

Modulation of drug resistance in *Staphylococcus aureus* by extract of mango (*Mangifera indica*) peel

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Abstract: In an ongoing project to evaluate natural compounds isolated from by-products or wastes from vegetables and fruits (edible plants) as modulators of antibiotic resistance, ethanol extract from mango peel was investigated using *Staphylococcus aureus* strains possessing efflux mechanisms of resistance to norfloxacin, erythromycin and tetracycline. The minimum inhibitory concentrations (MIC) of the antibiotics were determined by the micro dilution assay in the absence and in the presence of sub-inhibitory mango peel extract concentration. Although the extract did not display relevant antibacterial activity (MIC \geq 2048 μ g/mL), it modulated the activity of antibiotics, i.e. in combination with antibiotics (at 512 μ g/mL), a four-fold reduction in the MIC values for tetracycline and erythromycin was observed. The results presented here indicates that mango peel could serve as a source of potential adjuvant of antibiotics which add value to this mango by-product.

Introduction

Mango (*Mangifera indica* L., Anacardiaceae) is grown naturally or cultivated mainly in tropical and a subtropical regions and is one of the most important and popular edible fruits in the world (Kim et al., 2009 and references therein).

Extracts of diverse parts of *Mangifera indica* L., vis. stem bark, leaf, seed, kernel and peel, have been reported to possess several biological and pharmacological properties (Masibo & He, 2008; Barreto et al., 2008) and are worldwide used in folk medicine against diarrhea (Sairan et al., 2003), kidney and urinary disorders (Ballabh et al., 2008), hypertension (Ajibesin et al., 2008), and as complementary therapy to manage type-2 diabetes (Chacko, 2003).

In the Northeast region of Brazil the decoction of the leaves is used as stomachic, anti-diarrheic and against genito-urinary inflammations, bronchitis and asthmas, and externally in baths or washes against scabies and syphilis (Agra et al., 2008).

Mango peel is a major by-product of mango processing industry constituting 15-20% of the total

fruit weight and, apart from being a good source of dietary fiber (Ajila et al., 2008), its extract showed protection against oxidative stress (Ajila & Prasada Rao, 2008), possess prothyroid and hypoglycemic effects (Parmar & Kar, 2008, 2009) as well as showed anti-allergic and antimicrobial activities (Tewtrakul et al., 2008).

Efflux pumps are integral proteins of bacterial membrane which account for much of the bacterial resistance since they extrude antibiotics from the cell (Piddock, 2006). Modifiers of antibiotic activity/modulators of drug resistance are compounds that potentiate the activity of antibiotics against resistance strains, and some of these agents may act as efflux pump inhibitors (EPI). Plants provide a rich source of EPI and several extracts (Coutinho et al., 2009 a,b), essential oils (Costa et al., 2008) and chemical constituents (Stavri et al., 2007; Gibbons, 2008; Falcão-Silva et al., 2009; Silva et al., 2009) have been identified as potent inhibitors.

In an ongoing project to evaluate natural compounds isolated from by-products or wastes from vegetables and fruits (edible plants) as modulators of

antibiotic resistance, ethanol extract of mango peel was investigated using effluxing strains of *Staphylococcus aureus*.

Material and Methods

Preparation of the extract

Mangifera indica L., Anacardiaceae fruits (cv. 'Tommy Atkins') were purchased from the local market and authenticated by the Departamento de Sistemática e Ecologia (Setor de Botânica) of the Universidade Federal da Paraíba. The fruits, before being peeled, were hand washed with 1% solution of neutral detergent and thoroughly rinsed in distilled water. Fresh peels (435 g), free of any pulp residues, were extracted with 95% ethanol (3 x 1 L, 72 h each) and the crude extract was concentrated under reduced pressure at 40 °C to yield 20.16 g of a dark yellow residue. The stock extract solution was prepared in dimethyl sulfoxide (DMSO), which at its highest final concentration after dilution in the broth (4%) caused no bacterial growth inhibition.

Bacterial strains

Three strains of *S. aureus* were used: 1) SA-1199B, which over expresses the *norA* gene encoding the NorA fluoroquinolones (and other drugs) efflux protein (Kaatz et al., 1993; Kaatz & Seo, 1995); 2) RN4220 harboring plasmid pUL5054, which carries the gene encoding the MsrA macrolide efflux protein (Ross et al., 1989); and 3) IS-58, which possesses the TetK tetracycline efflux protein (Gibbons & Udo, 2000). The strains, kindly provided by Professor Simon Gibbons (University of London), were maintained in blood agar base (Laboratórios Difco Ltda., Brazil) slants and, prior to use, the cells were grown overnight at 37 °C in brain heart infusion broth (BHI-Laboratórios Difco Ltda., Brazil).

Antibiotics

Norfloxacin, erythromycin and tetracycline were obtained from Sigma Chemical Co., USA, and their stock solutions were prepared according to CLSI guidelines (2005).

Drug susceptibility testing and modulation assay

The minimum inhibitory concentrations (MIC) of the antibiotics and of the mango peel extract were determined in BHI by the micro dilution assay using a suspension of ca. 10^5 cfu/mL and a drug concentration range of 1024-1 µg/mL (two-fold serial dilutions). The MIC is defined as the lowest concentration at which no

growth is observed. For the evaluation of mango peel extract as a modulator of drug resistance, the MIC of the antibiotics were determined in the presence of the mango peel extract at a sub-inhibitory concentration.

Results and Discussion

The mango peel ethanol extract showed no antibacterial activity at 1024 µg/mL against any *S. aureus* strains used (MIC \geq 2048 µg/mL). When the extract was incorporated into the grown medium at 512 µg/mL (\leq 1/4 MIC), a four-fold reduction in the MIC was observed for tetracycline and erythromycin and no reduction for norfloxacin (Table 1).

Table 1. Minimum inhibitory concentrations (MIC) of antibiotics in the absence and (presence) of mango peel extract (512 µg/mL) against effluxing strains of *Staphylococcus aureus*.

Strain (efflux protein)	Norfloxacin	Tetracycline	Erythromycin
SA-1199B (NorA)	64 (64)	-	-
IS-58 (TetK)	-	32 (8)	-
RN4220 (MsrA)	-	-	256 (64)

The extract modified the tetracycline and erythromycin activities by reducing the antibiotic concentration required to inhibit the growth of the drug resistant (effluxing) strains. Mango peel is a rich source of polyphenolic compounds (Berardini et al., 2004; Masibo & He, 2008; Barreto et al., 2008), and so, the modifying activity may be related to these compounds, since several of them have already been reported as putative EPI (Gibbons, 2005, 2008; Falcão-Silva et al., 2009). It is worth noting that no antibiotic modifying activity of mango pulp extract was observed against any effluxing strains here used (unpublished results), but in contrast to mango peel the pulp is a poor source of polyphenolic compounds (Berardini et al., 2004). The result obtained with the strain SA-1199B, *i.e.* no modulation of resistance to norfloxacin, indicates that the extract do not acts as an inhibitor of the NorA efflux protein.

The synergistic interaction of crude extract of *M. indica* leaves with tetracycline has already been demonstrated (Ahmad & Aqil, 2007), but the results presented here represent the first report of a crude extract of *M. indica* as a putative EPI. The present finding indicates that mango peel could serve as a source of potential adjuvant of antibiotics, which add value to this mango by-product.

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