



Brazilian Journal of
OTORHINOLARYNGOLOGY

www.bjorl.org



ORIGINAL ARTICLE

In vitro* antimicrobial activity of *Luffa operculata^{☆,☆}



Rodolfo Alexander Scalia^{a,b,*}, José Eduardo Lutaif Dolci^c, Suely Mitoi Ykko Ueda^d,
Suzethe Matiko Sassagawa^d

^a Department of Otorhinolaryngology, Irmandade da Santa Casa de São Paulo, São Paulo, SP, Brazil

^b Postgraduate Program in Surgery Research, Medical Sciences School, Santa Casa de São Paulo (FCMSCSP), São Paulo, SP, Brazil

^c Medical Sciences School, Santa Casa de São Paulo (FCMSCSP), São Paulo, SP, Brazil

^d Department of Pathological Sciences, Medical Sciences School, Santa Casa de São Paulo (FCMSCSP), São Paulo, SP, Brazil

Received 23 June 2014; accepted 24 July 2014

Available online 25 December 2014

KEYWORDS

Luffa operculata;
Sinusitis;
Products with
antimicrobial action;
Phytotherapeutic
drugs

Abstract

Introduction: *Luffa operculata* is probably one of the most popular herbal medicines used in the treatment of rhinitis and rhinosinusitis. However, its specific mechanism of action is still unknown.

Objective: To evaluate *in vitro* antibacterial activity of *L. operculata* against three ordinary agents of upper respiratory tract infection: *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*.

Methods: Different concentrations of *L. operculata* alcoholic extract were applied to bacterial broth containing reference and community strains of the three described agents. After a 24-h incubation period, the bacterial culture turbidity was measured. The samples were then inoculated onto Mueller-Hinton and human blood agar plates. Bacterial growth was analyzed after 24- and 48-h incubation period. The test was considered negative when there was no environmental turbidity, confirmed by the absence of bacterial growth into the inoculated plates. Tests were considered positive when either turbidity changes were observed on the bacterial broth or when bacterial growth was detected on inoculated plates. Appropriate statistical analysis of the data was performed.

Results: *L. operculata* extracts showed antibacterial activity mainly to *S. pyogenes* followed by *S. pneumoniae* and *S. aureus*.

Conclusions: *L. operculata* extract showed promising antibacterial activity *in vitro* against the studied agents.

© 2014 Associação Brasileira de Otorrinolaringologia e Cirurgia Cérvico-Facial. Published by Elsevier Editora Ltda. All rights reserved.

[☆] Please cite this article as: Scalia RA, Dolci JEL, Ueda SMY, Sassagawa SM. *In vitro* antimicrobial activity of *Luffa operculata*. Braz J Otorhinolaryngol. 2015;81:422–30.

[☆] Institution: Study conducted at Medical Sciences School, Santa Casa de São Paulo (FCMSCSP), to obtain Doctorate's degree in Surgery Research.

* Corresponding author.

E-mail: rodolfo Scalia@yahoo.com.br (R.A. Scalia).

PALAVRAS-CHAVE

Luffa operculata;
Sinusite;
Produtos com ação
antimicrobiana;
Medicamentos
fitoterápicos

Atividade antimicrobiana *in vitro* da *Luffa operculata***Resumo**

Introdução: A *Luffa operculata* é, provavelmente, o fitoterápico mais utilizado no tratamento das rinites e rinossinusites. Apesar de amplamente utilizada pela população, seus mecanismos de ação ainda não estão completamente estabelecidos.

Objetivo: Avaliar a atividade antimicrobiana *in vitro* da *Luffa operculata* em agentes causadores de infecções de vias aéreas superiores: *Staphylococcus aureus*, *Streptococcus pneumoniae* e *Streptococcus pyogenes*.

Método: Foram utilizadas diferentes concentrações de extrato alcoólico de *Luffa operculata* em caldo de bactérias dos agentes avaliados. Após incubação de 24 horas foi realizada a leitura de turvação do meio, e posteriormente, semeadura em placas de ágar-sangue e ágar Muller-Hinton, após 24 e 48 horas de incubação. Foram considerados testes negativos aqueles em que não houve a turvação do meio, confirmados pela ausência do crescimento das bactérias nas semeaduras. Foram considerados positivos os testes que apresentaram turvação do caldo ou positividade nas semeaduras de 24 ou 48 horas. Os resultados foram submetidos à análise estatística pertinente.

Resultados: Os extratos de *Luffa operculata* apresentaram atividade antimicrobiana, especialmente para *Streptococcus pyogenes*, seguido dos *Streptococcus pneumoniae* e *Staphylococcus aureus*.

Conclusões: O extrato de *Luffa operculata* apresentou promissora atividade antimicrobiana *in vitro* contra os agentes estudados.

© 2014 Associação Brasileira de Otorrinolaringologia e Cirurgia Cérvico-Facial. Publicado por Elsevier Editora Ltda. Todos os direitos reservados.

Introduction

The widespread use of antibiotics to treat bacterial rhinosinusitis, besides the elimination of the offending organism, aims to restore the normal function of the nasal mucosa and the paranasal sinuses, shortening the duration of symptoms and preventing local and intracranial complications.¹

Although widely used, antibiotics are not the only therapeutic option for rhinosinusitis.² Adjuvant treatments should be used, in accordance with the symptoms, needs and limitations of each patient. In general, nasal washes with saline solutions, topical and systemic corticosteroids, antihistamines, nasal decongestants, and mucolytics, among others, may also be used.¹⁻⁴

One of the therapeutic options employed by a large portion of the population in the treatment of rhinosinusitis is the use of phytotherapy, or herbalism. Traditionally used by the poor segment of the Brazilian rural population, especially in North, Northeast and Midwest regions of the country, herbal medicine has been used increasingly in urban centers across the country, by patients of various cultural and socioeconomic levels.⁴

Among the medicinal plants used informally for the treatment of rhinitis and rhinosinusitis, *Luffa operculata*, known in Brazil as “cabacinha” or “buchinha-do-norte”, is probably the most commonly used.^{5,6} *L. operculata* possesses a number of therapeutic properties, according to the Brazilian population. Although several studies in the literature sought to identify the mechanisms by which *L. operculata* exhibits such properties, no evidence of antihistamine, vasoconstrictor, anti-inflammatory, or antiviral activity of this

herbal medicine was found.⁷⁻¹⁰ Structural changes in the respiratory mucosa were described by experimental studies; however, the concentration and the way in which *L. operculata* was used were not clearly stated.^{4,11}

In a recent systematic review on *L. operculata* effectiveness in the clinical treatment of rhinosinusitis, it was concluded that many clarifying scientific data on the subject are still missing, and that the data currently available are still very controversial.¹² Thus, new, insightful and reliable studies are in order, so that *L. operculata* can be used safely and effectively in the treatment of sinonasal diseases.^{12,13}

In an attempt to elucidate these properties, the aim of this study was to evaluate the *in vitro* antimicrobial activity of *L. operculata* against some causative agents of rhinosinusitis.

Methods

The study was conducted in the Laboratory of Microbiology, Department of Pathological Sciences and Department of Otorhinolaryngology, after approval of the relevant Scientific Committees. The Research Ethics Committee of the institution waived the evaluation of the project, because there is no involvement of human beings or experimental animals.

Obtaining microorganisms

To conduct the microbiological assay, specimens of clinical origin were selected and stored in the Laboratory

Table 1 McFarland nephelometric scale with the estimated number of bacteria in each tube.

Tube no.	0.5	1	2	3	4	5	6	7	8	9	10
Approximate number of bacteria ($\times 10^8$)	1.5	3	6	9	12	15	18	21	24	27	30

of Microbiology of the institution. In addition, strains of American Type Culture Collection (ATCC) origin were also selected.

ATCC strains are standard strains with a known susceptibility profile (*i.e.*, they have a serial number that allows us to know, regardless of where they are evaluated, their response from previously tested antibiotics), thereby ensuring the reproducibility of the study.

Despite ATCC strains present a known profile, it is not possible to evaluate the antimicrobial activity of a new drug without also using strains of clinical origin, because ATCC strains do not represent bacteria causing diseases in daily life. Strains of clinical origin are necessary, since they have different susceptibility profiles, which ensure greater fidelity for the evaluation of antimicrobial action with respect to those organisms of interest.

ATCC strains may be sensitive or resistant. Those sensitive strains, used in this study, show sensitivity to antibiotics used in clinical practice, but this is not the case with resistant strains. Conceptually, ATCC strains, when presenting resistance to two or more classes of antimicrobials to which they would be originally sensitive, are classified as multi-resistant strains.

The most prevalent etiologic agents of bacterial rhinosinusitis are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*; but there are many factors involved in the acquisition, culture, and maintenance of each of these bacterial organisms, which influenced the choice of agents in our study.

H. influenzae and *M. catarrhalis* are fastidious slow-growing bacteria that require special culture media and nutrients for their development. This, together with the fact that most of our patients who had cultures sent to the laboratory had been treated with antibiotics prior to specimen collection, resulted in the isolation of only a few strains of *M. catarrhalis* and *H. influenzae* during the 26-month period of establishing our bacterioteca. Even after their isolation, due to preservation difficulties, none of the strains remained viable, precluding their use in our research.

Given that *Staphylococcus aureus*, *S. pneumoniae* and *Streptococcus pyogenes* are prevalent in upper airway infections and less fastidious bacteria and do not require special culture media to maintain their viability and growth, these organisms were selected for this study.

The selection of the strains occurred as follows:

- a. *S. aureus* (25 strains):
 - 1 ATCC-25923 sample – standard strain, with a known sensitive susceptibility profile.
 - 24 isolates of clinical origin with a variable susceptibility profile.
- b. *S. pneumoniae* (25 strains):
 - 1 ATCC-49619 sample – standard strain, with a known sensitive susceptibility profile.

24 isolates of clinical origin with a variable susceptibility profile.

- c. *S. pyogenes* (25 strains):

- 1 ATCC-19615 sample – standard strain, with a known sensitive susceptibility profile.

- 24 isolates of clinical origin with a variable susceptibility profile.

With the aid of a sterilized platinum loop, strains of *S. aureus*, *S. pneumoniae* and *S. pyogenes* were transferred to test tubes containing 10 mL of casein-peptone soymeal-peptone broth, *i.e.*, Tryptic Soy Bean (TSB), an environment that allows preservation of bacterial viability. Suspensions of the microorganisms were standardized by their comparison to 0.5 in a McFarland scale, corresponding to approximately 1.5×10^8 CFU/mL (Colony Forming Units per milliliter).

The McFarland scale (Table 1) is a nephelometric scale of 11 tubes, numbered from 0.5 to 10; its turbidity standard is the most often used in microbiology laboratories in Brazil and in the United States to determine the intensity of bacterial multiplication in liquid culture media. The bacterial multiplication precludes the passage of light, which causes turbidity and opacification of the culture medium. The greater the number of bacteria present in the sample, the greater the turbidity of the culture medium. Recommended by the Clinical and Laboratory Standards Institute (CLSI), United States, the McFarland nephelometric scale was also adopted and recommended by ANVISA in Brazil.¹⁴ With this regulation, it is possible to establish a standard that can be gauged at any location in the country, generating correspondence in the reading of their results.

Obtaining concentrations of *L. operculata*

The evaluation of the *in vitro* antimicrobial activity of *L. operculata* with respect to the tested agents was performed by the broth macrodilution technique, with *L. operculata* concentrations of 0.2%; 0.3%; 0.4%; 0.5%; 1% and 2% in TSB culture medium.

An alcoholic extract of *L. operculata* (Schraiber®), batch number 5343, with 125 mL, alcohol strength of 65%, with *L. operculata* concentration of 10% was used. This product was manufactured on October 6, 2010 with validity period until October 6, 2015.

For obtaining these dilutions, a graded pipette, which allowed accurate samplings of the 10% alcoholic extract of *L. operculata*, with a minimum content of 0.1 mL, was used, as well as sterile and dry 10-mL test tubes for solution. Several 10 mL tubes were prepared at different concentrations; these tubes were used as needed, with no need to keep them stored. That is to say, a new test tube containing TSB and *L. operculata*, at different concentrations, was prepared only after the previous tube was finished. Hence, our aim was to avoid any contamination from their storage (Table 2).

Table 2 Calculation of dilutions from the initial concentration of 10% alcoholic extract of *Luffa operculata*.

Concentrations of <i>Luffa operculata</i> (%)	<i>Luffa operculata</i> 10% (mL)	TSB (mL)	<i>Luffa operculata</i> + TSB (mL)
0.2	0.2	9.8	10
0.3	0.3	9.7	10
0.4	0.4	9.6	10
0.5	0.5	9.5	10
1	1	9	10
2	2	8	10

TSB, Tryptic Soy Bean.

After this dilution, 1 mL of alcoholic extract in several concentrations was deposited into different dry and sterile 10 × 1.5-cm test tubes. Next, 1 mL of bacterial broth (concentration of 0.5 McFarland, which corresponds to approximately 1.5×10^8 CFU/mL) was deposited in these test tubes. All turbidity tests, 24- and 48-h cultures, and controls were performed in duplicate for all concentrations, and controls were performed in duplicate for all concentrations, in order to exclude any sample contamination.

Controls

To avoid bias in the interpretation of data, three sample controls were performed: a negative control, a positive control and an alcoholic control.

For negative control, a 2-mL tube containing *L. operculata* was used; and for positive control, another 2-mL tube with bacteria broth was used. These tubes were taken to the incubator, where they were incubated at about $37^\circ\text{C} \pm 2^\circ\text{C} \times 24$ h. After 24 h, a tube reading was performed, to ascertain whether or not there was bacterial growth, which was indicated by the occurrence of medium turbidity. All tubes were plated on 90-mm blood agar plates to assess growth and viability of *S. pneumoniae* and *S. pyogenes*, and on 150-mm Mueller Hinton agar plates to assess growth and viability of *S. aureus*. Plate reading was performed after 24 and 48 h.

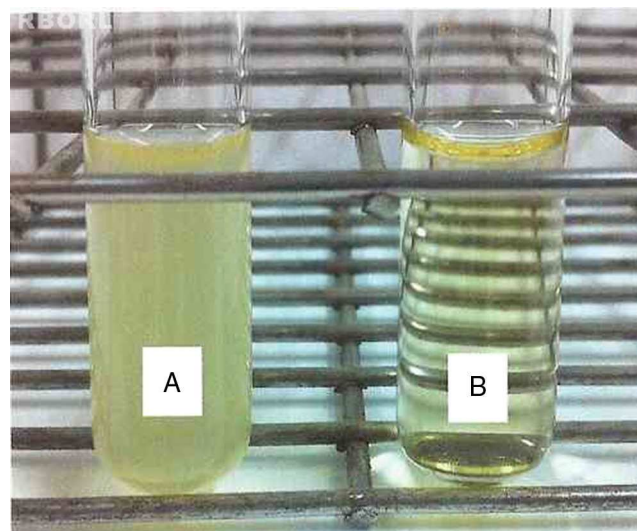
With respect to our analysis criteria, tests not presenting medium turbidity after bacterial inoculation were considered negative, and this was confirmed by the absence of bacterial growth in 24- and 48-h inoculations. Tests exhibiting broth turbidity or positivity in 24- and 48-h inoculations were considered positive (Figs. 1 and 2).

The simple absence of turbidity in the test tubes does not make it possible to assert whether or not there was bacterial eradication in a medium containing *L. operculata* and in bacterial broth. On the other hand, this finding allows us to state that there was a minimum inhibitory concentration (MIC), *i.e.*, the minimum concentration at which bacterial growth was inhibited. If bacterial growth in 24- and 48-h inoculations occurred, this suggests that a greater *L. operculata* concentration will be required to kill all bacteria. On the other hand, when, during the reading, the absence of turbidity is confirmed by bacterial growth absence in 24- and 48-h inoculations, we can establish the minimum bactericidal concentration (MBC), *i.e.*, the minimum concentration required for killing all bacteria.

After dilution of *L. operculata* extract to 10% and with an alcoholic content of 65% for *L. operculata* extracts at concentrations of 0.2%; 0.3%; 0.4%; 0.5%; 1% and 2%, the alcohol content of the same concentrations was calculated (Table 3).

The higher alcoholic content found was 13% at a concentration of 2% of *L. operculata*. To confirm the absence of antimicrobial action by ethanol in these different concentrations, ethanol tubes with different alcohol strengths (between 1.35% and 13%) and with bacterial broth were taken to the incubator, where the tubes were incubated at about $37^\circ\text{C} \pm 2^\circ\text{C} \times 24$ h. After 24 h, a tube reading was performed to verify whether there was bacterial growth, indicated by medium turbidity; culturing of all tubes on 90-mm agar-blood plates was performed to assess *S. pneumoniae* and *S. pyogenes* growth and viability. We did the same for *S. aureus* on 150-mm Muller-Hinton agar plates. This scheme was repeated after 48 h incubation.

To assess the agreement between the methods of turbidity and of 24- and 48-h cultures, the Kappa method, which aims, by a measure of association, to establish an agreement between qualitative variables, was used. Its value can be equal to zero, or non-zero. When a non-zero value is obtained, the agreement is calculated. The agreement is deemed poor when $p < 0.4$; moderate, when $0.4 < p < 0.6$; and strong when $p > 0.6$. All samples were performed in

**Figure 1** (A) Presence of turbidity; (B) absence of turbidity.

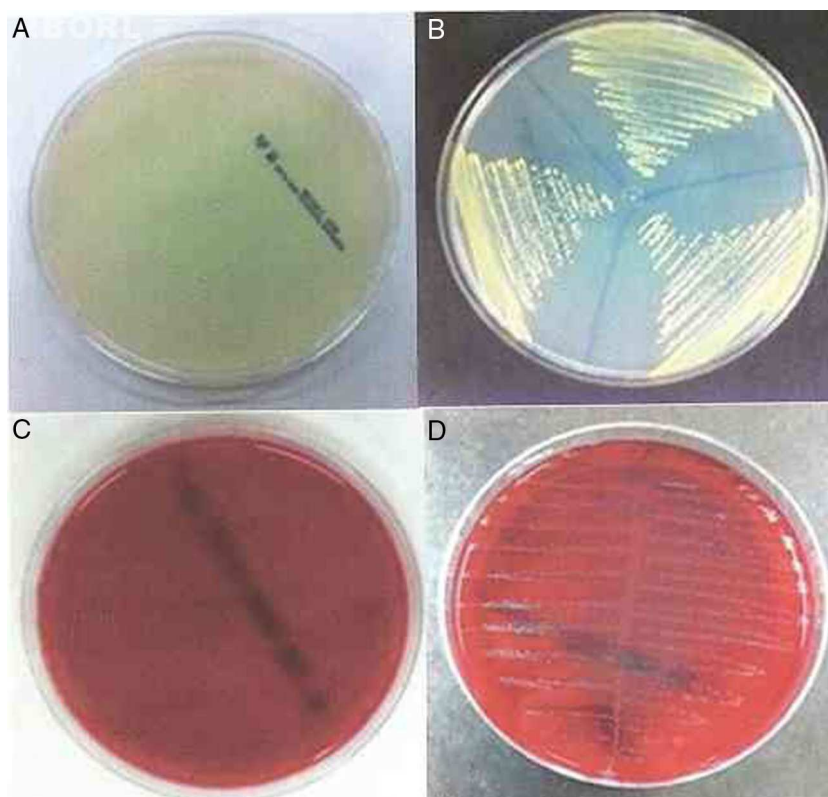


Figure 2 Muller-Hinton agar plates: (A) no bacterial growth; (B) with bacterial growth. Blood-Agar plates: (C) no bacterial growth; (D) with bacterial growth.

duplicate. The program used for statistical analysis was SPSS 13.0 for Windows.

Results

Controls

In all concentrations considered, none of the negative control tubes with *L. operculata* showed visible turbidity or growth of any bacteria strain.

All bacterial broth and all alcoholic controls showed visible turbidity and specific bacterial growth of each broth, without sample contamination.

All negative, positive and alcoholic controls were performed in duplicate for all samples, having confirmed their results.

Microorganisms

All tubes containing *L. operculata* at concentrations of 0.2% and 0.3% and with *S. pyogenes*, *S. pneumoniae* and *S. aureus* bacterial broth showed turbidity after a 24-h incubation period. The 24- and 48-h seedings confirmed the presence of bacteria, showing growth of just those bacteria tested, which excluded any sample contamination.

All tubes containing *L. operculata* at a concentration of 0.4% and with *S. pyogenes* bacterial broth showed no

Table 3 Calculation of dilutions and alcohol strength from the initial concentration of 10% alcoholic extract of *Luffa operculata*, and with alcoholic strength of 65%.

Concentrations of <i>Luffa operculata</i> (%)	<i>Luffa operculata</i> 10% (mL)	TSB (mL)	<i>Luffa operculata</i> + TSB (mL)	Alcoholic strength (%)
0.2	0.2	9.8	10	1.30
0.3	0.3	9.7	10	1.95
0.4	0.4	9.6	10	2.60
0.5	0.5	9.5	10	3.25
1	1	9	10	6.50
2	2	8	10	13.00

TSB, Tryptic Soy Bean.

Table 4 Turbidity and seeding results on bacterial agents for *Luffa operculata* in different concentrations.

Agent	Concentration	Turbidity (n = 25)	24- and 48-h seedings (n = 25)
<i>Streptococcus pyogenes</i>	<i>Luffa operculata</i> 0.4%	25 Negatives (100%)	24 Negatives (96%)
	Kappa = 0 (p-value not calculated)	0 Positive (0%)	1 Positive (4%)
<i>Streptococcus pneumoniae</i>	<i>Luffa operculata</i> 0.4% (Kappa $\neq$ 0; $p < 0.449$)	8 Negatives (32%)	0 Negative (0%)
		17 Positives (68%)	0 Positive (0%)
	<i>Luffa operculata</i> 0.5% (Kappa $\neq$ 0; $p < 0.528$)	18 Negatives (72%)	3 Negatives (12%)
		7 Positives (28%)	5 Positives (20%)
<i>Staphylococcus aureus</i>	<i>Luffa operculata</i> 1% (Kappa = 0 (p-value not calculated)	17 Positives (68%)	0 Negative (0%)
		18 Negatives (72%)	12 Negatives (48%)
		4 Negatives (16%)	6 Positives (24%)
		21 Positives (84%)	0 Negative (0%)
			21 Positives (84%)

turbidity after a 24-h incubation period. However, among those 25 samples of *S. pyogenes* (ATCC-19615 and 24 clinical isolates) tested, a clinical isolate (4%) showed bacterial growth in 24- and 48-h seedings (Table 4).

Among the 25 tubes containing *L. operculata* at a concentration of 0.4% and with *S. pneumoniae* bacterial broth (ATCC-49619 and 24 clinical isolates), the ATCC sample and two clinical isolates (12%) showed no turbidity after a 24-h incubation period and no bacterial growth in 24- and 48-h cultures. Five other clinical samples (20%) showed no turbidity after a 24-h incubation, but showed growth in 24- and 48-h cultures. Seventeen clinical isolates (68%) showed turbidity after a 24-h incubation period and bacterial growth in 24- and 48-h cultures. These 25 cultures showed growth only for *S. pneumoniae*, which excluded any type of sample contamination. Statistical analysis showed Kappa $\neq$ 0 and $p < 0.449$, indicating a moderately suggestive result of antimicrobial response (Table 4).

All tubes containing *L. operculata* at concentrations of 0.4% and 0.5% and with *S. aureus* bacterial broth showed turbidity after a 24-h incubation period. 24- and 48-h cultures confirmed the presence of these bacteria.

All tubes containing *L. operculata* at concentrations of 0.5%; 1% and 2% and with *S. pyogenes* bacterial broth showed no turbidity after a 24-h incubation period and no bacterial growth in 24- and 48-h cultures.

Among the 25 tubes containing *L. operculata* at a concentration of 5% and with *S. pneumoniae* bacterial broth (ATCC-49619 and 24 clinical isolates), the ATCC sample and 11 clinical isolates (48%) showed no turbidity after a 24-h incubation period, or bacterial growth in 48-h cultures. Six other clinical isolates (24%) showed no turbidity after a 24-h incubation period, but with growth after 24- and 48-h cultures. Seven clinical isolates (28%) showed turbidity after a 24-h incubation period and bacterial growth in 24- and 48-h cultures. These 25 cultures showed growth only of *S. pneumoniae*, which excluded any type of sample contamination. Statistical analysis showed Kappa $\neq$ 0 and $p < 0.528$,

indicating a moderately suggestive result of antimicrobial response (Table 4).

The test tubes containing *L. operculata* at concentrations of 1% and 2% and with *S. pneumoniae* bacterial broth showed no turbidity after a 24-h incubation period and no bacterial growth in 24- and 48-h cultures.

Among the 25 tubes containing *L. operculata* at a concentration of 1% and containing *S. aureus* (ATCC-25923 and 24 clinical isolates), the ATCC sample and three clinical isolates (16%) showed no turbidity after a 24-h incubation period; however, all showed growth after 24- and 48-h cultures (Table 4).

The tubes containing *L. operculata* at a concentration of 2% and with *S. aureus* bacterial broth showed no turbidity after a 24-h incubation period and no bacterial growth in 24- and 48-h cultures.

Discussion

Extract choice

One of the major difficulties in the use of herbal medicine in controlled studies is the lack of standardization of herbal drugs. None of the experimental studies published between 2001 and 2010 had the same method of obtaining *L. operculata* extracts.^{4,11-13}

Another difficulty for the use of plants *in natura*, especially when it comes to evaluating the antimicrobial action of an herbal drug, is to ensure the origin of the material and absence of contamination. In a study published in 2001, the quality of *L. operculata* dried fruits from the Municipal Market of São Luiz do Maranhão was evaluated, and contamination of all samples of *L. operculata* fruits acquired in this market has been proven; some fruits were contaminated by fungi and some by bacteria, making them unsuitable for its use.¹⁵

Aiming to avoid the presence of contaminated fruits that could contaminate the samples in our study, as well as to ensure that the same extract was used throughout the work, we chose to use an industrialized alcoholic extract of *L. operculata*, whose access was available for replication of the study, if necessary.

It is relevant to note that the reproducibility of the research is restricted to the batch specifically used in this study. Depending on the conditions of soil, sunlight and rainfall index, we cannot guarantee that other batches produced in other seasons or with origin in other crops would have the same results found in our study. It is important to ensure some comparison level in the conduction of other studies using different *L. operculata* extracts at the same concentrations and with the same bacterial agents.

Concentrations used

Since 1960 a few articles have attempted to evaluate the mechanisms of action by which *L. operculata* acts on the respiratory epithelium, and also to prove its therapeutic efficacy in the clinical treatment of rhinosinusitis.¹² The authors could not identify anything that would establish reliable criteria for its safe use¹² and some actually reported that the improper use of this herbal product, especially at high concentrations of *L. operculata*, can lead to a worsening of sinonasal conditions.^{4,6} Nevertheless, some clinical and experimental studies list concentrations of 0.5% and 1% for the treatment of rhinosinusitis.^{13,16,17}

As we found no references on antimicrobial activity of *L. operculata* in the literature before 2008, when, in a single study, only one screening procedure with different bacteria and fungi was conducted,¹⁸ we chose to start our investigation with a concentration of 1%, the same level present in this commercial extract, released by ANVISA and tested in most studies cited in the literature.^{11,13,17} We used twice the recommended concentration (2%), thus setting a limit to this antimicrobial activity, without approaching LD50^{7,16} or causing an increase of its deleterious effects to the mucosa. Finally, other concentrations (0.2%; 0.3%; 0.4% and 0.5%) were evaluated, in an attempt to establish a minimum concentration in which *L. operculata* exhibited an antimicrobial effect, so as to minimize any side effects.

Bacterial agents

All tubes containing *L. operculata* at concentrations of 0.2% and 0.3% and with *S. pyogenes*, *S. pneumoniae* and *S. aureus* bacterial broth showed turbidity after a 24-h incubation period, indicating bacterial growth in these concentrations, and hence, no antimicrobial action to these microorganisms.

All tubes containing *L. operculata* at a concentration of 0.4% and with *S. pyogenes* bacterial broth showed no turbidity after a 24-h incubation period, demonstrating antimicrobial activity for this agent, in this concentration. The absence of visual turbidity *per se* led to the conclusion that, at a concentration around 0.4%, it was possible to determine MIC for *S. pyogenes*.

Considering that the absence of visual turbidity, confirmed by an absence of growth in 24- and 48-h cultures, only occurred in those tubes with *L. operculata* at concentrations

greater than 0.5%, it was inferred that, with concentrations around this level, MBC of these agents can be established.

Some samples containing *L. operculata* at concentrations of 0.4% and 0.5% and with *S. pneumoniae* bacterial broth, showed no medium turbidity. However, the fact that some samples presented bacterial growth in the cultures confirmed that, with a concentration around 0.4% or 0.5%, we can establish MIC for *S. pneumoniae*, in the same line as for *S. pyogenes*.

At *L. operculata* concentrations higher than 1%, neither turbidity in tubes containing *S. pneumoniae* nor bacterial growth in 24- and 48-h cultures was perceived; however, considering that no intermediate concentrations between 0.5% and 1% were tested, we cannot conclude that MBC for *S. pneumoniae* is equal to 1%. It may be that, at some concentration between 0.5% and 1%, the killing of all tested bacteria occurs, and in order for this to happen, clinical studies with a greater *L. operculata* concentration variety would be needed to establish its MBC.

Some tubes containing *L. operculata* at a concentration of 1% and with *S. aureus* bacterial broth showed no visual turbidity after the 24-h reading, suggesting that, at this concentration, *L. operculata* showed antimicrobial activity for this agent, thereby establishing its MIC. Only at a concentration of 2% there was no turbidity and no bacterial growth in 24- and 48-h cultures; however, as also occurred with *S. pneumoniae*, we cannot guarantee that this concentration represents its MBC. The lack of intermediate concentrations between 1% and 2% is no guarantee that a value higher than 1% and lower than 2% also may be able to eradicate all *S. aureus* organisms (Table 5).

In spite of being Gram-positive cocci, *S. pyogenes*, *S. pneumoniae* and *S. aureus* showed different susceptibility profiles, when in a milieu containing *L. operculata*. Lower concentrations of around 0.5% were able to kill all *S. pyogenes* organisms, while *S. pneumoniae* organisms were eradicated only at a concentration of 1%; as for *S. aureus*, its eradication occurred only at a concentration of 2%. This response to the presence of *L. operculata* was equivalent to the response of these microorganisms to conventional antibiotics. There are multiple mechanisms by which these bacteria develop resistance to antibiotics.

S. pneumoniae, for example, has a capsule that impairs the action of antimicrobial agents on its cell wall. Usually, this bacterium is more resistant to antibiotics than *S. pyogenes*. On the other hand, *S. aureus* has a greater ability to generate bacterial resistance than those microorganisms previously cited, in part because it is a high beta-lactamase inducer.

Study limitations

The paucity of studies on the antimicrobial activity of *L. operculata* made it difficult to find references for the discussion of our study. The only study with an antimicrobial activity screening for some ATCC strains of different bacteria also found for *S. aureus* at MIC = 100 mg/mL.¹⁸ In our study, as in the previous study, we evaluated the ATCC-25923 strain, demonstrating its MIC at a concentration of 1%, together with three other clinical isolates. The other 21 clinical isolates tested showed visual turbidity after a 24-h culture and

Table 5 Presence or absence of bacterial growth in different concentrations of *Luffa operculata* extract.

Bacteria	Concentrations of <i>Luffa operculata</i>					
	0.2%	0.3%	0.4%	0.5%	1%	2%
<i>Streptococcus pyogenes</i>	+	+	+	—	—	—
<i>Streptococcus pneumoniae</i>	+	+	+	+	—	—
<i>Staphylococcus aureus</i>	+	+	+	+	+	—

+, presence of bacteria (turbidity or seeding-positive); —, absence of bacteria (turbidity or seeding-negative).

bacterial growth after 24- and 48-h cultures, not replicating the results of the ATCC sample. The lack of studies with extracts at intermediate concentrations between 0.5% and 1% does not allow us to state that its MIC is 1%, but allows us to deduce that MIC may be situated between 0.5% and 1%. Likewise, an MBC for *S. aureus* also cannot be established, but we can state that, for these samples used in our study, MBC lies at concentrations of *L. operculata* between 1% and 2%.

No previous study has evaluated the antimicrobial activity of *L. operculata* against *S. pyogenes* or *S. pneumoniae*. It became clear in our study that *S. pyogenes* samples were more susceptible to the antimicrobial action of *L. operculata*, compared to *S. pneumoniae* samples, and the latter organism, in turn, was more susceptible than *S. aureus*.

It is important to note that a number of substances, such as cucurbitacines B and D, glycosides, saponins, free sterols, organic acids and phenols, were isolated from the phytochemical analysis of *L. operculata*.^{13,16,19} While a number of substances exhibit therapeutic effects, others may have toxic effects, leading even to a worsening of the clinical presentation upper airway infections.⁴

Thus, even with the promising antimicrobial effect of this herbal medicine,¹⁸ further studies should be conducted with the aim to establish which of these components have a therapeutic effect and what are the most appropriate concentrations, so that its use does not cause more harm than good. These studies should seek to recognize and separate the therapeutic and toxic components of *L. operculata*, so one can accept or discard its use in the treatment of upper airway inflammatory and infectious conditions.^{4,9,11–13}

Considerations for the future

Many studies have been conducted to elucidate the therapeutic properties of *L. operculata*. The task of establishing the tenuous relationship between toxicity and tractability is the challenge to be confronted. The finding of an antimicrobial action in this herbal agent can be of great value in the therapeutic arsenal against upper airway diseases, considering that, every day, mechanisms of bacterial resistance increase the complication in the treatment of these diseases.

It is a fact that herbal medicines present variations in their characteristics and also exhibit changes in their components, depending on variations in the composition of the soil, rainfall index and seasonality of their harvest; this results in an ever different composition having unique characteristics that make it difficult to induce bacterial resistance.

From the moment of the establishment of a safe dose of *L. operculata* for use in the oral cavity, its antimicrobial effect against *S. pyogenes* could be of great importance, in creating an oral spray for prophylaxis of rheumatic fever. Patients with a positive rapid test and no signs or very severe symptoms could use this spray, to eradicate the bacterial agent without use of systemic antibiotics.

The same can be said for the treatment of sinonasal diseases, where the presence of biofilm is proved. The caustic and detergent effects of this herbal drug, through the action of its cucurbitacines and saponins (if their side effects are controlled) could, in low doses, be useful in the destruction of polysaccharides' structure, thus facilitating the action of an associated antibiotic to resolve these diseases.

It is important that we continue searching for information about these and other herbal remedies, in an attempt to find solutions to problems that arise every day, and that often result in failure with the resources currently available.

Conclusion

L. operculata extract showed promising antimicrobial activity *in vitro* against the causative agents of upper airway bacterial infections tested in this study, both in those microorganisms with known susceptibility and profile (ATCC), and in those of clinical origin. This can be an important step in creating new drugs to treat these diseases.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Anon JB, Jacobs MR, Poole MD, Ambrose PG, Benninger MS, Hadley JA, et al. Antimicrobial treatment guidelines for acute bacterial rhinosinusitis. *Otolaryngol Head Neck Surg.* 2004;130:1–45.
2. Associação Brasileira de Otorrinolaringologia e Cirurgia Cérvico-Facial (Departamento de ORL da Associação Médica Brasileira). Diretrizes Brasileiras de Rinossinusites. *Rev Bras Otorrinolaringol (Supl.)*. 2008;74:S1–59.
3. Fokkens WJ, Lund VJ, Mullol J. European position paper on rhinosinusitis and nasal polyps. *Rhinol Suppl.* 2007;20:1–136.
4. Menon-Miyake MA. Efeitos da infusão de *Luffa operculata* sobre o epitélio e a atividade mucociliar do palato isolado de rã [thesis]. São Paulo: Faculdade de Medicina da Universidade de São Paulo; 2004.
5. Niggemann B, Grüber C. Side-effects of complementary and alternative medicine. *Allergy*. 2003;58:707–16.

6. Menon-Miyake MA, Caniello M, Balbani APS, Butugan O. Inquérito sobre o uso de plantas medicinais para tratamento de afecções otorrinolaringológicas entre pacientes de um hospital público terciário. Caderno de Debates. Rev Bras Otorrinolaringol. 2004;70:43–55.
7. Silva JB. Algumas pesquisas sobre saponinas da *Luffa operculata*. Rev Farm Bioquím São Paulo. 1964;2:153–60.
8. Champney R, Ferguson NM, Ferguson GG. Selected pharmacological studies of *Luffa operculata*. J Pharm Sci. 1974;63:942–3.
9. Silva EA. Contribuição para o estudo farmacológico da *Luffa operculata* cogniaux [dissertation]. Fortaleza: Universidade Federal do Ceará; 1983.
10. Glatthaar-Saalmüller B, Fallier-Becker P. Antiviral action of *Euphorbium compositum* and its components. Forsch Komplementarmed Klass Naturheilkd. 2001;8:207–12.
11. Roncada PRA. Estudo analítico das alterações estruturais e ultra-estruturais da superfície do epitélio da mucosa nasal (concha inferior e septo nasal) de coelhos após o uso tópico do extrato do fruto da *Luffa operculata* [thesis]. São Paulo: Faculdade de Ciências Médicas da Santa Casa de São Paulo; 2001.
12. Sousa Neto PS. *Luffa operculata*: mecanismo de ação no epitélio respiratório e eficácia terapêutica no tratamento clínico das rinossinusites: revisão sistemática [thesis]. São Paulo: Faculdade de Ciências Médicas da Santa Casa de São Paulo; 2006.
13. Campos CAC. Eficácia de solução tópica nasal de extrato de *Luffa operculata* para tratamento de rinossinusite bacteriana em coelhos [dissertation]. São Paulo: Faculdade de Ciências Médicas da Santa Casa de São Paulo; 2008.
14. The National Committee for Clinical Laboratory Standards (NCCLS). Metodologia dos testes de sensibilidade a agentes antimicrobianos por diluição para bactéria de crescimento aeróbico: norma aprovada–6^a ed. M7-A6, 23; 2011. p. 1–81.
15. Amaral FMM, Coutinho DF, Ribeiro MNS. Controle de qualidade de frutos de *Luffa operculata* Cogn. Comercializados em Mercados de São Luiz/Maranhão, Brasil. Rev Hosp Univ UFMA. 2001;3:9–13.
16. Vasques CAV, Vasques NV, Arraes LA, Geller M. Revisão farmacognóstica da cabacinha (*Luffa operculata* Cogn.). Folha Med. 1986;93:185–7.
17. Salviano PA. Tratamento da sinusite com preparação contendo *Luffa operculata* e solução fisiológica. Rev Bras Med. 1992;49:681–2.
18. Caribé RA. Abordagem da atividade biológica do extrato de *Luffa operculata* Cogn. (cucurbitaceae) [dissertation]. Pernambuco: Universidade Federal de Pernambuco; 2008.
19. Matos FJA. Farmocognosia de *Luffa operculata* Cogn. Rev Bras Farm. 1979;60:69–76.