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# Antimicrobial and Cytotoxic Activity of Fruit Extract from *Syzygium cumini* (L.) Skeels

Ketylin F. MIGLIATO [1,3*], João C.P. MELLO [4,5], Olga Z. HIGA [7], Andrea C.D. RODAS [6], Marcos A. CORRÊA [1], Maria J.S. MENDES-GIANNINI [2,3], Ana M. FUSCO-ALMEIDA [2,3], Antonio C. PIZZOLITTO [2,3], Hérida R.N. SALGADO [1,3]

[1] *Departamento de Fármacos e Medicamentos &* [2] *Departamento de Análises Clínicas, Faculdade de Ciências Farmacêuticas, University of São Paulo State (UNESP), Rodovia Araraquara-Jaú, km 1Araraquara – SP, Brazil*
[3] *Programa de Pós-Graduação em Ciências Farmacêuticas – UNESP*
[4] *Departamento de Farmácia e Farmacologia, Universidade Estadual de Maringá (UEM), Maringá, PR, Brazil*
[5] *Programa de Pós-Graduação em Ciências Farmacêuticas, UEM, Maringá*
[6] *Instituto de Pesquisas Energéticas e Nucleares, Centro de Biotecnologia, São Paulo, Brazil*

***SUMMARY*. The aim of the present study was to evaluate the antimicrobial and cytotoxic activity of the ethanolic extract of *S. cumini* according to the Clinical and Laboratory Standards Institute reference method (with modifications), determining the minimal inhibitory and lethal concentration. Activity against Gram-positive *(Staphylococcus aureus* and *S. epidermidis)*, Gram-negative *(Pseudomonas aeruginosa)* and yeast of *Candida* sp and *Cryptococcus neoformans* was evaluated. The effects of the fruit extract were examined in hamster cells ovaries in concentrations ranging from 1250.0 a 4.9 µg/ml, measuring the reduction of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium. The extract showed both bactericidal and fungicidal activity among the various microorganisms tested and the MIC ranging from 7.8 to 250 µg/ml. The MIC, MBC and MFC should values that were similar for all the microorganisms. Cytotoxicity index of the dried extract corresponded to the concentration of 400 µg/ml. The extract could potentially be used in topical antimicrobial products. Thus, the activity of extract was potent to bacteria and mainly to non-*albicans* species and *C. neoformans*.**

## INTRODUCTION

*Syzygium cumini* (L.) Skeels (Synonym: *S. jambolanum, Eugenia jambolana, S. jambos)* (Myrtaceae), popularly known in Brazil as "jambolão" (jambolan or java plum), is a native tree of the tropics, originally from India and SE Asia. It is widespread in some states of North, Northeast and Southeast Brazil [1-7] and is used as a popular treatment against various diseases. In Brazil, the bark, fruits, seeds and leaves of this plant are used for the diabetes treatment and administered in various pharmaceutical preparations (*e.g.,* aqueous or alcoholic extract, decoctions or crude plant juice) [8]. Prince *et al.* [9] have shown hypoglycemic and antioxidant activities in *S. cumini* seeds. A decoction of the bark is also used for dysentery and diarrhea [10]. Moreover, *S. cumini* has been shown to have sedative and anticonvulsant effects [11] and a potent central nervous system depressant effect [12].

*Syzygium* species are reported to be very rich in tannins, flavonoids, essential oils, anthocyanins and others phenolic constituents [3,4,5,7,12-15]. Scalbert [16] reviewed the antimicrobial properties of tannins in 1991.

A multitude of plant compounds (often of unreliable purity) is readily available over the counter from herbal suppliers and natural-food stores, and self-medication with these substances is commonplace. The use of plant extracts, as well as other alternative forms of medical treatment, has been enjoying great populari-

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* Author to whom correspondence should be addressed. *E-mail:* migliato@fcfar.unesp.br

ty since the late 1990s. On the other hand, the search for new antimicrobial agents has been intense in recent decades, owing to the development of resistance to existing agents among pathogenic microorganisms [17-19].

According to Shafi *et al.* [20], leaf plant extracts of *Syzygium* species have known antibacterial activity. In addition, Chandrasckaran & Venkatesalu [21] showed that aqueous and methanol seed extracts inhibited the growth of some of the fungal microorganisms implicated in skin diseases, such as *Candida albicans, Trichophyton rubrum, T. mentagrophytes* and *Microsporum gypseum*. However, the fruit extracts from this plant have never been carried out to verify its antimicrobial activity.

Cellular toxicity tests have always been among the methods of bioassays and may predict the toxicity of substances to various tissues. Many techniques have been developed for in vitro assessments of cytotoxicity and genotoxicity of toxic compounds [22].

The discovery of new chemical agents with biological activity is undoubtedly a multidisciplinary activity, where the efficacy studies, mechanisms of action, potential toxic and genotoxic depend on bioassays drug-toxicology *in vitro* and *in vivo* for these substances may have confirmed their activities against these aspects. Despite the cell *in vitro* tests do not replace the tests in animals to evaluate the biological activity of various substances, initially; these tests are applied in pilot studies of new drugs serving as economic model for rapid screening of xenobiotics.

The aim of the present investigation was to evaluate the antibacterial, antifungal and cytotoxic activity of the ethanolic extract of fruits of *S. cumini* cultivated in Brazil.

## MATERIAL AND METHODS

### *Plant material*

*S. cumini* (L.) Skeels fruits ('jambolão') were collected in Araraquara, São Paulo State, Brazil, in December 2005 and January 2006 and a voucher specimen was deposited in the herbarium of the Department of Botany of IBILCE, UNESP, São José do Rio Preto-SP (Herbarium # SJRP 19586), by Dr. Neusa Taroda Ranga .

### *Preparation of extract*

The crude extract was obtained by percolation of 50 °GL aqueous ethanol through 10 % (w/v) of the powdered dried fruit sample. The filtrate was evaporated at low pressure and 40 °C in a rotary evaporator and then lyophilized.

### *Preparation of test solution and microplate*

A test solution was prepared with a known weight of crude lyophilized extract, dissolved in 5 % aqueous dimethyl sulphoxide (DMSO), and another was prepared with 80 % propylene glycol: water. Microtiter (plastic, with 96 u-bottomed wells; Sarstedt, Australia) plates were prepared with wells containing 100 µl of two-fold serial drug dilutions, the final concentrations ranging from 3.9 to 1,000 µg/ml of plant extract.

### *Microorganisms*

The test organisms included were: *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (ATCC 27853) were originally obtained from the Microbiology Laboratory of the Department of Clinical Analysis at Universidade Estadual Paulista (UNESP). *Candida albicans* ATCC 90028, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019 and *Cryptococcus neoformans* ATCC 90012, originally obtained from the Mycology Laboratory of the Department of Clinical Analysis at Universidade Estadual Paulista (UNESP). The bacterial and fungal stock cultures were maintained, respectively, on Mueller-Hinton (M-H) Agar at 4 °C and Sabouraud Dextrose Agar at 30 °C. These standard strains were obtained from the American Type Culture Collection (ATCC).

### *Bacterial and Fungal concentration*

Bacterial suspension was standardized as described by CLSI (M07-A6, 2003) [23] guidelines. Inocula were prepared in M-H Broth (Merck) for bacteria CLSI (M07-A6, 2003) [23] at a density adjusted to a 0.5 McFarland turbidity standard [$10^6$ colony-forming units (CFU)/ml] and diluted 1:10 for the broth microdilution procedure.

The yeast inoculums was adapted and standardized according to the CLSI (M27-A2, 2002) [24] guidelines. Working suspensions of yeast were prepared in RPMI 1640 to a final density of $1 \times 104$ to $5 \times 10^4$ CFU/ml on the micro plates.

### *Drugs and plant extract*

Standard powders of ciprofloxacin and amphotericin-B were used as the controls, respectively, for antibacterial and antifungal tests. The stock solutions were prepared as described in the CLSI guidelines (M07-A6, 2003) [23] for bacteria and CLSI (M27-A2, 2002) [24] for yeast. Stock solutions of ciprofloxacin and amphotericin-B were diluted in 5 % DMSO (Sigma Aldrich Quimica) at concentrations of 3,200 µg/ml for both. For the microdilution test, ciprofloxacin

and amphotericin-B were diluted serially to give a concentration range at 16 to 0.031 µg/ml. The test extract was dissolved in 5 % DMSO and 80 % propylene glycol to obtain 5,000 µg/ml stock solutions. Stock solution (100 µl) was mixed with 100 µl of M-H Broth and RPMI 1640 broth (with L-glutamine but without bicarbonate), supplemented with glucose (2 %), and buffered to pH 7.0 with 0.165 mol $l^{-1}$ morpholinepropanesulfonic acid (MOPS- Difco American Bioorganic obtain) for both bacteria and fungi to obtain a concentration of 1,000 µg/ml and two- fold serial dilutions were used to give a final concentration 500, 250, 125, 62.5, 31.25, 15.62, 7.8, and 3.9 µg/ml in sequential wells.

### *Antibacterial and Antifungal Susceptibility Testing*

Broth microdilution method was used in accordance with the CLSI (M07-A6, 2003) [23] for bacteria and CLSI (M27-A2, 2002) for yeast 24 guidelines. All stocks for bacteria were diluted with Mueller-Hinton Broth and those for yeasts with modified RPMI 1640 broth. Minimum inhibitory concentration (MIC) of the plant extract was tested by the two-fold serial dilution method. The final concentrations ranged from 0.03 to 16.0 µg/ml for ciprofloxacin and amphotericin-B, and 3.9 to 1.000 µg/ml for *S. cumini* (L.) Skeels fruit extract. Tests were performed in 96-well round-bottom microtiter plates. Bacterial and yeast inocula were prepared and absorbance measured 490 nm to determine cell number, as recommended in CLSI (M27-A2, 2002) [24]. Each plate contained 9 serial dilutions of the fruit extract arranged in rows as well as a growth control well (without drug) and a purity control well, which contained medium but no yeasts. The plates were incubated at 37 °C ± 1 °C, with shaking, and the endpoints were determined spectrophotometrically 630 nm after 24-48 h. The MIC of the test extract, ciprofloxacin and amphotericin-B was defined as the lowest concentration of each solution at which there was 90 % inhibition of growth. *Staphylococcus aureus* (ATCC 25923) and *Candida parapsilosis* (ATCC 22019) were included in the test as quality control strains. The whole series of tests was performed in triplicate.

### *Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of plant extract*

All samples tested in the MIC study, whether showing or not showing any microbial growth, were transferred to plates of M-H Agar, for bacteria or Sabouraud Dextrose Agar for fungi. The plates were incubated at 37 °C ± 1 °C for 24 h (bacteria) or 35 °C ± 1 °C for 48 h (yeast). The MBC and MFC were defined as the lowest concentration of the extract that did not permit any visible bacterial and fungal colony growth on the appropriate agar plate after the period of incubation. The whole series of tests was performed in triplicate.

### *Cytotoxic assay*

The test was conducted in a single culture of ovarian cells of Chinese hamsters (CHO), (ATCC CCL-61). Culture of these cells has been separated by a single tripsinization. The suspension was centrifuged and resuspended in RPMI 1640 broth, containing 10 % fetal bovine serum (SFB). After resuspension, the suspension was washed with saline solution buffer (PBS) sterile. After cell monolayer preparation, the increasing dilutions of the extract of *S. cumini* fruit (50 µL/ wells) were added serially to give a concentration range from 1,250 to 4.9 µg/ml. Then, the plate was stabilized at 37 °C + 1 °C in humid atmosphere of 5 % $CO_2$, to make the tripsinization of cells. The test was developed using microplates of 96 wells and 50 µL of suspension (of 3000 cells) were pipetted in each well. As control, we used four wells containing media without cells (blank) and media with cells only (negative control). The mean absorbance (of four replicates) generated by the "medium-only" control is denoted as IC0. The mean absorbance generated by the "cell-only" control is denoted as IC100.

After 72 h, for the MTS [tetrazolium (5-(3-carboxymethoxyphenyl)-2-(4,5-dimetilthiazol)-3(4-sulphophenyl)] assay, 20 µL of the dye mixture of the compound supravital (MTS) to 0.2 % and PMS (phenazine methosulfate) to 0.09 % (20:1) were added to test wells. After 2 h at 37 °C ± 1 oC, the OD was read at a wavelength of 490 nm. The quantity of formazan product in both methods is directly proportional to the number of living cells in culture.

## RESULTS

The fruits ethanol extract of *S. cumini* had antibacterial and antifungal activities against all the microorganisms tested. The antibacterial and antifungal susceptibility test, using ciprofloxacin for the bacteria and amphotericin-B for the yeasts, exhibited MICs of 1.0 µg/ml and 0.25 µg/ml, respectively. The blind controls

| Microorganisms | MIC (µg/ml) /MFC and MBC | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1000 | 500 | 250 | 125 | 62.5 | 31.25 | 15.6 | 7.8 | 3.9 |
| *S. aureus* | -/- | -/- | -/- | -/- | */† | +/† | +/† | +/† | +/† |
| *P. aeruginosa* | -/- | -/- | -/- | */† | +/† | +/† | +/† | +/† | +/† |
| *S. epidermidis* | -/- | -/- | -/- | -/- | -/- | */† | +/† | +/† | +/† |
| *C. albicans* | -/ Δ | -/ Δ | -/ Δ | */ Δ | +/ Δ | +/ Δ | +/ Δ | +/ Δ | +/ Δ |
| *C. krusei* | -/ Δ | -/ Δ | -/ Δ | -/ Δ | -/ Δ | -/ Δ | -/ Δ | -/ Δ | */ Δ |
| *C. parapsilosis* | -/ Δ | -/ Δ | -/ Δ | -/ Δ | -/ Δ | -/ Δ | */ Δ | +/ Δ | +/ Δ |
| *C. neoformans* | -/- | -/- | -/- | -/- | -/- | -/† | */† | +/† | +/† |

**Table 1**. Minimum inhibitory concentration and minimum bactericidal/ fungicidal concentration of *Syzygium cumini* (L.) Skeels fruit extract. Δ: Fungistatical/ bacteriostatical activity; †: Fungicidal/bactericidal activity; * Values of minimum inhibitory concentration; (-) absence of growth microorganisms; (+): growth of microorganisms.

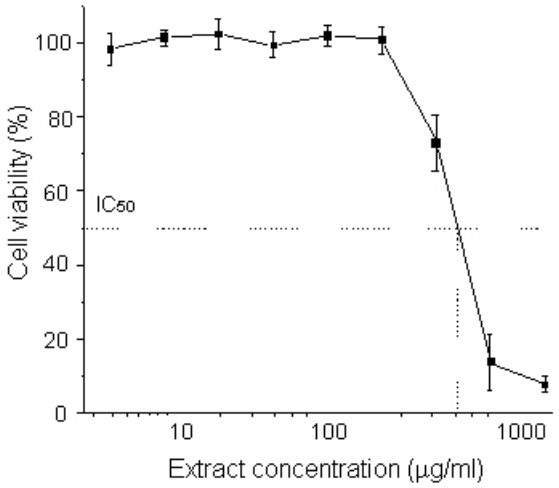


**Figure 1**. Cytotoxicity index (CI) of dry extract of *S. cumini fruit* by (tetrazolium (5-(3-carboxymethoxyphenyl)-2-(4,5-dimetilthiazol)-3(4-sulphophenyl)) (MTS) test developed with ovarian cells of Chinese hamsters (CHO).

(dimethyl sulphoxide and propylene glycol) did not inhibit any of the microorganisms tested.

The antimicrobial concentrations of 3.9-125.0 µg/ml were obtained for the ethanol extract in the tests with the bacterial and fungal strains (Table 1). The MBC of the *S. cumini* extract was 62.5 µg/ml, 31.25 µg/ml, and 125.0 µg/ml for the bacteria *S. aureus, S. epidermidis,* and *P. aeruginosa,* respectively, while the yeasts *C. albicans, C. krusei* and *C. parapsilosis* the fungistatical activity was > 1000 µg/ml. The MFC to *C. neoformans* was 31.25 µg/ml.

Cytotoxicity index that causes cell death by 50% was achievement in the concentration of 400 µg/ml (Fig. 1).

## DISCUSSION

The search for new drugs with antibacterial and antifungal activity led to test the dry extract of fruits of *S. cumini* for evidence of such activity. Other components of this plant have already tested, but fruits extracts had never been evaluated for antimicrobial activity. This interest is related to the popular uses described for the jambolan. The plants of this family are rich in some active constituents and much used in popular medicine. Our results show that ethanol extracts of *Syzygium cumini* are active against all the microorganisms tested, with MIC values range to 7.8 and 250.0 µg/ml. Tannins and related compounds have long been recognized to possess quite potent antibiotic activities, reflected by the records of traditional herbal medicines, rich in polyphenols, being used as effective antiseptic drugs. Tannin toxicity to bacteria and yeast had been discussed and some reasonable mechanisms were presented to explain tannin antimicrobial activity [25].

The majority of the studies involving antimicrobial activity of extracts derived from plants have not used standardized techniques following the NCCLS guidelines for the determination of the minimum inhibitory concentration [20,21]. Therefore in this study, it was intended to evaluate the antimicrobial potential of the *S. cumini* extracts, using the methodology specified by the CLSI (M07-A6, 2003) [23] for bacteria and CLSI (M27-A2, 2002) for yeast [24] guidelines. The testing procedure was robust and the results were reproducible and the MICs obtained in all triplicates were the same.

The results showed that ethanolic extracts of *S. cumini* fruit had appreciable activity against all tested microorganisms, with MBC and MFC

values range to 7.8 and 250µg/ml, confirming the MIC values. In the present study, the Gram-positive bacteria *(S. aureus* and *S. epidermidis)* were more susceptible than the Gram-negative *(P. aeruginosa)*. Our results showed similar results to the other authors that have tested methanol and aqueous extracts of the leaves against microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Candida albicans, Cryptococcus neoformans, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Rhizopus* sp., *Trichophyton mentagrophytes, Trichophyton rubrum* and *Microsporum gypseum* [20,21,26]. The essential oils of *S. cumini* leaves were tested against bacteria by the disk diffusion method [21]. According to these authors, these oils showed considerable antibacterial activity especially against *Salmonella typhimurium*.

In agreement with Chandrasekaran and Venkatesalu [21], our results showed the potential of *S. cumini* extracts to be used for the treatment of fungal infections. Additionally, our extract was more effective to fungal strains, mainly non-albicans species and *C. neoformans,* since presented lowest MIC and MFC values against these fungal strains. The use of plants to treat infectious diseases is common practice in populations worldwide. Data from the literature, as well as our results, reveal the great potential of plants for therapeutic treatment, despite the fact that they have not been completely investigated. Plant extracts, before being used in new therapeutic treatments, should have their toxicity tested *in vivo*. In particular, chemical and pharmacological investigations may be recommended for *Syzygium cumini*.

The cytotoxic index (IC) was developed, using a monolayer cell bioassay and the extract of dried fruits *S. cumini* presented IC to the concentration of 400 µg/ml (Fig. 1).

The statement will be studied later used for incorporation into cosmetic formulations to be administered topically, such as anti-septic chlorhexidine, chlorhexidine +benzalkonium chloride + benzyl alcohol. Although, they exhibit cytotoxicity against cultures of human cells, as fibroblasts and keratinocytes, even thus, they were approved by the bodies responsible and are used by the population, in contrast, what happens with iodine-povidine that when their cytotoxicity in the murine fibroblasts is compared to that of digluconate chlorhexidine, presents itself as an antiseptic for better tolerability, promoting cytotoxicity contact after 30 min, but with the regeneration of cells after 24 h [27].

Our studies have shown that the values of IC50 are much larger than the minimum inhibitory concentration for all tested microorganisms showing that the fruit extract obtained from *S. cumini* can be mainly used in cosmetic formulations for topical use with security since this route of absorption encourages their use.

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