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Short Communication

Assessment of the Antibacterial and Antifungal Activities of Baccharis trinervis (Lam.) Pers. and Vernonia cinerea (L.) Less. (Asteraceae)

"In memorian to Lcda. Adela Estaba de Caserta"

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Abstract

Background and Aim: *Baccharis trinervis* (Lam.) Pers and *Vernonia cinerea* (L.) Less are used in traditional medicine in the treatment of several infectious diseases, including typhus, cholera, hepatic diseases, malaria, pulmonary, skin infections and conjunctivitis. The present research aimed to investigate the antimicrobial activity of extracts from *B. trinervis* and *V. Cinerea*. We compared the results obtained in this study with the folkloric medicinal use of these species.

Materials and Methods: Antibacterial and antifungal tests were performed using the disc-diffusion method. The tested extracts were obtained by partitioning, with solvents of increasing polarity of the residual aqueous layer from the methanol-aqueous extracts obtained from the powdered plant material. The use of the examined species in the popular pharmacopoeia was investigated from a wide literature review.

Results: The results of this study revealed that *V. cinerea* had the broadest antimicrobial effect. The most polar extract, obtained by partition with n-butanol, was active against the Gram + bacteria *Staphylococcus aureus* and *Bacillus cereus* and the fungal pathogens *Fusarium oxysporum* and *Aspergillus Niger*. On the other hand, the activity of *B. trinervis* was evidenced only against *B. cereus* in the extract obtained by partition with the solvent of lower polarity, CH_2Cl_2.

Conclusion: The present study indicated that *V. cinerea* and *B. trinervis* contains metabolites that exhibit varying degrees of antimicrobial activity. Moreover, our findings confirm that Vernonia cinerea is a promising source of biologically active compounds potentially useful for the treatment of infectious diseases. In fact, the use of this plant in folklore medicine has been validated.

Keywords: *Baccharis trinervis*, *Vernonia cinerea*, Antimicrobial activity, Traditional medicine

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Introduction

The folkloric knowledge about medicinal properties of plants is generally a combination of empirical observations, speculation and fantasy. *Baccharis trinervis* (Lam.) Pers. and *Vernonia cinerea* (L.) Less are two vegetable species widely distributed in tropical zones that are used in folk medicine for the treatment of many diseases. Decoction of either leaves or whole plant is the most common method for the preparation of remedies based on *B. trinervis* and *V. Cinerea* (1-12). Traditional medicine describes the use of *B. trinervis* and *V. cinerea* in a variety of ailments including, mainly infectious diseases such as typhus (1), fever (3, 7, 9), typhoid fever (4), ulcer (3, 12), wounds (6), conjunctivitis (6), malaria (8, 9), cholera (9), gonorrhea and skin infections (11). This popular knowledge has stimulated many phytochemical and pharmacological studies on *B. trinervis* and *V. Cinerea*, which have included the evaluation of the activities against fungi and bacteria.

The antibacterial activities of extracts of different polarity from *V. cinerea* were tested against *Staphylococcus aureus* using the cup-plate agar diffusion method (13). This study indicated that chloroform and alcoholic extracts from aerial parts of *V. cinerea* could have antibacterial activities against *S. aureus.* More recently, an antibacterial study conducted in 2016 on n-hexane, chloroform and ethyl acetate fractions obtained by solvent partitioning from methanol extract of *Vernonia*, revealed that the most polar ethyl acetate fraction could demonstrate the highest activity against Gram + and Gram – bacteria, and the yeast *Candida albicans* (14).

The antimicrobial activity of the *B. trinervis* species has been investigated mainly in polar alcoholic extracts and non-polar volatile essential oil. Hence, Albuquerque *et al.* reported the activity of the essential oil obtained from aerial parts of *B. trinervis* against four bacteria and one fungus (15). On the other hand, the antimicrobial capacity of *Baccharis trinervis* ethanol extract was evidenced against the bacteria *Streptococcus pneumoniae* and *Staphylococcus aureus*, and against the fungus *Candida albicans* and *Cryptococcus neoformans*. Another study conducted to evaluate the antifungal activity of the ethanol extracts of the species against *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Candida albicans* showed that dermatophytes presented sensitivity at a dose of 150 mg/ml, while *Candida albicans* was not sensitive to any of the concentrations evaluated (16). In the present study, we compared the folkloric medicinal use of *B. trinervis* and *Vernonia Cinerea* (L.) with their antimicrobial activities against bacteria and fungi.

Materials and Methods

Plant Material

The plants were collected in San Juán de Macarapana, south of Cumaná, State of Sucre, Venezuela, from February to March, 1995. The plants were identified as *Baccharis trinervis* (Lam.) Pers. (No.1442, collector J.L. Cumana) and *Vernonia cinerea* (L.) Less. (No. 1712, collector J.L. Cumana) by Professor J.L. Cumana of I.R.B.R. Herbarium at Universidad de Oriente, Cumaná, Venezuela, where voucher specimens were deposited.

The dried and milled aerial parts of *B. trinervis* and *V. cinerea* were extracted with a 3:1 MeOH-H2O mixture (1:20 g/ml). The organic solvent was removed under reduced pressure from MeOH-H2O extract, and then the remaining aqueous phase was successively extracted with CH_2CL_2, AcOEt and n-butanol. Solvents were removed under reduced pressure to give the crude extracts. The ethnobotanical data were obtained from a bibliographic review.

Antimicrobial Assays

To estimate the antibacterial activity, a group of microorganisms were used that included *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 9634), which were provided by the Microbiology Laboratory of the Department of Biology, Universidad de Oriente, Cumaná, Venezuela. The antifungal effect was evaluated on spores of the phytopathogenic fungi, *Fusarium oxysporum*, *Aspergillus niger* and *Aspergillus flavus*, which were provided by the Phytopathology Laboratory of the Department of Biology, Universidad de Oriente, Cumaná, Venezuela.

A modified version of the disc-diffusion method was

used to carry out the antimicrobial test (17). In a typical assay, 5 mm-diameter Whatman No. 3 filter paper discs were dosed with 10 µl of a solution prepared by dissolving 40 mg of the extract in 5 ml of a 3:2 H2O-DMSO mixture. Sterilized Müller Hinton agar medium was poured hot into each sterilized Petri plate. The Petri plates were left for cooling and solidification of the medium. To inoculate the petri plates with the bacterial strains, samples from fresh cultures were suspended in sterile saline solution to give turbidity equivalent to McFarland 0.5 standard. The solvent free discs impregnated with the extract solution were then applied on the Petri dishes inoculated with the different strains of microorganisms. The Petri plates were placed in an incubator at 37°C for 24 h to allow the maximum growth of the organisms.

Prior to antifungal studies, fungi were maintained in PDA agar slopes and incubated at room temperature over a seven-day period. Subsequently, the cultures were resuspended in sterile saline solution and then passed through sterile gauze. The filtrates were used to inoculate Petri plates for the antifungal test using the disc-diffusion method described above. The Petri plates were incubated at room temperature for 72 h and then observed for fungus growth and inhibition zone.

Standard discs of amikacin (30 µg/disc) for bacteria and nystatin (100 UI/disc), positive and blank discs that were impregnated with solvents followed by evaporation were utilized as the negative control. The activity was measured as the diameter of the inhibition zones.

Results and Discussion

The folkloric uses of *Baccharis Trinervis* and *Vernonia cinerea* have been indicated in Table 1. Several types of information were reported for every species. These information included scientific name, vernacular name, parts of the plant used, ways of preparing (when known), and popular medicinal use. Decoction of either leaves or the whole plant is the most common method for the preparation of remedies based on *B. trinervis* and *V. Cinerea* (1, 2, 6, 9, 8, 10). This ethnobotanical information suggests that the active compounds, against pathogen microorganisms, present in *V. cinerea* and *B. trinervis* are probably polar compounds.

Antimicrobial activity of extracts obtained from *B. trinervis* and *V. cinerea* were tested against four bacterial (two Gram + and two Gram -) and two fungal strains. The bioassay results (Table 2) showed that polar n-butanol extract from *V. cinerea* was more active than dichloromethane nom-polar extract, while the intermediate polarity extract in ethyl acetate was inactive against all microorganisms tested. Hence, the extract in n-butanol was active against the Gram + bacteria *Staphylococcus aureus*, *Bacillus cereus*, the fungus strains *Fusarium oxysporum* and *Aspergillus niger*. The dichloromethane extract was also activated, but its spectrum of action was limited only to *Bacillus cereus* bacteria.

Sonibare *et al.* obtained a fraction by partitioning a methanolic extract from *V. cinerea* using solvents with increasing polarity. They tested antimicrobial activities against human pathogenic microorganisms using the agar diffusion method (14). In the present research, the maximum antimicrobial activity was detected in the ethyl acetate fraction followed by chloroform fraction against the bacteria *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus subtilis, and Proteus vulgaris* and the yeast *Candida albicans,* while *Aspergillus flavus* was found to be resistant to all the fractions. Thus, our findings confirm that the polar and medium polar solvent fractions obtained from *Vernonia cinerea* have the highest potential as sources of antimicrobial agents. This fact is indeed in accordance with the popular use of decoction from *Vernonia cinerea* for the treatment of infectious diseases (8, 9).

However, the nom-polar dichloromethane extract from *Baccharis trinervis* was active only against one bacterial strain. This finding demonstrates that active metabolites in this species are concentrates in low-polarity extracts. Consequently, formulations as decoction should not contain active principles against bacteria and fungi. Accordingly to the biological test, it is necessary to use the whole crushed plant as cataplasm in order to use this plant against infectious diseases in traditional medicine.

A comparison of our results with that of the studies conducted by Albuquerque, *et al.* (15) and Alvares, *et al.* (16) show that there are substantial differences

Table 1: Determination of MIC of Thymus vulgaris essential oil (the diameter of each disc was 7 mm, the diameter of growth inhibition zones is reported in millimeters).

Scientific name	Vernacular name	Parts used	Preparation	Popular use
Baccharis trinervis	Chilca, Santa María	Whole plant, Stems	Decoction	Impotence (1), Typhus (1), Hepatic diseases (1, 2),
		Leaf	N/I	Fever (3, 4), Stomach ache (2-4), Ulcers (3), Inflammation (3)
		Leaf	N/I	Edema (4), Typhoid fever (4)
		Aerial parts	Ointment, Vapor, Bathing	Snake bites (5)
		Leaf, Whole plant, Flowers	Juice, Decoction, Cataplasm	Dysentery (6), Cough (6, 10), Wounds (6, 9), Conjunctivitis (6)
Vernonia cinerea	Abangak Purple fleabane Kala-jeera	Leaf	N/I	Fever (7, 9)
		Leaf	Decoction	Malaria (8, 9)
		Leaf	Decoction	Cholera (9)
		Whole plant	Decoction	Insomnia (10)
		Whole plant, Seeds	N/I	Amenorrhea, gonorrhea, female sterility, pulmonary and skin infections (11)
		N/I	N/I	Diarrhea, Ulcers (12)

N/I, Not indicated

Table 2: Antimicrobial activity of *Baccharis trinervis* (Lam.) Pers. and *Vernonia cinerea*.

Specie	Extract	Zone of inhibition (mm)						
		E.c.	*P.a.*	*S.a.*	*B.c.*	*F.o.*	*A.n.*	*A.f.*
Baccharis trinervis	CH_2Cl_2	-	-	-	14	-	-	-
	AcOEt	-	-	-	-	-	-	-
	n-butanol	-	-	-	-	-	-	-
Vernonia cinerea	CH_2Cl_2	-	-	14	13	-	-	-
	AcOEt	-	-	-	-	-	-	-
	n-butanol	-	-	14	19	20	14	-
Positive control	Amikacin	14	-	-	16	ND	ND	ND
	Nystatin	ND	ND	ND	ND	20	19	28
Negative control	Evapored solvent	-	-	-	-	-	-	-

E.c., Escherichia coli; *P.a., Pseudomonas aeruginosa*; *S.a., Staphylococcus aureus*; *B.c., Bacillus cereus*; *F.o.., Fussarium oxysporum*.; *A.n., Aspergillus niger*; *A. f., Aspergillus flavus*.; -, no inhibition zone; ND, Not determined.

between the inhibitory effects of polar extracts from *B. trinervis*. The disparity in the results might be due either to differences in the way the extracts were prepared or to the use of more concentrated solutions of the extracts in bioassays of previous studies.

Our research indicated a greater antimicrobial effect of the polar extract of *V. cinerea* compared to *B. trinervis*. The activity of the former against Bacillus cereus was even greater than that of the commercial antibiotic used as a positive control. Moreover, the antifungal effect of *V. cinerea* against *Fusarium oxysporum* and *Aspergillus niger* was comparable to that of the antifungal nystatin. The high activity of this plant species against pathogenic microorganisms demonstrates that its aerial parts have potentially useful antibacterial metabolites that require further investigations.

Conclusion

The present study revealed that *Vernonia cinerea* and *Baccharis trinervis* extracts could exhibit varying degrees of antimicrobial activity. This phenomenon indicate that some potential antibacterial and antifungal agents might be identified and isolated from these vegetal species, particularly from *V. cinerea*, thereby suggesting that the medicinal potential of this species in the treatment of infectious diseases should be explored. Furthermore, the antimicrobial screening validates the use of *V. cinerea* in folkloric medicine.

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Conflict of Interest

The authors declare that there is no conflict of interest in this study.

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