GC/MS Analysis of Some Extractives from *Eichhornia crassipes*

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Eichhornia crassipes (water hyacinth) is an invasive weed that causes serious issues for rivers, lakes, and other reservoirs around the world, although it can be an excellent source for bioactive compounds such as phytosterols and some steroids found in many plants. In this study, water hyacinth samples from both Durango and Distrito Federal in Mexico were collected. Ascendant extracts (cyclohexane, hexane, acetone, and methanol) from their leaves, stems, and roots were analyzed. Using boron trifluoride (~10% [~1.3 M] in 1-butanol), all extracts were derivatized. Twenty-four derivatized samples were analyzed using a gas chromatography-mass spectrometry (GC/MS) method. Twenty carboxylic acids were found, as well as squalene, which was found in nine extract samples: four cyclohexane extracts, one hexane extract, three acetone extracts, and two methanol extracts. A compound not reported before, β-stigmasterol, was identified on three hexane extracts, an acetone extract, and a methalonic extract. Spirostane in acetone root extract and cholestane in cyclohexane stem-leaf extract were also identified.

Keywords: Extractive substances; Steroids; GC/MS; Water hyacinth

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INTRODUCTION

Water hyacinth (*Eichhornia crassipes*) is a plant from Brazil that has been spread around the world by man. It is considered an invasive weed; however, it could be an excellent source of chemical compounds because of its chemical composition, which includes chemical species that can be utilized for several applications. At present, this plant has been detected in more than 62 countries, and it is considered the most important floating weed in tropical and subtropical regions (Groote *et al.* 2003). The main issues caused by *E. crassipes* is that it blocks navigation pathways; decreases oxygen in water, causing fish species to die; and causes eutrophication in rivers and lakes (Dandelot *et al.* 2008). This aquatic plant has the highest growing rate in the world, providing 0.26 tons of dried biomass per hectare in all seasons. Because of global warming, which has caused a rise in global temperatures, this plant has spread to higher latitudes (Huber *et al.* 2006; Hellmann *et al.* 2008).

In recent years, *E. crassipes* has been studied with a lot of interest because of its effects on habitats, but to be eradicated, a big investment is required (Lata and Dubey 2010). Water hyacinth is a source of many compounds with radical-scavenging activity, such as vitamins, terpenoids, phenolic acids, lignin, stilbens, alcaloids, sterols, and other metabolites with high antioxidant activity (Jayanthi and Lalitha 2011). Plants such as water hyacinth synthesize chemical compounds, which have chemical relevance due its functional groups into primary and secondary metabolites (Jayanthi *et al*. 2011). Phytosterols are steroidal molecules that show a similar structure to cholesterol found in many vegetables such as water hyacinth. The most common phytosterol compounds are β-sitosterol, campesterol, and β-stigmasterol. Those compounds comprise 98% of all the vegetable sterols identified in plants (Nair *et al.* 2006).

Both aqueous and organic extracts from several plants have been used as medicines because of their beneficial properties against several diseases. Compounds in plant extracts have shown the ability to reduce plasmatic cholesterol levels by inhibiting enzymatically cholesterol esterification and avoiding linking to chylomicrons (Nair *et al.* 2006).

The aim of this study was to analyze organic water hyacinth extracts through gas chromatography-mass spectrometry (GC-MS) to elucidate their chemical composition and determine their potential applications.

EXPERIMENTAL

Materials

Water hyacinth was collected from Chapultepec Lake located in Mexico City, Mexico (latitude 19º25'22'' longitude 99º11'18'') and from El Tunal River, located in Durango, Mexico (latitude 26º48' longitude 102º28'). The harvest was done in 2010 between September and November. Samples from Chapultepec Lake were donated by Universidad Metropolitana de México, Campus Iztapalapa. Samples collected from El Tunal River were separated into three groups: leaves, stems, and roots. Each respective sample group was washed to remove dust and then cut into small pieces, dried, milled, and finally sieved with a number 40 mesh to obtain a homogenous particle size. Samples were stored in plastic bags and sealed at room temperature.

Extractions

Successive extracts from the sample groups were obtained using a Soxhlet extractor. The solvents used in the extractions were cyclohexane, hexane, acetone, and methanol. Each sample group was extracted for 6 h using each of the above solvents. After that, the solvents were evaporated using a rotary evaporator at 40 ºC. In small flasks, the extracts were stored and protected from light at 7 ºC until they were derivatized.

GC/MS Analysis

Derivatization

BF3-butanol (10% boron trifluoride in butanol) was used to derivatize the extracts of *E. crassipes*. 10 mg of extract was put into a 5-mL "V" vial glass, and 0.3 mL of derivatization reagent was added with a syringe.

The vials were heated at 60 ºC on a block heater for 10 min. After that, the samples were cooled and transferred to a separatory funnel along with 3 mL of hexane. Samples were washed two times with saturated NaCl solution. Anhydrous $NaSO_4$ (sodium sulfate) was used to dry the samples.

To evaporate the solvents, a nitrogen stream was used. After this, the samples were resuspended in 1 mL of hexane in a 1.5-mL vial and injected into the GC-MS system. A blank vial was prepared using the same procedure and was injected as well.

GC/MS injection

The *E. crassipes* extracts were analyzed using an Agilent 5975 MSD (USA) with a 7890 GC. The GC column was 30 m long with 0.25 mm internal diameter and 0.25 mm film thickness DB-5 (Agilent, USA). The GC injector temperature was 220 °C. The oven temperature was held at 40 °C for 3 min, then programmed from 40 °C to 250 °C at 5 °C/min, and held at 250 °C for 2 min. The transfer line temperature was 250 °C. Helium was the carrier gas.

The carrier gas flow rate was 1 mL/min. The MS source was operated in electron impact (EI) mode at 70 eV. The MS was scanned from 40 to 500 m/z.

RESULTS AND DISCUSSION

Twenty organic acids were identified from the samples, as well as three steroids: spirostane, cholestane, and β-stigmasterol; and one terpenoid: squalene. Table 1 shows all the compounds found in water hyacinth extracts *via* GC/MS.

Carboxylic acids were identified in the leaf and stem extracts (Table 1). Carboxylic acids, such as malonic acid, which was identified in the methanol extracts from the leaves, stems, and roots (Table 1), are very important in the biochemistry and physiology of plants because of their role in the Krebs cycle. These substances can function as carbon dioxide (CO_2) storage. Other functions of carboxylic acids inside plants include acting like reductors transporters, buffering pH inside cells, and controlling citosol osmolarity, as well as chelation of toxic cations to solubilize phosphate groups (Camacho and Calderón 2005).

The Krebs cycle is more important in the leaves and stems than in the roots, thus explaining the higher concentration of carboxylic acids in the samples analyzed in this work. Extracts using cyclohexane as solvent showed higher carboxylic acids concentrations, followed by those using acetone as solvent.

Squalene was identified in nine samples (Table 2). This compound is a hypocholesterolemic terpenoid found in insaponificable fractions of oleaginous seeds and animal fat. This compound acts like a cholesterol precursor, similar to other sterols. Squalene in plants such as water hyacinth is one of the primary steroid precursors. It has several antioxidant properties and carries out cardio-protective activity, reducing esterified cholesterol by oxidizing low-density lipoproteins in blood (Sabeena *et al.* 2004). Kandukuri *et al*. (2009) reported alkaloids, phenols, and steroids in methanol extracts from water hyacinth.

Table 1. Compounds Identified in Water Hyacinth Extracts by GC/MS

Compound	Extraction solvent	Part of plant	Retention time (min)
Levulinic acid	Methanol	Root	12.96
Oxalic acid	Hexane, methanol	Stem	14.44
Caprilic acid	Methanol	Laef, root	14.83
Malonic acid	Methanol	Leaf, stem, root	15.46
Nonanoic acid	Methanol	Leaf, root	16.12
Succinic acid	Methanol	Stem, root	17.01
Miristic acid	Cyclohexane, hexane, methanol	Leaf, stem, root	18.92
Lauric acid	Methanol	Stem, root	19.57
Linolenic acid	Hexane, methanol	Leaf, stem, root	20.83
Palmitic acid	methanol	Stem, root	21.04
Tetradecanoic acid	Acetone	Leaf, stem	21.62
Oleic acid	Hexane, acetone	Leaf, stem	22.08
Pentadecanoic acid	Methanol	Leaf, stem, root	22.24
Vaccenic acid	Hexane, methanol	Leaf, stem	23.4
Linoleic acid	Hexane, Methanol	Leaf, stem	24.96
10,12-octadecadienic acid	Hexane	Leaf, stem, root	24.98
Octadecanoic acid	Methanol, hexane	Leaf, stem, root	25.21
Arachidonic acid	Cyclohexane	Leaf, stem, root	26.24
Phtalic acid	Methanol	Stem, root	26.41
Tetracosanoic acid	Acetone	Leaf, stem	27.88
Squalene	Acetone, methanol, cyclohexane, hexane	Leaf, stem	28.5
Cholestane	Cyclohexane	Leaf	31.47
β-stigmasterol	Hexane, acetone, methanol	Leaf, stem	31.51
Spirostane	Acetone	Root	32.22

Table 2. Organic Extracts where Squalene was Identified

Part of the plant	Extraction solvent
Leaf	Cyclohexane
Stem	Cyclohexane
Leaf/stem	Cyclohexane
Root	Hexane
Leaf	Acetone
Stem	Acetone
Leaf/stem	Acetone
Leaf	Methanol
Root	Methanol

Squalene has been reported in other aquatic plants, such as duckweed (Duan *et al.* 2012), although no references were found regarding the presence of this terpenoid in water hyacinth.

β-stigmasterol was identified in five samples (Table 3), and three of them were from extracts that used the hexane solvent. β-stigmasterol has not been reported in water hyacinth before. This compound has a regulatory function inside plants and has a role as an anti-inflammatory molecule over those compounds involved in such processes (Pérez *et al.* 2008).

Table 3. Organic Extracts where β-stigmasterol was Identified

Part of the plant	Extraction solvent
Root	Hexane
Stem	Hexane
Leaf/stem	Hexane
Leaf/stem	Acetone
Leaf	Methanol

All samples where β-stigmasterol was identified were collected at El Tunal River. This sterol could be used as a precursor to produce several compounds for medical purposes. In other studies, β-stigmasterol inhibited carcinogenic tumors on laboratory rats, and exhibited anti-inflammatory activity when used in topical applications (Gómez *et al.* 1999).

On the other hand, spirostane and squalene were found in all samples collected at Chapultepec Lake. Plant sterols like those act as protective agents against insect and phytopathogens by forming part of the cell membrane, thus providing viscosity and elasticity. Spirostane-type sterols from some plant methanolic extracts have been shown to have anti-diabetogenic activity (Yoshikawa *et al.* 2007).

Despite *E. crassipes* being considered an invasive weed, it can potentially be an excellent raw material source that can provide high-value added compounds for the food, energy, and pharmaceutical industries, as well as other industries. The secondary metabolites in water hyacinth, phytosterols and terpenoids, could be used to give value to this plant, which is currently considered a plague.

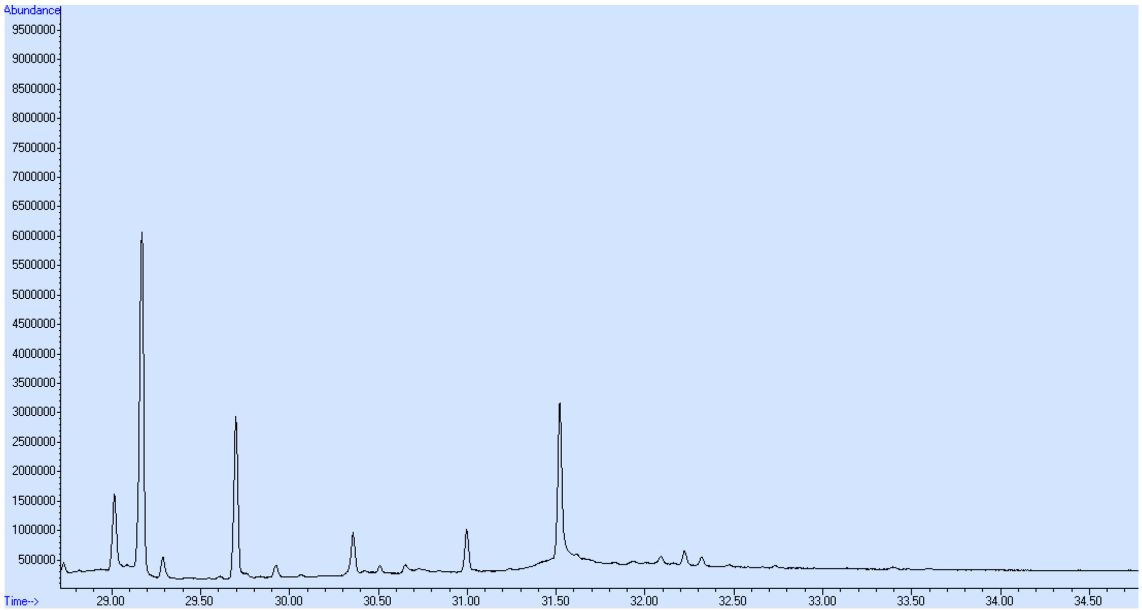

Fig. 1. Chromatogram for leaf hexane extract from Durango. β-stigmasterol (retention time: 31.51 min)

Figure 1 shows the chromatogram for leaf hexane extract from the Durango sample. Most compounds on the chromatogram are carboxylic acids that have been reported before (Camacho and Calderón 2005). β-stigmasterol (retention time: 31.51 min) has not been reported for water hyacinth. Although water hyacinth β-stigmasterol is in low concentration (0.07%), it has a high relevance because it can be used as steroids precursor and has showed anti-inflammatory activity on higher organisms (Jing 2013). Squalene (retention time: 28.5) is an important steroids precursor (Sabeena *et al*. 2004) that could be potentially used to manufacture several steroids for medical purposes.

CONCLUSIONS

1. Organic extracts obtained with the hexane solvent showed a high quantity of phytosterols such as β-stigmasterol, cholestane, and spirostane. Those compounds could be used to increase interest in metabolite precursors in the biotechnological industry. These compounds also have potential in other industries, such as the pharmaceutical industry.
2. Extracts obtained by use of the methanol solvent showed higher carboxylic acids concentrations, which was consistent with the solvent's polarity.
3. Carboxylic acids identified from water hyacinth could be potentially used as precursors to manufacture some interesting compounds for multiple industries.

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