Algerian Journal of Biosciences

Journal homepage: http://www.ajbjournal.periodikos.com.br

Original Article

Antimicrobial and antioxidant activity of methanol extract of *Echinophora spinosa* L. from Jijel, Algeria

GHADBANE Mouloud[a,b,*], BOUNAR Rabah[a,c] and REBBAS Khellaf[a,d]

[a] *Department of Natural and Life Sciences, Faculty of Sciences, University of Mohamed Boudiaf-M'sila, M'sila , Algeria*

[b] *Laboratory of Applied Microbiology, Department of Microbiology, Faculty of Natural and Life Sciences, University of Ferhat Abbas, Setif 1, Algeria*

[c] *Laboratory of Biodiversity and biotechnological techniques for the valuation of plant resources, Department of Natural and Life Sciences, Faculty of Sciences, University of Mohamed Boudiaf-M'sila, M'sila , Algeria*

[d] *Laboratoire d'Agro-Biotechnologie et de nutrition en zones arides et semi-arides, université Ibn Khaldoun, Tiaret, Algérie.*

ARTICLE INFO

Article history:
Received 03 Aug 2020
Revised 20 Aug 2020
Accepted 30 Aug 2020

Keywords:
Antioxidant ;
Echinophora spinosa ;
Flavonoid ;
methanol extracts;
Antimicrobial

ABSTRACT

In this study, the antioxidant and antimicrobial activities of *Echinophora spinosa* were investigated. Antimicrobial activity of methanol extracts obtained from *Echinophora spinosa* was examined using the disc diffusion method. Antioxidant activity of the methanol extracts was examined using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test. Methanol extract of ripe fruits of *E. spinosa* showed highest total phenolic content (69.17 ± 1.2 µg GAE/mg extract) and the major flavonoid contents (12.122 ± 0.44 µg QE/mg extract) was found in leaves of the plant. In disk diffusion antimicrobial assay, *E. spinosa* manifested broad spectrum of activity. The largest capacity to neutralize DPPH radicals was found for ripe fruits methanol extract of *E. spinosa* plant. The results shows that the various parts of *E. spinosa* extracts promising antioxidant and antimicrobial activities have potential bioactivities due to high content of phenolic and flavonoid compounds.

1. Introduction

The genus *Echinophora* L. (Apiaceae) in the Mediterranean area is represented by seven species [1,2]. In North Africa, only *Echinophora spinosa* L. was reported from Algeria and Tunisia as a very rare taxon [3,1].

Echinophora spinosa L. is a psammophilous species growing on maritime sands. The plant is edible with a pleasant taste: thornless young and tender leaves are used for salads and the roots as carrots [4].

The plants genera *Echinophora* species are also used in folk medicine to heal wounds and to treat gastric ulcers due to its antifungal, carminative, and digestive properties [4]. *E. spinosa*, though other members of this genus have been used as wound healing, antispasmodic, and digestive agents [5,6].

From a phytochemical composition of *E. spinosa*, only a few works have been conducted on the phytochemical profiles of this species gathered in the Mediterranean area [7,4,8,9,6].

Antibacterial properties of essential oil of *E. spinosa* have been demonstrated against *Clostridium difficile*, *C. perfringens*, *Enterococcus faecalis*, *Eubacterium limosum*, *Peptostreptococcus anaerobius* and *Candida albicans* [4]. In addition, Pavela et al., [9]; Pavela et al., [6], mentioned that the essential oil of leaves and ripe fruits of *E. spinosa* are rich in phenolic compounds and exhibited insecticidal activity.

The aim of our work was to investigate the chemical composition of the methanol extract of leaves and ripe fruits of *E. spinosa* collected from Jijel (Algeria), and to evaluate the antioxidant and antimicrobial activities.

2. Materials and Methods

2.1. Collection of plant material

The plant material (leaves and ripe fruits) of *Echinophora spinosa* L. (Figure 1) was collected from Jijel, Algeria

* *Corresponding author*. E-mail address: *mouloud.ghadbane@univ-msila.dz*

Peer review under responsibility of University of Echahid Hamma Lakhdar.

DOI: http://dx.doi.org/10.5281/zenodo.4076344

(Figure 2), during the month of May and June, 2018. The identity was subsequently confirmed by Dr. BOUNAR Rabah, University of M'sila. The fresh material was kept in perforated poly bags and immediately brought to the laboratory.

Fig 1. *Echinophora spinosa* L. plant.

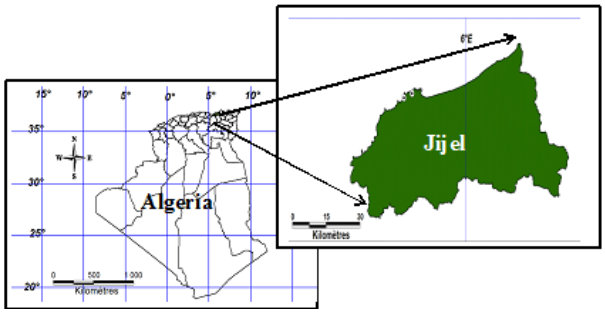

Fig 2. The study area.

2.1. Preparation of plant extracts

Plant leaves and ripe fruits of *E. spinosa* were washed thoroughly with distilled water and shade-dried at room temperature. The dried leaves and ripe fruits were uniformly ground using an electric grinder. The powdered plant material (200 g) was extracted for 2 days in 1 L 100% methanol. The separated extracts were then filtered through Whatman No. 1 filter paper and the methanol filtrate evaporated to dryness using a rotary evaporator at room temperature (30 °C). The thick extracted mass was then dried at room temperature, and the dried extract stored in an air-tight container at 4 °C until further use [10].

2.2. Determination of plant extract yield (%)

Yield percentage (*w*/w) from the dried extracts was calculated by formula (1):

$$Yied\,(\%) = \left[\frac{W1}{W2}\right] \times 100 \qquad (1$$

Where W1 is the dry weight of extract after evaporating the solvent and W2 is the weight of the soaked plant powder.

2.3. Determination of total phenolic content

The total phenolic content in methanol extract of leaf and ripe fruits of *Echinophora spinosa* were quantified by using a Folin-Ciocalteu colorimetric method spectrophotometrically as described by Khouchlaa et *al*., [11] with slight modification. Gallic acid was used as standard (concentration: 5.00–40 µg/mL) while *E. spinosa* whole plant extract was 100 µg/mL. Firstly, 1 mL of the extract or standard gallic acid solution was used in screw cap tube and 5 mL of Folin-Ciocalteu reagent was added. Then, 2 mL (2 %) of anhydrous sodium carbonate (Na_2CO_3) was added followed by 30 in incubation at 30 °C. The vehicle solvent was used as blank solution. UV absorbance was taken with a UV–VIS spectrophotometer at 760 nm. The amount of total phenolics was calculated as gallic acid equivalent (GAE) in mg per g of dry weight extract.

2.4. Determination of total flavonoid content

Total flavonoid contents in methanol extract of leaves and ripe fruits of *Echinophora spinosa* were quantified using the aluminum chloride ($AlCl_3$) colorimetric method [12], was used to determine total flavonoid contents, using quercetin as a standard. The absorbance was measured at 415 nm and total flavonoid contents were expressed as quercetin equivalents in milligrams per gram sample (average of the triplicate analysis). Crude extracts that have been attuned to come under the linearity range and different dilution of standard solution of Quercetin (20–100 µg/ml).

2.5. Antimicrobial activity assay

The plant extracts were tested for antimicrobial activity using the disk diffusion method. The antimicrobial activities of the methanol extracts of leaves and ripe fruits of *E. spinosa* were evaluated against microorganisms from the American Type Culture Collection (ATCC), namely two strains of Gram positive bacteria: *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923; three strains of Gram negative bacteria: *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 35659 and *Pseudomonas aeruginosa* ATCC 27853; one strain of yeast: *Candida albicans* ATCC 24333 and one strain of filamentous fungi: *Aspergillus niger* ATCC 16404.

2.5.1. Determination of antibacterial activity

The Disk diffusion assay was done in accordance with the National Committee for Clinical Laboratory Standards guidelines for bacteria [13]. Antibacterial activities were evaluated by measuring the diameters of zones of

inhibition in mm against the test organism. This assay was done in triplicate.

2.5.2. Determination of antifungal activity

Antifungal susceptibility testing by disk diffusion was done as per Clinical and Laboratory Standards Institute (CLSI) guidelines for filamentous fungi [14] and National Committee for Clinical Laboratory Standards guidelines for yeasts [15]. The antifungal activity was observed with inhibited growth of the microorganisms giving a clear, distinct zone of inhibition around the discs. The diameter of zone of inhibition was measured. The data was obtained from three individual experiments.

2.6. DPPH radical scavenging assay

The antioxidant activity methanol extract of leaves and ripe fruits of *E. spinosa* was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [16]. Briefly, serial dilutions were carried out with the stock solution (1 mg/mL) of the extracts. Diluted solutions (1 mL of each samples) were reacted with 1 mL of a freshly prepared DPPH (2,2-diphenyl-1-picryl hydrazyl) methanol solution (80 µg/mL) for 30 min in the dark at room temperature. Absorbance values of these solutions were determined with a spectrophotometer at 517 nm. Methanol was used as a blank. butylated hydroxytoluene (BHT) was used as a positive control. The experiment was carried out in triplicate. The percent (%) inhibition of DPPH radical was calculated by the following formula (2):

$$Percent\quad DPPH\quad Scavenging = \left[\frac{A_C \times A_S}{A_C}\right] \times 100 \quad .....2)$$

where A_C is defined as the absorbance of the control reaction (comprising all reagents without the test compound (Sample)) and A_S is the absorbance of the test compounds. The antiradical activity was expressed as IC_{50} (µg/mL), the extract dose required to cause a 50% decrease of the absorbance at 517 nm. A lesser IC_{50} value corresponds to a greater antioxidant activity.

2.7. Statistical analysis

The results were analyzed by one way ANOVA. Duncan's multiple range test was used to identify significant differences among the mean (SAS 9.0). Difference among means at 5% level ($p < 0.05$) was considered statistically significant.

3. Results and discussion

3.1. Extract yield

Extraction yield of the samples expressed as percentage of dry weight plant material is presented in Table 1, were varied in different parts of plant. Methanol extract of leaves of *E. spinosa* presented the highest amount of extraction yield (17.75 % *w*/*w*), whereas methanol extract of ripe fruits the lowest (7 % *w*/*w*). The increase in the extraction yield of methanol extract of leaves might be due to the conditions for harvesting the plant. However, it is difficult to compare these results with those of the bibliography, because the extraction yield is only relative and seems to be linked to the extraction methods applied, to the genetic properties of the species used, the geographical origin and the conditions for harvesting the plant material [17].

3.2. Total phenolic content

The total phenolic content of *E. spinosa* was evaluated as expressed by gallic acid equivalents per mg of extract. The value was obtained from regression equation of the calibration curve (y = 1.07x + 1.09; r^2 = 99.82). There was a significant difference in total phenolic content in all parts of *E. spinosa* (Figure 3). Highest phenolic content (69.17 ± 1.2 µg GAE/mg extract) was observed in methanol extract of ripe fruits while lowest content (53.75 ± 0.58 µg GAE/mg extract) was found in methanol extract of leaves. The total phenolic contents in essential oils of different parts of *E. spinosa* were also previously reported; however, since those results are in a methanol extract, it is difficult to compare them with the obtained in this work [6,3,8,5].

3.3. Total flavonoid content

The aim of this study is to highlight the differences in secondary metabolite contents between the methanol extract from leaves and ripe fruits of *E. spinosa* in term of flavonoid contents (Figure 4).

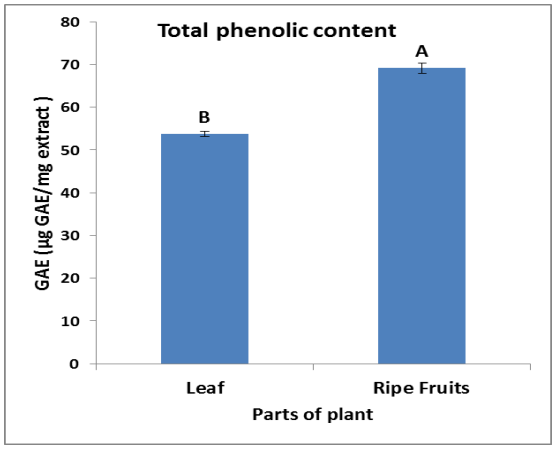

Figure 3. Total phenolic content in methanol extracts of leaves and ripe fruits of *Echinophora spinosa*. Data are mean ± SD ($n = 3$). Columns in figure that are headed with the different letter are significantly different ($P < 0.05$) according to the Duncan's multiple range test.

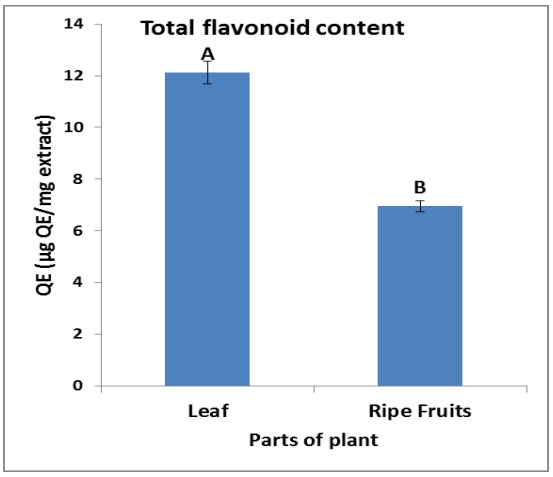

Figure 4. Total flavonoid content in methanol extracts of leaves and ripe fruits of *Echinophora spinosa*. Data are mean ± SD ($n = 3$). Columns in figure that are headed with the different letter are significantly different ($P < 0.05$) according to the Duncan's multiple range test.

Difference in total flavonoid content among leaves and ripe fruits of *E. spinosa* was statistically significant ($p < 0.05$).The highest flavonoids content was detected in methanol extract of the leaves (12.122±0.44 µg QE/mg extract) and the lowest content was seen in methanol extract of the ripe fruits (6.95±0.22 µg QE/mg extract) in (Figure 4). Several agents such as plant age, pretreatment of plants, parts of plant and extraction method also are effective on the contents of phenolic and flavonoids compounds [18,19]. The flavonoid plays an important role in the defense of plants towards pathogens, parasites, diseases, and predators [20]. Plants rich in secondary metabolites such as phenols and favonoids are rich in antioxidant activities. Flavonoids are the most important phenolics which are responsible for various biological activities [21].

3.4. Free Radical Scavenging Activity

The DPPH test was widely used as a fast, reliable and reproducible parameter to investigate *in vitro* the total antioxidant activity of plant extracts [20]. Radical-scavenging activity of the methanol extracts from the leaves and a ripe fruits of *E. spinosa* was evaluated by DPPH radical assay. In the present study, the antioxidant potential of the methanol extract of leaves and ripe fruits as well as BHT was explored in a dose-dependent (0.1–1.2 mg/mL) manner and the concentration with a 50% radical scavenging percentage (IC_{50}) was calculated by the diagrams as shown in Figure 5.

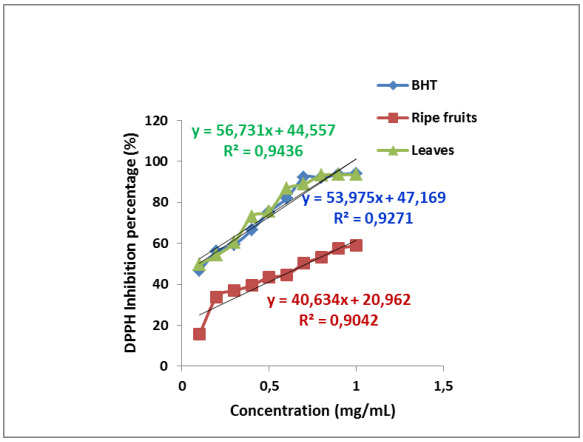

Figure 5. DPPH free radical scavenging activity (%) of BHT and of methanol extract of ripe fruits and leaves *Echinophora spinosa*.

The concentrations that led to 50% inhibition (IC_{50}) are presented in Table 1. Then, the antioxidant activities were compared with that of BHT. After analyzing the percentage of inhibition, the IC_{50} was calculated for each sample. The obtained results indicate that the methanol extract of ripe fruits from *E. spinosa* with an IC_{50} value of 0.087 ± 0.003 µg/mL proved to be an effective free radical scavenger than BHT and methanol extract of leaves of *E. spinosa*. This suggests that the plant extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. These results were consistent with the findings of many studies who described such correlation between total phenolic content and antioxidant activity besides indicated that the antioxidant effect of extracts, is due to the contribution of phenolic compounds in these extracts [22], and flavonoids [23].

Table 1. DPPH radical scavenging assay (IC_{50}) and extract yield of methanol extracts of *Echinophora spinosa*.

	Extract yield (%)	IC_{50}
Leaves	17.75	0.728 ± 0.028a
Ripe fruits	7	0.087 ± 0.003b
BHT	-	0.095 ± 0.001b

Data are mean ± SD (n = 3). Columns in table that are headed with the different letter are significantly different (P < 0.05) according to Duncan's multiple range test. BHT used as a positive control.

3.5. Antimicrobial activity of extracts

The results of agar disc diffusion are presented in Table 2. Both methanol extracts of *E. spinosa* showed antimicrobial effects against studied pathogens except for *S. aureus*, and *P. aeruginosa*. Antibacterial activity of methanol extracts of leaves and ripe fruits of *E. spinosa*

was tested against Gram positive and Gram negative bacteria. It was found that the methanolic extract of ripe fruits of *E. spinosa* showed the highest antibacterial activity against *B. subtilis* (30.5 ± 0.50 mm), *E. coli* (23.67 ± 1.53 mm) and *P. mirabilis* (20.50 ± 0.50 mm) as compared to methanol extracts of leaves and Gentamycin (Table 2). The antifungal activity of methanol extracts of leaves and ripe fruits of *E. spinosa* were assessed against two fungal species, *C. albicans* and *A. niger* in terms of zone of inhibition. The results indicated that both plants extracts showed antifungal activities at variable degrees against tested organisms (Table 2).

Table 2. Antimicrobial screening test of methanol extract of *Echinophora spinosa* against some microbial strains.

Part of plants	Inhibition zones (mm)						
	Gram positive bacteria		Gram negative bacteria			Pathogenic fungi	
	B. subtilis	*S. aureus*	*E.coli*	*P. mirabilis*	*P. aeruginosa*	*C. albicans*	*A. niger*
Leaves	13.67 ± 1.5c	00±0.00b	13.50±0.87c	9±1.00b	00.00±0.00b	7.67±0.29c	2,0±0.50c
Ripe fruits	23.67±1.53 a	00±0.00b	30.50±0.50 a	20.50±0.50a	00.00±0.00b	19.67±1.53b	4,67±0.58b
Gentamycin (5 µg)	20.17±0.29b	18.50±0.50a	27.33±1.15b	19.00±1.00a	21.67±0.58a	_	_
Amphotericin B (20 µg/mL)	_	_	_	_	_	27.83±0.29a	19.33±1.15a

Values in the table are means of three independent experiments and error bars indicates standard deviation of the mean. Letters show significant deference using Duncan's test ($p < 0.05$).

The methanol extract of ripe fruits and leaves of *E. spinosa* are found to have antimicrobial properties along with higher quantities of phenolics and flavonoids [24].

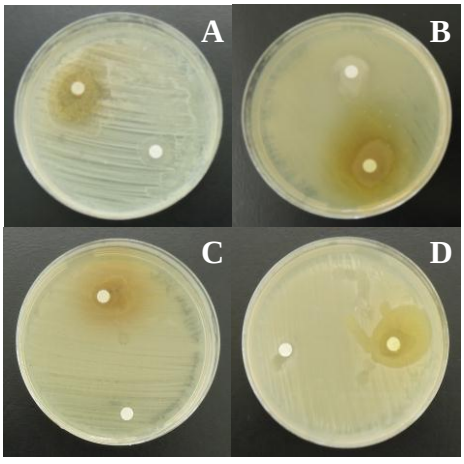

Fig 6. Zone of inhibition of different extracts of *Echinophora spinosa* on different microbial strains (mm): A) *C. albicans;* B) *P. mirabilis;* C) *S. aureus;* D) *E. coli.*

4. Conclusion

This study suggested that methanol extract of *E. spinosa* have significant effect in free radical scavenging and antimicrobial activities. The ripe fruits of methanol extract from *E. spinosa* have strong antioxidant and antimicrobial activities than the leaves methanol extract as they contain phenol and flavonoids which are known as potent antioxidants and antimicrobial agents. According to obtained results, it can be recommended that the ripe fruits of *E. spinosa* who was a relationship with the best phenolic and less flavonoids compounds quantity, as well as antimicrobial and antioxidant activities. The results provide evidence that supports the traditional uses of *E. spinosa* and can be applied for further pharmacological and phytochemical investigations.

Acknowledgement

The University of Mohamed Boudiaf, M'sila, Algeria and the Laboratory of Applied Microbiology, Department of Microbiology, Faculty of Natural and Life Sciences, University of Ferhat Abbas Setif 1, Algeria are gratefully acknowledged for financial support.

References

1. El Mokni R. *Echinophora spinosa* L. (Apiaceae), a new species in the flora of Tunisia and second report from North Africa. Hacquetia. 2020, 19 ;1: 137–142.
2. Khennouf H, Chefrour A, Corcket E, Alard D, Véla E. La végétation dunaire du littoral de Jijel (Algérie) : proposition d'une nouvelle zone importante pour les plantes. Revue d'Ecologie (Terre et Vie). 2018, 73; 3: 345-362.
3. Quézel P, Santa S. Nouvelle flore d'Algérie et des régions désertiques méridionales. Ed. CRNS, Paris (FR). 1962-1963.
4. Fraternale D, Genovese S, Ricci D. Essential Oil Composition and Antimicrobial Activity of Aerial Parts and Ripe Fruits of Echinophora spinosa (Apiaceae) from Italy. Natural Product Communications. 2013, 8;4: 527 – 530.
5. Cakilcioglu U, Turkoglu I. An ethnobotanical survey of medicinal plants in Sivrice (Elazıg-Turkey). J Ethnopharmacol. 2010, 132:165–175.

6. Pavela R, Maggi F, Cianfaglione K, Canale A, Benelli G. Promising insecticidal efficacy of the essential oils from the halophyte *Echinophora spinosa* (Apiaceae) growing in Corsica Island, France. Environmental Science and Pollution Research. 2019, https://doi.org/10.1007/s11356-019-04980-y.
7. Glamoclija JM, Sokovic MD, Šiljegovic JD, et al. Chemical Composition and Antimicrobial Activity of *Echinophora spinosa* L. (Apiaceae) Essential Oil. Rec. Nat. Prod. 2011, 5;4: 319-323.
8. Ngahang Kamte SL, Ranjbarian F, Cianfaglione K. et al. Identification of highly effective antitrypanosomal compounds in essential oils from the Apiaceae family. Ecotoxicology and Environmental Safety. 2018, 156: 154–165.
9. Pavela R, Maggi F, Cianfaglione K, Bruno M, Benelli G. Larvicidal Activity of Essential Oils of Five Apiaceae Taxa and Some of Their Main Constituents Against Culex quinquefasciatus. Chem. Biodiversity. 2018, 15, e1700382.
10. Naz R Ayub H Nawaz S. et al. Antimicrobial activity, toxicity and antiinflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. BMC Complementary and Alternative Medicine. 2017, 17:302 DOI 10.1186/s12906-017-1815-z
11. Khouchlaa A, Talbaoui A, El Yahyaoui El Idrissi A. et al. Détermination des composés phénoliques et évaluation de l'activité litholytique in vitro sur la lithiase urinaire d'extrait de *Zizyphus lotus* L. d'origine marocaine. Phytothérapie. 2017, https://doi.org/10.1007/s10298-017-1106-3.
12. Elija K, Adsul VB, Kulkarni MM, Deshpande NR, Kashalkar RV. Spectroscopic determination of total phenol and flavonoid contents of *Ipomoea carnea*. International Journal of ChemTech Research. 2010, 2;3: 1698–1701.
13. NCCLS. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard—Second Edition. NCCLS document M31-A2 (ISBN 1-56238-461-9). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA. 2002.
14. CLSI. Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Approved Guideline. CLSI document M51-A (ISBN 1-56238-752-1). Clinical and Laboratory Standards Institute. 2010.
15. NCCLS. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline. NCCLS document M44-A (ISBN 1-56238-532-1). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA. 2004.
16. Chahardoli A, Karimi N, Ma X, Qalekhani F. Effects of engineered aluminum and nickel oxide nanoparticles on the growth and antioxidant defense systems of *Nigella arvensis* L. Sci Rep. 2020, 10; 3847. https://doi.org/10.1038/s41598-020-60841-6.
17. Tamert A, Latreche A, Aouad L. Phytochemical Screening and Antimicrobial Activity of Extracts of *Thymus serpyllum* and *Thymus vulgaris* from the Mount of Tessala (Western Algeria). Phytothérapie. 2017, 15:384-394; DOI 10.1007/s10298-017-1132-1
18. Chávez-González ML, Sepúlveda L, Verma DK, et *al*. Conventional and Emerging Extraction Processes of Flavonoids. Processes. 2020, 8, 434; doi:10.3390/pr8040434
19. Najjaa H, Abdelkarim BA, Doria E, et al. Phenolic composition of some Tunisian medicinal plants associated with anti-proliferative effect on human breast cancer MCF-7 cells. The EuroBiotech Journal. 2020, 4;2: 104-112.
20. Yahia Y, Bagues M, Zaghdoud C, Al-Amri SM, Nagaz K, Guerfel M. Phenolic profile, antioxidant capacity and antimicrobial activity of *Calligonum arich* L., desert endemic plant in Tunisia. South African Journal of Botany. 2019, 124:414–419. https://doi.org/10.1016/j.sajb.2019.06.005
21. Das P, Hasnu S, Lahkar L, Mahanta S, Borthakur SK, Tanti B. Antimicrobial activity and antioxidant properties of *Brucea mollis* Wall. ex Kurz: a medicinal plant of Northeast India. Advances in Traditional Medicine. 2020, https://doi.org/10.1007/s13596-020-00494-y.
22. Lahmass I, Ouahhoud S, Elmansuri M, et al. Determination of Antioxidant Properties of Six By-Products of *Crocus sativus* L. (Saffron) Plant Products. Waste Biomass Valor. 2018, 9:1349–1357.
23. Abdel-Aleem ER, Attia EZ, Farag FF, Samy MN, Desoukey SY. Total phenolic and flavonoid contents and antioxidant, anti-inflammatory, analgesic, antipyretic and antidiabetic activities of *Cordia myxa* L. leaves, Clin Phytosci. 2019, 5; 29. https://doi.org/10.1186/s40816-019-0125-z.
24. Goswami S, Das R, Ghosh P, Chakraborty T, Barman A, Ray, S. Comparative antioxidant and antimicrobial potentials of leaf successive extract fractions of poison bulb, *Crinum asiaticum* L. Industrial Crops & Products. 2020, 154 112667, https://doi.org/10.1016/j.indcrop.2020.112667.

Recommended Citation

Ghadbane M., Bounar R., Rebbas K. Antimicrobial and antioxidant activity of methanol extract of *Echinophora spinosa* L. from Jijel, Algeria. *Algerian Journal of Biosciences*. 2020, 01;01:024-029.

http://dx.doi.org/10.5281/zenodo.4076344