

Studying the Antimicrobial Activity of Tamarind Extract (*Tamarindus indica* L.)

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Abstract

The present study included the preparation of *Tamarindus indica* extract (pulp and seeds) at different concentrations (25, 50, 70 and 100) mg/ml in the manner of 1:1 water –alcohol extraction. To determine the sensitivity of some positive and negative Gram bacteria to the different concentration mentioned above of the extract such as *E.coli* , *Pseudomonas*, *Staph aureus*, *Bacillus ceres* compared with antimicrobial Ampicillin, Tetracycline and Amoxicillin. Concentration of 100 mg/ml gave the highest rates of inhibition (22, 19, 18 and 17 mm) for the pulp extract respectively and for the same previous arrangement of the bacteria, while the diameter of the transparent halo decreased with the reduction of concentration of the tamarind extract compared with the control treatment, its inhibition was 5mm. On the other hand, the results of tamarind seed extract showed the highest inhibition for all types of bacteria used in the experiment with the highest rates of inhibition diameters (21, 22, 25 and 17 mm) for the highest concentration 100 mg/ml and the previous bacteria sequence. Tetracycline was given the highest rates of inhibition of bacteria in the same order (30, 24, 28 and 25 mm) respectively, while no inhibitory effect was observed for Ampicillin and Amoxicillin.

Keywords: antimicrobial, Tamarind extract, antibiotics

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1. Introduction

Tamarinds indica belongs to the legume, a corpus of horny of a rapidly growing evergreen tree (Kumar and Bhattacharya, 2008). The Tamarinds plant consists about 30 -50% pulp, 11-30% shell and 25- 40% seeds (El-Siddig *et al.*, 2006). They are becoming more important due to their used to treat different disease disturbed digestive systems dipter and other disease (Diapali *et al.*, 2010).

Food contaminated by different microbes that effect the food become unsecure health, for that reason many chemical types used as preservative for example acetic acid, lactic, ascorbic and benzoic acid .These acids are acumen lactase in the materials which become harmful. The used of plant extract are and more save to used in food (Chen *et al.*, 2013). For that reason the health environment control groups were materials for more save and secure. (Gupta *et al.*2010).

There were also side effect of antibiotics used to mix different strains of pathogenic may have a negative effect. Recent studies have given great importance to use alternatives to plant extract in inhibition (Cowan,1999).

bacterial because they contain chemical compounds that Tamarinds fruit is one of these fruits which is containing high percentage of the activity material for example organic acid (citric, tartaric and malic acid); potassium citrate and minerals (phosphorus, magnesium, iron, manganese, calcium, sodium, chlorine; also vitamin B3 and volatile oil also it contains antibacterial for different kinds of bacterial (El-Siddig *et al.*, 2006). The present study aimed at using extraction of Tamarind plant with antimicrobial activity, including positive and negative bacteria and comparing with antibiotics for their great importance present in order to use their extraction in food preservation, inhibition of pathogenic bacteria and in preserving the public health of the individual in our society.

2. Martials and methods

2.1 Tamarind extraction:

Tamarind fruits were brought from Basrah market. The pulp was hands crapped from the seeds .The pulp and crushed seed were dried at 40C° for 72 hours. Then 100g of each were soaked with 400 ml of water-alcohol solution (1:1) and left for 24 hours in the refrigerator with intermittent shaking. The mixtures were centrifuged at 3500r for 20 min. and finality filtered through what Mann filter paper No. 1 (Azoro, 2000) The supernatant were collected and concentrated under pressure in a rotary vacuum evaporator until semisolid residue were obtained according to the method mention by (Martinello *et al.*, 2006). There were dried inside the crucible under to obtain solid powder (Jonathan and Fasidi, 2003). One gram from each pulp and crush seeds powder extraction were dissolved by 5 ml of alcohol solution (1:1). These stokes solution become as 100% concentrate extraction. Different concentration levels 25, 50, 75 and 100 mg /ml were prepared from pulp and seed crushed solution extraction.

2.2 phytochemical analysis

The phytochemical tests were performed using various reagents. The purity of polysaccharide was determined by prescribed phytochemical tests, which indicated the absence of tannins, alkaloids, flavonoids, sugars, steroids,

phenols and saponins (Molan,1992).

2.3 Antibacterial activity

The propagation method was used on the center of Mueller-Hinton Agar (Okeke *et al.*, 2001). The pure isolates were activated for types of Gram positive and negative bacteria are *E.coli*, *Pseudomonas*, *Staph aureus*, *Bacillus cereus* in Nutrient broth medium Oxoid.

Antibacterial activity was test for the efficiency of antibacterial agents in the pulp and crushed seed extraction concentration and compared with amoxicillin, ampicillin, tetracycline and control. *Pseudomonas*; *Bacillus*; *E. coli* and *Staph aureus* were used as test microorganisms.

They were activated in nutrient broth by fermentation at 37 C° for 24 hours. A loop full of the bacteria that activated and enriched in nutrient broth were transferred to pepton water and emulsified to turbidity of McFarland 0.5 density. The final bacterial cell concentration approximated to 10⁸ g cfu /ml. leave the dishes to dry for 5-10 minutes after which the hole was pierced with 5mm diameter hole and 50 microliters of the prepared concentrations of tamarind pulp and seed extract (100, 75, 50 and 25 mg /ml) were placed in each hole. The control was only soul of alcohol solution (1:1). The dishes were then incubated at 37 C° for 24 hours and the inhibitory diameter was measured for each treatment (Gupta *et al.*, 2014). The test for inhibition of the samples obtained from pulp and seed extraction in relation to antibiotic and control was carried out with three repetitions. The average of the results for each were compared statistically.

2.4 Statistical analysis

Data in current study were subject to one-way analysis of variance (ANOVA) using XL Stat program for Windows. Differences Between the means were tested by Duncan’s multiple range tests. The level of significance was chosen at P<0.05 and the results are presented as mean (Steel *et al.*, 1996).

3. Results and Discussions

Phytochemical evaluation of pulp fruit extract Table 1 show the phytochemical screening of the tamarind pulp fruit extract . then samples revealed only tannins and flavonoids were positive , the tannins are mostly responsible for antioxidant properties and flavonoids are responsible for other properties . and all reagents are negative in these results . this results agreement with (GHONGADE, 2013).

Table 1 : Phytochemical evaluation of pulp fruit extract

phytoconstituent	results
Steroids	-
Tannins	+
Alkaloids	-
Spanning	-
Flavonoids	+
Glycoside	-
Protein and amino acid	-

Table 2 showed significant differences (**p> 0.05**) between the region of microbial inhibition zones of the different treatment.

Table (2) Effect of tamarinds fruits extract and antibiotics on pathogenic bacteria inhibitory

Con Type	Control	25	50	75	100	Amoxicillin	Ampicillin	Tetracycline
<i>Pseudomonas</i>	5 ⁱ	7 ^{ghi}	9 ^f	15 ^e	17 ^{de}	5 ⁱ	5 ⁱ	25 ^b
<i>Bacillus</i>	5 ⁱ	8 ^{fgh}	10 ^f	15 ^e	19 ^d	5 ⁱ	5 ⁱ	24 ^{bc}
<i>E.coli</i>	5 ⁱ	6 ^{hi}	10 ^f	15 ^e	18 ^d	5 ⁱ	5 ⁱ	28 ^a
<i>Staph aureus</i>	5 ⁱ	8 ^{fgh}	15 ^e	18 ^d	22 ^c	5 ⁱ	5 ⁱ	30 ^a
% of inhibitory								
<i>Pseudomonas</i>	-	40	80	200	240	-	-	400
<i>Bacillus</i>	-	60	100	200	280	-	-	380
<i>E.coli</i>	-	20	100	200	260	-	-	460
<i>Staph aureus</i>	-	60	200	260	340	-	-	500

Means with different letters in the same are significantly different (p<0.05) for each sample

The a gradual were increased of pulp extracted 25, 50, 75 and 100 mg/ml as compared with the control were 8, 15, 18, 22 mm. The best effected at 100 mg/ml. *Staph aureus* in the diameter of the inhibition area zones as compared with *Pseudomonas*, *Bacillus*, *E.coli* and the control. *Staph aureus* had a highly significant effectiveness at 100 mg/ml concentrated to all microbial diameter inhibition (mm) percentage. It was 340% as compared with 280%, 260%, 240% and zero *Bacillus*, *E.coli*, *Pseudomonas* and the control respectively. Table 2 also showed a

significantly differences in the rates of tetracycline diameter inhibitors area to *Staph aureus* as compared with *E.coli*, *Pseudomonas*, *Bacillus* and the control while there was no inhibition of the Ampicillin and Amoxicillin antibiotic used in the inhibition diameter parentage to the tetracycline was 500% as compared with 460%, 400%, 380% and zero in the following image 1 showed diameter (mm) the inhibition area of *E.coli*, *Pseudomonas*, *Bacillus* and *Staph aureus*. These results were in agreement with Gungumjee *et al.*, (2012) who indicated that the tamarinds leaves extract inhibited effectiveness (25, 15, 15 and 20 mm) to bacteria *Pseudomonas aeruginosa*, *E. coli*, *Staph aureus* and *Bacillus subtilis* respectively. Abdul Kapur and Ahmed John (2014), indicated that the extract of tamarind plant has anti-bacterial positive and negative effects suggested that organic acids, hydrogen peroxide and other substances were responsible for the inhibition. The results in table 2 showed the inhibitory effect of the extract of seeds of tamarind, which increased significantly by increasing the concentration of 100 mg/ml gave the highest significant difference ($p > 0.05$) in the trans of inhibition 25 mg \ml of *Staph aureus* bacteria as compared with other 22, 21, 17 and zero mm diameters inhibition to *Pseudomonas*, *E.coli*, *Bacillus* and control, then image 2 showed diameter (mm) the inhibition area at seed extraction.

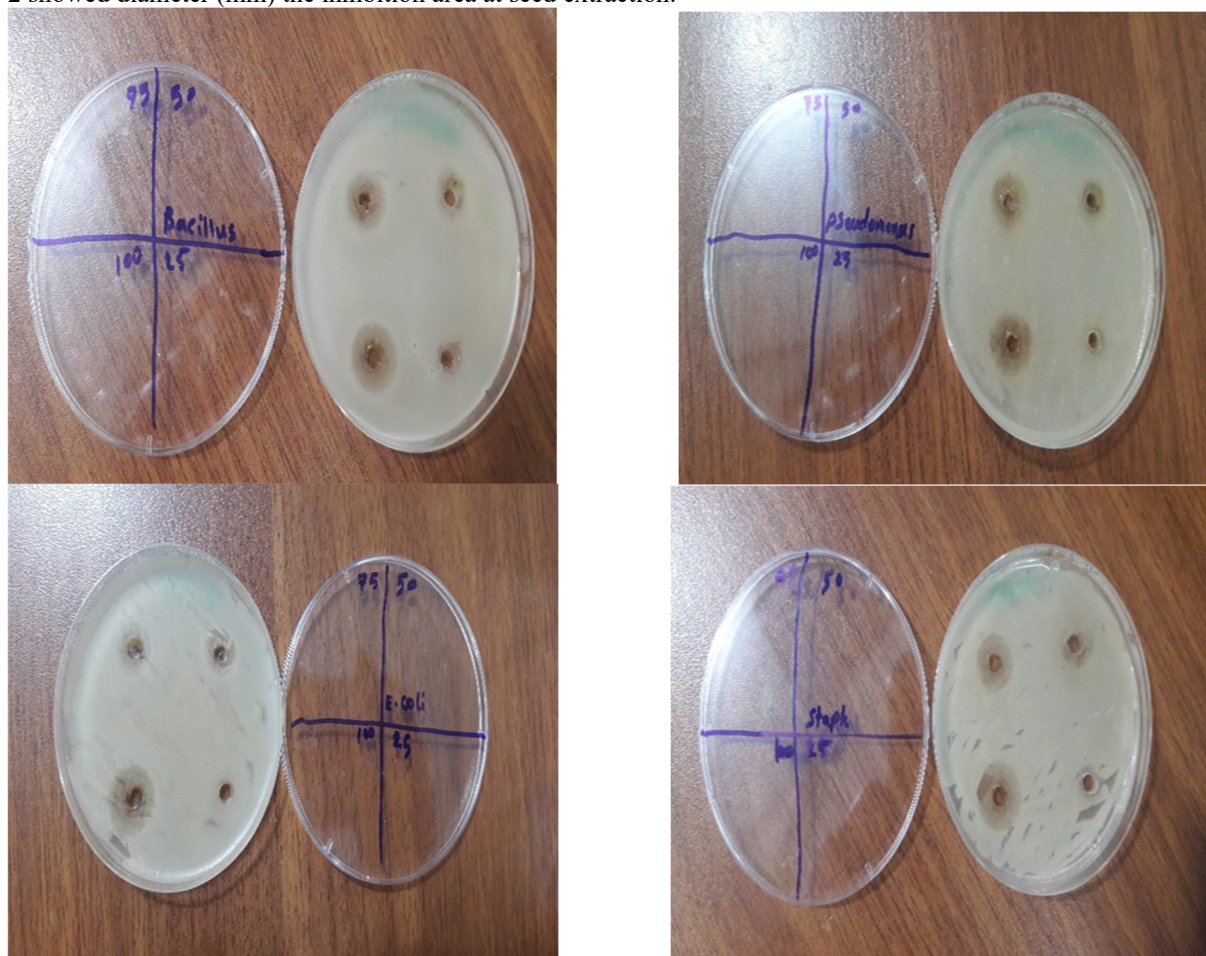


Figure (1) the effect of different concentrations of tamarind extract on pathogenic bacteria

Abdul kapur and Ahmed john (2014) indicated that the pulp and seed extract in diameter (mm) of the inhibition of all bacteria may be due to the inhibition of the presence of antibacterial compounds found in the pulp of fruits and seeds tamarind likes saponins, glycosides, alkaloids work of enzyme. In addition to be working to formation of complexes with sugar and citric acid is one of the main components of the tamarind, which has the ability to reduce the pH, which prevents the absorption of essential nutrients by microorganism and thus get a change in the permeability of cell wall, the death time of bacteria (cowan, 1999).

In seed extract treatment may be due to active ingredients in tamarind seeds, it contained pectin and polysaccharides by 46-48% which is one of the main reasons that increase of the antimicrobial activity as well as containing tartaric acid which increases the acidity of the extract and thus the inhibitory effect of microbes (EL-Sidding *et al.*, 2006). When compared the extracts of the pulp and the tamarind seeds, it was observed that extract of tamarind seeds gave the best rates of inhibition in comparison with the pulp extract on all species of bacteria. This may be due to containing the seeds on palmitic acid, fatty acid (14-20) %, oleic (15-27) % and linoleic acid (36-49) %. These acids are the most important antioxidants that increase antibacterial active (khairunnuur *et al.*, 2009).

Table (3) Effect of tamarind seeds extract and antibiotics on pathogenic bacteria inhibitor

Con type	Control	25	50	75	100	Amoxicillin	Ampicillin	Tetracycline
<i>Pseudomonas</i>	5 ⁱ	16 ^{gi}	18 ^{fgh}	20 ^{def}	22 ^{cd}	5 ⁱ	5 ⁱ	25 ^b
<i>Bacillus</i>	5 ⁱ	12 ^k	13 ^f	15 ^{ij}	17 ^{ghi}	5 ⁱ	5 ⁱ	24 ^{bc}
<i>E.coli</i>	5 ⁱ	13 ^{jk}	16 ^{hi}	19 ^{egh}	21 ^{de}	5 ⁱ	5 ⁱ	28 ^a
<i>Staph aureus</i>	5 ⁱ	18 ^{fgh}	20 ^{def}	22 ^{cd}	25 ^b	5 ⁱ	5 ⁱ	30 ^a
% of inhibitory								
<i>Pseudomonas</i>	-	220	260	300	340	-	-	400
<i>Bacillus</i>	-	140	160	200	240	-	-	380
<i>E.coli</i>	-	160	220	280	320	-	-	460
<i>Staph aureus</i>	-	260	300	340	400	-	-	500

Means with different letters in the same are significantly different ($p < 0.05$) for each sample

Table 3 shows the effect of the different concentration levels of the alcoholic water extract of the sour tamarind pulp (25, 50, 75 and 100) mg/ml to test effectiveness against the sensitivity of both positive and negative gram bacteria. The results showed that all the levels of concentrations used in the experiment gave a graduated positive effect of all gram positive and negative bacteria, *Staph aureus*, *Bacillus*, *Pseudomonas* and *E. coli*. Gungumjee *et al.* (2012) indicated that the *tamarindus indica* leaves extract inhibited effectiveness (25, 15, 15 and 20) mm of the bacteria *Pseudomonas aeruginosa*, *E.coli*, *Staph aureus* and *Bacillus subtilis* respectively. The results also showed that *Staph aureus* bacteria had significantly effectiveness ($p > 0.05$) as compared with other bacteria. The pulp extraction of 100% concentrations increasing order for *Staph aureus*, *Bacillus*, *Pseudomonas* and *E. coli*. It showed that 340, 280, 260 and 200 respectively inhibitory decreased effectiveness compared with other concentrations the effectiveness of some antibiotics are decreased of growth different types of bacteria (Saga and Yamaguchi, 2009). Then the 100 concentrated had more significantly effect on the increasing inhibitory by 240% as compared with 280, 260, 240 and zero to *Bacillus*, *Pseudomonas*, *E.coli* and control respectively this way agree with the finding as Ahulkiapur and Ahmed (2014) who indicated that the extract at the tamarind plant has anti- bacteria positive and negative effect of their bacteria.

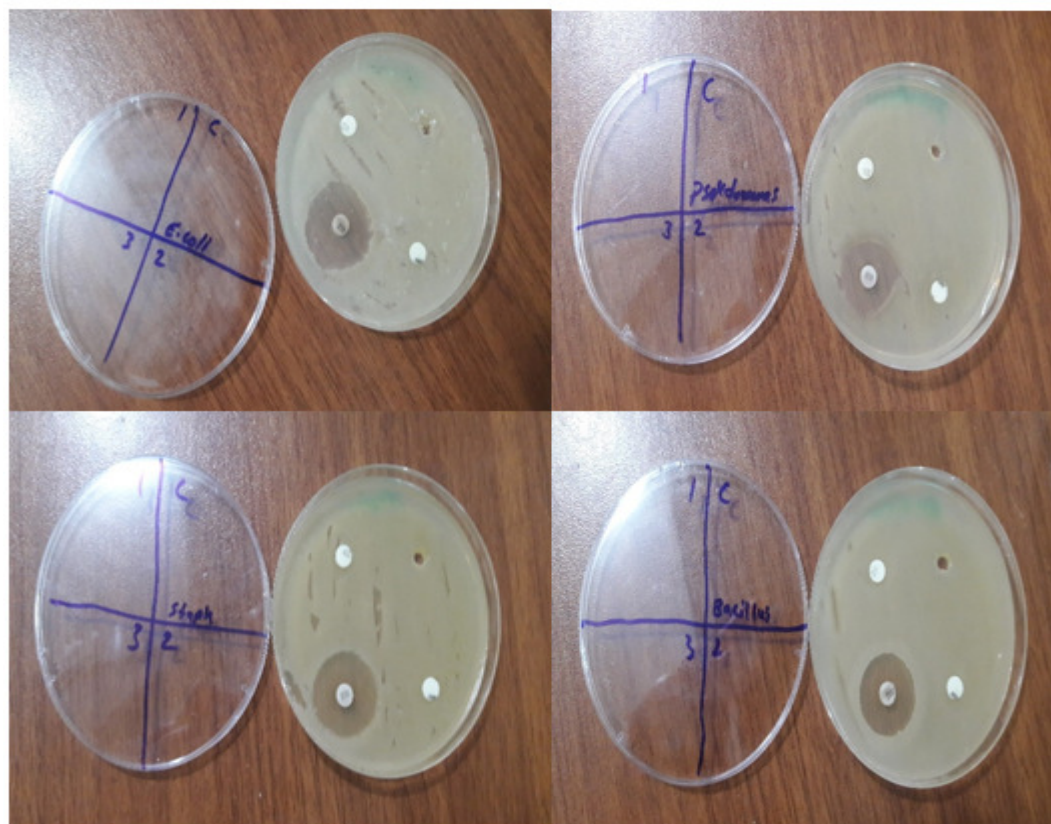


Figure (2) Effect of antibiotic and control treatment on pathogenic bacteria the conclusion from this study that tamarind seeds extract and the pulp extract were associated with antagonistic mechanisms against the pathogens bacteria.

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