodo, Grace E. Obiyeke, Vincent N. Chigor and Anthony I. Okoh, (2011). Assessment of *Tamarindus indica* Extract for Antibacterial Activity; 12, 6385-6396.
28. Ugoh, Sylvanus Chukwudi and Haruna, Isa Mohammed, (2013). Phytochemical Screening and Antibacterial Activity of the Fruit and Leaf Extracts of *Tamarindus indica* Linn. *Rep. opinion*; 5(8):18-27.

Antibiotics	Concentration	Diameter of inhibition zone (in mm)													
		L. acidophilus		B. subtilis		S. aureus		S. typhi		E. coli		P. aeruginosa		P. mirabilis	
Amp	10mcg	30.4±1.21	++	-	-	-	-	11±3.08	++	-	-	-	-	-	-
Cep	30mcg	24.6±1.86	++	26±3.11	++	9.2±0.37	++	14.4±0.68	++	14±0.55	++	9.0±1.00	+	8.5±0.50	++
CD	2mcg	21.4±0.93	++	16.8±1.39	++	-	-	7±0.32	+	10.4±0.51	++	7.5±0.50	+	-	-
E	15mcg	23.6±1.12	++	24.4±0.51	++	-	-	12.4±0.51	++	11.4±0.24	++	-	-	9.5±0.50	++
OX	1mcg	21.8±1.71	++	13±0.84	++	-	-	-	-	-	-	-	-	-	-
VA	30mcg	17±1.87	++	17±1.67	++	-	-	-	-	5.4±0.40	+	-	-	-	-

Sensitivity levels- : +=5.0 to 7.0 mm; ++ = 7.1 to 9.0; +++ =9.1 to 11.0, ++++=11.1 above.

Table-1: Differential sensitivity test for Bacterial cultures against Antibiotic disk Hexa G +7.

Conc.	Antifungal drug	Diameter of inhibition zone (in mm)									
		C. albicans		C. glabrata		C. krusei		Candida sp.		A. niger	
100µg	Streptomycin	10.2±3.80	+++	12.4±0.51	++++	-	-	15.2±0.37	++++	-	-
	Fluconazole	18.2±0.58	++++	11.0±0.50	+++	17.40±0.51	++++	11.6±0.24	++++	16.2±0.37	++++
	Itraconazole	-	-	-	-	9.20±0.37	+	-	-	-	-
	Ketoconazole	-	-	-	-	-	-	6.8±0.37	+	-	-
	Metroconazole	6.4±0.51	+	10.4±0.81	+++	-	-	-	-	5.4±0.24	+

Sensitivity levels- : +=5.0 to 7.0 mm; ++ = 7.1 to 9.0; +++ =9.1 to 11.0, ++++=11.1 above.

Table-2: Differential sensitivity test for Fungal cultures against standard antifungal.

trains	Zone of Inhibition Ethanolic extract of leaves (in mm)					
	Concentration					
	50µg		100µg		200µg	
L. acidophilus	0±0.0	-	0±0.0	-	0±0.0	-
B. subtilis	0±0.0	-	0±0.0	-	0±0.0	-
S. aureus	0±0.0	-	0±0.0	-	0±0.0	-
S. typhi	0±0.0	-	0±0.0	-	0±0.0	-
E. coli	0±0.0	-	0±0.0	-	0±0.0	-
P.aeruginosa	0±0.0	-	0±0.0	-	0±0.0	-
P. mirabilis	0±0.0	-	0±0.0	-	0±0.0	-
C. albicans	0±0.0	-	0±0.0	-	0±0.0	-
C. glabrata	0±0.0	-	0±0.0	-	0±0.0	-
C. krusei	0±0.0	-	0±0.0	-	0±0.0	-
Candida sp.	0±0.0	-	0±0.0	-	0±0.0	-
A. niger	0±0.0	-	0±0.0	-	0±0.0	-

Sensitivity levels- : +=5.0 to 7.0 mm; ++ = 7.1 to 9.0; +++ =9.1 to 11.0, ++++=11.1 above.

Table-3: Differential sensitivity test for Bacterial and fungal cultures against Ethanol extract of T. indica leaves.

Strains	Zone of Inhibition Methanolic extract of leaves					
	Concentration					
	50µg		100µg		200µg	
L. acidophilus	5±0.0	+	9.4±0.40	+++	11.8±0.37	++++
B. subtilis	5±0.0	+	5±1.26	+	9.4±0.81	+++
S. aureus	0±0.0	-	10±0.55	+++	11±0.32	+++
S. typhi	0±0.0	-	0±0.0	-	0±0.0	-
E. coli	0±0.0	-	7.2±0.20	++	9.4±0.60	+++
P.aeruginosa	0±0.0	-	0±0.0	-	0±0.0	-
P. mirabilis	0±0.0	-	0±0.0	-	6.5±0.71	+
C. albicans	0±0.0	-	0±0.0	-	0±0.0	-
C. glabrata	0±0.0	-	0±0.0	-	0±0.0	-
C. krusei	0±0.0	-	7.50±0.50	++	11±1.00	+++
Candida sp.	0±0.0	-	5.5±0.50	+	9±1.00	++
A. niger	0±0.0	-	0±0.0	-	0±0.0	-

Sensitivity levels- : +=5.0 to 7.0 mm; ++ = 7.1 to 9.0; +++ =9.1 to 11.0, ++++=11.1 above.

Table-4: Differential sensitivity test for Bacterial and fungal cultures against methanol extract of T. indica leaves.

Strains	Zone of Inhibition aqueous extract of leaves					
	Concentration					
	50µg		100µg		200µg	
L. acidophilus	0±0.0	-	0±0.0	-	0±0.0	-
B. subtilis	0±0.0	-	0±0.0	-	0±0.0	-
S. aureus	0±0.0	-	0±0.0	-	5±1.26	+
S. typhi	0±0.0	-	0±0.0	-	0±0.0	-
E. coli	0±0.0	-	10±0.55	+++	10±0.77	+++
P.aeruginosa	0±0.0	-	0±0.0	-	0±0.0	-
P. mirabilis	0±0.0	-	0±0.0	-	0±0.0	-
C. albicans	0±0.0	-	0±0.0	-	0±0.0	-
C. glabrata	0±0.0	-	0±0.0	-	0±0.0	-
C. krusei	0±0.0	-	0±0.0	-	0±0.0	-
Candida sp.	0±0.0	-	0±0.0	-	0±0.0	-
A. niger	0±0.0	-	0±0.0	-	0±0.0	-

Sensitivity levels- : +=5.0 to 7.0 mm; ++ = 7.1 to 9.0; +++ =9.1 to 11.0, ++++=11.1 above.

Table-5: Differential sensitivity test for Bacterial and fungal cultures against Aqueous extract of T. indica leaves.