ISSN:2716-9227 Algerian Journal of Engineering and Technology 05 (2021) 049–054 e-ISSN: 2716-9278

Algerian Journal of Engineering and Technology

Journal homepage: https://jetjournal.org/index.php/ajet

Original Article

Phytochemical investigation, antibacterial and antioxidant activities of *Sideritis incana* L extracts.

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ARTICLE INFO

Article history:
Received 07 February 2021
Revised 01 July 2021
Accepted 21 October 2021
Keywords:
Sideritis incana L;
Antioxidant activity;
Antibacterial activity;
Phenolic content;
Alkaloids.

ABSTRACT

According to the phytochemical investigation conducted on the *Sideritis incana* L. plant, it was found to be rich in secondary metabolites, in particular tannins, flavonoids and alkaloids, based on what we found by preliminary tests for chemical constituents. The content of phenols, flavonoids was estimated by spectrophotometric methods. Also, the antioxidant activities of the extracts of *Sideritis incana* L were investigated by DPPH scavenging, and phosphomolybdenum method. The results of the quantitative estimation of total phenols showed the highest content in chloroform where it was estimated 585.64 mg GAE /g followed by n-butanol 282.22 mg GAE /g and the lowest content recorded in petroleum ether 17.32 mg GAE /g. The results of the quantitative evaluation of flavonoids showed that petroleum ether fraction contained an excess of flavonoids in the unusual results. For the DPPH assay, *n*-butanol fraction showed the best result followed by the ethyl acetate fraction and finally chloroform fraction the lowest activities (IC_{50} = 0.0534, 0.071 and 0.0756 µg/ml) respectively. Finally, result of antibacterial activity of *S.incana* showed that the chloroform extract gave the highest diameter inhibition among all extracts (13 mm) at a concentration of 50 mg/ml against *Escherichia coli* ATCC25921 and *Staphylococcus aureus* ATCC25923.

1. Introduction

In recent decades, interest in new sources of health-promoting compounds has become a major research issue. Considerable attention has been paid to edible plants, especially those rich in bioactive phytochemicals [1,2]. In Algeria, plants are of great importance in traditional medicine. Herbal remedies are cheaper and have no side effects [3]. The continued use of traditional medicine is attributable not only to cultural and poverty reasons but also to the ineffectiveness of many existing medicines [4].The need to restore it has been demonstrated through efforts to develop and take care of it. Although human use of plants remains the main source of treatment for many of the diseases affecting it, herbal medicines are a long-standing phenomenon in the Arabian Peninsula, where it is called the World Health Organization has confirmed that 80% of the world's populations rely on the treatment of diseases using plants [5]. Despite the wide range of more than 150 species available, the researchers demonstrated that all the plants of the species *Sideritis* have anti-microbial, anti-inflammatory, antioxidant and antispasmodic properties. They are rich in a number of natural antioxidants, including flavonoids, and almost all

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Peer review under responsibility of University of El Oued.

http://dx.doi.org/10.5281/zenodo.5602084

species also contained essential oils [5, 6]. These chemical defense substances have received considerable attention due to their potential as pharmaceuticals, food additives and valuable chemicals for human use [7]. *Sideritis incana* L. belong to family Lamiaceae. It's used in folk medicine as anti-inflammatory, anti-ulcerous, anti-microbial agents, antioxidants; antispasmodic, anti-convulsive, analgesic and carminative [8].

The objective of this research is to estimate the quantitative, polyphenols and flavonoids by Folin-Ciocaleu and aluminium trichloride reagents respectively, and studying the biological activity of the *Sideritis incana* L plant.

2. Materials and Methods

2.1. Extraction method

Sideritis incana L was collected during the flowering phase in May 2015 in the east of Algeria. The drying process of this plant material was carried out in a region away from moisture. They cut small parts. Air-dried leaves and flowers (370 g) of *Sideritis incana* L were macerated at room temperature with ethanol (70:30, v/v) for 24 h, three times. After filtration, the filtrate was concentrated and dissolved in H_2O (200 ml). The resulting solution was filtered and successively extracted with chloroform, ethyl acetate and *n*-butanol. The organic phases were dried with Na_2SO_4 filtered and concentrated *in vacuo*at room temperature to obtain the following extracts: petroleum ether (0.37 g)**,** chloroform (1.39 g), ethyl acetate (2.94g) and *n*-butanol (3.18 g).

Protocol of Preliminary tests to detect the chemical families in the ethanol extract [9]:

- Alkaloids: Add Dragerouf Wagner Reagent Drops.
- Flavonoids: Add concentrated HCl + Mg chip.
- Tannins: Add $FeCl_3$1%.
- Sugars: Add Fehling's reagent (A + B).
- Saponins: Agitation vigorously.
- Terpenes: Add CH_3Cl + H_2SO_4.
- Coumarins: Add NH_4OH 10%.

2.2. Total phenolic content

The total phenolic content in the extracts of *Sideritis incana* was estimated by using Folin Ciocalteu method [10]. A volume of 100 µl of the extract solutions at different concentrations were added to 1.5 ml of Folin-Ciolcalteu reagent (10%). After 5 min, 1.5 ml of sodium carbonate (6%) were added. The mixture was allowed to react for 90 minutes at room temperature and then the reading was taken at 725 nm. Gallic acid (0.03 - 0.3 mg / ml) was the standard used to establish the calibration curve, from which the concentration of the total polyphenols in the extracts is calculated [11]. The results were expressed in mg equivalents of gallic acid per gram of extract (mg/g) as shown in Figure 1.

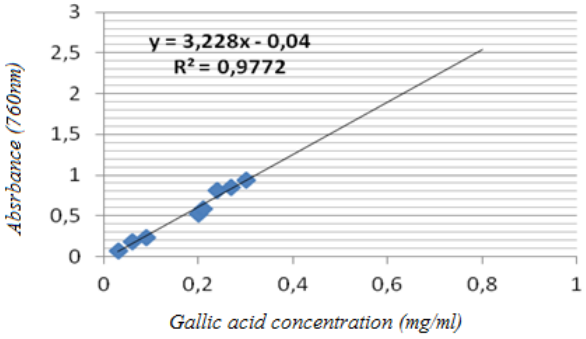

Fig 1.The standard calibration curve of gallic acid

2.3. Total flavonoid content

Total flavonoid content in crude extract and *Sideritis incana* L fractions was estimated using aluminum chloride method. 0.5 ml of a 2% ethanol $AlCl_3$ solution was added to 0.5 ml of extract. After 30 min of incubation at ambient temperature, the absorbance was as shown in Figure 2, was measured at 430 nm and the results were expressed in mg of quercetin equivalent per gram of dry weight of plant (mg EQ/g) [12].

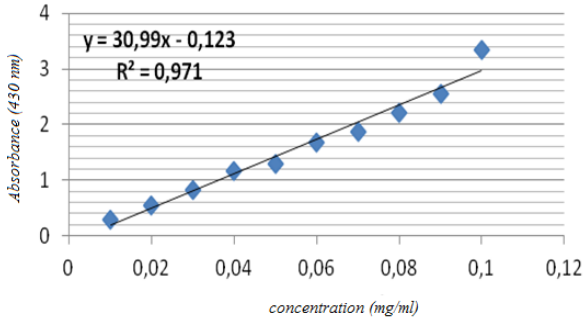

Fig 2. The standard calibration curve of quercetin

2.4. Determination of antioxidant activity

The evaluation of the antioxidant power of the various extracts was carried out by the DPPH test which is considered as a relatively stable free radical. The principle of this method was based on the measurement of the free radical scavenging of DPPH (1, 1-diphenyl-2 picrylhydrazyl) of the violet color. In the presence of the so-called antioxidant molecule, the DPPH is converted into its reduced form (1,1-diphenyl-2-pyryl hydrazine) of yellow color, which leads to a decrease in the absorbance. The discoloration of DPPH is directly proportional to the

ability of bioactive molecules to reduce it [13-14].

The ascorbic acid (Vitamin C) is used as reference with different concentration (0.01- 0.1) mg / ml. The DPPH solution (0.1 mM) is prepared by solubilization of 4 mg of this product in 100 ml of ethanol. 150ul of each ethanolic solution extracts at different concentrations were added to 3 ml of DPPH solution. The mixture was left in the dark for 30 min and the absorbance reading was made against a blank at 517 nm [15].

Figure 3 showed the results of the inhibition of free radicals were expressed as percent inhibition (I %) estimated according to the following equation: [16].

$$I\% = \frac{(A_0 - A_i)}{A_0} \times 100 \quad (1)$$

Where

I%: percentage of inhibition

A_0: absorbance of control

A_i: the absorbance of the extracts

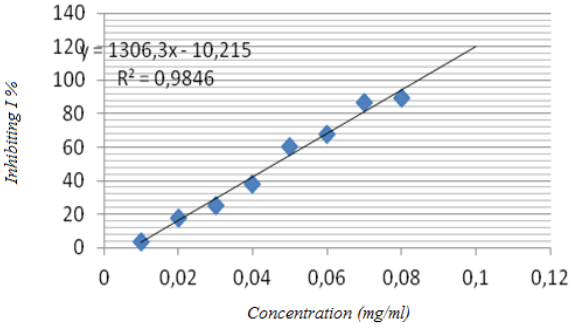

Fig 3.The standard calibration curve of ascorbic acid

2.5. Determination of total antioxidant activity

This technique was based on the reduction of Mo (VI) molybdates in the presence of an antioxidant with the formation of a green complex (phosphate / Mo (V)) at acidic pH diluted solution of ascorbic acid (control solution) at concentration between (0.2- 0.02) g / l.

The phosphomolybdate reagent was prepared from a mixture of sulfuric acid (H_2SO_4, 0.6M) of phosphate sodium ($NaPO_4$.28Mm) and of ammonium molybdate (((NH_4) $8Mo_7O_{24}.H_2O$, 4Mm).

A 1 ml dose of this reagent is added to 100 µl of each diluted concentration. The tube is incubated at 95 ° C for 90 min after standing at room temperature. The absorbance is measured at 695 nm. Against a blank that contains ethanol instead of the extract [15-17]. The results were expressed in equivalent of ascorbic acid as shown in Figure 4.

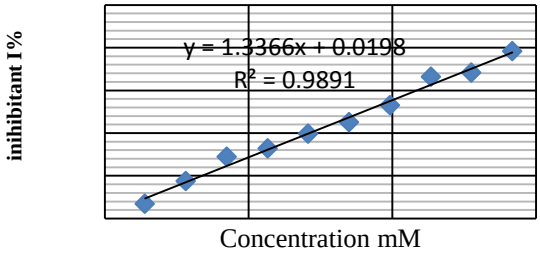

Fig 4. The standard curve of ascorbic acid

2.6. Antibacterial activity

The test was performed according to the disk diffusion method [18]

- Bacterial strains

The antibacterial activity of isolated microbial silver-based nanoparticles pellet was tested by standard well cutting method. *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC11303), *Pseudomonas aeruginosa* (ATCC27853) were included in this study to assess the susceptibility pattern of the compounds.

- Preparation of extracts

The plant extracts (chloroform, ethyl acetate, *n*-butanol and aqueous phase) were fully dried and weighed to dissolve in a dimethylsulfoxide DMSO solvent with different volumes with specific concentrations: 100 mg / ml; 50 mg / ml; 25mg / ml [19].

3. Results and Discussion

3.1. The yield of the extraction

The yield of the extraction of ethyl acetate and the *n*-butanol very close and they were the highest yield (0.7946 % & 0.8598 % respectively), while the petroleum ether and chloroform gave the least yielding 0.1% & 0.3767% respectively) . In general, the yield was somewhat weak, which explains the plant's poverty quantitatively rather than qualitatively.

3.2. Total phenolic of S.incana extracts

The results showed the highest percentage of chloroform where it was estimated 585.64 mg GAE/g followed by butanol 282.22 mg GAE/g and the lowest percentage recorded in petroleum ether 17.32 mg GAE/g which confirmed the presence of a high percentage of polyphenols in the chloroform phase.

3.3. Total flavonoid of the extracts S.incana

According to the results obtained the most petroleum ether contained 29.55 mgQE/g of flavonoids followed by *n*-butanol 16.97 mgQE/g, ethyl acetate with 9.707 mgQE/g and finally chloroform with 5.317 mgQE/g.

3.4. Antioxidant activity by DPPH assay

In these results the ascorbic acid is greatest antioxidant activity if compared to the natural extracts studied. Results had showed that n-butanol extract had shed by 0.0534 µg/ml followed by ethyl acetate 0.071 µg/ml and finally chloroform the least effective with 0.0756 µg/ml. These results correspond to the quantitative estimate of phenols. We know that many phenols are responsible for clearing free radicals, the rate of inhibition of the phase of the butanol is close to the inhibition ratio of the reference ascorbic acid and this explains why this phase contains donor groups of hydrogen atoms [20]. The results obtained are shown in Table 1 and Figure 5 and Figure 6. Total antioxidant activity TAC, it is known that the reduction capacity is greater than 1 of the extract was better, where acetate was most effective at 1.073 mM against n-butanol with 0.7798 mM and the minimum is chloroform with 0.268 mM.

Table 1. Results of total phenolic, flavonoid, DPPH scavenging, and total antioxidant activity

	Yield %	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)	DPPH IC50 (µg/ml)	TAC (mM)
Petroleum ether	0.1	17.32	29.55	-	-
Chloroform	0.3767	585.64	5.317	0.0756	0.268
Ethyl acetate	0.7946	197.88	9.707	0.071	1.073
n-butanol	0.8598	282	16.97	0.0534	0.7798
ascrobic Acid	-	-	-	0.0046	-

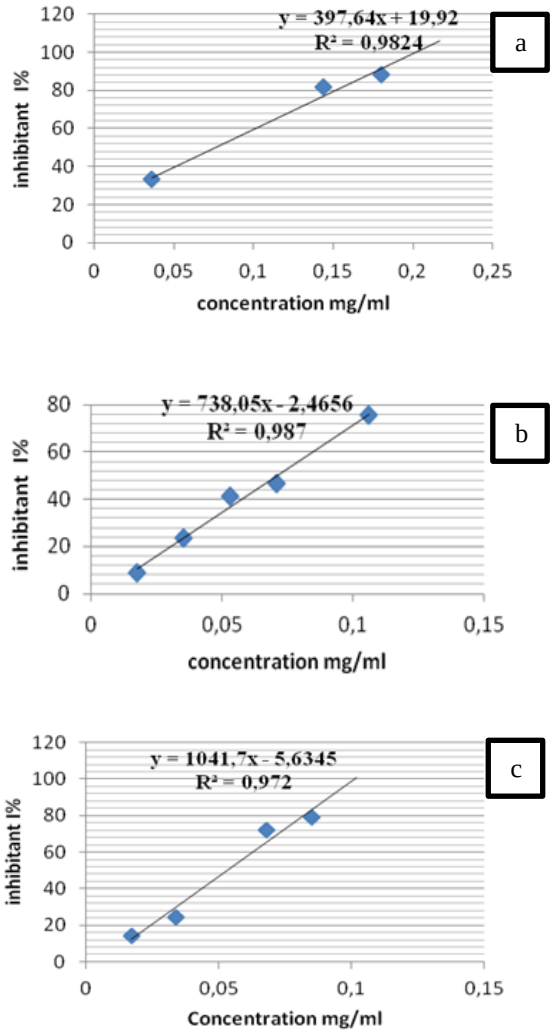

Fig 5.The inhibitory ratios curves **(**IC_{50}**)** of *S.incana* plant extracts, (a) chloroform, (b) ethyl acetate and (c) n-butanol

Note through the curves results, It's clearly showed that there is a positive relationship between the content of phenolic compounds and plant extracts and reticular effect on the DPPH root, in agreement with the results of many researchers The mechanism of interaction between the antioxidant compounds and the DPPH root is its chemical structure as well as the number of hydroxyl groups it contains [21].

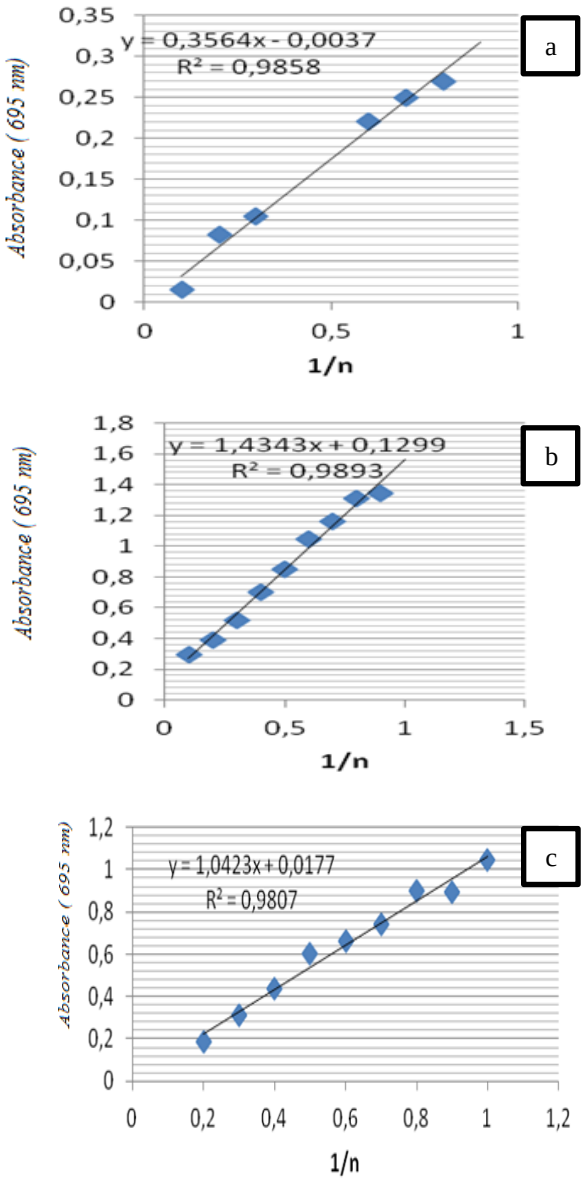

Fig 6.The curves of the total antioxidant capacity of *S.incana* extracts, (a) chloroform, (b) ethyl acetate and (c) n-butanol

The results showed that the chloroform extract gave the highest diameter inhibition among all extracts (13 mm) at a concentration of 50 mg / ml against *Escherichia coli* ATCC25921 bacteria and *Staphylococcus aureus* ATCC25923 bacteria (9 mm) at 100 mg / ml as shown in Table 2. For the remaining species, no extracts from the extracts were given any resistance against these colonists (*Pseudomonas aeurginosa* ATCC29733).

Table 2. Result of antibacterial activity of *S.incana* extracts

Inhibitor diameter (mm)			Concentration (mg/ml)	
Escherichia coli ATCC25921	Staphylococus aureus ATCC25923	Pseudomonas aeurginosa ATCC29733		
-	4.5	-	A= 100	Raw extract
-	0.3	-	B=50	
-	-	-	C=25	
-	4	-	A=100	Aqueous extract
-	7	-	B=50	
-	5	-	C=25	
10	9	-	A=100	Chloroform extract
13	7	-	B=50	
-	6	-	C=25	
7	-	-	A=100	AcoEt extract
6	-	-	B=50	
-	-	-	C=25	
-	7	-	A=100	n-butanol extract
-	8	-	B=50	
-	-	-	C=25	

4. Conclusion

As a result of this investigation, the aerial parts of *sideritis incana* L have been rich in secondary metabolites, especially phenolic, and flavonoids Results of total antioxidant TAC, acetate activity were more effective at 1.073 mM, n-butanol with 0.7798 mM and minimal chloroform with 0.268 mM. Which show the antiradical activity as well as its antibacterial activity to *Escherichia coli* ATCC25921 (at a concentration of 50 mg/ml) bacteria and *Staphylococcus aureus* ATCC25923 (9 mm) at 100 mg/ml). The results obtained for the first time at the level of *sideriteis incana* L extracts, according to our bibliographic research, as preceded by the level of volatile oils, enhance the therapeutic benefits of traditional medicine of this type pending further studies in this plant *sideritis incana* L

Conflict of Interest

The authors declare that they have no conflict of interest.

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Recommended Citation

Khelassi-Sefaoui A, Zaoui-Djelloul-Daouadji M , Mekhelfi T , Naili I, Zaiter L, Benarima A, Khechekhouche A. Phytochemical investigation, antibacterial and antioxidant activities of *Sideritis incana* L extracts.. *Alger. J. Eng. Technol. 2021*, 5:49-54.
http://dx.doi.org/10.5281/zenodo.5602084