The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM)
ISSN: 2602-3199

The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 2019

Volume 8, Pages 1-11

ICVALS 2019: International Conference on Veterinary, Agriculture and Life Science

Evaluation of cytotoxic Effect, anticholinesterase, antioxidant, antiarthritic and antibacterial activities of the Algerian species *Scabiosa stellata* L.

Mouffouk CHAIMA
University of Batna-2

Mouffouk SOUMIA
University of Batna-1

Hambaba LEILA
University of Batna-2

Haba HAMADA
University of Batna-1

Mouffouk SARA
University of Batna-2

Abstract: The present study investigates the evaluation of *in vitro* pharmacological activities of crude extracts (petroleum ether, ethyl acetate and *n*-butanol) obtained from the plant *Scabiosa stellata* L. The cytotoxicity of extracts was tested by Brine shrimp lethality method; the acetylcholinesterase inhibitory activity was performed using Ellman's colorimetric method. The anti-arthritic activity was conducted by bovine serum albumin denaturation method, and the antioxidant activity was evaluated by six different methods including DPPH and ABTS radicals scavenging activities, reducing power, CUPRAC assay, ferrous ions and metal chelating assay. Furthermore, the antibacterial activity was estimated by agar disk diffusion assay against ten bacterial strains. The phytochemical screening of all the extracts revealed the presence of several types of secondary metabolites. The ethyl acetate extract recorded the highest content of polyphenols, flavonoids and tannins. All the crude extracts (PE, EtAOc and *n*-BuOH) had antioxidant activities in various assays and prevent the denaturation of bovin serum albumin in dose depending manner. A significant cytotoxic effect was observed for the *n*-butanolic extract with 57.2 ± 0.2% of mortality at 80 µg/mL, the ethyl acetate extract displayed a moderate anticholinesterase activity at 200 µg/mL. All the crude extracts showed antibacterial activity against most tested strains, with zones of inhibition ranging from 9 to 20 mm. The results indicate that the species *S. stellata* could be an important source of therapeutic agents against neurodegenerative inflammatory and infectious diseases.

Keywords: *Scabiosa stellata* L., Cytotoxicity, Anticholinesterase, Antioxidant, Anti-arthritic

Introduction

The supplementation of the body by exogenous bioactive compounds is one of the new therapeutic strategies to prevent the appearance of cancers oxidative, inflammatory, infectious and neurodegenerative diseases [1-2]. In this context, many researchers are interested in medicinal plants as an alternative and important source of natural compounds to find new antioxidant and anticancer agents with few side effects, which act according to several modes of action to reduce the effects of free radicals and the appearance of oxidative stress-related diseases (cancers, neurodegenerative and inflammation). These species are found in many families.

The family Dipsacaceae is represented by three tribes and comprises nine genera.The genus *Scabiosa* comprises about 100 species [3]. Plants of this genus are widely used in traditional medicine for the treatment of various

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

diseases including hypoglycemia [4], respiratory diseases and menstrual regulation [5] and for their diuretic effects [6]. They are also recommended in dermatoses and against ulcers [7]. *Scabiosa stellata* L. is an annual plant with bluish flowers, distributed in North Africa [8] and used in Moroccan popular medicine against heel cracks [9] and for the treatment of various respiratory diseases including bronchitis, bronchial pneumonia, influenza and asthma [10].

The present study investigated the biological evolution of crude extracts (petroleum ether, ethyl acetate and *n*-butanol) obtained from the plant *Scabiosa stellata* by the estimation of total phenolic and flavonoid contents and the assessment of its *in vitro* cytotoxic, anticholinesterase, antioxidant, antiarthritic and antibacterial activities.

Material and Methods

Plant material

The plant *Scabiosa stellata* L. was collected in May 2014 in the Aures region (Bellezma, Algeria) and was identified by Professor Bachir Oudjehih, Agronomic Institute of the University of Batna-1, under the number 2224/LCCE.

Preparation of plant extract

The powder of the whole plant *Scabiosa stellata* (500 g) was macerated twice (5 Lx 2 each 48h) with a solvent mixture of ethanol- H2O (70:30) at room temperature. After filtration, the filtrate was concentrated under vacuum at room temperature to obtain a hydro-alcoholic extract (400 mL). This solution was submitted to liquid-liquid fractioning using organic solvents PE, EtOAc and *n*-BuOH successively (100 mLx 5 of each organic extract) until the separation of both phases aqueous and organic. The obtained phases were dried over anhydrous sodium sulfate, filtered and evaporated to to provide dryness extracts PE (1.03 g), EtOAc (6.52 g) and *n*-BuOH (30.38 g).

Phytochemical Screening

In order to detect the different secondary metabolites present in crude extracts of *Scabiosa stellata*, a phytochemical screening was carried out using the methods described by Fransworth (1966) [11] based on the observation of color changes in the initial mixture or the formation of a precipitate.

Total Bioactive Content

The quantification of total phenolic content of the methanolic extract was assessed spectrophotometrically using the Folin-Ciocalteu method [12]. 200 µL of the sample was added to 1 mL of Folin-Ciolcalteu solution (10 %). After 4 minutes, a volume of 800 µL of sodium carbonate solution (Na_2CO_3, 75 g/L) was added. The mixture was incubated for two hours in darkness at room temperature. After incubation, the absorbance was read at 765 nm using UV-Vis spectrophotometer (VIS-7220G). Gallic acid (50-500 µg/mL) was used as a standard to establish the calibration curve from which the concentration of polyphenols was calculated and the results were expressed in microgram equivalents of gallic acid per milligram of extract (µg GA/mg of extract).

The total flavonoid content of MeOH extract from *Nonea vesicaria* was performed by the trichloroaluminum method [13]. 1 mL of trichloroaluminum solution prepared in water ($AlCl_3$, 2 %) was added to 1 mL of the crude extract. The mixture was vigorously agitated and incubated for 10 minutes at room temperature and then the absorbance of the sample was measured at 430 nm. Quercetin (25 - 200 µg/mL) was used to realize the calibration curve to determine the concentration of flavonoids and the results were expressed in microgram equivalents of quercetin per milligram of extract (µg QE/mg of extract).

The analysis of condensed tannins was processed using the method described by Heimler et al [14]. 3 mL of vanillin solution (4 %) were added to 50 µL of tested extracts and then 1.5 mL of concentrated hydrochloric acid were added to the mixture. After 15 min of incubation, the absorbance was measured at 500 nm. Catechin (10 - 300 µg/mL) was used to establish the calibration curve to calculate the concentration of condensed tannins in the extracts. The results were expressed in micrograms equivalent of catechin per milligram of extract (µg EC/ mg extract).

Antioxidant Activities

DPPH free radical-scavenging assay

The antioxidant activity of extracts was evaluated using the method of Blois et al [15]. 25 µL of different dilutions of the extracts (PE, EtOAc and *n*-BuOH) or standards (BHT, BHA and ascorbic acid) were added to 975 µL of DPPH (0.025 mg/mL). Then, the mixture was kept in the dark place at room temperature for 30 min. The absorbance was measured at 517 nm and the percentage of DPPH radical-scavenging activity of each extract was calculated as follows:

$$\text{DPPH scavenging effect (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

$A_{control}$ is the absorbance of blank and A_{Sample} is the absorbance of positive control or sample.

ABTS scavenging activity

The spectrophotometric analysis of ABTS scavenging activity was done by the method of Re et al [16]. The $ABTS^{\cdot+}$ was produced by the reaction between 7 mM of ABTS prepared in H_2O and 2.45 mM of $K_2S_2O_8$, stored in the dark at room temperature for 12 h before use. The ABTS solution was diluted with ethanol to get an absorbance of 0.70 ± 0.20 at 734 nm. Then, 2 mL of the diluted ABTS solution were added to 1 mL of sample solution (extracts, BHT and BHA) at different concentrations (0.0156 - l mg/mL). After 30 min, absorbances were measured at 734 nm and the percentage of inhibition was calculated for each concentration relative to a control absorbance. The scavenging capacity of ABTS was estimated using the following equation:

$$\text{Scavenging effect (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

$A_{control}$ is the absorbance of control and A_{Sample} is the absorbance of positive control or sample.

Reducing power assay

The reducing power was determined according to the method of Oyaizu et al [17], 100 µL of sample solution at different concentrations were added to 0.5 mL of phosphate buffer (0.2 M; pH 6.6) and 0.5 mL of potassium ferricyanide (1 %). The mixture was incubated at 50 °C for 20 min. Then 0.5 mL of trichloroacetic acid (10 %) was added to the mixture which was centrifuged for 10 min at 3000 rpm. The supernatant (0.5 mL) was mixed with 0.5 mL of distilled water and 125 µL of $FeCl_3$ (1 %) freshly prepared. The absorbances were measured at 700 nm and the results were calculated as $A_{0.5}$ (µg/mL) which indicated the concentration corresponding to the absorbance at 0.50 nm. The reducing power of the different extracts was compared to those of BHA, BHT, ascorbic and tannic acids and α-tocopherol as standards.

Cupric reducing antioxidant capacity (CUPRAC)

The cupric reducing antioxidant capacity was determined according to the method of Apak et al [18]**.** 50 µL of a solution of $CuCl_2$ (10 mM) were added to 50 µL of the neocuprine solution (7.5 mM) and 60 µL of NH_4Ac buffer solution (1 M, pH = 7.0). Different concentrations of extracts and standards were added to the initial mixture to make a final volume of 200 µL. The samples were shielded from light and the absorbance was measured at 450 nm after 1h. The results were calculated as $A_{0.5}$ (µg/mL) and the reduction capacity of the extracts was compared with those of α-tocopherol, BHA and BHT as standards.

Chelation of ferrous iron

The chelation of ferrous iron was estimated by the method of Le et al [12]**.** 500 µL of the samples (extracts and EDTA) were initially mixed with 100 µL of $FeCl_2$ (0.6 mM) and 900 µL of methanol. After 5 min, 100 µL of Ferrozine (5 mM) were added. The obtained solutions were agitated and incubated for 10 min at room temperature and the absorbance was measured at 560 nm. The results of ferrous iron chelation were transmitted as percentage of inhibition (%) according to the equation:

$$\text{Activity (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

$A_{Control}$ is the absorbance at 560 nm of the control and A_{sample} is the absorbance of positive control or sample.

Chelation of the metal ions

The chelation of metal ions was assessed according to the method of Decker et al [19]**.** 40 µL of the samples (extracts and EDTA) at different concentrations were added to 40 µL of $FeCl_2$ (0.2 mM) and 40 µL of ethanol. The reaction was initiated by the addition of 80 µL of ferene solution (0.5 mM). The mixture was agitated vigorously and incubated for 10 min at room temperature. The absorbance was measured at 562 nm and the results were given as a percentage of inhibition using the following equation:

$$\text{Activity (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

$A_{Control}$ is the absorbance at 562 nm of the control and A_{sample} is the absorbance of positive control or sample.

***In vitro* cytotoxicity**

The cytotoxicity of the crude extracts prepared from the species *Scabiosa stellata* was evaluated according to brine shrimp *lethality essay* described by Meyer *et al* (1982) [20]**.** Brine shrimp eggs were hatched in a shallow rectangular dish (150 mm × 5 mm) filled with sea water (38 g marine salts dissolved in 1L of distilled water). The dish was divited into two unequal compartments using a plastic divider. The shrimp eggs (50 mg) were sprinkled into the larger darkened compartment, while the smaller compartment was illuminated. After 48 hours the phototropic nauplii were collected using pastor pipette from the illuminated compartment. The extracts in various concentrations (0.5, 1, 2, 4, 8 mg/mL) were prepared in dimethyl sulfoxide (DMSO). 100 µL of each prepared solution were transferred into vial tubes containing 4.9 mL of sea water and ten larvae of brine shrimps (nauplii) to obtain final concentrations of 10, 20, 40, 80 µg/mL. The vials were maintained under illumination. After 24 hours the survived shrimps were counted, the total death and the percentage of mortality of each dose were determined.

Acetylcholinesterase Inhibitory Activity

The inhibition of AChE by crude extracts was evaluated using the method described by Ellman et al [21]. 750 µL of sodium phosphate buffer (100 mM , pH 8.0) were added to 50 µL of crude extracts (PE, AcOEt and *n*-BuOH) at different concentrations and 100 µL of AChE (5.32×10^{-3} U) solution prepared in phosphate buffer salin pH = 8. The mixture was incubated at 25 °C for 15 minutes then 50 µL of DTNB (5,5′-dithio-bis-2-nitrobenzoate) (0.5 mM) were added with 50 µL of acetylthiocholine iodide (0.71 mM). The absorbance of the mixture was read at 412 nm, after 5, 10, 15 and 20 min. Galantamine was used as a positive control. The percentage of inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = (E - S/E) \times 100$$

Where E is the activity of the enzyme without extract and S is the activity of the enzyme with the extract.

Antibacterial Activity

The antibacterial activity of the crude extracts (PE, AcOEt and *n*-BuOH) was estimated by the agar disk diffusion assay [22] against ten bacterial strains including four Gram-positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus albus*, *Enterococcus* sp, *Streptococcus* D) and six Gram-negative (*Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 15442, *Acinetobacter baumannii*, *Proteus mirabilis*, *Salmonella* Typhimurium and *Enterobacter sakazaki).* The bacterial strains used were initially isolated from patient affected with infections, identified in the Laboratory of Microbiology University Hospital Center (Batna) by microscopic examination stain reaction according to their morphology, Gram character, colony aspect, oxygen requirement, physiological and biochemical characters and using serological methods and purified using the method of streaking the four quadrants in sterile conditions and at optimum temperatures according to the strain concerned for 24 h. One or several colonies from each pure culture were transferred into 5 mL of nutrient broth. The bacterial suspension was homogenized and incubated at 37 °C for 10-24 hours. After incubation, a reading of the optical density (OD) of 1 mL of inoculum was made by a spectrophotometer at 625 nm. Opacity must be

equivalent to 0.5 McFarland. A sample from each inoculum was used to inoculate Petri disks containing Mueller Hinton by swabbing technique. Wathman paper disks (6 mm) were impregnated with 10 µL of the extract solutions at different concentrations (1000, 500, 250, 125, 62.5 and 31.25 mg/mL and filed carefully on the surface of the inoculated agar with sterile forceps. The discs of the negative controls were impregnated with DMSO. The Petri dishes were incubated at 37 °C for 24 h. The tests were performed in triplicate (three boxes for each concentration of extract and for each strain). The results were expressed by the diameters of zones of inhibition around the discs produced.

In vitro anti-inflammatory activity

The anti-inflammatory activity of *S. stellata* extracts was achieved by bovine serum albumin denaturation method [23]. 500 µL of sample solutions or standard drug (Ibuprofen) at different concentrations (125, 250, 500 and 1000 µg/mL) were added to 500 µL of BSA solution (0.2%) prepared in Tris Buffer Saline (pH 6.6). A control tube contains a volume of 0.5 mL of BSA and 0.5 mL of ethanol was also prepared. The tested tubes were incubated at 37 °C for 10 min then heated at 72 °C for 5 min. After cooling, the absorbances of these solutions were read at 660 nm. Each experiment was performed in triplicate and the average absorbance was recorded. The percentage of inhibition of denaturation was determined as follows:

$$\text{Inhibition of denaturation (\%)} = ((\text{A Control} - \text{A sample}) / \text{A Control}) \times 100$$

AControl is the absorbance at 660 nm of the control and Asample is the absorbance of positive control or sample.

Statistical Analysis

The results were given as the means ± S.D (p <0.05) for three replicates for each sample. The $A_{0.5}$ (reducing power and CUPRAC assay) and IC_{50} (DPPH, ABTS, anticholinesterase inhibition activity), LC_{50} (brine shrimp lethality test) and EC_{50} (chelation of ferrous iron, chelation of the metal ions) values were calculated by linear regression analysis.

Results and Discussion

Phytochemical screening

The phytochemical screening of crude extracts (PE, EtOAc and n-BuOH) obtained from the plant *S. stellata* L. reveals the presence of several classes of secondary metabolites well-known to possess pharmacological proprieties including : steroids, flavonoids, saponins, tannins, alkaloids, quinons and carotenoids. All the detected compounds were previously isolated from species of the same genus [24-28]. Flavonoids, saponins, triterpenoids and sterols have been previously purified and identified from this species [29-31].

Total bioactive contents

Regarding the importance of phenolic compounds as bioactive products doted of biological activities, the quantification of phenolic, flavonoid and tannin contents of *S. stellata* crude extracts were assessed. And the results are summarized in Table 01.

Table 1. Total bioactive contents of *Scabiosa stellata* extracts

Extracts	Total phenolic content [a] (µg GAE/ mg of extract)	Total flavonoids content [b] (µg QE/ mg of extract)	Total Condensed tannins content [c] (µg EC/mg of extract)
PE	30.33 ± 0.15	13.83 ± 0.45	0.07 ± 0.02
EtOAc	117.66 ± 0.52	72.90 ± 0.76	32.05 ± 0.15
***n*-BuOH**	77.12 ± 0.29	57.83 ± 0.25	0.95 ± 0.015

[a]Total phenolic content was expressed as µg gallic acid equivalent/ mg of plant extract ; [b] Flavonoid content was expressed as µg quercetin equivalent / mg of plant extract ; [c]Total condensed tannins content was expressed as µg catechin equivalent/ mg of plant extract.

A difference in total bioactive contents depending on solvent polarities used for the extraction procedures were observed**.** The highest phenolic, flavonoid and tannin contents were found in the ethyl acetate extract, followed by *n*-butanolic and petroleum ether extracts respectively.

Previous research conducted on the same species [29] and on species of the same genus including : *S. tschiliensis* [32] and *S. arenaria* [33] indicated lower levels of polyphenol and flavonoid contents compared to the results of the present study.

The variation in total phenolic content between species of the same genus could be due to various intrinsic factors (genetic potential of the individual species for the biosynthesis of polyphenols) and extrinsic factors (environment, stage of maturation and storage period) [34].

Antioxidant Activities

The results of the antioxidant activity are presented in Table 2. All the crude extracts exhibit an interesting antioxidant activity at different systems including: DPPH and ABTS radicals scavenging activities, reducing power, CUPRAC assay, ferrous ions and metal chelating assay. This result was expected, since several studies reported the considerable antioxidant potential of plants belonging to the same genus [32-33].

Table 2. Antioxidant activities of the species *Scabiosa stellata*

	Antioxidant activities					
	DPPH assay[a]	**ABTS assay[a]**	**CUPRAC assay[a]**	**Reducing power[a]**	**chelation in ferrous iron assay [a]**	**chelation in metal chelate assay [a]**
Extract and standards	IC_{50} (µg/mL)	IC_{50} (µg/mL)	$A_{0.50}$ (µg/mL)	$A_{0.50}$ (µg/mL)	EC_{50} (mg/mL)	EC_{50} (mg/mL)
***n*-BuOH**	21.22 ± 0.30	24.99 ± 0.36	42.16 ± 3.06	12.13 ± 0.52	1.65 ± 0.01	145.35 ± 0.34
EtOAc	25.15 ± 0.68	14.00 ± 0.8	28.50 ± 1.24	6.54 ± 0.48	5.03 ± 0.07	> 200
PE	171.61 ± 0.12	64.10 ± 0.38	100.9 ± 8.06	> 50	ND	ND
BHA[b]	6.82 ± 0.49	1.81 ± 0.10	3.64 ± 0.19	8.41 ± 0.67	NT	NT
BHT[b]	22.32 ± 0.02	1.29 ± 0.30	9.62 ± 0.87	> 50	NT	NT
Tannic acid[b]	7.74 ± 0.19	1.01 ± 0.16	3.76 ± 0.73	4.57 ± 0.30	NT	NT
Ascorbic acid[b]	3.1 ± 0.002	1.74 ± 0.10	12.43 ± 0.09	9.01 ± 0.46	NT	NT
α-Tocopherol[b]	13.02 ± 0.17	7.59 ± 0.53	19.92 ± 1.46	34.93 ± 0.38	NT	NT
EDTA[b]	NT	NT	NT	NT	0.063 ± 0.01	8.57 ± 0.14

[a]Values expressed are means ± SD of three measurements ($p < 0.05$); [b]Reference compounds; NT : not tested; ND: not determined because the inhibition at highest screened concentration (200 µg /mL) was less than 50%.

.

All the crude extracts (PE, EtOAc and *n*-BuOH) possessed radical scavenging properties in both DPPH and ABTS assays that varies in a dose-dependent manner. In DPPH free radical scavenging assay, the *n*-butanolic extract (21.22 ± 0.30 µg/mL) exhibited a higher antioxidant activity then BHT (22.32 ± 0.02 µg/mL), but this activity was relatively low compared to the BHA (6.82 ± 0.49 µg/mL), ascorbic acid (3.1 ± 0.002 µg/mL) and α-tocopherol (13.02 ± 0.17) used as standards. In ABTS radical scavenging test, the EtOAc extract had strong antioxidant activity with a value of IC_{50} at 14.00 ± 0.8 µg/mL, followed by the *n*-butanolic and PE extract respectively.

In order to relate the antioxidant potential of the species *Scabisa stellata* to the presence of bioactive compounds that serve as electrons donor inducing the reduction of transition metals, the antioxidant activity was assessed by CUPRAC and reducing power assays (Table 2). The results of all the tested samples (extracts and standards) in both tests were affected in a dose-dependent manner. The EtOAc extract was the most potent extract, this

extract had high reducing power better than that of ascorbic acid, α-tocopherol and BHA known as common standards.

The results of the ferrous ion-chelating and chelation of the metal ions tests (Table 2) revealed that *n*-BuOH and EtOAc extracts had moderate metal chelating activities compared to EDTA as a positive control.While, PE extract does not possess any chelation activities at all the tested concentrations.

The interesting antioxidant activities of crude extracts at different systems can be attributed to the phenolic and flavonoids contents. In fact, several studies noticed that phenolic compound constitute the most powerful antioxidant agents isolated from plants [35].

In vitro cytotoxicity

All the extracts obtained from the plant *Scabiosa stellata* were subjected to Brine Shrimp lethality bioassay in order to evaluate their possible cytotoxic action and anti-tumor properties. The results are given in Table 3.

The result indicates that no mortality was observed with the control group treated with DMSO, while a concentration-dependent increment in a mortality rate of the brine shrimp nauplii was observed in groups treated with the *Scabiosa stellata* crude extracts. The *n*-butanolic extract was the most toxic to Brine Shrimp nauplii, with 57.2 ± 0.2 % of mortality at 80 µg/mL followed by the ethyl acetate extract (28.5 % of mortality) and the petroleum ether extract (42.8 % of mortality) at the same concentration (80 µg/mL).

Table 3. Result of *in vitro* cytotoxicity by Brine shrimp lethality bioassay

sample	**% Mortality under the studied concentrations (µg/mL)** [a]					**Toxicity Profile**
	100	**80**	**40**	**20**	**10**	
DMSO	**0**	**0**	**0**	**0**	0	Non-toxic
***n*- BuOH**	70.14±0.22	57.14±0.32	42.86±0.1	39.29±0.47	21.34±0.57	Toxic
EtOAc	32.02±0.92	28,57± 0.2	21,43± 0.26	17,86±0.3	14,29±0.3	Non-toxic
PE	47.2±0.83	42.86± 0.5	32.14± 0.3	21.43± 0.57	17.86± 0.2	Non-toxic

[a]Values expressed are means ± SD of three measurements ($p < 0.05$).

The significant cytotoxicity of the *n*-butanolic extract could be related to the chemical profile of the species *Scabiosa stellata*. Indeed, saponins isolated previously from this plant are known as potential antiproliferative and antitumor agents doted of apoptic and cytotoxic effects [36].

In vitro anti-inflammatory activity

The anti-inflammatory activity of *S. stellata* extracts was achieved by bovine serum albumin denaturation method and the results are presented in Table 4.

Table 4. In-vitro anti-inflammatory activity of *S. stellata* extracts

	Concentration (µg/ml)		
Extracts/standard	1000	500	250
PE	16 ± 0.1[a]	5.6 ± 0.1	4.0 ± 0.1
EtOAc	78.8 ±0.1	69.5 ±0.1	58.9 ±0.1
n-BuOH	3.2 ± 0.3	1.8 ± 0.1	-
Iboprofen[b]	100 ± 0.3	92 ±0.2	61 ± 0.2

[a]Values expressed are means ± SD of three measurements ($p < 0.05$); [b]Reference compounds; (-): not determined

All the crude extracts and the standard drug (Ibuprofen) had the ability to inhibit thermally-induced protein denaturation in a dose-dependent manner. Ibuprofen showed the maximum percentage of inhibition (100%) at the concentration of 1000 PPM. For the extracts, the maximum percentage of inhibition was observed in EtOAc (78.86%) extract followed by PE (16.02%) and *n*-BuOH (3.21%) extracts at the same concentration.

This result indicated the ability of various extracts obtained from the species *Scabiosa stellata* to prevent the alteration of the electrostatic, hydrogen, hydrophobic and disulfide bonds leading to maintain the three-dimensional structure of the proteins, and their capability to control the production of self-antigen in case of rheumatoid arthritis disease . The interesting anti-inflammatory activity of EtOAc extract can be attributed to the presence of phenolic and flavonoid compounds. Known to possess interesting biological properties [37].

Acetylcholinesterase inhibitory activity

The crude extracts of *S. stellata* were tested for their AChE inhibitory activities by spectrophotometric Ellman method . results showed that The ethyl acetate extract has a moderate activity (30.8%) at the concentration of 200 µg/mL, the *n*-BuOH extract has a low AChE inhibitory activity (10.9%) at the same concentration. While the PE extract did not show any AChE activity at all the tested concentrations.

Previous research conducted on crude extracts of the species *S. arenaria* [33] showed strong inhibition of AChE compared to the results of the present study, this variation in AChE activities among the species of the same genus could be explained by the difference in their chemical composition especially in polyphenols and flavonoids known for their anticholinesterase activities [38].

The results of the antibacterial activity are presented in Tables **5** . All the tested extracts showed inhibition of bacterial growth against at least two strains. The strains *Staphylococcus albus*, *Pseudomonas aregionosa* (ATCC 15442) and *Salmonella* Typhimurium are the most resistant strains to all plant extracts.

Table 5. Antibacterial activity of crude extractrs prepared from the species *Scabiosa stellata*

			Extracts		
	Bacterial strains	**Concentration (mg/ml)**	**PE**	**EtOAc**	***n*-BuOH**
Gram-negative strains	*Escherichia coli ATCC 35218*	1	12 ± 0.1[a]	10± 0.1	-[b]
		0.5	10 ±0.2	09± 0.2	-
		0.25	09 ± 0.2	-	-
		0.125	08± 0.1	-	-
		0.0625	-	-	-
	Pseudomonas aregionosa ATCC 15442	1	-	-	-
		0.5	-	-	-
		0.25	-	-	-
		0.125	-	-	-
		0.0625	-	-	-
	Acinetobacter baumannii	1	-	14 ± 0.1	10± 0.1
		0.5	-	10 ± 0.3	09 ± 0.1
		0.25	-	09 ± 0.1	08± 0.5
		0.125	-	-	-
		0.0625	-	-	-
	Proteus mirabilis	1	-	20± 0.1	-
		0.5	-	18± 0.4	-
		0.25	-	17± 0.1	-
		0.125	-	17± 0.3	-
		0.0625	-	16 ± 0.2	-
	Salmonella Typhimurium	1	-	-	-
		0.5	-	-	-
		0.25	-	-	-
		0.125	-	-	-
		0.0625	-	-	-
	Enterobacter sakazaki	1	-	-	10± 0.1
		0.5	-	-	09 ± 0.1
		0.25	-	-	08± 0.1
		0.125	-	-	-
		0.0625	-	-	-
Gram-positive strains	*Staphylococcus aureus ATCC 25923*	1	18 ± 0.2	13 ± 0.2	-
		0.5	15 ± 0.1	10 ± 0.1	-
		0.25	12 ± 0.3	09 ± 0.1	-
		0.125	09 ± 0.1	-	-
		0.0625	-	-	-
	Staphylococcus albus	1	-	-	-
		0.5	-	-	-
		0.25	-	-	-
		0.125	-	-	-
		0.0625	-	-	-
	Enterococcus sp	1	09± 0.1	09± 0.3	-
		0.5	-	-	-

	0.25	-	-	-
	0.125	-	-	-
	0.0625	-	-	-
Streptococcus D	1	-	09 ± 0.1	-
	0.5	-	-	-
	0.25	-	-	-
	0.125	-	-	-
	0.0625	-	-	-

[a]Values expressed are means ± SD of three measurements ($p < 0.05$) as the diameter of the zones of inhibition (mm) . [b] (-) no zones of inhibition around the discs.

The ethyl acetate extract was found to be the most effective extract against six strains .The greatest zone of inhibition was observed against the clinical strain *P. mirabilis* which is considered as a common cause of nosocomial and urinary infections and one of the most resistant strains [39].

The *n*-BuOH extract has an antibacterial activity against two Gram-negative bacteria *A. baumannii* and *E. sakazaki*. While the petroleum ether extract exhibited an inhibitory effect against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 35218).

The antibacterial activity observed in the tested extracts could be due to different classes of secondary metabolites purified and identified previously including flavonoids, saponins, triterpenoids and sterols, known for their antimicrobial activities [40].

Conclusion

The present study reported the pharmacological evaluation of the species *Scabiosa stellata*. The phytochemical screening of crude extracts indicated the presence of various types of secondary metabolites with important biological activities. important antioxidant activities in all the tested methods were observed and all the crude extracts inhibit thermally-induced protein denaturation in a dose-dependent manner and showed antibacterial and anticholinesterase activities.

Furthermore, it can be concluded that *S. stellata* extracts could be used as a good source of alternative natural products helpful in preventing oxidative and neurodegenerative diseases and as a source of antibacterial, anti-inflammarory and antiproliferative compounds.

Acknowledgements or Notes

The authors wish to express thanks to the DGRSDT of the Algerian ministry of higher education and scientific research for providing a research grant (Project 8/u05/853).

References

Nobili, S., Lippi, D., Witort, E., Donnini, M., Bausi, L., Mini, E., & Capaccioli, S. (2009). Natural compounds for cancer treatment and prevention. *Pharmacological research*, *59*(6), 365-378.

Uttara, B., Singh, A. V., Zamboni, P., & Mahajan, R. T. (2009). Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current neuropharmacology*, *7*(1), 65-74.

Carlson, S. E., Linder, H. P., & Donoghue, M. J. (2012). The historical biogeography of Scabiosa (Dipsacaceae): implications for Old World plant disjunctions. *Journal of Biogeography*, *39*(6), 1086-1100.

Bonet, M. À., Parada, M., Selga, A., & Valles, J. (1999). Studies on pharmaceutical ethnobotany in the regions of L'Alt Emporda and Les Guilleries (Catalonia, Iberian Peninsula). *Journal of Ethnopharmacology*, *68*(1-3), 145-168.

Bussmann, R. W., Glenn, A., Meyer, K., Kuhlman, A., & Townesmith, A. (2010). Herbal mixtures in traditional medicine in Northern Peru. *Journal of Ethnobiology and Ethnomedicine*, *6*(1), 10.

Bonet, M. À., & Valles, J. (2007). Ethnobotany of Montseny biosphere reserve (Catalonia, Iberian Peninsula): plants used in veterinary medicine. *Journal of Ethnopharmacology*, *110*(1), 130-147.

Hotta, M.; Ogata, K.; Nitta, A.; Hoshikawa, K.; Yanagi, M.; Yamazaki, K. *Useful plants of the world*, 1st ed.; Tokyo: Heibonsha LTD, 1989.

Quezel P, Santa S (1963) Nouvelle flore de l'Algérie et des régions désertiques méridionales, vols 1–2. In: CNRS (Ed), Paris, pp 892

Bammi, J., & Douira, A. (2002). Medicinal plants in the forest of Achach (central plateau, Morocco). *J. Acta Bot. Mal*, *27*, 131-145.

Wannes, W. A., & Marzouk, B. (2016). Research progress of Tunisian medicinal plants used for acute diabetes. *Journal of Acute Disease*, *5*(5), 357-363.

Farnsworth, N. R. (1966). Biological and phytochemical screening of plants. *Journal of pharmaceutical sciences*, *55*(3), 225-276.

Le, K., Chiu, F., & Ng, K. (2007). Identification and quantification of antioxidants in Fructus lycii. *Food Chemistry*, *105*(1), 353-363.

Turkoglu, A., Duru, M. E., Mercan, N., Kivrak, I., & Gezer, K. (2007). Antioxidant and antimicrobial activities of Laetiporus sulphureus (Bull.) Murrill. *Food Chemistry*, *101*(1), 267-273.

Heimler, D., Vignolini, P., Dini, M. G., Vincieri, F. F., & Romani, A. (2006). Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. *Food chemistry*, *99*(3), 464-469.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, *26*(9-10), 1231-1237.

Oyaizu, M. (1986). Studies on products of browning reaction: antioxidative activity of products of browning reaction. *Jpn. J. Nutr*, *44*(6), 307-315.

Apak, R., Güçlü, K., Özyürek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of agricultural and food chemistry*, *52*(26), 7970-7981.

Decker, E. A., & Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and food Chemistry*, *38*(3), 674-677.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. J., & McLaughlin, J. L. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta medica*, *45*(05), 31-34.

Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology*, *7*(2), 88-95.

Medini, F., Fellah, H., Ksouri, R., & Abdelly, C. (2014). Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant Limonium delicatulum. *Journal of Taibah University for science*, *8*(3), 216-224.

Karthik, I. (2013). Evaluation of anti-inflammatory activity of Canthium parviflorum by in-vitro method.

Al-Qudah, M. A., Otoom, N. K., Al-Jaber, H. I., Saleh, A. M., Abu Zarga, M. H., Afifi, F. U., & Abu Orabi, S. T. (2017). New flavonol glycoside from Scabiosa prolifera L. aerial parts with in vitro antioxidant and cytotoxic activities. *Natural product research*, *31*(24), 2865-2874.

Zheng, Q., Koike, K., Han, L. K., Okuda, H., & Nikaido, T. (2004). New Biologically Active Triterpenoid Saponins from Scabiosa t schiliensis. *Journal of natural products*, *67*(4), 604-613.

JI, M., LI, S. J., & MA, C. M. (2014). Chemical constituents of the inflorescence of Scabiosa comosa Fisch and their antioxide and α-glucosidase inhibitory activities. *Journal of Inner Mongolia University (Natural Science Edition)*, *4*, 398-403.

Kuril'chenko, V. A., Zemtsova, G. N., & Bandyukova, V. Y. (1971). A chemical study of Scabiosa bipinnata. *Chemistry of Natural Compounds*, *7*(4), 519-519.

Christopoulou, C., Graikou, K., & Chinou, I. (2008). Chemosystematic value of chemical constituents from Scabiosa hymettia (Dipsacaceae). *Chemistry & biodiversity*, *5*(2), 318-323.

Lehbili, M., Magid, A. A., Hubert, J., Kabouche, A., Voutquenne-Nazabadioko, L., Renault, J. H.,Kabouche, Z. (2018). Two new bis-iridoids isolated from Scabiosa stellata and their antibacterial, antioxidant, anti-tyrosinase and cytotoxic activities. *Fitoterapia*, *125*, 41-48.

Lehbili, M., Magid, A. A., Kabouche, A., Voutquenne-Nazabadioko, L., Morjani, H., Harakat, D., & Kabouche, Z. (2018). Triterpenoid saponins from Scabiosa stellata collected in North-eastern Algeria. *Phytochemistry*, *150*, 40-49.

Rahmouni, N., Pinto, D. C., Santos, S. A., Beghidja, N., & Silva, A. M. (2018). Lipophilic composition of Scabiosa stellata L.: An underexploited plant from Batna (Algeria). *Chemical Papers*, *72*(3), 753-762.

Wang, J., Liu, K., Xu, D., Wang, Q., Bi, K., Song, Y., ... & Zhang, L. (2013). Rapid micropropagation system in vitro and antioxidant activity of Scabiosa tschiliensis Grunning. *Plant growth regulation*, *69*(3), 305-310.

Hlila, M. B., Mosbah, H., Mssada, K., Jannet, H. B., Aouni, M., & Selmi, B. (2015). Acetylcholinesterase inhibitory and antioxidant properties of roots extracts from the Tunisian Scabiosa arenaria Forssk. *Industrial Crops and Products*, *67*, 62-69.

Mouffouk, C., Mouffouk, S., Dekkiche, S., Hambaba, L., & Mouffouk, S. (2019). Antioxidant and Antibacterial Activities of the species Silene inflata Sm. *PSM Biological Research*, *4*(2), 74-86.

Mouffouk, S., Marcourt, L., Benkhaled, M., Boudiaf, K., Wolfender, J. L., & Haba, H. (2017). Two New Prenylated Isoflavonoids from Erinacea anthyllis with Antioxidant and Antibacterial Activities. *Natural Product Communications*, *12*(7), 1934578X1701200716.

Ayaz, M., Junaid, M., Ullah, F., Sadiq, A., Subhan, F., Khan, M. A., ... & Ahmad, S. (2016). Molecularly characterized solvent extracts and saponins from Polygonum hydropiper L. show high anti-angiogenic, anti-tumor, brine shrimp, and fibroblast NIH/3T3 cell line cytotoxicity. *Frontiers in pharmacology*, *7*, 74.

Mouffouk, C., Hambaba, L., Haba, H., Mouffouk, S., Bensouici, C., Hachemi, M., & Khadraoui, H. (2018). Acute toxicity and in vivo anti-inflammatory effects and in vitro antioxidant and anti-arthritic potential of Scabiosa stellata. *Oriental Pharmacy and Experimental Medicine*, *18*(4), 335-348.

Mouffouk, C., Hambaba. L., Haba. H. , Mouffouk.,S , Bensouici.,C.(2018). Evaluation of Cytotoxic Effect, Anti-cholinesterase, Hemolytic and Antibacterial activities of the Species Scabiosa stellata L. Current Bioactive Compounds,10.2174/1573407214666180730102338

Wang, M., Guo, Q., Xu, X., Wang, X., Ye, X., Wu, S., ... & Wang, M. (2009). New plasmid-mediated quinolone resistance gene, qnrC, found in a clinical isolate of Proteus mirabilis. *Antimicrobial agents and chemotherapy*, *53*(5), 1892-1897.

Benli, M., Bingol, U., Geven, F., Guney, K., & Yigit, N. (2008). An Investigation on the antimicrobial activity of some endemic plant species from Turkey. *African Journal of Biotechnology*, *7*(1).

Author Information

Mouffouk Chaima
Laboratory of Biotechnology of Bioactive Molecules and Cell Physiopathology. University of Batna 2 Mustapha Benboulaid, 53, Constantine Road. Fesdis, Batna 05078, Algeria.
Contact E-mail: *chaimamouf@gmail.com*

Mouffouk Soumia
Laboratoire de Chimie et Chimie de l'Environnement (L.C.C.E), Département de Chimie, Faculté des Sciences de la Matière, Université de Batna-1, Batna, Algeria
Batna-1 University; Faculty of Matter Sciences; Department of Chemistry. (L.C.C.E) Batna, Algeria

Hambaba Leila
Laboratory of Biotechnology of Bioactive Molecules and Cell Physiopathology. University of Batna 2 Mustapha Benboulaid, 53, Constantine Road. Fesdis, Batna 05078, Algeria

Haba Hamada
Laboratoire de Chimie et Chimie de l'Environnement (L.C.C.E), Département de Chimie, Faculté des Sciences de la Matière, Université de Batna-1, Batna, Algeria.
Batna-1 University; Faculty of Matter Sciences; Department of Chemistry. (L.C.C.E) Batna, Algeria

Mouffouk Sara
Laboratory of Biotechnology of Bioactive Molecules and Cell Physiopathology. University of Batna 2 Mustapha Benboulaid, 53, Constantine Road. Fesdis, Batna 05078, Algeria.