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Phytochemical Characterization, *In-Vitro* Cytotoxic and Antibacterial Activity of *Cotula cinerea* (Delile) Vis Essential Oil

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Abstract

Cotula cinerea is a traditional Algerian medicinal plant that has many biological properties, such as analgesic, antiseptic and antibacterial activities. Essential oil of *Cotula cinerea* was subjected to chromatographical and spectroscopical studies to determine the chemical composition. The analysis highlighted that the oil contains trans thujone (51.86%), santalina triéne (10.6%), α- pinéne (2.02%), sabinene (6.17%), cineole <1.8> (5.34%), δ - terpinene (1.57%), camphor (2.63%), β- terpineol (1.39 %) and terpin-4-ol (1.73%) as the major constituents. The essential oil was tested for antibacterial activity. The oil exhibited substantial antibacterial activity against both the tested Gram negative and Gram positive bacteria. In search of better anticancer agent the essential oil of *Cotula cinerea* was also subjected for *in-vitro* cytotoxic activity on two cancerous human cell lines; colon (HCT116) and hepatic (HePG2) cancer cell lines. The results indicates that the essential oil of *Cotula cinerea* has a significant cytotoxic activity on the tested colon cancer cell lines with a 66.9% inhibition and a minimal inhibition (33.9%) on liver cancer cell line.

Keywords: Essential Oil Composition, GC/MS, HCT116, HePG2

1. Introduction

For better knowing the phytochemical and microbiological properties of the plants of "El-Oued" region, *Cotula cinerea* [Syn. *Brocchia cinerea* (Del) Vis], locally known as (Shihia/Shihit El Ebel)[1], belonging to the family "Asteraceae" was chosen; as it is among the plants mostly used by the local population for TS medicinal properties. *Cotula cinerea* is used against insolation, colic, cough and bronchopulmonary cooling. This species is widely used in traditional Moroccan medicine for its biological properties such as anti-inflammatory, analgesic, antiseptic, antibacterial, antipyretic activities[2]. It is also used as an infusion to facilitate digestion[3]. The species *Cotula cinerea* is one of three species belonging to the genus *Cotula* (Asteraceae) existing in South-Algeria[3]. It is a woolly whitish plant, with thick leaf divided in their upper part to a 3-5 obtuse teeth, stems are 10–40 cm, slept then raised; capitulum from 6 to 10 mm in diameter, woolly involucres with a tubular flower, and brown buds which would become golden yellow[4,5]. Its stems are diffuse or erect. The leaves and whitish-green stems are covered with tiny hairs thick[6], velvety small leaves, whole are cut into three to seven teeth or "fingers"[7], and the shaft of high branch yellow inflorescences[8]. The terpenes in the essential oils present are responsible for the characteristic odor[8]. The literature studies highlights that the leaf extracts of *Cotula cinerea* Del are effective against pathogenic fungi, and also have insecticidal activity on the larvae[9]. It finds importance in the management of stomach pain, fever, headache, migraine, cough and joint inflammation[10].

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In the present investigation, the chemical components of *Cotula cinerea* Del essential oil was analyzed and screened for antibacterial activity against some pathogenic and food-borne bacteria. The essential oil was also evaluated for anticancer activity against HePG2 (Hepatic) and HCT116 (Colon) human cancerous cell lines.

2. Materials and Methods

2.1 Plant Material

Aerial parts of *Cotula cinerea* Del., were collected from the Hassi Khalifa City in the wilaya of Eloued, north east of Algeria during the period of December 2015 to January 2016 for the investigation. Voucher specimens were deposited to the Herbarium of the Chemistry Laboratory, University of El-Oued.

2.2 Isolation of the Essential Oils

The aerial parts (100 g) were washed, sorted and dried for a month at room temperature, in the shade and then were finely pulverized by using a mill blade. Clevenger-type apparatus was used for extraction, and hydrodistillation was performed for 4 h[11]. The distilled essential oil was dried over anhydrous sodium sulfate, filtered and stored at 4°C.

2.3 Gas Chromatography - Mass Spectrometry (GC/MS) Analysis Conditions

The oil was analyzed by GC on a Hewlett Packard GC-MS system, Model 6890 equipped with Flame Ionization Detector (FID), HP-5, 30m × 0.25mm ID, 0.25 mm film thickness, fused capillary column. The carrier gas was nitrogen (0.8mL/min). Temperature programming was done from 60–250˚C at 4˚C /min with initial and final hold time of 8 minutes. The injection volume was 0.4 µL neat and Split ratio was 1:20. The percentage of the constituents was calculated by electronic integration of FID peak areas without the use of response factor correction and the sample indices were calculated following Van den Dool and Kratz[12].

The analysis of the volatile constituent was run on a Hewlett-Packard GC-MS system Hewlet Packard 5973 A, equipped with an non polar capillary column HP5MS, 30 m × 0.25 mm, phase thickness 0.25 µm. The detection of elutent was done using an electron ionization system with ionization current: 70eV. Inert helium gas (99.999%) was used as a carrier gas at a constant flow rate of 0.7 mL/min. The injector temperature was 60 and 280°C, respectively. The temperature programmed for oven was from 2°C/min then held at isothermal condition for 8 min and finally raised to 280°C at 2°C/min, split ratio of 1:20, and volume injected was 0.1 to 0.2 µl of the isolated essential oil[13].

2.4 Identification of Compounds

The identification of the essential oil constituents was based on a comparison of their retention times to *n*-alkenes function (C_9–C_{28})[14]. The identification of the oil components were based on MS search Data Library (Wiley & Nist) or with authentic compounds or with the data published in the literature, and Retention Indices (RI) (Adams, 2010)[15]. The chromatographic conditions were identical to those used for GC-MS analysis[16].

2.5 Antibacterial Activity

The Minimal Inhibitory Concentration (MIC) of essential oil values were evaluated in the Mueller Hinton Broth (MHB) by dilution method[15,16] against *Bacillus subtilis* (ATCC6663), *Micrococcus luteus* (ATTC9314), *Listeria monocytogene* (CIP82110), *Escherichia coli* (CIP54.8), *Klebsiella pneumonia* (CIP82.91), *Pseudomonas aeruginosa* (CIPA22), *Agrobacterium tumefaciens* (N°2410), *Salmonella enteric* (CIP 81.3). An aliquot (1 mL) of this suspension was transferred to a sterile tubes of MHB containing various concentrations of oils (0.1-15µL)[18] and the volume was adjusted to 10 mL with ethanol (5%, w/v) to obtain with 10 µl bacterial inoculums adjusted a concentration of 106 CFU/mL[17,18]. They were incubated under shaking conditions (100–120 rpm) for 24 h at 37 °C[19,20].

2.6 In-Vitro Cytotoxic Activity

Cytotoxic activity on human cell line (HePG2–HCT116) was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan[21].

3. Results

3.1 Chemical Composition of the Essential Oil

The yield of essential oil isolated by hydrodistillation of *Cotula cinerea* was 0.74% (w/w), based on the dry weight of the sample. Forty one constituents were identified by GC-MS (Figure 1), representing 88% of the oil (Table 1). The major compounds were trans thujone (51.86%), santalina triéne (10.6%), α- pinéne (2.02%), sabinene (6.17%), cineole <1.8> (5.34%), δ - terpinene (1.57%) and camphor (2.63%).

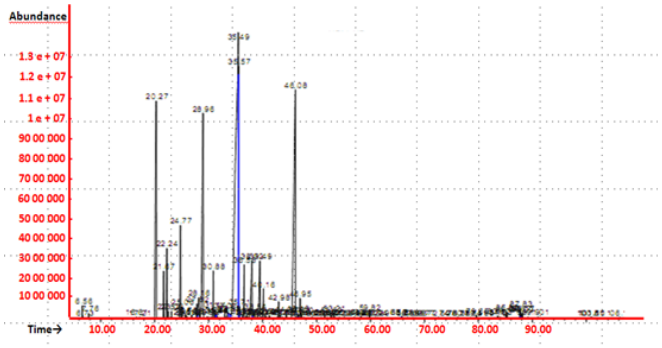

Figure 1. GC-MS of *Cotula cinerea* essential oil.

Table 1. Chemical composition, percentage composition of *Cotula cinerea* essential oil and Retention Indices (IR)

S. No.	IR_a	Compounds	IR_d	%
01	855	cis salven	-	-
02	908	**santalina triéne (santolina triene)**	914	**10.6**
03	931	α-**thujen**	935	0.88
04	939	α-pinene	943	**2.02**
05	953	camphene	956	0.85
06	976	**sabinene**	976	**6.17**
07	980	β- pinene	981	0.58
08	991	cineole dehydro 1.8	988	0.64
09	991	myrcene	993	0.07
10	999	mentha-1(7)-8dien <meta>	997	0.06
11	1018	α - terpinene	1017	0.83
12	1022	**ortho cymen**	1020	0.43
13	1026	para cymen	1029	0.6
14	1033	**cineole <1.8>**	1033	**5.34**
15	1062	δ - **terpinene**	1057	**1.57**
16	1068	sabinene hydrate (cis)	1069	0.46
17	1088	terpinolene	1088	0.36
18	1102	cis thujone	1100	0.52
19	1114	**trans thujone**	1117	**51.86**
20	1121	menth-2en-1-ol <cis para>	-	-
21	1133	thujanol <iso-3->	-	-
22	1143	**camphor**	1140	**2.63**
23	1163	β- terpineol	1160	**1.39**
24	1177	**terpin-4-ol**	1178	**1.73**
25	1189	α - terpineol	1190	0.58
26	1193	cis piperitol	-	-
27	1194	myrtenol	1195	0.13
28	1217	trans carveol	-	-

S. No.	IR_a	Compounds	IR_d	%
29	1226	dihydro carveol <neo iso>	1224	0.53
30	1239	cumin aldehyde	-	-
31	1246	carvotanacetone	1247	0.9
32	1262	**chrystanthenyl acetate cis**	1264	**5.07**
33	1273	iso pulegol acetate	1274	0.06
34	1287	cymen-7-ol-<para>	1285	0.08
35	1298	carvacrol	-	-
36	1314	decadienal <(E,E)-2,4->	-	-
37	1340	terpin-4-ol acetate	-	-
38	1365	neryl acetate	1367	-
39	1394	cis jasmone	1390	0.15
40	1458	farnesen <(E)- beta->	-	-
41	1480	germacrene D	1481	0.06

IR_a Retention Index.
IR_d Experimentally Determined Retention Indices.

Table 2. MIC of essential oil from *Cotula cinerea*

Concentration (µL/mL)	0.1	0.2	0.3	0.4	0.5	1	2	3	4	5	10	15
Gram–positive												
Bacillus subtilis (ATCC6663)	+	+	+	+	+	+	+	+	+	+	–	–
Micrococcus luteus (ATTC9314)	+	+	+	+	+	+	+	+	+	+	+	–
Listeria monocytogene (CIP82110)	+	+	+	+	+	+	+	+	+	+	–	–
Gram–negative												
Escherichia Coli (CIP54.8)	+	+	+	+	+	+	+	+	+	+	–	–
Klebsiella pneumonia (CIP82.91)	+	+	+	+	+	+	+	+	+	+	–	–
Pseudomonas aeruginosa (CIPA22)	+	+	+	+	+	+	+	+	+	+	–	–
Agrobacterium tumefaciens (N°2410)	+	+	+	+	+	+	+	+	+	+	+	–
Salmonella enterica (CIP 81.3)	+	+	+	+	+	+	+	+	+	+	+	–

Note: (–), total inhibition; (+), growing

3.2 Antibacterial Activity

The antibacterial activity of the *Cotula cinerea* essential oil was tested against three Gram positive and five Gram negative pathogenic bacterial strains. The results of the antibacterial activity are shown in Table 2.

3.3 In-Vitro Cytotoxic Activity

The cytotoxic activity of the *Cotula cinerea* essential oil was tested against two cell line HCT116 and HePG2. The results are as shown in Table 3.

Table 3. Cytotoxic activity of oil of *Cotula cinerea* against cultured different cell

Cell lines	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	LC_{90} (µg/mL)	LC_{50} (µg/mL)	Doxorubicin	DMSO at 100 ppm
HCT116	66.9 + 0.86	21.33 + 1.858	6.0 + 0.65	0	122.3	86.7	37.6	1%
HePG 2	33.9 + 0.56	11.1 + 0.12	0	0	-	21.6	21.6	1%

4. Discussion

Djellouli et al.,[22] and Sieniawska et al.,[28] have reported that trans-thujone (41.4%), cis-verbenyl acetate (24.7%), 1,8-cineole (8.2%) and camphor (5.5%) as the major components of the plants collected in Morocco, while Atef et al.,[9] have reported that santolina triene (18.58%), thujone (21.73%), 3-carene (30.99%) and camphor (6.21%) as major constituents[24]. Current study has shown that the oil contains trans thujone (51.86%), santalina triéne (10.6%), α- pinéne (2.02%), sabinene (6.17%), cineole <1.8> (5.34%), δ - terpinene (1.57%), camphor (2.63%), β- terpineol (1.39%) and terpin-4-ol (1.73%) as the major constituents.

The four bacterial strains manifested the same sensitivity vis-à-vis to the *Cotula cinerea* essential oils. They were all inhibited at 15 µL/mL concentration. As for mold, they were less sensitive than bacteria and their growth was stopped at 10 µL/mL concentration. The current study is in agreement with study of Boussoula et al[25].

The essential oil showed potent cytotoxic activity on HcT116 colon cancer cell line with a 66.9 % inhibition at 100 µg/mL concentration (LC50 86.7 µg/mL and LC90 122.3 ug/mL). The result is very promising compared with positive control doxorubicin 37.6 µg/mL, while the results indicated week activity on HePG2 (33.9 %) inhibition.

Cotula cinerea has also been reported for anticandidal activity[26]. Medicinal plants rich with volatile oil represent an important source of antioxidant and anticancer drugs[27]. The use of essential oil in combination with cancer therapy decreases the side effect of drugs[28]. Essential oil of *Ricinus communis* containing thujone and 1.8-cinole has been reported for antiproliferative activity[29]. Cotula *cinerea* showed potent cytotoxic activity especially for colon cancer and this may also be corresponding to the chemical composition of essential oil which is rich in trans thujone 50% and santolina triene, as they are reported for anticancer activity.

5. Conclusion

The present study reveals antibacterial potency of essential oil of *Cotula cinerea* of eastern Algeria. *C. cinerea* can also be an inexpensive source of natural antibacterial substance for use in pathogenic systems to prevent the growth of bacteria and extend the shelf life of the processed foods. *C. cinerea* showed better cytotoxic activity against colon cancer cell line (HCT116) than liver cancer cell line (HePG2).

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