Rami Ali Taqi. / *International Journal of Biological & Pharmaceutical Research.* 2013; 4(12): 1147-1150.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

EFFECT OF *FICUS RELIGIOSA* PHENOLICS ON THE FORMATION OF BIOFILM AND SWARMING OF *PROTEUS MIRABILIS*

Rami Ali Taqi

Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq.

ABSTRACT

Urinary tract infection by *Proteus mirabilis* depends on several virulence properties such as ability of adherence and swarming. Here we report the antiadherence, antibiofilm and antiswarming effects of phenolic compounds of *Ficus religiosa* leaves and bark. The results showed that phenolic compounds of leaves was more effected than bark in reduction of adherence, biofilm formation and swarming motility especially at concentration 2 mg/ml , the reduction of adherence of bacteria was obvious in glass tubes. The ability of leaves phenolics to decreasing the biofilm formation in microtiter plate was significant in contrast with control. The results which suggests that *Ficus religiosa* phenolics can potentially be developed as UTI drugs.

Key Words: *Ficus religiosa*, *Proteus mirabilis*, Phenolics, Biofilm, Swarming.

INTRODUCTION

Bacteria growing in a biofilm on a surface are generally more resistant to many antimicrobial agents than the same bacteria growing in a free-swimming state (Dontan and Costerton, 2002). Swarming, a process wherein short, oligoflagillated vegetative swimmer cells. differentiate into a separate multinucleate, filamentous, hyperflugellated swarm cells arranged in palisades or rafts enables bacteria to migrate rapidly and collectively over surfaces (Hernandez *et al*., 1999).

P. mirabilis can cause severe UTI particularly in patients with urinary catheters or in people with structural abnormalities of the urinary tract (Warren *et al*., 1982) *Ficus* species are rich source of polyphenolic compounds, flavonoids which are responsible for strong antioxidant properties that help in prevention and therapy of various disorders (Ephuraim *et al*., 2008). It has been observed in some studies that the plant-produced phenolic metabolites attenuates biofilm formation in *P. aeraginosa* (Yang *et al*., 2009). With an increasing difficulty in treating UTI infections, there has been a growing interest in alternative therapies and the use of natural, especially plant based products. The aim of this study are evaluation the effect of phenolic compounds extracted from leaves and bark of *Ficus religiosa* against the formation of biofilm and swarming activity of *Proteus mirabilis* isolated from Urinary Tract Infections (UTI).

Corresponding Author

Rami Ali Taqi
Email: raminaqi@yahoo.com

MATERIALS AND METHODS

Plant material

Park and leaves of *F. religiosa* were procured from Al Jadiria, Baghdad, Iraq. Authentication and identification of the plant was carried out by Prof. Dr. Ali Al- Mosawy, Department of Biology, College of Science, University of Baghdad.

Sample preparation

Park and leaves of *F. religiosa* were cleaned and cut into small pieces, and then oven dried at 50°Cfor a day. The dried sample was then pulverized into fine powder in a grinder, and then stored at 4°C until use.

Extraction and purification of phenolics

A dried sample of leaves and park l0 g extracted for 30 min. by stirring at 4°C with 200 ml of cold aqueous

ethanol 65 % containing 0.5 % Sodium metabisulphite. The homogenate was filtered through four layers of cheesecloth, and the residue was then extracted with two additional portions (100 ml each) of the same extraction solution as described above. The combined filtrate was centrifuged at 7000 rpm for 15 min. at 4C and residue was discarded. Ethanol was removed from the supernatant by rotary evaporator under vacuum at 35°C, and the mass is measured. Pigments were eliminated by two successive extractions with petroleum ether. After addition of 20% ammonium sulphate and 2% metaphosphoric acid to the aqueous phase, the compounds were extracted three times with ethyl acetate. The extracts were combined, evaporated and then dried under vacuum at 35*P*o*P*C. The residue was redissolved in methanol (1:1) for analysis(Zhu *et al*., 2008).

The effects of plant extraction on adhering of bacteria to glass tube

This test was used to detect the ability of bacteria to adhere to smooth surface (glass tubes). The test was done by cultivating the tested bacteria in glass tubes contain (1ml) nutrient broth. The control tube contains (1ml) nutrient broth only. All the tubes were incubated for 24 hrs at 37 °C. After incubation period, the contents of tubes were removed then 1m of plant extracted were add to each tubes with different concentration (1 mg/ml, 2 mg/ml) after that all tubes were incubated for 24 hrs at 37 °C then the contents of tubes were removed and the contents of tubes were removed and tubes were stained by adding (1 ml) of 0.1% crystal violet for 1 minute then the effect of plant extraction on adhering growth was seen. Slime production was visible through a film that occurred on tube walls. We estimated the amount of slime production as absent (score 0), weak (score 1), moderate (score 2), strong (score 3) or very strong (score 4) (Christensen *et al*., 1983).

Biofilm inhibition assay

Biofilm inhibition carried out in 96 wall plates adopting modified method of biofilm inhibition spectrophotometric assay (Regev-Shoshani *et al*.,2010) .100µl of cell suspension of *E.coli* thus prepared was added into 96 well titre plate and different concentration of plant extracts as 1 and 2 mg/ml was added and incubated at 37º C for 3 days. After the incubation, the liquid suspension was removed and 100 µl of 1% w/v aqueous solution of crystal violet was added. Following staining at room temperature for 30 minutes the dye was removed and the wells were washed thoroughly, 95% ethanol was added and incubated for 15 minutes. The reaction mixture was read spectrophotometrically at 570nm. Inhibition mediated reduction of biofilm formation was calculated by the following formula:

$$\% \text{ of inhibition} = \frac{\text{OD in control} - \text{OD in treatment} \times 100}{\text{OD in control}}$$

Swarming behavior assay

The effect of the extract on swarming migration was assessed as described by Liaw *et al.* (2000), Briefly an overnight bacterial culture (5 µl) was inoculated centrally onto the surface of dry LB swarming agar plates without or with phenolic compounds at 1 and 2 mg/ ml which were then incubated at 37 ºC for 24 hrs. The swarming migration distance was assayed by measures of the swarming areas fronts.

RESULTS AND DISCUSSION

The ability of bacteria to adherence to glass tubes and the effect of F. religiosa phenolics on adhering of bacterial isolate P. mirabilis were summarized in figure 1 – a, b.

Results indicated that *P. mirabilis* gave positive result (presence of adherent growth on smooth surface or glass tubes). It was found that phenolics of leaves and bark of *F. religiosa* (1 mg/ml and 2 mg/ml) decrease the adherent growth of bacterial isolate on glass tubes as shown in figure 1.b. and c, It was obvious that the effect of leaves phenolics at concentration 2mg/ml gave the better result in contrast with control with more decreasing of adherent growth. Microbial adhesion is considered the first step in the sequence of events leading to colonization, the ability to adhere is weakened by exposure to sublethal doses of antibacterial agents (Sharma and Sabris, 2010). Some studies referred to the effect of polyphenolic compounds on the enzymatic activity of glucosyltransferase which is the essential virulence factor that allows the colonization of bacteria and adherence (Yanagida *et al*., 2000, Gregoire *et al*., 2007).

The results of the effect of phenolic compounds of F.religiosa leaves and park on biofilm formation of *P. mirabilis* showed in figure 2. It was found that phenolic compounds of leaves exhibited reduction in biofilm.

Formation of *P. mirabilis* , where the absorbance 2 mg\ml of phenolics leaves was 0.019 in comparison with control (0.075) .The effect of park phenolic was less than leaves but it good effect in comparison with control. Biofilm formation plays avery important role in the pathogenesis of UTI pathogens (Jones *et al*., 2005) .

Some studies referred that phenolic compounds such as catecnin-derived flavanols protect the fruit surface against pathogenic attack (Feucnt *et al*., 1994 and Feucnt and Schwalb, 2000). The obvions interfering of polyphenols such as epigallocatechingallate (EGCG), ellagic acid and tannic acid with biofilm formation and Quorum-sensing reported Huber *et al*., (2003). The inhibitory action of phenolics on biofilm formation may be due to iron starvation, since some of phenolic have moderate iron chelating properties (Devoss *et al*., 1999).

The figure 4. Summarized the effect of phenolic compounds of leaves and park of *F. religiosa* on swarming of p. mirabilis. The results revealed that ther is no significant effect of 1mg\ml of leaves and park phenolics

on swarming zone diameter, in contrast swarming zones more susceptible to the concentration 2mg\ml of phenolics specially from leaves , where the reduction of swarming was from 9cm to 1cm. Some studies suggested that the phenolic acids such as tannic acid and tannin –containing materials blocked swarming motility without impairing *P. aeruginosa* growth capacities (Omay and Tufenkji, 2011). The mechanism of inhibition swarming may be due to binding and precipitating of phenolics to many different types of proteins (Haslam, 1996; Pratt and Kloter, 1998).

Fig 1. The ability of *P. mirabilis* to adherence to glass tubes (A) , the effect of phenolics of *F.religiosa* bark on adhering of *P. mirabilis* to glass tubes (B) and the effect of phenolics of *F.religiosa* leaves on adhering of *P. mirabilis* to glass tubes (at 2 mg/ml)

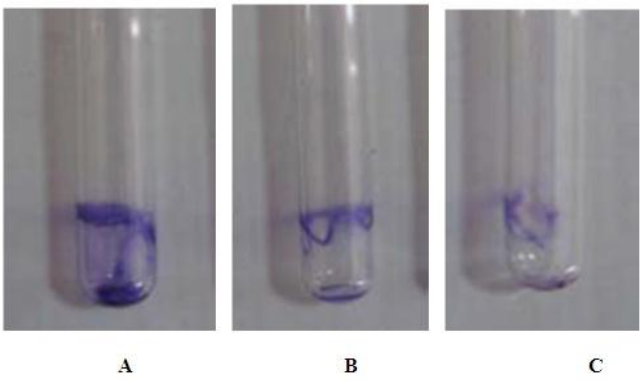


Fig 2. Antibiofilm activity of *F. religiosa* phenolics by using stained biofilms of *P. mirabilis* in a microtiter plate reader

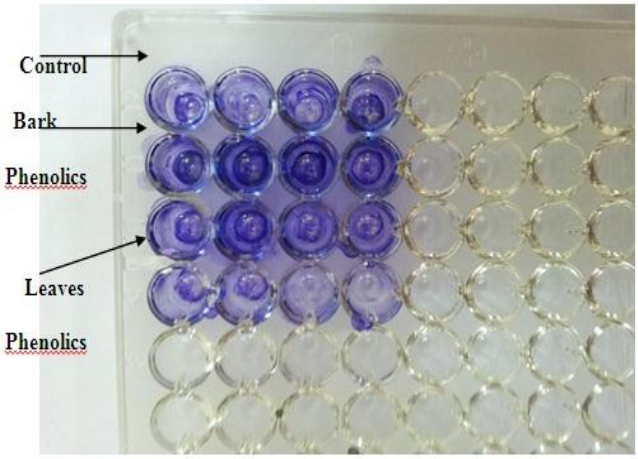


Fig 3. Effect of phenolic compounds of *Ficus religiosa* leaves and bark on biofilm formation of *Proteus mirabilis*

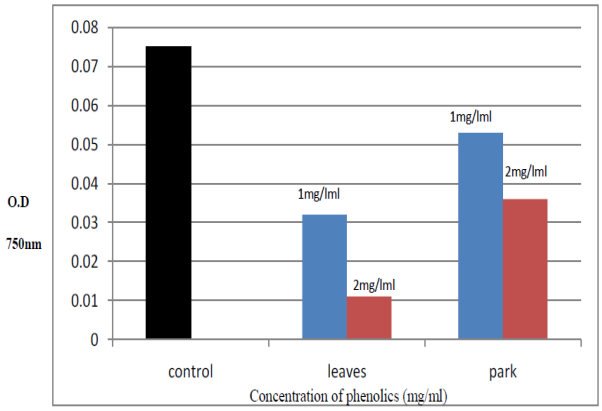
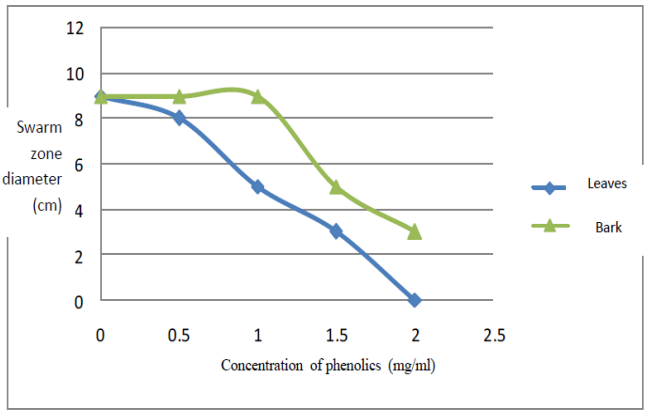


Fig 4. Effect of phenolic compounds of *Ficus religiosa* leaves and bark on swarming of *Proteus mirabilis*



REFERENCES

Christensen GD, Purisi JT, Bisno AL, Sineson WA and Beachey EH. Characterization of clinically significant strains of coagulase-negative Staphylococci. *J. Clin. Microbiol.* 1983; 18: 258-264.

Devoss JJ, Rutter K, Schroeoder BG and Barry CE. Iron acquisition and metabolism by mycobacteria. *J. Bacterial.* 1999; 181: 4443-4451.

Donlan RM and Costerton JW. Review biofilms survival mechanisms of clinically relevant microorganisms. *Clin. Microbial Rev.* 2002; 15: 167-193.

Ephuraim L, Helena MP, Alison D, Newman A. Ficus Spp. (Fig.): Ethnobotany and potential as anticancer and anti-inflammatory agents. *J. Ethanopharmacology.* 2008; 119(2): 195-213.

Feucht W and Crist ET. Flavonols as defend barriers of the fruit surface. *Angew. Bot*. 1994; 68: 122-126.

Feucht W and Schwalb P. Complexation of Fungal structure with monomoric and oligomeric flavonols. *J. Plant Did. and Protact.* 2000; 107: 106-110.

Grgoire S, Singn AP, Vorsa N and Koo H. Influence of Cranberry phenolics on glucan synthesis by glucosyltransterase and *Streptococcus mutans* acidigenicity. *J. Appl. Microbiol.* 2007; 103:1960-1968.

Haslum E. Natural poly phenols (vegetable tannins) as drugs: possible modes of action . *JNat prod.* 1996; 59: 205-215.

Hernandez E, Ramisse F and Cavallo JD. Abolition of swarming of proteus. *J. Clin. Microbial.* 1999; 37: 343-343.

Huberb, Eberl L, Feucnt W, Polster G. Influence of polyphenols on bacterial Biofilm for mation and Quorum-sensihg. Z. *Naturforscn.* 2003; 58: 879-884.

Jones BV, Mahentniralingam E, Sabbaba NA, Sticler DJ. Role of Swarming in the formation of crystalline *Proteus mirabilis* biofilms on urinary catheters. *Journal of Medical Microbiology*. 2005; 54: 807-813.

Liaw SJ, Lai HC, Ho SW, Luh KT, Wang WB. Inhibition of virulence factor expression and swarming differentiation in *Proteus mirabilis* by p-nitrophenylglycerol. *J Med Microbiol.* 2000; 49: 725-731.

Omay C and Tufenkji N. The swarming motility of Pseudomonas aeruginosa is blocked by cranberry proanthoeyanidins and other tannin-containing materials. *Aeelized Environ microbial.* 2011; 77(a): 3061- 3067.

Pratt LA and Kotter R. Genetic analysis of Escherichia coli biofilm formation iroles of flagella , motility and chemotaxis. *Mol microbial.* 1998; 30: 285-293.

Regev-Shoshani G, Ko M, Miller C, Av-Gay Y. Slow release of nitric oxide from charged catheters and its effect on biofilm formation by *Escherichia coli*. *Antimicrob Agents Chemother.* 2010; 54: 273-279.

Sharma S and Sabois S. Study of anti-adhesive properties of fruit juices and plant extract on urine tract pathogens. *Asian J. Exp. Biol. Sci*. 2010; 2: 100-103.

Warren JW, Tenney JH, Hoopes JM, Kass EH. A prospective microbiologic study of baceriuria in patients with chronic indwelling catheters. *J. Infect. Dis.* 1982; 146: 719-726.

Yang L, Ryotke MT, Jakobsen TH, Tolker-Nielsen T. Computer-aided identification of recognized drugs as Pseudomanus aeruginosa quorum-sensing inhibitors. *Antimicrob Agents Chemother.* 2009; 53: 2432-2443.

Zhu C, Deng X and Shi F. Evalution of the antioxidant activity of Chinese Hickory (*Carya cathayensis*) kernel ethanol extraction. *African Journal of Biotechnology.* 2008; 7(3): 2169- 2173.