International Journal of Research Studies in Biosciences (IJRSB)
Volume 9, Issue 2, 2021, PP 15-21
ISSN No. (Online) 2349-0365
DOI: https://doi.org/10.20431/2349-0365.0902003
www.arcjournals.org

The Effect of Seed Extract from *Mangifera indica* L. on the Growth and Biofilm Formation of Uropathogenic *Escherichia coli*

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Abstract: *The extracts of plant origin have shown multiple antibacterial properties. One of the advantages of using plants as an alternative therapeutic is that it has a wide range of antimicrobial activity because it contains a lot of active ingredients that make it toxic to microorganisms. In this study, the effect of M. indica L. seed extract on the growth and biofilm formation of uropathogenic E. coli was determinated.*

Keywords: *Mangifera indica, extract, antibacterial, biofilm, E. coli, uropathogenic*

1. INTRODUCTION

Antibiotic resistance is a current problem that occurs in different countries of the world (Odonkor and Addo, 2011). As is known, acute respiratory infections and diarrheal infections occupy the first places in diseases of infectious origin, however systemic infections are also present among the human population, being difficult to treat due to the multiple cases of resistance to antibiotics (Atiaa *et al*., 2020). Currently, the search for new substances with antimicrobial activity is frequent by different research groups in the world, especially the study of compounds of natural origin with antimicrobial properties. In this context, various extracts of plant origin have shown multiple antibacterial and antibiofilm properties (Dogruoz *et al.*, 2008). It has been reported that medicinal plants have been used for therapeutic purposes since ancient times (Flores-Encarnación *et al*., 2016*b*). One of the advantages of using plants as an alternative therapeutic is that it has a wide range of antimicrobial activity because it contains a lot of active ingredients that make it toxic to microorganisms (Ali *et al.*, 2015; Diao *et al*., 2013; Flores-Encarnación *et al*., 2016*b*; Patra and Baek, 2016). It has been reported that fruit and leaf extracts of some seed plants have shown antibacterial effects against *Bacillus subtillis, B. megaterium, Pseudomonas aeruginosa, Staphylococcus aureus, E. epidermidis* (Dogruoz *et al*., 2008). Many examples of colorful fruits are known for their nutritional and antioxidant properties. One of them is *Mangifera indica* L. (mango), sometimes called "The king of fruits", which is by volume the second largest tropical fruit crop in the world after bananas (Govindan, 2019). *M. indica* L., is a good source of sugars, vitamins A and C and minerals. The fruit pulp contains vitamins A and C, β-carotene and xanthophylls (Shibahara *et al*., 1993). Manzur *et al*., (2019) reported that the leave extracts of *M. indica* L. reduced the biofilm formation of *Staphylococcus* spp. in stainless steel. In the present work, the effect of seed extract from *M. indica* L. on the growth and biofim formation of uropathogenic *E. coli* was studied.

2. MATERIAL AND METHODS

2.1 Biological material

A strain of uropathogenic *E. coli* CFT073 was used. Bacterial strain was stored in cryovials at -40°C until analysis. The *Mangifera indica* L. var. Manila fruits used in this study were purchased from a local market of Puebla city, México.

2.2 Culture conditions

The trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md) was used for bacterial culture. Test strain that had been cultured at 37°C for 24 to 48 hours in trypticase soy broth were seeded crosswise in a Petri dish containing trypticase soy agar, and the plate was incubated at 37°C for 24 hours.

2.3 M. indica seed preparation

To obtain extracts, 10 seeds from *M. indica* L. were used. The peels were manually removed, while *M. indica* L. pulps by knife were removed. Then seeds were dried using an oven at 60°C until it attained constant weight (36 to 96 hours). *M. indica* L. seeds were stored at 0°C until analysis. Then, seeds were mechanically fractionated and 5 grams of them was added in 100 mL of sterile distilled water. To obtain the homogenate, the previous preparation was placed in a 200 mL-glass for blender with stainless steel blades and pulverized during 5 to 10 minutes at 4°C; immediately the microwave-assisted extraction was performed.

2.4 Microwave-assisted extraction

A modified domestic microwave oven was used for the extraction purpose according to the methodology reported by Thakker *et al.*, (2016). The oven has an output power range of 600 W with a operating frequency of 2500 Hz. The oven was modified by making a hole at the top to accommodate a reflux condenser. To start the extraction, the homogenate of *M. indica* L. seed was placed in a round bottom flask (250 mL). The flask was then placed in the central position of microwave oven and subsequently subjected to microwave power (according to the conditions mentioned above), applying 10 pulses during 1 minute with break intervals of 1 minute (total extraction time: 20 min). After extraction, the resulting extracts were filtered using compressed sterile cotton gauges to remove large plant debris and then filtered with filter paper. After filtration, the samples were aliquoted and kept at -5 ° C until use.

2.5 Antibacterial activity

Antibacterial activity of *M. indica* seed extract was determined using technique of well diffusion in agar. Briefly, tripticasein soy agar plates containing 20 mL and 40 mL of medium were prepared. Sterile Petri dishes (150 mm) were used. Plates were inoculated by crossstriation with uropathogenic *E. coli*. Each inoculum contained approximately 10^6 CFU mL^{-1}. Subsequently, 5 wells were made on the tripticasein soy agar plate with the aid of the mouthpiece of a sterile glass Pasteur pipette. Then, different volumes of *M. indica* seed extract (5 to 750 µL) were added in each well. The agar plates were allowed to stand for about 20 min at room temperatura and then they were incubated at 37°C for 24 h. The halo diameter of bacterial growth inhibition was measured using a caliper ruler. The analyses were conducted in triplicate.

2.6 Detection of biofilm

For biofilm detection of uropathogenic *E. coli*, the calcofluor white staining was used according to modified methodology described by Ramos *et al*., (2006) and Flores-Encarnación *et al*., (2016a). Briefly, Luria Bertani agar plates containing 0.02% calcofluor White and the technique of well diffusion in agar were used. So, 20 µL of uropathogenic *E. coli* incubated overnight was inoculated by crossstriation. Subsequently, 4 wells were made on agar with the aid of the mouthpiece of a sterile glass Pasteur pipette. Then, two volumes of *M. indica* seed extract were added in each well: 200 and 750 µL. The agar plates were incubated at 37°C during 48-72 h into a chamber to keep moisture. Then, Luria Bertani agar plates containing 0.02% calcofluor White were exposed to UV light and the fluorescence emitted by exopolysaccharides of cells forming biofilm was observed. Assays on all samples were repeated in duplicate.

3. RESULTS

As described above, the effect of *M. indica* seed extract on the growth and biofim formation of uropathogenic *E. coli* was determined. For this, the mango seeds were pulverized and extracted by a microwave-assisted method as indicated in Materials and Methods. Antibacterial activity of extract was determined using technique of well diffusion in agar using tripticasein soy agar plates containing 20 mL and 40 mL of medium. Plates were inoculated with uropathogenic *E. coli* and 5 wells were made on the tripticasein soy agar. Then, different volumes of *M. indica* seed extract were added in each well: 5 to 750 µL and the agar plates were incubated at 37°C for 24 h. The results obtained are shown in Fig. 1. As shown in Fig. 1A, uropathogenic *E. coli* was grown in trypticase soy agar at 37°C for 24 h in the presence of 500 µL of *M. indica* seed extract, which was added in one quadrant of the surface of trypticase soy agar. As can be seen in the figure, uropathogenic *E. coli* had a good growth on trypticasein soy agar, except in the area where the *M. indica* seed extract was placed. This indicated that the seed extract obtained had antibacterial properties. Next, some tests were performed using the agar diffusion technique. For this, some paper discs were impregnated with 1 to 10 µL of *M. indica* seed extract. At these low amounts of the extract, effect was not observed on the growth of uropathogenic *E. coli* (data not shown). Due this, the well diffusion tests were used. Thus, different amounts of *M. indica* seed extract were placed in each well. Fig. 1B shows the results obtained when 5, 10, 20, 30 and 50 µL of *M. indica* seed extract were placed in wells. As shown in Fig. 1B, the *M. indica* seed extracts did not inhibit the growth of uropathogenic *E. coli*. Similar results were observed using 75, 100, 125 and 150 µL of *M. indica* seed extract (Fig. 1C). Using larger amounts of the *M. indica* seed extract such as 400, 500, 600, 700 and 750 µL, the growth inhibition of *E. coli* was observed. The results obtained are shown in Fig. 1D. As shown in this figure, the *M. indica* seed extracts produced the growth inhibition halo. In most cases, the halo came to measure around 18 to 25 mm in diameter. Due to these results, it was proposed that the *M. indica* seed extracts did not diffuse in the best way due to agar thickness. As mentioned in Materials and Methods, the trypticasein soy agar plates containing 40 mL of medium were used.

On the other hand, the effect of *M. indica* L. seed extract on biofilm formation of uropathogenic *E. coli* was determined. For that, Luria Bertani agar plates containing 0.02% calcofluor White were used as described in Materials and Methods. The results obtained are shown in Fig. 2. As seen in Fig. 2A, the production of expolysaccharides in biofilm was inhibited by presence of *M. indica* seed extract. The fluorescence emitted by calcofluor White was only observed surrounded all wells containing the *M. indica* extracts (outside of bacterial growth inhibition halos). This suggested that there was no presence of exopolysaccharides in the growth inhibition area of uropathogenic *E. coli*. Outside the growth inhibition halos, the fluorescence due to calcofluor white was observed, indicating that uropathogenic *E. coli* produced exopolysaccharides on the agar surface in the absence of *M. indica* seed extract. As a reference, uropathogenic *E. coli* was cultived cross-streaked in a Luria Bertani agar plate containing 0.02% calcofluor White in absence of *M. indica* seed extract. The production of exopolysaccharides was observed (Fig. 2B).

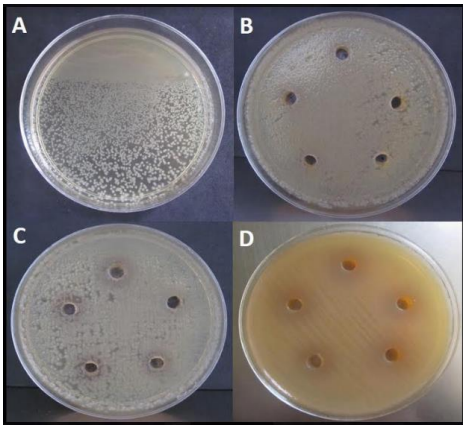

Fig1. *The effect of M. indica L. seed extract on the growth of uropathogenic E. coli. A. Antibacterial activity of an extract aliquot. B. 5 to 50 µL of extract were added to wells. C. 50 to 150 µL of extract were added to wells. D. 400 to 750 µL of extract were added to wells. In all cases, extracts were placed in increasing concentrations in the counterclockwise direction, starting with the top well.*

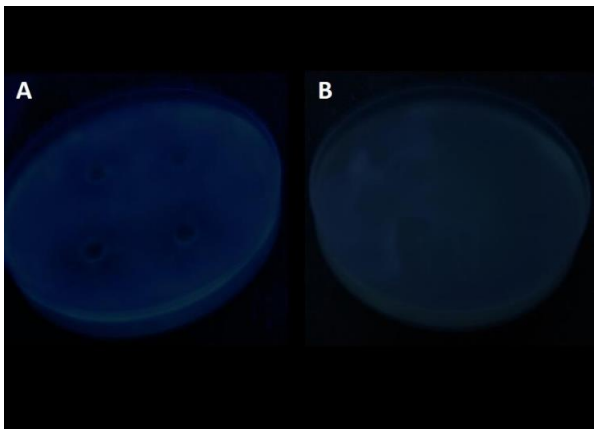

Fig2. *The effect of M. indica L. seed extract on biofilm formation of uropathogenic E. coli. A. Inhibition of production of biofilm (expolysaccharides) in Luria Bertani agar plate containing 0.02% calcofluor White. B. Control condition.*

4. DISCUSSION

Resistance to antibiotics by bacteria is a growing problem. As a result, the treatment of many infectious diseases is difficult and expensive (Odonkor and Addo, 2011). At present, various products of plant origin are known to possess antimicrobial properties. Attacking not only bacteria, but also viruses, fungi and even parasites (Abubakar, 2009; Akinpelu and Onakoya, 2006; Castaño *et al*., 2010; Dogruoz *et al.*, 2008). One of the advantages of using plants as an alternative therapeutic is that it has a wide range of antimicrobial activity because it contains a lot of active ingredients that make it toxic to microorganisms (Flores-Encarnación *et al*., 2016*b*). In them, a mixture of aldehydes, alcohols, terpenoids and other compounds have been found (Diao *et al*., 2013; Flores-Encarnación *et al.*, 2016*b*). The plant products have found also wide application as alternatives to conventional therapy and food preservation (Carhuapoma *et al*., 2009; Castaño *et al*., 2010; Dogruoz *et al*., 2008; Patra and Baek, 2016; Rincón-Mejía *et al*., 2012). Among those plant products, extracts of *M. indica* L. obtained from the leaves have shown antibacterial properties. *M. indica* L, commonly called as mango, is a plant belonging to the family *Anacardiaeceae*; it is one of the most popular tropical fruit trees in the world (Akinpelu and Onakoya, 2006; Kabuki *et al*., 2000). In the present study, the effect of seed extract from *M. indica* L. on the growth and biofilm formation of uropathogenic *E. coli* was studied. The results obtained shown that the presence of *M. indica* seed extract inhibited the growth of uropathogenic *E. coli*, indicating that the seed extract obtained had antibacterial properties (Fig. 1A). This was confirmed using the well diffusion technique in agar plates, where it was observed that using 400 to 750 µL of the *M. indica* seed extract the growth inhibition of *E. coli* was observed (Fig. 1D). As it was shown in this figure, all samples of *M. indica* seed extracts produced the growth inhibition halo around 18 to 25 mm in diameter, probably because the seed extracts did not diffuse in the best way due to agar thickness. At amounts lower than 400 µL of the *M. indica* seed extract, no effect was observed on the growth of uropathogenic *E. coli*. As other authors have reported, *M. indica* L. seed kernel extract showed its inhibitory effect against coliform and *E. coli* isolates and it has been used in food products or cosmetics due to its bacteriostatic and antibacterial properties and antifungal and antioxidant activities (Abdalla *et al*., 2007; Hannan *et al.*, 2013). It has been reported that *M. indica* L. plant has also several biological properties as like to treat mouth infections in children, diarrhea, dysentery, gastrointestinal tract disorders, typhoid fever, sore throat and scurvy. It has been reported the antibacterial efficacy of stem bark extracts of *M. indica* against some bacteria associated with respiratory tract infections (Abubakar, 2009; Hannan *et al.*, 2013). *M. indica* L. is indigenous to eastern Asia, Myanmar (Burma), and Assam state of India. Cultivated in many tropical regions, *M. indica* L. has special significance in Africa, and a large part of Asia including Pakistan, India, Bangladesh and Philippines and other regions in the world (Abubakar, 2009). In the present study, the extracts were obtained from *M. indica seeds*, which is relevant because most people, at least in Mexico, consider this material as waste and throw it away. However, as could be seen, *M. indica* seeds have broad biotechnological potential as a source of antimicrobial substances. It has been reported that the *M. indica* seed represents 10% to 25% of the entire fruit weight, depending on the mango variety. Studies have shown that *M. indica* seed kernel can be a rich source of different

phenolic and antioxidant compounds (Abdalla *et al*. 2007; Dorta *et al.* 2013; Maisuthisakul and Gordon, 2009). Lim *et al*., (2019) reported that gallic acid, caffeic acid, rutin and penta-O-galloyl-b-D-glucose were identified to be present in *M. indica* seed kernel. On the other hand, in this study the effect of *M. indica* L. seed extract on biofilm formation of uropathogenic *E. coli* was determined. The results obtained shown that the production of expolysaccharides in biofilm of uropathogenic *E. coli* was inhibited by presence of *M. indica* seed extract. The fluorescence emitted by calcofluor White was only observed surrounded all wells containing the *M. indica* extracts (there was no presence of exopolysaccharides in the growth inhibition area of uropathogenic *E. coli*) (Fig. 2). The presence of biofilm gives certain advantages to bacteria as protection from the environment, resistance to the bactericidal action of the antimicrobials, altered host defense mechanisms (hinders macrophage phagocytic activity by interfering with the coating antibodies to block opsonization and phagocytosis) (Flores-Encarnación *et al*., 2014; Kostakioti *et al*.,, 2013; Shiau and Wu, 1998). The results obtained in this study are in agreement with that reported by other authors. Adesina *et al*., (2015) reported that *M. indica*, *Psidium guajava* and *Ocimum gratissimum* leaf extracts prevented the *E. coli* biofilm formation on catheters. Manzur *et al*., (2019) reported that extract of *M. indica* L. leaves reduced biofilms of *Staphylococcus* spp. Finally, it is important to mention that the microwave-assisted extraction method allowed obtaining the *M. indica* seed extracts with a good antibacterial activity and reduced the extraction time to 20 min under the conditions tested. Major conventional techniques being employed like Soxhlet extraction, heating–stirring method, heat-refluxing, consume large quantity of solvents and require longer extraction time. Compared to traditional techniques, microwave-assisted extraction is characterized by reduced solvent consumption, shorter extraction time, and increased pollution prevention (Cui *et al*., 2015; Cvjetko Bubalo *et al*., 2016; Pal and Jadeja, 2020).

5. CONCLUSION

At present, the search for new substances with antibacterial activity is carried out in different parts of world, with plant extracts being an option for the extraction of their active ingredients. In this study, the extracts of the *M. indica* L. seed showed antibacterial and antibiofilm activities. It is important to carry out more studies to know about the possible mechanisms of its antimicrobial action.

ACKNOWLEDGEMENT

We appreciate the enthusiastic collaboration and technical support of IB. Melchor-Jiménez N. and MC. Nava-Nolazco R.M. Thank to Facultad de Medicina-BUAP and Grupo de Académicos de Puebla SC for the facilities provided for the development of this work.

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Citation: *Flores-Encarnación, M, et.al., The Effect of Seed Extract from Mangifera indica L. on the Growth and Biofilm Formation of Uropathogenic Escherichia coli. International Journal of Research Studies in Biosciences. 2021; 9(2): 15-21. DOI: https://doi.org/10.20431/2349-0365.0902003.*