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A Comparative study of the antibacterial and the antioxidant Activity of *Atriplex halimus* L.

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ABSTRACT

This study underscored the antimicrobial activity of one chenopodiaceae species namely: *Atriplex halimus L*. using three different solvents : Methylene chloride, Ethyl acetate, and n-butanol with increasing polarity. These extracts against six types of gram negative and gram positive bacteria: *Escherichia coli ATCC 8739 G(-), Salmonella typhimurium ATCC 14028 G(-)*, *Staphylococus aureus ATCC 6538 G(+)*, *Enterococcus feacium ATCC 19434 G(+)*, *Streptocoque B (Streptococcus agalactiae) G(+)* and *Candida albicans ATCC 10231*. Methylene chloride extract is more potent compared to ethyl acetate and butanol extracts. The study of the antioxidant activity by the DPPH method showed that the greatest activity is obtained with the methylene chloride, with IC50 equal to 11.75 mg/ml, followed by the ethyl acetate extract, with a value of 23.20 mg / ml and finally the butanol extract which seems to be the least efficient with an IC50 of 57.16 mg / ml. The EC_{50s} are inversely proportional to the Trolox equivalent whose low values reflect a high Trolox equivalent value.

Keywords: antioxidant activity, *Atriplex halimus L*, *Candida albicans*, antibactrial activities.

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INTRODUCTION

Worldwide, 80% of people use medicinal plants to treat themselves, for lack of access to medicines prescribed by medicine modern, but also because these plants are often very effective. Today, the knowledge of traditional healers is less and less transmitted and tends to disappear. This is why ethnobotany and ethnopharmacology are working to identify, all over the world, plants that are known to be active and for which it is up to modern research to specify the properties and validate their use [1]. The search for new ones molecules must be undertaken within plant biodiversity using ethnopharmacological data. This approach makes it possible to select potentially active plants and significantly increase the number of discoveries of new active substances. An increasing number of reports dealing with the evaluation of the antimicrobial effects of different extracts of various medicinal plants are frequently available [2,3]. However, due to the emergence of new strains of bacteria and weak chemotherapeutic agents and antibiotic resistance of pathogens, several medicinal plants have been tested for their potential antimicrobial activity [4-6].

This has led us to focus more on the study of medicinal plants. The literature concerning the plant Atriplex halimus L. contains little or no information about its antibacterial and antioxidant activities, except its use for extracting salt from the soil and has therefore been used in land reclamation projects to de-salt the soil ground. Ash from the burned plant is used as an alkaline soap. The shoots are burned to produce antacid powder. Among the Atriplex species in North Africa [7], is Atriplex halimus L. which is an evergreen shrub growing at 2 m by 3 m at an average rate. It is not sensitive to frost; It is extremely tolerant to pruning and regrowing even when cut in old wood. Suitable for sandy and loamy soils; prefers well-drained soil and can grow in nutrient-poor soil; it can grow in acidic, neutral and basic soils and even in very alkaline and saline soils [8]. It cannot grow in the shade; it prefers dry or wet soils and can tolerate drought and heavy metal stress [9].

So we wish to report the study and evaluation of the antibacterial and antioxidant activities of *Atriplex halimus* against several *Gram-positive* and *Gram-negative* bacterial strains *in vitro*.

Atriplex halimus known locally as "G'tef." In Algeria, *Atriplex halimus* L. is spontaneous in the semi-arid and arid areas and can be found in coastline zone. More in the South, it was identified in the salty soils, in Hoggar, Tassili and Salah Timimoun. *Atriplex halimus* is in leaf in January and in flower in July. The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by wind.

EXPERIMENTAL

Antibacterial activity

Materials and methods

The aerial parts of *Atriplex halimus L*. were collected randomly from the Ouargla desert, south of Algeria in April 2015. The medicinal plant was deposited at Laboratoire de Dynamique Interaction et Réactivité des Systèmes, Department of Process engeneering, Faculty of Applied Sciences, University of Kasdi Merbah-Ouargla. Fresh *plant* material was washed under running tap water, air dried under dark and then homogenized to fine powder using an electrical mixer "Panasonic Type" for 20 minutes, and stored in closed container away from light and moisture.

Preliminary Phytochemical Analysis

The preliminary phytochemical analysis of the crude powder of *Atriplex halimus L*. showed that this plant contains many active ingredients: *Coumarins, tannins, unsaturated* sterols, saponiside, cardinolides, *volatile oils, terpenes and alkaloids*, one of the antioxidants of the bacteria responsible for the effect of microbs, also contains *flavonoids* including *glycosides* antioxidant, *phenols* and *saponins.* As for the nature of the extracts were characterized by strength viscous dark green color and aromatic smell, due to the emergence of green chlorophyll pigment and material xanthine.

Extraction of plant material

The extracts were prepared by soaking 200 g of the plant powder in a mixture of EtOH/H_2O (70/30) for 24 hours. The procedure was repeated three times and the filtrates were combined before being evaporated under reduced pressure. The resulting extracts were diluted with distilled water and left overnight. The filtrates were subjected to extraction by various solvents with increasing polarity (methylene chloride, ethyl acetate and butanol). The organic phases were separated and evaporated and the resulting residues were stored at 4°C.

Microorganisms

All bacterial standard strains: *Escherichia coli ATCC 8739 G(-), Salmonella typhimurium ATCC 14028 G (-)*, *Staphylococus aureus ATCC 6538 G(+)*, *Enterococcus feacium ATCC 19434 G(+)*, *Streptocoque B (Streptococcus agalactiae) G(+)* and *Candida albicans ATCC 10231* were obtained Laboratoire de Biotechnologie "INRAP" (**I**nstitut **N**ational de la **R**echerché et d'**A**nalyse **P**hysico-chimique) de Tunisie.

Preparation of the bacterial culture media

3.7 of muller Hilton agar were mixed with hot distilled water and autoclaved at 121°C and 2 atm for 15 minutes. After autoclaving, it was allowed to cool to 45°C in a water bath. Then the medium was poured into sterilized Petri dishes with a uniform depth of approximately 5 mm [10].

Preparation of plant extract impregnated discs

Whatman N°1 filter paper was used to prepare discs of 6 mm in diameter. They were sterilized by autoclaving and then dried during the autoclaving cycle. The discs were then impregnated with extract of the plants [11].

Disc diffusion method

Disc diffusion method for antimicrobial susceptibility test was carried out according to the standard method by Kirby-Bauer to assess the presence of antibacterial activities of plant extracts [11,12]. The resulting residue of all extracts stored at 4°C was tested at concentrations 10^{-1} g/ml and were prepared in DMSO.

Standard antimicrobs:

A standard antimicrobs: Ampicillin/ Nystatine were obtained from Italian company "*Liofilchem.*"

Antioxidant activity:

Generalities:

The oxidative stress resulting from the imbalance created by the excessive production of reactive oxygen species (ROS) is considered to be critically involved in the normal aging process but also in the development and progression of various human pathologies, including cancers. Indeed, initiation and progression of cancer have been associated with oxidative stress by increasing DNA mutations or inducing DNA damage, genome instability, and cell proliferation. Normal cells are hypersensitive to reactive oxygen species (ROS) if they are not sufficiently protected by antioxidant mechanisms and can lead to cancer formation [12, 13].

The evaluation of antioxidant activity in vitro is done by several techniques. These methods are based exclusively on the reducing capacity or trapping of radicals as an indicator of its antioxidant potential.[13]The demonstration of the in vitro antioxidant activity of the compounds tested was carried out by trapping the free radical DPPH. The antioxidant capacity is expressed in Trolox equivalents (TEAC); it corresponds to the concentration of Trolox having the same activity as the test substance at a concentration. The result is given in µM of Trolox equivalent per g of product.

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Trolox or 3,3-dihydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-carboxylic acid, is a hydrophilic analogue of vitamin E. Like the latter, it is an antioxidant, which is used in biology and biochemistry to limit damage due to oxidative stress.

The antiradical activity of DPPH· was determined based on the assays described by Brand-Williams with some modifications. Thus, in a volume of 1 ml, different concentrations of the test extract are prepared in methanol, then 2 ml of the solution of DPPH· 0.1 mM concentration. After vigorous stirring, the mixture is incubated for 1 hour in the dark and at room temperature, then the absorbance is measured at 515 nm by a UV vis spectrophotometer (JASCO-V530). A solution containing 1 ml methanol and 2 ml of DPPH· considered as analytical white is prepared in parallel.

The percentage inhibition (% I) formula:

The estimate of the free radical activity is expressed as the percentage inhibition value (% I) calculated using the following formula:

$$\%I = [(Abs_0 - Abs_1)/Abs_0)] \times 100$$

With Abs_0: absorbance of analytical white.

Abs_1: absorbance of the solution in the presence of extract.

The curve giving the variation of (% I) as a function of the different concentrations of the extract makes it possible to determine the antiradical activity or EC_{50} (Efficient Concentration 50%), defined as the quantity of extract necessary to halve the initial concentration of DPPH.

RESULTS AND DISCUSSION

Antibacterial activity

Table 1 summarized the microbial growth inhibition of these standard antibiotics and and clarified by **Figure 1** and **Figure 2** respectively.

Table 1: Antibacterial activity of methylene chloride, ethyl acetate and butanol extracts of screened plant *Atriplex halimus L*.

	Diameter of Inhibition zone (mm)			
	Ampicillin/ Nystatine	Methylene chloride Extract	Ethyl acetate Extract	Butanol Extract
Charge of the disc (µg) / Bacteria strains	10µg/100µg	15µl	15µl	15µl
Escherichia coli ATCC 8739 G(-)	11,75±0.3	12,5±0.7	-	10±0.0
Salmonella typhimurium ATCC 14028 G(-)	13,75±1.0	11,75±1.0	-	9,75±1.0
Staphylococus aureus ATCC 6538 G(+)	35,5±0.7	11,5±0.7	-	9,5±0.7
Enterococcus feacium ATCC 19434 G(+)	37.5±0.7	19,75±0.3	9,25±0.3	15,25±0.3
Streptocoque B (Streptococcus agalactiae) G(+)	27,75±1.0	13,5±0.7	-	11,5±0.7
Candida albicans ATCC 10231	36±0.3	14,25±1.0	-	11,5±0.7

Results for antibacterial activity as obtained with different solvent extracts of *Atriplex halimus L*. revealed that the two Methylene chloride and butanol extracts exhibited a positive effect against six types of gram negative and gram positive bacteria *Escherichia coli ATCC 8739 G(-), Salmonella typhimurium ATCC 14028 G(-), Staphylococus aureus ATCC 6538 G(+), Enterococcus feacium ATCC 19434 G(+), Streptocoque B (Streptococcus agalactiae) G(+)* and *Candida albicans ATCC 10231* where the maximum activity was recorded against *Enterococcus feacium ATCC 19434 G(+)* and a maximum inhibition diameter of 19.75 mm with the dichloromethane extract. Moreover the ethyl acetate extract showed significant bacterial activity against *Enterococcus feacium ATCC 19434 G(+)* with an inhibition diameter of 16 mm). On the other hand the ethyl

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acetate extract was ineffective with the remaining bacteria strains *Escherichia coli ATCC 8739 G(-), Salmonella typhimurium ATCC 14028 G(-)*, *Staphylococus aureus ATCC 6538 G(+)*, *Streptocoque B (Streptococcus agalactiae) G(+)* and *Candida albicans ATCC 10231*. These significant effects may be due to the extract effect on the permeability of the cell membrane and the function of the bacterial cell [14, 15].

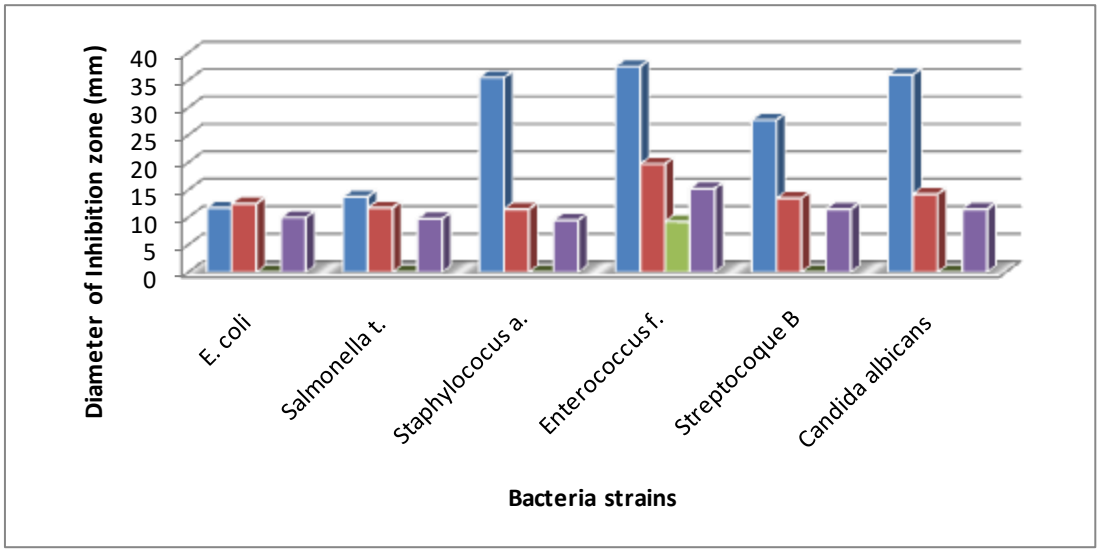

Fig 1: The diameters of inhibition zone (mm) of *Atriplex halimus L*. extracts on the tested bacteria.

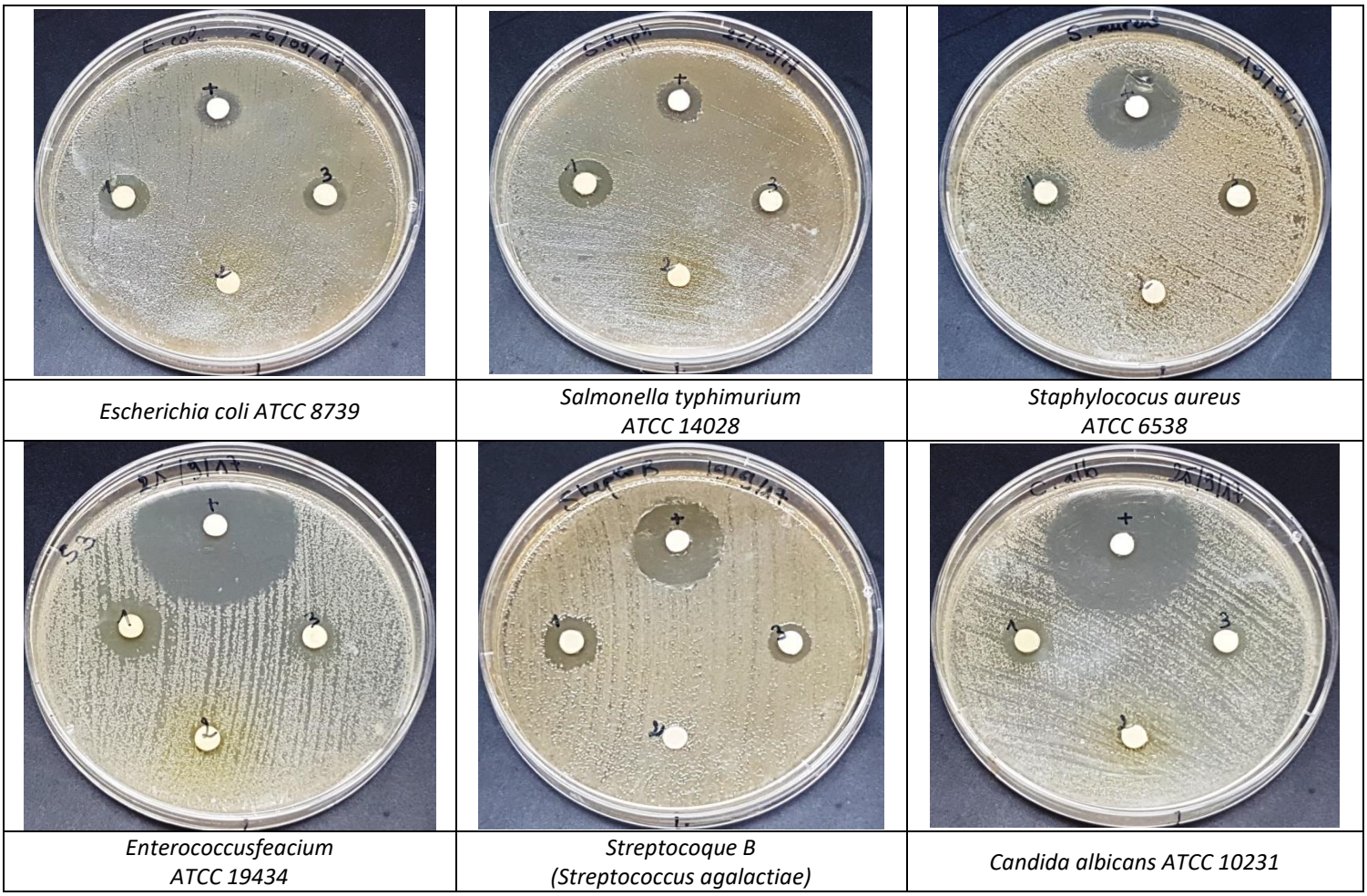

Fig 2: The diameters of inhibition zone of *Atriplex halimus L*. extracts on the six types of tested gram negative and gram positive bacteria

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Generally, the extracts of the plant is more or less effective towards the tested bacteria and methylene chloride extract is more potent compared to ethyl acetate and butanol extracts.

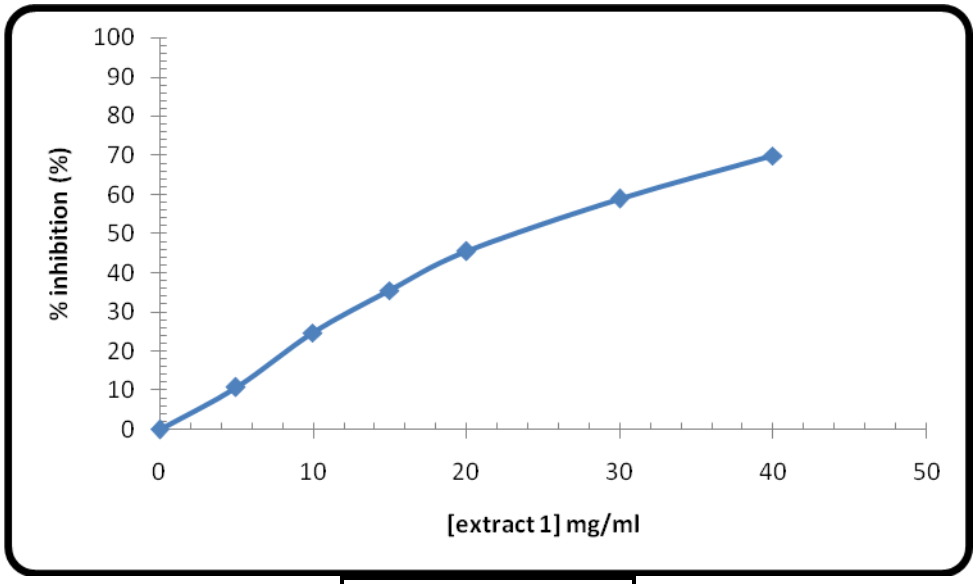
Antioxidant activity:

The anti-free radical activity was evaluated using DPPH, which was one of the first free radicals used to study the relationship structure antioxidant activity. 2,2-diphenyl-1-picrylhydrazil (DPPH), a stable, violet radical in solution and having a characteristic absorption maximum at 517 nm. The protocol applied routinely relies on the disappearance of this maximum when the DPPH is reduced by a compound having antiradical property, thus causing discoloration towards the yellow color [16].

The effectiveness of Ext1, Ext2, and Ext3 are summarized in **Table 2**, **Table 3** and **Table 4** and clarified by **Figure 3, Figure- 4,** and **Figure 5** respectively to trap the radical DPPH, expressed by the inhibition rate (I%) depending on the different concentrations.

Table 2: Antiradical activity of methylene chloride extract (extract1)

[Extrait 1] mg/ml	0	5	10	15	20	30	40
Absorbance à 515nm	0,7084	0,63155	0,53365	0,4567	0,38665	0,2911	0,21315
%inhibition	0	10,85	24,67	35,53	45,42	58,91	69,91



EC50= 23.20 ± 0.39 mg/ml

Figure 3: Antiradical activity of extract1

Table 3: Antiradical activity of ethyl acetate extract (extract2)

[Extrait 2] mg/ml	0	5	10	15	20	30	40
Absorbance à 515nm	0,7084	0,536	0,408	0,2834	0,1838	0,0777	0,0773
%inhibition	0	24,34	42,41	59,99	74,05	89,03	89,09

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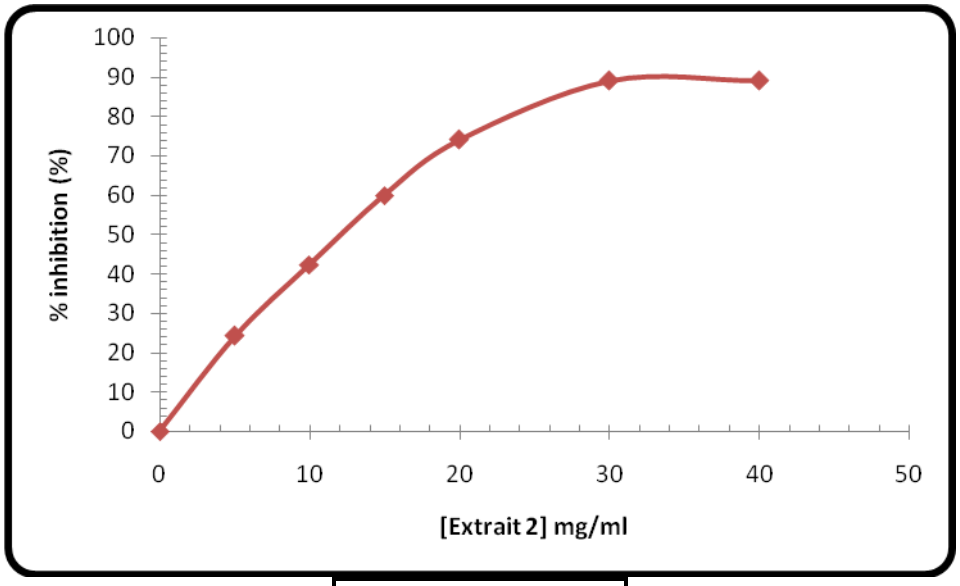
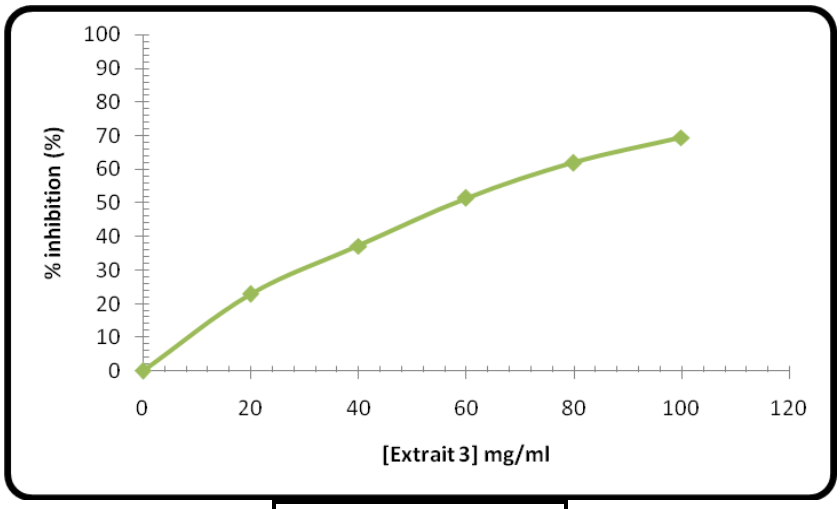


EC50= 11.75 ± 0.18 mg/ml

Figure 4: Antiradical activity of extract2

Table 4: Antiradical activity of butanol extract (extract3)

[Extrait 3] mg/ml	0	20	40	60	80	100
Absorbance à 515nm	0,7084	0,54605	0,44475	0,3436	0,2696	0,2163
%inhibition	0	22,92	37,22	51,50	61,94	69,47



EC50= 57.16 ± 0.19 mg/ml

Figure 5: Antiradical activity of extract3

Figure 6 clarified the effectiveness of grouped Extract1, Extract2, Extract3 and Trolox respectively to trap the radical DPPH, expressed by the inhibition rate (I%) depending on the different concentrations.

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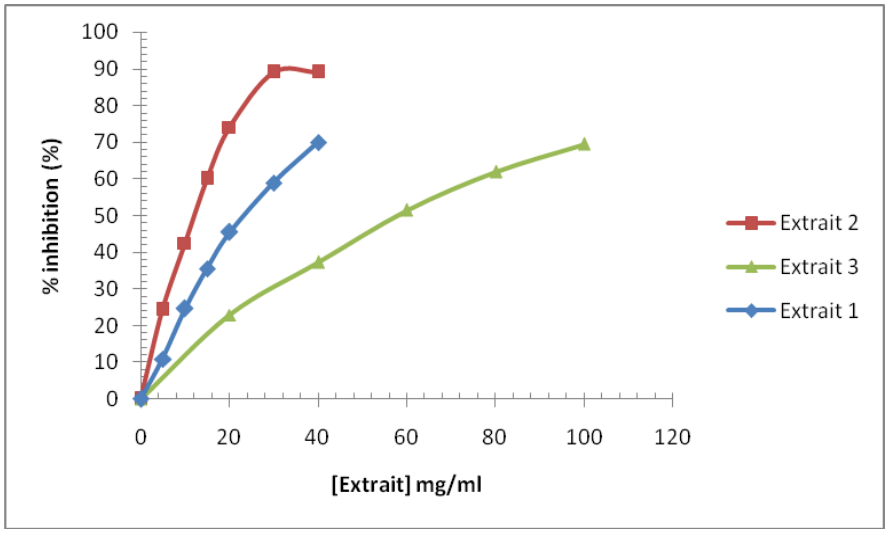
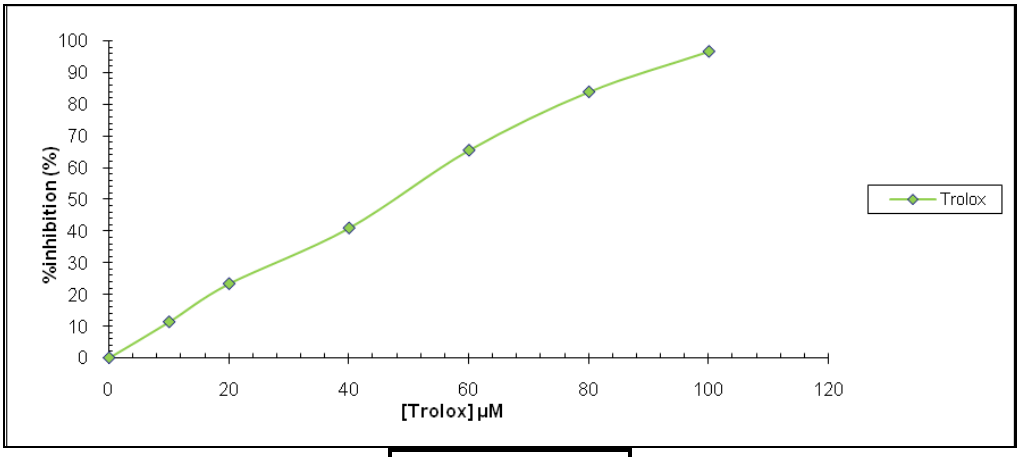


Figure 6: Antiradical activity of Ext1, Ext2 and Ext3.

The IC50 value (otherwise known as 50% inhibitory concentration) or EC50 Efficient Concentration at 50% is determined and summarized in **Table- 5** ; clarified by **Figure 7** for the used standard "Trolox".

Table 5: Antiradical activity of Trolox

[Trolox] µM	0	10	20	40	60	80	100
Absorbance à 515 nm	0,706	0,626	0,541	0,417	0,244	0,114	0,024
%inhibition	0	11,33	23,37	40,93	65,44	83,85	96,6



$EC50_{Trolox}$= 45.642 µM

Figure 7: Trolox antiradical activity.

The IC50 value (otherwise known as 50% inhibitory concentration) or EC50 Efficient Concentration at 50% is determined and summarized in **Table 6** for our extracts and the used standard. It is defined as the concentration of the sample required to give a 50% decrease in the absorbance of the initial DPPH solution. The EC50s are inversely proportional to the scavenger effect, the low values of which reflect a strong antiradical effect.

Table 6: Inhibitory concentration at 50% of extract1, Etxtract2 and Exttract3

	Trolox	Extract 1	Extract 2	Extract 3
EC_{50} (mg/ml) µM	45.642	23.20±0.39	11.75±0.18	57.16±0.19

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The values of the antioxidant capacity expressed in Trolox equivalents (TEAC) are determined and grouped together in **Table 7** and clarified by **Figure 8**.

Table 7: Values of antioxidant capacity expressed in Trolox equivalent

	EC_{50} (mg/ml)	Equivalent Trolox (µmoles Trolox/g of extract)
Extract 1	23.20	1.967
Extract 2	11.75	3.884
Extract 3	57.16	0.798

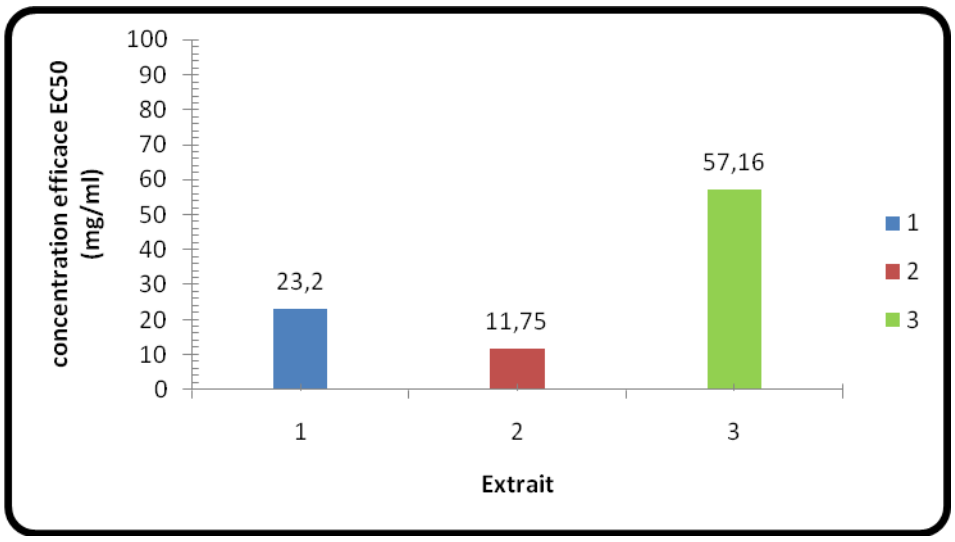

Figure 8: Antioxidant capacity expressed in Trolox equivalent

According to the results, the antiradical activity increased by the increase of the concentrations. It seems that Trolox is the most effective antioxidant than the the three extracts studied with a value of 45.642 µM. It has been reported by several authors that the synthetic antioxidants have more ability to trap the DPPH radical than the plant extracts [17-18].

Among the three extracts, the extract1 is the most active with IC50 equal to 11.75 mg / ml, followed by the extract2, with a value of 23.20 mg / ml and finally the extract3 which seems to be the least efficient with an IC50 of 57.16 mg / ml. The EC_{50s} are inversely proportional to the Trolox equivalent whose low values reflect a high Trolox equivalent value.

In several reports, the antioxidant activity of the plants can be linked to the phenolic content. Indeed, the comparative study on the DPPH radical's reducing ability by different chemotypes has proved that the phenolic chemotypes had showed in-vitro, more expressed and much stronger antioxidant capacities than those of the non-phenolic chemotypes [19, 20]. In our case, the three extracts are reach with phenolic compounds, especiall extract2 (Preliminary), and this plant has showed an antioxidant activity which sparks an iterest for its therapeutic use and cosmetic applications.

CONCLUSION

This study underscored the antimicrobial activity of one chenopodiaceae species namely: *Atriplex halimus L*. using three different solvents : Methylene chloride, Ethyl acetate, and n-butanol with increasing polarity against six bacteria strains. This plant averred to be effective against six types of gram negative and gram positive bacteria *Escherichia coli ATCC 8739 G(-), Salmonella typhimurium ATCC 14028 G(-), Staphylococus aureus ATCC 6538 G(+)*, *Enterococcus feacium ATCC 19434 G(+)*, *Streptocoque B (Streptococcus agalactiae) G(+)* and *Candida albicans ATCC 10231* and methylene chloride extract is more potent compared to ethyl acetate and butanol extracts.

The study of the antioxidant activity by the DPPH method shows that the greatest activity is obtained with the methylene chloride, with IC_{50} equal, followed by the ethyl acetate extract, and finally the butanol extract which seems to be the least efficient.

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REFERENCES

[1] Ashakkumar R, and Ramaswamy, M J. Chem Bio Physci Sec., 2013; 3(2):1279-1289.
[2] Bindu J, Anjali K, Vibhor Kj. Asian Journal of Biochemical and Pharmaceutical research 2011; 2(1): 437-452.
[3] Pelt JM. Les noveeaux actifs naturels. Marabout, Paris 2001**.**
[4] Ishrak K, Ahmed D. The effecincy of random versus ethno-directed research in the evaluation of sinai medicinal plants for bioactive compounds. J. of Ethnopharmacology 2000**;** 71: 365-376.
[5] Benkeblia N, Antimicrobial activity of essential oil extracts of various onions (allium cepa) and garlic (Allium stivum). Lebensm-Wiss u-Technol 2004; 37: 263-275.
[6] Babamer Zohra Y, Sekhri L, Al-Jaber Hala I, Al-Qudah Mahmoud A, Abu Zarga Musa H. Journal of Asian Natural Products Research 2012; 1-7.
[7] Haddioui A, Baaziz M, Population of Atriplex halimus of Moroco Euphyca, *Atriplex halimus L*. 2001; 121: 99-106.
[8] Ortiz-Dorda J, Martinez-Mora C, Correal E, Simon B, Cenis JI. Genetic structure of *Atriplex halimus* populations in the Mediterranean Basin. Ann.Bot. 2005; 95: 827-834.
[9] Lutts S, Almansouri M, Kinet JM, Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. Plant Sci., 2004; 167: 9-18.
[10] Harbone J, Phytochemical methods Chapman and Hall. London 1973.
[11] Swarnamoni D, Mukundam B, and Shagufa A. Asian Pharm Clin Res., 2013; 6 (4): 136-139.
[12] Harbone JB**.** Phytochemical methods. A guide to modern techniques of plant analysis. 2nd Ed., Chapman and Hall. London. 1984; P288.
[13] Bayala B. Etude des propriétés anti-oxydantes, anti-inflammatories, anti-prolifératives et anti-migratoires des huiles essentielles de quelques plantes médicinales du burkinafaso sur des lignées cellulaires du cancer de la prostate et de glioblastomes, Thèse Doctorat 2014, Université Blaise Pascal-Clermont-Ferrand II.
[14] Allaoul A, Cherity A, Al-Gharabli S, Gherraf N, Chebouat E, Dadamoussa, BA, Al-Lahhm A. Research Journal of pharmaceutical and Biological and Chemical Sciences 2014, 5 (5): 85-89.
[15] Al-Abed KF. Antibacterial activity in the volatile oils of some medicinal plants in Saudi Arabia. Microbiology, aureus, Bacillus, Escherichia coli, outside the body of the organism. M. Sc Thesis, Faculty of Education, University of Riyadh 2008; Faculty of Education, University of Riyadh.
[16] Liang N, Kitts DD. Antioxidant property of coffee components: assessment of methods that define mechanisms of action. Molecules 2014 ; 19 (11) : 19180-19208.
[17] Tepe BM, Sokmen HA, Akpulat D, Dafera M, Polissiou A, Sokmen A. J. Food Chem. Toxicol., 2005 ; 43 : 345-363.
[18] Kizil S, Hasimi N, Tolane V, Kilin E,Yuksel U. Turk. J. Field Crops 2010 ; 15: 148-153.
[19] Nanasombat S, Wimuttigsol P. Food Sci, Biotechnol., 2011, 20 : 45-53.
[20] Jukie M, Milos, M. Croatica Acta 2005 ; 78 : 105-110.