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Phytochemical Analysis and Antibacterial Activity of *Lantana camara* L. Leaf Extract

Bondy Jorge Lourenço[1*], Asimbawe kiza[1], Abrão Amândio João[1], Clemência Félix Odala Niconte[2], Pompílio Armando Vintuar[3], Lázaro Gonçalves Cuinica[4]

[1]Department of Health Sciences, Lúrio University, Nampula, Mozambique, [2]Department of Health Research and Training Center, National Health Institute, Nampula, Mozambique, [3]Department of Food and Agrarian Sciences, Rovuma University, Nampula, Mozambique, [4]Department of Natural Sciences, Mathematics and Statistics, Rovuma University, Nampula, Mozambique

ABSTRACT

Antibacterial effects screening of plant extracts is strategic, to discover new species of plants with pharmacological activity, which can be used to develop new antibacterials or to potentiate the action of already clinically known antibacterials, in an attempt to combat to antibacterials resistant infections. In this study, we performed a qualitative analysis of *Lantana camara* L. leaves compounds and its antibacterial activity against *Escherichia coli* and *Staphylococus aureus*. Extract were obtained by leaves maceration with 90% ethanol. Qualitative phytochemical analysis was performed using specific reagents for each class of secondary metabolites. Antibacterial activity against *Escherichia coli* and *Staphylococus aureus* was evaluated using disc diffusion method according to Kirby-Bauer. The classes of Alkaloids, flavonoids, tannins and saponins were identified. The diameters of the inhibition halos ranged from 0.7 to 12 mm, in extract concentrations of 200 mg/ml to 300 mg/ml, respectively, with an average of 7.940 mm for *S. aureus* strains and there was no inhibition halo for *E. coli*. The extract from the leaves of *L. camara* contains bioactive compounds, alkaloids, flavonoids, tannins and saponins, which is why this plant is used for various medicinal purposes and has antibacterial activity against *S. aureus* and not against *E. coli*.

Keywords: Antibacterial activity; *Escherichia coli*; Phytochemical analysis; *Lantana camara* L; *Staphylococcus aureus*

INTRODUCTION

Antibacterial resistant infections cause at least 50,000 deaths each year in the United States and Europe alone, with more than hundreds of thousands of deaths in other regions of the world. Although, in modern, well-funded health care systems, gaining access to second- and third-line treatments may often not be a problem, mortality rates for patients with infections caused by resistant bacteria are significantly higher [1].

The situation is worse, especially in developing countries that have a high burden of infectious diseases. Studies conducted on rates of antibacterial resistance in Mozambique have revealed high levels of resistance to common drugs used to treat serious bacterial diseases [2].

Plants represent an important source of chemical products with biological activities [3]. *L. camera* is a native plant in tropical America, being commonly used for various medicinal purposes, as the whole plant has biological or pharmacological activity, such as antimicrobial, fungicidal, insecticidal, nematicidal, antioxidant, anti-inflammatory and diuretic activity [4].

In Mozambique, the species *L. camara* is called in the local Bantu language: m'bulimuthi, chauwunké, chamarenke, n'teja, n'toja or n'tuja [5].

The *E. coli* species is the most common and clinically important, causing more than 80% of all community-acquired urinary tract infections, as well as many nosocomial infections, and gastroenteritis in developing countries. The *S. aureus* bacteria can cause purulent infections and abscesses that commonly affect children, the elderly, and people with weakened immune systems [6].

Given this scenario, the present work analysed the chemical composition and the antibacterial activity of the extract from the leaves of *L. camara* against *E. coli* and *S. aureus*.

METHODOLOGY

Sample collection

The collection of plant matter was carried out in July 2019, in Nampula city-Mozambique, at coordinates (15° 07'09.5"S 39° 12'56.3"E). Identification of the species *L. camara* was made

Correspondence to: Bondy Jorge Lourenço, Department of Health Sciences, Lurio University, Nampula, Mozambique, E-mail: bondylourenco@gmail.com

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by comparing its characteristics with other species in the literature and using the plantsnap application [7]. Also, this species was confirmed by a specialist in Agricultural Sciences–Agroecology). Exsiccate was prepared and preserved in the herbarium of the Pharmacy Course at the Lúrio University. Damaged parts of plant matter were separated, washed with water to remove impurities, dried out at the sun for seven days and then crumpled and sieved, from which 200 g of fine and homogeneous powder was obtained.

Obtaining plant extract

Preparation plant extract was carried out at the Laboratory of Ethnobotany and Phytochemistry of the Faculty of Health Sciences at Lúrio University. Through the maceration method, 200 g of the powder of the leaves of *L. camara* was mixed with 2 L of ethanol at the final concentration of 90%, in the proportion of 1 g/10 mL, and occasionally stirred for 7 days. The mixture was stored in a dry environment protected from direct light and daily temperature average of 26˚C. Subsequently, the mixture was filtered with cotton twice and the fluid extract was obtained.

To determine the yield of the dry extract, three measurements of the liquid extract were made, removing 2 mL of extract and placing it in each of the three porcelain crucibles previously tared on the analytical pressure balance, and then placed in the oven at 105˚C for 2 hours, then cooled in a desiccator and weighed. The yield of the dry extract was determined according to the following formula:

$$\text{Dry Extract Yield} = \frac{\text{Mass of dry extract} \times 100\%}{\text{Mass of liquid extract}}$$

Then, the remaining liquid extract was divided in half, one part was dried in an oven at 80˚C for 8 hours, and the other part was placed in a rotary evaporator at reduced pressure and at a temperature of 42˚C for 6 hours.

Qualitative phytochemical analysis

A part of the extracts was used to identify the following secondary metabolites: alkaloids, flavonoids, tannins, saponins and anthraquinones, based on the following procedures:

For tannins: 1 mL of extract diluted with water in a proportion of 1:2 was added to a test tube, then 2 drops of 10% lead acetate were added. In this reaction, the formation of a dense reddish brown precipitate indicates the presence of tannins. With the same procedure, a control test was carried out with only the solvent without extract.

For alkaloids: the extract was dissolved in 6.25 ml of 5% HCl and heated for 10 minutes. After cooling, it was filtered through cotton. Then, 5 drops of wagner's reagent (iodine/potassium iodide, (I2/KI)) were added. The presence of a slight turbidity or precipitate respectively purple to orange, white to cream and brown,

evidences the possible presence of the alkaloids.

For saponins: 2 ml of ethanol extract was added to a test tube, then 5 ml of distilled water was added, shaken vigorously for 2 to 3 minutes and left to stand for 20 minutes. The existence of persi-stent and abundant foam suggests the presence of saponins.

For flavonoids: The extract was diluted 1:5 with water, then 5 ml of the diluted extract was placed in a test tube and a drop of 2% ferric chloride was added through the wall of the test tube. In this reaction, the presence of a color that varies between green, yellow brown and violet, showed the presence of flavonoids. Then, a control test was performed with only the solvent.

For anthraquinones: 0.5 g of the extract was weighed into a watch glass and 3 drops of 0.5% NaOH were added. The presence of a yellowish color shows the presence of anthraquinones in the reduced form or a reddish color that indicates the presence of anthraquinones in the oxidized form.

Evaluation of antibacterial activity

The evaluation of the antibacterial activity of the ethanolic extract from the leaves of *L. camara* was carried out using the Kirby-Bauer disk diffusion method. *E. coli* strains obtained from a stool sample cultured on TCBS agar (thiosulfate, citrate, bile and sucrose) and *S. aureus* strains obtained from a pus wound sample cultured on blood agar were used. The bacterial suspension was prepared with 0.9% NaCl, with density close to Mac Farland's standard scale n˚0.5. Then, using the sheet technique, the suspension was seeded on Muller Hington agar. Then, impregnation in triplicate, in 0.4 cm diameter filter paper discs at concentrations of 100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml and 500 mg/ml of ethanol extract. As a negative control, discs only impregnated with 90% ethanol solvent were used. For positive control 10 µg gentamicin was used for both bacteria and 30 µg nalidixic acid only for *E. coli*. Then, the impregnated discs were left in an oven to dry for 1 minute and then placed on muller hington agar, spaced 2 cm apart and incubated in the oven for 24 hours at 37˚C. After incubation, the diameters of the halos in millimeters were determined using a millimeter ruler [8].

Data analysis

Analysis of the results was performed using the SPSS statistical package version 22. And were calculated the average of the inhibition halos of concentrations in triplicate and the respective standard deviation, the minimum and maximum inhibitory halo and the average of the diameters of inhibition halos, through descriptive statistics. Also, the relationship between the increase in extract concentrations and the increase in inhibitory halo diameters was tested through statistical tests.

RESULTS

Rotary evaporator extraction yielded 4.61% and the oven-dried extract was 3.92%, these results were considered positive in relation to the dry residue in both methods. According to the Brazilian Pharmacopoeia, generally, the ethanolic extract has a dry residue of no more than 5% [9].

Phytochemical analysis has shown presence of alkaloids, tannins, flavonoids, and saponins. While for quinones the result was negative in Table 1. Also, the qualitative phytochemical analysis carried out in the study by Faria Goes, et al., identified the classes of flavonoids, tannins, saponins and alkaloids in the extract of the leaves of *L. camara*, using the maceration method and ethanol as extracting solvent [10]. Generally, the presence of these metabolites is influenced by many external factors that stimulate the production to defend the plant itself. Thus, the absence of these metabolites may indicate that the plant produced in undetectable amounts, or that there was still no external attack that could influence its production [11].

Table 1: Secondary metabolites identified in the extract of *L. camara* leaves according to the extract drying method.

Secondary Metabolites	Dry extract oven	Dry extract rotational evaporator
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Saponins	+	+
Quinones	-	-

Note: (-) absent; (+) present.

Presence of classes of flavonoids, alkaloids, tannins and saponins indicates that the analyzed extract may have antibacterial action.

Regarding to antibacterial analysis, result showed diameters of inhibition halos ranging from 0.7 mm to 12 mm for concentrations of 200 mg/mL and 300 mg/mL, respectively, and had the total average of the diameters of inhibition halos equal to 7.940 mm for *S. aureus* strains. However, for *E. coli* strains there was no inhibition halo detected for all extract concentrations (Table 2). In this case, when the extract shows a difference in activity against Gram-negative and Gram-positive bacteria, it may be because of the constitution of the bacterial cell wall and the components of the plant extract [12]. Several authors state that there is a relationship between the amount of tannins and the activity against Gram-positive bacteria, whose cell structure is more rigid, cell wall chemically less complex and less amount of lipids than in Gram-negative bacteria [13].

Table 2: Concentrations of extract of *L. camara* and diameters of inhibition halos against *S. aureus* and *E. coli*

Concentration (mg/ml)	Inhibition diameter halo (mm)		
	S. aureus		*E. coli*
C1	100	6.66±0.57	-
C2	200	0.70±0.00	-
C3	300	12.00±0.00	-
C4	400	10.00±0.00	-
C5	500	10.33±0.58	-

S. aureus				
Sample (n)	Minimum	Maximum	Average	Standard deviation
15	0.7	12	7,940	4.1646

Note: (-) Undetected inhibition halo; (C) concentration; ± standard deviation.

In this study, the diameter of the inhibition halo at C1 concentration (100 mg/ml) was different, comparing with the study carried out by Rasyid SA, et al., using a methanolic extract from the *Lantana camara* leaves, and inhibition halo is equal to 0.6 cm at a concentration of 100%, against *S. aureus* [14]. Due to the amount of secondary metabolite extracted, as many studies have shown methanol as a potent extracting solvent [15]. Also, it may be, because of the resistance of the microorganism tested, because depending on the resistance of the microorganism it may or may not be susceptible to the product to which it is exposed. Therefore, the same species coming from different samples can present different diameters of inhibition halos [15].

These results confirm the use of the leaves of this plant in some preparations, in the treatment of certain infectious diseases that afflict communities in Mozambique.

The Shapiro-Wilk test for the *S. aureus* data had P<0.05, indicating that the data are not normally distributed.

Spearman's correlation coefficient was equal to rho=0.571 and p-value=0.026, indicating that there is a positive correlation and there is a statistically significant association between the increase in concentration and the increase in the diameter of the inhibition halo. This association has been analysed in several previous studies, because the ability of an antimicrobial material to inhibit the living capacity of microorganisms depends on the concentration of the antimicrobial material [16]. The higher the concentration of the extract is, the higher the extract's ability to inhibit microbial growth will be.

The results of the positive control, gentamicin 10 µg had an inhibitory halo diameter greater than or equal to 15 mm, being considered sensitive to *S.aureus* and *E. coli*. For nalidixic acid 30 µg had an inhibitory halo of about 7.33 mm for *E. coli*, being considered resistant is shown in Table 3. However, according to the halo table standardized by the Manual for Antibiogram-2019, these results do not imply the low quality of the controls, because the test bacteria were exposed to several factors that can lead to resistance to antibacterials [17].

Table 3: Positive controls and the respective diameters of the inhibition halo in *S.aureus* and *E. coli*

Positive controls	Inhibition halo diameter (mm)	
	S.aureus	*E. coli*
Gentamicin 10µg	15.66 ± 0.577	17.33 ± 1.15
Nalidixic Acid 30µg	No	7.33 ± 0.57

Note: No: not tested.

DISCUSSION

Phytochemical analysis has shown presence of alkaloids, tannins, flavonoids, and saponins.While for quinones the result was negative in Table 1. Also, the qualitative phytochemical analysis carried out in the study by Faria Goes, et al., identified the classes of flavonoids, tannins, saponins and alkaloids in the extract of the leaves of *L. camara*, using the maceration method and ethanol as extracting solvent [10]. Generally, the presence of these metabolites is influenced by many external factors that stimulate the production to defend the plant itself [11]. Thus, the absence of these metabolites may indicate that the plant produced in undetectable amounts, or that there was still no external attack that could influence its production [11].

Presence of classes of flavonoids, alkaloids, tannins and saponins indicates that the analyzed extract may have antibacterial action.

In this study, the diameter of the inhibition halo at C1 concentration (100 mg/ml) was different, comparing with the study carried out by Rasyid SA, et al., using a methanolic extract from the *Lantana camara* leaves, and inhibition halo is equal to 0.6 cm at a concentration of 100%, against *S. aureus* [14]. Due to the amount of secondary metabolite extracted, as many studies have shown methanol as a potent extracting solvent [15]. Also, it may be, because of the resistance of the microorganism tested, because depending on the resistance of the microorganism it may or may not be susceptible to the product to which it is exposed. Therefore, the same species coming from different samples can present different diameters of inhibition halos [15].

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CONCLUSION

We concluded that the extract of the leaves of *L. camara* contains bioactive compounds, alkaloids, flavonoids, tannins and saponins, which is why this plant is used for various medicinal purposes and has antibacterial activity against *S. aureus* and not against *E. coli*. Also, it showed which there is a positive correlation and there is a statistically significant association between the increase in concentration and the increase in the diameter of the inhibition halo.

DECLARATIONS

Conflict of interest

There is no conflict of interest.

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Availability of data and materials

The data sets used and/or analysed during the current study are available from the corresponding author, upon reasonable request.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competitive interests

The authors declare that they have no conflicting interests

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