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Antibacterial and Antifungal Activities of Gossypitrin from *Talipariti elatum* Sw. (Fryxell)

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A B S T R A C T

The present study investigated the antibacterial and antifungal activities *in vitro* of gossypitrin, a flavonoid glucoside isolated and characterized from the ethanolic extracts of *Talipariti elatum* Sw. (Fryxell). The inhibition activity against bacteria was 53.85%, while inhibition activity against fungal was 15.38%. The antibacterial activity of gossypitrin against *Staphylococcus epidermidis*, *Proteus vulgaris* and *Klebsiella pneumoniae*, showed varying degrees of inhibition on the tested organisms. Only two of 13 fungal strains were inhibited, *Candida albicans* and *Candida subtilis.*

Introduction

Flavonoids are known to be synthesized by plants in response to microbial infection; thus it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Flavonoid rich plant extracts from different species have been reported to possess antibacterial activity (Mishra *et al*., 2013; Mishra *et al*., 2013, 2011; Pandey *et al*., 2010). Several flavonoids including apigenin, galangin, flavone and flavonol glycosides, isoflavones, flavanones, and chalcones have been shown to possess potent antibacterial activity (Cushnie *et al*., 2005). Antibacterial flavonoids might be having multiple cellular targets, rather than one specific site of action. One of their molecular actions is to form complex with proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, and so forth. Lipophilic flavonoids may also disrupt microbial membranes (Cowan, 1999; Mishra *et al*., 2009).

Catechins, the most reduced form of the C3 unit in flavonoid compounds, have been extensively researched due to their antimicrobial activity. These compounds are reported for their *in vitro* antibacterial activity against *Vibrio cholerae*, *Streptococcus mutans*, *Shigella*, and other bacteria (Borris, 1996). The catechins have been shown to inactivate cholera toxin in *Vibrio cholera* and inhibit isolated bacterial glucosyl transferases in *S. mutans*, probably due to complexing activities (Borris, 1996). Robinetin, myricetin, and (−)-epigallocatechin are known to inhibit DNA synthesis in *Proteus vulgaris*. Mori *et al*. (1987) suggested that the B ring of the flavonoids may intercalate or form hydrogen bond with the stacking of nucleic acid bases and further lead to inhibition of DNA and RNA synthesis in bacteria. Another study demonstrated inhibitory activity of quercetin, apigenin, and 3, 6, 7, 3', 4'-pentahydroxyflavone against *Escherichia coli* DNA gyrase (Ohemeng *et al*., 1993).

Naringenin and sophoraflavanone G have intensive antibacterial activity against methicilline resistant *Staphylococcus aureus* (MRSA) and *streptococc*i. An alteration of membrane fluidity in hydrophilic and hydrophobic regions may be attributed to this effect which suggests that these flavonoids might reduce the fluidity of outer and inner layers of membranes. The correlation between antibacterial activity and membrane interference supports the theory that flavonoids may demonstrate antibacterial activity by reducing membrane fluidity of bacterial cells.

Talipariti elatum is native to the islands of Cuba, Jamaica, US Virgin Islands, Puerto Rico and Martinica. In wetter areas it will grow in a wide range of elevations, up to 1200 meters (3900 Ft.) and is often used in reforestation. It is the national tree of Jamaica. *Talipariti elatum* tree is quite attractive with its straight trunk, broad green leaves and hibiscus-like flowers. The attractive flower changes color as it matures, going from bright yellow to orange red and finally to crimson (Figure 1). It grows quite rapidly, often attaining 20 meters (66 Ft.) or more in height. The name mahoe is derived from a Caribe word. The “blue” refers to blue-green streaks in the polished wood, giving it a distinctive appearance.

Whereas the pattern of flavonoids and polyphenol derivatives in *T. elatum* has been studied in detail and showed qualitative conformity among these species (Cuéllar *et al*., 2001), only very few data on flavonoids in *T. elatum* are available. Until now, only gossypitrin (gossypetin-7-*O*-β-Dglucoside) has been reported in Cuba (Cuéllar *et al*., 2010). In Blue Mahoe trees that are harvested in Martinica, Martinican researchers revealed the presence for the first time of gossypetin-3'- *O*-glucoside, an isomeric molecule of gossypitrin.

The aim of this study was to evaluate the antimicrobial activity *in vitro* of gossypitrin against bacterial and fungal strains.

Materials and Methods

Plant Material

Flowers were collected in January 2015 in the gardens of the Faculty of Pharmacy and Foods at Havana University, and identified at the herbarium of National Botany Garden of Havana, where the voucher specimen no. HAJB 82587 has been deposited.

Extract and Samples Preparation

Dark red flowering types were collected daily. The isolated petals used were dried in an oven with controlled temperature, at

40˚C, during 5 days. The extracts were prepared with the ground material (60 g) without screen extracted in a Soxhlet apparatus with 675 mL of ethanol at 95% during 20 hours. The ethanolic extracts were concentrated and evaporated under vacuum to 200 mL at 120 rpm, a temperature of 70˚C and 500 mbar. For to the purification, 1 g of solid was dissolved in 25 mL of diethyl ether and the volume was completed to 100 mL with ethanol. The sample was refrigerated until an abundant solid appear and it was recuperated to filtration. This process was done twice, to obtain only a yellowish-green solid monitoring by TLC on silica gel with fluorescent indicator 254 nm on aluminum cards (layer thickness 0.2 mm) (10 × 20 cm) using n-butanol: acetic acid: water (4:1:5) as eluent (v/v/v) (Yaque *et al*., 2016).

Antibacterial activity assay

In the antimicrobial activity evaluation were used 26 strains of bacteria (Gram + and Gram -), where only one was from clinical origin, and 13 strains of fungus of *Candida* genus (all of them collection strains). The maximum concentration of gossypitrin employed in the research to determinate MIC and MBC was 50 mg/mL in base of the solubility of the flavonoid. The value of MBC or MFC corresponding to the minimum concentration in which gossypitrin inhibited the microorganism grows.

The antibacterial activity of the extracts was determined using the agar cup diffusion as described by Biavati *et al*., 2008. A 1 mL of an overnight culture of each bacterial isolate (equivalent to 107 to 108 CFU mL-1) was used to seed sensitivity test agar plates maintained at 45°C. The seeded plates were allowed to set, and a sterile cork borer of 8 mm diameter was used to cut equidistant wells on the surface of the agar. The wells were filled with 0.1 mL solution of each extract reconstituted with methanol at a concentration of 10 mg mL-1. Gentamycin and Nistatin at 5 μg mL-1 were included as positive control. The plates were incubated at 37°C for 24 h after which the diameter of zones of inhibition were measured.

Statistical analysis

All analysis were conducted in triplicate (n = 3), and an ANOVA test (using SPSS statistical software) was used to compare the mean values of each treatment. Significant differences between the means of parameters were determined by using the Duncan test ($p< 0.05$).

Results and Discussion

Antibacterial activity test showed that gossypitrin had growth inhibitory effect on several tested microorganism. Inhibition zone was wide against *S. epidermidis*, *P. vulgaris*, *K. pneumoniae*, *E. coli*, *S. aureus*, *E. faecium*, *Sh. flexneri* and *C. freundii*. The flavonoid glucoside of *T. elatum* showed equal inhibition ability against *E. coli* and *S. aureus* (40 mm) which was highest than *B. subtilis* (28 mm) and *S. typhi* (27 mm). The phytochemical component exhibited higher inhibition ability against *S. epidermidis* (47 mm) than against *P. vulgaris* (35 mm) and *K. pneumoniae* (33 mm).

In the present study, a variety of gram positive (*S. aureus* and *S. epidermidis*) and gram negative (*E. coli* and *P. vulgaris*) bacteria were used in screening antimicrobial activity of gossypitrin, the main isolated and characterized flavonoid glucoside from the ethanolic extracts of *Talipariti elatum* Sw in Cuba. The results of the current study clearly indicated that this flavonol glucoside inhibit the growth of some tested microorganisms, however, the effectiveness varied against the different tested microorganisms as shown in Table 1.

Table.1 Antibacterial evaluation of gossypitrin isolated from the petals of *T. elatum* Sw.

Strains	ATCC	Screening	MIC (mg/mL)	MBC (mg/mL)
Salmonella typhi	7251	+	50	40
Serratia marcescens	14056	-		
Klebsiella pneumoniae	13883	+	10	10
Providencia sp.	c-3450	+	40	40
Alcalaigenes faecalis	1460	-		
Streptococcus faecalis	29212	-		
Escherichia coli	35150	+	20	20
Escherichia coli	25922	-		
Staphylococcus aureus	25923	+	50	50
Shigella flexneri	12022	+	30	30
Citrobacter freundi	8090	+	50	40
Bacillus subtilis	6633	+	50	50
Proteus mirabilis	12453	-		
Streptococcus cloacae	23353	-		
Salmonella typhimunium	14028	-		
Salmonella enteriditis	13076	-		
Enterococcus aerogenes	13048	-		
Proteus mirabilis	7202	-		
Staphylococcus aureus	6538	-		
Salmonella typhi	19430	-		
Staphylococcus aureus	33862	+	20	20
Proteus vulgaris	13315	+	10	5
Enterococcus faecium	6056	+	30	20
Citrobacter freundii	10625	+	30	30
Staphylococcus aureus	clinical	+	20	20
Staphylococcus epidermidis	12228	+	5	5

Fig.1 Flowers of *Talipariti elatum* Sw.

The best results found in from of the different gram positive and gram negative bacterias used in the research were against *S. epidermidis*, *P. vulgaris* and *K. pneumoniae*, where the MIC and MBC were the lowest values at 5-5 mg/mL, 10-5 mg/mL and 10-10 mg/mL, respectively. The chemical component had an antibacterial effect of 53, 85 %, being positive its inhibition effect in front of 14 of the 26 strains of evaluated microorganisms. Only in front of two of 13 strains of fungus had a good effect (15, 38%). Antifungal activity of gossypitrin against *Candida subtilis* and *Candida albicans* showed values at 20 mg/mL. The last one is an opportunistic fungus present in inmunodepression conditions and it which is very resistant to the treatment with conventional medication.

The antibacterial activity of the cycle extracts of *H. sabdariffa* can be attributed to the action of the phytochemical compounds it contains (Babayi *et al*., 2004). These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. These results agree with Garcia- Alonso *et al*., (2006) who found that plant polyphenols have been demonstrated as potential antibacterial. Polyphenolic compounds and/or volatile oils are known to inhibit a wide range of organisms (Cheesbrough, 1984). Antibacterial activity of gossypetin isolated from *H. sabdariffa* was investigated and the activity may be due to Polyphenolic nature of the flavonoid gossypetin (Mounnissamy *et al*., 2002; Al-Hashimi, 2012).

Is important to remind that, up to now, only four flavonoid glycosides have been found in nature derivatives from gossypetin:

Gossypetin-8-*O*-glucoside or gossypin (*H. vitifolius* and *H. sabdariffa*); Gossypetin-7-*O*-glucoside or gossypitrin or gossypetrin (*H. sabdariffa*, *T. elatum* and *T. tiliaceum*); Gossypetin-3-*O*-glucoside or gossytrin (*H. sabdariffa* and *T. tiliaceum*) and Gossypetin-3'-*O*-glucoside (*Abelmoschus manihot* and *T. elatum*)(18). Antibacterial effects of this plant flavonoid against *Escherichia coli*, *S. epidermidis* and *S. aureus* suggest that they may possess remarkable therapeutic action in the treatment of gastrointestinal infection and diarrhea in man and skin diseases (Rogger *et al*., 1990). The good result of the antibacterial evaluation against *K. pneumoniae* leads to futures opportunities in th treatment of asthma and bronchial infections, especially in tropical countries.

In conclusion, the present study indicated that gossypitrin from the petals of *T. elatum* (Sw.) Fryxell, have significant antimicrobial and antifungal activity. Gossypitrin, the isolated glucoside flavonol from alcoholic extracts of Blue Mahoe in Cuba, effectively inhibited the growth of *S. epidermidis*, *P.vulgaris*, *K. pneumoniae*, *E. coli*, *S. aureus*, *C. albicans* and *C. subtilis*. Over another 11 different strains of bacteria possess a remarkable inhibitory effect. This fact lead us to the conclusion that gossypitrin might be an alternative source of antimicrobial compounds.

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