ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Antibacterial activity of *Mangifera indica* (mango) leaves against drug resistant bacterial strains

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Manuscript Info

Manuscript History:

Received: 21 July 2013
Final Accepted: 29 July 2013
Published Online: August 2013

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Key words:
Mangifera indica leaf extract,
Antibacterial activity,
Disc diffusion method,
Minimum inhibitory concentration.

Abstract

Antibacterial activities of leaf extract of *Mangifera indica* were studied against bacteria some as *Proteus vulgaris*, *Pseudomonas fluorescens*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Salmonella typhi*. The methanol, ethanol and benzene extract of *M. indica* leaf showed effectiveness against bacterial strains but the benzene extract showed the maximum growth inhibition 85.1% against *Pseudomonas fluorescens* bacteria at 100µl/ml extract concentration which are drug resistance for Tabramycin, Cephaloridine, Lincomycin, Norfloxacin and Oleandomycin. Minimum inhibitory concentration of benzene extract was determined against *Pseudomonas fluorescens* 50×10^{-1}µl/ml. The present study depict that the *Mangifera indica* leaf extract showed good antibacterial activity against drug resistance bacterial strains.

Introduction

At present nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly form plants and in home or ayurvedic medicines, medicinal plants, their parts and extracts dominate the scenes. The commercial value of various innumerable drugs and pharmaceuticals derived from tropical forest systems on worldwide basis is projected at 20 billion dollars a year (Sharif *et al.,* 2006). *Mangifera indica* is a large evergreen tree, long living, 10-45 m high with a strong trunk and heavy crown. 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). *Mangifera indica* (Anacardiaceae) is one of the important tropical fruits in the world and India contributes major part of the world production. Mango is considered as a king of fruits in Indian delicacy. There are many traditional medicinal uses for the different parts of *M. indica* throughout the globe. The ripe pulp posses numerous therapeutic uses including ripe pulp used as rheological properties (Dak *et al.,* 2007)**,** unripe pulp used as antibacterial activity against food borne bacteria (Gupta *et al.,* 2008). Mango pulp contains vitamins, organic acids, carbohydrates, amino acids, polyphenols and certain volatile compounds (Pino *et al.,* 2005). The leaves possess antibacterial activity (Doughari and Manzara, 2008) antiulcerogenic action (Severi, et al., 2009), hypoglycaemic activity (Aderibigbe *et al.,* 2001), atherogenicity (Muruganandan *et al.,* 2005). Seed kernels possess anti-diarrhoeal activity (Sairam, *et al.,* 2003), effectiveness for dyslipidemia (Anila and Vijayalakshmi, 2002). Bark and stem possess immunomodulatory (Makare, *et al.,* 2001), anti-inflammatory and neuroprotective activity (Lemus-Molina *et al*., 2009). In contrast the present investigation were carried out various extract of M*angifera indica* leaf against drug resistant pathogens to possible development of new antimicrobial against drug resistant pathogens.

Materials and Methods:

Plant and preparaion of extracts:

The green leaves of *Mangifera indica* collected from Meerut region India in June 2012. Dried leaves of *Mangifera indica* were crushed in to powder and suspended in to organic solvent as methanol, ethanol and benzene for 24 hours, after filtration the extract were evaporated by the help of rotary evaporator. For

stock, each extract was re-dissolved with 5ml DMSO (dimethyl sulphoxide).

Microbial test strains:

All tested bacterial strains *viz Proteus vulgaris* (MTCC 1744), *Pseudomonas fluorescens* (MTCC 1748), *Shigella flexneri* (MTCC 1457), *Klebsiella pneumoniae* (MTCC 109), *Salmonella typhi* (MTCC 3216) were collected from MTCC (Microbial type culture collection), IMTECH, Chandigarh. These microorganisms were maintained on Nutrient Agar media (NAM) at 30°C for further investigation.

Determination of the strains sensitivity to antibiotics:

The susceptibilities of the bacterial strains to different antibiotics were tested using disc diffusion method (Aboaba and Efuwape, 2001; Reynolds, and Martindale, 1996). Antibacterial agents from different classes of antibiotics were used which included tabramycin, cephaloridinee, kanamycine, lincomycin, norfloxacin and oleandomycin (Himedia Labs, Mumbai, India) to find out the sensitivity of the strains.

Antibacterial screening of *Mangifera indica* leaves extracts against test pathogens:

The antimicrobial screening of the bacterial strains were carried out disc diffusion method (Grover and Moore, 1962). The plant extracts of 0.1 ml were mixed in 0.9 ml of pre-sterilized Nutrient broth and then added 0.1 ml bacterial culture suspension. In control sets, DMSO (in place of the plant extract) were used in the medium in appropriate amount. Culture tubes were incubated for 24 hour at 30^0C.

After incubation, sterile disc of 6 mm (Hi Media) were dip in to the broth (treated as well as control seperately), disc were aseptically inoculated on the agar surface of the Nutrient agar medium in plates. Inoculated petriplates were incubated at 30^0C and the observations were recorded after 24 hr. Percentage of bacterial growth inhibition (BGI%) was calculated as per formula.

$$BGI\ (\%) = \frac{dc - dt}{dc} \times 100$$

where,

dc =diameter in control

dt =diameter in treatment

Determination of MIC of extracts by microtiter plate assay:

MIC (minimum inhibitory concentration) expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube. A broth microdilution assay was adopted using 96 well micro titer plates with resazurin. It was carried out to assess the microbial growth and determine the Minimal Inhibitory Concentration (Sarker, *et al.,* 2007). The resazurin (oxydation-reduction indicator) solution was prepared by dissolving a 270 mg tablet in 40 ml of sterile distilled water. A sterile 96 well microtiter plate was taken for the test. 50 µl of test extracts were pipetted into the first row of the microtiter plate A1. Wells from A2 to H2 till A12 to H12 were dispensed with 50 µl of nutrient broth. 50 µl of test extract was transferred from test solution (A1-H1) to next wells (A2-H2) and so on to create serial dilutions. 30µl of the test culture were mixed in serially descending concentrations to each well, from A2 to H2 till A12 to H12. In last 20 µl of resazurin solution was added in all tested as well as control set. A11, A12 and H11, H12 served as controls, 50 µl of DMSO was used in place of extracts. The plates were incubated at 30^0C for 24 hours. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value. The average of two values was calculated.

Results:

Some bacterial strains were drug resistant for different antibiotics viz *Pseudomonas fluorescens* bacteria was resistant for many drugs as Tabramycin, Cephaloridine, Lincomycin, Norfloxacin and Oleandomycin (Table 1). The results of antibacterial activity of *Mangifera indica* leaves determined by disc diffusion method. All the extract of *Mangifera indica* leaves showed the antibacterial activity against the pathogenic bacterial strains. The Methanol extract of *Mangifera indica* leaves showed maximum growth inhibition 18.5% against *Salmonella typhi* and also showed minimum growth inhibition 0% against *Pseudomonas fluorescens*. The Ethanol extracts of *Mangifera indica* leaves showed maximum growth inhibition 20% against *Pseudomonas fluorescens* and also showed minimum growth inhibition -28.75% against *Klebsiella pneumoniae.* The Benzene extract of *Mangifera indica* leaves showed maximum growth inhibitions 85.1% against *Pseudomonas fluorescence* and also showed minimum growth inhibition 18.51% against *Shigella flexneri* (Table 2). The MIC value of Methanol extract against *Salmonella typhi* was

50×10^{-1} µl/ml, in Ethanolic extract against *Pseudomonas fluorescens* was 0 µl/ml, in Benzene extract against *Pseudomonas fluorescens* was 50×10^{-1} µl/ml (Table 3).

Table1: Zone of inhibition by different drugs against bacterial strains (Sahrawat and Shahi, 2013).

Antibiotics	*Zone of drug against Bacterial strains (mm)*				
	Proteus vulgaris	*Pseudomonas fluorescens*	*Shigella flexneri*	*Klebsiella pneumoniae*	*Salmonella typhi*
Tabramycin	21	-	19.5	23	10
Cephaloridine	17.5	-	-	14.5	8.5
Kanamycin	22.5	11.5	16.5	12.5	7.5
Lincomycin	-	-	-	-	-
Norfloxacin	13	-	18.5	17.5	17.5
Oleandomycin	-	-	-	-	-

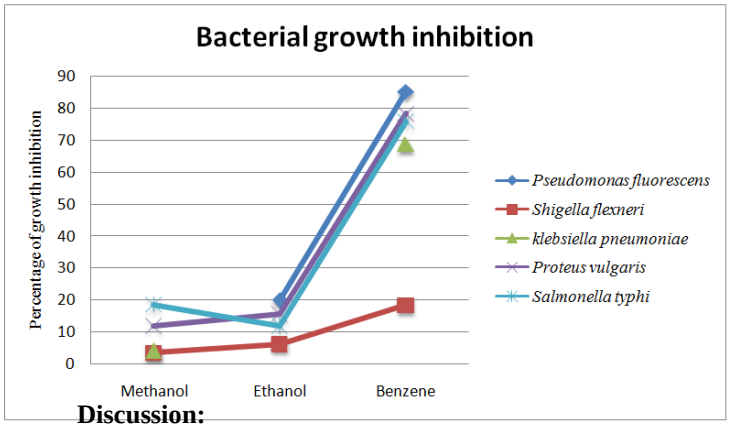
Table 2: Antibacterial screening of different extracts of *Mangifera indica* against pathogenic bacteria by disc diffusion method.

Pathogens	Percentage of growth inhibition at 100µl/ml		
	Methanol	Ethanol	Benzene
Pseudomonas fluorescens	0%	20%	85.1%
Shigella flexneri	3.57%	6.25%	18.51%
Klebsiella pneumoniae	4.16%	-28.75%	68.83%
Proteus vulgaris	12%	15.62%	78.23%
Salmonella typhi	18.5%	12%	75.83%

Table 3: Minimum Inhibitory Concentration of *Mangifera indica leaves* against bacterial strains.

Bacterial strains	*MIC of Mangifera indica extract (µl/ml) against pathogens*		
	Methanol extract	*Ethanol extract*	*Benzene extract*
Salmonella typhi	50×10^{-1}	-	-
Pseudomonas fluorescens	-	-	-
Pseudomonas fluorescens	-	-	50×10^{-1}

Figure: 1 Effect of different extracts on Bacterial growth



Discussion:

The use of higher plants and preparations made from them to treat infections is a longstanding practice in a large part of the population, especially in the developing countries, where there is dependence on traditional medicine for a variety of ailments (Ahmad and Mohammad, 1998). The antimicrobial effects of various plant extracts against certain pathogens have been reported by a number of researchers (Ahmed and Beg, 2001; Erasto, *et al.*, 2004; Nair and Chanda, 2007; Carneiro. *et al.*, 2008; Liasu and Ayandele, 2008; Parekh and Chanda, 2008). A 50% ethanol extract of the leaves of *M. indica* produced a significant hypoglycemic effect at a dose of 250 mg/kg, both in normal and streptozotocin induced diabetic animals. The stimulation of β-cells to release insulin was thought to be part of the mechanism of

action (Sharma, *et al.,* 1997). The leaves of *M. indica* used for antidiabetic properties using normoglycaemic, glucose-induced hyperglycaemia and streptozotocin (STZ) induced diabetic mice. The aqueous extract of the leaves of *M. indica* possess hypoglycaemic activity (Aderibigbe, *et al.,* 2001). In vitro the effect of mangiferin was studied against Herpes simplex virus type 2; mangiferin does not directly inactivate HSV-2 but inhibits the late event in HSV-2 replication (Zhu, *et al.,* 1993). In vitro mangiferin was also able to inhibit HSV-1 virus replication within cells (Zheng and Lu, 1990). Ethanolic flower extract of *Mangifera indica* showed the presence of alkaloids, phenols, flavonoids and carbohydrates and absence of saponins (Parvathi, *et al.,* 2012), ethanolic leaves extract revealed the existence of alkaloids, carbohydrates, phytosterols, flavonoids and protein (Luka and Mohammed, 2012).

Conclusions:

After this research we conclude that the benzene extract of *Mangifera indica* leaves show the maximum antibacterial activity against *Pseudomonas fluorescens* bacterial strain which is drug resistance. In future after investigation the benzene extract could be used as effective antimicrobial agent against drug resistant pathogens.

Acknowledgement:

All thanks are department of microbiology C.C.S. University Meerut, providing the facilities for this research.

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