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RESEARCH ARTICLE

THE ANTIMICROBIAL EFFECT OF AQUEOUS EXTRACT OF TAMARIND (*TAMARINDUS INDICA*) LEAVES

Aram Abuzied[1], Mawadda Adam[1], Rashid Eltayeb Abdalla[2], Abu baker Osman Uro[3], Amel Ali Suleiman[4]

[1]School of Pharmacy, Ahfad University for women, Sudan

[2]School of Pharmacy, Ahfad University for Women, Sudan

[2]Faculty of Medicine, Shendi University, Sudan

[3]Centre of science & Technology, Ahfad University for women, Sudan

[4]Centre of Science & Technology, Ahfad University for women, Sudan

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ABSTRACT

This study is comparative-experimental work. It was conducted in National Center for Research (Sudan) during the period from 28th of August to 20th December 2013 to investigate the antimicrobial activity of aqueous extract of *Tamarindus indica* leaves In vitro using diffusion method. *Tamarindus indica* belongs to Super division Spermatophytes (Seed plants) of the family Leguminosae (Fabaceae).

To evaluate the scientific basis for the use of the plant, The leaves was collected from Acacia Forest Trees in Khartoum and extracted using sterile water as a solvent, the antimicrobial activities of extracts of the leaves was evaluated. Serial concentrations MICs (Minimum Inhibitory Concentrations) of the extract were tested against *Bacillus subtilis , Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli, Salmonella paratyphi, Candida albicans and* Aspergillus *niger* then comparing the results obtained with standard antibiotic Gentamicin and antifungal Clotrimazole. Furthermore, the combined effect of the extract with Gentamicin and Clotrimazole was tested.

The results indicate that the aqueous extract has promising antimicrobial activity against gram positive, gram negative bacteria and fungi. The MIC shows increase in the zone of inhibition when increasing the concentration. In addition, the combination of extract with Gentamicin showed synergistic effect with *Salmonella paratyphi* while Gentamicin and Clotrimazole showed antagonistic effect with *Pseudomonas ariogenosa* and *Candida albicans* respectively.

Key words: *Tamarindus indica*, antibacterial activity, antifungal activity, minimum inhibitory concentration.

INTRODUCTION:

Medicinal plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for (80) % of world population in Asia, Latin America and Africa and is reported to have minimal side effects [(1)]. During the last decade, the use of traditional medicine has expanded globally and is gaining popularity. It has continued to be used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system [(3)].

According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About (80) % of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency [(4)].

Traditionally, the use of plant preparations as sources of drug are based on the experience and superstitions passed from generation to generation, virtually by the word of mouth [(5)]. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. The rising incidence in multidrug resistance amongst pathogenic microbes has further

**Corresponding author:* Aram Abuzied, Mawadda Adam | *E-mail:* aramabuzied90asheery@gmail.com, mody_qatar@hotmail.com

necessitated the need to search for newer antibiotic sources [1].

Tamarind is a tropical evergreen tree native to fertile areas throughout Africa and Southern Asia [1], It belongs to the dicotyledonous family Leguminosae which is the third largest family of flowering plants with a total of (727) genera recognized and the number species is estimated at (19,327) [6]. Tamarind, *Tamarindus indica L*., is a multipurpose tropical fruit tree used primarily for its fruits, which are eaten fresh or processed, used as a seasoning or spice, or the fruits and seeds are processed for non-food uses. The species has a wide geographical distribution in the subtropics and semiarid tropics and is cultivated in numerous regions [7].

Tamarind has been used for centuries as a medicinal plant; its fruits are the most valuable part which has often been reported as curative in several pharmacopoeias. The leaves have a proven hap to protective activity associated with the presence of polyhydroxylated compounds with many of them of a flavonolic nature [2]. Leaves also present good levels of protein, fat, fiber, and some vitamins such as thiamine, riboflavin, niacin, ascorbic acid and B-carotene [7].

MATERIALS AND METHODS

Preparation of the plant extract:

The freshly collected fresh mature leaves were chopped into pieces and shade dried at room temperature to constant weight for (10) days. Leaves of the plant were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. (100) grams of the powdered plant sample were soaked in (500) mL hot distilled water, and left till cooled down with continuous stirring at room temperature. Extract was then filtered and freezed in a deep. Freezed extract was dried using Freezed drier till powdered extract obtained [1].

Preparation of the test organisms [10]:

Preparation of bacterial suspensions:

Using sensitive balance (2.8) grams of nutrient agar was weighted and transferred to the bottle. After that, the volume was completed to (100) mL distilled water then was shook. The bottle that contains the media was put in autoclave for (15) minutes/ (121) °C for sterilization. After cooling, (20) mL of media and (0.2) μl of microorganism were poured onto the plates; which sterilized in hot air oven for (1) hour/ (180) °C with gentle shaking.

Preparation of fungal suspension:

The same method as for bacteria was adopted. Instead of nutrient agar, (6.5) grams of Sabouraud dextrose agar was used.

In vitro testing of extracts for antimicrobial activity [11]:

Testing for antibacterial Activity:

The agars was left to set and in each of these plates (2) cups (10) mm in diameter were cut using a sterile cork borer and agar discs were removed. Alternate cups were filled with (0.1) µl sample of extract using automatic microlitre pipette. The plates were then incubated at (37) °C for (24) hours. Two replicates were carried out for extract against each of the organisms. After incubation the diameters of the resultant growth inhibition zones were measured, and values were tabulated.

Testing for antifungal activity:

The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at (25) °C (room temperature) for (24) hours.

Determination of minimum inhibitory concentration (MIC) by agar diffusion method [11]:

The principle of the agar plate diffusion is the inhibition of the growth on the surface of the agar by the plant extracts incorporated into the medium. Test tubes were prepared in the series of decreasing concentrations of the plant extraction in the following order (50, 25, 12.5, and 6.25) mg/mL. The bottom of each plate was marked off into (4) segments, and then incubated at (37) °C for (24) hours for bacteria and at (25) °C (room temperature) for (24) hours for fungi. The end point (MIC) is the least concentration of antimicrobial agent that completely inhibits the growth.

Results are reported as the MIC in mg/mL.

RESULTS:

Antimicrobial activity:

Table 1: Antimicrobial activity of aqueous extract of *Tamarindus indica* leaves:

Tested organisms		Zones of inhibition (mm)						
		Gram –ve			Gram +ve		Fungi	
		Ec	Ps	Sal	Bs	Sa	As	Ca
Aqueous extract	10 mg/mL	20-21	20-21	20-20	18-19	10-20	25-26	26-27

Key:

Ec: *Escherichia coli*, **Ps:** *Pseudomonas aerugenosa*, **Sal:** *Salmonella paratyphi*, **Bs:** *Bacillus subtilis*, **Sa:** *Staphylococcus aureus*, **As:** *Aspergillus niger*, **Ca:** *Candida albicans*.

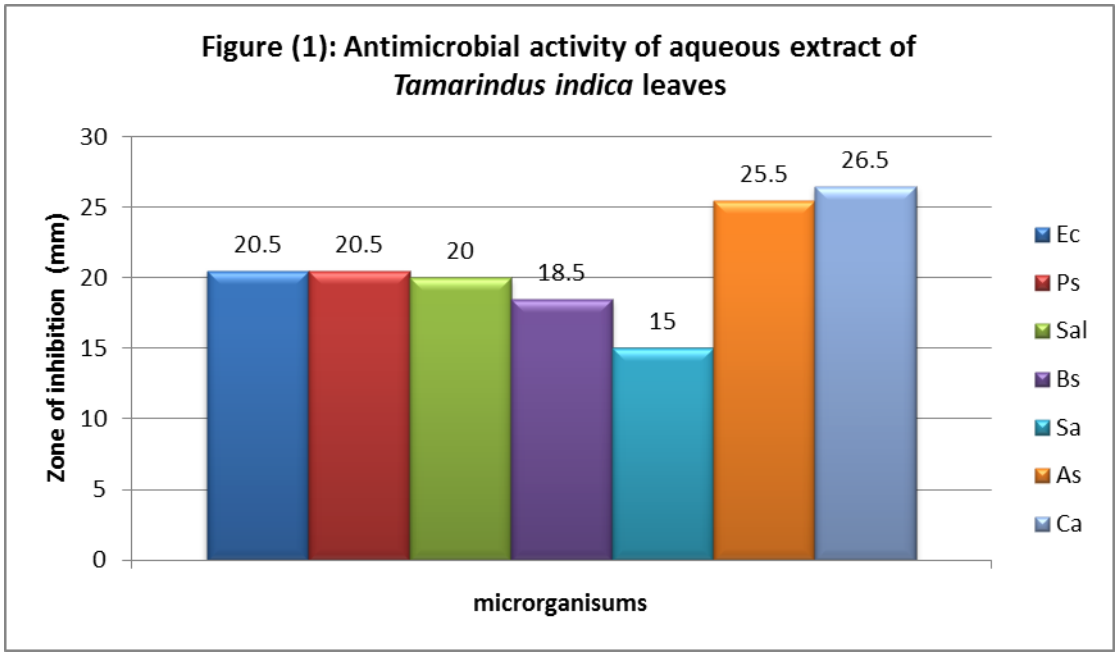


Table 2: MIC of aqueous extract of *Tamarindus indica* leaves:

		Zones of inhibition (mm)						
Tested organisms		**Gram –ve**			**Gram +ve**		**Fungi**	
		Ec	**Ps**	**Sal**	**Bs**	**Sa**	**As**	**Ca**
MIC	**50 mg/mL**	17	17	19	17	18	24	20
	25 mg/mL	16	16	16	16	16	21	17
	12.5 mg/mL	15	15	14	14	15	20	16
	6.25 mg/mL	-	14	13	13	14	17	15

Key:

Ec*: Escherichia coli*, **Ps:** *Pseudomonas aerugenosa*, **Sal:** *Salmonella paratyphi*, **Bs:** *Bacillus subtilis*, **Sa:** *Staphylococcus aureus*, **As:** *Aspergillus niger*, **Ca:** *Candida albicans*, **MIC:** Minimum inhibitory Concentration.

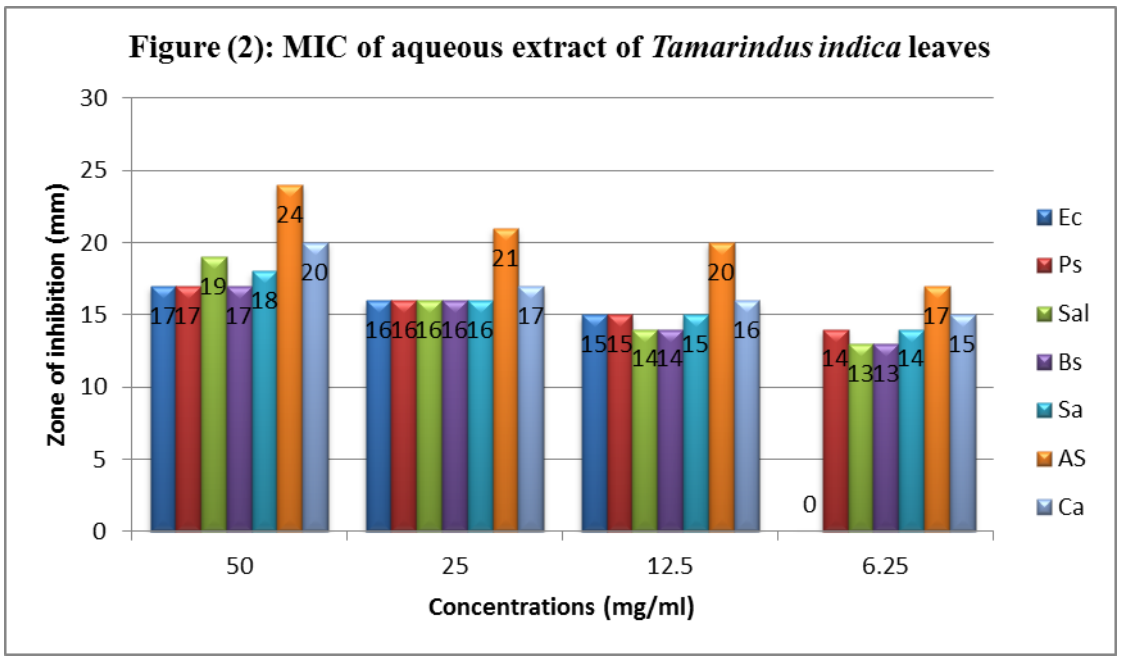


Table 3: Antibacterial activity of reference drug against the tested organisms:

Tested organisms		Zones of inhibitions (mm)				
		Gram –ve			Gram +ve	
		Ec	Ps	Sal	Bs	Sa
Gentamicin	40 mg/mL	24	24	32	25	27
	20 mg/mL	22	20	30	23	25
	10 mg/mL	20	17	25	20	20
	5 mg/mL	19	15	23	18	18

Key:
Ec: *Escherichia coli*, **Ps:** *Pseudomonas aerugenosa*, **Sal:** *Salmonella paratyphi*, **Bs:** *Bacillus subtilis*, **Sa:** *Staphylococcus aureus*.

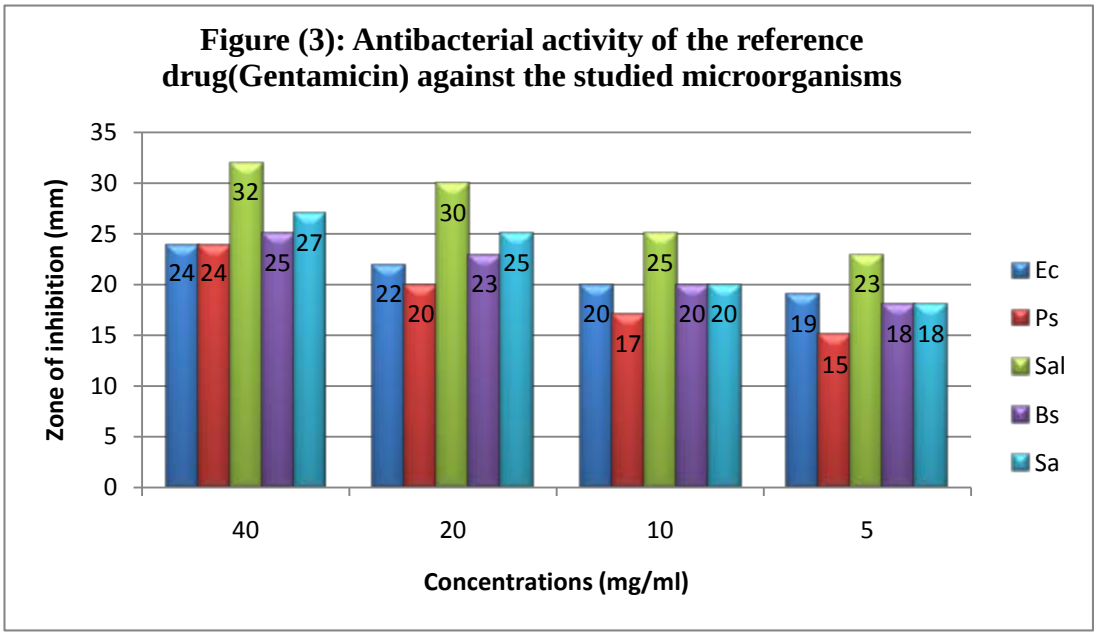


Table 4: Antifungal activity of reference drug against the tested organisms:

Tested organisms		Zones of inhibition (mm)	
		Fungi	
		As	Ca
Clotrimazole	40 mg/mL	Nil	Nil
	20 mg/mL	Nil	Nil
	10 mg/mL	Nil	Nil
	5 mg/mL	Nil	Nil

Key:
As: *Aspergillus niger*, **Ca:** *Candida albicans*.

Table 5: Combination of aqueous extract of *Tamarindus indica* leaves and antibacterial reference drug:

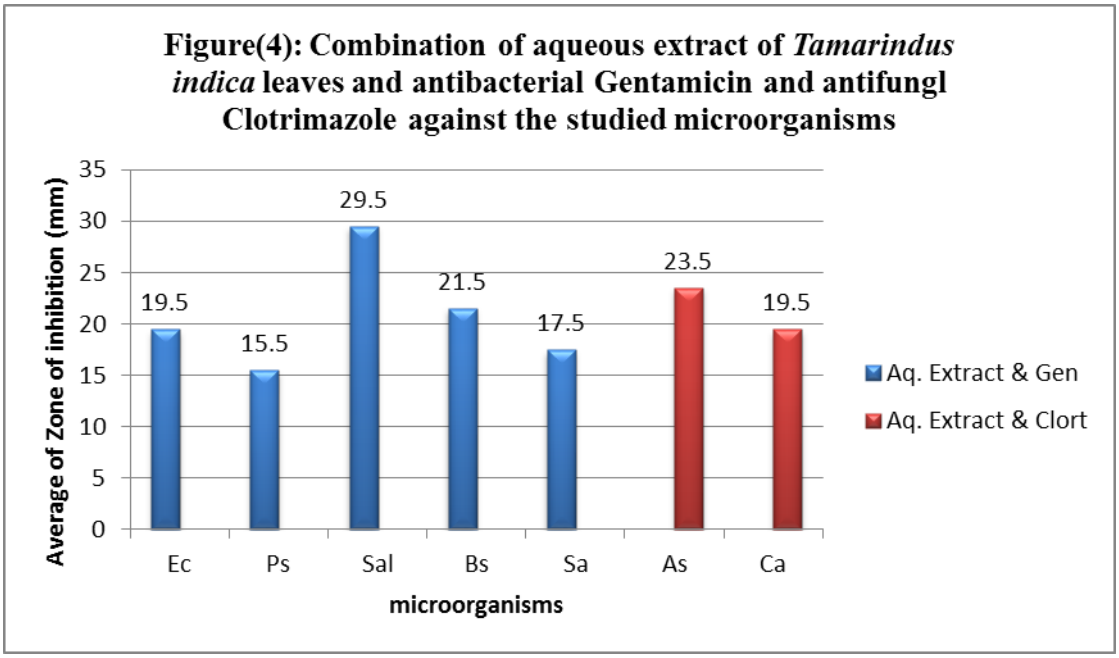
Tested organisms	Zones of inhibition (mm)				
	Gram -ve			Gram +ve	
	Ec	Ps	Sal	Bs	Sa
Combination of AE & Gen	19-20	15-16	29-30	21-22	17-18

Key:
Ec: *Escherichia coli*, **Ps:** *Pseudomonas aerugenosa*, **Sal:** *Salmonella paratyphi*, **Bs:** *Bacillus subtilis*, **Sa:** *Staphylococcus aureus*, **AE:** aqueous extract, **Gen:** Gentamicin.

Table 6: Combination of aqueous extract of *Tamarindus indica* leaves and antifungal reference drug:

	Zones of inhibitions (mm)	
Tested organisms	**Fungi**	
	As	**Ca**
Combination of AE & Clot	23-24	19-20

Key:
As: *Aspergillus niger*, **Ca:** *Candida albicans*, **AE:** aqueous extract, **Clot:** Clotrimazole.



DISCUSSION:

The current study was carried out to investigate the antimicrobial activity of aqueous extract of *Tamarindus indica* in vitro. This extract was studied in different concentrations using the diffusion method to identify the antimicrobial activity against seven microbes namely *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella paratyphi, Candida albicans and Aspergillus niger.*

Table (1) shows that the aqueous extract had antimicrobial activities against all microorganisms. The highest inhibition zone was found to be in the fungi species (compared with previous studies done which found to be resistant to the extracts) [(1) (2)] followed by gram negative bacteria then gram positive bacteria.

On other hand, The MIC of aqueous extraction on Table (2) shows that when the concentrations increased the inhibition zones increased; this can be explained by natural effect of the increased in dose leading to increasing effect. While, on Table (3) Gentamicin exhibited high inhibition zones comparing with the extract even though of using low concentrations in serial dilution. On Table (4) the fungi species found to be resistant when using clotrimazole; this may be due to improper handling or inappropriate storage conditions, even though it was unexpired 2015 but ear drops should be thrown away four weeks after opening [(8)].

The combination of Gentamicin and Clotrimazole respectively with the extract on Table (5) and (6) gave synergistic effect with the *Salmonella paratyphi* (29-30) mm indicate that the efficacy of the extract increased while antagonized with *Pseudomonas aeruginosa*, *Candida albicans* (15-16) mm and (19-20) mm respectively compared with Table (1).

The demonstration of antimicrobial activity against gram positive, gram negative bacteria and fungi may be indicative of the presence of broad spectrum chemotherapeutic compounds [(9)].

CONCLUSIONS:

This study showed that the aqueous extract of the Tamarindus indica was found to have promising antimicrobial activity against all gram positive, gram negative bacteria and fungi. This demonstration of broad spectrum may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease, chemotherapy and control. Also, this investigation has opened up the possibility of using

the plant in drug development for human consumption; possibly for the treatment of gastrointestinal, urinary tract and wound infections and typhoid fever. The effect of this plant on more pathogenic organisms, toxicological investigations and further purification needs to be carried out.

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