Therapeutic effects of Mangifera indica extract against Proteus mirabillis isolated from dogs with UTI, Invivo and invitro study

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Abstract

The aim of this study was for detection the therapeutic effects of Mangifera indica extract against Proteus mirabillis isolated from dogs with UTI, Invivo and invitro study. Fresh Mangifera indica were purchased from local market in Fallujah city. Plant shell were washed under tap water, and then dried at room temperature at shade. The dried shell was crushed by a laboratory blender. Organic solvents were used for the extraction of Mangifera indica shells by using absolute ethanol (Ethyl alcohol) which was considered as very effective in extracting the active ingredients of the plant according to the method described by many authors previously. Analysis of Mangifera indica by HPLC for detection of active ingredients. Determination the activity of Mangifera indica in vitro was done by using Agar well diffusion method was used to check the activity of plant extract in vitro for 3 concentration (100,150,200). Induction of infection in mice and Invivo study done on sixty (60) Mice were selected for infection which divided into 3 groups (each group contain 20 animals),Infected group (control positive) with dose 2×108 CFU/ml, Infected group with dose 2×108 CFU/ml then treatment with Mangifera indica extract at dose of 200mg/ml, control negative group which given only normal saline. Three concentrations of extracts were used for determination activity of M. indica, 100,150,200 mg/ml were used. The results of HPLC showed that analysis of extracts contains Qurcetine , Gallic acid, Rutin, Kaempferol, Apigenin,Catechin. The main component were Gallic acid as well as Qurcetine. A concentration of 200 mg/ml were showed to be more active against bacteria. The present results of invivo study revealed that mice in G1 exhibited signs of UTI which diagnosed by isolation of bacteria during all the period of experiment, while mice in G2 showed that decrease of bacterial count till its subsids after 2 weeks of treatment by Mangifera indica.

In conclusion, Mangifera indica have antibacterial effects against virulent Proteus mirabillis isolated from dogs with UTI.

Keywords: *Proteus, dogs, Mangifera indica, invivo, invitro.*

INTRODUCTION

Herbs, herbal materials, herbal preparations, and finished herbal products that contain whole plants, parts of plants, or other plant materials like barks, leaves, flowers, roots, berries, or their extracts as active ingredients are referred to as herbal medicine. These products are intended for use therapeutically or for other purposes in humans (WHO 2019).

A biennial tree from the Anacardiaceae family, Mangifera indica, may reach heights of 15 to 30 meters. Simple, alternating, without stipules, and typically oblong with tips that range from round to acuminate, the leaves' size and form are diverse. Having a glossy top surface and a glabrous, lighter green below surface, mature leaves have a dark green color. Depending on the species, the juvenile leaves may range in color from pale tan to purple. A single seed is contained in a leathery endocarp in the mango fruit, which is a drupe. Initially a dark green color, the fruit ripens to a golden color. Typically, the seeds are single and ovoid or oblong in form (Lawson et al., 2019).

The ethanolic extract of mango seed kernels contains 79.5% polyphenols, which have antimicrobial properties. The extract preserved activity and remained heat and pH stable when heated at 1210C for 15 minutes, frozen at -20 0C for 16 hours, and subjected to a 3–9 pH treatment. The extract also includes 0.5% fat, 3.1% nitrogen, 1.6% ash, and 21.7% carbs. The presence of polyphenols is shown by the extract's formation of a brown precipitate with ferric chloride and a yellow precipitate with an alkaline solution. When exposed to lead acetate, it splits into two layers: a top layer that is white and denotes tannins, and a bottom layer that denotes flavones. It releases hydrogen gas when reacting with MgCl2, which demonstrates the existence of flavones (Maldonado-Celis et al., 2019).

According to research conducted in the northern Kibale region of Uganda, Mangifera indica is used as a decoction to alleviate cough (Namukobe et al., 2011).

Mangiferin, flavonoids, and tannins have antibacterial effect against certain microorganisms. The extract is used to treat illnesses brought on by Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Bacillus pumilus, and Bacillus cereus (Bonev et al., 2010). Salmonella typhi was completely inhibited at a dose of 50mg/ml, Staphylococcus aureus was inhibited at a concentration of 10mg/ml, and 90% of multi-drug resistant salmonella typhi strains were inhibited at values lower than 40mg/ml (Hannan et al., 2013).

Staphylococcus aureus, both the clinical isolate and the methicillin-resistant strain, showed no resistance to the seed extract with concentrations of the extract ranging from 5-1.25mg/ml of both the ethanolic and methanolic extract, and only Bacillus cereus and Rhodococcus equi demonstrated resistance to the seed extract among the 22 bacterial strains tested for sensitivity to Mangifera indica seed extract (Awad El-Gied et al. 2012).

The maximum inhibitory activity of the Mangifera leaf extract in acetone against Salmonella typhi, Streptococcus pneumoniae, and Staphylococcus aureus was observed to occur at 250 mg/ml. In both gram-positive and gram-negative bacteria, the MIC of the acetone leaf extract ranged from 12.5 to 175 mg/ml. Additionally, it has been charged with causing typhoid fever, urethritis, pneumonia, otitis media, and gastroenteritis (Length, 2008).

Staphylococcus aureus was also shown to be more susceptible to the ethanolic extract from mango leaves in research conducted in Uganda, with a mean MIC of 5.48 mg/ml and a reported yield of 3.88%. (Bbosa and others, 2007). Many researchers in Iraq work on isolation of this bacteria from these (Al-Samarrae, 2011 ; Sabeeh and Hatem,2013).

The aim of this study was for detection the therapeutic effects of Mangifera indica extract against Proteus mirabillis isolated from dogs with UTI, Invivo and invitro study.

Materials and Methods:

Fresh Mangifera indica were purchased from local market in Fallujah city. Plant shell were washed under tap water, and then dried at

room temperature at shade. The dried shell was crushed by a laboratory blender.

Preparation of Alcoholic Extract of Mangifera indica :

Organic solvents were used for the extraction of Mangifera indica shells by using absolute ethanol (Ethyl alcohol) which was considered as very effective in extracting the active ingredients of the plant according to the method described by (Doughari and Manzara, 2008). This was done by using soxhlet apparatus , which around bottom glass flask placed that fitted to an extraction unit. The extracting unit contained the solvent and cellulose (thumble) located inside it that contained the dry plant powder.

A distiller unit was fitted on to the extraction unit. For condensation of vapor solvent , 30 g of plant powder were placed inside the thumble and 300 ml of absolute ethanol were placed inside the flask. The extraction was carried out for 24 hrs. by heating temperature that kept the solvent at 50- 60 co until a clear and colorless solvent appeared in the extracting unit . After that , the extract was dried by using a rotary evaporator 40-45 co.

The dry extract was placed in an incubator under 38-40 co for complete dryness. The final extract was kept frozen at -20 co until use.

Analysis of Mangifera indica by HPLC

Shimadzu's LC-2030 C Prominence-i (Japan) system was used to perform HPLC.

The LabSolutions software was used to control the equipment and evaluate the results. The Kinetex XB-C18 column (100 Ao, 250 mm 4.6 mm, 5 m pore size) was used for a separation. The mobile phase is made by isocratic extraction at a low-pressure gradient using 0.1% formic acid: acetonitrile, with a flow rate of 1.5 ml/min and an input volume of 10 l. (87:13). After being degassed, each solution was run through a 0.45 m pore size filter. Throughout the study, the column was kept at 26°C, and the UV detector was adjusted at 254 nm. The HPLC analysis utilized a diluent of over 70% methanol, and the entire liquid chromatography (LC) run took 15 minutes.

Before the analysis, the equipment was certified and calibrated. By injecting a matching standard separately under similar chromatographic conditions, it was feasible to validate the retention period of mangiferin. The hydroalcoholic extract of M. indica was also examined using LC-mass spectrometry (Shimadzu LC-MS 8040), and its composition was determined by comparing its M/Z value to the mangiferin reference standard.

Determination the activity of Mangifera indica in vitro:

- Agar well diffusion method

Agar well diffusion method was used to check the activity of plant extract in vitro for 3 concentration (100,150,200) (Kavanagh, 1972). Bacterial colonies were inoculated into 4 ml of nutrient broth and incubated for 2-8 hrs. at 37co, the turbidity of inoculums compared with standardized MacFarland tube number (one) containing (1.5 ×10^8) cfu/ ml, with a sterile cotton swab, the inoculum was spread evenly on the surface of Muller Hinton agar in Petridish, A five wells were made in Muller Hinton agar plates using a sterile cork borer (6mm) , 0.1 ml of different concentrations of plant extract (100, 150, 200mg /ml) were poured in the three wells while the other two wells were filled with 0.1 ml of DMSO and with ethyl alcohol as a control, the plates were incubated up down at 37 co for 24 hrs. Three replicates were carried out for each concentration extract, the diameter of inhibition zone was measured and the average values were recorded. The results and standard errors mean values were tabulated.

Induction of infection in mice and Invivo study:

Sixty (60) Mice will be selected for infection which will be divided into 3 groups (each group contain 20 animals),

Infected group (control positive) with dose 2×108 CFU/ml.

Infected group with dose 2×108 CFU/ml then treatment with Mangifera indica extract at dose of 200mg/ml.

control negative group which given only normal saline.

Results and Discussions:

Active ingredient detection by HPLC

The results of HPLC showed that analysis of extracts contains Qurcetine , Gallic acid, Rutin, Kaempferol, Apigenin ,Catechin. The main component were Gallic acid as well as Qurcetine (Table 1).

Table 1. shows active ingredients of the M. indica extract

Name (ppm)	Qurcetine	Gallic acid	Rutin	Kaempferol	Apigenin	Catechin
Manga	29.58	40.15	22.56	24.99	18.97	26.59

Two polyphenols found in high concentration in specific foods, such grapes and wine, these are gallic acid and catechin (Monagas et al., 2005). In some meals, the content of the non-flavonoid polyphenol gallic acid can exceed 220 mg/kg (Obreque-Slier et al., 2010). As compared to catechin, gallic acid had a greater inhibitory impact on both H. pylori strains (Díaz-Gómez et al., 2013). An important plant flavonoid called quercetin has a wide range of pharmacological effects. Many studies examine its antibacterial effects and potential mechanisms of action. It has been demonstrated that quercetin prevents the growth of several Gram-positive and Gram-negative bacteria, fungi, and viruses (Nguyen & Bhattacharya, 2022).

Determination the activity of Mangifera indica extract invitro against selected Proteus mirabilis isolate:

Three concentrations of extracts were used for determination activity of M. indica, 100,150,200 mg/ml were used. A concentration of 200 mg/ml were showed to be more active against bacteria (Figure 1).

Figure 1. shows the activity of Mangifera indica extract against Proteus mirabilis bacteria

According to reports, M. indica has outstanding anti-influenza action thanks to the bioactive component mangiferin that was isolated from the plant. M. indica has also been shown to have antibacterial activity against E. coli and other members of the enterobacteriaceae family of bacteria (Neon, 1984). The plant's antibacterial properties may be due to the presence of phytoconstituents in the leaf extracts (Marjorie, 1999). The presence of both bactericidal and bacteriostatic action in the M. indica extract may account for

the growth of S. aureus that was isolated from optimum, supraoptimal, and suboptimal concentrations of the extract (Othman et al., 2019). All bacteria would be eliminated by its bactericidal action, while its bacteriostatic activity would stop the development or multiplication of any remaining bacteria (Article, 2004).

The results of this investigation showed that the methanol extract of Mangifera indica leaves was efficient against antibiotic-resistant E. coli, Contrary to other extracts, Mangifera indica methanol extracts (MJLM) shown greater efficacy against P. aeruginosa. This implies that Mangifera indica has unique and powerful inhibitory substance(s) that are effective against P. aeruginosa. (Doughari and Manzara, 2008). This suggests that Mangifera indica leaves have inhibitory compounds that are effective against E. coli (De and Pal, 2014).

Induction of infection in dogs and In vivo study against selected Proteus mirabilis:

The present results revealed that mice in G1 exhibited signs of UTI which diagnosed by isolation of bacteria during all the period of experiment, while mice in G2 showed that decrease of bacterial count till its subsids after 2 weeks of treatment by Mangifera indica.

It had been reported that the aqueous and methanolic extracts of M. indica are assumed to have antibacterial and antifungal properties because they include secondary metabolites such phenols, alkaloids, saponins, favonoids, and triterpenes. In fact, favonoids and certain phenolic chemicals may prevent microbial enzymes from crosslinking, which prevents the development of bacterial and fungal infections; alkaloids can affect the permeability of the cell membrane and change how pathogens use their mitochondria (Joana et al. 2014; Khan et al. 2017). Mangiferin, a substance found in M. indica preparations, has also been demonstrated to have antibacterial and antifungal effects by Wauthoz et al. (2007) and Xiao et al. (2008).

The findings showed that fecal EPEC growth was significantly inhibited by the aqueous and methanolic extracts of M. indica. Additionally, the bactericidal action of the extracts may be shown by the decrease in bacterial load seen starting on the fourth day in all the feces of sick animals treated with them. Additionally, investigations have shown that certain plant extracts containing triterpenes or other chemicals may prevent E. coli from producing flagellin, which is a crucial component of a bactericidal effect (Chao and Yin 2009), these may potentiate the results of current study.

Also, another study reported that the aqueous extract of M. indica is efficacious against both the Gram positive and negative bacteria examined, according to El-Mahmood (2009) in a separate experiment.

Conclusion:

Mangifera indica have antibacterial effects against virulent Proteus mirabillis isolated from dogs with UTI.

Reference

Al-Samarrae, E. A. A. (2011). Evaluation of Proteus vulgarisfimbriae antigen by delayed type hypersensitivity (DTH)-skin test in rabbits. The Iraqi Journal of Veterinary Medicine, 35(1), 100-106.

Article, R. (2004). Clinical Relevance of Bacteriostatic versus Bactericidal Mechanisms of Action in the Treatment of Gram- Positive Bacterial Infections. 38.

Awad El-Gied, A., R. P. Joseph, M., M. Mahmoud, I., M. Abdelkareem, A., M. Al Hakami, A., and E. Hamid, M. (2012). Antimicrobial Activities of Seed Extracts of Mango (<i>Mangifera indica</i> L.). Advances in Microbiology, 02(04), 571–576.

Bbosa, G. S., Kyegombe, D. B., Ogwal-Okeng, J., Bukenya-Ziraba, R., Odyek, O., and Waako, P. (2007). Antibacterial activity of Mangifera indica (L.). African Journal of Ecology, 45(SUPPL. 1), 13–16.

Bonev, B., Hooper, J., Review, T., Chaired, A. R., December, N., Ajayi, C. O., … José, P. (2010). Mangifera Indica (Mango). Pharmacognosy Reviews, 4(7), 42–48.

Chao, C. Y., & Yin, M. C. (2009). Antibacterial effects of roselle calyx extracts and protocatechuic acid in ground beef and apple juice. Foodborne Pathogens and Disease, 6(2), 201-206.

Díaz-Gómez, R., López-Solís, R., Obreque-Slier, E., & Toledo-Araya, H. (2013). Comparative antibacterial effect of gallic acid and catechin against Helicobacter pylori. LWT-Food Science and Technology, 54(2), 331-335.

Doughari, J. H., & Manzara, S. (2008). In vitro antibacterial activity of crude leaf extracts of Mangifera indica Linn. Afr J Microbiol Res, 2(4), 67-72.

Doughari, J. H., & Manzara, S. (2008). In vitro antibacterial activity of crude leaf extracts of Mangifera indica Linn. Afr J Microbiol Res, 2(4), 67-72.

El-Mahmood, M. A. (2009). Antibacterial efficacy of stem bark extracts of Mangifera indica against some bacteria associated with respiratory tract infections.

Hannan, A., Asghar, S., Naeem, T., Ullah, M. I., Ahmed, I., Aneela, S., and Hussain, S. (2013). Antibacterial effect of mango (Mangifera indica Linn.) leaf extract against antibiotic sensitive and multi-drug resistant Salmonella typhi. Pakistan Journal of Pharmaceutical Sciences, 26(4), 715–719.

Joana M, Ana C, Abreu AC et al (2014) Antimicrobial Activity of Selected Phytochemicals against Escherichia coli and Staphylococcus aureus and Their Biofilms. Pathogens 3:473–498

Khan S, Imran M, Imran M et al (2017) Antimicrobial activity of various ethanolic plant extracts against pathogenic multi drug resistant Candida spp. Bioinformation 13:67–72.

Lawson, T., Lycett, G. W., Ali, A., & Chin, C. F. (2019). Characterization of Southeast Asia mangoes (Mangifera indica L) according to their physicochemical attributes. Scientia Horticulturae, 243, 189-196.

Length, F. (2008). In vitro antibacterial activity of crude leaf extracts of Mangifera indica Linn. African Journal of Microbiology Research, 2(4), 67–72.

Maldonado-Celis, M. E., Yahia, E. M., Bedoya, R., Landázuri, P., Loango, N., Aguillón, J., ... & Guerrero Ospina, J. C. (2019). Chemical composition of mango (Mangifera indica L.) fruit: Nutritional and phytochemical compounds. Frontiers in plant science, 10, 1073.

Monagas, M., Bartolomé, B., & Gómez-Cordovés, C. (2005). Updated knowledge about the presence of phenolic compounds in wine. Critical reviews in food science and nutrition, 45(2), 85-118.

Namukobe, J., Kasenene, J. M., Kiremire, B. T., Byamukama, R., Kamatenesi-Mugisha, M., Krief, S., … Kabasa, J. D. (2011). Traditional plants used for medicinal purposes by local communities around the Northern sector of Kibale National Park, Uganda. Journal of Ethnopharmacology, 136(1), 236–245.

Neon, B. (1984). Medicinal plants in Nigeria. Private Nigerian Collage Arts Sciences Technology. 1-84.

Nguyen, T. L. A., & Bhattacharya, D. (2022). Antimicrobial activity of quercetin: an approach to its mechanistic principle. Molecules, 27(8), 2494.

Obreque-Slier, E., Peña-Neira, Á., López-Solís, R., Zamora-Marín, F., Ricardo-da Silva, J. M., & Laureano, O. (2010). Comparative study of the phenolic composition of seeds and skins from Carménère and Cabernet Sauvignon grape varieties (Vitis vinifera L.) during ripening. Journal of Agricultural and Food Chemistry, 58(6), 3591-3599.

Othman, S. N. N., & Seka, M. (2019). In-Vitro Antioxidant and Cytotoxic Activities of Silver Nanoparticles of Mangiferin Isolated from Mangifera indica. Journal of Global Pharma Technology, 11(6), 10-15.

Pal, N., Sharma, N., Sharma, R., Hooja, S., & Maheshwari, R. K. (2014). Prevalence of multidrug (MDR) and extensively drug resistant (XDR) Proteus species in a tertiary care hospital, India. Int. J. Curr. Microbiol. Appl. Sci, 3, 243-252.

Sabeeh, J. A. A., & Hatem, Z. A. (2013). Study of the inhibitory effect of the ethanolic extract of a number of local medicinal plants on the growth of proteus spp. in vitro. The Iraqi Journal of Veterinary Medicine, 37(1), 40-46.

Wauthoz, N., Balde, A., Balde, E. S., Van Damme, M., & Duez, P. (2007). Ethnopharmacology of Mangifera indica L. bark and pharmacological studies of its main C-glucosylxanthone, mangiferin. International Journal of Biomedical and Pharmaceutical Sciences, 1(2), 112-119.

World Health Organization. (2019). WHO global report on traditional and complementary medicine 2019. World Health Organization.