BJP

Bangladesh Journal of Pharmacology

Research Article

A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index;
Bangladesh J Pharmacol 2016; 11: 531-544
ISSN: 1991-0088

Anti-oxidant, antimicrobial and anti-acetylcholinesterase activities of organic extracts from aerial parts of three Tunisian plants and correlation with polyphenols and flavonoids contents

Imen Sellem, Fatma Kaaniche, Ahlem Chakchouk Mtibaa and Lotfi Mellouli

Laboratory of Microorganisms and Biomolecules of the Center of Biotechnolgy of Sfax-Tunisia. Road of Sidi Mansour, Km 6, B.P. 1177, 3018 Sfax-Tunisia.

Article Info

Received: 17 December 2015
Accepted: 30 March 2016
Available Online: 7 May 2016

DOI: 10.3329/bjp.v11i2.26001

Cite this article:
Sellem I, Kaaniche F, Mtibaa AC, Mellouli L. Anti-oxidant, antimicrobial and anti-acetylcholinesterase activities of organic extracts from aerial parts of three Tunisian plants and correlation with polyphenols and flavonoids contents. Bangladesh J Pharmacol. 2016; 11: 531-44.

Abstract

In goal of searching new active compounds with important biological activities, a screening of several plants from salt-marsh region of Chebba-Tunisia had been realized. Three species had been selected: *Calendula arvensis*, *Chenopodium murale* and *Nicotiana glauca*. The organic extracts of different aerial parts of these plants (stems, leaves and flowers) displayed variable contents of total polyphenols (TPP) and total flavonoids (TF). Flowers acetone extract from *N. glauca* contained the higher quantity in TPP (264.8 ± 1.6 µg GA/mg), while stems dichloromethane extract exhibited the best TF content (49.8 ± 2.2 µg QE/mg). The important TPP and TF contents reflected a good anti-oxidant and antimicrobial activities. The best acetylcholinesterase inhibitory activity had been shown in the fractions obtained after extraction with low polarity solvents. Whence, a correlation of flavonoids contents with biological activities had been shown, while, there was no correlation with acetylcholinesterase inhibitory activity.

Introduction

For centuries, extracts from plants have been used in traditional medicine to solve various health problems (Montiel Ruiza et al., 2013). Now-a-days, plants still play an important role in terms of prevention and cure of several diseases. They are the base for the development of modern medicine (Ameyaw and Duker-Eshun, 2009), and they have provided a great number of drugs (Kintzios and Barberaki, 2004).

The medicinal value of plants lies in the bioactive constituents, such as alkaloids, tannins, coumarins, terpenoids, phenolic compounds, etc (Chethan et al., 2012), that have been known to bear valuable therapeutic activities (Raina et al., 2014). Several studies showed significant variations of plants secondary metabolites production between spices and even for the same spices under different environmental conditions (Selmar and Kleinwaëchter, 2013). Furthermore, in arid and semi-arid regions, plants are often subjected to severe environmental conditions (salinity, drought, extreme temperatures etc), that influence secondary metabolites biosynthesis, especially the rise of phenolics production and distribution (Jallali et al., 2012).

The aims of this work were firstly, the gain of more information about total polyphenols and flavonoids quantities, the study of the potential natural anti-oxidant, antimicrobial and acetylcholinesterase inhibitory effect of diverse crude extracts from various areal parts of the three plants species from salt-marsh region of Chebba-Tunisia; *Calendula arvensis*, *Chenopodium murale* and *Nicotiana glauca* belonging respectively to *Asteraceae*, *Chenopodiaceae* and *Solanaceae* families, secondly to carry on the relationships between phenolics and flavonoids quantities and the different biological activities.

Materials and Methods

Chemicals and reagents

The following chemicals and reagents were used: Cyclohexane (Riedel-deHaën), dichloromethane (Loba Chemie), ethyl acetate (Loba Chemie), acetone (Loba Chemie), acetonitrile (Loba Chemie), Folin–Ciocalteau reagent (Fluka), Na_2CO_3 (Merck), ascorbic acid (Bio Basic), butylated hydroxytoluene (BHT) (Supelco), gallic acid (Bio Basic), $AlCl_3$ (Merck), quercetin (HWI Analitik), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Aldrich), DMSO (Scharlau), 2, 2′-azino-bis-(3-ethylbenz -thiazoline-6-sulfonic acid) (ABTS) (Fluka), $MK_2S_2O_8$ (Merck), β-carotene (Sigma), linoleic acid (Acros Organics), Tween 40 (Scharlau), peptone (Accumix), Yeast extract (Accumix), NaCl (Suvchem), agar (Biokar), glucose (Accumix), electric eel AChE (Type-VI -S, EC 3.1.1.7, Sigma), acetylthiocholine iodide (ATCI) (Sigma), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (Sigma), Tris (Vivantis) and galanthamine (Sigma).

Plants collection and extracts preparation

C. arvensis, *N. glauca* and *C. murale* were collected from the marsh area of Chebba (Mahdia, Tunisia, latitude 35.23° and longitude 11.11°), this region strong saline and had a semi-arid climate with scarce rainfall (Mabrouk et al., 2014; Saidi et al., 2009). The identification of the three plants species was performed according to the flora of Tunisia deposited in the Center of Biotechnology of Sfax.

The plants were washed and air-dried in the shade at room temperature. The areal parts were separated and grounded to fine powder using a grinder. Each material resulted was extracted by maceration in 5 solvents successively in increasing polarity (cyclohexane–dichloromethane-ethyl acetate-acetone-acetonitrile) for 24 hours with a mass to volume ratio of 1:10 (g/mL), at room temperature. The resulting crude extracts were evaporated under rotary evaporator to dryness at 37°C. The dried extracts were kept at 4°C in the dark until a further analysis.

Total polyphenols content

Total polyphenols content was determined according to Folin-Ciocalteau method (Waterman and Mole, 1994) with some modifications. 10 µL of diluted extract solution was shaken for 5 min with 50 µL of Folin-Ciocalteau reagent. Then 150 µL of 20% Na_2CO_3 was added and the mixture was shaken once again for 1 min. Finally, the solution was brought up to 790 µL by adding distilled water. After 2 hours, the absorbance at 760 nm was evaluated using a spectrophotometer (Thermo Scientific / Genesys 20, Germany). Gallic acid was used as a standard for the calibration curve. Total polyphenolics content (TPP) of the extracts was calculated according to the following equations: y = 0.012 x + 0.017 (r2 = 0.997), expressed as µg gallic acid equivalent per milligram of dry extract (µg GA/mg) using the linear equation based on the calibration curve.

Flavonoids content

The flavonoids content in extract was determined by spectrophotometric method (Quettier Deleu et al., 2000). Briefly, 1 mL of $AlCl_3$ was added to 1 mL diluted extract solution and vortexed, and then incubated for 15 min in the dark. The absorbance at 430 nm was evaluated for the samples and the quercetin as standard for the calibration curve. Total flavonoids (TF) content of the extracts was calculated according to the following equations: y = 0.051 x + 0,003 (r2 = 0.999), and expressed in µg of quercetin equivalent per milligram of dry plant extract (µg QE/mg).

Anti-oxidant assays

All anti-oxidant activities were expressed as EC_{50} (the concentration that causes 50% of effect). The free radical scavenging activity of the three plants extracts had been assessed by 2.2-diphenyl-1-picrylhydrazyl (DPPH) and 2.2'- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^+$) radicals assays. Gallic acid (GA), ascorbic acid (AA) and butylated hydroxytoluene (BHT) were used as controls, they are well known anti-oxidant compounds.

DPPH radical scavenging activity (Video clip)

DPPH is one of the compounds that possess a proton free radical, when DPPH encounters proton radical scavengers its purple color fades rapidly. This assay determines the scavenging of stable radical species according to the method of Kirby and Schmidt, (1997) with slight modifications. Briefly, extracts were dissolved in 10% DMSO and diluted with ultrapure water at different concentrations (500, 250, 125, 60.25, 30.125 µg/mL). Then, 500 µL of a 4% (w/v) solution of DPPH radical in methanol was mixed with 500 µL of samples. The mixture was incubated for 30 min in the dark at room temperature. The scavenging capacity was determined spectrophotometrically by monitoring the decrease in absorbance at 517 nm against a blank. The percentage of antiradical activity (%ArA) had been calculated as follows:

%ArA = **[**(absorbance of control - absorbance of test sample) / absorbance of control**]** x 100

$ABTS^+$ radical scavenging activity

The potential of extracts to scavenge free radicals was also assessed by their ability to quench $ABTS^+$ anti-radical activity done by using the $ABTS^+$ free radical discoloration assay developed by Re et al. (1999) with some modifications. Briefly, the preformed radical monocation of $ABTS^+$ was generated by reacting 2.2′ azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) solution (7 mM) with 2.45 mM $K_2S_2O_8$. The mixture was allowed to stand for 15 hours in the dark at room

temperature. The solution was diluted with ethanol to obtain the absorbance of 0.7 ± 0.2 units at 734 nm. Samples were separately dissolved in ethanol to yield the following concentrations (500, 250, 125, 60.25, 30.125, 15.625 µg/mL). In order to measure the anti-oxidant activity of extracts and standards, 20 µL of each sample at various concentrations was added to 180 µL of $ABTS^+$ and vortexed for 30 sec, and then incubated for 6 min in dark at room temperature. The absorbance was measured spectrophotometrically at 734 nm. The percentage of scavenging activity of $ABTS^+$ (% SA) was calculated by the following formula:

% SA = **[**(absorbance of control - absorbance of test sample) /absorbance of control**]** x 100

β-Carotene–linoleic acid bleaching assay

The extracts anti-oxidant activity was determined according to the β-carotene bleaching method described by Pratt (1980): 0.5 mg of β-carotene was dissolved in 1 mL chloroform, and then 25 µL of linoleic acid and 200 µL of Tween 40 were added. The chloroform was then evaporated under rotary evaporator, and the residue was dissolved in 100 mL of H_2O. Extracts were diluted at different concentrations (500, 250, 125, 60.25, 30.125 µg/mL), and for 500 µL of each sample, 2.5 mL of β-carotene-linoleic acid solution was added. The absorbance was read at 470 nm firstly at zero time and after 120 min of incubation in dark at 50°C. The anti-oxidant activity in β-carotene bleaching model in percentage (AA%) was calculated using the following equation:

$$AA\% = [1- (A^0_S-A^{120}_S)/ (A^0_C-A^{120}_C)] \times 100$$

where A^0_S and A^0_C are absorbance of the sample and the control respectively measured at zero time, and A^{120}_S and A^{120}_C are absorbance of the sample and the control respectively measured after 120 min

Antimicrobial activities

Microorganisms and growth conditions

Bacteria and *Candida* species were obtained from International Culture Collections (ATCC) and local culture collection of Laboratory of Microorganisms and Biomolecules of the Centre of Biotechnology of Sfax - Tunisia. They included Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* LB14110 and *Listeria monocytogenes* ATCC 19117 and Gram-negative bacteria: *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 49189 and *Enterobacter aerogenes* ATCC 13048 and two *Candida* strains: *Candida tropicalis* R2 CIP 203 and *C. albicans* ATCC 10231.

The bacterial cultures were performed in Luria-Bertani (LB) agar medium composed of (g/L): peptone 10; yeast extract 5; NaCl 5 and agar 20 at pH 7.2, then the bacterial strains were incubated at 37°C, except *M. luteus* and *P. aeruginosa* which are incubated at 30°C. The *Candida* species were cultured on Sabouraud agar medium composed of (g/L): peptone 10; glucose 10 and agar 20 at pH=5.6 and incubated at 28°C. Working bacterial cultures were prepared by inoculating a loopful of each test bacteria in 3 mL of LB broth. *C. tropicalis* was cultured in YP10 medium composed of (g/L): yeast extract 10; peptone 10; glucose 100 and 15 mL of 2 g/L adenine solution, while *C. albicans* was cultured in YEPD medium composed of (g/L): yeast extract 10; peptone 20 and dextrose 20 at pH 5.6. All strains were incubated at adequate temperature for 12 hours. For the test, final inoculum concentrations of 10^6 CFU/mL were used.

Agar diffusion method

In this research, the antimicrobial activities of each crude extract were assessed by diameters of inhibition areas (halo) in mm, by means of agar-well diffusion assay according to Güven et al. (2006) with some modifications. Fifteen milliliters of the molten agar (45° C) were poured into sterile petri dishes (Ø 90 mm). Working cell suspensions were prepared and 100 µL was evenly spreaded onto the surface of the agar plates of LB agar medium for bacteria and sabouraud agar medium for *Candida*. Once the plates had been aseptically dried, 5 mm wells were punched into the agar with a sterile pasteur pipette. The extracts were dissolved in dimethylsulfoxide (DMSO)/water (1/9; v/v) to a final concentration of 100 mg/mL and then filtered through 0.22 µm pore-size black polycarbonate filters (Millipore). Thus, 100 µL were placed into the wells and the plates were incubated at adequate temperature overnight for bacterial strains and 72 hours for *Candida* strains.

Acetylcholinesterase inhibitory potential

AChE inhibitory activity was measured by slightly modified spectrophotometric method of Ellman et al. (1961). Electric eel AChE was used, while acetylthiocholine iodide (ATCI) was employed as substrate of the reaction. 5.5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was used for the measurement of the anti-acetylcholinesterase activity. Briefly, in this method, 100 µL of Tris buffer at 50 mM (pH 8.0), 30 µL of sample or standard and 5 µL of AChE enzyme (0.5 U/mL) were added in a 96 well microplate and incubated for 10 min at 25°C. Then, 142 µL of DTNB (3 mM) and 23 µL of substrate (75 mM) were added. Hydrolysis of ATCI was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at 405 nm utilizing a 96-well microplates reader (Thermo Scientific/Varioskan Flash, Germany). The kinetic reaction has been followed until the AChE activity decreased, and then the reaction has been stopped. Percentage of inhibition of AChE was determined by

comparison of rates reaction of samples relative to control (10% DMSO in Tris buffer) using the following formula:

$$\% \text{ AChEI} = 1 - (\delta A_{\text{sample}} / \delta A_{\text{control}}) \times 100$$

Where δA_{sample}: Sample absorbance at zero time – Sample absorbance at the end of reaction, and $\delta A_{\text{control}}$: Control absorbance at zero time - Control absorbance at the end of reaction.

Galanthamine, the antiacetylcholinesterase alkaloid-type of drug obtained from the bulbs of snowdrop (Galanthus sp.), was used as standard.

Firstly, all extracts have been tested at 100 µg/mL of concentration. Then, dose-dependent AChE inhibitory activity (of extracts that display an activity with 100 µg/mL of concentration) was further studied and the results are shown in the IC50 values.

Statistical analyses

All tests are assayed in triplicate and expressed as the average ± standard deviation of the measurements. The statistical program SPSS version 21.00 for Windows (SPSS Inc., Chicago, IL) was used to analyse data. Variance was analysed by one-way ANOVA and Duncan's multiple range tests were calculated for the significant data at $p<0.05$. Correlation between the phenolic and the flavonoids contents and biological activities in plant extracts were analysed by Pearson correlation coefficient.

Results

Total polyphenols and flavonoids contents

TPP contents of the extracts from aerial parts of the three plants were tabulated in Table I. Overall, the values appear to vary between 16.2 ± 0.4 and 264.8 ± 1.1 µg GA/mg. Concerning TPP contents in *C. arvensis* extracts, the values are ranged from 26.8 ± 0.2 µg GA/mg (Flowers cyclohexane extract) to 188.3 ± 0.2 µg GA/mg (leaves dichloromethane extract). For *C. murale*, polyphenols lowest concentration has been shown with stems acetonitrile extract (16.2 ± 0.4 µg GA/mg), while flowers acetonitrile extract showed the highest concentration (215.3 ± 0.3 µg GA/mg). *N. glauca* extracts presented a polyphenols contents ranged from 20.8 ± 0.2 µg GA/mg for the stems cyclohexane extract to 264.8 ± 1.1 µg GA/mg for the flowers acetonitrile extract.

TF contents of the extracts are shown in Table I. Globally, the values varied between zero to 49.8 ± 0.0 µg QE/mg. For *C. arvensis* values were ranged from 0 µg QE/mg (stems cyclohexane extract), to 43.0 ± 0.0 µg QE/mg (flowers acetonitrile extract). Concerning *C. murale* the lowest flavonoids content had been obtained in the flowers cyclohexane extract (0.4 ± 0.0 µg QE/mg) and the highest content had been shown in the leaves dichloromethane extract (49.2 ± 0.1 µg QE/mg). While, *N. glauca* had the lowest content of flavonoids in the stems dichloromethane extract (5.4 ± 0.3 µg QE/mg) and the highest one in the flowers dichloromethane extract (49.8 ± 0.0 µg QE/mg).

Anti-oxidant power

The results of anti-oxidant activities are shown in Table II. As regard DPPH radical scavenging activity, EC50 values of the extracts are ranged from 41.4 ± 1.6 µg/mL (Flowers acetonitrile extract from *N. glauca*) to >500 µg/mL for several extracts. The EC50 values of GA, AA and BHT content were found to be 3.6 ± 0.0, 4.2 ± 0.1 and 28.6 ± 0.3 µg/mL respectively. Discoloration of $ABTS^+$ reflects the capacity of anti-oxidant species to donate electrons or hydrogen atoms to inactivate this radical cation. In this finding, the EC_{50} of the different extracts in $ABTS^+$ assay are widely variable, they are ranged between 10.8 ± 0.2 µg/mL (Stems dichloromethane extract from *C. arvensis*) to 100.5 ± 0.6 µg/mL (Stems cyclohexane extract from *C. murale*).

There was a correlation between degradation rate and the bleaching of b-carotene; where, the extract with the lowest β-carotene degradation rate exhibits the highest anti-oxidant activity. In this work, extracts showed a large variation of their protective action against bleaching of β-carotene, the EC_{50} values are ranged from 38 µg/mL (Ethyl acetate extract from *C. murale* leaves) to >500 µg/mL for several extracts. The EC_{50} value of BHT is 72.7 ± 0.3 µg/mL. Several extracts from these 3 plants exhibited better activities than BHT.

In this work, extracts showed a large variation of their protective action against bleaching of β-carotene, the EC_{50} values are ranged from 38 µg/mL (Ethyl acetate extract from *C. murale* leaves) to >500 µg/mL for several extracts. The EC_{50} value of BHT is 72.7 ± 0.3 µg/mL. Several extracts from these 3 plants exhibit better activities than BHT.

These results revealed that considerable antiradical components were contained in the different plants parts. Differences in species and in solvents polarities and thus different extractability of the anti-oxidant components may explain the differences in anti-oxidant activity of the extracts from the three plants.

Antimicrobial activities

The results against both bacterial and *Candida* species are presented in Figure 1-3. For *C. arvensis*, leaves extracts showed the best activities (Figure 1B), especially in acetonitrile extract, which gave inhibition diameters of 20.4 ± 0.6, 15.0 ± 0.0 and 15.6 ± 0.2 mm respectively against *Candida tropicalis*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes*. While, the leaves

Table I

Anti-oxidant and acetylcholinesterase inhibitor activities of extracts

	Plant	*C. arvensis*	*C. arvensis*	*C. arvensis*	*C. murale*	*C. murale*	*C. murale*	*N. glauca*	*N. glauca*	*N. glauca*	GA	AA	BHT	Galan-thamine
	Extract	Stems	Leaves	Flowers	Stems	Leaves	Flowers	Stems	Leaves	Flowers	3.6 ± 0.1	4.2 ± 0.1	28.6 ± 0.3	-
±EC 50% DPPH	Cyclohexane	360.1 ± 1.1^{Be}	160.3 ± 0.5^{Cc}	>500^{Ad}	>500^{Cd}	92.5 ± 1.3^{Aa}	451.4 ± 1.2^{Be}	>500^{Ae}	>500^{Ae}	>500^{Ac}	3.6 ± 0.1	4.2 ± 0.1	28.6 ± 0.3	-
	Dichloromethane	358.2 ± 0.3^{Bd}	104.4 ± 2.7^{Aa}	>500^{Cd}	>500^{Cd}	270.1 ± 0.3^{Bd}	124.6 ± 3.1^{Ac}	97.3 ± 2.2^{Bc}	83.3 ± 0.7^{Ac}	>500^{Cc}	3.6 ± 0.1	4.2 ± 0.1	28.6 ± 0.3	-
	Ethyl acetate	197.4 ± 0.5^{Bb}	110.4 ± 0.8^{Ab}	430.2 ± 1.4^{Cc}	350.2 ± 0.6^{Cb}	248.4 ± 0.9^{Bc}	85.2 ± 0.1^{Ab}	80.4 ± 2.1^{Bb}	47.1 ± 0.6^{Aa}	>500^{Cc}	3.6 ± 0.1	4.2 ± 0.1	28.6 ± 0.3	-
	Acetone	213.2 ± 0.2^{Bc}	>500^{Ce}	185.0 ± 0.7^{Ab}	304.6 ± 2.1^{Ca}	179.3 ± 0.5^{Bb}	82.7 ± 1.3^{Aa}	70.2 ± 0.3^{Aa}	83.5 ± 2.5^{Ad}	190 ± 3.2^{Bb}	3.6 ± 0.1	4.2 ± 0.1	28.6 ± 0.3	-
	Acetonitrile	179.4 ± 0.7^{Ba}	302.1 ± 0.6^{Cd}	168.2 ± 0.2^{Aa}	470.2 ± 0.1^{Cc}	275.8 ± 0.1^{Be}	269.0 ± 0.4^{Ab}	152.1 ± 0.2^{Cd}	79.3 ± 1.9^{Bb}	41.4 ± 1.6^{Aa}	3.6 ± 0.1	4.2 ± 0.1	28.6 ± 0.3	-
±EC 50% ABTS	Cyclohexane	69.1 ± 0.5^{Ce}	25.7 ± 0.6^{Aa}	35.4 ± 0.3^{Ba}	100.5 ± 0.6^{Be}	57.3 ± 1.5^{Ad}	95.0 ± 0.6^{Ce}	94.3 ± 1.2^{Ce}	22.5 ± 2.4^{Ac}	40.7 ± 0.3^{Bb}	1.9 ± 0.5	5.4 ± 0.1	17.3 ± 0.4	-
	Dichloromethane	10.8 ± 0.2^{Aa}	31.7 ± 0.4^{Bb}	85.6 ± 0.1^{Ce}	82.4 ± 2^{Cb}	23.8 ± 0.6^{Ab}	38.2 ± 0.5^{Bc}	23.5 ± 0.5^{Ba}	12.7 ± 0.9^{Aa}	79.1 ± 0.6^{Cc}	1.9 ± 0.5	5.4 ± 0.1	17.3 ± 0.4	-
	Ethyl acetate	43.8 ± 0.3^{Cd}	32.5 ± 0.2^{Ac}	43.2 ± 0.3^{Bb}	91.4 ± 1.1^{Cc}	70.2 ± 0.7^{Be}	46.9 ± 2.1^{Ad}	39.7 ± 2.8^{Cc}	16.8 ± 1.5^{Ab}	34.2 ± 2.6^{Bd}	1.9 ± 0.5	5.4 ± 0.1	17.3 ± 0.4	-
	Acetone	28.2 ± 0.4^{Ab}	40.1 ± 0.1^{Bd}	49.8 ± 0.2^{Cc}	79.2 ± 1.5^{Ca}	42.2 ± 2.6^{Bc}	30.1 ± 0.6^{Aa}	29.7 ± 1.4^{Bb}	69.6 ± 0.9^{Ce}	13.7 ± 0.9^{Aa}	1.9 ± 0.5	5.4 ± 0.1	17.3 ± 0.4	-
	Acetonitrile	42.8 ± 1.1^{Ac}	49.7 ± 0.3^{Be}	52.5 ± 0.5^{Cd}	98.6 ± 1.3^{Cd}	11.7 ± 1.4^{Aa}	31.3 ± 0.9^{Bb}	51 ± 0.6^{Cd}	38.2 ± 2.9^{Bd}	13.2 ± 0.4^{Aa}	1.9 ± 0.5	5.4 ± 0.1	17.3 ± 0.4	-
±EC 50% β-caroten	Cyclohexane	47.3 ± 0.9^{Aa}	102 ± 1.3^{Bb}	495.3 ± 3.1^{Cd}	246.1 ± 4.1^{Ab}	359.0 ± 0.9^{Bb}	399.2 ± 4.1^{Cd}	>500^{Ce}	410.2 ± 2.7^{Be}	357.5 ± 1.2^{Ad}	-	-	72.7 ± 0.3	-
	Dichloromethane	90.2 ± 1.2^{Ac}	96.1 ± 2.2^{Bb}	158.8 ± 3.2^{Ca}	408.7 ± 1.2^{Cc}	257.1 ± 4.1^{Bc}	73.9 ± 1.5^{Ab}	62.5 ± 2.4^{Aa}	89.0 ± 1.6^{Bb}	400 ± 0.0^{Ce}	-	-	72.7 ± 0.3	-
	Ethyl acetate	52.1 ± 0.2^{Ab}	54.9 ± 0.4^{Ba}	183.3 ± 1.2^{Cb}	160.1 ± 0.4^{Ca}	38.5 ± 1.2^{Aa}	50.0 ± 0.9^{Ba}	250.8 ± 3.1^{Bd}	40.6 ± 2.1^{Aa}	308.7 ± 1.0^{Cc}	-	-	72.7 ± 0.3	-
	Acetone	95 ± 0.0^{Ad}	300.3 ± 0.5^{Bc}	>500^{Ce}	470.6 ± 0.3^{Cd}	293.2 ± 1.0^{Bd}	265.8 ± 3.3^{Ac}	140 ± 3.3^{Ab}	374.4 ± 0.8^{Cd}	252.3 ± 2.2^{Bb}	-	-	72.7 ± 0.3	-
	Acetonitrile	470.2 ± 2.1^{Ae}	>500^{Cd}	482.0 ± 1.2^{Bc}	>500^{Be}	>500^{Be}	430.1 ± 2.3^{Ae}	207.0 ± 1.4^{Ac}	289.6 ± 1.1^{Bc}	216.5 ± 0.3^{Aa}	-	-	72.7 ± 0.3	-
% AChEI *	Cyclohexane	41.3 ± 1^{Cb}	20.2 ± 0.0^{Bb}	0 ± 0.0^{Aa}	0 ± 0.0^{Aa}	0 ± 0.0^{Aa}	53.08 ± 0.0^{Bc}	0 ± 0.0^{Aa}	83.33 ± 0.0^{Bb}	0 ± 0.0^{Aa}	-	-	-	-
	Dichloromethane	0 ± 0.0^{Aa}	0 ± 0.0^{Aa}	47.8 ± 0.1^{Bb}	100 ± 0.0^{Cb}	0 ± 0.0^{Aa}	46.27 ± 0.3^{Bb}	100 ± 0.0^{Bb}	100 ± 0.0^{Bc}	0 ± 0.0^{Aa}	-	-	-	-
	Ethyl acetate	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{Aa}	100 ± 0.0^{Bb}	0 ± 0.0^{Aa}	0 ± 0.0^{Aa}	0 ± 0.0^{Aa}	68.63 ± 0.2^{Bb}	-	-	-	-
	Acetone	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	-	-	-	-
	Acetonitrile	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	-	-	-	-
IC 50% AChEI **	Cyclohexane	>100	>100	-	-	-	95.7 ± 1.3	-	12.5 ± 0.01	-	-	-	-	0.1 ± 0.03
	Dichloromethane	-	-	>100	40,9 ± 0.5	-	>100	6.2 ± 0.5	2.5 ± 0.7	-	-	-	-	0.1 ± 0.03
	Ethyl acetate	-	-	-	-	31.7 ± 0.6	-	-	-	72.3 ± 0.06	-	-	-	0.1 ± 0.03
	Acetone	-	-	-	-	-	-	-	-	-	-	-	-	0.1 ± 0.03
	Acetonitrile	-	-	-	-	-	-	-	-	-	-	-	-	0.1 ± 0.03

[A] Values in the same row (for the same specie) followed by the same lower case letter are not significantly different between the plant parts, by Duncan's multiple range test ($p<0.05$); [a] Values in the same column followed by the same lower case letter are not significantly different among extracting solvent, by Duncan's multiple range test ($p<0.05$); ± Extract concentration that give 50% of anti-oxidant effect (EC 50%); *Percentage of acetylcholinesterase inhibitory activity (% AChEI) of the crude extracts at 100 µg/mL of concentration; ** Acetylcholinesterase inhibitory concentration at 50% of activity (IC50 in µg/mL) of the active crude extracts

Table II

Total polyphenols and flavonoids content

	Plant	*C. arvensis*			*C. murale*			*N. glauca*		
	Extract	Stems	Leaves	Flowers	Stems	Leaves	Flowers	Stems	Leaves	Flowers
TPP *	Cyclohexane	43.1 ± 0.1^{Ba}	59.2 ± 0.7^{Ca}	26.7 ± 0.2^{Aa}	16.2 ± 0.4^{Aa}	77.0 ± 1.2^{Cb}	39.3 ± 0.1^{Ba}	20.8 ± 0.2^{Aa}	98.0 ± 0.2^{Ce}	80.2 ± 0.1^{Ba}
	Dichloromethane	59.0 ± 0.3^{Bb}	188.3 ± 0.2^{Ce}	43.8 ± 0.2^{Ab}	18.5 ± 0.1^{Ab}	182.3 ± 0.6^{Ce}	96.1 ± 0.2^{Bb}	92.0 ± 0.6^{Be}	54.8 ± 0.5^{Ac}	138.5 ± 0.2^{Cb}
	Ethyl acetate	96.6 ± 0.8^{Bc}	163.3 ± 0.8^{Cd}	68.2 ± 0.3^{Ac}	69.7 ± 0.2^{Bc}	49.1 ± 0.2^{Aa}	159.7 ± 0.1^{Cd}	91.2 ± 0.3^{Bd}	69.2 ± 0.2^{Ad}	196.1 ± 0.5^{Cc}
	Acetone	129.6 ± 0.3^{Bd}	149.2 ± 0.2^{Cb}	$121.0.2^{Ad}$	109.1 ± 0.5^{Be}	105.5 ± 0.4^{Ac}	146.0 ± 0.9^{Cc}	60.2 ± 0.4^{Bb}	49.1 ± 0.3^{Ab}	264.7 ± 1.6^{Cd}
	Acetonitrile	171.4 ± 0.3^{Ce}	157.5 ± 0.6^{Bc}	104.4 ± 0.4^{Ae}	33.0 ± 0.3^{Ac}	167.3 ± 1.3^{Bd}	215.3 ± 0.3^{Ce}	64.5 ± 0.2^{Ac}	47.5 ± 0.3^{Aa}	254.3 ± 0.8^{Bd}
TF**	Cyclohexane	24.4 ± 0.1^{Cd}	0 ± 0^{Aa}	19.8 ± 0.3^{Ba}	4.9 ± 0.3^{Bb}	14.2 ± 2.4^{Ca}	0.4 ± 0.2^{Aa}	8.4 ± 0.2^{Aa}	28.0 ± 1.4^{Cd}	10.3 ± 0.2^{Bb}
	Dichloromethane	3.8 ± 0.9^{Aa}	3.6 ± 0.3^{Ab}	35.1 ± 0.1^{Bb}	3.8 ± 1^{Aa}	49.2 ± 0.7^{Cd}	24.7 ± 0.6^{Bb}	49.8 ± 2.2^{Ce}	42.9 ± 0.2^{Bc}	5.4 ± 0.3^{Aa}
	Ethyl acetate	9.7 ± 0.5^{Ab}	41.7 ± 0.2^{Ce}	38.7 ± 0.1^{Bc}	19.4 ± 0.2^{Bc}	20.0 ± 0.1^{Cb}	12.5 ± 0.3^{Ad}	49.0 ± 0.7^{Bd}	49.3 ± 0.2^{Be}	20.2 ± 0.3^{Ac}
	Acetone	19.1 ± 0.2^{Ac}	25.7 ± 0.3^{Bc}	40.1 ± 0.2^{Cd}	19.4 ± 0.5^{Ac}	20.2 ± 0.1^{Bb}	26.4 ± 0.2^{Ce}	45.8 ± 2.4^{Bc}	9.2 ± 0.6^{Aa}	36.7 ± 0.1^{Cd}
	Acetonitrile	27.0 ± 0.2^{Ae}	30.6 ± 0.2^{Bd}	43.0 ± 0.0^{Cd}	20.6 ± 0.4^{Be}	48.8 ± 0.5^{Cc}	15.8 ± 0.4^{Ac}	38.6 ± 0.4^{Ab}	46.9 ± 0.2^{Bd}	39.8 ± 2.1^{Ad}

[A] Values in the same row (for the same specie) followed by the same lower case letter are not significantly different between the plant parts, by Duncan's multiple range test ($p<0.05$); [a] Values in the same column followed by the same lower case letter are not significantly different among extracting solvent, by Duncan's multiple range test ($p<0.05$); *TPP contents in µg equivalent of gallic acid to 1 mg of extract (µg GA/mg); ** TF content in µg equivalent of quercetin to 1 mg of extract (µg QE/mg)

acetone and ethyl acetate extracts were the most active respectively against *Salmonella typhimarium* (27.1 ± 0.2 mm) and *Candida albicans* (20.0 ± 0.1 mm). Flowers extracts carried out good activities against some pathogens species (Figure 1C), indeed, ethyl acetate extract exhibited the best activity against *L. monocytogenes* (40.7 ± 1.1 mm), and acetone extract was the most active against *M. luteus* (34.9 ± 0.2 mm), while the best activity against *S. aureus* has been shown in acetonitrile extract (20.0 ± 0.0 mm). *C. murale* extracts seemed to be less active than the two others species extracts. Indeed, the best activities were shown for flowers acetonitrile extract (Figure 2C), especially for *C. albicans* (19.1 ± 0.1 mm), *P. aeruginosa* (30.2 ± 0.1 mm), *S. typhimarium* (25.0 ± 0.0 mm), *L. monocytogenes* (34.7 ± 0.6 mm), *M. luteus* (30.9 ± 1.1 mm) and *S. aureus* (22.1 ± 1.8 mm). While leaves acetonitrile extract (Figure 2B) exhibited the best activities against *C. tropicalis* (5.1 ± 0.3 mm) and *E. aerogenes* (33.93 ± 0.09 mm). *N. glauca* leaves crude extracts (Figure 3B) were the most active: Ethyl acetate extract presents the best activities against *S. typhimurium* (29.7 ± 1.3 mm), *E. aerogenes* (47.2 ± 0.7 mm), *L. monocytogenes* (50.1 ± 0.2 mm), *M. luteus* (54.0 ± 0.0 mm) and *S. aureus* (26.2 ± 1.0 mm). While, acetonitrile and ethyl acetate extracts had fairy the same activities against *C. tropicalis* (10.0 ± 0.0 and 10.1 ± 0.1 mm, respectively). Dichloromethane extract had the best activities against *C. albicans* (12.1 ± 0.5 mm) and *P. aeruginosa* (31.0 ± 0.2 mm).

Globally, by comparing the three species aerial parts extracts, *N. glauca* leaves ethyl acetate extract was the most active against the three Gram+ bacteria strains and against *E. aerogenes*, while, *N. glauca* leaves was the best against *P. aeruginosa*. Moreover, *C. arvensis* leaves ethyl acetate, acetone, and acetonitrile extracts were the most active respectively against *C. albicans*, *S. typhimarium* and *C. tropicalis*. In comparison with findings in other studies, we remark that several extracts from the three plants exhibit a good activity against the used microbial strains.

Acetylcholinesterase inhibition

The results of AChEI are tabulated in Table II. Extracts from *C. arvensis* displayed the weakest inhibitory activities, even the most active one (flowers dichloromethane extract) had an IC_{50} above 100 µg/mL. Extracts from *C. murale* exhibited moderate activity. The most potent one was the leaves ethyl acetate extract (31.7 ± 0.6 µg/mL). While, extract from *N. glauca* seemed to be most powerful AChE inhibitors, especially leaves dichloromethane extract which displayed the highest activity (IC50% = 2.5 ± 0.8 µg/mL). The % AChEI is remarkably influenced by the solvent type, indeed dichloromethane extract exhibited the best activities, followed by cyclohexane extracts then ethyl acetate extracts, while none of acetone or acetonitrile extracts has shown activity with a concentration less than 100 µg/mL. Thus, the best AChEI activity appears in extracts obtained by maceration in solvents with weak polarity.

Correlation

In this study, there was not a correlation between TPP content and TF content (R2 = 0.225), and for the anti-oxidant assays, only $ABTS^+$ activity had correlation with TPP content (R2 = -0.525). Concerning antimicrobial activities, there was a weak correlation between TPP content and the activities against *C. albicans*, *S. typhimarium*, *E. aerogenes* and *S. aureus* (R2 were 0.337, 0.334, 0.322 and 0.320, respectively) $p<0.1$, the correlation was more important with *M. luteus* (R2 = 0.410) $p<0.01$. Whereas, a distinct correlation between the amounts of TF content with the anti-oxidant and antimicrobial activities was revealed. Although a trend can be found, relating the higher content of TF to the DPPH activity (R2 = -0.421) and $ABTS^+$ activity (R2 = -0.460) $p<0.01$, therefore, this good correlation suggests that flavonoids compounds, present in the extracts from the three plants play an important role as anti-radicals. While, TF and β-carotene test were not correlated (R2 = -0.058). Furthermore, TF content and the antimicrobial activities were highly correlated ($p<0.01$) with all the pathogenic strains. As regards the AChEI activity, no correlation was noticed with any of the other parameters.

Discussion

Our results show that TPP and TF contents varied depending on solvent used for the extraction and plant part. Acetonitrile extracts seemed to be the richest in TF, and leaves extracts seemed to contain the major quantities of TPP and TF, except for *N. glauca* that exhibited the highest content in TPP in flowers extracts. Concerning the antiradical capacity assayed by the most commonly used test based on DPPH and $ABTS^+$ radicals scavenging (Liu et al., 2014), results reveal flatulence antiradical potential, which statistically dependent on flavonoids content, while the results of β-carotene-linoleic acid bleaching system had correlation neither with TPP content nor with TF content.

Then, the assessment of extracts antimicrobial activity had been performed and their positive relationship with TTP and TF contents had been likewise highlighted by studying their correlation.

In this study we have also assessed the acetyl-cholinesterase inhibitory power; results reveal that the active extracts were those obtained by extraction with low polarity solvents, and the higher activity had been shown with the cyclohexanic extract from *N. glauca* flowers.

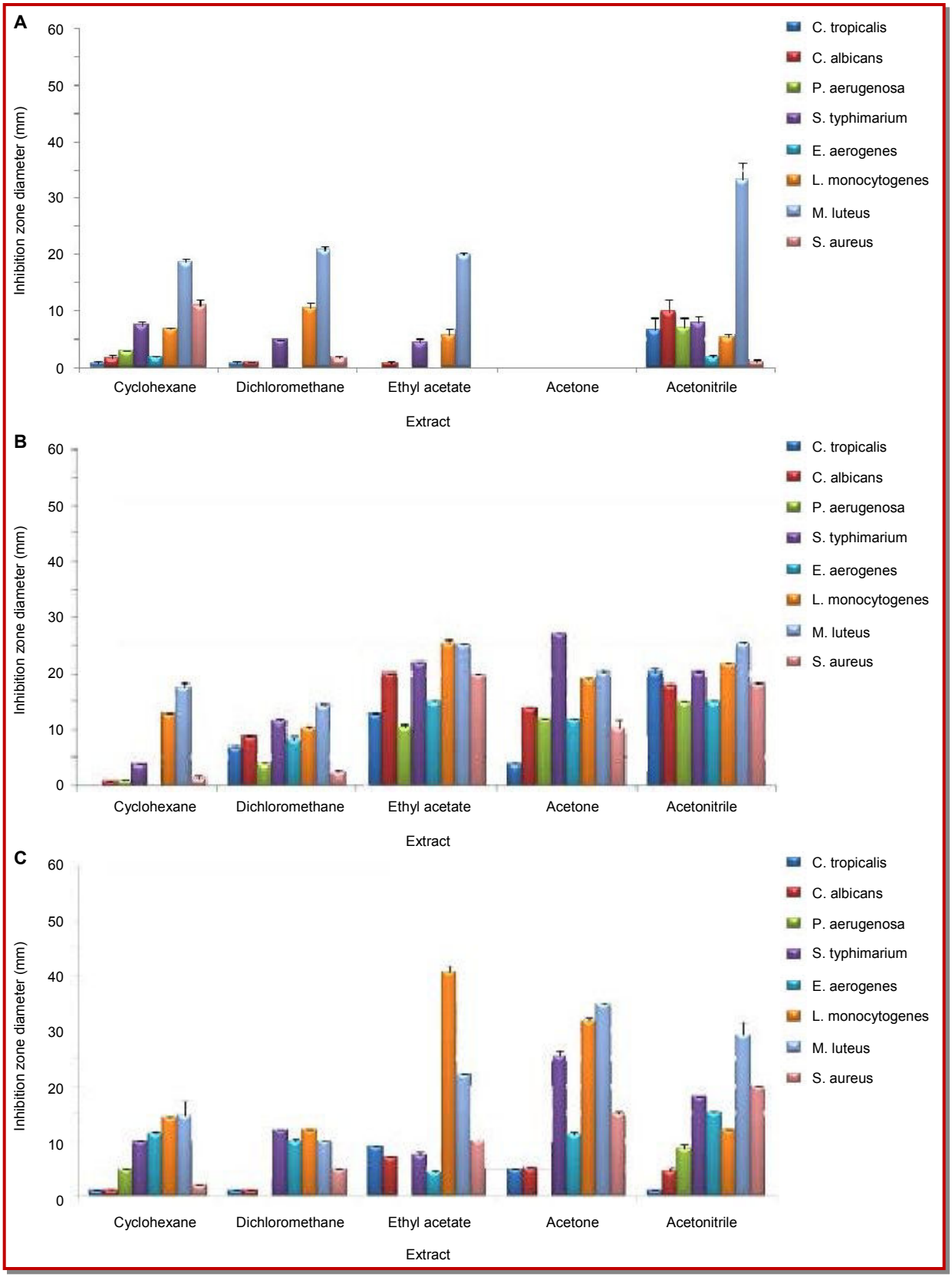

Figure 1: *C. arvensis* extracts antimicrobial activities by agar diffusion test (inhibition zone in mm). (A) stems extracts activities, (B) leaves extracts activities, (C) flowers extracts activities

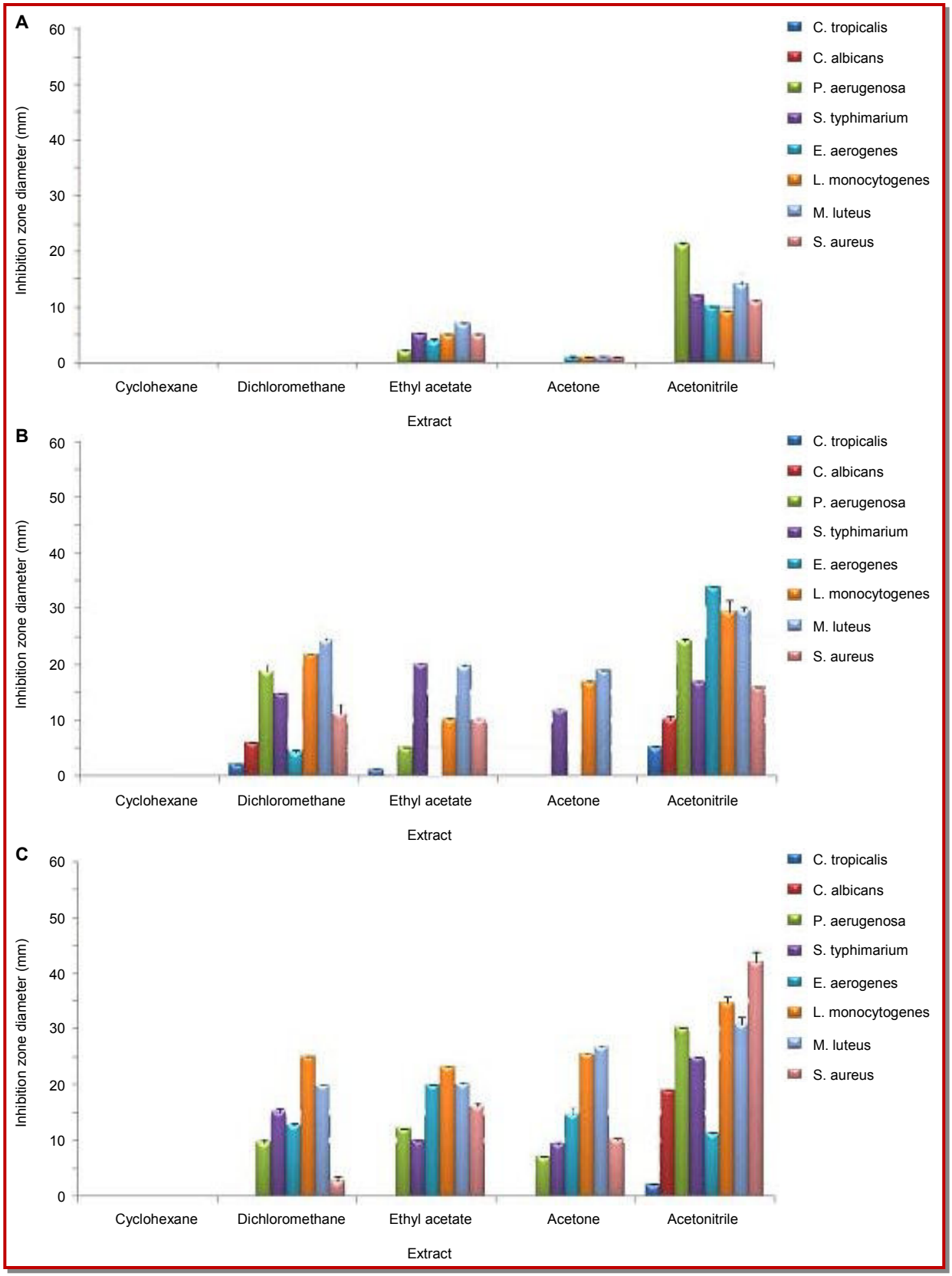

Figure 2: *C. murale* extracts antimicrobial activities by agar diffusion test (inhibition zone in mm). (A) stems extracts activities, (B) leaves extracts activities, (C) flowers extracts activities

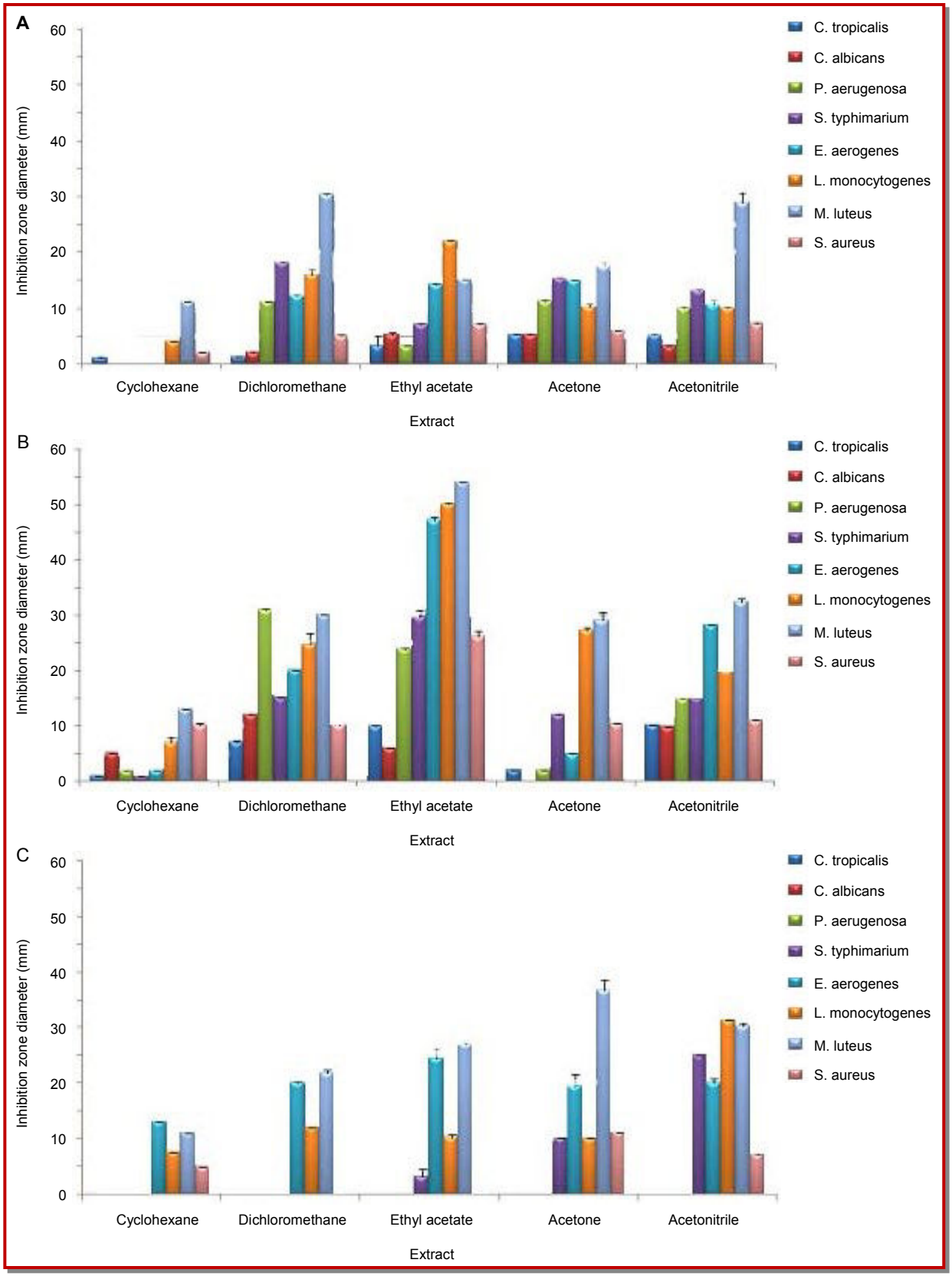

Figure 3: *N. glauca* extracts antimicrobial activities by agar diffusion test (inhibition zone in mm). (A) stems extracts activities, (B) leaves extracts activities, (C) flowers extracts activities

In comparison with other studies for the same species, much more important quantities of TPP and TF have been remarked in our findings. Indeed, Ercetin et al. (2012) found a TPP content of 43 µg GA/mg approximately in flowers dichloromethane extract from *C. arvensis*, which is similar to our result (43.8 ± 0.1 µg GA/mg), however, for the same extract, they found a weak quantity of TF (5 µg QE/mg) while we found 35.1 ± 0.1 µg QE/mg. Other study showed that a methanolic extract of flowers from *C. arvensis* exhibited a TPP and TF contents of 14.5 ± 0.4 µg GA/mg and 5.3 ± 0.4 µg QE/mg, respectively (Cetkovic et al., 2004), and this quantities are much fewer than the contents found with all flowers extracts in our work. For *N. glauca*, Tabana et al. (2015) reported that an organic fraction from this plant contain 108.2 µg GA/mg of TPP and 20.8 µg QE/ mg of TF, in our work we found a much higher quantities, for example, the TPP content in flowers acetone extract was 264.8 ± 1.6 µg GA/mg and the TF for the same extract was 36.7 ± 0.1 µg QE/mg. grown.

Concerning anti-oxidant activities, our findings seem to be better than results reported from other works on these three plants, indeed, Ercetin et al. (2012) findings showed that extracts from *C. arvensis* exhibits a weak DPPH anti-radical activity (EC_{50}% >1000 µg/mL for all extracts), while in our work we found that extracts from this same plant present a potent activity. Furthermore, Tabana et al. (2015) indicated that an extract from *N. glauca* had been exhibit an anti-radical activities against DPPH and ABTS of 86.7 µg /mL and 67.6 µg /mL respectively, besides, we found a much better activities with several extracts from this same plant. For antimicrobial activities, it had been demonstrated that *C. arvensis* methanol and hexane extracts from flowers are able to inhibit some pathogen such as *S. aureus*, *L. monocytogenes* and *P. aeruginosa*, while the aqueous extract is unable to inhibit them (Abudunia et al., 2014), moreover the inhibitory capacity of these extracts against *S. aureus* and *L. monocytogenes* remain weaker than our results found with *C. arvensis* flowers extracts. Indeed in our work the largest zones of inhibition of the pathogens were respectively 20.0 ± 0.1 mm (Acetonitrile extract), 40.7 ± 1.1 mm (Ethyl acetate extract), while the best result against *P. aeruginosa* in our work was found in acetonitrile extract (Zone of inhibition reached 8.7 ± 0.6 mm of diameter) and it is less active than *C. arvensis* flowers methanolic extract (Abudunia et al., 2014). No data about antimicrobial activity from other areal parts of this plant. Concerning *C. murale* antimicrobial potential, Zain et al. (2012) found that methanolic extract of *C. murale* leaves is active against *P. aeruginosa* and *C. albicans*, but unable to inhibit *S. typhimurium*, *M. luteus* and *S. aureus*. Awadh Ali et al. (2001) found the lack of activity of the whole plant methanolic extract against *S. aureus* and also *P. aeruginosa*. In other study (Ahmad et al., 2003), they found that the whole crude methanolic extract is inactive against *C. albicans*, *S. aureus* and *S. typhimurium*, but exhibited a medium activity against *P. aeruginosa* (Zone of inhibition reached 16 mm). Nevertheless in our work, *C. murale* extracts had a good inhibitory activity against these pathogens, for example, leaves acetonitrile extract gave important activity with zones of inhibition that reached 10.2 ± 0.6 mm, 24.3 ± 0.4 mm, 17.0 ± 0.2 mm, 29.7 ± 0.6 mm and 15.9 ± 0.2 mm respectively against *C. albicans*, *P. aeruginosa*, *S. typhimurium*, *M. luteus* and *S. aureus*. As regards *N. glauca*, studies reported its cytotoxic activities against pathogens, indeed, Abdel Rahman et al. (2011) found that hexane and methanol extracts from *N. glauca* whole aerial part inhibit weakly different strains of *S. aureus* but don't inhibit *L. monocytogenes*, while in our case all extracts from each areal part (Stems, leaves and flowers) had strongly inhibiting both *S. aureus* and *L. monocytogenes*.

Concerning AChEI activity, although the potent activities of organic extracts from *C. arvensis* flowers and leaves reported by Ercetin et al. (2012), they remain weak comparing with our findings. Literature didn't reported AChEI activity for *C. murale* and *N. glauca*.

Phenolics are very important plant constituents; they exhibit anti-oxidant activity by inactivating free radicals or preventing decomposition of hydroperoxides into free radicals. The intermediate compounds formed by the action of the phenolic anti-oxidants are relatively stable due to charge distribution throughout the aromatic ring system (Costamagna et al., 2013; Maisuthisakul et al., 2007). This large variation of TPP and TF contents in the different extracts from the three plants can be explained by the varying solubility of the phenolic compounds in the used solvents, and this due to the difference of solvent polarities (Marinova and Yanishlieva, 1997). Furthermore, the different parts of plants are known to contain varying quantities of bioactive compounds, indeed, most phenolic compounds and flavonoids were found in the leaf extract, due to photosynthesis in this organ (Andersen and Markham, 2006).

The high correlation between flavonoids content and anti-radical capacity is due to hydroxyl groups in flavonoids molecules that confer scavenging ability on them (Emeka et al., 2015). As regard β-carotene bleaching system, linoleic acid produces hydroperoxides as free radicals during incubation at 50° C. Beta-carotene undergoes rapid discoloration in the absence of anti-oxidants, while, the presence of anti-oxidants in the extracts will minimize its oxidation by the hydroperoxides (Kchaou et al., 2016). This hydroperoxides formed in this system will be neutralized by anti-oxidants. Thus, the degradation rate of β-carotene depends on the anti-oxidant capacity. In the present work, several extracts from the three plants

had shown an excellent capacity to protect β-carotene from discoloration, thus, there are able to neutralize hydroperoxides. It is important to develop new compounds that protect β-carotene from degradation because of the strong biological activity and physiological important role of this pigment (Maisarah et al., 2014).

The antimicrobial ability of the extracts in this work can be attributed to levels of phenolics contents, especially flavonoids levels, which was making out by numerous studies (Özçelik et al., 2011). Indeed, the effectiveness of flavonoids against several pathogenic bacteria and fungi had been carried out (Rauha et al., 2000; Sohn et al., 2004), and researches had make evidence that flavonoids are able to form complexes with extracellular soluble proteins which bind to the bacterial cell wall (Tsuchiya et al., 1996).

Inhibition of acetylcholinesterase (AChE), the key enzyme in the breakdown of acetylcholine, is considered as a promising strategy for the treatment of neurological disorders such as Alzheimer's disease, senile dementia, ataxia and myasthenia gravis (Mukherjeea et al., 2007). In our findings, extracts obtained by low polarity solvents like cyclohexane and dichloromethane exhibited the strongest AChEI activity which hadn't any correlation with TPP and TF contents. This may due to power of other compounds presents in the three plants such as alkaloids, a nonpolar compounds. Indeed, many studies make out the effectiveness of alkaloids like a strong AChE inhibitors (Ahmed et al., 2013; Murraya et al., 2013). Moreover, many Alzheimer's drugs are made from plants alkaloids or their derivatives (Ng et al., 2015; Dall' Acqua, 2013).

The great activity from *N. glauca* (in leaves dichloromethane extract) can be explained by the fact that this plant contains some compounds that inhibit the AChE, such as anabisine (Lisko et al., 2013). Indeed, it had been reported that the anabisine, an alkaloid, and a principal compound of *N. glauca*, reversibly inhibits AChE by binding a serine in the active site of acetylcholinesterase, inhibiting the enzyme completely (Dobren'kov et al., 1987; Karadsheh et al., 1991).

We can finally deduce that the important contents in phenolics, and the good biological profiles of *C. arvensis*, *C. murale* and *N. glauca*, are probably due to the hostile conditions of the marsh area, where these three plants had grown. Indeed the high level of salinity causes stress in the plants from which, they produce much more quantities of secondary metabolites to survive in this conditions (Chanwitheesuk et al., 2005), especially flavonoids to improve a good anti-oxidative response (Reginato et al., 2014; Rezazadeh et al., 2012).

Conclusion

Globally, these screening investigations indicate that extracts from different aerial parts with different solvent polarities (cyclohexane, dichloromethane, ethyl acetate, acetone and acetonitrile) of the three species *C. arvensis*, *C. murale* and *N. glauca*, exhibit different biological activities. Then, confirm the great potential of plants from Chebba marshy region for the production of bioactive compounds, under salinity stress condition, especially polyphenolics and flavonoids.

Conflict of Interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgement

This research was funded by the Tunisian Ministry of Higher Education and Scientific Research.

References

Abdel Rahman SM, Abd-Ellatif SA, Deraz SF, Khalil AA. Antibacterial activity of some wild medicinal plants collected from Western Mediterranean coast, Egypt: Natural alternatives for infectious disease treatment. Afr J Biotechnol. 2011; 10: 10733-43.

Abudunia A-M, Ansar M, Taoufik J, Ramli Y, Essassi EM, Ibrahimi A, Khedid K. Evaluation of antibacterial activity of extracts from Calendula aventis flowers. J Chem Pharma Res. 2014; 6: 156-61.

Ahmad B, Jan Q, Bashir S, Nisar M, Shaheen F, Ahmad M. Pharmacological and biological investigations of *Chenopodium murale* Linn. Asian J Plant Sc. 2003; 2: 1107-11.

Ahmed F, Ghalib RM, Sasikala P, Mueen Ahmed KK. Cholinesterase inhibitors from botanicals. Phcog Rev. 2013; 7: 121-30.

Ameyaw Y, Duker-Eshun G. The alkaloid contents of the ethno -plant organs of three antimalarial medicinal plant species in the Eastern region of Ghana. Int J Chem Sci. 2009; 7: 48-58.

Andersen OM, Markham KR. Flavonoids chemistry, biochemistry and applications. CRC Press, Tylor and Francis, 2006, pp 145-54.

Awadh Ali NA, Jülich WD, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J Ethnopharmacol. 2001; 74: 173-79.

Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N. Screening of anti-oxidant activity and anti-oxidant compounds of some edible plants of Thailand. Food Chem. 2005; 92: 491-97.

Chethan J, Sampath Kumara KK, Shailasree S, Prakash HS.

Anti-oxidant, antibacterial and DNA protecting activity of selected medicinally important *Asteraceae* plants. Int J Pharm Pharm Sci. 2012; 4: 257-61.

Ćetković GS, Djilas SM, Canadanovic-Brunet JM, Tumbas VT. Anti-oxidant properties of marigold extracts. Food Res Inter. 2004; 37: 643-50.

Costamagna MS, Ordoñez RM, Zampini IC, Sayago JE, Isla MI. Nutritional and anti-oxidant properties of Geoffroea decorticans, an Argentinean fruit, and derived products (flour, arrope, decoction and hydroalcoholic beverage). Food Res Inter. 2013; 54: 160-68.

Dall'Acqua S. Plant-derived acetylcholinesterase inhibitory alkaloids for the treatment of Alzheimer's disease. Bot Targets Ther. 2013; 3: 19–28.

Dobren'kov AG, Tilyabaev Z, Dalimov DN, Abduvakhabov AA. Anabasine and cytisine derivatives as reversible cholinesterase inhibitors. Chem Nat Compd. 1987; 1: 97-100.

Ellman GL, Courtney KD, Andres Vjr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7: 88-95.

Emeka EJ, Oluwatoyin AE, Adekunle OI, Ignis IO. Preliminary phytochemical screening and evaluation of hypoglycemic properties of the root extract of *Uveria chamae*. Bangladesh J Pharmacol. 2015; 10: 326-31

Ercetin T, Sezer Senolb F, Erdogan Orhanb I, Tokerb G. Comparative assessment of anti-oxidant and cholinesterase inhibitory properties of the marigold extracts from *Calendula arvensis* L. and *Calendula officinalis* L. Ind Crop Prod. 2012; 36: 203-08.

Güven K, Yücel E, Çetintaş F. Antimicrobial activities of fruits of *Crataegus* and *Pyrus* species. Pharm Biol. 2006; 44: 79-83.

Jallali I, Megdiche W, M'Hamdi B, Oueslati S, Smaoui A, Abdelly C. Changes in phenolic composition and anti-oxidant activities of the edible halophyte *Crithmum maritimum* L. with physiological stage and extraction method. Acta Physiol Plant. 2012; 34: 1451-59.

Kaileh M, Berghe WV, Boone E, Essawi T, Haegeman G. Screening of indigenous Palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity. J Ethnopharmacol. 2007; 113: 510-6.

Karadsheh N, Kussie P, Linthicum DS. Inhibition of acetylcholinesterase by caffeine, anabasine, methyl pyrrolidine and their derivatives. Toxicol Lett. 1991; 55: 335-42.

Kchaou M, Ben Salah H, Mhiri R, Allouche N. Anti-oxidant and anti-acetylcholinesterase activities of *Zygophyllum album*. Bangladesh J Pharmacol. 2016; 11: 54-62.

Kirby AJ, Schmidt, RJ. The anti-oxidant activity of Chinese herbs for eczema and of placebo herbs. J Ethnopharmacol. 1997; 56: 103-08.

Kintzios SE, Barberaki MG. Plants that fight cancer. CRC Press, Beth Budny, USA, 2004, pp 195-241.

Lisko JG, Stanfill SB, Duncan BW, Watson CH. Application of GC-MS/MS for the analysis of tobacco alkaloids in cigarette filler and various tobacco species. Anal Chem. 2013; 85: 3380-84.

Liu YR, Li WG, Chen LF, Xiao BK, Yang JY, Yang L, Zhang CG, Huang RQ, Dong JX. ABTS•+ scavenging potency of selected flavonols from *Hypericum perforatum* L. by HPLC-ESI/MS QQQ: Reaction observation, adduct characterization and scavenging activity determination. Food Res Inter. 2014; 58: 47-58.

Mabrouk L, Ben Brahim M, Hamza A, Bradai MN. Diversity and temporal fluctuations of epiphytes and sessile invertebrates on the rhizomes *Posidonia oceanic* in a seagrass meadow off Tunisia. Mar Ecol. 2014; 35: 212-20.

Maisarah AM, Asmah R, Fauziah O. Proximate analysis, anti-oxidant and anti-proliferative activities of different parts of *Carica papaya*. J Tissue Sci Eng. 2014; 5: 1-7.

Maisuthisakul P, Suttajit M, Pongsawatmanit R. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chem. 2007; 100: 1409-18.

Marinova EM, Yanishlieva NV. Anti-oxidative activity of extracts from selected species of the family *Lamiaceae* in sunflower oil. Food Chem. 1997; 58: 245-48.

Montiel-Ruiza M, González-Trujanob ME., Déciga-Campos M. Synergistic interactions between the anti-nociceptive effect of Rhodiolarosea extract and B vitamins in the mouse formalin test Rosa. Phytomedicine 2013 ; 20: 1280-87.

Mukherjeea PK, Kumarb V, Malb M, Houghton PJ. Acetylcholinesterase inhibitors from plants. Phytomedicine 2007; 14: 289-300.

Murraya AP, Faraonia MB, Castroa MJ, Alzaa NP, Cavallaro V. Natural AChE inhibitors from plants and their contribution to Alzheimer's dsease therapy . Curr Neuropharmacol. 2013; 11: 388-413.

Ng YP, Tsun Or TCh, Ip NY. Plant alkaloids as drug leads for Alzheimer's disease. Neurochem Inter. 2015; 89: 260-70.

ÖzçelikB, KartalM, OrhanI. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. Pharm Biol. 2011; 49: 396-402.

Pratt DE. Natural anti-oxidants of soybean and other oil-seeds. In: Simic, M.G., Karel, M. (Eds.), Aut-oxidation in food and biological systems. Plenum Press, New York, 1980, pp 283-92.

Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M, Cazin M, Cazin JC, Bailleul F, Trotin F. Phenolic compounds and anti-oxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. J Ethnopharmacol. 2000; 72: 35–42.

Raina H, Soni G, Jauhari N, Sharma N, Bharadvaja N. Phytochemical importance of medicinal plants as potential sources of anticancer agents. Turk J Bot. 2014; 38: 1027-35.

Rauha JP, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P. Antimicrobial effects of finnish plant extracts containing flavonoids and other phenolic compounds. Int. J Food Microbiol. 2000; 56: 3-12.

Re P, Proteggente R, Pannala N, Yang A, Rice-Evans CMA. Anti-oxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med. 1999; 26: 1231-37.

Reginato MA, Castagna A, Furlàn A, Castro S, Ranieri A, Luna V.Physiological responses of a halophytic shrub to salt stressby Na2SO4 and NaCl: Oxidative damage and the role of polyphenols in anti-oxidant protection. AoB Plants. 2014; 6.

Rezazadeh A, Ghasemnezhad A, Barani M, Telmadarrehei T. Effect of salinity on phenolic composition and anti-oxidant activity of artichoke (*Cynara scolymus* L.) leaves. Res J Med Plant. 2012; 6: 245-52.

Saidi S, Bouri S, Ben Dhia H, Anselme B. A GIS-based susceptibility indexing method for irrigation and drinking water management planning: Application to Chebba Mellouleche Aquifer, Tunisia. Agric. Water Manage. 2009; 96: 1683-90.

Selmar D, Kleinwächter M. Stress enhances the synthesis of secondary plant products: The impact of stress-related over-reduction on the accumulation of natural products. Plant cell Physiol. 2013; 54: 817-26.

Sohn HY, Son KH, Know CS, Kan SS. Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: Morus alba L., Morus mongolica Schneider, *Broussnetia papyrifera* (L.) Vent, Sophora flavescens Ait and Echinosophora koreensis Nakai. Phytomedicine 2004; 11: 666-72.

Tabana YM, Dahham SS, Ahmed Hassan LE. *In vitro* anti-metastatic and anti-oxidant activity of *Nicotiana glauca* fraction against breast cancer cells. Adv Biol Res. 2015; 9: 95-102.

Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma, M. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. J Ethnopharmacol. 1996; 50: 27-34.

Waterman PG, Mole, S. Analysis of phenolic plant metabolites. Blackwell Scientific Publications, Oxford, UK, 1994, pp 85-87.

Zain ME, Awaad AS, Al-Outhman MR, El-Meligy RM. Anti-microbial activities of Saudi Arabian desert plants. Phytopharmacology 2012; 2: 106-13.

Author Info
Lotfi Mellouli (Principal contact)
e-mail: lotfi.mallouli@cbs.rnrt.tn

Your feedback about this paper

1. Number of times you have read this paper 0

2. Number of times you have seen the video clip 0

3. Quality of paper

○ Excellent ○ Good ○ Moderate ○ Not good

4. Your comments