Phytochemical Analysis of Bioactive Extracts and Seed Oil of Three Euphorbia Species from Algerian Flora by LC-MS and GC-MS

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Abstract: Euphorbia species possess pharmacological properties that have been widely used for medical purposes worldwide. In this paper, three plants belonging to the Euphorbia genus growing in North-East of Algeria were studied. The phenolic contents were identified using LC-MS, while the fatty acid composition of their fixed oils was determined with GC-MS. The quantification of the total condensed tannins and the leaves’ entire anthocyanin content were performed using photometric methods. The main constituents of the polyphenolic compounds identified by LC-MS were ascorbic, chlorogenic, and ellagic acids. The oil yield of the seeds of E. terracina, E. biumbellata, and E. dendroides was 17.48%, 18.5%, and 20.05%, respectively. Quantitative analyses of these oils using GC-MS showed variations in the species’ fatty acid constituents’ concentrations and compositions. Besides, the phytochemical screening results showed that E. terracina possessed a high amount of tannin and anthocyanin content compared with other studied plants.

Keywords: Euphorbia; polyphenols; oil; LC-MS; CG-MS

INTRODUCTION

More attention has been given to plants as a human medicaments source, especially in some developing countries, as plant-derived medicines have made significant contributions to human health and wellbeing [1-3]. Among the natural products found in plants, polyphenols constitute one of the largest classes of the plant’s secondary metabolite, which has an important role in preventing and restricting free radicals.

The plant Euphorbia belongs to the family Euphorbiaceae that is widespread worldwide and widely present in the tropical region. It is a large family of flowering plants, including 300 genera and over 5000 species [4-5].

The Euphorbia species are considered critical medicinal plants used mainly in folk medicine all over the world. In Africa, Euphorbia species were used to treat various diseases such as skin disease, migraines, gonorrhea, fungal and inflammatory disorders, and sexual transmission [6-7]. In Nepal, the latex of E. milii is used to cure sprains [8], while in China, it is used to cure hepatitis [9]. In Pakistan E. hellioscopia leaves are used to relieve constipation [10]. In India, E. neriifolia is known for its medicinal value, such as antibacterial, antifungal, antiviral, and antioxidant [11]. In Nigeria, essential oils from the leaves of E. milii are used for insecticidal action [12].

Few previous works are reported on the use of E. biumbellata, E. terracina, and E. dendroides in folk medicine. According to ethnobotanical investigations, it was observed that E. terracina leaves have been used as a remedy for paralysis and fever [13]. And E. dendroides was used as a fish poison and as a cathartic, while E. biumbellata latex has been used to cure warts [4].

Phytochemical studies of Euphorbia dendroides exhibited the presence of polyphenols, flavonoids, and
jatropane esters [4], while in previous research studies, *Euphorbia terracina* was found to contain a variety of bioactive chemical compounds such as saponins, tannins [13], triterpenes, flavonoids and coumarins [14].

This research aims to identify the chemical constituents contained in the seeds and leaves of the three Algerian plants: *E. biumbellata*, *E. dendroides*, and *E. terracina*, with LC-MS. An additional goal is to determine the fatty acid composition of oil obtained from their seeds using GC-MS analysis.

**EXPERIMENTAL SECTION**

**Materials**

*E. biumbellata*, *E. dendroides*, and *E. terracina* were collected from El Kala National Park in the northeast of Algeria in March 2019. The identification was carried out by the botanists of the Biology Department, Badji Mokhtar University. *ph005_48, ph005_49, ph006_04*, voucher specimens of *E. biumbellata, E. dendroides*, and *E. terracina*, respectively, were deposited in the Herbarium of Gérard De Belair.

The plants were cleaned and air-dried in the shade at room temperature for three weeks. The dry seeds and sheets were ground at the mill until they obtain fine homogeneous powders and stored in dark glass flasks to protect them from humidity and light till theirs use in further analysis.

**Instrumentation**

The main apparatus used in the research were rotary evaporator (Buchi R-124), analytical balance, Shimadzu Spectrophotometer UV-1800, gas chromatography-mass spectrometer (GC-MS) (Shimadzu QP-2010S), liquid chromatography-mass spectrometry (Shimadzu LCMS-2020S).

**Procedure**

**Extract preparation**

The weighed amount of each part (sheets and seeds) of the samples was extracted in a known volume of the solvent MeOH-H₂O (70/30: v/v) for 24 h with intermittent shaking. Each extracted material is filtered through Whatman filter paper No.1 and centrifuged at 4000 rpm for 15 min. The supernatant is then stored at -4 °C for further use in the phytochemical analysis, total anthocyanin contents, determination of antioxidant activity, and LC-MS analysis.

**Analysis of chemical composition**

**Total condensed tannin contents (TCT).** The tannin contents were estimated with the vanillin-HCl method [15] with slight modification, using catechin as a reference compound. In brief, 1 g of dry residue was added to 15 mL of MeOH–HCl (1%) in a test tube. The tube was placed in a water bath at 35 °C for 30 min, centrifuged at 1532 × g, and finally filtered. Aliquots (0.5 mL) of extract were mixed with 3 mL of vanillin-HCl reagent (4% in methanol) in test tubes. After 20 min of incubation at room temperature, the absorbance was read at 500 nm, using a UV–visible spectrophotometer. The estimation of condensed tannins was carried out in triplicate, and the results were expressed as mg catechin equivalent/g of dry matter.

**Total anthocyanin compounds (TAC).** The samples’ total anthocyanins compounds were determined using a Vis- spectrophotometer according to the pH-differential method [16]. Two buffer systems, potassium chloride buffer, pH 1.0 (0.025 M), and sodium acetate buffer, pH 4.5 (0.4 M), were used. Absorbance was measured at 520 and 700 nm. TAC Results were expressed as cyanidin-3-glucoside equivalents and calculated using the formula:

\[
TA (\text{mg/g}) = \Delta A \times \frac{\text{MW} \times \text{DF} \times 1000}{\epsilon \times 1}
\]

where \(\Delta A\): difference of absorbance; \(DF\) = dilution factor; \(MW\) (molecular weight) = 449.2 g/mol; \(1 =\) cuvette pathlength in cm; \(\epsilon = 26,900 \text{ L/mol.cm}\).

**Evaluation of total antioxidant capacity by phosphomolybdate (PPM) method.** The total antioxidant capacity assay of plant extract was carried out by the phosphomolybdenum method [17-18]. A 0.1 mL of sample solution was mixed with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Against a blank containing 0.1 mL of methanol mixed with 1 mL of reagent solution. The test tube contained the reaction mixture was covered with aluminum foil and incubated at 95 °C for 90 min. After the samples were cooled to room temperature, the absorbance was measured at 695 nm.
Ascorbic acid was used as a standard. The antioxidant capacity was estimated using the following formula:

\[
\text{Tot. antioxidant cap. (\%)} = \left( \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \right) \times 100
\]

Total antioxidant activity was expressed as mg vitamin C equivalent/1g dry weight, and the values are presented as the means of triplicate analysis.

**Fixed oil analysis**

**Oil extraction.** Seeds powder of different plants *E. biumbellata*, *E. dendroides*, and *E. terracina* was extracted by Soxhlet method with hexane solvent for 8 h. After solvent evaporation under reduced pressure, the oil was recovered and stored in glass vials at 4 °C for further analysis.

**Chemical characteristics of oil.** Oil characteristics, namely saponification number (SP), iodine value (IV), and acidity, were determined according to AOCS standard methods [19].

**GC-MS analysis**

The fatty acid composition is determined as the methyl esters of fatty acids by gas-liquid chromatography. Methyl esters of the fatty acids (FAME) of the three seed oils were prepared according to a convenient method [20], using BF₃-methanol at 60 °C.

The FAME was recovered using *n*-hexane, and an aliquot (2 µL) was injected automatically in splitless mode into the GC-MS apparatus. The carrier gas was helium, the temperatures of the injector and detector were held at 300 °C. Electron impact ionization was achieved with ionization energy IE of 70 eV. The identification of the components was based on the comparison of their mass spectra with those of the NIST 11 mass spectral library.

**LC-MS analysis**

The concentration of standards solution was 1 mg in 1 mL of methanol-water (1:1) solution. Measurements were carried out at 280 nm wavelength. Standards and extracts were filtered through a Millipore membrane (0.45 µm). LC-MS method was used for the determination of bioactive ingredient content in our plant’s hydro-methanolic extracts.

An aliquot of extract (20 µL) was injected onto a Gemin C-18 column (4.6 mm i.d. × 150 mm). The solvents used were formic acid–acidified water at 0.5% (A) and acetonitrile (B). The elution gradient was 35 min with a flow rate of 0.6 mL/min (Table 1). Spectra were recorded in negative ionization mode in full scan between 0 and 1000 Da.

### RESULTS AND DISCUSSION

**Chemical Composition**

Previous phytochemical screening of the phytochemical constituents of various extracts from the three plants [21] showed that they are very rich in polyphenols, lipids, tannins, flavonoids, fats and oils, anthocyanins, sterols, and terpenes. In contrast, alkaloids and starch were not detected, and *E. dendroides* extract gave a negative result for cardenolides.

**TAC, TTC, and phosphomolybdate assay**

The results of TAC, TTC, and PPM tests of leave extracts are shown in Table 2. The quantitative estimation of chemical constituents revealed that *E. terracina* leaves contained the highest tannins and anthocyanin levels with 15.54 mg catechin/g and 1.070 cy-3-gly/g, respectively.

<table>
<thead>
<tr>
<th>TEST</th>
<th><em>E. biumbellata</em></th>
<th><em>E. dendroides</em></th>
<th><em>E. terracina</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>TTC (CE mg/g Dm)</td>
<td>6.41 ± 0.13</td>
<td>7.31 ± 0.33</td>
<td>15.54 ± 0.56</td>
</tr>
<tr>
<td>TAC (AC mg/g Dm)</td>
<td>0.23 ± 0.42</td>
<td>0.35 ± 0.32</td>
<td>1.070 ± 0.98</td>
</tr>
<tr>
<td>PPM (VCE mg/g Dm)</td>
<td>9.05 ± 0.02</td>
<td>8.17 ± 0.03</td>
<td>8.75 ± 0.07</td>
</tr>
</tbody>
</table>

*Dm*: dry matter
exceeding the contents of *E. dendroides* and *E. bumbellata*. It was reported that these phytochemical compounds (tannins and anthocyanin) are known to provide support for the bioactive properties of plants [22-23].

The results obtained from the phosphomolybdic acid assay of the three *Euphorbia* leaves extracts show that all the tested extracts possessed a scavenging activity (Table 2). *E. biumbelatta* sheets extract exhibited a higher activity than the other two extracts, while *E. dendroides* extract was the least active.

**Fixed Oil Analysis**

The yields of oils extracted from *E. terracina*, *E. biumbellata*, and *E. dendroides* were 17.48%, 18.5%, and 20.05%, respectively.

**Chemical characteristics of the oils**

Results of the chemical characteristics of the three samples are displayed in Table 3. The data indicate considerable variations in acid values, saponification values, and iodine values between the three seed oils.

The acid index is a parameter that demonstrates the quality of the oil. However, the acid value of the oil must not be too high; an acid value of 0.00 to 3.00 mg KOH/g oil is recommended for oil to find application in cooking [24]. All the vegetable oils understudy with a high acid value of 5.83 to 10.99 mg KOH/g, this denotes a high content of free fatty acids, which causes the oil to turn sour and a less storage quality [25].

Iodine value is a measure of the degree of unsaturation. It is used to quantify the number of double bonds present in the oil. Among the different oils analyzed, *E. dendroides* seed oil displayed the highest iodine value, which reflected a high concentration of unsaturated fatty acids in the oil. On the other hand, *E. biumbelatta* seeds oil showed a low degree of unsaturated fatty acid, which indicated that the oil could be used as a nondrying oil, which helps manufacture soap [26].

The saponification value is inversely compared to the molecular weight of the oil. It helps assess the chain lengths of fatty acids in the oil. All of the analyzed oils showed a high SP number, but *E. biumbelatta* recorded the highest value.

**GC-MS Reports**

The GC-MS analysis of seed oils from the three *Euphorbia* species allowed us to identify numerous compounds belonging to different chemical families. The active compounds with their molecular mass, retention time, and concentration are presented in Table 4.

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**Table 3. Chemical properties of the seeds oil**

<table>
<thead>
<tr>
<th>Chemical characteristics</th>
<th><em>E. biumbellata</em></th>
<th><em>E. dendroides</em></th>
<th><em>E. terracina</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid value: AV (mg KOH/g)</td>
<td>5.83</td>
<td>10.09</td>
<td>10.99</td>
</tr>
<tr>
<td>Iodine value: IV (gI_2/100 g)</td>
<td>47.58</td>
<td>90.41</td>
<td>63.45</td>
</tr>
<tr>
<td>Saponification: SP (mg KOH/g)</td>
<td>266.47</td>
<td>210.30</td>
<td>162.32</td>
</tr>
</tbody>
</table>

**Table 4. Principal compounds in oils from seeds**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Molar mass (g/mol)</th>
<th>Retention time (min)</th>
<th><em>Euphorbia biumbelatta</em></th>
<th><em>Euphorbia dendroides</em></th>
<th><em>Euphorbia terracina</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexadecanoic acid, methyl ester</td>
<td>270</td>
<td>19.35</td>
<td>2.08 ± 0.2</td>
<td>10.05 ± 0.7</td>
<td>8.5 ± 1.2</td>
</tr>
<tr>
<td>8,11-Octadeca-dienoic acid, methyl ester</td>
<td>294</td>
<td>21.22</td>
<td>14.6 ± 2.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11,14,17-Eicosatrienoic acid, methyl ester</td>
<td>320</td>
<td>21.64</td>
<td>11.3 ± 1.7</td>
<td>-</td>
<td>13.55 ± 0.3</td>
</tr>
<tr>
<td>Linoleic acid, methyl ester</td>
<td>294</td>
<td>21.19</td>
<td>-</td>
<td>37 ± 1.2</td>
<td>31.05 ± 1.2</td>
</tr>
<tr>
<td>Linolenic acid, methyl ester</td>
<td>292</td>
<td>21.27</td>
<td>-</td>
<td>2.3 ± 0.9</td>
<td>-</td>
</tr>
<tr>
<td>Stearic acid, methyl ester</td>
<td>298</td>
<td>21.47</td>
<td>-</td>
<td>14.35 ± 0.5</td>
<td>-</td>
</tr>
<tr>
<td>Ascorbic acid, dipalmitate</td>
<td>652</td>
<td>19.92</td>
<td>-</td>
<td>-</td>
<td>3.95 ± 0.2</td>
</tr>
</tbody>
</table>

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Nadia Toudert et al.
The GC-MS chromatogram of the compounds detected is shown in Fig. 1. Results showed that hexadecanoic acid and linoleic acid are present in the various oils. The predominant unsaturated fatty acid is linoleic acid (37% in *E. dendroides*, 31.05% in *E. terracina*, and 14.06% in *E. buimbelatta*). Besides, only *E. terracina* oil contains a rare and important compound of l-(+)-ascorbic acid 2,6-dihexadecanoate (Fig. 1). Ascorbic acid, dipalmitate has been reported to have many biological properties such as antioxidant, anti-inflammatory, and anti-nociceptive properties [27-28].

**LC-MS Analysis**

Different peaks at different retention times were obtained through LC-MS analysis in all three samples, and ten phenolic compounds were identified, and the chromatographic profile of standards and their retention times are represented by Fig. 2 and Table 5.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>3.00</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>4.70</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>8.70</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>10.55</td>
</tr>
<tr>
<td>Rutin</td>
<td>12.53</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>13.35</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>13.85</td>
</tr>
<tr>
<td>Colchecin</td>
<td>17.30</td>
</tr>
<tr>
<td>Quercitin</td>
<td>19.90</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>20.70</td>
</tr>
</tbody>
</table>

**Fig 1.** Chromatographic profiles of seeds oil of *Euphorbia terracina*

**Fig 2.** Chromatographic profiles of standards at 280 nm at 1 mg/mL: 1: ascorbic acid; 2: gallic acid; 3: chlorogénic acid; 4: syringic acid; 5: rutin; 6: ellagic acid; 7: ferulic acid; 8: colchicin; 9: quercitin; 10: cinnamic acid
Table 6. Important compounds identified by LC-MS

<table>
<thead>
<tr>
<th>Standards</th>
<th>Euphorbia biumbelatta</th>
<th>Euphorbia dendroides</th>
<th>Euphorbia terracina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Seeds</td>
<td>Leaves</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rutin</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Colchicin</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Quercetin</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

different leaves and seeds extracts are compiled in Table 6. The compound’s chemical structures are elucidated from the standard reference graphs, using the molecular weight data.

From the standard reference graphs, the compounds are elucidated using the molecular weight. The results showed the presence of ascorbic acids, chlorogenic acids, and ellagic acids in the different extracts. The species that contains the more standards are *Euphorbia biumbelatta*.

Most of the phytoconstituents identified are very important in medicine. They exhibit various pharmacological activities such as antioxidant, antidiabetic, anti-inflammatory, and anticancer [29-30].

**CONCLUSION**

The present work has demonstrated the potential of *Euphorbia* species growing in Algeria as an important source of secondary metabolites.

Hydro-alcoholic extracts from sheets of the three plants were subjected to quantitative estimation of chemical constituents and antioxidant activity essay. Results showed that total tannin and anthocyanin contents were higher in *Euphorbia terracina* extract, compared to the two other generators. Whereas, the total antioxidant activity was higher in *Euphorbia biumbelatta* extract, and was found to be equal in *Euphorbia dendroides* and *Euphorbia terracina* extract. In addition, chemical compositions of hydro-methanolic extracts of leaves were analyzed by the LC-MS technique, which leads to the identification of ten compounds with considerable concentrations.

On the other hand, fixed oils of seeds were obtained with the Soxhlet method and analyzed by GC-MS apparatus. Linoleic acid was detected as the main FAME among the identified FAMEs in the various oils. From LC-MS and GC-MS results, an appreciable abundance of secondary metabolites in the studied plants was noticed. These results demonstrated the importance of *Euphorbia* genre as a promising source of new bioactive compounds.

**ACKNOWLEDGMENTS**

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