



Evaluation of Antimicrobial Activity of *Tamarindus Indica* Fruit Extract Growing Up in Yemen against Pathogenic Microorganisms

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Abstract

Introduction: *Tamarindus indica* is a genus of plant belongs to family *Fabaceae*, subfamily *caesalpinaceae*. In this current study *T. indica* represent as one of the selected plants used traditionally as a treatment for many illnesses such as fever, headache and jaundice in Yemen.

Aims: the aim of this study to determine the phytoconstituents and antimicrobial activity of *Tamarindus indica* Fruit.

Methods: well diffusion assay and phytochemical tests were used to evaluate antimicrobial activity and phytoconstituents. The antimicrobial activity of the concentrated extracts was evaluated by measuring the diameter of inhibition zone against gram positive, gram negative and *Candida albicans*.

Results: Phytoconstituents such as flavonoids, alkaloids, saponins and carbohydrates present in the extract. The maximum zone of inhibition of about 15mm was shown on both *E. coli* and *P. aeruginosa* in the ethanolic extract (150mg/ml) when compared with standard drug (Gentamycin).

Conclusion: the results showed antimicrobial activity against bacteria, so *T. indica* is a promising plant to be used antimicrobial.

Keywords: Antimicrobial Activity; Aqueous; DMSO; Ethanol; Phytochemicals; *Tamarindus Indica*; Yemen

Introduction

Tamarindus indica is plant belongs to family *Fabaceae*, subfamily *caesalpinaceae* and a tropical evergreen tree native to fertile areas throughout Africa and Southern Asia [1]. It remains the most common source of antimicrobial agents. Their usage as traditional health remedies are the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects [2,3]. While most reports estimated that of 80–90% of rural population of the world still relies on herbs for their healthcare. This plant is used as varied as the cultures that use it. It is often more difficult to determine which use is more important, as food and beverage or as folklore medicine [4–6]. It is widely cultivated as an ornamental tree and for its acidic fruits used in making drinks and a popular component of many decoctions used as health remedies. In Northern

and southern, Yemen the fresh fruits and fresh leaves are used as decoction mixed with potash for the treatment of stomach disorder, general body pain, jaundice, yellow fever and as blood tonic and skin cleanser. Because of its wide usage and availability, this study was set out to investigate the antimicrobial activity of the plant and to investigate the presence of phytochemical constituents that may possess therapeutic effect as antimicrobial and other effects in the fruit pulp of *Tamarindus indica*.

Materials and Methods

Sample Collection

The fruits of *T. indica* were obtained from Dhamar city, Yemen. The analysis has been carried out in May 2020 at the Laboratory of science Faculty of science Sana'a University

Yemen.

Preparation of Plant Extract

Pulp covering of in fruit was dried in shade for two weeks. About forty (40) gram of the pulp was macerated in 200ml of purified water and ethanol respectively at room temperature for 24 hours. The macerations were filtered through a filter paper (Whatman No. 2) and the filtrate was evaporated to dryness in oven at 40°C. The obtained residue was treated with 20% Dimethylsulfoxide (DMSO) to form 300mg/ml stock solution of extract from which different concentrations of 150, 100, and 50mg/ml were produced.

Phytochemical Screening

Existing bioactive ingredients of extract were identified by carrying out some phytochemical tests. Wagner's, Lead acetate reagent, saponins and Molish's test, were used to test presence/absence of alkaloids, flavonoids, saponins and carbohydrates respectively.

Antimicrobial Activity

This current study performed to examine the antimicrobial activity of this extract by using five bacteria and one fungi were tested. Gram negative bacteria encompass *Escherichia coli*, *Pseudomonas aeruginosa*, *Kelibsellia pneumonia* and Gram positive bacteria likewise *Bacillus subtilis* and *Staphylococcus aureus*, and fungi include *Candida albicans*. All the used strains were locally isolated and were obtained from Division of Microbiology, Department of Biology, Faculty of science, Sana'a university.

The bacteria prepared as suspension which contain 106 CFU/ml of bacteria that compared with McFarland solution. Five wells were formed in each of these plates by utilizing a standard corn borer (7mm). Approximately, 70µl from

each of the three prepared concentrations that mentioned above were added in each wells, as standard Gentamicin, Nystatin and DMSO were used as positive and negative control respectively. All the plates were incubated at 37 Co for 24h. After that bioactivity was evaluated by measuring the inhibition zone in a way of agar well diffusion method.

Results

The phytochemical screening revealed that the presence of Alkaloids, Flavonoids, Saponins and carbohydrates. While the antimicrobial activity of *Tamarindus indica* Fruit Pulp extracts against pathogenic bacteria and fungi is shown in a (Table 1). These results displayed that both ethanolic and aqueous extract showed similar activity against *staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* and *Kelibsellia pneumonia*. The antimicrobial activity was proportional concentration (150 >100>50). Whereas, the activity against *B. subtilis* was very low at concentration of 150mg/ml, and no activity against *Candida albicans*. In this research, ethanolic extract showed higher antimicrobial activity than aqueous extract because the phytoconstituents are organic compounds and their solubility in organic solvent are greater than water, and the antimicrobial activity was higher against *P. aeruginosa* and *E. coli* where was the diameter of inhibition zone was 15mm and 14mm for ehtanolic and aqueous extract respectively. The extract was compared with Gentamycin as positive control for gram positive and gram negative bacteria, the results were showed that the diameter of inhibition zone was 20mm and 21mm for *E. coli* and *P. aeruginosa* respectively for both ethanolic and aqueous extract. While Nystatin was used as positive control for *C. albicans*, these results displayed that diameter of inhibition zone was 23mm and 20mm with ethanolic and aqueous extract respectively. The Placebo was 20% DMSO solution, and used as negative control that revealed no inhibition zone with all tested organisms (Figures 1-3).

Conc. & Zone	Ethanol					Purified Water				
	Zones/Mm		Standard		Plac*	Zones/Mm		Standard		Plac*
Organisms	150 mg	100 mg	50 mg	Gentamycin		150 mg	100 mg	50 mg	Gentamycin	
<i>Staphylococcus aureus</i>	13 mm	12 mm	11 mm	27 mm	NG*	13 mm	11 mm	NG*	17 mm	NG*
<i>Bacillus subtilis</i>	10 mm	NG*	NG*	25 mm	NG*	12 mm	10 mm	8 mm	24 mm	NG*
<i>Pseudomonas aeruginosa</i>	15 mm	14 mm	12 mm	21 mm	NG*	14 mm	12 mm	11 mm	21 mm	NG*
<i>E- Coli</i>	15 mm	12 mm	10 mm	21 mm	NG*	14 mm	12 mm	11 mm	20 mm	NG*
<i>klebsiella pneumoniae</i>	13 mm	12 mm	12 mm	20 mm	NG*	13 mm	12 mm	10 mm	21 mm	NG*
<i>Candida albicans</i>	NG*	NG*	NG*	Nys23 mm	NG*	NG*	NG*	NG*	Nys20 mm	NG*

* NG = No Growth, Plac= Placebo, Nys = Nystatin

Table 1: Showed that the zones of *Tamarindus indica* Fruit Pulp by using ethanol and purified water solvents.

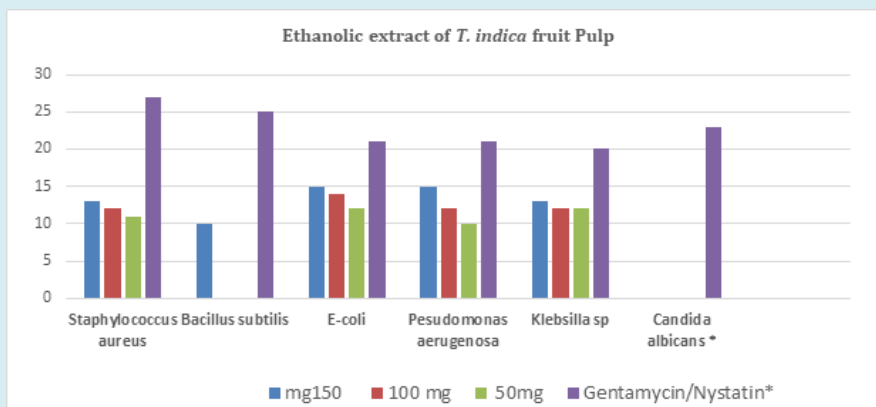


Figure 1: Zones inhibition for ethanolic extract of *T. indica* pulp versus standards.

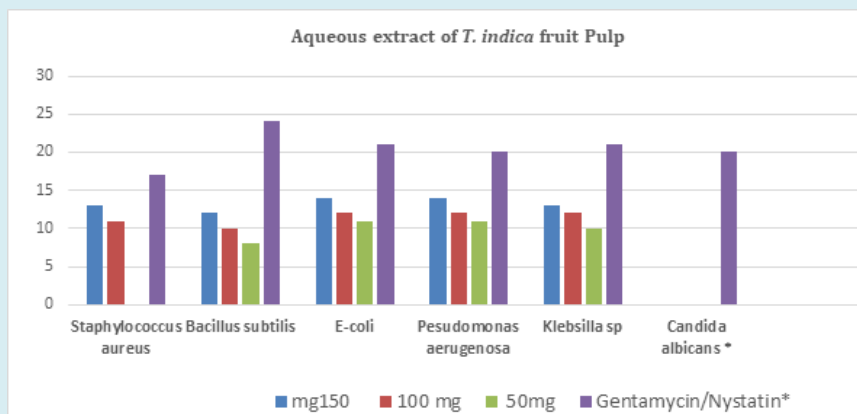


Figure 2: Zones inhibition for aqueous extract of *T. indica* pulp versus standards.

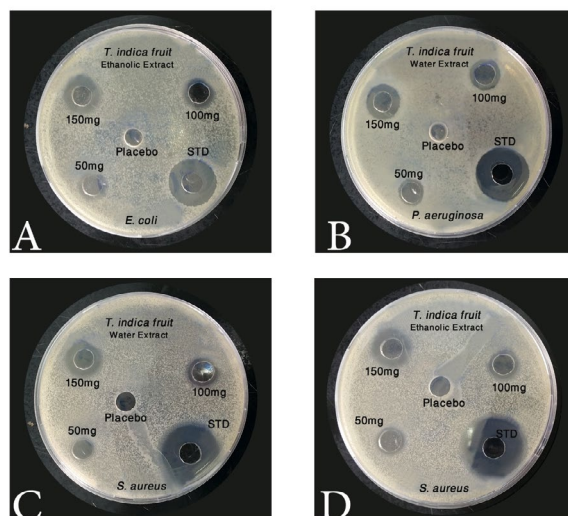


Figure 3: Inhibition zones observed with ethanolic and aqueous extract of *T. indica* fruit.

Discussion

Discovering the ability of plant in healing many health issues is an ancient concept and for long time ago, people have been trying to develop and explore various drugs from plants that have accepted activity and less side effects. In literature, medicinal plants act as the backbone that traditional medicine based on and different chemical ingredients are responsible about the activity of plant extract. Secondary metabolites such as alkaloids, tannins, flavonoids, essential oil and several other aromatic compounds are the phytochemical constituents of plant that serve as defense mechanisms against attack by many insects, microorganisms and herbivores and represent as a phytoprotectants and endure the environmental stress condition. This may so clarify that antimicrobial activity was demonstrated by fruit pulp of *Tamarindus indica* [7]. Some gram positive and gram-negative pathogenic bacteria have been used in this research, such as *P. aeruginosa*, *S. aureus*, *E. coli*, *B. subtilis* and *Klebsilla Pneumoniae* and one fungi (*C. albicans*).

Tamarindus indica is a plant growing up in Yemen, and called «Homar» traditionally, it is used to relief the headache and high body temperature, furthermore, infusion of fruits pulp used as drink or as a poultice on the head. To our knowledge the phytochemical screening of *Tamarindus indica* fruits extract indicate the presence of alkaloids, flavonoids, saponins and carbohydrates, so the antimicrobial activity was due to presence of phytochemicals in plant parts. Many previous studies have been showed the flavonoids possess anti-inflammatory, anti-hepatotoxic and antimicrobial activity, also saponins have antimicrobial activities. In addition, tannins act as astringent and play important role in wound healing [8]. The result of our study appeared that the presence of alkaloids, flavonoids, saponins and carbohydrates. The phytochemical results of this study were agreed with previous studies that conducted in Nigeria and in India [7-9].

The result of this current study showed higher antimicrobial activity with ethanolic extract than aqueous extract. The inhibition zone of ethanolic and aqueous extract at concentration of 150mg/ml was 15mm and 14mm against *E. coli* and *P. aeruginosa* respectively when compared with standard drug (Gentamycin). Our result was agree to previous studies conducted in Nigeria that reported to possess antimicrobial activity against *E. coli* and agree with studies which reported *Tamarindus indica* show antimicrobial activity against both of *P. aeruginosa*, *S. aureus* and *B. subtilis*, however these studies stated the extract showed antibacterial against *B. Subtilis* that disagree with our findings.

In our study, we found that the ethanolic and aqueous extract of *Tamarindus indica* Fruit Pulp has antimicrobial

activity against gram negative bacteria more than gram positive bacteria and resistant against *Candida albicans*.

Conclusion

In conclusion, our result revealed that fruit pulp of *T. indica* extract showed effective antibacterial activity for both gram positive and negative bacteria and can be used traditionally for treatment various infections. Furthermore, this plant can be promising, from which many compounds can be isolated and studied to develop novel antibiotics with low side effects. Based on results of this study, we recommend further studies to fractionate the components of extract by using different solvents and study effects of every compound individually and draw the structure of promising compounds. After that further studies required in trying to synthesis that compound chemically.

Limitations of Study

Further studies should be done to develop a pharmaceutical formula as gel, cream or ointment, but we get difficulties in the study of their stability because we should have reference materials, separating techniques like HPLC and climate chambers. Also DPPH for antioxidant study was not available.

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