Algerian Journal of Natural Products 10:2 **(2022)** 934 - 938

OPEN ACCESS

Online ISSN: 2353-0391

Algerian Journal of Natural Products
www.univ-bejaia.dz/ajnp

Type of the Paper (short Communication)

Evaluation of the Antibacterial Activity of the *Sesamum alatum* plant through Phytochemical Screening

Benedita Paula Custódio Bila[1], Valeria Dias[2], Jorge Sumbana[2] and Julião Monjane[1*]

[1]Department of Chemistry, Faculty of Sciences, Eduardo Mondlane University, PO BOX 257, Maputo - Mozambique
[2]Department of Biological Sciences, Faculty of Sciences, Eduardo Mondlane University, PO BOX 257, Maputo - Mozambique

Author to whom correspondence should be addressed: juliao.monjane@uem.mz, Tel: +258828676497

Received:18/11/2022 /Accepted:28/12/2022

Abstract: The present work aimed to evaluate the antibacterial activity of the *Sesamum alatum* plant based on the screening of secondary metabolites present on the aqueous (H) and ethanolic (A) extracts of the leaves (F) and seeds (S). The biological assay was performed on the following microorganisms: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, sensitive *Escherichia coli*, and multi-resistant *E. coli* using the disk diffusion method and the phytochemical analysis were performed based on the layer chromatography technique. From the crude extracts prepared and tested, a positive result was observed in the extracts SA and FH against *E. coli* mult and FA against *P. aeruginosa*, while the SH crude extract was inactive against all the strain tested. The active extracts were fractionated. The following fractions: FH-DCM (*P. aeruginosa* and *S. aureus*), SH-Et OAc (*E. coli* mult), and SH-DCM (*S. aureus* and *E. coli* mult) were active. Ciprofloxacin was used as a positive control. Phytochemical screening revealed the presence of flavonoids, alkaloids, glycosides, and terpenes in the plant. These results could justify the use of this plant in the traditional medicine.

Keywords: *Sesamum alatum; Antibacterial activity, Phytochemical screening*

I. Introduction

The majority of the African population depends on the use of plants for their food and for therapeutic purposes, as well as relies on the use of traditional remedies, as well as on the services provided by the respective practitioners. Plants are an alternative for the treatment of infections, currently; they are bets for obtaining new drugs. In Mozambique, medicinal plants are a valuable instrument of traditional medicine, being widely used in rural areas as the main source of medicines for primary health care, as well as in urban areas. Its sociocultural value is vast and its commercial potential is still unknown. Popular knowledge makes it clear that plants are natural remedies and do not constitute a danger to health, however, plants have the power to heal, calm down, increase the production of blood cells, reduce weight, among other uses, but have, in addition to the principle desired active, toxic, allergic action and contamination by heavy metals. These interact with other medication, causing reactions contrary to those expected, hence the need for a deep reflection on their use by communities. The action of some plants used in communities has not been scientifically proven, that is, they are used arbitrarily and inappropriately, which may even represent a risk to their health and even to public health in general [1].

Sesamum alatum belongs to the Pedaliaceae family, which this family consists in 16 genera and 85 species. The genus *Sesamum* has about 36 species; *S. indicum* is the best known and cultivated specie of this genus. Most species are wild herbs that grow preferentially in humid regions; some

species grow in arid climates and occur in tropical Africa and Asia. The leaves of *S. alatum* are used in rural communities areas for primary health care, specifically for wound treatment and hair washing. The seeds contain a considerable amount of unsaturated fatty acids such as oleic and linoleic acids, antioxidants, flavonoids and 2-episesalatine[2]. Saponins such as alatoside A-C, verbacoside and 2-Cyclohexyethanol derivatives were isolated from leaves extracts [3]. In the present study aimed to evaluate the antibacterial activity of the crude aqueous (H) and Ethanolic (A) extracts of the leaves (F) and seeds (S) of the plants using standardized isolates, as well as the phytochemical screening of active fractions

II. Experimental Section

The whole plant sample of the *S. alatum* plant (Figure 1) was collected in December 2020, in Moamba District, Maputo Province, Mozambique and then transported to the laboratory of Natural Products of the Department of Chemistry at UEM; it was identified at Biological Sciences Department herbarium of UEM, where the voucher specimen is kept. The plant material was separated into leaves and pods containing the seeds, was dried in the shade at room temperature for 30 days, and then ground into a powder. Powdered samples were stored in amber bottles and kept in a cool place until use.

Figure 1 S. alatum plant collected in Moamba district, Maputo Province, Mozambique

II.1 Preparation of crude extracts and microorganisms

II.1.1 Preparation of crude extracts

The dried and powdered leaves and seeds (80.0 g, each material) of the *S. alatum* plant were macerated, separately, with water (H) and ethanol 70% (A). The mixtures were kept under agitation for 6 hours at room temperature and left overnight for 24 hours, and then filtrated. The following extracts were obtained: SA (seeds + alcohol), FA (leaves + alcohol), SH (seeds + water) and FH (leaves + water). The extracts were concentrated using a Rotavapor and kept in a refrigerator at -5^{o}C until use. All the prepared crude extracts were evaluated for their antibacterial activity in the biological assay 1.

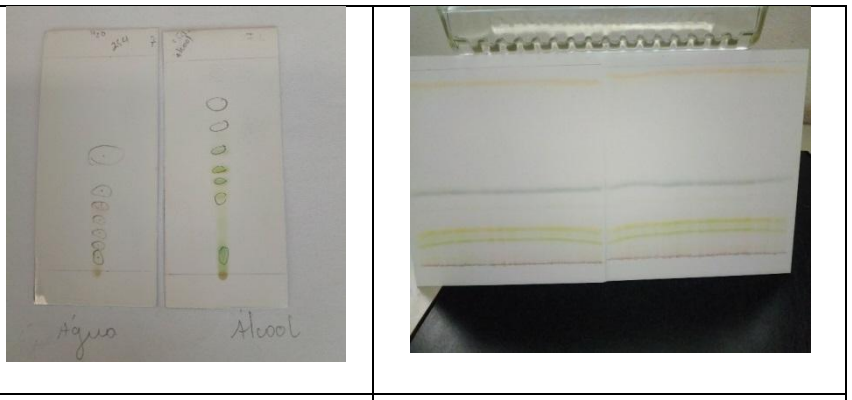
II.1.2 Preparation of microorganisms

The investigation of the antibacterial activity of *S. alatum* was carried out in four bacterial species: *S. aureus* (ATCC 25923), *P. aeruginosa*, *E. coli* (ATCC 25922) sensitive and multi-resistant that are part of the collection of the Department of Microbiology at the Faculty of Medicine at Eduardo Mondlane University. The microorganisms were reanimated and incubated at 37^{o}C for 24 hours in the respective culture medium. *S. aureus* was cultivated on blood agar and the other microorganisms on MacConkey agar. The culture media were prepared according to the manufacturer's instructions and kept in the refrigerator.

II.2 Fractionation of the crude extracts

The active crude extracts (SA, FA and FH) were subjected to liquid – liquid fractionation using solvents with increasing polarity: *n*-Hexane, DCM, and EtOAc. Twelve fractions were obtained (named: SA -Hex, SA-DCM, SA-EtOAc and SA-Aq; FA-Hex, FA-DCM, FA-EtOAc and FA-Aq; FH-Hex, FH-DCM, FH-EtOAc and FH-Aq). The obtained fractions were evaluated for their antibacterial

activity in the biological assay 2. The active fractions were subjected to preparative TLC using silica gel aluminum plates. The mobile phase was n-Hex/DCM (2.5:7.5). The plates were developed with the vanillin solution, iodine chamber and UV lamp, as shown in Figure 2.



TLC-FH-DCM	PTLC-FH-DCM

Figure 2 TLC of the DCM fraction derived from the aqueous extract of the leaves

II.3 Evaluation of the antibacterial activity

The biological assays ware carried out using the disk diffusion method. Petri dishes containing Muller Hilton agar were spread with fresh microbial culture of 0.5 CFU. To obtain 0.5UFC, a colony was identified on the plate, inoculated and calibrated. The procedure was repeated for each of the cultures. The discs, previously identified with the number of the sample to be applied, were placed in a Petri dish, sterilized and each corresponding disc was moistened with 5µL of each extract. In the central position, the disk containing the positive control of the antibiotic Ciprofloxacin was placed, which is an antibacterial used for the treatment of infections caused by microorganisms, and acts on the metabolism of bacteria, blocking the synthesis of some enzymes that are determinant for their reproduction [4]. The plates were covered and placed inside out in the bacteriological oven at 37^{o}C for 24 hours. This procedure was repeated for the other biological assays with the fractions and the chromatographic sub-fractions.

II.4 Phytochemical analysis

The compounds resulting from the chromatography were submitted to phytochemical tests. 2ml of each solution was placed in an identification tube and then the respective identification reagents of each class of substances were added, based on the standard protocols. The UV lamp was used to observe the coumarins.

III. Results and Discussion

III.1. Biological assays 1 (Crude extracts evaluation)

The crude extracts were submitted to biological assay 1 using the disk diffusion method. The results are presented in Table 1. The results showed that the crude extracts SA and FH were active against *E. coli* mult, and FA was active against *P. aeruginosa*. While the crude extract of SH was inactive in all bacterial strain tested. These differences could be attributed to the various factors which the most important in the presence of different secondary metabolites in the obtained crude extracts and the bacterial sensitivity (Gram-positive and Gram-negative). *S. aureus* is a Gram-positive bacterium, has a thick cell wall made up of peptidoglycan that confers mechanical resistance but is easily penetrated by small molecules of antibiotics when compared to *E.coli* (Gram-negative). However in recent years *S. aureus* has been developing resistance to antibiotics such as penicillin, amoxicillin, and ampicillin. Biological assays involving some of these strains with the crude extracts of *S. alatum* show negative results for *S. aureus* and *E. coli* sens [5]. The number of microorganisms resistant to antibiotics reinforces the need to seek a new line of effective treatment for infectious diseases in which plants are considered a promising source [4].

Table 1. Biological assays 1 of crude extracts of the leaves and seeds of S. alatum

	SA	FA	SH	FH	Ciprofloxacin
S. aureus	-	-	-	-	+
P. aeruginosa	-	+	-	-	+
***E. coli* sens**	-	-	-	-	-
***E. coli* mult**	+	-	-	+	-

III.2 Biological assays 2 (fractions derived from crude extracts)

In the biological assays of the fractions, the number of susceptible isolates increased when compared to the crude extract. A negative result with a crude extract does not exclude the possibility of a positive result with fractions resulting from different fractionations, since some fractions will be more enriched in active substance(s). The SH-EtOAc fraction showed inhibition against *E. coli* mult, while DCM fractions of the leaves and seed were active for 2 isolates each one. FH-DCM against *P. aeruginosa* and *S. aureus*; SH-DCM against *S. aureus* and *E. coli* sens. These results could be attributed for the possibility of the presence of one or class of compounds that could decrease the viability of the other compound(s) cannot be ruled out. As well as, the hypothesis that the compounds act synergistically within the plant, so the same test *in vivo* and *in vitro* can give different results. So, in order to reach a final result or to have the effectiveness of the tested material, the experiment must be repeated using different methods to avoid false positives.

Table 2. Biological assays of the fractions

FH					
		Fractions			
Isolates	n-Hexane	DCM	AE	Residue	Ciprofloxacin
P. aeruginosa		+	-	-	-
S. aureus		+	-	-	+
E. coli		-	-	-	+
E. coli mult		-	-	-	-

SH					
		Fractions			
Isolates	n-Hexane	DCM	AE	Residue	Ciprofloxacin
P. aeruginosa	-	-	-	-	-
S. aureus	-	-	-	-	+
E.coli	-	-	-	-	+
E. coli mult	-	-	+	-	-

FA					
		Fractions			
Isolates	n-Hexane	DCM	AE	Residue	Ciprofloxacin
P. aeruginosa	-	-	-	-	-
S. aureus	-	+	-	-	+
E. coli	-	+	-	-	+
E. coli mult	-	-	-	-	-

III.3 Biological assay 3 (Sub-fractions)

The test revealed a negative result for all compounds resulting from the chromatography. As stated by Shittu and co-workers [4], the disk and agar diffusion methods are widely used to study the antibacterial activity in plant extracts. However, for solutions with low antibacterial activity, it is essential to increase the concentration of the sample, even this procedure is limited, because depend on the amount of the sample. In addition to this limitation, there is also the difficulty of measuring the concentration of extracts of natural products and visualization, which may be due to low concentration or color identification. So it may be that the active compounds could not be visualized, so it could be possible by using higher concentrations than recommended in this study. However, other methods are needed to confirm the results.

III.4 Phytochemical analysis

The TLC revealed the presence of 7 spots, which were confirmed by the UV analysis. This analysis revealed the presence of four classes of secondary metabolites namely: flavonoids, alkaloids, glycosides, and terpenes in the leaves of *S. alatum*.

IV. Conclusions

The crude extracts of the plant have a synergy of compounds and which could act as antimicrobial agents. Qualitative analysis of the antibacterial activity of *S. alatum* was observed in the crude extracts of the leaves. The phytochemical study points to the existence of flavonoids, alkaloids, glycosides and terpenes in the DCM fraction of the aqueous extract of the leaves, responsible for the inhibition of *S. aureus* and *E. coli* sens. The study allows us to state that there is a relationship between the application of the crude extract of the leaves, by rural communities, in the treatment of wounds related to antibacterial action for sensitive and resistant species. This research experimentally proved the reason why some communities use *S. alatum* as a medicinal plant in the treatment of wounds and that it can be a line of research for the discovery of new drugs to combat multi-resistant bacteria.

V. References

[1]. Piriz M, Lima C, Jardim V, Mesquita M, Souza A, Heck R. Plantas Medicinais No Processo De Cicatrização De Feridas: Uma Revisão De Literatura. Rev Expressão Católica Saúde. 2018;3(2):64.

[2]. Sundarakumar M, Karmegam N. Antibacterial Activity of Ethanol Extracts of Sesamum alatum Thonn. Leaves. Int J Curr Res Biosci Plant Biol. 2018;5(3):38–41.

[3]. Kamal-Eldin A, Yousif G. A furforan lignan from Sesamum alatum. Phytochemistry. 1992;31(8):2911–2.

[4]. Shittu LAJ, Bankole MA, Ahmed T, Bankole MN, Shittu RK, Saalu CL, et al. Antibacterial and antifungal activities of essential oils of crude extracts of Sesame radiatum against some common pathogenic micro-organisms. Iran J Pharmacol Ther. 2007;6(2):165–70.

[5]. Atef NM, Shanab SM, Negm SI, Abbas YA. Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causing wound infection. Bull Natl Res Cent. 2019;43(1):144–56.