P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Short Communication

Antibacterial Activity of *Mangifera indica* Leaves Aqueous and Alcoholic Extract

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Article History: Received: March 24, 2018 Revised: July 08, 2018 Accepted: July 14, 2018

ABSTRACT

Antibacterial activities of aqueous and alcoholic extracts of *Mangifera indica* leaves were studied against pathogenic *Streptococcus pyogens*, *Enterococcus faecalis* and *Escherichia coli* bacterial strains that obtained from university of Baghdad, college of veterinary medicine and compared with Ampicillin (0.2µg/µ) by using agar well diffusion method. The present study reveals that the pattern of inhibition varied with the solvents used for extraction. Plant extracts prepared in ethanol solvents provided more consistent antibacterial activity as compared to aqueous extracts. Gallotannin, mangiferin, flavonoids, alkaloids and tannins make ethanol extract more active against this bacterial species. Gram positive and Gram-negative bacteria were inhibited by almost all the extracts even in very low concentrations. Results of the present study sign the interesting assurance of designing a potentially active antibacterial agent from *Mangifera indica* leaves.

Key words: Antibacterial activity, *Mangifera indica*, Extract

INTRODUCTION

The general population is increasingly using herbal medicines in the treatment of many disorders. In present many modern drugs are derived directly or indirectly from plants **(S**harif and Banik, 2006). *Mangifera indica* is a large evergreen tree, long living, native from tropical Asia, it has been introduced wherever the climate is sufficiently warm and damp and is now completely naturalized in many parts of tropics and subtropics (Ross, 1999). There are many traditional medicinal uses for the different parts of *M. indica* throughout the world. The leaves possess antibacterial, antifungal, antiviral, anti-amoebic, anthelmintic, antimalarial activity **(**Masud, 2016), antiulcerogenic action **(**Severi *et al*., 2009 and Neelima *et al*., 2012), hypoglycaemic activity (Reda *et al*., 2010), atherogenicity Muruganandan *et al*., 2005), anticancer (Ali *et al*., 2012 and Timsina & Kilingar, 2015), hepatoprtective (Nithitanakool *et al*., 2009), antidiarhoea (Alkizim *et al*., 2012) and antiinflamatory (Dhananjaya & Shivalingaiah, 2016). The objective of the study was to evaluate the antibacterial properties of *Mangifera indica* leaves aqueous and alcoholic extracts against *Streptococcus pyogens*, *Enterococcus faecalis* and *Escherichia coli.*

MATERIALS AND METHODS

Collection of plant

The fresh green leaves of *Mangifera indica* collected from local gardens in Baghdad /al-Amirya in April 2017, dried leaves were ground to fine powder by electrical grinder. This powder was stored in airtight bottles and subjected to various extraction procedures.

Extraction

Two types of extracts, aqueous and ethanolic extract from *Mangifera indica* leaves were prepared. 10 g powder was soaked in 100 ml of distilled water in falsk and another in ethanol separately. The flasks were put on magnetic stirrer for 72 hours with shaking at 45°C. The solution was then filtered using muslin cloth. The supernatant was again filtered using Whatman filter paper under strict aseptic conditions and the filtrate was collected in fresh sterilized bottles and stored at 4°C until further use. The crude extracts were centrifuged at 3000 rpm for 10 minutes at 25°C. The ethanol extracts were evaporated at 50°C while the aqueous extracts were evaporated at 80°C in rotary evaporator. All dried extract samples were stored at 4°C **(**Aderibigbe *et al*., 2001).

Bacterial isolates

Bacterial cultures were activated in screw capped tubes containing 10 ml of brain heart infusion agar slants and incubated for 24 hours at 37ºC. For maintenance of isolates, nutrient and brain heart infusion agar were stored at 4ºC and were sub -cultured once every two-weeks for further investigation (Quinn *et al*., 2004). Pathogenic bacteria isolate was obtained from the College of Veterinary Medicine/ University of Baghdad.

Cite This Article as: Saliem AH, 2018. Antibacterial activity of *Mangifera indica* leaves aqueous and alcoholic extract. Inter J Vet Sci, 7(3): 117-120. www.ijvets.com (©2018 IJVS. All rights reserved)

Antibacterial assay:

Preparation of standard bacterial suspension

The mean numbers of the viable bacterial cell / ml of the stock suspensions was measured by the average of the standard McFarland solution No.0.5. By taking 1 ml from over-night cultures [brain heart infusion broth (BH)] of bacterial suspension mixing with 9 ml of pepton water, then taking 1 ml of this suspension and making serial ten-fold dilution (Quinn *et al*., 2004).

Preparation the concentration of antibacterial

Stock solution of Ampicillin was prepared by mixing 0.1 gram with10 ml of distilled water (10mg/ml), then concentrations of (0.2 µg/ µl) were prepared by mixing known volume from the stock solution with distilled water.

Preparation of different concentrations of *Mangifera indica* leaves extract

Stock solution of each extracts was prepared by mixing 0.1 gram with 10 ml of distilled water (10mg/ml), then concentrations of (50, 100 and 150µg/ µl) were prepared by mixing known volume from the stock solution with distilled water. These concentrations extracts and antibacterial were used in sensitivity test to determine the bacterial sensitivity to extracts and Ampicillin.

Sensitivity test

Sensitivity test of the aqoues and ethanolic extract of Mangifera indica leaves was compared with antibacterial agent. The method of agar well diffusion was adopted according to Kavanagh (1972) and Perez *et al*., (1990). 0.5ml of standardized stock suspensions (1.5 ×108 cfu/ml) of each type of bacteria was mixed to 500 ml of sterile Mueller Hinton agar at 45oC. Fifteen milliliters of the inoculated Mueller Hinton agars were diffused into the sterile Petri dishes of each. The agars were left to set for 10 minutes to permit solidifying the agar and making wells in these plates, in diameter 6 mm were cut by a sterile Pasteur pipette and the discs of agar were removed by sterile forceps, after that the wells were filled with 55 microliters of each concentrations of extracts (50, 100 and 150µg/ µl) using micropipette. The plates of this test were then incubated at 37oC in the upright position for 24 hours. Three replicates were done for every concentration of extracts and the activity was dictated by measuring the distance across (diameter) of inhibition zone around every well by millimeter against Streptococcus pyogens, Enterococcus faecalis and Escherichia coli at the same time. The observations and standard errors meanes values were tabulated. Ampicillin was used as a reference to determine the sensetivity test for Streptococcus pyogens, Enterococcus faecalis and Escherichia coli. The same technique which was used for Mangifera indica leaves extracts was used for determination of antibacterial agent with concentration (0.2 µg/ µl).

RESULTS

Different concentrations of aqueous and ethanolic Mangifera indica leaves extracts (50, 100,150 µg/µl) and antibacterial (Ampicillin 0.2 µg/µl) were used in agar well diffusion assay, caused different degrees of zones of inhibition against Streptococcus pyogens, Enterococcus faecalis and Escherichia coli. The size of inhibition zones was different according to concentration of the extracts and antibacterial agent, the size of inhibition zone proportionally increased with increasing of concentration of the agents, Table 1 and Figure 1.

The results showed that Streptococcus pyogens, Enterococcus faecalis and Escherichia coli were sensitive significantly ($P<0.05$) to ethanol extract more than aqueous extract in the concentrations (50 µg/ µl, 100 µg/ µl and 150 µg/ µl) using in this study. In this concentrations, there was a significant increase ($P<0.05$) in diameter of zone of inhibition in Streptococcus pyogen, Enterococcus faecalis and Escherichia coli growth as compared with zone of inhibition of aqueous extract.

The results of inhibitory zone diameter indicated the sensitivity of bacteria towards different tested concentrations. Extracts and antibacterial activities were observed to be concentration dependent and this appeared significantly in the concentrations 50, 100 and 150 µg/ µl.

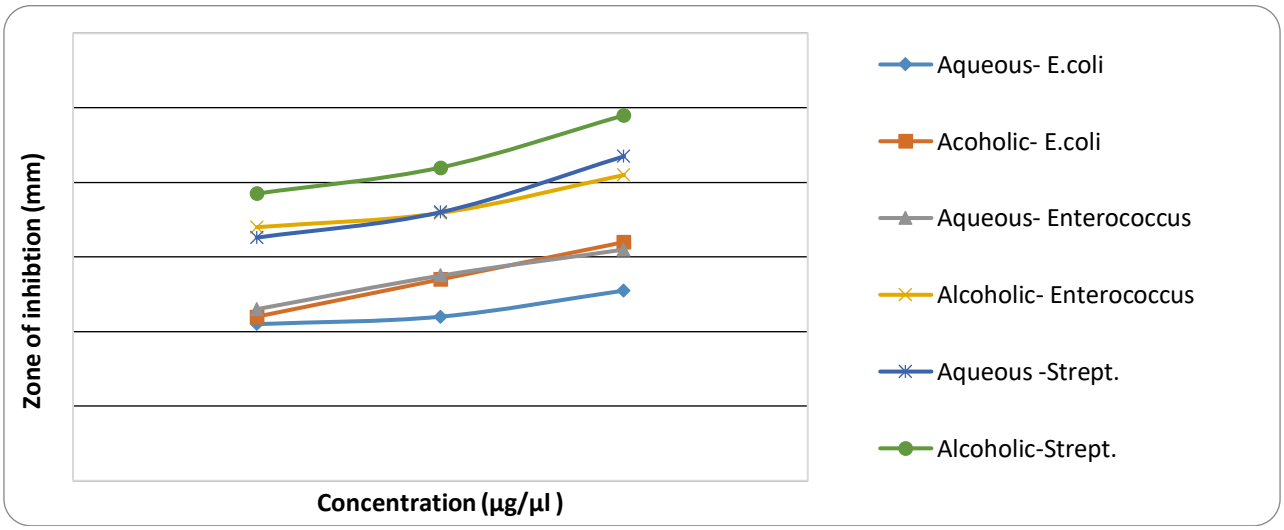

Fig. 1: Relationship between concentrations of aqueous and alcoholic extract and mean diameter of inhibition zone (mm.) against *Streptococcus pyogens, Enterococcus faecalis* and *Escherichia coli*.

Table 1: Antibacterial activity of *Mangifera indica* leaves extracts and reference antibiotic in different concentrations on *Streptococcus pyogens, Enterococcus faecalis* and *Escherichia coli* (diameter of inhibition zone in mm.)

Extract	Concentration µg/µl	Zone of inhibition (mm)		
		Streptococcus pyogens	*Enterococcus faecalis*	*E. coli*
Aqueous	50	6.52±0.10Fa	4.60±0.07Fb	4.20±0.07Dc
	100	7.20±0.14Ea	5.50±0.07Eb	4.40±0.07Dc
	150	8.70±0.14Ca	6.20±0.14Db	5.10±0.09Cc
Alcoholic	50	7.70±0.14Da	6.80±0.14Cb	4.40±0.11Dc
	100	8.40±0.14Ca	7.18±0.13Cb	5.40±0.11Cc
	150	9.80±0.14Ba	8.20±0.17Bb	6.40±0.14Bc
Ampicillin	0.2	23.20±0.09Aa	21.10±0.07Ab	18.30±0.7Ac

LSD=0.38. The different capital letters refer to significant differences between different groups (concentrations) at ($P<0.05$). The different small letters refer to significant differences between different bacteria species at ($P<0.05$).

DISCUSSION

Antibacterial activity of Mangifera indica leaves extracts was demonstrated upon gram-positive and gram-negative bacteria was in agreement with Stoilova *et al*., (2005), and this may due to the presence of gallotannin and mangiferin(Engels *et al*.,2011).The differences in the observed activities of various extracts may be due to varying degrees of solubility of the active constituents in the solvent used. It has been documented that different solvents have different solubility capacities for different phytoconstituents (Abdul Hannan *et al*., 2013) and ehanolic leaves extract revealed the existence of alkaloids, carbohydrates, phytosterols, flavonoids and protein (Luka and Mohammed, 2012).

Previous studies have revealed that mango leaf extract is more affective against Gram positive bacteria than Gram negative (Doughari and Manzara, 2008). This may be attributed to presence of lipopolysaccharides (LPS) in the Gram negative bacteria (Zakaria *et al*., 2006).

Conclusions

1. The extract of Mangifera indica leaves have antibacterial activity against gram positive bacteria (Streptococcus pyogens and Enterococcus faecalis) and gram negative bacteria (Escherichia coli).
2. Antibacterial activity concluded that the alcoholic extract effective for the three types of bacteria when compared with aqueous extract and antibacterial reference.
3. Antibacterial activity of *Mangifera indica* leaves extract was concentration dependent.

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