



Chemical composition, antioxidant, anticholinesterase, antimicrobial and antibiofilm activities of essential oil and methanolic extract of *Anthemis stiparum* subsp. *sabulicola* (Pomel) Oberpr

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ABSTRACT

Anthemis species are traditionally used to treat infectious and inflammatory processes, among others clinical disturbances. In the current study, the chemical composition, the total phenolic and flavonoid contents, the antioxidant, anticholinesterase, antimicrobial, and antibiofilm activities of *Anthemis stiparum* subsp. *sabulicola* aerial parts methanolic extract (As-ME) and essential oil (As-EO) were investigated. The chemical composition of As-EO was established by GC-MS and GC-FID. Total phenolic and flavonoid contents of As-ME were spectrophotometrically determined. Diphenyl-1-picrylhydrazyl (DPPH[•]) radical scavenging, cupric reducing antioxidant capacity (CUPRAC) and β -carotene bleaching assays were applied to evaluate the antioxidant potential. The anticholinesterase activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes were carried out spectrophotometrically. The antimicrobial activity was assessed by Minimal Inhibitory Concentration (MIC) using broth microdilution method against 7 ATCC[®] bacterial and one ATCC[®] yeast reference strains. The antibiofilm effect was determined quantifying the percentage of adhesion inhibition. GC-MS and GC-FID identified 72 compounds (99.02%), being As-EO predominantly constituted by germacrene D (11.13%), α -cadinol (11.01%), camphor (6.73%), spathulenol (6.50%) and isoamyl salicylate (6.45%). The total phenolic and flavonoid contents of As-ME were 13.6 ± 0.03 and 5.9 ± 0.04 pyrocatechol equivalents and quercetin equivalents, respectively. In β -carotene-linoleic acid assay, As-ME showed the best lipid peroxidation inhibition activity with an $IC_{50} = 9.96 \mu\text{g/mL}$ followed by As-EO with an $IC_{50} = 619.98 \mu\text{g/mL}$. In contrast, in DPPH assay, As-ME and As-EO showed moderate to low activity with an $IC_{50} = 92.69 \mu\text{g/mL}$ for As-ME and $917.69 \mu\text{g/mL}$ for As-EO. While in CUPRAC assay, As-EO and As-ME indicated a less to moderate reducing activity. As-ME inhibited AChE ($IC_{50} = 490.46 \mu\text{g/mL}$) and BChE ($IC_{50} = 142.07 \mu\text{g/mL}$), while As-EO was inactive against AChE and revealed a discreet inhibitory action against BChE ($IC_{50} = 212.14 \mu\text{g/mL}$). As-ME displayed better antimicrobial activity than As-EO, being active against *Staphylococcus aureus* (ATCC[®] 25923) and *Bacillus subtilis* (ATCC[®] 6633), with MIC of 1.56 mg/mL. An expressive fungal adhesion inhibition (80.02%) on *Candida albicans* (ATCC[®] 10239) was detected with As-ME at 6.25 mg/mL. These results showed that *A. stiparum* subsp. *sabulicola* is a natural source of active compounds with antibiotic and antibiofilm effects against *S. aureus* and *B. subtilis*, and *C. albicans*, respectively, and also presents antioxidant and anticholinesterase properties.

1. Introduction

Reactive oxygen species (ROS) are chemical species that comprise of molecular oxygen [1]. We can find ROS in every aerobic cell and they balance fairly with biochemical antioxidants. In the event that ROS

become excessive, or antioxidants become reduced, or both occur, that oxidative stress occurs because the balance gets altered. And oxidative stress is considered as a precursor to Alzheimer's disease (AD), which might be responsible for the dysfunction or death of neuronal cells that contributes to disease pathogenesis [2]. The brain is considered to be

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Table 1

Chemical composition (%) of the essential oil from aerial parts of *A. stiparum* subsp. *sabulicola* using Gas Chromatography with Flame Ionization Detector (GC-FID) and Gas Chromatography Mass Spectrometry (GC-MS).

N°	Compound	Composition (%)	RI ^a	RI ^{lit.} on DB5	Identification method
1	Cumene	0.49	906	924	1, 2
2	α-Pinene	0.49	936	932	1, 2, 3
3	Camphene	0.24	950	946	1, 2
4	6-Methyl-5-heptene-2-one	4.34	976	981	1, 2
5	β-Pinene	0.05	978	974	1, 2, 3
6	o-Cymene	0.05	1009	1022	1, 2
7	Eucalyptol (1,8 Cineol)	0.26	1024	1026	1, 2, 3
8	Limonene	0.06	1025	1024	1, 2, 3
9	γ-Terpinen	0.15	1051	1054	1, 2
10	Camphor	6.73	1123	1141	1, 2, 3
11	Borneol	0.28	1150	1165	1, 2
12	Isogeraniol	0.99	1156	1174	1, 2
13	α-Terpineol	0.10	1176	1186	1, 2, 3
14	2-Decanone or 3-Decanone	0.13	1178	1190	1, 2
15	n-Dodecane	0.10	1200	1200	1, 2
16	δ-Elementene	0.49	1340	1335	1, 2
17	Neryl acetate	0.17	1342	1359	1, 2
18	β-Damascenone	0.18	1348	1383	1, 2
19	Decanoic acid	0.29	1349	1364	1, 2
20	α-Copaene	0.62	1379	1374	1, 2
21	Methyleugenol	0.12	1385	1403	1, 2
22	β-Bourbonene	0.12	1388	1387	1, 2
23	β-Elementene	0.28	1390	1389	1, 2, 3
24	β-Caryophyllene	1.97	1408	1417	1, 2, 3
25	β-Gurjunene	0.15	1431	1431	1, 2, 3
26	Seychellene	0.64	1447	1444	1, 2
27	α-Humulene	0.33	1455	1452	1, 2
28	Alloaromadendren	0.57	1460	1458	1, 2
29	Aromadendrane < dehydro- >	0.41	1462	1460	1, 2
30	Sesquiceneole < dehydro- >	4.46	1469	1469	1, 2
31	Sesquiceneole < 7-epi-1,2-dehydro- >	0.16	1471	1471	1, 2
32	Germacrene D	11.13	1481	1484	1, 2, 3
33	δ-Selinene	0.34	1492	1492	1, 2
34	β-Himachalene	5.19	1500	1500	1, 2
35	(E,Z)-α-Farnesene	0.50	1501	1505	1, 2
36	1,5-Cycloundecadiene, 8,8-dimethyl-9-methylene-	0.84	1504	1485	1, 2
37	β-Bisabolene	0.18	1505	1505	1, 2
38	γ-Cadinene	1.60	1510	1513	1, 2
39	δ-Cadinene	1.15	1520	1522	1, 2
40	Isoamyl salicylate	6.45	1523	1535	1, 2
41	cis-3-Hexenyl Benzoate	0.74	1545	1565	1, 2
42	γ-Elementene	0.05	1570	1434	1, 2
43	Spathulenol	6.50	1572	1577	1, 2, 3
44	Caryophyllene oxide	2.56	1578	1582	1, 2, 3
45	Globulol	0.44	1589	1590	1, 2
46	Isoaromadendrene epoxide	1.22	1592	1639	1, 2
47	Ledene oxide-(II)	0.65	1604	1646	1, 2
48	Cubenol	0.56	1630	1645	1, 2
49	Cedren-3-one < 2-epi-α >	2.22	1631	1626	1, 2
50	Hexenyl Phenyl acetate < (3Z)- >	1.50	1635	1632	1, 2
51	t-Cadinol	11.01	1640	1638	1, 2
52	β-Eudesmol	0.53	1641	1649	1, 2
53	α-Cadinol	2.56	1643	1652	1, 2
54	Edusmol < 7-epi-α >	1.41	1653	1662	1, 2
55	Khusinol	0.88	1658	1679	1, 2
56	α-Bisabolol	1.39	1673	1685	1, 2
57	cis-Z-α-Bisabolene epoxide	3.80	1704	–	1, 2
58	Nuciferol < (Z)- >	0.32	1713	1724	1, 2
59	Farnesol < 2Z,6E)- >	0.42	1720	1722	1, 2
60	γ-Costol	0.26	1746	1745	1, 2
61	Cedryl acetate	0.26	1769	1767	1, 2
62	Farnesyl acetate < (2Z,6E)- >	0.28	1812	1821	1, 2
63	E – 10-Pentadecenol	0.13	1908	–	1, 2
64	Pimaradiene	0.08	1962	1948	1, 2
65	Hexadecanoic acid	0.43	1981	1959	1, 2
66	Thunbergol	0.35	2002	2032	1, 2
67	Hexadecanoic acid, trimethylsilyl ester	0.30	2040	2047	1, 2
68	Heneicosane	0.67	2100	2100	1, 2
69	1,18-Nonadecadien-7,10-dione	0.10	2168	2062	1, 2
70	Epimanol	0.55	2265	2057	1, 2
71	Tricosane	3.29	2306	2300	1, 2
72	Pentacosane	0.78	2500	2500	1, 2
	Total identified:	99.02			

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