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Correspondence

Evaluation of antibiotic & antibiotic modifying activity of pilocarpine & rutin

Sir,

Secondary metabolites are small molecules[1], responsible for various biological activities, acting as antibiotic, antifungal and antiviral agents to protect plants from pathogens. There are large groups of secondary metabolites, which include alkaloids and flavonoids[2], described mainly by their antimicrobial[3,4] and antioxidant potential[5]. Pilocarpine is an alkaloid present in the leaves of jaborandi (*Pilocarpus microphyllus*)[6], which is utilized as a phytotherapeutic agent in the treatment of glaucoma and xerostomia[7]. Rutin is a flavonoid belonging to the subclass of flavones found in various plant sources[8,9], which has shown antioxidant activity[10], efficacy in the control of *Aspegillus oschraceus*[11], anticonvulsivant effects in rats[12], suppression of cellular immunity[13], anticarcinogenic activity[14] and antiinflammatory effect[15]. The irrational use of antibiotics and antifungals has resulted in the development of drug resistance. The rapid development of drug resistance and the slow-down in the development of new active drugs, have drawn attention to treatment with drug combinations[16]. The aim of this study was to evaluate the antibacterial and antifungal activity of rutin and pilocarpine and to determine their possible modifying effect when combined with aminoglycoside antibacterials and the antifungal amphotericin B.

The experiments were carried out in Laboratory of Microbiology and Molecular Biology, Department of Biological Chemistry, Regional University of Cariri, Crato, Brazil, with standard and multiresistant strains of *Staphylococcus aureus* and *Escherichia coli*: *S. aureus* ATCC 12692, *S. aureus* 358 (MRSA), *E. coli* ATCC 25922 and *E. coli* 27 (EC27). All strains were maintained on heart infusion agar slants (HIA; Difco, USA), and before the assays, the cells were grown in brain heart infusion broth (BHI; Difco) for 24 h at 37°C. Clinical isolate EC27 was resistant to neomycin and gentamicin (low level) and to amikacin and kanamycin[17]. *S. aureus* 358 (MRSA) showed resistance to methicillin. All strains were obtained from the collection of microorganisms of the Mycology Laboratory, UFPB, Paraiba, Brazil. Three standard yeast strains were utilized: *Candida albicans* ATCC 40227, *C. krusei* ATCC 6538 and *C. tropicalis* ATCC 13803. All these strains were maintained on HIA, and before the assays, the cells were grown in BHI for 24 h at 37°C. The antibiotics tested were the aminoglycosides amikacin, kanamycin, gentamicin and neomycin (Sigma, USA). The antifungal agent was amphotericin B (Sigma, USA). The antibiotic and antifungal solutions were prepared following the recommendations of the Clinical and Laboratory Standards Institute – CLSI[18]. The test compounds (pilocarpine and rutin) (obtained from Merck & Company, Germany), was dissolved (10 mg) in 1 ml dimethylsuphoxide (DMSO- Merck, Darmstadt, Germany), giving an initial concentration of 10 mg/ml. Starting with this concentration, a dilution was made to 1024 µg/ml,

Table I. Evaluation of antifungal and antibacterial activities of test compounds

	Minimal inhibitory concentration - MIC (µg/ml)		
Cepas	Rutin	Pilocarpine	Negative DMSO control
C. albicans (ATCC 40227)	32	32	32
C. krusei (ATCC6538)	32	32	32
C. tropicalis (ATCC 13803)	32	32	32
E. coli 27	128	128	128
E. coli (ATCC 10536)	128	128	128
S. aureus 358	128	128	128
S. aureus (ATCC 25923)	128	128	128

Table II. Evaluation of antibiotic modifying activity of test compounds

	Concentrations (µg/ml)							
	S. aureus 358				*E. coli* 27			
	AMI	KAN	GEN	NEO	AMI	KAN	GEN	NEO
Rutin	156.25	2.500	19.53	312.5	156.25	312.5	19.53	156.25
Pilocarpine	156.25	2.500	≤1.22	39.06	156.25	312.5	19.53	156.25
DMSO	156.25	2.500	19.53	312.5	156.25	312.5	19.53	156.25
C+	156.25	2.500	19.53	312.5	156.25	312.5	19.53	156.25
C+, only antibiotic								

and further dilutions were made serially 1:2 in culture medium, obtaining concentrations of 512 to 8 µg/ml. The minimal inhibitory concentration (MIC, µg/ml) was determined in 10 per cent BHI by the broth microdilution method, using a suspension of 10^5 cfu/ml and a drug concentration of 1024-1 µg/ml[19]. To evaluate the test compounds for drug modifying activity when combined with antibiotics and antifungals, a subinhibitory concentration was determined as the MIC/8 values of 16 µg/ml for EC27 and MRSA, and 10 µg/ml for *C. albicans, C. krusei* and *C. tropicalis*. The plates were incubated for 24 h at 37 °C, utilizing resarzurin to read bacterial growth and no stain for fungi.

The MIC values for rutin and pilocarpine showed no antibacterial or antifungal activity against the strains tested (Table I). Pilocarpine, however, increased bacterial sensitivity to the aminoglycosides gentamicin and neomycin, when added concomitantly (Table II). Pilocarpine combined with these aminoglycosides altered synergistically the MIC values against *S. aureus* but not *E. coli*. Neither of the compounds tested showed an antifungal modifying effect (> 1024 µg/ml) with amphotericin B against the *Candida* strains tested.

The MIC values determined in the antifungal and antibacterial assays were equal to that of the negative DMSO control, suggesting that this activity was due to DMSO which is considered toxic only at higher concentration[20]. Rutin has been extensively studied for its various pharmacological properties, such as its anti-candida activity[9,21]. Missau *et al* [22] used the direct bioautography method and showed anti-fungal activity against three strains of *Candida*, with a significant effect against only *C. krusei*. Bolle *et al*[23] found that this method could result in decomposition of the test substances during the assay. Our findings corroborated with that of Pereira *et al*[11] who showed that rutin, isolated from the plant species *Solanum palinacanthum* does not inhibit the growth of *Staphylococcus aureus*. The use of drugs in combination has been extensively studied, mainly because of the emergence of resistant strains[24].

The synergistic effect of pilocarpine indicates a new therapeutic possibility for the treatment of diseases associated with *S. aureus* infection and an alternative for the resistance shown by this microorganism against certain aminoglycosides. Further studies are required to evaluate the toxicity and antibacterial activity of this compound *in vivo*.

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