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GC-MS Characterization of Ethanolic Extract from *Croton wagneri* Müll. Arg.

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Abstract

An ethanolic extract at 50% obtained by maceration process was characterized by GC-MS using a Shimadzu QP2010 spectrometer and the Silylation agent was *N*,*O*-bis (trimethylsilyl) trifluoroacetamide (**BSTFA**). The compounds were analyzed using GC/MS NIST21 and NIST107 libraries and having into account the results obtained after phytochemical screening and comparing the results with NISTDEMO Software. After 78 minutes of running 859 chemical components were identified from the sample, and among them, the presence of five essential oils, six triterpenes and/or steroids, an anthracene derivative, six aminoacids or their derivatives, and one flavonoid were tentatively characterized. The sample also contains a lot of amount of reductants sugars and their derivatives, hydrocarbons, and aromatic and/or aliphatic acids (unsaturated and saturated).

Subject Areas

Plant Science

Keywords

Croton wagneri, GC-MS, Ethanolic Extract, Chemical Composition, Phytochemical Screening

1. Introduction

The genus *Croton* belonging to the family Euphorbiaceae, is characterized by its species, having a lot of ethnobotanical uses. This information has been validated by the ancestral stories and bibliographic reports that have been found in the last

forty years. These species are located very close to the tropics and are distributed mainly in Central America, South America, Asia and North Africa; from there you can observe the many uses it has and which have led to further study of the genus study as discussed in [1].

Croton genus has more than 1500 spices and among them, *Croton wagneri* Müll. Arg. is considered a good source of flavonoids, terpenes, unsaturated fatty acids and alkaloids. Its main folkloric ethnomedicinal uses are cicatrizing agent, gastroprotective, antiinflammatory, antiseptic and hemostatic according to [2] [3].

The plant is a bush or a little tree very branched up to 4 m of high, spade of 5 cm cream color, greenish coffee inflorescent and green yellowish spigot (**Figure 1**) according to [4]. To the best of our knowledge, the analysis of the ethanolic extract of whole plant has not been carried out. The aim of this research was to determine qualitatively the chemical composition of an ethanolic extract at 50% of *C. wagneri* using a non-toxic, accessible, and cheap solvent for potential application in the nutraceutical, medical, and pharmaceutical industries.

2. Materials and Methods

2.1. Plant Material and Reagents

The vegetable material consisted in all plant, when they were starting flowered. The collection was between 10 and 11 AM in a sunny day. The sample collection was done manually using a machete and scissors. Plants collected were deposited in an appropriated container to avoid their damage. The dried was done at shadow at room temperature. All reagents used were of analytical grade (Merck). All solvents were degassed prior to use in an ultrasonic bath without filtration.

2.2. Extract Preparation

The extracts were prepared with the ground material (100 g) without screen extracted in a Soxhlet apparatus with 675 mL of ethanol at 50% for 20 hours. The ethanolic extracts were concentrated and evaporated under vacuum to 200 mL at

Figure 1. Leaves and flowers of *Croton wagneri* Müll. Arg.

120 rpm, a temperature of 70˚C and 500 mbar.

2.3. Phytochemical Screening

The chemical constituents were screened according to Chhabra [5] and Miranda y Cuéllar [6] to ascertain the presence of chemical components in diethyl ether, ethanol and water, respectively.

2.4. Procedures, Instrumentation and Parameters

The sample was subjected to chromatographic analysis in equipment GC/MS; brand Shimadzu QP2010, equipped with a splitter split/splitless. With a BP5 (30 m × 0.25 mm × 0.25 microns) capillary column under the following chromatographic conditions: Helium gas carrier obtained by electron impact fragments to a power of 70 eV rate of 1.2 mL/min, 1:50 split flow and the volume of injected sample of 1 µL. Programmed oven temperature: initial temperature was 70˚C with a heating ramp of 10˚C/min to 300˚C and remained stable at this temperature for 10 minutes. Subsequently the temperature was increased at a rate of 10˚C/minute to 300˚C for a total time of 78 minutes with an injector temperature 250˚C and the interface temperature 300˚C. The compounds were analyzed using GC/MS NIST21 and NIST107 library and having into account the results obtained after phytochemical screening according with González *et al*., 2019 [7]. Silylation agent was *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (**BSTFA**), CAS 25561-30-2, Lot: 0901-1 Macherey-Nagel GmbH & C. KG.

3. Results and Discussion

Table 1 summarized the preliminary phytochemical screening suggesting the presence of flavonoids, tannins, reductants sugars, fat and/or volatile oils, triterpenes

Table 1. Phytochemical screening of *C. wagneri* ethanolic extract.

Test for constituents groups	Metabolite	Results
Dragendorff	Alkaloids	+/−
$HCl_{(conc)}$/amilic alcohol	Anthocyanins	+/−
Baljet	Coumarins	+/−
Foam	Saponins	+/−
Shinoda	Flavonoids	+++
Liebermann-Burchard	Triterpenes or steroids	+++
Fehling	Reductants sugars	++
$FeCl_3$	Tannins	++
Sudan III	Fats or volatile oils	+++
Ninhydrin	Aminoacids or amines	+++
Börntrager	Quinones	−
Kedde	Cardiotonic glycosides	−
Resin test	Resins	−

and/or steroids, and aminoacids or amines. Alkaloids, anthocyanins, coumarins and saponins test were found doubt and the absence of resins, cardiotonic glycosides and quinones, may be attributed to the false positive/negative results usually observed with preliminary photoscreening of plants.

Figure 2 shows the TIC chromatogram with the retention times of different kind of chemical components present in the sample, indicating that the main amount of chemical compounds in the extract are between 12.5 and 44 minutes of retention time. The most intense peaks are between 28 and 32 minutes of retention time.

After 78 minutes of running, 859 chemical components were automatically identified from the sample, and among them, the presence of five essential oils, six triterpenes and/or steroids, an anthracene derivative, six aminoacids or their derivatives, and one flavonoid were tentatively characterized.

At 3.265 and 3.290 min of retention time two derivate from exo-Norbornanol were tentatively identified by their corresponding molecular masses, exo-Nor-bornanol, dimethyl (trimethylsilylmethyl) silyl ether (256 u) and exo-Norbornanol, pentamethyldisilyl ether (242 u), respectively, suggesting the presence of exo-Norbonanyl alcohol in the sample as is shown in Figure 3.

Borneol (8.570 min) with a molecular mass of 154 u and a glycosylated Thymol derivative at (37.615 min) with MW of 600 were also identified in the extract, confirming the results of phytochemical screening, suggesting the presence of volatile oils in the sample.

L-Alanine (7.760 min), Valine (8.065 min), an L-Proline derivative (16.510 min), an L-Lysine derivative (19.485 min) and D-L-Alanine derivative at 20.705 min of retention time were also tentatively identified. L-Glutamic acid was detected at 23.050 min suggesting the presence of several aminoacids in the sample.

Several steroidal derivatives were automatically identified starting at 43.515 min of retention time. The first one was 5. beta.-Pregnan-20-one, 17-hydroxy-3. alpha., 11. beta., 21-tris(trimethylsiloxy)-(*m/z* 582). The second one was Cholest-8(14)-en-3-one, 7,15-bis(acetyloxy)-, (5. alpha., 7. alpha., 15. alpha (44.335

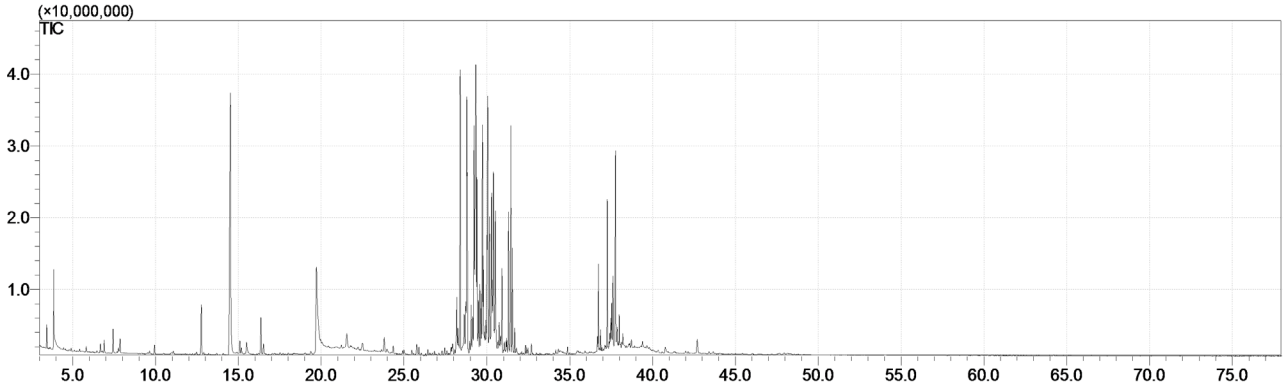

Figure 2. Current chromatogram of ethanolic extract at 50% from *C. wagneri.*

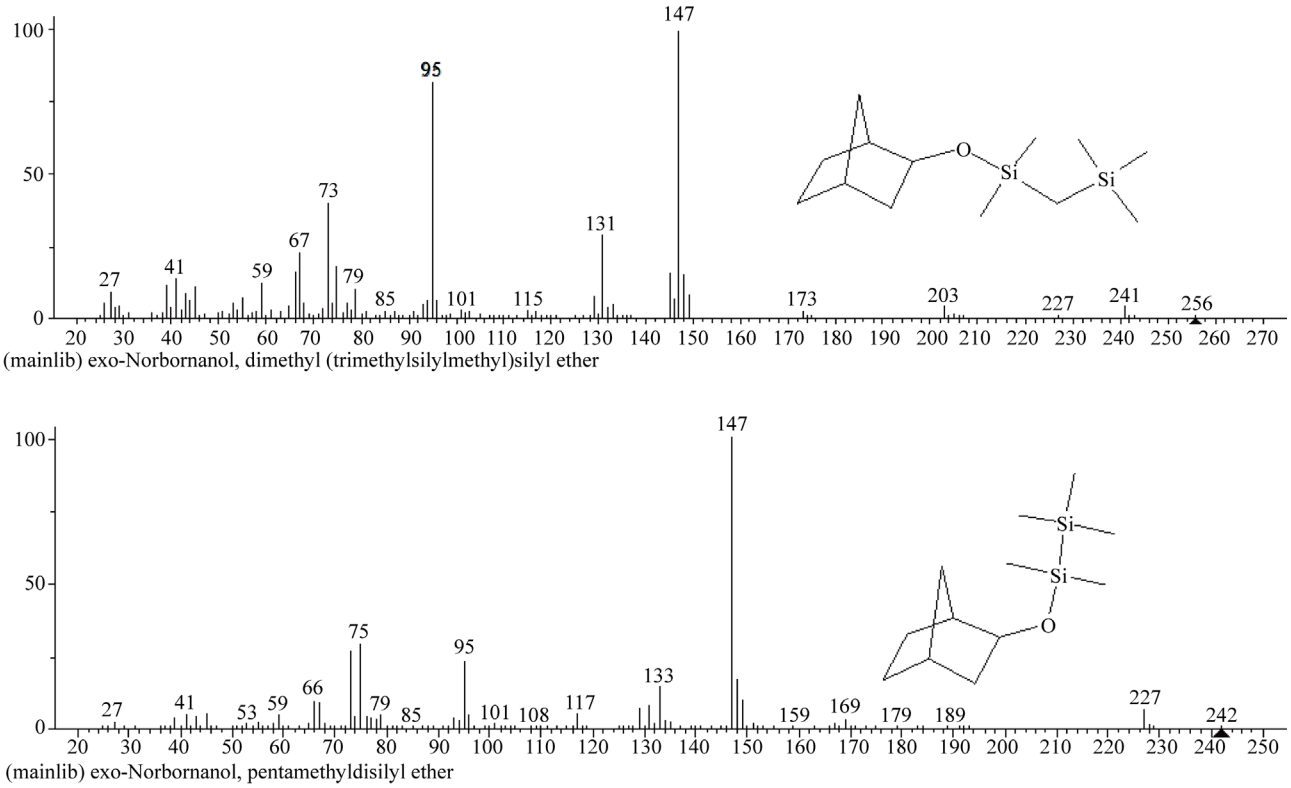

Figure 3. Mass spectrums of both exo-Norbornanol derivatives from *C. wagneri.*

min and *m/z* 500), the third one was Stigmastane, 23,24-epoxy-, (5.alpha.) at 49.950 min and *m/z* 414, respectively.

Another three derivatives related with steroidal compounds were tentatively identified in the extract. At 50.150 min of retention time and *m/z* 671 was detected Pregnan-20-one, 3,11,21-tris[(trimethylsilyl)oxy]-, O-(phenylmethyl) oxime, (3. alpha., 5. beta., 11. beta.); at 51.190 min 5.beta.-Pregnane-17,20. alpha. -diol, 3. alpha.-(trimethylsiloxy)-, cyclic methaneboronate (*m/z* 432) and finally, at 52.700 min and with a *m/z* of 724 Ergostane-5,25-diol, 3,6,12-tris[(trimethylsilyl)oxy]-, 25-acetate, (3. beta., 5. alpha., 6. beta., 12. beta.).

An anthracene derivative was identified at 44.390 min of retention time (anthracene, 9-ethyl-9,10-dihydro-9,10-dimethyl) and a MW of 236. At last, a flavonoid belonging to rotenoid group was identified. Rotenone was the unique flavonoid component detected at 57.800 min of retention time and a MW of 394 u as is shown in **Figure 4**.

Our results are in general agreed with the results of Altamirano Pérez, 2015 [2] when she presented her results of phytochemical screening in *C. wagneri.* Our research group found out the presence of at least one example of chemical compound that belongs to anthraquinones.

Pino *et al*., 2018 [8], evaluating the chemical composition of the essential oil from leaves of *Croton wagneri* Müll. Arg. grown in Ecuador reported that a total of 135 volatile compounds were identified in the essential oil, of which the most prominent were cis-chrysanthenol (27.5%) and myrcene (19.2%). Our results are

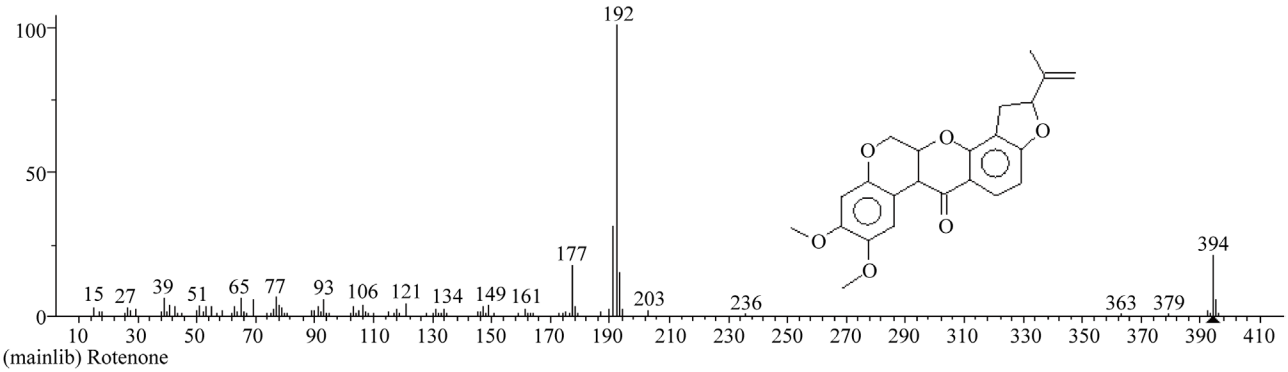

Figure 4. Mass spectrum of Rotenone.

agreeing with their research only with the presence of Borneol and Thymol, but differ with them by the presence of exo-Norbornanol in the extract.

4. Conclusion

GC-MS is frequently applied to characterize the chemical complexity of analytical samples based on its separation and identification capacity. GC-MS-based metabolite profiling of TMS derivatives does not only generate vast chemical information about primary metabolism, but includes also extensive MS data about secondary metabolites and "unknowns". The phytochemical investigation of ethanolic extracts at 50% of *C. wagneri* revealed the presence of several chemical components related with some steroids, anthraquinones, volatile oils, aminoacids and flavonoids that possess antioxidant, antiinflammatory, anticarcinogenic, antibacterial, antiviral and neuroprotective activities. These properties could be closely related to all kinds of chemical compounds contained in its chemical structures, a variable number of hydroxy groups that react with free radicals. Although some of them are still unknown, this study could be used as a diagnostic tool for the standardization of this medicinal plant and will be helpful in characterization of the crude drug.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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