International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)
Journal Home page: www.iscientific.org/Journal.html

Rosmarinus officinalis, Artemesia mesatlantica and Syzygium aromaticum on SARS-CoV-2 viral activity

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Abstract

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has led to a global health crisis. Current treatment options are limited, emphasizing the need for effective antiviral agents. This study explores the antiviral potential of essential oils from Thymus vulgaris, *Rosmarinus officinalis*, *Artemesia mesatlantica*, and Syzygium aromaticum against SARS-CoV-2. Additionally, the antioxidant activities of these essential oils are investigated due to their potential role in mitigating oxidative stress associated with COVID-19. The antiviral activities are assessed using a direct yield reduction assay on human epithelial lung cancer Calu-3 cell lines infected with SARS-CoV-2. Essential oils are extracted from plant specimens, and their chemical compositions are analyzed through gas chromatography. The antioxidant capacity is determined by the DPPH radical scavenging assay.Thymus vulgaris essential oil exhibits noteworthy antiviral activity, with potential attributed to high thymol content. *Rosmarinus officinalis* and *Artemesia mesatlantica* essential oils show moderate antiviral effects, while Syzygium aromaticum essential oil demonstrates potent antiviral activity, likely linked to its substantial eugenol content. Antioxidant assessments reveal Syzygium aromaticum essential oil as the most potent, surpassing commonly used standards. The chemical analysis of essential oils provides insights into their diverse compositions, with major compounds identified. The variations in chemical profiles contribute to differing antiviral and antioxidant activities observed among the essential oils. Thymol and eugenol emerge as key contributors to the observed biological effects. This study highlights the potential of essential oils, particularly from Syzygium aromaticum, as antiviral agents against SARS-CoV-2. Their antioxidant properties further suggest a role in alleviating oxidative stress associated with COVID-19. These findings contribute to the exploration of natural products as potential therapeutic options for COVID-19 management.

Keywords: SARS-CoV-2, *Rosmarinus officinalis*, *Artemesia mesatlantica*, *Syzygium aromaticum*, DPPH radical scavenging assay.

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1. Introduction

The Coronavirus Disease 2019 (COVID-19) is an outbreak caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), still spreading and has led to unprecedented health emergency all over the world. It is characterized by an acute respiratory distress syndrome (ARDS) accompanied with clinical pathologies, including various coagulopathies that may be associated by hyper-coagulation and platelet hyper-activation [1]. Therefore, the involvement of platelet function in the pathophysiology of COVID-19 is well documented. While vaccination campaign is underway, only few treatments showed efficacy against SARS-CoV-2 which is probably the biggest challenge for public health systems in most countries [2]. The effective antiviral activities of natural products have been proved in different studies [3, 4].

2. Materiel and methods

2.1. SARS-CoV-2 replication

Human epithelial lung cancer Calu-3 (ATCC® HTB-55) cell line was used to determine the antiviral activity of candidate compounds in the direct yield reduction assay (DYRA). Both VERO E6 and Calu-3 cell lines have been shown to support SARS-CoV-2 replication. VERO E6 cell line was maintained in high-glucose Dulbecco's Modified Eagle's Medium with sodium pyruvate and L-glutamine (DMEM; Euroclone, Milano, Italy), while Calu-3 was maintained in Minimum Essential Medium Eagle (EMEM; Sigma, Darmstadt, Germany) supplemented with 2 mM L-glutamine (L-glut, Euroclone, Milano, Italy). Both culture media were supplemented with 10% Fetal Bovine Serum (FBS; Euroclone, Milano, Italy) and 1% Penicillin/Streptomycin (Pen/Strep, Euroclone, Milano, Italy). The same medium with a lower FBS concentration (1%) was used for the viral propagation and drug susceptibility testing. Cells were incubated at 37 °C in a humidified incubator supplemented with 5% CO2. All the virus stocks were titrated by plaque reduction assay (PRA), as previously described. Briefly, VERO E6 cultures were infected with SARS-CoV-2 and monitored by microscopy every 24h. In the presence of large cytopathic effects induced by viral replication, cell cultures were subjected to one cycle of freezing and thawing, with cellular debris then being cleared through centrifugation for 30 min at 1300× g, and virus stock titrated through PRA. Viral titer was expressed as plaque-forming units (PFU)/mL.

2.2. Antiviral Assays

To determine the antiviral activity of candidate compounds against SARS-CoV-2, a DYRA, based on the infection of cells in the presence of serial drug dilutions, was performed. Briefly, 25,000 Calu-3, pre-seeded in the 96-well plates, were treated with serial dilutions of each tested compound, and incubated for 30′ at 37 °C with 5% CO2. The virus stock was added at a concentration of 250 PFU/well, then, after 1 h of adsorption, the medium was removed, and fresh dilutions of each tested compound were added to the cells. After an incubation of 48h at 37 °C with 5% CO2, the antiviral activity was measured on the cell monolayers by an immunodetection assay (IA), consisting of the fixation and permeabilization of cells, followed by 1 h incubation with a monoclonal SARS Nucleocapsid Protein Antibody (Novus, Milano, Italy, cat. AP201054), diluted 1:1000 in blocking buffer (PBS containing 1% BSA and 0.1% Tween 20) [Ref]. After washing, monolayers were incubated for 1 h with a polyclonal HRP-coupled anti-mouse IgG secondary antibody (Novus Bio, Milano, Italy, NB7570), diluted 1:5000 in blocking buffer. After cell washing, the 3,3′,5,5′-Tetramethylbenzidine substrate (Sigma Aldrich, Darmstadt, Germany) was added to each well and the reaction was stopped with one volume of 0.5 M sulfuric acid. Absorbance was measured at 450 nm optical density (OD450) using the Absorbance Module of the GloMax® Discover Multimode Microplate Reader (Promega).

2.3. Extraction of the essential oil

The four species of aromatic and medicinal plants, representing several families and genera, were collected in various regions of Morocco. The essential oils were obtained by hydrodistillation of the aerial parts (stems, leaves and flowers) in fractions of 250 g for a duration of 3h, using a Clevenger type extractor according to the method recommended in the *European pharmacopoeia* [58]. Essential oils were separated from the aqueous phase and then dried with anhydrous Na_2SO_4 and stored in an amber bottle at 4 °C until use. The essential oil yield (%) was calculated as follows:

Yield (%) = EO (g)/Dry matter (g) x 100

2.4. Analysis by GC and GC/MS

Analyses were carried out using a Perkin-Elmer Clarus 690 (Walthon, MA, USA) equipped with dual flame ionisation detection (FID) system and fused-silica capillary columns, namely, Rtx-1 (polydimethylsiloxane) and Rtx-wax (poly-ethyleneglycol) (60 m × 0.25 mm i.d; film thickness 0.25 μm). The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C for 30 min: hydrogen was employed as carrier gas (0.7 mL/min). The injector and detector temperatures were maintained at 280°C, and samples were injected (0.5 μL HE diluted in ethanol grade FID) in the split mode (1/80). Retention indices (RI) of compounds were determined relative to the retention times of a series of n-alkanes (C5–C30) by linear interpolation using the Van den Dool and Kratz equation with the aid of software from Perkin-Elmer (Total Chrom navigator). The relative percentages of the oil constituents were calculated from the GC peak areas, without application of correction factors. Samples were also analysed with a Perkin-Elmer Autosystem XLClarus SQ8 C, T, Scoupled to a Perkin-Elmermass detector Turbo Mass, equipped with fused-silica capillary column Rtx-Wax. The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C (30 min): hydrogen was employed as carrier gas (0.7 mL/min). The following chromatographic conditions were employed: injection volume, 0.5 μL of HE diluted in ethanol grade FID; injector temperature, 280°C; split, 1:80; ion source temperature, 150°C; ionisation energy, 70 eV; MS (EI) acquired over the mass range, 35–350 Da; scan rate, 1 s. Identification of the components was based on: (a) comparison of their GC retention indices (RI) on non-polar and polar columns, determined from the retention times of a series of n-alkanes with linear interpolation, with those of authentic compounds or literature data; (b) on computer matching with commercial mass spectral librarie and comparison of spectra with those of our personal library; and (c) comparison of RI (Retention indices) and MS spectral data of authentic compounds or literature data.

2.5. Antioxidant test by DPPH

The free radical scavenging activity of the extracts was measured by DPPH. Briefly, 0.2 mM solution of DPPH in methanol was prepared, 0.5 mL of this solution was added to 2.5 mL of extract and was allowed to stand at room temperature for 30 min. The absorbance was then read at 517 nm against blank samples. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity [51]. The free radical scavenging activity determined by DPPH was expressed using IC_{50} values (the concentration of extract required to inhibit 50% of the initial DPPH free radical). The results were compared to those of Ascorbic acid [1 - 20 μg/mL].

2.6 Statistical Analysis

Results are presented as mean ± SD of at least 5 independent experiments. Statistical comparisons were done using a one-way ANOVA, followed by a Dunnett's test for comparison against a single group. Data with $P \leq 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Essential oil yields

The mean yield of essential oil was calculated as a function of plant material. The plants of Thymus vulgaris, *Rosmarinus officinalis*, *Artemesia mesatlantica* and Syzygium aromaticum yielded approximately 1.35, 1.29, 0.89 and 2.89%, respectively (Table 1). The EO yield of T. vulgaris is close to that reported by [2], where the yield ranges from 1.75 to 2.05 % [10]., reported a yield of 1.5% of T. vulgaris EO. Additionally, lower yields than that obtained in this study were found in other studies in Morocco (1%) (9 [4],The EO of rosemary had a pale yellow color and a strong odor, and the obtained yield of the essential oil was 1.03% (v/w in dry matter). The average EO of *A. mesatlantica* content of this species is 0.5% on a dry matter basis [19]. Another study [20] showed that the yield of EO of the aerial part (stems, leaves and flowers) of *A. mesatlantica* is 0.97% [20] .The highest yield found in this study is that of EO of S. aromaticum (2.89%), our finding was in agreement with those of [35] [36] [37] [38] and [39] who obtained varying yield from 0.18 to 7.6%. Although, these results are different from those acquired by [33] and [34] 11.5% and 10.54%, respectively. Figure 1 shows a histogram representing the different effects of the 4 essential oils on the activity of SARS-CoV-2.

3.2 Essential oil chemical composition

The results of GC-FID and GC-MS analyses of the essential oils studied are presented in Tables 2-5.

3.2.1 Thymus vulgaris EO

The results obtained by GC-FID and GC-MS analyses of the essential oils T. vulgaris are presented in Table 1. Thirty-five compounds were identified in the essential oils of *T. vulgaris.* The results of GC/FID and GC/MS analysis of *T. vulgaris* essential oil show that the major components are Endo-borneol (23.48%) and thymol (19.54%) respectively. Other minor compounds were detected such as: □-terpineol (9.29 %), p-cymene (9.44 %), carvacrol (7.10) and camphene (5.92 %). From the chemical composition of this oil, it can be deduced that it belongs to the endo-borneol chemotype. These results are different to those reported by [54] with carvacrol (34.62%) and thymol (27.43%) as the majority compounds identified in the EO of T. vulgaris from Algeria. [4] investigated the composition of the EO of T. vulgaris, grown in Morocco allowed the identification of 99% of constituents. Thymol has the highest content of the order 41.4%, γ-terpinene 22.25% and p-cymene 15.59%. Similar [10] founded that the main compounds of this EO are Thymol (36.70%), p-Cymene (30.00%), γ-Terpinene (9.00%) and Carvacrol (3.60%). According to Roman (2009), analyses showed that the majority substances for T.vulgaris were thymol 60.3% and p-cymene 10.1%.[6]), reported to have extracted an oil characterized by a high content of thymol (34.6%), γ-terpinene (17.6%) and p-cymene (17.6%). On the other hand, they differ from those published by [7], whose oil is characterized by a high content of thymol (36.6%), α- thujone (23.2%) and 1,8-cineole (13.4%). Alexandre et al., (2008) also reported that thymol (44.77%), p-cymene (18.6%) and γ-terpinene (16.5%) are major compounds of T. vulgaris grown in Rio de Janeiro State (Brazil). The GC-MS analysis showed the presence of 16 chemical constituents, and thymol (55.88 %), linalool (13.71 %), carvacrol (8.36 %), and p-cymene (6.00 %) were found as major components [11]. The T. vulgaris species from Ain Defa region (Northern Algeria) is characterized by Carvacrol (71.35%) as major compound, followed by other constituents with much lower contents: p-Cymene (8.17%), γ-Terpinene (5.48%), Thymol (3.46%), Linalool (1.89%), α-Pinene (1.25%) and α-Terpinene (1.22%) [52]. Moreover, in Cameroon, [53] noted that the EO of T. vulgaris is dominated by Thymol (40.1%), p-Cymene (23.4%), γ-Terpinene (15.1%), with the presence of Carvacrol in low content (2.4%); the number of identified compounds is 23 chemical compounds. The variation of the chemical composition of essential oils is influenced by edaphic and climatic conditions as well as the cultivation conditions of the plants and by the method of extraction and preservation [3,1].

3.2.2 Rosmarinus officinalis EO

The results obtained by GC-FID and GC-MS analyses of the essential oils R. officinalis are presented in Table 2. Fifty-two compounds were identified in the essential oils of R. officinalis. The main compound identified in the essential oil of R. officinalis is 1,8-cineole (47.08%), followed by camphor (16.24%), α-pinene (11.13%), Borneol (4.58%), □-terpineol (3.97%), □-pinene (2.84%), □-myrcene (1.09%), p-cymene (2.01%) and Linalol (1.26%). These results are similar to those reported by [55] in the Bibans region (Algiers), the majority compound is 1,8-cineole (52.4%), followed by camphor (12.6%). Comparatively, Moroccan rosemary has a high content of one of the 3 compounds: α-pinene (37.0 - 40.0%, Rabat), 1,8-cineole (58.7 - 63.7%, El Ateuf), camphor (41.7 - 53.8%, Taforhalt) [56]. The major compounds identified by GC-FID and GC-MS were 1,8-cineole (43.77%), camphor (12.53%), α-pinene (11.51%), β-pinene (8.16%), camphene (4.55%) and β-caryophyllene (3.93%) [44] [13] have reported that the essential oil of rosemary containing mostly 1,8-cineole (46.4%), camphor (11.4%) and α-pinene (11.0%). [14] investigated the composition of rosemary EO which mainly consists of 1,8-cineole (26.54%) and α-pinene (20.14%). [15] have observed that the major components of rosemary EO are camphor (37.6%), 1,8-cineole (10.0%), p-cymene-7-ol (7.8%) and borneol (5.4%). [16] reported that the major compounds of EO were 1,8-cineole, camphor, α-pinene, camphene, β-pinene, and terpinen-4-ol, with some minor compounds such as β-myrcene, α-terpinene, cis-sabinene hydrate, linalool, borneol, α-phellandrene, α-terpineol, and β-caryophyllene. Sixteen compounds were identified in rosemary EO, dominated by 1,8-cineole (32.18%), camphor (16.20%) and α-pinene (15.40%) in the cultivated type. The α-pinene (51.19%) presents the majority compound in the rosemary samples [17].

Table 1: Yields of the studied essential oils

Oil	Yield (%)
Thymus vulgaris EO	1.35 ± 0.13
Rosmarinus officinalis EO	1.29 ± 0.04
Artemesia mesatlantica EO	0.89 ± 0.20
Syzygium aromaticum *EO*	2.89 ± 0.40

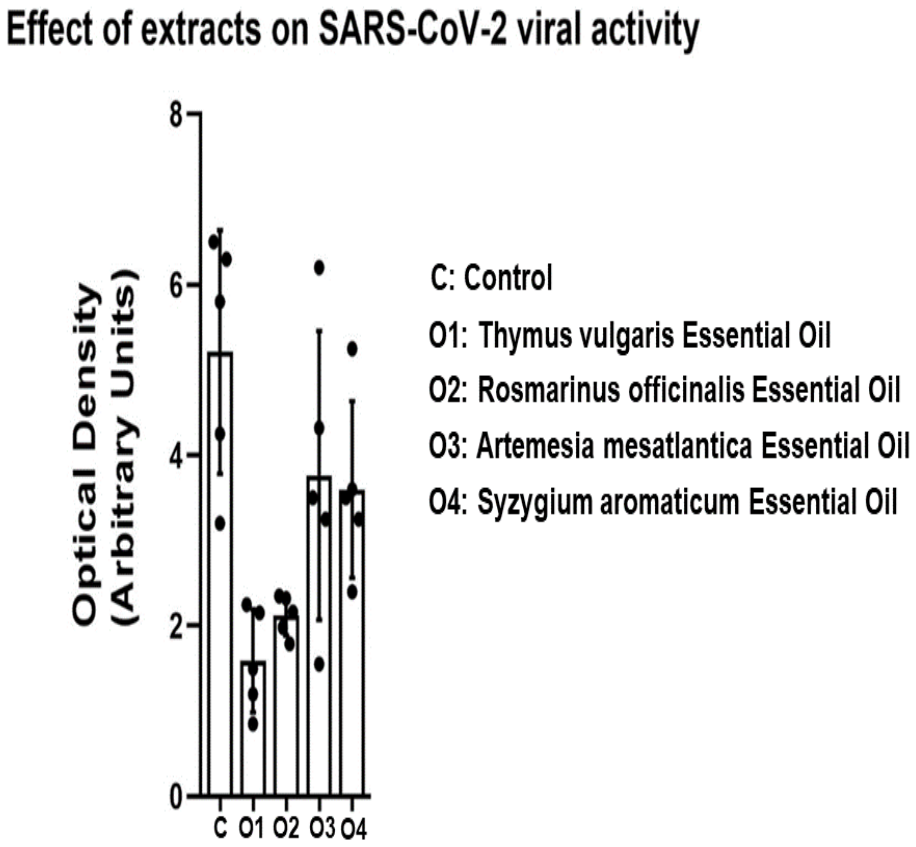

Figure 1: The effect of essential oils of the 4 plants on the viral activity of SARS-CoV-2

Table 2: Chemical composition of essential oil of *T. vulgaris*

No	Compound Name	RI l	RI a	%
1	α-thujene	932	927	0.59
2	α-pinene	936	936	3.27
3	Camphene	950	950	5.92
4	β-pinene	978	976	0.57
5	α-myrcene	987	984	0.99
6	Phellandrene	998	1002	0.06
7	α-3-carene	1010	1010	0.03
8	β-terpinene	1013	1014	0.37
9	Para-cymene	1015	1018	9.44
10	Limonene	1025	1026	1.06
11	Trans-α-ocimene	1038	1039	0.03
12	α-terpinene	1051	1053	1.86
13	Trans-sabinene hydrate	1051	1058	0.02
14	Cis-linalol oxide	1072	1062	0.05
15	1-nonen-3-ol	1058	1065	0.02
16	Para-cymenene	1076	1077	0.11
17	Terpinolene	1086	1083	0.14
18	Linalol	1086	1088	2.34
19	α-campholenal	1105	1110	0.03
20	Cis-p-meth-2-en-1-ol	1108	1112	0.04
21	Camphre	1123	1127	1.43
22	Isoborneol	1142	1147	0.02
23	Endo-borneol	1150	1162	23.48
24	Terpinen-4-ol	1164	1169	1.47
25	α-terpineol	1179	1182	9.29
26	Carvacrol	1278	1205	0.02
27	Bornyl formate	1208	1217	0.26
28	Isothymol methyl ether	1230	1230	1.23
29	Carvenone	1236	1244	0.07
30	Anethol	1264	1265	0.28
31	Thymol	1267	1278	19.54
32	Bornyle acetate	1273	1279	0.16
33	Carvacrol	1278	1286	7.10
34	Eugenol	1331	1334	0.12
35	β-copaene	1379	1380	0.09
	Total composés identifiés			91.52

a : Retention indices on apolar column (Rtx-1).

l : Retention indices from literature.

Table 3: Chemical composition of essential oil of *R. officinalis*

No	Compound Name	RI l	RI a	%
1	Tricylene	921	924	0.15
2	α-pinene	936	936	11.13
3	Camphene	950	948	3.57
4	1-octen-3-ol	963	965	0.27
5	α-pinene	978	975	2.84
6	β-myrcene	987	983	1.09
7	α-phellandrene	998	1001	0.13
8	β-3-carene	1006	1009	0.03
9	α-terpinene	1013	1013	0.15
10	p-cymene	1015	1015	2.01
11	1,8-cineole	1024	1031	47.08
12	Ocimene	1028	1039	0.01
13	α-terpinene	1051	1052	0.26
14	Cis-Linalol oxide	1064	1060	0.02
15	Fenchone	1076	1073	0.02
16	Para-cymenene	1076	1076	0.07
17	Terpinolene	1086	1082	0.14
18	Linalol	1086	1088	1.26
19	Fenchol	1099	1106	0.09
20	Cis-p-menth-2-en-1-ol	1108	1113	0.05
21	Camphor	1123	1131	16.24
22	Isopulegol	1144	1134	0.13
23	Camphene hydrate	1138	1138	0.08
24	Pinocarvone	1137	1143	0.10
25	Neo-isopulegol	1176	1146	0.06
26	Borneol	1150	1156	4.58
27	Terpinene-4-ol	1164	1167	1.10
28	α-terpineol	1179	1179	3.97
29	Myrtenol	1178	1184	0.05
30	Verbenone	1183	1186	0.15
31	Trans-piperitol	1192	1201	0.03
32	Citronellol	1213	1211	0.04
33	Carvone	1214	1219	0.04
34	Perilla aldehyde	1252	1236	0.01
35	1-decanol	1257	1256	0.03
36	Anethol	1264	1263	0.12
37	Thymol	1267	1270	0.02
38	Bornyl acetate	1273	1272	0.50
39	Carvacrol	1278	1279	0.07
40	Eugenol	1331	1331	0.04
41	1-undecanol	1358	1357	0.01
42	Methyl eugenol	1370	1372	0.04
43	Caryophyllene	1421	1421	0.55
44	Geranyle acetate	1429	1429	0.07
45	β-Humulene	1451	1453	0.09
46	α-bisabolene	1483	1501	0.02
47	Caryophyllene alcool	1557	1562	0.01
48	Caryophyllene oxide	1578	1573	0.26
49	Humulene epoxide	1591	1597	0.07
50	Methyl jasmonate	1612	1610	0.03
51	Humulenol II	1630	1619	0.09
52	Caryophyllene-14-hydroxy-9-epi	1672	1643	0.26
	Total composés identifiés			99.23

a : Retention indices on apolar column (Rtx-1).
l : Retention indices from literature.

Table 4: Chemical composition of essential oil of *A. mesatlantica*

No	Compound Name	RI l	RI a	%
1	α-thujene	932	926	0.16
2	β-pinene	936	934	0.25
3	Camphene	950	948	0.46
4	Sulcatone	964	963	0.06
5	Sabinene	967	969	0.45
6	α-pinene	978	975	0.18
7	2,3-dehydro-1,8-cineol	979	982	0.06
8	α-phellandrene	998	1001	0.02
9	β-terpinene	1013	1012	0.09
10	Para-cymene	1015	1016	1.71
11	1,8-cineole	1024	1025	4.05
12	β-terpinene	1051	1052	0.26
13	Cis-sabinene hydrate	1060	1058	0.51
14	Terpinolene	1086	1083	0.14
15	α-thujone	1089	1095	15.66
16	α-thujone	1097	1109	32.38
17	Cis-p-menth-2-en-1-ol	1108	1113	0.48
18	Camphre	1123	1129	9.57
19	Sabinaketone	1143	1133	0.30
20	Isothujol	1124	1137	0.10
21	Pinocarvone	1137	1144	0.10
22	Borneol	1150	1155	0.87
23	Myrtenal	1172	1163	0.05
24	Terpinen-4-ol	1164	1167	2.53
25	α-terpineol	1179	1177	0.70
26	Myrtenol	1178	1179	0.10
27	Cis-piperitol	1183	1184	0.14
28	Trans-piperitol	1192	1194	0.25
29	Fragranol	1196	1198	0.13
30	Carveol	1206	1201	0.10
31	Cis-p-mentha-1(7),8-dien-2-ol	1201	1204	0.04
32	Nerol	1215	1214	0.45
33	Carvone	1214	1219	0.05
34	Carvotanacetone	1218	1225	0.04
35	Piperitone	1232	1231	0.75
36	Carvenone	1236	1234	0.02
37	Phellandral	1252	1252	0.01
38	Anethol	1264	1262	0.05
39	Para-cymen-7-ol	1270	1267	0.36
40	Carvacrol	1278	1280	0.46
41	1,4-p-menthadien-7-ol	1310	1307	0.12
42	Piperitenone	1315	1314	0.08
43	Eugenol	1331	1331	0.08
44	Cis-jasmone	1368	1370	0.02
45	α-copaene	1379	1378	0.04
46	β-cedrene	1411	1416	0.03
47	Trans-caryophyllene	1419	1421	0.04
48	Germacrene D	1479	1479	0.07
49	Epicubebol	1489	1483	0.03
50	Palustrol	1563	1564	0.23
51	Spathulenol	1572	1568	0.45
52	Caryophyllene oxide	1578	1574	1.33
53	Ledol	1586	1597	1.34
54	δ-cadinol	1633	1623	2.54
	Total composés identifiés			80.47

a : Indices de rétention sur colonne apolaire (Rtx-1).
l : Indices de rétention issus de la littérature.

Table 5: Chemical composition and Percentage of essential oil constituents of *S. aromaticum*

No	Compound Name	RI l	RI a	%
1	Eugenol	1331	1332	67.28
2	3-Allylguaiacol	1362	1362	9.15
3	β-caryophyllene	1421	1423.6	5.36
4	α-Humulen	1451	1453	2.13
5	Gemacrene D	1479	1479.7	0.13
6	Eugenyl acetate	1524	1523	12.58
7	γ-Cadinene	1534	1532	0.28
8	β-caryophyllene oxyde	1578	1574.4	0.74[2]

Table 6: Antioxidant activity of EOs in reducing power and DPPH assays

EOs	IC_{50} (µg/mL)
Thymus vulgaris	203.889 ± 7.539
Rosmarinus officinalis	> 2000
Syzygium aromaticum	5.318 ± 0.155
Artemisia mesatlantica	1376.204 ± 9.302
Ascorbic acid	1.907 ± 0.038
Trolox	1.298 ± 0.022

3.2.3 Artemisia mesatlantica EO

The results obtained by GC-FID and GC-MS analyses of the essential oils *A. mesatlantica* are presented in Table 2. Fifty-four compounds were identified in the essential oils of *A. mesatlantica*. The EO was characterized by high quantities of β-thujone (32.38%). Other molecules are present in different proportions namely: α-thujone (15.66%), Camphor (9.57%), 1,8-cineole (4.05%) and The other 50 compounds are presented in low quantities in the EO of *A. mesatlantica* from the region of Ifrane. The main components identified by [18] of *A. mesatlantica* essential oil, were β-thujone (33.9%) followed by camphor (7.5%), 1,8-cineole (6.9%), sabinene (6%) and α-thujone (5.5%). Sixty compounds were identified by GC and GC/MS; β-thujone (56.33%) is the major compound identified followed by camphene (7.48%) and camphor (4.17%) [19] A previous study [20] reported that the essential oil of *A. mesatlantica* is rich in β-thujone (77.77%), followed by 1,8-cineole (6.31%) and camphor (3.52%). Other compounds are present in small quantities such as cis-β-dihydroterpineol (2.94%), terpinen-4-ol (2.10%) and Camphene (1.44%).

In fact, [21] have found that the predominant components of the EO of *A. mesatlantica* collected in the Boulmane region were: ß-thujone (56.33%) followed by camphene (7.48%) and camphor (4.17%). Another study by [22]) reported that the EO of *A. mesatlantica* is dominated by ß-thujone (60%). In contrast, [24] found a chemotype composed mainly by ß-thujone and camphor (34% and 32%, respectively) in *A. mesatlantica* EO from the Ifrane region. [23] extracted the essential oil of *A. mesatlantica* using a conventional method by microwave and steam entrainment. Where β-thujone is the predominant compound for both techniques steam entrainment (62.05%) and microwave (51.81%), followed by camphor (14.39%) for microwave and α-thujone (4.68%) for steam entrainment.

3.2.4 Syzygium aromaticum EO

The results obtained by GC-FID and GC-MS analyses of the essential oils S. aromaticum are presented in Table 4. Characterization of S. aromaticum EO by gas chromatography coupled with mass spectrometry indicates that the major components are: eugenol (67.28%), eugenyl acetate (12.58%) 3-Allylguaiacol (9.15%), β-caryophyllene (5.36%) and α-Humulen (2.13%). The content of the other constituents is often less than 1%. [25] reported the presence of 18 components in clove bud essential oil. The major compounds characterized were eugenol (87%), chavibetal (19.7%), β-caryophyllene (13%), eugenol acetate (8.01%), trisiloxane 1,1,1,5,5,5-hexamethyl-3, 3-bis [(trimethylsilyl) oxy] (1.7%) etc. Further studies [27,28,26] found the eugenol (74.32%) followed by the β-caryophyllene (15.94%) and eugenol acetate (5.8%) as main compounds of clove bud EO. Another study [30] also studied the chemical composition of clove bud EO through GC-MS and reported the presence of eugenol, β-caryophyllene, caryophyllene oxide, eugenol acetate, α-selinene, cadinene. [31] detected total 9 components in clove bud essential oil among them eugenol (49.0%), 3-phenylprop-2-enal (14.32%) and β-caryophyllene (7.5%) were major compounds. [32]) showed the presence of oxygenated monoterpenes (89.06%), monoterpenes (0.04%),

sesquiterpenes (10.6%) and linear components (0.03%) in clove bud EO and eugenol (87.62%) as main compound.

3.3. Antioxidant test by DPPH

In this study, reducing power and DPPH radical scavenging capacity has been used to evaluate antioxidant activity of different essential oil studies. Table 6 summarized the IC50 values of DPPH scavenging activity of EOs. The IC50 value of different essential oils (T. vulgaris, R. officinalis, *A. mesatlantica* and S. aromaticum) were 203.889 ± 7.539 µg/mL, up to 2000 µg/mL, 1376.204 ± 9.302 µg/mL and 5.318 ± 0.155 µg/mL, respectively, for comparison we used two standards (Trolox and ascorbic acid) with an IC50 of 1,298 ± 0.022 µg/mL and 1.907 ± 0.038 µg/mL respectively. In comparison with standards, S. aromaticum EO show a high antioxidant activity, higher than the other EO, this could be due to the high content of eugenol, which had a very high antioxidant power [47]. Several authors found a lower IC50 values of S. aromaticum EO in comparison with our study [40] (0.2 µg/mL), [38] (4.5 µg/mL) and [43] (0.048 µg/mL)), but others found it higher [39] (23.17 µg/mL), [41] (26 µg/mL) and [42] (380 µg/mL). The present study found that thyme had a higher antioxidant capacity than R. officinalis and *A. mesatlantica* but lower than S. aromaticum (IC50 = 203.88). This could be due to the presence of a high thymol content in T. vulgaris. Previous studies found that thymol has a higher antioxidant activity due to the greater steric hindering effects of the phenolic group in thymol, which is greater than that of carvacrol [57], our T. vulgaris had higher antioxidant activity than those found by [48] (IC50 = 437 µg/mL) but lower antioxidant activity than those found by [49], (IC50 = 41.4 µg/mL). In the third place, comes *A. mesatlantica* with an IC50 value of 1376.204, very high value which mean a very low antioxidant activity, To the best of our knowledge, this is the first study of the antioxidant activity of essential oil from the *A. mesatlantica*, in comparison to the other Artemisia species, according to [50] antioxidant (DPPH) radical scavenging activities were determined, and weak activities were found for Artemisia oils. R. officinalis shows the very low antioxidant activity, the IC50 value exceeds 2000 µg/mL, in contrary to the result of [44] who found that R. officinalis EO from Serbia has a high antioxidant effect (IC50 = 77.6 µL/mL). [45] ,found also a high antioxidant activity for R. officinalis EO collected in Pakistan (IC50 = 20.9 ± 0.9 µg/mL), [46] report that the IC50 of R. officinalis EO from five places in Palestine (Hebron, Ramallah, Tulkarm, Jenin and Nablus) were 107.15, 158.48, 37.15, 10.23, and 38.9 µg/mL, respectively.

References

[1] S. Echeverrigaray, G. Agostini, L. Atti-Serfini, N. Paroul, G. Pauletti, A. Atti dos Santos. (2001). Correlation between the chemical and genetic relationships among commercial thyme cultivars. Journal of agricultural and food chemistry. 49(9): 4220-4223.

[2] M. El Ajjouri, M. Ghanmi, B. Satrani, F. Amarti, M. Rahouti, A. Aafi, M.R. Ismaili, A. Farah. (2010) Chemical composition and antifungal activity of the essential oils of Thymus algeriensis Boiss. & Reut. and Thymus ciliatus (Desf.) Benth. against wood-rotting fungi. Acta botanica gallica . 157(2): 285-294.

[3] R. Kulkarni, K. Baskaran, S. Ramesh, S. Kumar. (1997). Intra-clonal variation for essential oil content and composition in plants derived from leaf cuttings of rose-scented geranium (Pelargonium sp.). Industrial crops and products. 6(2): 107-112.

[4] F. El-Akhal, H. Greche, F.O. Chahdi, R. Guemmouh, A.E.O. Lalami. (2015). Chemical composition and larvicidal activity of Culex pipiens essential oil of Thymus vulgaris grown in Morocco. Journal of Environmental Sciences. 6(1): 214-219.

[5] R. Pavela. (2009). Larvicidal property of essential oils against Culex quinquefasciatus Say (Diptera: Culicidae). Industrial crops and products. 30(2): 311-315.

[6] J.A. Pino, M. Estarrón, V. Fuentes. (1997). Essential oil of thyme (Thymus vulgaris L.) grown in Cuba. Journal of Essential Oil Research. 9(5): 609-610.

[7] N. Naguib. (2002). Thyme (Thymus vulgaris L.) growth, oil quality, yield and chemical composition as affected by of chelated iron and two potassein forms. Arab Universities Journal of Agricultural Sciences. 10 (3), 893-918.

[8] A. Porte, R.L. Godoy. (2008). Chemical composition of Thymus vulgaris L.(Thyme) essential oil from the Rio de Janeiro state, Brazil. Journal of the Serbian Chemical Society. 73(3): 307-310.

[9] B. Imelouane, H. Amhamdi, J.-P. Wathelet, M. Ankit, K. Khedid, A. El Bachiri. (2009). Chemical composition and antimicrobial activity of essential oil of thyme (Thymus vulgaris) from Eastern Morocco. International Journal of Agriculture And Biology. 11(2): 205-208.

[10] H. Niksic, F. Becic, E. Koric, I. Gusic, E. Omeragic, S. Muratovic, B. Miladinovic, K. Duric. (2021). Cytotoxicity screening of Thymus vulgaris L. essential oil in brine shrimp nauplii and cancer cell lines. Scientific reports. 11(1): 13178.

[11] A. Ali. (2021). Chemical Composition, α-Glucosidase Inhibitory and Anticancer Activity of Essential Oil of Thymus vulgaris Leaves. Journal of Essential Oil Bearing Plants. 24(4), 695-703.

[12] A. Rašković, I. Milanović, N. Pavlović, T. Ćebović, S. Vukmirović, M. Mikov. (2014). Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. BMC complementary and alternative medicine. 14(1), 1-9.

[13] M. Sienkiewicz, M. Lysakowska, M. Pastuszka, W. Bienias, E. Kowalczyk. (2013). The potential of use Basil and Rosemary essential oils as effective antibacterial agents. Molecules. 18, 9334–9351.

[14] Y. Jiang, N. Wu, Y.-J. Fu, W. Wang, M. Luo, C.-J. Zhao, Y.-G. Zu, X.-L. Liu. (2011). Chemical composition and antimicrobial activity of the essential oil of Rosemary. Environmental toxicology and pharmacology. 32(1): 63-68.

[15] M.S. Bendeddouche, H. Benhassaini, Z. Hazem, A. Romane. (2011). Essential oil analysis and antibacterial activity of Rosmarinus tournefortii

from Algeria. Natural Product Communications. 6(10): 1934578X1100601026.

[16] S. Rathore, S. Mukhia, S. Kapoor, V. Bhatt, R. Kumar, R. Kumar. (2022). Seasonal variability in essential oil composition and biological activity of *Rosmarinus officinalis* L. accessions in the western Himalaya. Scientific Reports. 12(1), 1-13.

[17] M. Elyemni, F. El Ouadrhiri, A. Lahkimi, T. Elkamli, A. Bouia, N. Eloutassi. (2022). Chemical composition and antimicrobial activity of essential oil of wild and cultivated *Rosmarinus officinalis* from two Moroccan localities. Journal of Ecological Engineering. 23(3).

[18] K. Boumhara, M. Tabyaoui, C. Jama, F. Bentiss. (2015). Artemisia Mesatlantica essential oil as green inhibitor for carbon steel corrosion in 1 M HCl solution: Electrochemical and XPS investigations. Journal of Industrial and Engineering Chemistry. 29, 146-155.

[19] H.K. Bencheqroun, M. Ghanmi, B. Satrani, A. Aafi, A. Chaouch. (2012). Antimicrobial activity of the essential oil of an endemic plant in Morocco Artemisia mesatlantica. Bulletin de la société Royale des sciences de Liège.

[20] D. Hinane, S. Oubaha, F. Hama, D. Hmouni, B. Satrani, M. Ghanmi, B. Bourkhiss. (2020). Chemical and microbiological characterization of the essential oil of Artemisia mesantlantica domesticated endemic species of Morocco. International Journal of Innovation and Applied Studies. 30(1), 246-250.

[21] M. Ghanmi, B. Satrani A. Aafi, M.R. Isamili, H. El Monfalouti, K.H. Bencheqroun, M. Aberchane, L. Harki, A. Boukir, A. Chaouch, Z. Charrouf. (2010).Effect of harvest date on the yield, chemical composition and bioactivity of essential oils of white anoise (Artemisia herba alba) from the Guercif region (eastern Morocco). Phytothérapie 8, 295-301.

[22] M. Holeman, A. Idrissi, M. Berrada. (1991). Flavonoids and Sesquiterpene Lactones of Artemisia mesatlantica. Planta Medica. 57 (2), 198-9.

[23] Z. Sekkat, M. Rouani, S. Ghazi, A. Il Idrissi, M. Arahou, R. Hassikou. (2017). Chemical characterization of Artemisia mesatlantica essential oils and evaluation of their anticandidosic potential. Phytothérapie. 15: 253-262.

[24] A. Ouyahya, R. Negre, J. Viano, Y. Lozano, E.M. Gaydou. (1990). Essential oils from Moroccan Artemisia negrei, *A. mesatlantica* and A. herba alba. Lebensmittel-Wissenschaft & Technologie. 23(6): 528-530.

[25] M.H. Alma, M. Ertaş, S. Nitz, H. Kollmannsberger. (2007). Chemical composition and content of essential oil from the bud of cultivated Turkish clove (Syzygium aromaticum L.). Bio Resources. 2(2): 265-269.

[26] H. Kasai, M. Shirao, M. Ikegami-Kawai. (2016). Analysis of volatile compounds of clove (Syzygium aromaticum) buds as influenced by growth phase and investigation of antioxidant activity of clove extract. Flavour and Fragrance Journal. 31:178-184.

[27] M.S.A. Khan, M. Zahin, S. Hasan, F.M. Husain, I. Ahmad. (2009). Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. Letters in applied microbiology. 49(3): 354-360.

[28] C.M. Marya, G. Satija, R. Nagpal, R. Kapoor, A. Ahmad. (2012). In vitro inhibitory effect of clove essential oil and its two active principles on tooth decalcification by apple juice. International journal of dentistry. 2012.

[29] O. Kerdchoechuen, N. Laohakunjit, S. Singkornard, F.B. Matta. (2010). Essential oils from six herbal plants for biocontrol of the maize weevil. Horticultural Science. 45(4): 592-598.

[30] J.-G. Xu, T. Liu, Q.-P. Hu, X.-M. Cao. (2016). Chemical composition, antibacterial properties and mechanism of action of essential oil from clove buds against Staphylococcus aureus. Molecules. 21(9): 1194.

[31] S. Lee, M. Najiah, W. Wendy, M. Nadirah. (2009). Chemical composition and antimicrobial activity of the essential oil of Syzygium aromatcum flower bud (Clove) against fish systemic bacteria isolated from aquaculture sites. Frontiers of Agriculture in China, 3:332-336.

[32] P.M. Fankem, S.N. Kwanga, M. Sameza, F. Tchoumbougnang, R. Tchabong, T. Ngoune, P. Dongmo. (2017). Antioxidan t and antifungal activities of cocoa butter (Theobroma cacao), essential oil of Syzygium aromaticum and a combination of bot h extracts against three dermatophytes. American Scientific Research Journal for Engineering Technology and Sciences. 37: 255-272.

[33] W. Guan, S. Li, R. Yan, S. Tang, C. Quan. (2007). Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods. Food chemistry. 101(4): 1558-1564.

[34] W.L. Nana, P. Eke, R. Fokom, I. Bakanrga-Via, D. Begoude, T. Tchana, N.S. Tchameni, J. Kuate, C. Menut, F. Fekam Boyom. (2015). Antimicrobial activity of Syzygium aromaticum and Zanthoxylum xanthoxyloides essential oils against Phytophthora megakarya. Journal of Phytopathology. 163(7-8): 632-641.

[35] G. Ayoola, F. Lawore, T. Adelowotan, I. Aibinu, E. Adenipekun, H. Coker, T. Odugbemi. (2008). Chemical analysis and antimicrobial activity of the essential oil of Syzigium aromaticum (clove). African Journal of. Microbiology Research. 2(7): 162-166.

[36] G.A. Alitonou, F.P. Tchobo, F. Avlessi, B. Yehouenou, P. Yedomonhan, A.Y. Koudoro, C. Menut, D.K. Sohounhloue. (2012). Chemical and biological investigations of Syzygium aromaticum L. essential oil from Benin. International Journal of Biological and Chemical Sciences. 6(3): 1360-1367.

[37] O.E. Rodriguez, R.M. Sánchez, M.J. Verde, M.A. Núñez, R. Castro, A.C. Montes. (2014). Obtaining of the essential oil of Syzygium aromaticum, identification of eugenol and its effect on

Streptococcus mutans. Journal of oral research. 3(4): 218-224.

[38] A. Saeed, D. Shahwar. (2015). Evaluation of biological activities of the essential oil and major component of Syzygium aromaticum. Journal of Animal and Plant Sciences. 25(4), 1095–1099.

[39] T.A. Sokamte, D.P.M. Jazet, N.L. Tatsadjieu. (2016). In vitro & nbsp; activity of Syzygium aromaticum against food spoilage fungi and its potential use as an antiradical agent. Journal of. Microbiology Research. 6(1), 1–7.

[40] K. Chaieb, T. Zmantar, R. Ksouri, H. Hajlaoui, K. Mahdouani, C. Abdelly, A. Bakhrouf. (2007). Antioxidant properties of the essential oil of Eugenia caryophyllata and its antifungal activity against a large number of clinical Candida species. Mycoses. 50, 403–406.

[41] M. Mahboubi, M. Mahboubi. (2015). Chemical composition, antimicrobial and antioxidant activities of Eugenia caryophyllata essential oil. Journal of essential oil-bearing plantS. 18(4), 967–975.

[42] M. Viuda-Martos, Y. Ruiz Navajas, E. Sánchez Zapata, J. Fernández-López, J.A. Pérez-Álvarez. (2010). Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. Flavour and Fragrance Journal. 25(1): 13-19.

[43] S.M.A. Selles, M. Kouidri, B.T. Belhamiti, A. Ait Amrane. (2020). Chemical composition, in-vitro antibacterial and antioxidant activities of Syzygium aromaticum essential oil. Journal of Food Measurement and Characterization. 14(4): 2352-2358.

[44] A. Rašković, I. Milanović, N. Pavlović, T. Ćebović, S. Vukmirović, M. Mikov. (2014). Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. BMC complementary and alternative medicine. 14(1): 1-9.

[45] A.I. Hussain, F. Anwar, S.A.S. Chatha, A. Jabbar, S. Mahboob, P.S. Nigam. (2010). *Rosmarinus officinalis* essential oil: antiproliferative, antioxidant and antibacterial activities. Brazilian journal of Microbiology. 41: 1070-1078.

[46] N. Al-Maharik, N. Jaradat, M. Hawash, S. Al-Lahham, M. Qadi, I. Shoman, S. Jaber, R.A. Rahem, F. Hussein, L. Issa. (2022). Chemical Composition, Antioxidant, Antimicrobial and Anti-Proliferative Activities of Essential Oils of *Rosmarinus officinalis* from five Different Sites in Palestine. Separations. 9(11): 339.

[47] I. Gülçin. (2011). Antioxidant activity of eugenol: A structure–activity relationship study. Journal of medicinal food. 14(9), 975-985.

[48] H. Miladi, R. Slama, D. Mili, S. Zouari, A. Bakhrouf, E. Ammar. (2013). Essential oil of *Thymus vulgaris* L. and *Rosmarinus officinalis* L.: Gas chromatography-mass spectrometry analysis, cytotoxicity and antioxidant properties and antibacterial activities against foodborne pathogens. Natural Science. 5, 729-739.

[49] I. Stoilova, S. Bail, G. Buchbauer, A. Krastanov, A. Stoyanova, E. Schmidt, L. Jirovetz. (2008). Chemical composition, olfactory evaluation and antioxidant effects of an essential oil of Thymus vulgaris L. from Germany. Natural Product Communications. 3(7), 1934578X0800300703.

[50] D. Lopes-Lutz, D.S. Alviano, C.S. Alviano, P.P. Kolodziejczyk. (2008). Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. Phytochemistry. 69(8), 1732-1738.

[51] H. El Moudden, Y. El Idrissi, W. Belmaghraoui, O. Belhoussaine, C. El Guezzane, T. Bouayoun, H. Harhar, M. Tabyaoui. (2020). Olive mill wastewater polyphenol-based extract as a vegetable oil shelf life extending additive. Journal of Food Processing and Preservation. 44(12): e14990.

[52] El Zahed, S. S., French, S., Farha, M. A., Kumar, G., & Brown, E. D. (2021). Physicochemical and Structural Parameters Contributing to the Antibacterial Activity and Efflux Susceptibility of Small-Molecule Inhibitors of Escherichia coli. Antimicrobial agents and chemotherapy, 65(4), e01925-20. https://doi.org/10.1128/AAC.01925-20

[53] Tchoumbougnang, F., Dongmo, P. M. J., Sameza, M. L., Mbanjo, E. G. N., Fotso, G. B. T., Zollo, P. H. A., & Menut, C. (2009). Larvicidal activity on Anopheles gambiae Giles and chemical composition of essential oils extracted from four plants grown in Cameroon. Base

[54] Cheurfa, M., Allem, R., Sebaihia, M., & Belhireche, S. (2013). Effect of essential oil of Thymus vulgaris on bacterial pathogens responsible for gastroenteritis. Phytothérapie, 11, 154-160.

[55] Boutekedjiret, C., Bentahar, F., Belabbes, R., & Bessiere, J. M. (1998). The essential oil from *Rosmarinus officinalis* L. in Algeria. Journal of Essential Oil Research, 10(6), 680-682.

[56] Elamrani, A., Zrira, S., Benjilali, B., & Berrada, M. (2000). A study of Moroccan rosemary oils. Journal of Essential Oil Research, 12(4), 487-495.

[57] Yanishlieva, N. V., Marinova, E. M., Gordon, M. H., & Raneva, V. G. (1999). Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chemistry, 64(1), 59-66

[58] COUNCIL, O. E. (1997). Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine. Oviedo: COE.

[59] Wang, S., Sakhatskyy, P., Chou, T. H., & Lu, S. (2005). Assays for the assessment of neutralizing antibody activities against Severe Acute Respiratory Syndrome (SARS) associated coronavirus (SCV). Journal of immunological methods, 301(1-2), 21–30. https://doi.org/10.1016/j.jim.2005.03.008