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ENVIRONMENTAL INTERFERENCE IN THE SYNTHESIS OF ANTIMICROBIAL SUBSTANCES FROM *Sida rhombifolia* L. (MALVACEAE)

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Abstract: The same plant species may present variation in its chemical composition depending on the stage of its development, place of collection and time of year in which it is collected. The objective of this work was to compare the antimicrobial activity of two specimens of *Sida rhombifolia,* collected at the same stage of development, in the same period of the year, in different locations in the State of Acre. Crude extracts were obtained from leaves, stem-twigs, flowers and roots by cold exhaustion with hexane, acetone and ethanol. The antimicrobial activity was evaluated against Gram-positive, Gram-negative and acid-fast bacteria, in addition to the yeast *Candida albicans.* Paper disks were impregnated with 25µL of each extract, at a concentration of 4mg/mL, and deposited on the previously inoculated medium. The experiments were incubated at 36ºC and the results evaluated after 24h of microbial growth. The extracts were also submitted to thin layer chromatography, using the solvent system TOLUOL/ETHYL ACETATE (85:15v/v), in order to verify differences in the chemical composition between the specimens. The chromatoplates were visualized under ultraviolet light and developed with resublimated iodine vapors. It was found that extracts of *Sida rhombifolia,* obtained from specimen I, showed antimicrobial activity against all microorganisms tested, while those obtained from specimen II were inactive against the same microorganisms. The leaves showed a higher concentration of active principles, which were extracted mainly by ethanol, producing inhibition halos of up to 17mm in diameter. The chromatographic tests revealed differences in the chemical composition between the two specimens, both with regard to the content of substances, which were more concentrated in specimen I, and the presence of possible antagonistic substances in specimen II. It can be concluded that the specimens of *Sida rhombifolia* showed qualitative and quantitative variations in their chemical composition and that these variations interfered in the antimicrobial potential of the species.
Keywords: *Sida rhombifolia.* Antimicrobian activity. Medicinal plants. Chemical composition.

INTRODUCTION

The species *Sida rhombifolia* L. belongs to the Malvaceae family, being popularly known as clock, field broom, broom, mallow, black mallow, guanxuma, among others, depending on the region where it is found (MING *et al.,* 1997). It is a subshrub, perennial, erect plant, 0.5 to 1.0 m high that propagates by seed. The leaves are simple, measuring 0.2 to 0.4 cm in length, and generally have stellate hairs. The flowers are perfect, with five sepals and five yellowish petals, without vinaceous spots. The fruit, in general, is dry and fragmented into mericarps. It is a species with pantropical distribution, being found throughout the Brazilian territory (BRANDÃO-NETO, 2014). Among the medicinal properties attributed to this species, one can mention its emollient, fortifying and tonic action, being used in folk medicine to combat menstrual cramps, hemorrhoids, kidney stones, cough, among other illnesses (http://www. plantamed.com. br/ESP/Sida_rhombifolia.htm).

In a study on medicinal plants in the state of Acre, Braga (2006) detected antimicrobial activity in crude extracts of *Sida rhombifolia.* Ferreira (2006) also attributed to this species the ability to inhibit bacteria with multidrug resistance to antibiotics used in medical practice. Although some studies report the medicinal properties of *Sida rhombifolia,* the majority of works in relation to this species make reference to the great problems that they cause to agriculture, being considered

as weeds. Weed species can germinate, grow, develop and reproduce in unfavorable environmental conditions, such as water stress, excessive humidity, unfavorable temperatures, unfavorable fertility, high salinity, acidity or alkalinity, constituting real pests for agriculture, as they have a high degree of adaptation in relation to the cultivated species (CONSTANTIN *et al.*, 2007; BIANCO *et al.*, 2014; KONZEN *et al.*, 2021).

The greater adaptability of a plant species in relation to others is related to factors that can change the shape and structure of the plant. Climatic and soil conditions, for example, are capable of determining or modifying plant organization. Although each living being has a pattern of development established and controlled by its genetic heritage, abiotic factors can act by modifying gene expression (SIMÕES *et al.*, 2003; MARTINS *et al.*, 2015). There are genotypes with a greater or lesser degree of phenotypic plasticity, allowing the occurrence of their representatives in the most varied environments. The greater adaptive capacity of weeds, such as *Sida rhombifolia,* for example, may be related to the ability of some organisms to change their morphology and physiology in response to changes in environmental conditions. For example, the same plant can exhibit morphologically different leaves, called sun and shade leaves, according to the degree of sun exposure to which they are subject (SIMÕES *et al.,* 2003).

Likewise, physiological changes can also occur. There are reports in the literature about the variation in the concentration of active principles in plant species, depending on the place and time in which they were collected. Globbo-Neto and Lopes (2007), studying factors that influence the content of plant metabolites, reported that they are synthesized, translocated and stored in different parts of plants, depending on the time of material collection. Plants with medicinal properties are collected by Amazonian caboclos, preferably in certain areas of a region; collection of latex or sap from medicinal trees is also carried out at certain times and times. It is believed that this is related to the variation in the concentration of bioactive compounds in these materials (DI STASI, 1996). In a phytochemical study carried out with *Sida rhombifolia,* Bortoluzzi et al. (1994) reported the presence of some constituents such as steroids, triterpenoids, alkaloids and flavanoids, but little is known about the relationship of these compounds with the medicinal properties of this species.

Taking into account the few studies on the chemical composition and biological activity of *Sida rhombifolia,* its ability to produce substances capable of inhibiting the growth of pathogenic microorganisms, including bacteria resistant to already commercialized antibiotics, and the knowledge that variations may occur in the concentration of active principles, depending on the environmental conditions to which a species is exposed, the present work aimed to compare the antimicrobial activity of *S. rhombifolia* collected in different locations in the State of Acre, verifying the occurrence of chemical variations and their influence on the inhibitory potential of the species.

MATERIAL AND METHODS

BOTANICAL MATERIAL

In this work, two specimens of *Sida rhombifolia* L. (Malvaceae) were evaluated: I – Specimen collected at the Guarany farm, located on Br-317, Rio Branco, State of Acre; II – Specimen collected in the Nova Olinda colony, located on Br-364, km 52, Espinhara branch, in the direction of Rio Branco-Sena Madureira, State of Acre. Representative samples of each specimen collected

were identified through morphological observations by Technicians in Botany from ''Universidade Federal do Acre" (UFAC) and deposited in the Herbarium of the Zoobotanic Park (HPZ) of the same institution. The remaining material was taken to the microbiology laboratory of the Food Technology Unit (UTAL) at UFAC and dried in an oven at 40ºC.

OBTAINING RAW EXTRACTS

The dry material was separated into leaves, stem-twigs, flowers and roots, which were crushed and subjected to cold exhaustion extraction, following the eluotropic series of the solvents: hexane, acetone and ethanol. The crushed material remained in infusion, at room temperature (2 ± 29 ºC), for 48 hours, in hexane. After this period, the material was filtered and the residue extracted with acetone. After 48 hours, the material was again filtered and the residue extracted with ethanol under the same conditions. The extracts obtained were evaporated in a rotary evaporator and kept in a desiccator until reaching a constant weight.

TEST MICROORGANISMS

The microorganisms used in this work were provided by the Department of Antibiotics of ''Universidade Federal de Pernambuco" (UFPEDA) and maintained at the Microbiology Laboratory of UTAL, at UFAC. Gram positive bacterial strains were used: *Staphylococcus aureus* (UFPEDA-02) and *Bacillus subtilis* (UFPEDA-16); Gram negative: *Escherichia coli* (UFPEDA-224) and *Klebsiella pneumoniae* (UFPEDA-396); and alcohol-acid-resistant bacilli (BAAR): *Mycobacterium phlei* (UFPEDA-71), cultivated in nutrient agar medium. In addition to the bacterial strains, *Candida albicans* (UFPEDA-1007) was used as a representative of yeast-like fungi, cultivated on Sabouraud agar medium.

DETECTION OF ANTIMICROBIAL ACTIVITY

To evaluate the antimicrobial activity of the extracts, the solid medium diffusion method was used, according to Bauer *et al.* (1966). Suspensions of microorganisms at 10^7 CFU/mL were inoculated in Petri dishes containing Mueller-Hinton agar medium for bacteria and Sabouraud agar for fungi. Paper disks, 6 mm in diameter, were impregnated with 25 µL of each crude extract, solubilized in DMSO (Dimethylsulfoxide) at a concentration of 4mg/mL, and deposited in Petri dishes over the previously inoculated medium. The Petri dishes were incubated at 36ºC and the results evaluated after 24h of microbial growth, through the formation and size of inhibition halos around the discs. Inhibition halos ≥ 8 mm in diameter were considered active. As a positive control, the antibiotics Oxacillin (30 µg) were used for Gram positive and negative gram bacteria, Amikacin (30 µg) for BAAR, and Amphotericin B for *Candida*; as a negative control, paper disks impregnated with DMSO were used. The experiments were carried out in triplicate and the results expressed as the average of the repetitions.

THIN LAYER CHROMATOGRAPHY (CCD)

The extracts of both specimens were submitted to thin layer chromatography (TLC), according to Wagner *et al.* (1984), with adaptations. Silica gel plates were impregnated with 5 µL of each crude extract and submitted to elution using the TOLUOL/ETHYL ACETATE (85:15 v/v) solvent system. The chromatoplates were visualized under ultraviolet light (UV) and developed with resublimated iodine vapors.

RESULTS AND DISCUSSION

Antimicrobial tests showed that only specimen I of *Sida rhombifolia* showed the ability to inhibit the microorganisms tested. It can be seen that the leaves of this specimen concentrate the highest content of active principles and that ethanol is the most effective solvent in its extraction, since the ethanolic extract of the leaves inhibited all microorganisms, with inhibition halos that varied from 9 to 17 mm in diameter. Still regarding this part of the plant, it was observed that acetone also has a good extracting potential, however, the acetone extract inhibited a smaller number of microorganisms, with smaller inhibition zones. The hexane extract, on the other hand, proved to be ineffective in extracting the active principles from this part of the plant, since it was inactive against all microorganisms evaluated (Table 1).

Similar results were obtained for the twigs-stem mixture, where the ineffectiveness of the hexane extract was also observed; the acetonic and ethanolic extracts produced inhibition halos of up to 13 mm and 15 mm, respectively, demonstrating greater efficiency of ethanol in the extraction of active principles from this part of the plant, as well as from the leaves. In the case of flowers and roots, the antimicrobial potential was lower and the most effective solvent was hexane, with inhibition halos that varied from 9 to 12 mm for the hexane extract of the flowers, and 8 to 11 mm for the hexane of the roots. (Table 1). These results demonstrate that different active principles can be concentrated in different parts of a plant and that the extractive potential of a solvent varies depending on the part of the plant used.

Regarding the extracts obtained from specimen II, the results revealed total ineffectiveness in the ability to inhibit microbial growth. Although some studies show that genetic variations, within the same species, can change the content of active principles, this may have occurred due to environmental influences, since this specimen was collected in a different location and subject to different environmental conditions. Genetic and environmental interferences are often cited in the literature as responsible for changes in the levels of active principles in medicinal plants (DI STASI, 1996; SIMÕES *et al.*, 2003; GASPARIN *et al.*, 2022).

	DIAMETER OF THE INHIBITION HALOS (mm)													
	SHEETS			BRANCHES/ STEM			FLOWERS			ROOTS				
MICRO-ORGANISMS	HE	AC	ET	HE	AC	ET	HE	AC	ET	HE	AC	ET	DMSO	ATB
Staphylococcus aureus	0	15	17	0	13	15	11	9	0	11	0	0	0	20
Bacillus subtilis	0	13	16	0	10	12	12	10	0	8	0	0	0	21
Escherichia coli	0	9	10	0	0	0	9	9	0	10	0	0	0	22
Klebsiella pneumoniae	0	10	14	0	9	10	0	11	0	9	0	0	0	19
Mycobacterium phlei	0	12	16	0	10	12	0	0	0	9	0	0	0	28
Candida albicans	0	0	9	0	0	9	0	0	0	0	0	0	0	22

HE: Hexane Extract; AC: Acetone Extract; ET: Ethanol Extract; DMSO: Dimethyl Sulphoxide; ATB: Antibiotic (Oxacillin (30 µg) for Gram positive and Gram negative bacteria, Amikacin (30 µg) for BAAR, and Amphotericin B for Candida).

Table 1 – Antimicrobial activity of extracts obtained from specimen I of Sida rhombifolia collected at the Guarany farm, located on Br-317, Rio Branco, State of Acre.

The chromatographic tests revealed differences in the chemical composition between the two specimens, both in terms of concentration and the number of substances found. A lower concentration of substances was observed in the extracts of specimen II, which in itself can explain the absence of antimicrobial activity of these extracts, however, it was also observed that some extracts, such as the ethanolic extract of the leaves, presented a number of substances to be more that may be related to the absence of antimicrobial activity of these extracts, since these substances may be acting in an antagonistic way, inhibiting the action of the active principle (Figure 1).

These data confirm that chemical variations can occur between individuals of the same species and that these variations interfere in their biological potential, since specimen II presented differences in its chemical composition, in relation to specimen I, and total absence of antimicrobial activity. This could have occurred both due to the absence or lower concentration of the active principle, as well as to the presence of antagonistic substances in the extracts of specimen II.

It is important to note that chemical variations in the same species can occur both between specimens from different locations, due to environmental variations, and between specimens from the same location, due to genetic differences and different stages of plant development (SIMÕES *et al.*, 2003). Thus, to achieve effectiveness in obtaining active products, in studies on the biological potential of plant species, all aspects that may affect the synthesis and concentration of these products must be taken into account.

CONCLUSIONS

Sida rhombifolia showed variations in its chemical composition, depending on where it was collected. These variations interfered with the antimicrobial activity of the species, since specimen I showed the ability to inhibit the growth of pathogenic microorganisms, while specimen II did not show any inhibitory effect. The absence of antimicrobial activity may be related to a lower concentration of active principles or the presence of antagonistic substances, as a result of genetic or environmental factors.

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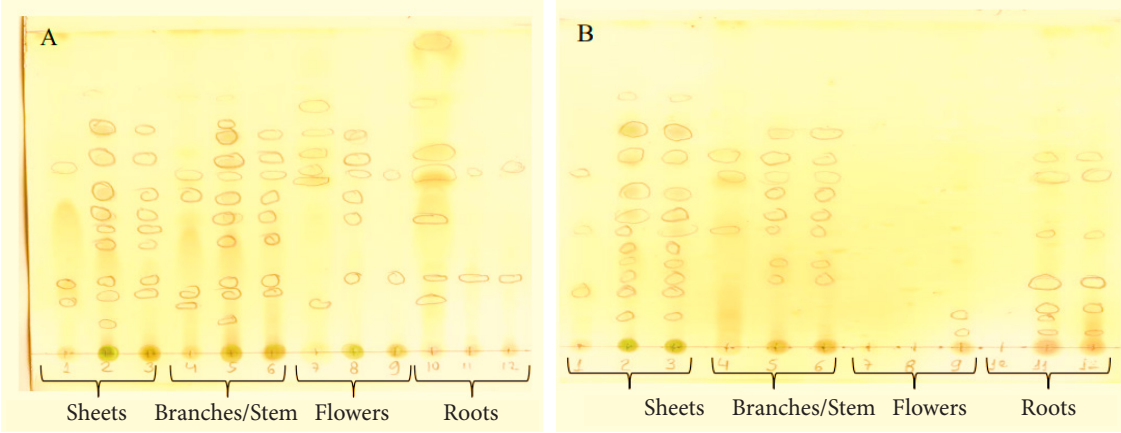

Figure 1 – Thin layer chromatography of *Sida rhombifolia extracts.* **A** - Extracts from specimen I; **B** - Extracts from specimen II; 1-4-7-10 (Hexan extracts); 2-5-8-11 (Acetonic extracts); 3-6-9-12 (Ethanolic extract).

REFERENCES

http://www.plantamed.com.br/ESP/Sida_rhombifolia.htm. Acesso em: 10 de novembro de 2022.

BAUER, A. W.; KIRBY, W.; SHERRIS, J. C.; TRUCK, M. Antibiotic susceptibility testing by a standardzed single disk method. **The American Journal of Clinical Pathology,** 45: 493-496, 1966.

BIANCO, S. *et al*. Growth and mineral nutrition of Sida rhombifolia. Planta daninha, 32 (2). 2014.

BORTOLUZZI, M.; SIQUEIRA, V. L.; LOPES, A. M.; PAIM, A. C. (1994). Estudos fitoquímicos de *Sida rhombifolia* L. (Malvaceae) e aspectos anatômicos da raiz. **Lecta,** 12(1): 81-97.

BRAGA, M. E. M. **Estudos da atividade antimicrobiana de Plantas Medicinais do Estado do Acre.** Orientador: Sheyla Mara de Almeida Ribeiro. Relatório semestral apresentado ao PIBIC/UFAC em janeiro de 2006.

BRANDÃO-NETO, J. L. S. **O gênero *Sida* L. (Malvaceae) no estado de Pernambuco, Brasil.** Orientador: Margareth Ferreira de Sales. 120f. Dissertação (Programa de Pós-graduação em Botânica), UFRPE, Recife. 2014.

CONSTANTIN, J. *et al*. Estimate of the period prior to weed interference in soybean (Glycine max), Variety Coodetec 202, through two-fold checks. **Planta daninha**, 25 (2). 2007.

DI STASI, L. C. **Plantas medicinais**: Arte e Ciência. UNESP – São Paulo, 1996.

FERREIRA, V. C. **Detecção da atividade antimicrobiana de plantas medicinais frente a amostras clínicas de bactérias multiresistentes**. Orientador: Sheyla Mara de Almeida Ribeiro. Relatório semestral apresentado ao PIBIC/UFAC, 2006.

GASPARIN, G. P. *et al*. Use of medicinal plants and their chemical and genetic diversity. **Brazilian Journal of Development**, v.8, n.4, p.27678-27690. 2022.

GLOBBO-NETO, L.; LOPES, N. P. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. **Quím. Nova**, 30 (2). 2007.

KONZEN, A. *et al*. Competitive interaction between soybean cultivars and *Sida rhombifolia*. **Revista Brasileira de Ciências Agrárias**, v. 16, n. 2. 2021.

MARTINS, A. C. F. *et al*. Capacidade adaptativa de espécies do cerrado utilizadas em áreas de recuperação ambiental. **Rev. Árvore**, 39 (3). 2015.

MING, L. C., GAUDÊNCIO, P.; SANTOS, V. P. **Plantas Medicinais – Uso Popular na Reserva Extrativista Chico Mendes/ Acre.** UNESP – São Paulo, 1997.

SIMÕES, C. M.; SCHENKEL, E. P.; GOSMANN, G.; MELLO, J. C.; MENTZ, L.; PETROVICK, P. **Farmacognosia: da planta ao medicamento.** Porto Alegre/Florianópolis: Editora da UFRGS / Editora da UFSC, 2003.

WAGNER H.M., BLADT S., ZGAINSKI E.M. (1984). **Plant drug analysis**. New York: Springer-Verlag. 320p