Chromatographic analysis of Vernonia polifanthes Less extract obtained by Energized Dispersive Extraction (EDGE).

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ABSTRATO

The present work proposes a new extraction procedure for Vernonia polifanthes Less using energized dispersive extraction and relative identification by gas chromatography coupled to mass spectrum (GC-MS). Lately, scientists have been interested in investigating constituents present in Vernonia polifanthes, as well as its pharmacological properties such as anti-inflammatory, immunomodulatory, antitumor, antioxidant, antibacterial, among others. In this sense, this study proposed the chromatographic analysis of the Vernonia polifanthes extract, in different polarities. According to the results presented, the vast amount of compounds identified by gas chromatography may be associated with the biological activities that the extracts of this plant present, making it necessary to apply other chromatographic techniques for a more in-depth analysis.

Keywords: *Vernonia polifanthes Less* 1. Powered dispersive extraction 2. Identification 3. Gas chromatography 4. Chemical constituents 5.

1 INTRODUCTION

Vernonia polifanthes Less (Asteraceae) is a plant popularly known as Assa-Peixe, Peixe Branco Assa. Belonging to the astareceae family, of the vernonia genus, *V. polifanthes is* a plant native to Brazil and widely distributed in the Northeast region of the country. It adapts easily to different soils and climates, for this reason it grows easily in pastures and less fertile soils, especially on roadsides, in open and well-ventilated places, and can be considered a weed in perennial crops [1]. It has a honey rich in bioactive compounds and widely produced by its species considered a supplier of good floral honey mainly due to its rich physical-chemical properties, being sought after by bees, both for nectar and pollen [10]. Of all parts of the plant, the leaves are most used in the treatment of respiratory system infections, kidney problems, injuries, bruises, diuretic among other properties [7,8,6] (Figure 1). Its phytochemical composition reports the presence of sesquiterpene lactones (piptocarfin A and glaucolide A), flavonoids and phenolic and terpenoid compounds as its main compounds. The study [4], comes from the presence of phenolic compounds, flavonoids and sesquiterpene lactones.

Figure 1. Aspects of the *Vernonia polyanthes Less* shrub.

The essential oil of *Vernonia polifanthes* has strong leishmanicidal potential [9]. we identified in their study that the oil obtained by hydrodistillation and applied by a combination of GC and GC/MS, presented more than 30 compounds identified in different classes, among these the largest classes represented with the most 20% are: monoterpenes, sesquiterpenes, oxygenated sesquiterpenes and myrcene. *V. polifanthes* oil has significant leishmanicidal activity, with IC50 values of 19.4 and 9.0 µg/ml. The extracts and essential oils of *V. polyanthes* are also trained for the treatment of infectious diseases [7]. investigating in their study the antibacterial activity of the rinse extract of the leaves of *V. polyanthes*, the identified compounds, glaucolide A, 3',4'-dimethoxyluteolin, acacetin and apigenin, innovation strong interaction in the prevention of Staphylococcus aureus, S. aureus, Escherichia coli, Salmonella Choleraesuis and Typhimurium, it can be understood that the plant presents itself as a potential source of phytochemicals with antibiotic effects. Chromatography is highlighted by [5] as an analytical method of great importance for the separation, identification and quantification of chemical compounds. Gas chromatography is consolidated as an analytical method capable of separating and identifying different species. However, its use requires understanding and adjusting several parameters to obtain accurate results. Given the benefits associated with the use of *V. polyphanthes*, the present work aims to characterize the volatile compounds found in extracts of different polarities, obtained by energized dispersive extraction, then the phenolic compounds present in these extracts will be quantified.

2 MATERIALS AND METHODS

For collecting material: The leaves of *Vernonia polifanthes* were collected on the campus of the Tiradentes University in Aracaju-Farolândia during periods of low rainfall (September and October), which allows for a greater concentration of these secondary metabolites between the months. to the laboratory, they were sanitized with a 200 mg/L sodium hypochlorite solution, dried in an oven at 40°C for a period of 72 hours and subsequently crushed in a knife mill and sieved to obtain uniform particles with a particle size of 16 mesh. The biomass thus processed was stored in a dry place and protected from light. Subsequent tests were carried out in triplicate and their results will be presented with means and standard deviations. For the extraction procedures, solvents such as water, ethyl acetate, ethanol, dichloromethane, petroleum ether and hexane were used.

Energized Dispersion Extraction (EDGE): The leaves of *Vernonia polifanthes* were extracted using the automated EDGE technique. Initially, the solvents water, ethyl acetate, ethanol, dichloromethane, petroleum ether and hexane were used, following the order of a solvent of lower polarity to higher polarity of each solvent for extraction as shown in table 1. The temperature used is the same boiling temperature of each solvent (Table 1). The ratio used for both plants was 1:10, so that it would be proportional for both. After extraction, it was separated from the biomass supernatant and this extract was stored in an amber bottle in a refrigerated environment for future analysis.

Table 1 Polarity and boiling temperature of each solvent.

Solvents	Polarity	Boiling temperature
Hexane	0.1	68.7°C
Petroleum ether	0.1	30 - 60°C
Dichloromethane	3.1	39.6°C
Ethanol	4.3	77.1°C
Ethyl acetate	4.4	78.37°C
Water	10.2	100°C

Chemical characterization by GC/MS: Chemical characterization was performed on a gas chromatograph coupled to a mass spectrometry detector with quadrupole type analyzer (GC/qMS from Shimadzu-Japan) model GCMS-QP2010-Ultra with automatic injector AOC-20i (Shimadzu, Japan). The column will be of the DB-5 type (30m x 0.25mm x 0.25µm) and helium as carrier gas with a linear velocity of 30cm.s -1. Injections will occur with 1µL of samples with a concentration of 1,000mg L -1 in dichloromethane, in split mode 1:15. The tentative identification of the compounds will compare the fragmentation profile with those present in the NIST 14 library (National Institute of Standards and Technology).

Total phenolic compounds: The quantification of phenolic compounds was carried out using the gallic acid calibration curve (Figure 6) at concentrations of 50; 100; 150; 200; 250; 300; 400; 500; 600; 700 and 800 ppm. The blank, obtained by the procedure described by replacing the sample with deionized water. The content of total phenolic compounds will be expressed in gallic acid equivalents (GAE mg/100g).

3 RESULTS AND DISCUSSION

In energized dispersion extraction processes, determining operational conditions is essential for applying the technique. Among these conditions, we can include the choice of the most appropriate solvent, which according to [2] the evaluation is also based on biocompatibility, toxicity, environmental safety and economic viability. In this sense, several experiments were carried out with different solvents, such as water, ethyl acetate, ethanol, dichloromethane, petroleum ether and hexane, following the order of lowest polarity to highest polarity of each solvent for extraction.

The qualitative and semiquantitative composition of the *Vernonia poliphanthes* extract is listed in figures 2, subdivided by compound classes. The content, expressed as a percentage, was calculated as the ratio between the peak area of the individual compound and the total area of the peaks in the GCMS chromatograms. Of the peaks detected in the chromatogram, all were identified at NIST. The predominant substance group in the extract were hydrocarbons (51.01%) and aldehydes (17.16%). In addition to these compounds, other groups of compounds contained fatty alcohol (5.3%), epoxide (7.15%), ketone (1.76%), fatty acid (2.5%). Of the compounds present in fish roast, 2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha .,9a.alpha.)]- (51.01%), was present in all extracts followed by Pentadecanal- (13.79%). In the literature, few studies address the recovery of volatile compounds in Vernonia polyphanthes extracts, most studies are related to its oil or honey. It was possible to observe that ethanol was the best solvent for extracting total phenolic compounds, according to [3] ethanol allows a good balance between the solvent and the plant cell as it presents a non-polar organic group and a radical highly polar hydroxyl. Maximum extraction was observed using ethanol (Table 2)

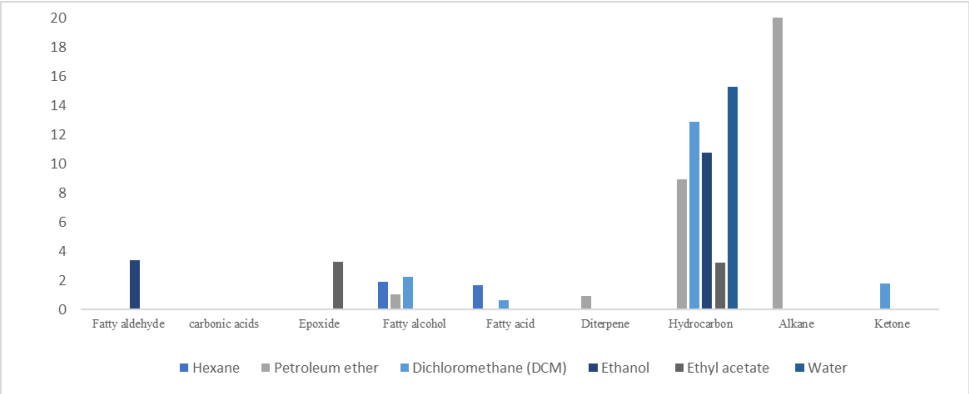

Figure 2. Main chemical classes of compounds identified from *Vernonia polyanthes Less*.

Table 2. Total phenolic concentration *Vernonia polyanthes Less*.

Vernonia polyphanthes	**Concentration mg/100g (GAE)**
Hexane	4.4± 1.0
Petroleum ether	6.8± 0.3
Dichloromethane (DCM)	11.6± 0.3
Ethanol	70.9± 0.9
Ethyl acetate	30.9± 0.9
Water	52.2± 1.2

4 CONCLUSION

It verified the presence of compounds of biotechnological interest, mainly in the pharmaceutical area with the antimicrobial and antioxidant activities present in some extracts of *Vernonia polyanthes* Less. In this way, more can be studied about these activities to identify possible antibiotic or antifungal alternatives that are of great importance for health, in addition, these extracts can be analyzed using other chromatographic techniques to identify more semi-volatile and non-volatile compounds.

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