ARTIGO ORIGINAL
ISSN 1677-5090

Annacardium occidentale stem bark extract can decrease the efficacy of antimicrobial drugs

O Extrato da Casca de Annacardium occidentale pode Reduzir a Eficácia de Fármacos Antimicrobianos

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Abstract

Background: *Due to the growing use of phytotherapy clinical strategies for infectious diseases, healthcare providers and patients must know the risks of interactions between such preparations and synthetic drugs. Cashew extracts are widely used in Brazil for complementary treatments of infectious diseases, and often are combined to antimicrobial drugs. However, the possible consequences of such combinations are unknown.* ***Objective:*** *To assess the interference of the Cashew stem bark hydroethanolic extract (CSBE) on certain antimicrobials against clinical isolates of staphylococcal species.* ***Methodology:*** *Antimicrobial susceptibility test and interference assays were performed using gentamycin, ampicilin, ciprofloxacin, G penicillin, neomycin, rifampicin and vancomycin disks.* ***Results:*** *Here we show that the CSBE might reduce the therapeutic activity of some antimicrobial drugs in Staphylococcal species. We found in controlled in vitro assays that the antagonistic effect was seen in all antimicrobial drugs tested for most of the strains and statistically significant differences were found when compared to the control antimicrobials. Our results also indicate the presence of tannins, flavonoids and saponins in the CSBE.* ***Conclusion:*** *Such combinations are suggested to be not adequate for clinical treatments. Further researches are needed for a better understating of such interactions at the molecular level.*
Keywords: *Cashew. Interference. Antimicrobials.*

Resumo

Introdução: Com o aumento do uso de recursos fitoterápicos na clínica de doenças infecciosas, profissionais de saúde e pacientes devem ter ciência do risco das interações destes com preparações antimicrobianas sintéticas. Os extratos do cajueiro são amplamente utilizados no Brasil como recursos complementares no tratamento de doenças infecciosas, e em alguns casos, são associados a fármacos antimicrobianos, entretanto, pouco se conhece sobre as consequências destas associações. **Objetivo:** Avaliar possíveis interferências do extrato hidroetanólico da casca do cajueiro (EHCC) em alguns fármacos antimicrobianos de uso clínico sobre bactérias do gênero *Staphylococcus*. **Metodologia:** Testes de susceptibilidade antimicrobiana e interferência foram realizados com discos de gentamicina, ampicilina, ciprofloxacim, penicilina G, neomicina, rifampicina e vancomicina. **Resultados:** Demonstrou-se que o EHCC reduziu a atividade de alguns fármacos antimicrobianos. Ensaios in vitro indicaram que o efeito antagônico visto em todas as substâncias testadas na maioria das cepas microbianas teve diferença estatística em comparação às drogas testadas sem interação com o extrato. Foram detectados taninos, flavonoides e saponinas no EHCC. **Conclusão:** Os dados sugerem que as combinações avaliadas são inadequadas para uso clínico. Mais estudos são necessários para a caracterização das interações encontradas a nível molecular.
Palavras-chave: Cajueiro. Interferência. Antimicrobianos.

INTRODUCTION

Staphylococcus aureus and Staphylococcus epidermidis are Gram-positive bacteria which have been frequently reported in hospital and community-acquired infections, and currently represent serious therapeutic challenges for drug therapy due to multidrug resistance (MDR) [1,2]. The irrational use and inadequate combinations of antimicrobials have been described as the main reasons for MDR [3,4]. Such drug interactions (DI) represent a common but challenging problem in antimicrobial drug therapy, and give rise to a large number of hospital admissions and deaths worldwide, beyond the rising microbial resistance problem[3].

In Brazil, Anacardium occidentale (cashew) extracts are used in oral and topical treatments of infectious and parasitic diseases, diabetes and wound healing[5]. Phy-

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tomolecules such as flavonoids, saponins and anacardic acids were previously detected on the cashew stem bark (CSBE), which is also prescribed for clinical use[5,6]. Such structures have evolved to bind unspecifically to targets and elicit biological effects which hardly induce resistance, once classical pathways like efflux pumps or enzymatic systems are avoided[6]. Nevertheless, patients' misinterpretation of this subject can lead to the idea that NP represent not only a cheaper but also a better option than prescribed medication does for clinical treatments, and a concern arises when they are combined to synthetic drugs, due to the idiosyncratic behavior of possible DI[3].

In order to overcome the MDR problem, some studies have proposed that natural products (NP) can potentiate the activity of antimicrobial drugs when combined, what could be explored for developing therapies for infectious diseases[7,8]. However, evidences to support this statement are still scarce, and possible consequences of DI of NP with antimicrobials remain not completely understood.

This study aimed to assess the interference of the CSBE on certain antimicrobials against clinical isolates of staphylococcal species. Here we show a simple and rapid method that can be used to predict DI with phytoextracts using antimicrobial disks. The scarcity of data regarding DI with NP makes our data even more relevant.

MATERIALS AND METHODS

Microorganisms

The clinical isolates of this study were provided by The Samaritan Hospital Clinical Laboratory (Governador Valadares, Minas Gerais, Brazil). They were isolated from haemodyalisis patients, from indwelling catheters (S. aureus) and nasal cavities (S. epidermidis). Reference strains of the organisms were obtained from the American Type Culture Collection (S. aureus ATCC 29213 and S. epidermidis ATCC 12228). All strains were saved in glycerol phosphate buffer and subcultured in Brain Heart Infusion (BHI) broth (Himedia) before testing. Isolates were identified by conventional biochemical and antimicrobial resistance tests[9]. All procedures were performed in triplicate. This research was approved by UNIVALE Ethics Committee (PQ 024/10-10).

CSBE preparation and analysis

The CSBE was purchased from All Chemistry (Brazil), in its hydroethanolic form. Aliquots of 100 ml were filtered and sterilized in Stericup & Steritop 220 nm vacuum bomb (Milipore, U.S.A.). The filtrate was concentrated in a vacuum process at 45°C to yield a dark brown paste, which weighed an average of 3 g and was stored in a refrigerator (4°C) until use.

The CSBE was qualitatively analyzed through classical methods for flavonoids, saponins and tannins[10]. For flavonoids, Shinoda test was performed and a pink color indicated the presence of these molecules. Saponins detection was based on the observation of stable foam formation after an aliquot was shaken in warm water. Tannins were detected by formation of precipitate in a 1% gelatin solution containing 10% sodium chloride.

Antimicrobial susceptibility test

The Antimicrobial susceptibility test was performed by the disk diffusion method [11]. The following antimicrobial disks (Sensifar) were used: gentamycin (10 µg), ampicillin (10 µg), ciprofloxacin (5 µg), G penicillin (10 IU), neomycin (30 µg), rifampicin (5 µg) and vancomycin (30 µg). All strains were standardized as presented in previous sections. The diameters (d) of the inhibition zones were measured, and strains were considered resistant if $d \leq 12$ mm.

Interference assay

Previously to this assay, the CSBE paste was dissolved in Dimethyl sulfoxide (DMSO), and serial dilutions were performed using DMSO as a diluent. In previous studies from our group (Dias-Souza et al. 2013, submitted to publication), the CSBE was tested at different concentrations, and the minimum bactericidal concentration (MBC) identified for planktonic cells was of 30 mg/ml. Therefore, this concentration was used for assessing possible interferences on antimicrobial disks diffusion results.

The interference assay was performed in triplicate as previously described[12], with some modifications. Briefly, 10 µl of the CSBE in its MBC was dispensed in triplicates of each disk, incubated for 24 h, and the grow inhibition halo mean diameter was compared with the control after 24 h incubation. Synergism was considered if the inhibition zone average diameter was at least 2 mm greater than the control, and antagonism was considered if the halo mean diameter was at least 2 mm shorter than the control.

Statistical Analysis

Differences between halo mean diameters of drugs with and without the dispensed CSBE were analyzed using ANOVA followed by Tukey test. The significance level was set at $p<0.05$, and highly significant values were set as $p<0.01$ using BioEstat 5.0 for Windows.

RESULTS

The qualitative analysis of the CSBE extract suggested the presence of tannins, flavonoids and saponins. Flavonoids analysis suggested the presence of flavonol and flavonone subtypes.

The antimicrobial susceptibility test indicated that most of the strains were sensible to the synthetic drugs used in this work (Tables 1 and 2). Resistant phenotypes were seen by this method for neomycin (S. aureus 4 and 11 strains) and vancomycin (S. aureus 10) (table 2).

The interference of the CSBE on the antimicrobial activity of tested drugs was analyzed in comparison to the disks results without the CSBE. Antagonism was detected in all antimicrobial drugs for most of the strains, and synergism was only seen for neomycin (S. aureus 2 and 4), rifampicin (S. epidermidis 2 and S. aureus 6) and vancomycin (S. epidermidis 1 and 2). Moreover, the sta-

Table 1. *Inhibition halos of antimicrobial drugs combined or not to the CBSE.*

Strain	GEN	GEN/CBSE (+ +)	AMP	AMP/CBSE (+ +)	CIP	CIP/CBSE (+ +)	PEN	PEN/CBSE (+ +)
S. epidermidis 1	18(0,47)	19(0,94)	24(0,94)	14 (0,82)**	24(0,94)	24(0,47)	20(0,94)	19(0,47)
S. epidermidis 2	26(0,47)	20 (0,47)**	24(0,94)	17 (0,47)**	26(0,94)	22(0,94)**	28(1,63)	17(0,47)**
S. aureus 4	20(0,47)	16 (0,47)**	16(0,94)	11 (0,82)**	26(0,94)	12(0,82)**	16(0,94)	15(0,82)
S. aureus 5	20(0,47)	17 (0,47)**	22(1,63)	9 (0,47)**	28(0,94)	21(0,47)**	24(0,82)	12(0,94)**
S. aureus 6	24(0,94)	19 (0,47)**	22(0,94)	12 (0,47)**	26(0,94)	19(0,94)**	22(0,94)	12(0,47)**
S. aureus 7	22(0,47)	17 (0,82)**	28(0,94)	10 (0,47)**	24(0,8)	19(0,94)**	26(0,82)	8(0,2)**
S. aureus 8	22(0,82)	20 (0,47)**	20 (1,89)	13(0,47)**	26(0,94)	24(0,47)**	24(0,94)	11(0,94)**
S. aureus 9	18(0,94)	17(0,94)	28(0,94)	8(0,82)**	22(0,82)	22(0,94)	30(0,82)	0**
S. aureus 10	22(0,82)	17 (0,47)**	18(0,94)	11(0,94)**	24(0,94)	19(0,94)**	18(0,94)	12(0,94)**
S. aureus 11	20(0,47)	18 (0,47)**	20(0,94)	9(0,94)**	30(0,47)	18(0,47)**	24(0,47)	11(0,47)**
S. aureus 14	26(0,82)	17 (0,94)**	22(0,94)	11(1,41)**	28(0,47)	18(0,94)**	28(0,82)	12 (0,82)**
S. epidermidis ATCC 12228	22(0,47)	16 (0,94)**	18(0,47)	11(0,94)**	18(0,94)	20*(0,47)*	22(0,82)	12(0,94)**
S. aureus ATCC 29213	22(0,94)	13 (0,94)**	20(0,94)	10(0,82)**	26(0,94)	12(0,94)**	28(0,94)	8(0,82)**

*GEN: gentamycin, AMP: ampicilin, CIP: ciprofloxacin, PEN: G penicillin, (–): No statistically significant difference when comparing CBSE-added disks to the same disk without the CBSE, (+): statistically significant difference when comparing CBSE-added disks to the same disk without the CBSE, (+ +) highly statistically significant difference when comparing to the same disk without the CBSE. *Synergism **Antagonism. Absence of * signals indicates no interference when comparing CBSE-added disks to the same disk without the CBSE. Data in parentheses are the standard deviation (SD).*

Table 2. *Inhibition halos of antimicrobial drugs combined or not to the CBSE (continued)*

Strain	NEO	NEO/CBSE (–)	RIF	RIF/CBSE (–)	VAN	VAN/CBSE (–)
S. epidermidis 1	18 (0,82)	19 (0,94)	15 (0,47)	16 (0,82)	14 (0,94)	18 (0,47) *
S. epidermidis 2	18 (0,94)	20 (0,47) *	15 (0,47)	17 (0,47) *	16 (0,94)	18 (0,94) *
S. aureus 4	**12 (0,94)**	17 (0,47) *	14 (0,47)	13 (0,82)	16 (0,47)	15 (0,82)
S. aureus 5	14 (0,94)	15 (0,47)	24 (0,82)	21 (0,47) **	18 (0,82)	17 (0,94)
S. aureus 6	14 (0,94)	7 (0,82) **	23 (0,47)	25 (0,47) *	16 (0,94)	15 (0,94)
S. aureus 7	24(0,8)	25 (0,47)	26 (0,47)	25 (0,47)	16 (0,47)	13 (0,94) **
S. aureus 8	18 (0,47)	13 (0,94) **	15 (0,94)	15 (0,47)	16(1,89)	17 (0,94)
S. aureus 9	22 (0,47)	15 (0,47) **	24 (0,47)	21 (0,82) **	16 (1,89)	14 (0,82) **
S. aureus 10	14 (0,82)	15 (0,47)	27 (0,47)	25 (0,94) **	**12 (0,94)**	15 (0,47) *
S. aureus 11	**12 (0,94)**	17 (0,47) *	26 (0,47)	24 (0,94) **	16 (0,94)	17 (0,94)
S. aureus 14	20 (0,94)	17 (0,94) **	22 (0,94)	19 (1,41)**	22 (0,94)	10 (0,82) **
S. epidermidis ATCC 12228	14 (0,94)	13 (0,47)	28 (0,47)	26 (0,94) **	16 (0,82)	15 (0,47)
S. aureus ATCC 29213	24 (0,82)	0**	21 (0,94)	22 (0,82)	16 (0,47)	15 (0,94)

*NEO: neomycin, RIF: rifampicin, VAN: vancomycin. (–): No statistically significant difference when comparing CBSE-added disks to the same disk without the CBSE, (+): statistically significant difference when comparing CBSE-added disks to the same disk without the CBSE, (+ +) highly statistically significant difference when comparing to the same disk without the CBSE. *Synergism **Antagonism. Absence of * signals indicates no interference when comparing CBSE-added disks to the same disk without the CBSE. Numbers in bold indicate resistance to the antimicrobial drug (d≤12 mm). Data in parentheses are the standard deviation (SD).*

tistical analysis (table 1) indicated that, with exception of neomycin (p=0.2603), vancomycin (p=0.6570) and rifampicin (p=0.6569), the grow inhibition halo mean diameter was significantly lower of the CSBE-added disks compared to the non CSBE-added disks.

DISCUSSION

DI potential is recognized as an important consideration in the evaluation of a new molecular entity and is a critical part of drug development[3]. In practice, pharmacological in vitro studies of DI are regarded as acceptable surrogates of in vivo effects [3,13]. Although the findings from such researches made many DI now predictable[3], few studies have addressed issues related to DI with NP[14]. Most of the evidences in this context are related to psychotropic, anti-clotting and anti-hypertensive drugs[13].

In Brazil, the CSBE is commonly prescribed by physicians for infectious diseases and wound healing treatments in drops for dilution in water prior to administration or in emulsions for topical use[5]. Despite this clinical use, data related to DI regarding the CSBE are often not available. Therefore, we developed an in vitro approach to evaluate potential DI and optimize knowledge regarding drugs safety.

Our data indicated that most of the drugs used were less effective when combined to the concentrated CSBE. The overall trend in the antimicrobial efficacy and interaction effect differed slightly among the antimicrobial drugs, with most combinations showing decreased activity.

The molecules detected at the CSBE are considered the main phytocomponents from varied A.occidentale extracts[5]. Because of the multiplicity of the detected phytocompounds, it is not possible to assume what chemical entity might have caused the interference effects, mostly antagonistic. However, our observations of this antagonistic profile, supported by statistical analysis, suggest that the combinations of the CSBE and the tested drugs should be avoided.

Beyond the incorrect use of antimicrobial drugs, NP interactions with antimicrobials have been characterized as the main causes of the growing rates of multidrug bacterial resistance specially in developing countries, where NP are largely used by patients and physicians[5].

CONCLUSION

Our study provided data about DI with phytocompounds and attempted to provide a simplified and reproducible method for rapid prediction of possible interferences of plant extracts with statistical support, an important data for clinical protocols which comprise the use of NP. The drugs-extract combinations exhibited reduced antibacterial activity when compared to non-combined drugs, what demonstrate the potential risk of a total set of phytochemical compounds within a single medicinal plant.

Undoubtedly, this study has limitations. NP provide a complex mixture of bioactive molecules which may interfere with drugs activity, and despite they can be useful in varied diseases, evidences of such risks are scarce. Therefore, establishing the clinical significance of phytocompounds-drug interactions and predicting the safety of such combinations is technically difficult, and we cannot ensure the generalisability of the results. However, it must be emphasized that due to the diversity and multitude of chemical compounds in this plant extract, its interaction effects may lead not only to the inefficacy of the antimicrobials, but also to toxic effects within the human body, what can not be assessed in vitro. Therefore, toxicological tests for such extract combinations are necessary to avoid detrimental effects. Further pharmacological tests using in vivo models are necessary for a better understanding of the molecular antagonism mechanisms, what would provide a new route to overcome the problem of drug safety when exploring such combinations in clinical treatments.

ACKNOWLEDGEMENTS

The authors are thankful to Gabriela Freitas for the important discussions, and to Elaine Oliveira and Adileia Regina, for providing most of the antimicrobial disks. MVDS is supported by grants from Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

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Submetido em 17.03.2013;
Aceito em 07.08.2013.