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ORIGINAL PAPER

Chemical characterization, antioxidant, anticholinesterase and alpha-glucosidase potentials of essential oil of *Rosmarinus tournefortii* de noé

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Abstract
The aim of the present work is to characterize the chemical composition of the essential oil and to determinate the antioxidant, anticholinesterase and α-glucosidase activities of *Rosmarinus tournefortii* de Noé. The essential oil of fresh aerial part of *Rosmarinus tournefortii* de Noé was analyzed by GC–MS and revealed the presence of 1,8-Cineole (55.26%), L-Borneol (9.57%) and β-Pinène (9.41%) and were identified as major compounds. The antioxidant activity was assessed using different methods, the anticholinesterase activity was determined by AChE and BChE and the α-glucosidase activity was evaluated using 4-Nitrophenyl-α-D-glucopyranoside as substrate. The antioxidant activity showed the good antioxidant activity of the butanolic and chloroform extracts in DPPH, $ABTS^{\cdot+}$, O_2^- DMSO alkalin, Reducing power, β-Carotene-linoleic acid and CUPRAC assays. Furthermore the anticholinesterase activity against acetylcholinesterase of the essential oil exhibited the highest activity, very close to the standard galantamine and for butyrylcholinesterase the chloroform extract exhibited activity higher than the standard galantamine. Moreover the α-glucosidase inhibitory of the chloroform extract showed a higher activity more than the standard acarbose. These results suggest a possible use of *Rosmarinus tournefortii* de Noé as a potential source of bioactive molecules with anticholinesterase antioxidant and antidiabetic properties

Keywords *Rosmarinus tournefortii* · Antioxidant activity · Anticholinesterase · α-glucosidase

Introduction

The Lamiaceae family comprises 6000 species about 210 genera, Rosmarinus includes two species *Rosmarinus eriocalyx* Jordan & Fourr (syn. *Rosmarinus tournefortii* de Noé) and *Rosmarinus officinalis* L (syn. *R. laxiflorus* de Noé) [1] of the family Lamiaceae plants native of Mediterranean Basin and part of Europe, it has been used for thousands of years [2].

Rosemary is cultivated on a large scale in Spain, Algeria, Tunisia, Morocco, Italy, Portugal and France, mainly to extract the essential oil. World production of rosemary essential oil reaches 200–300 tons [3]. The leaves of rosemary were used in traditional Mediterranean cuisine but also as antioxidant in foods, nutritional supplements and cosmetics which in addition to being used as a food flavoring [4, 5]. Rosemary species have been used in folk medicine against various symptoms. They have been reported for their anti-inflammatory [6], antioxidant [7], anti-proliferative [8], analgesic [9], anti-ulcerogenic [10, 11], hepatoprotective activity [12]. Secondary metabolites such as the essential oils of various species of rosemary have been the subject of considerable researches in recent years [13]. The literature reports, the principal volatile compounds of *Rosmarinus tournefortii* are camphor (17.3–41.2%), camphene (0.7–20%), α-pinene (1.8–18.2%) and 1,8-cineole (5.7–17.4%) followed by borneol (0.1–5.5%), tricyclene (0.5–14%) and *p*-Cymene-7-ol (0–7.8%) [14-18]. Several in vitro studies report the essential oils and main isolated compounds from rosemary have been investigated for their antioxidant, anti-diabetic and anticholinesterase activity [19-22].

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However in the Mediterranean region an area that has not received enough attention for the use of *Rosmarinus tournefortii* de Noé, also called in French as Romarin and in Arabic as Klil and Iklil el djebel [22]. It is present in large quantity at Bousaada region situated in the southeast of Algeria, it has been used in folk medicine against various symptoms. The aerial part of *Rosmarinus tournefortii de Noé* is used for the treatment of abdominal pain and the leaves are prescribed to reduce high blood pressure, also, the plant powder is used to treat diarrhea. Due to the multiple traditional uses of *Rosmarinus tournefortii* de Noé in the desert of southern region of Algeria, the purpose of this work was to investigate the chemical composition of essential oil and explore the in vitro antioxidant activities of the essential oil and various extracts of the aerial part of *Rosmarinus tournefortii* de Noé by using seven methods and compare them with six antioxidants references. Further, the anticholinesterase activity (Anti-Alzheimer) was evaluated using a combination of two complementary methods systems acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The antidiabetic effect was also investigated in vitro using α-glucosidase from Saccharomyces cerevisiae which is a key in the management and treatment of Noninsulin-dependent diabetes mellitus (NIDDM) or type 2 diabetes (T2D) contributes approximately 90–95% of all cases of diabetes.

Materials and methods

Plant material

The aerial parts of *Rosmarinus tournefortii* de Noé were collected in April 2012 at Djbal antar in Bechar (southern region of Algeria). A voucher specimen was deposited in the biotechnology research center, health division (CRbt/01/2016).

Spectral measurements and chemicals used

The measurements and calculations of the activity results were evaluated by using bioactivity measurements were carried out on a 96-well microplate reader, PerkinElmer Multimode Plate Reader EnSpire at National Center of biotechnology Research. The chemical products and reagents used were: Folin-ciocalteu's reagent (FCR), 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylatedhydroxylanisole (BHA), Butylatedhydroxyltoluene (BHT), α- Tocopherol, Ascorbic acid, Tannic acid, β-carotene, linoleic acid, polyoxyethylene sorbitan monopalmitate (Tween-40), Neocuproine, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonicacid) diammoniumsalt (ABTS), Trichloroacetic acid (TCA), Potassium ferricyanide, 3-(2-Pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5′,5″-disulfonic acid disodium salt (Ferrene), Ethylenediaminetetraacetic acid (EDTA), Nitro blue tetrazolium (NTB), Diméthyl sulfoxyde (DMSO), Acetylcholinesterase from electric eel (AChE, Type-VI-S, EC 3.1.1.7, 827,84 U/mg, Sigma), butyrlcholinesterase from horse serum (BChE, EC 3.1.1.8, 7,8 U/mg, Sigma), Acetylthiocholine iodide, S-Butyrylthiocholine iodide, 5,5′-Dithiobis (2-nitrobenzoic) acid (DTNB),Galantamine,4-Nitrophenylα-D-glucopyranoside (≥ 99%), α-Glucosidase from Saccharomyces cerevisiae (Type I, ≥10 units/mg protein), Acarbose (≥ 95%) were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Stern-heim, Germany), Sodium Carbonate, Aluminum Nitrate, Iron (III) chloride (FeCl3), Iron (II) chloride, Sodium bicarbonate, Copper (II) chloride, Potassium persulfate, Potassium acetate, were obtained from Biochem Chemopharma. All other chemicals and solvents were of analytical grade.

Extraction

The hydrodistillation of fresh aerial parts (100 g) of *Rosmarinus tournefortii* de Noé, for three hours in a Clevenger apparatus, according to the British Pharmacopeia method. Air-dried and powdered aerial parts (4 g) of *Rosmarinus tournefortii* de Noé were extracted with methanol–water (80:20, v/v) in the dark at room temperature under mechanical agitation for 24 h. The residue was suspended in water and extracted successively with chloroform and butanol respectively. The collected oil and organic phases thus obtained were weighed and kept in the dark at + 4 °C until its analysis.

GC–MS analysis

GC analysis was performed using an Agilent technologies GC 17A gas chromatograph equipped with a gross-linked HP 5MS column (30 m*0.25 mm, film thickness 0.25 µm). The oven temperature was programmed as isothermal at 60 °C for 8 min, helium was used as the carrier gas at a rate of 0.5 ml/min. GC/MS was performed using a HP Agilent technologies 6800 plus mass selective detector, the operating conditions were the same as for the analytical GC the MS operating parameters were as follows: ionization potential, 70 eV; ionization current, 2 A; ion source temperature, 280 °C; resolution,1000 scan time,5 s; scan mass range, 34–450 u; spit ratio, 50:1; injected volume, 1.0 µL. The identification of compounds of the essential oil was based on their retention times in comparison with matching spectral peaks available with NIST and Wiley mass spectral libraries, as well as by comparison of the fragmentation patterns of the mass spectra and their retention indices with those reported in the literature [23]. The retention indices were calculated for all constituents, using a series of n-alkanes.

Determination of total bioactive compounds

Determination of total phenolic content (TPC)

The total phenolic content of the extracts and essential oil of *Rosmarinus tournefortii* de Noé was determined spectrophtometrically following the Folin–Ciocalteu method [24] and results was expressed as micrograms of gallic acid equivalents per milligrams of extract (µg GAE/mg).

Determination of total flavonoid Content (TFC)

The total flavonoid content of the extracts and essential oil of *Rosmarinus tournefortii* de Noé was determined spectrophtometrically method described by Tel et al. [25] and the results was expressed as micrograms quercetin equivalents per milligram of extract (µg QE/mg).

Determination of antioxidant activity

DPPH scavenging assay The DPPH scavenging activity was determined spectrophotometrically by the method described by Blois [26]. BHA, BHT, α-Tocopherol, Ascorbic acid and Tannic acid were used as antioxidant standards for comparison of the activity. The results were given as 50% inhibition concentration (IC_{50}).

ABTS cation radical Assay The ABTS scavenging activity was determined according to the method of Re et al. [27], BHA, α-Tocopherol, BHT, α-Tocopherol, Ascorbic acid and Tannic acid were used as antioxidant standards for comparison of the activity. The results were given as 50% inhibition concentration (IC_{50}).

Superoxide radical scavenging assay by alkaline DMSO Superoxide scavenging activity of essential oil and extracts of *Rosmarinus tournefortii* de Noé was determined by the alkaline DMSO described by Madan [28] with slight modification adapted at microplate-reader. BHA, BHT, α-Tocopherol, Ascorbic acid and Tannic acid were used as antioxidant standards for comparison of the activity. The results were given as 50% inhibition concentration (IC_{50}).

Reducing power assay The reducing power of essential oil and extracts of *Rosmarinus tournefortii* de Noé was determined according to the method of Oyaizu [29] with slight modification adapted at microplate-reader. The results were given as absorbance and compared with BHA, BHT, α-Tocopherol, Ascorbic acid and Tannic acid used as antioxidant standards, the results were given as $A_{0.50}$, which corresponds to the concentration producing 0.500 absorbance.

β-carotene/linoleic Acid aleaching assay The antioxidant activity was evaluated by using β-carotene-linoleic acid test by method of Marco [30] BHA, BHT, α-Tocopherol, Ascorbic acid and Tannic acid were used as antioxidant standards for comparison of the activity. The results were given as 50% inhibition concentration (IC_{50}).

Cupric reducing antioxidant capacity (CUPRAC) The cupric reducing antioxidant capacity was determined according to the method of Apak [31] BHA, BHT, α-Tocopherol, Ascorbic acid and Tannic acid were used as antioxidant standards for comparison of the activity. The results were given as $A_{0.50}$, which corresponds to the concentration producing 0.500 absorbance.

Ferrous ions chelating assay The chelating activity of essential oil and extracts of *Rosmarinus tournefortii* de Noé on Fe^{2+} was measured as reported by Decker and Welch [32]. EDTA, BHA, BHT, α-Tocopherol, Ascorbic acid and Tannic acid were used as antioxidant standards for comparison of the activity.

Bioassays

Anticholinesterase activity Acetylcholinesterase and butyrylcholinesterase inhibitory activity were measured, by the method developed by Ellman et al. [33]. Galanthamine was used as reference compound. The results were given as 50% inhibition concentration (IC_{50}).

α-Glucosidase inhibitory activity The α-glucosidase inhibitory assay was performed by applying the literature procedure with minor modifications according to Sinéad Lordan [34].

Acarbose was used as a positive control. All experiments were carried out at least in triplicate.

Statistical analysis

Results are reported as mean value ± SD of three measurements; the IC_{50} and $A_{0.50}$ values were calculated by linear regression analysis, and one-way analysis of variance ANOVA to detect significant differences ($p < 0.05$) using XLSTAT.

Results and discussion

Chemical composition of the essential oil

The hydrodistillation of freshly wet matter of the aerial parts of *Rosmarinus tournefortii* de Noé, collected from Djbal antar Bechar (southern region of Algeria), yielded 2.1%

(w/w) of white good smell oil. The identified constituents of the essential oil of *Rosmarinus tournefortii* de Noé are presented in Table 1. Forty compounds were identified by GC/MS, representing 99.77% of the essential oil mainly characterized by 1,8-Cineole (55.26%), L-Borneol (9.57%) and β-Pinène (9.41%). α-Pinene (5.96%) and 1,8-Cineole (7.6–56.50%) was mainly detected in the essential oils of *Rosmarinus officinalis L*, *Rosmarinus eriocalyx* and *Rosmarinus tournefortii* de Noé [35-37].

Other studies showed a slightly different profile concerning the characterization of target compounds of *Rosmary* from 16 regions of Algeria. Dalila Meziane [38] found that α-Pinene (20.13–72.58%) and camphor (6.64–36.44%) were identified as the main constituents of *Rosmarinus officinalis* oils.

The composition of the rosemary extracts was qualitatively similar to those obtained by Christine Tschiggerl [39] for the leaves *Rosmarinus officinalis* from Austria with, 1,8-cineole (41.6%), camphor (17.0%), α-pinene (9.9%), α-terpineol (4.9%) and borneol (4.8%).

Total phenolics and flavonoids content

The results are presented in Table 3, the phenolic content of butanolic extract (168.60 ± 3.32 µg GAE per mg extract) was found to be higher than the other extracts. This is high when compared with other studies for ethanolic extract of *Rosmarinus eriocalyx* leaves, it was found to be 58.0 ± 3.6 mg GAE/g [18].

Regarding the content of total phenols of rosemary extract, our results are higher than that reported by other authors in aqueous extract from *Rosmarinus officinalis* (16.67 ± 0.40 mg GAE/g) [40], and other authors found almost similar results with (127.87 ± 2.1 mg GAE/g dw) [41]. We assume that the quantitative differences are the consequence of different extraction methods. Also the most flavonoid rich extract was found to be butanolic extract (49.72 ± 1.47 µg QE per mg extract), this is relative high when compared with flavonoids content from ethanolic extract of *Rosmarinus officinalis* (14.48 ± 1.5 mg QE/g dw) [41]. Variation of phenolic compounds content arises due to several factors, which include the environmental stresses, geographical positions of the plants, ecological conditions and climate [42].

Antioxidant properties

In the current study, antioxidant activity of essential oil and extracts of *Rosmarinus tournefortii* de Noé was evaluated by using DPPH scavenging, ABTS cation radical, Superoxide radical scavenging, Reducing power, β-carotene/linoleic acid bleaching, Cupric reducing

Table 1 Chemical composition, retention indices and percentage composition of the essential oil of *rosmarinus tournefortii* de noé

Pic	Compound[a]	RI[b]	RI[c]	(%)
1	*cis*-3-Hexen-1-ol	866	844	0.016
2	Tricyclene	920	926	0.022
3	α-Thujene	925	930	0.222
4	α-Pinene	933	939	5.960
5	Camphene	946	946	1.219
6	β-Pinène	977	979	9.410
7	3-Octanone	988	983	0.014
8	Myrcene	992	990	0.869
9	α -Phellandrene	1005	1002	0.063
10	δ-3-Carene	1010	1011	0.051
11	α-Terpinene	1018	1014	0.386
12	1,8-Cineole	1039	1031	55.268
13	β-Ocimene	1042	1044	0.332
14	γ-Terpinene	1060	1059	0.575
15	*trans*-Sabinene hydrate	1069	1070	0.085
16	Furfuranol	1073	1072	0.012
17	*cis*-Sabinene	1101	1098	0.067
18	L-Linalool	1107	1099	3.125
19	Fenchol	1117	1116	0.033
20	*cis*-2-Menthenol	1124	1121	0.066
21	Camphor	1143	1146	1.453
22	Pinocarvone	1161	1164	0.013
23	L-Borneol	1174	1169	9.573
24	Terpinen-4-ol	1182	1177	1.650
25	α-Terpineol	1199	1188	5.430
26	*cis*-Piperitol	1212	1196	0.022
27	β-Citronellol	1234	1225	0.019
28	Carvacrol methyl ether	1237	1244	0.029
29	Carvotanacetone	1248	1310	0.048
30	Isobornyl acetate	1285	1285	0.819
31	Carvacrol	1311	1299	0.019
32	*trans*-Caryophyllene	1418	1417	1.870
33	O-Methyleugenol	1410	1403	0.035
34	β-Selinene	1451	1490	0.274
35	Apofarnesol	1454	1591	0.019
36	δ -Germacrene	1475	1485	0.009
37	β-Bisabolene	1508	1505	0.010
38	δ-Cadinene	1522	1522	0.014
39	Caryophyllene oxide	1580	1583	0.672
40	Methyl jasmonate	1650	1648	0.019
	Identified compounds (%)	Total		99.792
	Monoterpene hydrocarbons			19.192
	Oxygen-containing monoterpenes			76.723
	Sesquiterpene hydrocarbons			2.177
	Oxygen-containing sesquiterpenes			0.691
	Others			1.009

[a]Compounds listed in order of their RI

[b]RI (retention indices experimental) measured relative to n-alkanes (C_6-C_{28}) using HP-5MS column

[c]RI (retention indices from literature) Robert P. Adams (2007)

Table 2 Antioxydants activity of essential oil and various extracts of *Rosmarinus tournefortii* by DPPH·, $ABTS^{\cdot+}$, $O_2^{\cdot}$ dmsoDMSO alkalin, Reducing power, β-carotene, CUPRAC and ferrous ions chelating assays

Extracts	DPPH· assay	$ABTS^{+}$ assay	O_2^{-} DMSO alkaline assay	Reducing power assay	β-Carotene-linoleic acid assay	CUPRAC assay	Ferrous ions chelating assay
	IC_{50} µg/mL	IC_{50} µg/mL	IC_{50} µg/mL	$A_{0.50}$ µg/mL	IC_{50} µg/mL	$A_{0.50}$ µg/mL	IC_{50} µg/mL
Essential oil	>200	>200	>200	>200	314,13±1,50[a]	>200	129.28±3.16[b]
Chloroform extract	31.11±1.54[a]	16.32±2.07[a]	10.09±0.14[c]	19.87±2.38[e]	9.67±0.88[c]	31.15±2.24[a]	>200
Butanolic extract	30.91±1.83[a]	11.58±0.68[b]	25.68±0.82[b]	38.96±0.06[a]	7.99±0.31[d]	25.17±1.83[b]	>200
BHA	6.14±0.41[c]	1.81±0.10[c]	>200	7.99±0.87[d]	0.90±0.02[e]	6.62±0.05[e]	NA
BHT	12.99±0.41[b]	1.29±0.30[c]	>200	>200	1.05±0.01[e]	8.97±3.94[e]	NA
α-Tocopherol	13.02±5,17[b]	7.59±0.53[b]	31.52±2.22[a]	34.93±2.38[b]	1.79±0.03[e]	19.92±1.46[e]	NA
Ascrobic acid	13.94±2.81[b]	1.74±0.10[c]	7.59±1.16[d]	6.37±0.42[d]	52.59±1.98 [b]	12.43±0.09[d]	NA
Tannic acid	7.74±0.19[c]	1.01±0.16[c]	0.94±0.22[e]	41.07±2.36[a]	7.46±0.26[d]	3.76±0.73[f]	NA
EDTA	NT	NT	NT	NT	NT	NT	1.77±3.61[a]

IC_{50} and $A_{0.50}$ values is defined as the concentration of 50% inhibition percentages and the concentration at 0.50 absorbance respectively. IC_{50} and $A_{0.50}$ were calculated by linear regression analysis and expressed as Mean±SD (n=3). The values with different superscripts (a, b, c,d or f) in the same columns are significantly different ($p < 0.05$)

BHA butylatedhydroxyanisole, *BHT* butylatedhydroxytoluene, *EDTA* ethylenediamine tetraacetic acid, *NT* not tested, *NA* not absorbance

Table 3 Total phenolics and flavonoids content of the various extracts of *Rosmarinus tournefortii*

Extracts	Total phenolics (µg GAE/mg)*	Flavonoids (µg QE/mg)**
Chloroform extract	37.17±7.33[b]	42.43±0.32[b]
Butanolic extract	168.60±3.32[a]	49.72±1.47[a]

Results are expressed as means±standard deviation of three measures (Tukey test, $p \leq 0.05$). The values with different superscripts (a, b, c or d) in the same columns are significantly different ($p < 0.05$)

*Total phenolics is expressed as µg Gallic acid equivalents/mg of extract

**Total flavonoids are expressed as µg Quercetin equivalents/ mg of extract

antioxidant capacity and Ferrous ions chelating assays. The results of antioxidant activity are shown on Table 2 and expressed in terms of IC_{50} and $A_{0.5}$.

The results of DPPH· scavenging (Table 2) show that the butanolic extract exhibited the highest antioxidant activity (IC_{50}: 30.91 ± 1.83 µg/mL) closer activity to that of BHT, α-tocopherol and ascorbic acid (IC_{50}: 12.99 ± 0.41, 13.02 ± 5.17 and 13.94 ± 2.81 µg/mL, respectively) and less than BHA and Tannic acid (IC_{50}: 6.14 ± 0.41 and 7.74 ± 0.19 µg/mL, respectively) and followed by chloroform extract (IC_{50}: 31.11 ± 1.54 µg/mL) but the essential oil exhibited week activity at 200 µg/mL. The analysis data of the ABTS assay showed that the butanolic extract give the best activity (IC_{50}: 11.58 ± 0.68 µg/mL) compared with standards, very closer with α-tocopherol (IC_{50}: 7.59 ± 0.53 µg/mL) and week activity compared with BHA, BHT, ascorbic acid and tannic acid (IC_{50}: 1.81 ± 0.10, 1.29 ± 0.30 1.74 ± 0.10 and 1.01 ± 0.16 µg/mL, respectively) followed by chloroform extract (IC_{50}: 16.32 ± 2.07 µg/mL) furthermore the essential oil did not give any activity at 200 µg/mL. These results of $DPPH^{\bullet}$ scavenging are in agreement with values previously reported by Hamdi Bendif (2017) for stems and leaves of ethanolic extract of *Rosmarinus eriocalyx* with (IC_{50}: 31.6 ± 0.9, 25.6 ± 0.4 µg/mL, respectively), however the results of ABTS assay of our studies are better than reported in the same study with (IC_{50}: 24.6 ± 0.4, 27.6 ± 1.3 µg/mL, respectively) [18]. Low IC_{50} values against DPPH radical have been reported by Naciye et al. (2008) on the methanolic extract of *Rosmarinus Officinalis* L. with (IC_{50}: 54.0 ± 1.4 µg/mL) [43].

The compared results in Table 2 for the β-carotene assay showed the highest activity of butanolic and chloroform extracts (IC_{50}: 7.99 ± 0.31, 9.67 ± 0.88 µg/mL, respectively), the same as the activity of tannic acid (IC_{50}: 7.46 ± 0.26 µg/mL) and better than the activity of ascorbic acid (IC_{50}: 52.59 ± 1.98 µg/mL) and not far compared with BHA, BHT, ascorbic acid and α-tocopherol (IC_{50}: 0.90 ± 0.02, 1.05 ± 0.01 and 1.79 ± 0.03 µg/mL, respectively) however the essential oil exhibited the low activity at 200 µg/mL. In terms of β-carotene bleaching test, the present results of all studied extracts were higher than the previously activities reported in the leaf extract of *Rosmarinus officinalis* with (IC_{50}: 42.41 µg/ml) [44].

For the CUPRAC assay the butanolic extract exhibited the best activity (IC_{50}: 25.17 ± 1.83 µg/mL) very closer to α-tocopherol (IC_{50}: 19.92 ± 1.46 µg/mL) and moderately lower than the BHA, BHT and ascorbic acid (IC_{50}:

Table 4 Acetylcholinesterase inhibitory activity of essential oil and various extracts of *Rosmarinus tournefortii*

Extracts	Acetylcholinesterase inhibitory activity							
	3.125 µg	6.25 µg	12.5 µg	25 µg	50 µg	100 µg	200 µg	IC_{50} µg/mL
Essential oil	41.77±2.81	47.98±0.58	49.28±0.13	60.49±0.38	61,66±0.00	78.57±0.13	87.52±0.63	13.80±1.87 [a]
Chloroform extract	NA	NA	NA	14.35±0.41	30.74±3.51	37.84±1.20	52,14±0.45	180.70±0.31[b]
Butanolic extract	NA	NA	NA	NA	NA	17.29±1.76	35.75±3.34	>200
Galantamine[b]	35.93±2.28	43.77±0.00	68.50±0.31	80.69±0.41	85.78±1.63	91.80±0.20	94.77±0.34	6.27±1.15[c]

IC_{50} values is defined as the concentration of 50% inhibition percentages and calculated by linerar regression analysis and expessed es Mean ± SD (n=3). The IC_{50} values with different superscripts (a, b, c or d) in the same column are significantly different ($p < 0.05$). NA: not absorbance. b: reference compounds

6.62 ± 0.05, 8.97 ± 3.94 and 12.43 ± 0.09 µg/mL, respectively) and showed a moderate activity compared with tannic acid (IC_{50}: 3.76 ± 0.73 µg/mL) however the essential oil doesn't give an activity. The result of Reducing power assay showed that the chloroform extract exhibited the best activity (IC_{50}: 19.87 ± 2.38 µg/mL) more than BHT, α-tocopherol and Tannic acid (IC_{50}: > 200, 34.93 ± 2.38 and 41.07 ± 2.36 µg/mL, respectively) and lower to BHA and ascorbic acid (IC_{50}: 7.99 ± 1.87 and 6.37 ± 0.42 µg/mL, respectively) followed by butanolic extract (IC_{50}: 38.96 ± 0.06 µg/mL) however the essential oil exhibited a week activity. From literature, our results are in accordance with those of previous works, indicating highest values of both CUPRAC and FRAP with (698 and 1947 mM Trolox equivalent/100 ml, respectively) [45]

For the Ferrous ions chelating assay only the essential oil exhibited the week activity (IC_{50}: 129.28 ± 3.16 µg/mL) when compared with EDTA (IC_{50}: 1.77 ± 3.61 µg/mL) furthermore the other references compounds showed being no actives. In addition the results of Superoxide radical scavenging assay showed that the chloroform extract has the best activity (IC_{50}: 10.09 ± 0.14 µg/mL) closer to ascorbic acid (IC_{50}: 7.59 ± 1.16 µg/mL), better than α-tocopherol (IC_{50}: 31.52 ± 2.22 µg/mL) and low than tannic acid (IC_{50}: 0.94 ± 0.22 µg/mL) followed by the butanolic extract (IC_{50}: 25.68 ± 0.82 µg/mL) which showed the good activity compared with α-tocopherol (IC_{50}: 31.52 ± 2.22 µg/mL) and lower compared with ascorbic acid and tannic acid (IC_{50}: 7.59 ± 1.16, 0.94 ± 0.22 µg/mL) furthermore the essential oil, BHA and BHT did not exhibit any activity.

The percentage of metal scavenging capacity of the methanol extract of *Rosmarinus officinalis* was found to be 38.31% at 200 µg/ml [46] which is slightly higher than value reported in the present work.

Our results revealed that butanolic and chloroform extracts were stronger than those reported in inhibitory of superoxide generation of *Rosmarinus officinalis* founded to be 69.12% at 300 µg/ml [46]

For the all assays, the results expressed in IC_{50} (µg/mL) and $A_{0.5}$ (µg/mL) presented in Table 2 showed for the seven methods used for evaluation of the antioxidants activities a close result between the butanolic and chloroform extracts. This result may be due to the type of the phenols present in the extracts and mode of action of each antioxidant methods. The strong antioxidant activity of rosemary extracts is primarily related to the presence two phenolic diterpenes, carnosic acid and carnosol [47]

Bioassays

Anticholinesterase activity

The anticholinesterase activity of essential oil and plant extracts was evaluated using a combination of two complementary methods systems acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The results for AChE and BChE inhibition were represented in Tables 4 and 5, respectively. Against AChE enzyme, the essential oil exhibited highly potent inhibition (IC_{50}: 13.80 ± 1.87 µg/mL), very close to the standard AChE enzyme inhibitors Galantamine (IC_{50}: 6.27 ± 1.15 µg/mL) this result is in good agreement with the results obtained from essential oil of *Rosmarinus officinalis* L, which exhibited inhibition value of (63.7 ± 1.2%) in AChE [37]. The chloroform (IC_{50}: 180.70 ± 0.31 µg/mL) and butanol extracts (IC_{50}: > 200 µg/mL) showed weak inhibitory activity against AChE.

The chloroform extract (IC_{50}: 10.03 ± 0.71 µg/mL) exhibited the highest inhibitory activity against BChE, even higher than galantamine (IC_{50}: 34.75 ± 1.99 µg/mL) and followed by butanol extract (IC_{50}: 73.94 ± 0.44 µg/mL). However the essential oil showed a weak inhibition activity (IC_{50}: 148.67 ± 1.54 µg/mL). Some studies confirmed that the majors components identified in rosemary oil by GC–MS, such as 1,8-cineole, camphor, and α-pinene are highly active monoterpenes on AChE [48].

For both enzymes (AChE and BChE), the essential oil and chloroform extract exhibited a strong activity compared with those reported in the inhibitory effect of Turkish *Rosmarinus officinalis* L [37]

Table 5 Butyrylcholinesterase inhibitory activity of essential oil and various extracts of *Rosmarinus tournefortii*

Extracts	Butyrylcholinesterase inhibitory activity							
	3.125 µg	6.25 µg	12.5 µg	25 µg	50 µg	100 µg	200 µg	IC_{50} µg/mL
Essential oil	NA	NA	NA	NA	NA	39,05±1.31	57.64± 2.46	148.67±1.54[a]
Chloroform extract	22.46±2.56	44.98±1.59	55.57±0.47	60.79±0.12	69.91±1.81	77.81±1.75	79.13±0.25	10.03±0.71[b]
Butanolic extract	NA	NA	8.30±1.64	21.82±2.70	49.12±3.27	56.88±1.68	63.95±2.85	73.94±0.44[c]
Galantamine [b]	3.26±0.62	6.93±0.62	24.03±2.94	45.13±2.60	63.87±2.85	73.57±0.77	78.95± 0.58	34.75±1.99[d]

IC_{50} values is defined as the concentration of 50% inhibition percentages and calculated by linerar regression analysis and expessed es Mean ± SD (n=3). The IC_{50} values with different superscripts (a, b, c or d) in the same column are significantly different ($p < 0.05$). NA: not absorbance. b: reference compounds

Table 6 α-glucosidase inhibitory assay of essential oil and various extracts of *Rosmarinus tournefortii*

Extracts	α-glucosidase inhibitory assay					
	31.25 µg	62.5 µg	125 µg	250 µg	500 µg	IC_{50} µg/mL
Essential oil	NA	NA	NA	NA	NA	NA
Chloroform extract	11.28±2.34	21.69±3.20	70.64±2.03	88.73±0.09	93.09±0.63	117.50±2.52[a]
Butanolic extract	NA	NA	8.42±0.24	17.35±2.96	17.55±3.62	>500
Acarbose[b]	78.125 µg	156.25 µg	312.5 µg	625 µg	1250 µg	275.43±1.59[b]
	27.43±2.18	38.91±3.20	54.86±1.79	67.29±2.63	80.19±1.66	

IC_{50} values is defined as the concentration of 50% inhibition percentages and calculated by linerar regression analysis and expressed as Mean ± SD (n=3). The IC_{50} values with different superscripts (a, b, c or d) in the same column are significantly different ($p < 0.05$). NA: not absorbance. b: reference compounds

α-Glucosidase inhibitory activity

As shown in Table 6, only the chloroform extract exhibited highest α-Glucosidase inhibitory activity (IC_{50}: 117.50 ± 2.52 µg/mL) more than acarbose (IC_{50}: 275.43 ± 1.59 µg/mL) used as a standard, this activity must be due to the presence of terpenoid such as ursolic acid which is identified in this extract as a major compound by other studies in our laboratory.

Furthermore, some studies reported that the enzyme inhibitory activity of Salvia extracts is attributed to the presence of ursolic acid, thus justifying the traditional use of Salvia in the management of diabetes [49]. Similarly, IC_{50} values determined in the chloroform extract of *Rosmarinus tournefortii* de Noé were comparable to the values reported of the diethyl ether fraction of *Rosmarinus officinalis* which showed a strong α-glucosidase inhibitory activity more than the acarbose (77% at 250 µg/ml) [50]

Conclusion

In conclusion and based on the results of antioxidant, anticholinesterase and α-glucosidase, it can be concluded that *Rosmarinus tournefortii* de Noé plant may be an effective source of antioxydants compounds and will probably be used for the development of additives food and protect of free radical damage. Furthermore it may be concluded that the inhibitory potentials against anticholinesterase and α-glucosidase action of *Rosmarinus tournefortii* de Noé can be used for the future therapeutic medicine such as neurodegenerative and antidiabetic diseases. The isolated bioactive compounds from essential oil and the extracts could provide more information necessary for desirable pharmacological.

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