Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231– 6345 (Online)
An Open Access, Online International Journal Available at www.cibtech.org/sp.ed/jls/2014/04/jls.htm
2014 Vol. 4 (S3), pp. 74-78/Abdallah
Research Article

IN VITRO ANTIBACTERIAL ACTIVITIES OF THE CRUDE METHANOL EXTRACT OF *TAMARINDUS INDICA* L FRUIT PULP, A NATIVE DRINK FROM SUDAN

Emad M. Abdallah
Department of Laboratory Sciences, College of Sciences and Arts at Al-Rass, Qassim University, P. O. Box 53, Saudi Arabia
**Author for Correspondence*

ABSTRACT
The crude methanol extract of *Tamarindus indica* L. edible fruit pulp collected from Sudan was examined against eight referenced bacterial strains, including three Gram positives and five Gram negatives using agar-well diffusion method. The most sensitive bacteria were *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10876, *Proteus vulgaris* ATCC 49132 and *Staphylococcus epidermidis* ATCC 49461, respectively. The least senstive bacteria were *Klebsiella pneumonia* ATCC 27736 and *Salmonella enterica* ATCC 5174. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the most senstive bacteria towards tested extract was also evaluated, which was varying between 25-50 mg/ml for MIC and 50-100 mg/ml for MBC. *Tamarindus indica* L. fruit pulp is a potential source for antimicrobial agents and it is recommended for food industries as a juice and natural preservative.

Keywords: *Tamarindus Indica, Tamarind, Ardeb, Methanol Extract, Antibacterial Activity*

INTRODUCTION
On searching for new antibiotics or antibacterial compounds, due to the dramatic global prevalence of multidrug resistant bacteria, efforts should focus on alternative sources from natural antibacterial derived from plants. Antibacterial compounds extracted from plants may have advantages over synthetic drugs, since it decrease the side effects and increase the biocompatibility (Viswanad *et al.*, 2011). However, it is amazing that between 25 to 50% of recent pharmaceutical drugs are from plant source and none of them are used as antibacterial drug (Cowan, 1999). Many edible plant foods reported having natural antibacterial properties, such as garlic and onion (Elnima *et al.*, 1983), cayenne, green pepper, parsley (Wahba *et al.*, 2010) and many others.

Tamarindus indica L. which is commonly known as Tamarind is belonging to family Fabaceae, subfamily Caesalpinioideae, indigenous to Africa and Southern Asia with many applications in traditional medicine. It is widely used in traditional medicine for treatment of a variety of ailments such as cough, sore throat, dysentery, hemorrhoids and malaria (Abdel-Gadir *et al.*, 2007). In Sudan, the ripe fruit pulps of *Tamarindus indica* L., locally known as Ardeb are a famous and popular native food which is mostly consumed as a juice. It is distributed in central and southern parts of Sudan and employed in Sudanese folk medicine in treatments of constipation, malaria and jaundice (Khalid *et al.*, 2012). Tamarind pulp are widely used in domestic and industrial purposes, it has a great nutritional value, rich in phenolic content and antioxidant capacity with distinguished sweet acidic taste (De caluwe *et al.*, 2010). This study aimed to evaluate the antibacterial activity of the methanol extract of the ripe fruit pulps of *Tamarindus indica* growing in Sudan.

MATERIALS AND METHODS
Plant Collection
Fruit pulps of *Tamarindus indica* were collected from local markets in Khartoum, Sudan, authenticated by botanists at the herbarium of Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

Plant Extraction

Extraction was performed by maceration method as described by Samie *et al.*, (2005) with minor modification. The fruit of *Tamarindus indica* were crusted and seeds were removed. Pulps were dried for up to one month and crushed into small parts. 50 g from the crushed pulps was soaked in 500 ml of absolute methanol (Merck, Garmany) for 3 days in dark tighten bottle with frequent shaking. The macerate was filtered using filter paper (Whatman No.1), and evaporated to dryness under reduced pressure at 40^{o}C. The crude extract was collected in a beaker, covered with Parafilm (Pechiney, Chicago), foiled and kept in refrigerator until used.

Referenced Microbial Strains

Eight referenced bacterial strains were used in testing the antibacterial activity of the fruit pulp methanol extract of *Tamarindus indica*, five of them are Gram negatives (*Escherichia coli ATCC 25922*, *Salmonella enterica ATCC 5174*, *Klebsiella pneumonia ATCC 27736*, *Pseudomonas aeruginosa ATCC 27853* and *Proteus vulgaris ATCC 49132*) and three are Gram positives (*Staphylococcus aureus ATCC 25923*, *Staphylococcus epidermidis ATCC 49461* and *Bacillus cereus ATCC 10876*). All referenced bacterial strains were previously purchased from Watin-Biolife, KSA.

Inoculum Preparation

The purchased referenced bacterial strains were cultured following the manufacture's instructions. Bacterial cultures were identified and sub-cultured in sterile bottles containing Nutrient Broth (Scharlab, S.L., Spain) and incubated for 24 h at 37°. Working bacterial samples were adjusted to 0.5 McFarland to be equivalent to about 1–2 × 10^{8} CFU/ml.

Antibacterial Assay

The working methanol extract of *Tamarindus indica* L fruit pulp was prepared by dissolving 2 g of the crude extract in 10 ml of 10% Dimethyl Sulphoxide DMSO (Techno Pharmchem Haryana, India) to give a concentration of 200 mg/ml. The antimicrobial activity of the plant extract was tested using agar-well diffusion method as described by Abdallah (2014) with minor modification. Briefly, 25 ml of hot-autoclaved Muller Hinton Agar (Watin-Biolife, KSA) was loaded on a sterile Petri-dish (100×15 mm), and left to solidify at room temperature. After solidification, plates were kept upside down in the refrigerator until used. For each bacterial strain, 100 µl from fresh working bacterial samples adjusted to 0.5 McFarland was loaded and spread over the solidified Muller Hinton Agar plate. Then, wells were punched into agar using a sterile cork borer (6 mm in diameter), 100 µl of the plant extract (200 mg/ml) was dropped into one well and 100 µl of Chloramphenicol (5mg/ml) was dropped into another well. Also 100 µl of 10% DMSO was loaded to another well as a negative control. Plates were incubated for 24 h at 37 ^{o} C and investigated for inhibition zone around wells. Tests were repeated 3 times in order to calculate the mean zone of inhibition.

Determination of Minimum Inhibitory Concentration (MIC)

Only bacterial strains revealed inhibition zone above 10 mm were tested for MIC. It was performed with the broth dilution method as mentioned by El-Mahmood and Ameh (2007) with some modifications. The required numbers of sterile and autoclaved test tubes containing 1 ml of Nutrient Broth (Watin-Biolife, KSA) were prepared. 1 ml of the methanol extract of *Tmarindus indica* pulps (200mg/ml) was loaded to the first tube and mixed. Subsequently, serial two fold dilutions were made by transferring 1 ml from first tube to another one and so on; giving extract concentrations of 200, 100, 50, 25, 12.5, 6.25 and 3.123 mg/ml. 1 ml of fresh adjusted bacterial sample was loaded to each dilution. Also, 1 ml of Chloramphenicol (5mg/ml) was loaded to a nutrient broth tube as a positive control and 1 ml of 10% DMSO was loaded to a nutrient broth tube as a negative control. All tubes were incubated at 37°C for 24 h. Then tubes were examined for microbial growth by observing the turbidity. The lowest dilution with no visible growth was recorded as minimum inhibitory concentration.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC test was conducted as reported by Doughari (2006) with slight modification. From the MIC test, 100 µl from the tubes that showed no visible growth was loaded above a new previously prepared sterile plates containing nutrient agar (Watin-Biolife, KSA) and spread over the agar with a sterile swap.

Similarly, from MIC tubes, another two plates were inoculated with Chloramphenicol as appositive control and with 10%DMSO as a negative control. All inoculated plates were then incubated overnight at 37 ^{o}C and investigated for bacterial growth. Plates with no visible growth were considered as the minimum bactericidal concentration.

Statistical Analysis

Results were expressed as Mean ± standard error of means. For graphing, the statistical software SPSS (Vertion17) was employed.

RESULTS AND DISCUSSION

Results obtained in the current study showed that the methanol extract of *Tamarindus indica* L fruit pulp revealed presence of antibacterial properties against most tested bacterial strains compared to the antibiotic chloramphenicol (Table 1 and Figure 1). Based on the mean zone of inhibition, the most sensitive bacteria were *Pseudomonas aeruginosa* ATCC 27853 (12.0±0.5 mm), *Escherichia coli* ATCC 25922 (11.6±0.6 mm), *Staphylococcus aureus* ATCC 25923 (11.3±0.6 mm), *Bacillus cereus* ATCC 10876 (11.0±1.5 mm), *Proteus vulgaris* ATCC 49132 (11.0±0.5 mm) and *Staphylococcus epidermidis* ATCC 49461 (10.6±0.8 mm), respectively. The least sensitive bacteria were *Klebsiella pneumonia* ATCC 27736 and *Salmonella enterica* ATCC 5174 (9.6±0.3). Zone of inhibition above 10 mm is considered as good antibacterial activity (Abdallah, 2014). Accordingly, these results of the crude extract are promising when compared with the antibiotic chloramphenicol which is in pure form. The findings of this study are in harmony- partially- with Adeola *et al.*, (2010) who showed that *S. aureus*, *E. coil*, *B. subtilis* and *K. pneumonia* were sensitive to methanol extract of *Tamarindus indica* L fruit pulp. Another study published that ethanol extract of *Tamarindus indica* L fruit pulp have strong antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi* A and *Pseudomonas aeruginosa*, whereas aqueous extract produce weak antibacterial activity except against *Pseudomonas aeruginosa* (Daniyan and Muhammad, 2008). As well, Abdel *et al.*, (2007) mentioned that ethanol extract of *Tamarindus indica* L fruit pulp revealed higher antibacterial activity among other extracts against four different bacterial strains. Thus, further studies using different extraction solvents, followed by fractionation and purification of the candidate active compound (s) may lead to discovery of new antibacterial agents. Also, other different parts of *Tamarindus indica* L. possesses antibacterial activities, Doughari (2006) cited that the acetone extracts from stem park and leaves of *Tamarindus indica* showed an interesting antibacterial activities against different pathogens. Gumgumjee *et al.*, (2012) stated that among six different extracts of *Tamarindus indica* leaves, ethanol extract recorded considerable activity against both Gram negative and positive bacteria and fungi.

Table 1: Antibacterial activity of methanol extract of *Tamarindus indica* fruit pulp and chloramphenicol

Test	**Mean zone of inhibition (mm) of microorganisms (Mean±SEM)***							
	Ec	**Pa**	**Pv**	**Kp**	**Sal**	**Sa**	**Se**	**Bc**
***Tamarindus indica* 200mg/ml**	11.6±0.6	12.0±0.5	11.0±0.5	9.6±0.3	9.6±0.3	11.3±0.6	10.6±0.8	11.0±1.5
Chloramphenicol 5 mg/ml	37.0±0.5	18.0±2.0	32.3±0.6	31.3±0.8	34.0±0.5	33.0±1.5	36.6±0.3	28.0±0.5

**Mean ± Standard error of means (SEM), mm=millimeter;*
Ec = Escherichia coli ATCC 25922, Pa = Pseudomonas aeruginosa ATCC 27853, Pv = Proteus vulgaris ATCC 49132, Kp = Klebsiella pneumonia ATCC 27736, Sal = Salmonella enterica ATCC 5174, Sa = Staphylococcus aureus ATCC 25923, Se = Staphylococcus epidermidis ATCC 49461, Bc = Bacillus cereus ATCC 10876.

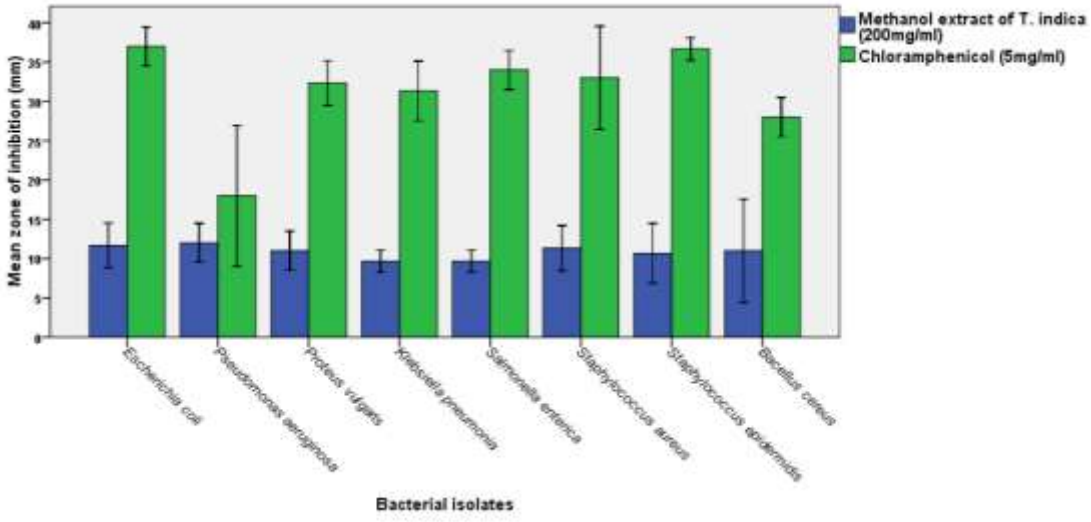

Figure 1: The antibacterial effect999+s of methanol extract of *Tmarindus indica* fruit pulp compared to chloramphenicol

As shown in Table 2, The MIC and MBC of sensitive bacteria 25-50 mg/ml for MIC and 50-100 mg/ml for MBC, confirming the antibacterial properties of the tested extract. The lowest MIC and MBC values indicated that the plant extract is highly effective and characterized by some inhibitory effects on bacteria. Although, ethanol extract of *Tamarindus indica* fruit pulps showed lower MIC and MBC than that reported in this study (Doughari 2006). Different extraction solvents collect different compounds or concentrations of antibacterial agents. However, it is believed that the effectiveness of the bioactive plants may be attributed to a combined action of different compounds present in these plants (Bai, 1990). Recently, the employment of plant products and extracts as remedies is enjoying great popularity even in developed countries due to increasing public awareness of the problems with the traditional antibiotics (Cowan, 1999). Therefore, scientists from different fields must give more attention to the applications of medicinal plants against different aliments.

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanol extract of *Tamarindus indica* fruit pulp

Referenced bacterial isolates	MIC (mg/ml)	MBC (mg/ml)
Escherichia coli ATCC 25922	25	50
Pseudomonas aeruginosa ATCC 27853	25	50
Proteus vulgaris ATCC 49132	25	50
Staphylococcus aureus ATCC 25923	50	100
Staphylococcus epidermidis ATCC 49461	25	50
Bacillus cereus ATCC 10876	25	50

Conclusion

This preliminary investigation describes the antibacterial activity of Sudanese Tamarind or Ardeb (*Tamarindus indica* L) fruit pulp, a famous indigenous plant in Sudan of many applications in food and Sudanese folk medicine. Tamarind was found to have antibacterial properties against broad spectrum bacteria. Intensive studies should be performed on this plant in order to obtain a new natural antibacterial agents form this plant of economic importance.

REFERENCES

Abdallah EM (2014). Antimicrobial properties and phytochemical constituents of the methanol extracts of *Euphorbia retusa* Forssk. and *Euphorbia terracina* L. from Saudi Arabia. *South Asian Journal of Experimental Biology* **4**(2) 48-53.

AbdelGadir WS, Mohamed F and Bakhiet AO (2007). Antibacterial activity of Tamarindus indica fruit and Piper nigrum seed. *Research Journal of Microbiology* **2**(11) 824-830.

Adeola AA, Adeola OO and Dosumu OO (2010). Comparative analyses of phytochemicals and antimicrobial properties of extracts of wild *Tamarindus indica* pulps. *African Journal of Microbiology Research* **4**(24) 2769-2779.

Bai D (1990). Traditional Chinese material: A respect and prospect. *Planta Medica* **56** 502.

Cowan MM (1999). Plant products as antimicrobial agents. *Clinical Microbiology Review* **12**(4) 564-582.

Daniyan SY and Muhammad HB (2008). Evaluation of the antimicrobial activities and phytochemical properties of extracts of *Tamaridus indica* against some diseases causing bacteria. *African Journal of Biotechnology* **7**(14) 2451-2453.

De Caluwé E, Halamová K and Van Damme P (2010). *Tamarindus indica* L. – A review of traditional uses, phytochemistry and pharmacology. *Afrika Focus* **23**(1) 53-83.

Doughari JH (2006). Antimicrobial Activity of *Tamarindus indica* Linn. *Tropical Journal of Pharmaceutical Research* **5**(2) 597-603.

El-Mahmood AM and Ameh JM (2007). In-vitro antibacterial activity of *Parkia biglobosa* (Jacq) root, bark extract against some microor-ganisms associated with Urinary tract infections. *African Journal of Biotechnology* **6**(11) 195-200.

Elnima EI, Ahmed SA, Mekkawi AG and Mossa JS (1983). The antimicrobial activity of garlic and onion extracts. *Pharmazie* **38**(11) 747-748.

Gumgumjee NM, Khedr A and Hajar AS (2012). Antimicrobial activities and chemical properties of *Tamarindus indica* L . leaves extract. *African Journal of Microbiology Research* **6**(32) 6172-6181.

Khalid H, Abdalla WE, Abdelgadir H, Opatz T and Efferth T (2012). Gems from traditional north-African medicine: medicinal and aromatic plants from Sudan. *Natural Products and Bioprospecting* **2** 92–103

Samie A, Obi CL, Bessong PO and Namrita L (2005). Activity profiles of fourteen selected medicinal plants from Rural Venda communities in South Africa against fifteen clinical bacterial species. *African Journal of Biotechnology* **4**(12) 1443–1451.

Viswanad V, Aleykutty NA, Zachariah SM and Prabhakar V (2011). Antimicrobial potential of herbal medicines. *International Journal of Pharmaceutical Sciences and Research* **2**(7) 1651-1658.

Wahba NM, Ahmed AS and Ebraheim ZZ (2010). Antimicrobial effects of pepper, parsley, and dill and their roles in the microbiological quality enhancement of traditional Egyptian Kareish cheese. *Foodborne Pathogens and Disease* **7**(4) 411-418.