American Journal of Essential Oils and Natural Products
Available online at www.essencejournal.com
AJEONP
American Journal of Essential Oils and Natural Products
AkiNik

ISSN: 2321-9114
AJEONP 2021; 8(3): 43-47

Received: 24-11-2020
Accepted: 26-12-2020

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Antimicrobial activity of the fixed oil extracted from *Ficus gomelleira* (Moraceae) seeds on *Escherichia coli* and *Staphylococcus aureus*

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Abstract
Bacterial resistance has gradually intensified and become a problem with the indiscriminate use of antimicrobials, reducing the potential for positive results in the control of infections through commercial drugs. Some of the most commonly-identified bacteria in cases of urinary tract and intestinal infections are *Escherichia coli* and *Staphylococcus aureus*. As such, this study aimed to evaluate the antimicrobial activity of the fixed oil extracted from *Ficus gomelleira* Kunth & C. D. Bouché (Moraceae) seeds on *E. coli* and *S. aureus*. The extraction of the fixed oil was performed using the Soxhlet extractor system with the solvents hexane and ethanol. The disc-diffusion technique was applied using white discs, 6.0 mm in diameter, soaked with the fixed oil extracted from the seeds, in Petri dishes containing Müeller-Hinton agar. This study showed the successful inhibition of both bacteria at concentrations of 100% and 50% using the solvent hexane. There was no effectiveness of the *Gameleira* extract as an antimicrobial with the ethanol solvent.

Keywords: Antimicrobial susceptibility, gameleira, essential oil

1. Introduction
Man has always used plants to fight pathologies. Throughout history, plants have been of fundamental medicinal importance, making them an increasing focus of research in studies that have proven essential to prevent, mitigate or cure diseases. Due to the difficulty to access the hospital environment and receive adequate treatment for diseases, medicinal plants have become an important and experimental resource in the treatment of pathogens due to the compounds found in vegetation [1].

The medicinal properties of some plants were found through the observation of the behavior of animals when feeding on these plants. Empirically, this information about these plants was passed along the population, and later these plants started to be used as medicinal resources and the results were transformed into popular knowledge [2].

In addition, the side effects of allopathic drugs and the increase in the number of resistant strains have led to the search for new treatments through ethnobotany, which is no longer only considered a primary source of health care for vulnerable people, but a criterion for selecting plants for pharmacological studies aimed at the discovery of antimicrobial active ingredients [3].

The *Gameleira* is a tree in the Moraceae family. Its scientific name is *Ficus gomelleira* (Kunth & C. D. Bouché). The tree is popularly known as fig tree, *gameleira-branca* and *apuí-preto* [4]. It is native to Brazil, although not exclusive to it. Its geographical distribution covers the biomes of the Amazon, Caatinga, Cerrado and Atlantic Forest [5] and it can be found in the North (Amapá, Pará, Amazonas, Acre, Rondônia), Northeast (Maranhão, Piauí, Bahia), Midwest (Mato Grosso, Goiás, Mato Grosso do Sul), Southeast (Minas Gerais, Espírito Santo, São Paulo, Rio de Janeiro) and South (Paraná, Santa Catarina, Rio Grande do Sul) of Brazil. Few studies regarding its properties have been carried out [6].

It is a semideciduous tree with a large, wide and dense crown; it can grow from eight to 18 meters in height and it is very important for riparian forests, because it holds the banks, soils and rocks, avoiding erosion. The name *gameleira* comes from *gamela*, which means nothing more than a vessel, bowl or dish made of light wood [7].

It is found near waterways and pastures [4] and it needs much sun exposure. If there is little sun,

it will always seek it out. It is adapted to soils with plenty of moisture. Its fruit ripens from February to March and it flowers from November to December [8].

Around 750 species of ficus can be found in tropical and subtropical regions. In Brazil, there are approximately 78 species, of which about 23 are native or endemic [6, 9, 10].

It is a plant that has good growth and provides food to native fauna, in addition to providing good shade. A pioneer in tropical forests, this tree is also used in landscaping [7]. A characteristic of the gameleira is the dense pubescence of its leaves, especially on the abaxial side (Fig. 1). It has moderate growth when young and produces small "fruits", which are actually inflorescences, called figs. This is why in Brazil it is also popularly called fig tree, but unlike the traditional fig tree, which produces edible figs, the *gameleira* figs are not for human consumption, and are only important for feeding the wild fauna. Their flowers are tiny and are enclosed inside the syconiums [11, 12].

Fig 1: (A) *Ficus gomelleira* exsiccate deposited in the herbarium of the Arboretum Program. (B) *Ficus gomelleira* seeds.

Standing out among some of the medicinal properties of the *Ficus gomelleira* is the latex obtained from the tree, which has a vermifuge characteristic and is widely used in folk medicine for intestinal diseases [13].

Its seed is best sown as soon as it is ripe in a partially shaded position in a seed nursery. The seed (Figure 1) is very small, and an easy way to get it from the tree is to collect the fruit, put it in a plastic bag and leave it until the fruit is partially decomposed. Subsequently, a suspension of the seeds and pulp should be made, using water as solvent (this suspension can be sown directly on the seedbed). A low germination rate should be expected, with the seed germinating within 20 to 30 days.

The indiscriminate use of antimicrobials has caused a significant increase in bacterial resistance, decreasing the chances of effective results in the control of infections by commercial drugs. Death by bacterial infection in the hospital environment has been observed all over the world; this fact is due *a priori* to the incorrect and indiscriminate use of medication. The bacteria *Escherichia coli* and *Staphylococcus aureus* are commonly found in infections in hospital environments. Much of this is due to the irregular use of medications, in addition to the easy contagion and transport of these bacteria through hospital corridors and treatment environments that are not properly sterilized. As such, they are responsible for a large part of the deaths caused by resistant bacteria [14].

The discovery of effective antimicrobials in the treatment of bacterial infections has resulted in enormous advances in medicine, considerably reducing the number of deaths caused by infectious diseases. However, the increasing use of antimicrobials has enabled the selection of bacterial strains resistant to these drugs, hindering the treatment of pathologies caused by bacterial agents and constituting a risk to the health of the affected individual and great costs to the public and private healthcare systems [15, 16, 17].

Bacterial resistance refers to the ability of bacteria to multiply in the presence of higher concentrations of antimicrobials than those found in the doses given to patients. This is a natural biological process that is driven by the use of these drugs in the treatment of infections and, due to the irrational and indiscriminate use of these in humans and other animals, it has been accelerating more and more [14, 16, 18, 19].

In this global context, the relevance of new phytotherapeutic studies increases as they can reveal and build information on antimicrobial activities, enabling the development of new substances capable of controlling and inhibiting infection and the proliferation of bacteria.

Research on the fixed oils taken from the seed of the *Ficus gomelleira* plant is of the utmost importance to develop and open new ways to develop drugs aimed at fighting pathogenic microorganisms, such as *Escherichia coli* and *Staphylococcus aureus*, and thus enable new research in the fields of pharmacology and microbiology.

The current work aims at fomenting the use of plants as a tool for the treatment of pathologies, using laboratorial research and investigations of the plant environment to collect data and information that may result in satisfactory numbers to search for an alternative to fight infections, in addition to focusing on the necessity of research geared to the fighting and control of bacteria due to the growth of cases, resistance and deaths caused by them. The use of plants to this end is important due to their low cost and easy access to less privileged social classes.

As such, this study aimed to evaluate the antimicrobial activity of the fixed oil extracted from *Ficus gomelleira* Kunth & C. D. Bouché (Moraceae) seeds on *Escherichia coli* and *Staphylococcus aureus*.

2. Materials and methods

The plant used in this study was the *gameleira*, or *Ficus gomelleira* (Moraceae). Its seeds were collected in April 2016 in the Núcleo Jequitibá by members of the Pedra Bonita settlement in the municipality of Itamaraju-BA. They were identified and cataloged in the herbarium of the Arboretum Program for Conservation and Restoration of Forest Diversity, located in the city of Teixeira de Freitas-BA.

The study was developed in the Microbiology Laboratory of Campus X of the Universidade do Estado da Bahia (UNEB), where the oil was extracted from the seeds of the *Ficus gomelleira* plant to analyze its antimicrobial activity.

The evaluation of the antimicrobial activity was performed for the bacterial microorganisms *Escherichia coli* ATCC 10536 (INCQS 00031) and *Staphylococcus aureus* ATCC 14458 (INCQS 00005), donated by the Collection of Reference Microorganisms in Health Surveillance (CRMVS) of the National Institute for Quality Control in Healthcare (INCQS) of the Oswaldo Cruz Foundation (FIOCRUZ). Once the microorganisms were received, they were reactivated and maintained in the Microbiology Laboratory of the Universidade Estadual da Bahia (UNEB), Campus X.

Petri dishes containing Müeller-Hinton agar were used in the assays seeded with *S. aureus* and *E. coli* cultures applying the disc-diffusion methodology [20, 21], using white discs, 6.0 mm in diameter, soaked with the fixed oil extracted from the seeds. The extraction of the fixed oil (FO) was performed using the Soxhlet extractor system with the solvents hexane and ethanol (absolute alcohol).

The *Ficus gomelleira* seeds were placed in plastic basins to

remove residues and dirt. An electronic scale was used to weigh 150 g of seeds, which were then taken to the drying oven with forced ventilation for 24 hours.

After drying, the seeds were ground in an industrial blender to facilitate the penetration of the solvent. Subsequently, 50 g of these seeds were weighed. After weighing, the seeds were placed in a cartridge which would later be deposited in the Soxhlet extractor. To perform the fixed oil extractions, 250 mL of each specific solvent (hexane and ethanol) were added to different extractors. The extraction process of the fixed oil occurred during three hours.

The yield was calculated from the volume of fixed oil obtained in the extraction system, divided by the dry mass of the seed sample. The FO yield obtained from each sample was then calculated through the formula: R% = (VFO / MAS) x 100. Where, R% = Fixed Oil Yield in Percent; VFO = Volume of the Fixed Oil in milliliters (mL) and MAS = Dry Mass of the Seed Sample in grams (g).

The bacteria were grown in test tubes containing 4.0 mL of tilted Nutrient Agar (NA) and incubated in an oven at 37°C for 24 h. After this period, a suspension was made from the NA tube containing the microbial inoculum, removing the bacteria colony and depositing it in 0.9% Saline Solution and comparing it to the 0.5 tube of the MacFarland scale. Using sterile swabs, the inoculum was then seeded onto the surface of sterilized Petri dishes containing Müeller Hinton Agar (MHA).

For the diffusion of the fixed oil from the gameleira seeds, white discs were deposited in microtubes (Eppendorf type) containing the fixed oil for 24 hours at room temperature.

For each bacterium (*E. coli* and *S. aureus*), six Petri dishes containing MHA were used, three dishes (triplicate) were used to evaluate the FO extracted using hexane and three dishes were used to evaluate the FO extracted using ethanol.

Each dish received the following disks: four disks soaked with the FO at concentrations of 100%, 50%, 25%, and 12.5%, respectively; a negative control disk (Tween); and a positive control disk (chloramphenicol for dishes seeded with *E. coli* and vancomycin for dishes seeded with *S. aureus*).

From the bacterial solution at 0.5 on the MacFarland scale, both *E. coli* and *S. aureus* were seeded on the surface of Petri dishes containing MHA with the help of a sterile swab.

After seeding the dishes, the white discs that were soaked in the fixed oils, extracted respectively by hexane and ethanol, were added using a sterile forceps. The positive control and negative control disks were also added. This entire procedure was performed near the flame of the Bunsen burner.

After these procedures, the dishes were incubated in a microbiological incubator at 37°C for 24 h. The antimicrobial activity was analyzed by measuring the diameter of the inhibition halos in millimeters using a halometer (millimeter ruler).

In this study, the ANOVA ("Analysis of Variance") test, also known as F-test, was performed using the *BioEstat® 5.3* software, with three different factors: type of solvent (ethanol and hexane); concentrations (1.0, 0.5, 0.25 and 0.125) and microorganisms (*E. coli* and *S. aureus*). The analysis of variance (Tukey's test) was also used to check if there was a significant difference ($p<0.001$) between the values found for the inhibition halos and the solvents [22].

3. Results & Discussion

The fixed oil (FO) yield from the seeds of the *gameleira* (*Ficus gomelleira*) using the hexane solvent was equal to 1% (from 50 g of seeds, 0.5 g of fixed oil was extracted). For the ethanol solvent, the yield was equal to 1.14% (from 50 g of seeds, 0.57 g of fixed oil was extracted). Based on the seed yield values, the 100% concentration of the extracted fixed oil was obtained, in µL/mL, as were its respective dilutions (Table 1).

Table 1: Concentrations in µL/mL of FO extracted from *gameleira* seeds according to the dilutions used in the research (100%, 50%, 25% and 12.5%) and respective solvents.

Concentration (%) of the dilutions	FO Concentration (µL/mL)	
	Hexane	Ethanol
100	10	11.4
50	5	5.7
25	2.5	2.85
12.5	1.25	1.425

Based on the analyses of the antimicrobial activities of the FO of the seeds of the *gameleira* (*Ficus gomelleira*) for the microorganisms *Staphylococcus aureus* and *Escherichia coli*, one can see that the FO extracted with hexane had antimicrobial action against the tested microorganisms, at concentrations of 100% and 50% (Table 2).

Table 2: Results of the halo diameters (mm) formed from the FO of the seeds extracted using hexane for *S. aureus* and *E. coli*.

FO Concentration (%) extracted through Hexane	*Staphylococcus aureus* Halo diameter (mm) (Triplicate)				*Escherichia coli* Halo diameter (mm) (Triplicate)			
	1	2	3	X±SD	1	2	3	X±SD
100	12	10	10	10.67 ± 1.15	28	28	30	28.67 ± 1.15
50	8	10	10	7.13 ± 1.15	10	16	16	14.0 ± 3.46
25	0	0	0	0 ± 0.00	0	0	0	0 ± 0.00
12.5	0	0	0	0 ± 0.00	0	0	0	0 ± 0.00
Control (+)	16	18	18	17.33 ± 1.15	30	30	30	30 ± 0.00
X = Mean; SD = Standard Deviation.								

The FO extracted from the seeds using the ethanol solvent did not show antimicrobial action against *Staphylococcus aureus* and *Escherichia coli*.

Using the F-test (ANOVA), a significant difference ($p<0.0001$) could be observed regarding the antimicrobial activity of the fixed oil for the tested microorganisms (*S. aureus* and *E. coli*) in the different concentrations (100%, 50%) used, because the calculated F value ($F_{calc.}=59.22$) for these treatments at the significance levels 1.0% (0.01) and 5.0% (0.05) was higher than the respective tabulated F values ($F_{tab.}=7.591$ at 1% and $F_{tab.}=4.066$ at 5%).

Amaral *et al.* [13] investigated the medicinal properties of *Ficus gomelleira* leaves, isolating the constituent flavonoids of the leaf, and they also cited medicinal properties of the latex of the leaves, which are used for intestinal problems, and also as a vermifuge.

Reschke *et al.* [23] used the *Ficus benjamina* species to observe antimicrobial activities, extracting the fixed oil from its leaves

and stems, with ethanol as solvent. They observed the appearance of an inhibitory halo for strains of 22 microorganism species, including *Staphylococcus aureus* and *Escherichia coli*.

Oliveira *et al*. [24] used *Ficus gomeleira* as a source for allelopathic, antimicrobial, antifungal and modulatory research with aqueous and ethanolic leaf extracts. Dilutions were made in the following proportions: 6.25%, 12.5%, 25%, 50% e 100%. They found that the ethanolic extract promoted inhibition of the germination of seeds of *Vigna radianta* (mung bean), but they did not find antimicrobial activity with the ethanolic extract. However, a synergistic effect was observed when combined with the antimicrobials amikacin, neomycin, and gentamicin against *E. coli* and *S. aureus* cultures.

Mandal *et al*. [25] found inhibition of bacterial growth with the aqueous extract of *Ficus bengalensis* bark against *Staphylococcus epidermidis* and *Bacillus subtilis*, Gram-positive bacteria. Mousa *et al*. [26], on the other hand, tested extracts from the fruit of *Ficus benjamina*, *Ficus sycomorus*, *Ficus benghalensis* and *Ficus religiosa* and found inhibitory activity on the growth of *S. aureus* and *E. coli*.

Annan and Houghton [27] also worked with the *Ficus* genus, testing aqueous extracts of *Ficus asperifolia*, reaffirming the bacterial inhibition results against resistant strains of *S. aureus* and *E. coli*.

The research described above points to the bacterial inhibition of the extracts and/or oils taken from various plant parts of the *Ficus* genus. Only one study used the *Ficus gomelleira* species, however, and it studied the leaves, in contrast to the current study, which used the seeds. Further research using various parts of the plants of this species and other parts of the *Ficus* genus is therefore important.

4. Conclusions

Positive data were obtained regarding the antimicrobial activity of the fixed oil from the seeds of the *gameleira* (*Ficus gomelleira*), obtaining better effects against the Gram-negative bacterium *Escherichia coli* than for the Gram-positive bacterium *Staphylococcus aureus*.

Considering that there are few studies with the fixed oil extracted from the *gamleira* regarding the antimicrobial activity of the FO from its seed when compared to studies with the FO from other plants, this work made important contributions, since the tested microorganisms showed sensitivity to the FO from the seed extracted with the hexane solvent. Knowing the existence of the antimicrobial activity of the *gameleira* seeds, further research is necessary in order to discover which compounds present in the fixed oil provide this action, in addition to finding out the best form to use it for phytotherapeutic purposes.

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