PROSPECTION OF BEAUVERIA BASSIANA BIOMOLECULES BY ENERGIZED DISPERSIVE GUIDED EXTRACTION (EDGE)

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ABSTRACT

The demand for products of natural origin has been growing in recent years, so that investigating different matrices, as well as more effective methods for recovering biomolecules, has become increasingly important. Biomass from entomopathogenic fungi has great potential, as several bioactive compounds can be found, which have both antimicrobial action and also against various agricultural pests. Due to the diversity of chemical compounds that can be explored, extraction methods based on unconventional techniques, as well as the investigation of factors that influence the recovery of compounds, are important. Energized Dispersive Guided Extraction (EDGE) is a new, simple, fast and more efficient technique than traditional methods for obtaining different chemical classes. To this end, the objective of the work was to evaluate the effect of different solvents on the extraction of compounds from *Beauveria bassiana* biomass. Extractions carried out using different solvents and characterized by gas chromatography associated with mass spectrometry demonstrated differences in the chemical compositions of the samples.

Keywords: Biomass 1. Secondary metabolites 2. Fungi 3. EDGE 4.

1 INTRODUCTION

The search for sources of biomolecules has increased in recent years, which is due to the need to use sustainable sources for the industrial and scientific sector. The exploration of different biomasses for the recovery and identification of biomolecules that have activity of interest for human activity has become increasingly important, especially for the pharmaceutical, cosmetics, food and agricultural sectors.[1,2].

Filamentous fungi are microorganisms with high secretion of secondary metabolites, which are poorly elucidated, making them good candidates for exploring compounds for the production of products [3]. *Beauveria bassiana* is an entomopathogenic fungus characterized by being capable of infesting a wide variety of insects, around 200 species, in six orders and fifteen families, with high specificity. When multiplying, it produces a series of toxins that cause exogenous infections that have different properties[4]. Furthermore, the respective species is capable of producing a range of bioactive metabolites, such as beauvericin, beauverolides, cyclosporine A, oxalic acid, which have antibacterial, antifungal, cytotoxic and insecticidal activities, which limit the growth of pathogens and can induce systemic resistance of plants against pathogenic bacteria. Although the literature presents some compounds, there is still little information on the volatile chemical composition produced by fungi.[5].

The extraction of bioactive compounds can be carried out using different techniques, which are based on the objective of efficient recovery of biomolecules, preserving their biological and pharmaceutical properties. Extraction methodologies can be classified as conventional, cold pressing, clevenger and soxhlet, and non-conventional, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE) and supercritical fluid (SFE)[6]. Traditional, or conventional extraction methods as they are also known, have several disadvantages, one of the most reported is the degradation of biomolecules since the raw material remains in contact with the solvent, normally at high temperatures for long periods[7].

The Energized Dispersive Guided Extraction (EDGE®) system was developed to combine pressurized liquid extraction (PLE) and solid phase extraction (SPE), in a way that is considered simpler than other extraction systems, being faster than Soxhlet is more automated than QuEChERS[8]. EDGE demonstrated to be efficient for samples from natural sources, including hinokinin from dry umburana bark, obtaining excellent recovery rates of the compound [9]. Despite the lack of exploratory work on the new equipment, recent work demonstrated the efficiency of the system for extracting important compounds, such as vitamin E, germacrone and spathulenol, from *Eugenia unifloraL* (pitanga), being considered an excellent source of antioxidant compounds.[10].

The different extraction methods, solvents and extraction conditions are of great importance, as they will define the degree of selectivity for each type or class of biomolecule. Despite the expansion of studies with extractions using fungi, there is a lack of more diverse studies for the recovery of compounds produced by entomopathogenic species and their applications, making it necessary to expand research to identify bioactive compounds.

2 MATERIAL & METHODS

The entomopathogenic fungus Beauveria bassiana was obtained from the Emdagro Entomopathogenic Fungal Isolate Bank, which is located at the Biotechnological Pest Control Laboratory (LCBiotec). For cultivation, semi-solid culture medium was initially

used, prepared from 19.5g of PDA medium (dextrose agar) and 0.12 of chloramphenicol, being dissolved in distilled water, using an Erlenmeyer flask (250mL). The fungus was inoculated in a laminar flow chamber, in petri dishes (80mm x 15mm) containing PDA medium that had been previously sterilized in an autoclave (120°C, 1atm and 20min). After inoculation of the fungus, the petri dishes were kept in a BOD-type germination chamber for colony development for a period of 7 days.

To prepare the potato broth, 26g of the culture medium containing dextrose was weighed and dissolved in distilled water, using an elernmeyer flask (250mL). Then, the culture medium was sterilized in an autoclave (120°C, 1 atm and 20 min) and transferred to a laminar flow chamber. After cooling, the medium was poured into five elernmeyer flasks (250mL). With the aid of a pourer, discs were removed from the fungus colony grown in the semi-solid medium and inoculated in the liquid dextrose medium. The elernmeyer flasks containing the fungus medium were transferred to the shaker (26ºC and 150rpm) for 7 days for colony growth.

After the development of the fungus and obtaining the wet biomass, the material produced was dried in an oven at 26ºC and freeze-dried for 2 days. Then, 0.5 grams of the dry biomass was inserted into the Q-Cup, which contained the S1 Q-Disc filter series (C9 + G1 + C9), where C9 were cellulose filters (40 µm pore size) and G1 was glass fiber filter (0.3 µm pore size). Later, the Q-Cup was allocated to the EDGE equipment. A serial extraction was carried out, with increasing polarity of the solvent used. The following solvents were used in the following order: petroleum ether, dichloromethane, ethanol and ethyl acetate, where the temperature was maintained at the boiling point of each solvent, respectively (40°, 60°, 78°, 77° C), for a period of 5 minutes, using 30 ml of solvent. At the end of the process, the extracts obtained were dried at room temperature to remove the solvent. The dried extracts were weighed to calculate yield and stored in the bottles until the next step, which was carried out on the gas chromatograph associated with the mass spectrometer.

Chemical characterization was carried out on a gas chromatograph coupled to a mass spectrometry detector with a quadrupole analyzer (GC/qMS from Shimadzu-Japan) model GCMS-QP2010-Ultra with AOC-20i automatic injector (Shimadzu, Japan). The column was type DB-5 (30m x 0.25mm x 0.25µm) and helium was used as carrier gas with a linear speed of 30cm.s -1. The injections took place with 1µL of samples with a concentration of 5,000mg L -1 in dichloromethane, in splitless mode. The temperatures of the injector, interface and ion source were maintained at 280ºC. The mass fragmentation profile was obtained between 50 and 450 Daltons. The analysis was carried out in gradient mode with an initial temperature of 50ºC, with heating of 5ºC min-1 until reaching a temperature of 190ºC, then 2ºC min-1 was maintained until 225ºC, ending with an increase of 5ºC min-1 until final temperature of 300ºC, maintained for 15 min.

To analyze the data obtained, the processed samples were applied to the GCMS Solution version 4.3 software. The tentative identification of the compounds compared the fragmentation profile with those present in the NIST 14 library (National Institute of Standards and Technology) with spectral similarity ≥ 70% and through the Van Den Dool and Kratz Index with a maximum difference of 10 units for columns with similar polarities. To calculate retention indices with linear temperature programming (LPTRI), linear hydrocarbon standards (C12-C34) were used.

3 RESULTS & DISCUSSION

The extraction yields carried out on the EDGE equipment are detailed in the following table (Table 1). The highest yields were observed using petroleum ether as a solvent for extraction, followed by ethanol.

Table 2 Yields obtained from extractions using different solvents in the EDGE equipment.

Species	Yield			
	Petroleum ether	Dichloromethane	Ethanol	Ethyl Acetate
Beauveria bassiana	5,14%	0,32%	0,4%	0,26%

The characterization of the extracts resulted in different chemical profiles. A total of 16 compounds were identified for extraction with petroleum ether (78.3%), with dichloromethane 18 compounds (67.32%), extraction with ethyl acetate 16 compounds (77.91%) and with ethanol 27 compounds were identified (86.26%).

In relation to the major compounds of the four extractions carried out, the *B. bassiana* extracts presented, for petroleum ether, n-hexadecanoic acid (16.05%) known as palmitic acid, oleic acid (8.8%), acid 11 -octadecenoic (Z)- (5.53%), linoleic acid (5.41%), as compounds with the highest percentages. The extract obtained by dichloromethane presented nonanal (9.06%), palmitic acid (7.87%), hexacosane (4.73%), pentacosane <n-> (4.47%), oleic acid (4. 43%). Extraction with ethyl acetate, palmitic acid (12.47%), hexacosane (8.53%), pentacosane <n-> (7.57%), heptacosane (7.28%) and nonanal (6.79%). Finally, the use of ethanol presented the highest concentration of palmitic acids, with 26.4% and oleic acid with 26.07%, these being the majority compounds. 5 main groups can be noted (Figure 1).

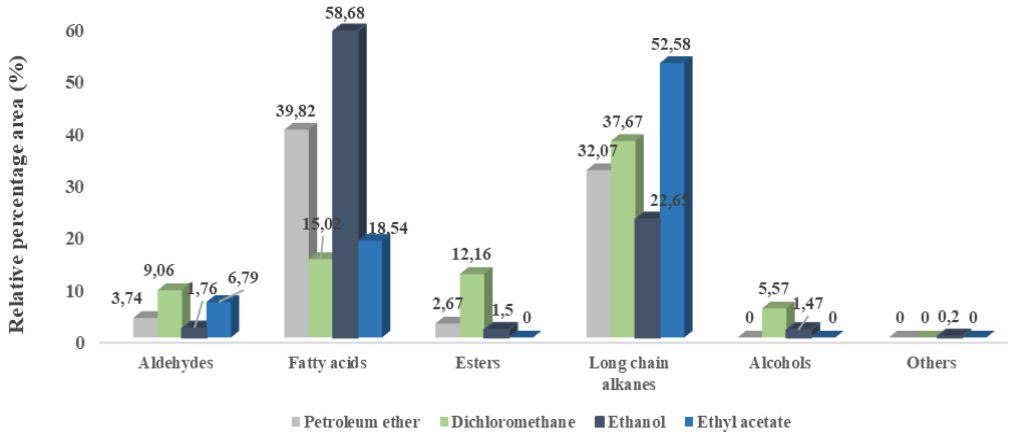

Figure 1 Main chemical classes (%) of compounds identified in the extracts, obtained from Beauveria bassiana biomass using different solvents, analyzed using GC/qMS. Solvents used: Petroleum ether, dichloromethane, ethyl acetate and ethanol.

The chemical groups of fatty acids and long-chain alkanes were the main classes of compounds found in extracts with different solvents, with aldehydes, esters and alcohols being found together. Extractions with ethanol presented the highest percentage concentration of fatty acids, with around 58.68%, with 22.65% of long-chain alkanes, being the second group with the highest percentage. Extraction with petroleum ether presented the second highest percentage of fatty acids, with 39.82%, followed by extractions with the solvents ethyl acetate (18.52%) and dichloromethane (15.02%). The chemical class of long-chain alkanes, on the other hand, presented the highest concentrations in extractions carried out with ethyl acetate solvents, with a percentage of 52.58%, followed by dichloromethane with 37.67% and petroleum ether 32.07%, with ethanol had the lowest percentage at 22.65%.

4 CONCLUSION

It is possible to conclude that, despite the changes in chemical classes, the samples obtained from the different solvents had the compound palmitic acid in common, as the majority in some of the samples, demonstrating that the different polarities influenced the obtaining of the chemical compounds. With the fungus *B. bassiana*, fatty acids and alkane hydrocarbons were the main groups obtained from its extracts.

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