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## Antioxidant and Antibacterial Activities of the species *Silene inflata* Sm

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### Abstract:

This study is devoted to the estimation of total phenolic content and the evaluation of *in vitro* antioxidant and antibacterial activities of crude extracts (petroleum ether, ethyl acetate and methanolic) obtained from the species *Silene inflata* Sm. The total phenolic content of the obtained extracts was estimated spectrophotometrically by the Folin-Ciocalteu method, the antioxidant activity was determined by scavenging of the free radical DPPH. Furthermore, the antibacterial activity was assessed by agar disk diffusion assay against four bacterial strains. The results of the phytochemical screening of crude extracts revealed the presence of several types of secondary metabolites including steroids, alkaloids, tannins, polyphenols and saponins. The ethyl acetate extract recorded the highest content of polyphenols ( $8.60 \pm 0.01$  µg GAE/mg of plant extract) followed by the methanolic and petroleum ether extracts respectively. The results of the antioxidant activity showed that all the tested extracts had the ability to scavenge the free radical DPPH and possess antioxidant activity in a dose-dependent manner. The methanolic extract has a strong antioxidant activity with an IC<sub>50</sub> value of  $23.53 \pm 0.15$  µg/ml. The crude extracts (petroleum ether and ethyl acetate) had no antibacterial effect against all the tested strains compared to penicillin and gentamicin as positive controls. While the methanolic extract revealed an antibacterial effect only against the clinical strain *Staphylococcus albus* with a value of the minimum inhibitory concentration at 0.5 mg/ml. In conclusion, the species *Silene inflata* Sm. could be an important source of new therapeutic agents against pathological damage due to free radicals and microbial infections.

**Keywords:** *Silene inflata*, phenolic content, phytochemical screening, antioxidant, antibacterial activity.

## INTRODUCTION

Reactive oxygen species are used by living organisms because of their beneficial reactivity in many processes, such as defense mechanisms or as signaling molecules (Ray *et al.*, 2012). These species are useful and beneficial in low concentrations in the cell, which is maintained by the balance between prooxidant/ antioxidant system (Rahal *et al.*, 2014). However, an imbalance between free radical sources and antioxidant systems induces oxidative stress (Birben *et al.*, 2012). This latter is the cause of several metabolic dysfunctions and the major reason for the damages observed in biological macromolecules (lipids, DNA and proteins) (Therond 2006; Mahjoub and Masrour-Roudsari, 2012). These damages could induce several diseases such as: cancer (Sosa *et al.*, 2013), cardiovascular disorders (Csányi and Miller, 2014), hypertension (Montezano *et al.*, 2015), neurodegenerative diseases (Tramutola *et al.*, 2017) and asthma (Aldakheel *et al.*, 2016).

The human body is also exposed to a multitude of microorganisms that can invade its tissues under certain conditions, causing serious infectious diseases. Antibiotics are the most commonly used antimicrobials against these infections, but the often abusive use of these molecules favors the evolution of bacterial resistance, frequently leading to therapeutic failures (Mégraud, 2017; Stewart and Costerton, 2001). In this context, many researchers are oriented towards the study of medicinal plants in order to find new alternative natural sources of antioxidants and antimicrobials more effective and safer than synthetic molecules.

The genus *Silene* is one of the largest genera of the family Caryophyllaceae, comprising about 700 species (Edalatiyan *et al.*, 2010). Species of this genus are often used in folk medicine for the treatment of various diseases. The species *Silene inflata* Sm. is a perennial plant, with oval or lanceolate leaves and white flowers, rarely pinkish. It is

distributed in North Africa, North America, Europe, Western and Central Asia. In the region of Marrakesh (Morocco) the decoction prepared from root parts of *S. inflata* is used at the low dose, like vomit and general antidote in cases of poisoning. This plant is also used as an infusion against constipation, to treat wounds, scabies and pruritus and various dermatosis. But it is considered toxic in high doses (Bellakhdar, 1997). This work is devoted to the estimation of total phenolic content and the evaluation of *in vitro* antioxidant and antibacterial activities of crude extracts (petroleum ether, ethyl acetate and methanolic) from the species *S. inflata* Sm.

## MATERIALS AND METHODS

### Plant material

The plant material was collected in May 2013 in the Aures region (high mountains of Bellezma, Algeria) and was identified by Professor Bachir Oudjehih, Agronomic Institute of the University of Batna-1.

### Extraction

The powder of the whole plant *Silene inflata* Sm (200 g) was extracted twice with 2L of petroleum ether (PE) at room temperature during 3 days. The residue was then extracted twice with 2L of ethyl acetate (EtOAc) and methanol (MeOH) successively. After filtration, the filtrates were evaporated to give 1.3 g of PE, 5.2 g of EtOAc and 8.3 g of MeOH extracts.

### Phytochemical screening

The crude extracts (PE, EtAOc and MeOH) were screened for their phytochemicals using the methods described by Fransworth (1966) to reveal the presence of many secondary metabolites including steroids, flavonoids, saponins, tannins, alkaloids quinones and cyanogenic glycosides.

### Steroids

Two milliliters of chloroform, 0.5 ml of acetic anhydride and 3 drops of sulfuric acid were added to 2 ml of crude extract. After agitation, the appearance of a blue color indicates the presence of steroids

### Flavonoids

The detection of flavonoids in the extracts is carried out by adding five drops of acetic acid, 0.5 ml of distilled water, 0.5 ml of concentrated hydrochloric acid, 5 drops of iso-amilic acid and magnesium slices (Mg) to 0.5 ml of the tested extract. The mixture obtained is stirred for one minute and the appearance of a pink orange or red-violet coloration in the supernatant layer indicates the presence of flavonoids.

### Saponins

A decoction was prepared from the plant *Silene inflata* (1% w/v), after boiling for 30 minutes. The solution was filtered and adjusted to a volume of 100 ml. Different dilutions of the prepared aqueous solution were made in clean tubes. Each tube was stirred lengthwise for 15 seconds and the height of the foam was measured after 15 min of rest.

### Tannins

Two milliliters of each extract was added to 1 ml of ferric chloride solution (FeCl<sub>3</sub>, 1%). After shaking, the appearance of a greenish or bluish color indicates the presence of tannoids in the extract.

### Alkaloids

Three to four drops of DRAGENDORFF reagent were added to 1 ml of the tested extract. The appearance of an orange-red precipitate indicates to the presence of alkaloids in the solution.

### Quinones

One milliliter of each extract was added to 0.5 ml of sodium hydroxide (NaOH, 1%). After

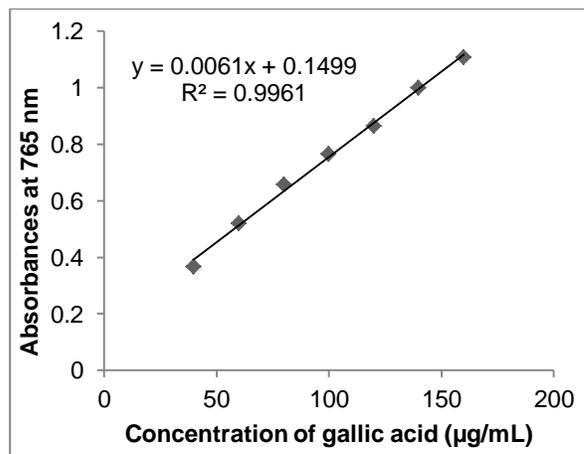
shaking, the appearance of a red color indicates the presence of quinones.

### Cyanogenic glycosides

In an Erlenmeyer flask, 2 g of the plant powder were mixed with 10 ml of distilled water and heated for 5 minutes. After boiling a strip of picrosidic paper was fixed in the Erlenmeyer. The change of picrosidic paper color from yellow to orange or red indicates the presence of cyanogenic glycosides.

### Determination of the total phenolic content

The total phenolic content of the crude extracts obtained from *Silene inflata* Sm. was estimated by Folin-Ciocalteu method described by Li and his collaborators (2007). Two hundred microlitres of diluted sample were added to 1 ml of diluted Folin–Ciocalteu reagent (10 %). After 4 min, 800 µl of saturated sodium carbonate (75 g/l) was added, the mixture was then incubated for 2 hours at room temperature. The absorbances at 765 nm were measured by UV–Vis spectrophotometer (Vis-7220G). Gallic acid (0–175 µg/ml) was used for the standard calibration curve (Figure 1) and the results were expressed as gallic acid equivalent (GAE) per mg of extract (µg of EAG/ mg of extract).



**Fig. 1.** Straight calibration of gallic acid for total phenolic content (mean ± SD of three trials)

### DPPH free radical scavenging assay

The antioxidant activity of PE, EtOAc and MeOH extracts was evaluated using the stable free radical 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) according to the method described by Menaceur and his collaborators (2014). 25 µl of different dilutions of the extracts or standards (butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tannic and ascorbic acid) were added to 975 µl of DPPH solution (0.025 mg/ml), 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:

$$\% = [(A_{\text{Blank}} - A_{\text{Sample}}) / A_{\text{Blank}}] \times 100$$

$A_{\text{Blank}}$  is the absorbance of blank and  $A_{\text{Sample}}$  is the absorbance of positive control or sample. The experiments were performed in triplicate and the results were expressed as mean values  $\pm$  SD.

### Antibacterial activity

The antibacterial activity of the crude extracts obtained from the plant *S. inflata* was estimated by agar disk diffusion assay (Falleh *et al.*, 2008) against four bacterial strains, including two Gram-positive (*Staphylococcus albus* and *Staphylococcus aureus* ATCC 25923) and two Gram-negative bacteria (*Escherichia coli* ATCC 35218 and *Enterobacter sp.*). The bacterial strains used were initially isolated by the method of streaking the four quadrants in sterile conditions and at optimum temperatures according to the strain concerned for 24h. 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 h. 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 is used to inoculate Petri disks containing Mueller Hinton by swabbing technique. Whatman paper disks (6 mm) were impregnated with 10 µl of the extract solutions and filed carefully on the surface of the inoculated agar with sterile forceps. The discs of the negative controls were impregnated with DMSO and the discs of positive controls contain the reference antibiotic (penicillin and gentamicin, 10 µg/disk). The Petri dishes were incubated at 37 °C for 24 h. The tests were performed in triplicate (three boxes for each concentration of extract, antibiotic and for each strain). The results were expressed by the diameters of zones of inhibition around the discs produced.

## RESULTS

### Phytochemical screening

The results of the phytochemical screening of the crude extracts (PE, EtOAc and MeOH) of *Silene inflata* showed the presence of various secondary metabolites known for their pharmacological properties such as steroids, alkaloids, tannins, polyphenols and saponins, as well as the absence of cyanogenic glycosides, flavonoids, and quinones has been reported in (Table 1).

**Table 1.** Phytochemical screening of *Silene inflata* extracts.

| Phytochemicals        | Extracts |       |      |
|-----------------------|----------|-------|------|
|                       | PE       | EtOAc | MeOH |
| Tannins               | -        | -     | +    |
| Polyphenols           | -        | +     | +    |
| Flavonoids            | -        | -     | -    |
| Saponins              | -        | -     | +    |
| Quinones              | -        | -     | -    |
| Cyanogenic glycosides | -        | -     | -    |
| Alkaloids             | -        | +     | -    |
| Steroids              | +        | -     | -    |

(+) presence of phytochemicals, (-) absence of phytochemicals.

### Total phenolic content

The results of the total phenolic content of *S. inflata* extracts are presented in Table 2. The extracts indicated differences in their total phenolic content depending on solvent polarities used for the extraction procedure. The highest phenolic content was found in the ethyl acetate extract, followed by the methanolic extract. Petroleum ether extract exhibited the lowest content.

**Table 2.** Total phenolic content of *Silene inflata* extracts

| Extracts | Total phenolic content (µg GAE/ mg of plant extract) <sup>a</sup> |
|----------|---|
| PE       | 1.806 ± 0.02  |
| EtOAc    | 30.13 ± 0.02  |
| MeOH     | 8.6 ± 0.01  |

<sup>a</sup>Total phenolic content was expressed as µg equivalent of gallic acid per mg of plant extract

### Antioxidant activity

The DPPH free radical scavenging properties of crude extracts (PE, EtOAc and

MeOH) are shown in Table 3. All the tested extracts had the ability to scavenge the DPPH free radical and possess an antioxidant activity that varies in a dose-dependent manner (Figures 2, 3 and 4). The methanolic extract has an antioxidant activity very close to the BHT, but this activity is relatively lower than that of BHA, ascorbic acid, tannic acid and α-tocopherol used as standards. The extracts PE and EtOAc showed moderate anti-radical activity.

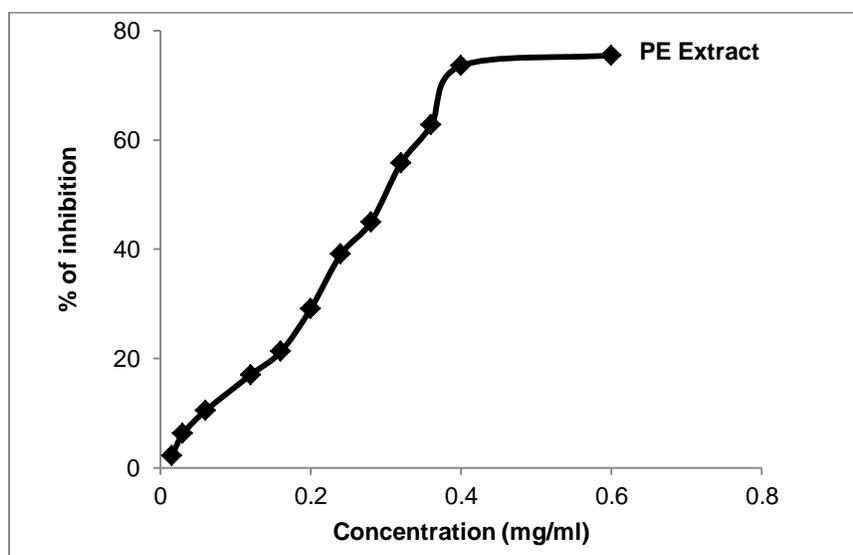
### Antibacterial activity

The results of the antibacterial activity of the species *Silene inflata* are shown in Table 4.

**Table 3.** Antioxidant activities of the species *Silene inflata* Sm.

| Extracts and standards     | IC <sub>50</sub> (µg/ml) <sup>a</sup> |
|----------------------------|---------------------------------------|
| PE                         | 30.20 ± 0.624                         |
| EtOAc                      | 31.03 ± 0.664                         |
| MeOH                       | 23.53 ± 0.15                          |
| BHA <sup>b</sup>           | 6.82 ± 0.49                           |
| BHT <sup>b</sup>           | 22.32 ± 0.02                          |
| tannic acid <sup>b</sup>   | 7.74 ± 0.19                           |
| Ascorbic acid <sup>b</sup> | 3.1 ± 0.002                           |
| α-Tocopherol <sup>b</sup>  | 13.02 ± 0.17                          |

<sup>a</sup>Values expressed are means ± SD of three measurements (p < 0.05), <sup>b</sup>Reference compounds.



**Fig. 2.** DPPH radical scavenging activity of PE extract (Mean ± SD of three trials)

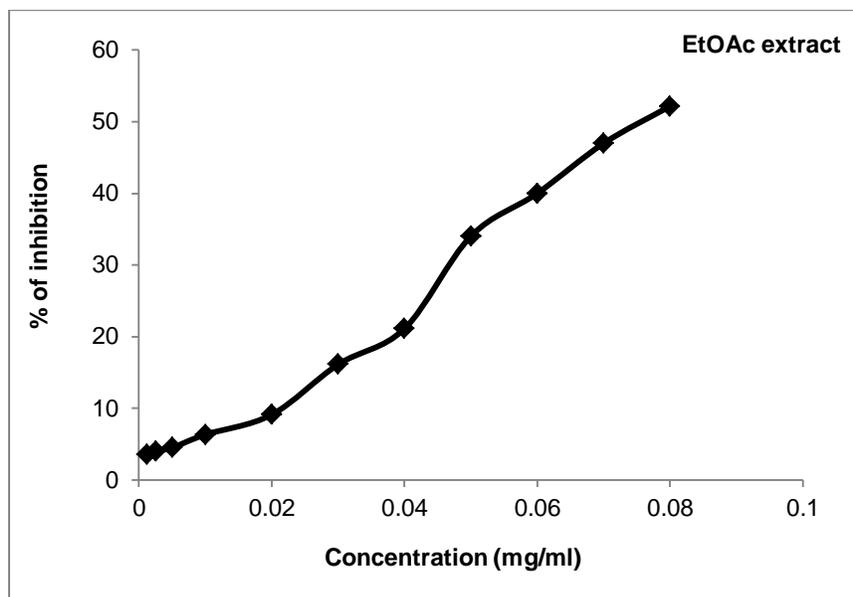


Fig. 3. DPPH radical scavenging activity of EtOAc extract (Mean ± SD of three trials)

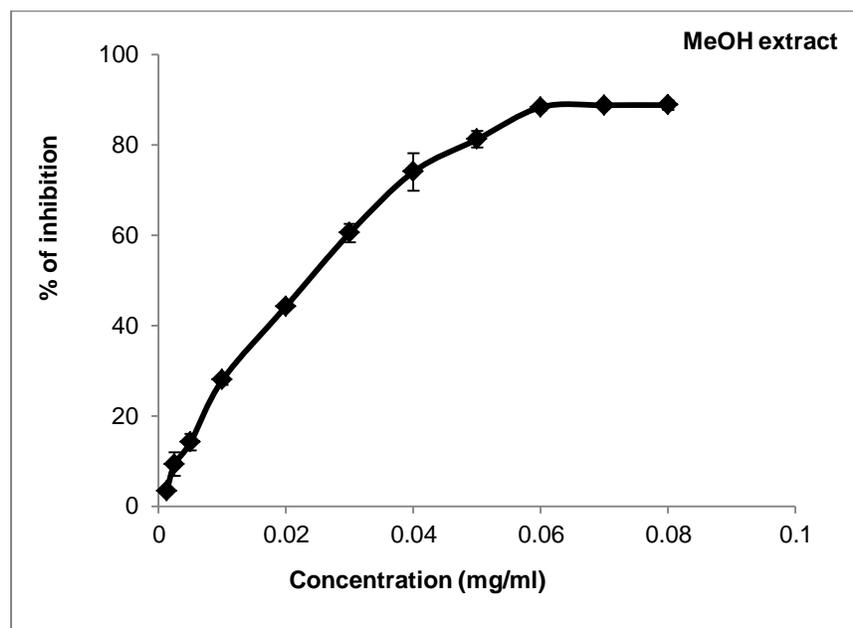


Fig. 4. DPPH radical scavenging activity of MeOH extract (Mean ± SD of three trials)

**Table 4.** Diameter of the zones of inhibition (mm) against bacterial strains

| Extracts/<br>Antibiotic | Dilutions* | Zone of inhibition                    |  |                             |                            |
|-------------------------|------------|---------------------------------------|--|-----------------------------|----------------------------|
|                         |            | <i>Escherichia coli</i><br>ATCC 35218 | <i>Staphylococcus aureus</i> ATCC<br>25923 | <i>Staphylococcus albus</i> | <i>Enterococcus</i><br>sp. |
| Gentamicin              | 0.1        | 29                                    | 22   | 20                          | -                          |
| Penicillin              | 0.1        | -                                     | 12   | 15                          | -                          |
| MeOH                    | 1          | -                                     | -  | 12                          | -                          |
|                         | 1/2        | -                                     | -  | 8                           | -                          |
|                         | 1/4        | -                                     | -  | -                           | -                          |
| EtOAc                   | 1          | -                                     | -  | -                           | -                          |
|                         | 1/2        | -                                     | -  | -                           | -                          |
|                         | 1/4        | -                                     | -  | -                           | -                          |
| PE                      | 1          | -                                     | -  | -                           | -                          |
|                         | 1/2        | -                                     | -  | -                           | -                          |
|                         | 1/4        | -                                     | -  | -                           | -                          |

Values expressed are means  $\pm$  SD of three measurements ( $p < 0.05$ ). (-) No zones of inhibition around the discs. \* Stock solution had the concentration of 1 mg/ml.

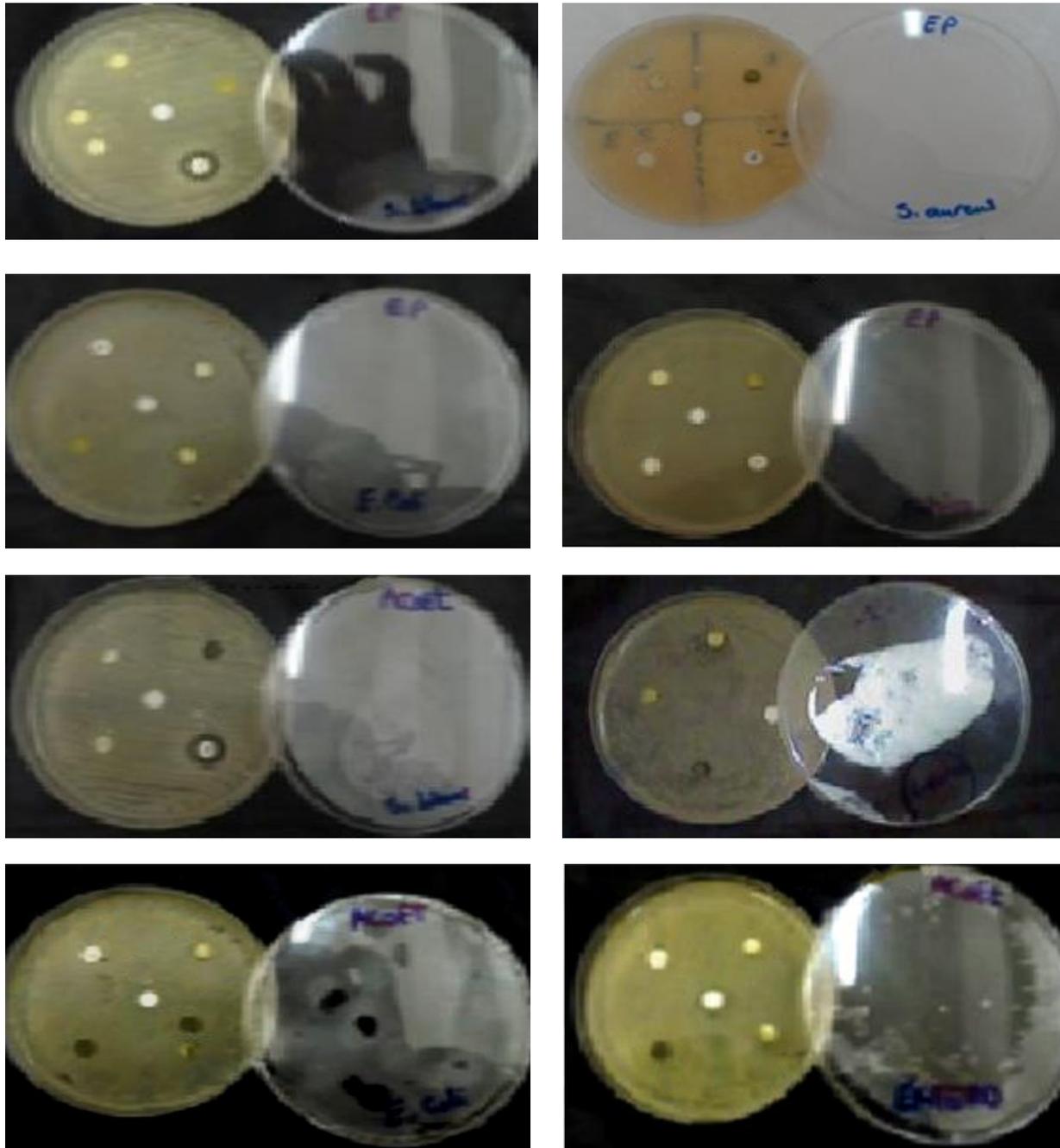
The crude extracts (petroleum ether and ethyl acetate) did not display any antibacterial effects against all the tested strains (*Enterococcus* sp, *Staphylococcus albus*, *Staphylococcus aureus* and *E. coli*) (Figure 5). The methanolic extract showed sensitivity only to the clinical *Staphylococcus albus* with a value of MIC at 0.5 mg/ml (Figure 6).

## DISCUSSION

The results of the phytochemical screening showed the richness of crude extracts obtained from *Silene inflata* in secondary metabolites. This species could be an important source of bioactive compounds with antioxidant and antimicrobial effects. Indeed, all the detected secondary metabolites in the present study were previously isolated from species of the same genus (Golea *et al.*, 2017; Azadi *et al.*, 2015; Alarcón and García, 2006; Jurgens *et al.*, 2004; Mamadaliyeva *et al.*, 2003; Jürgens *et al.*, 2002; Witt *et al.*, 1999). The results of previous studies carried out on the species *Silene swertiifolia*, *S. spergulfoliana* and *S. gynodioca*

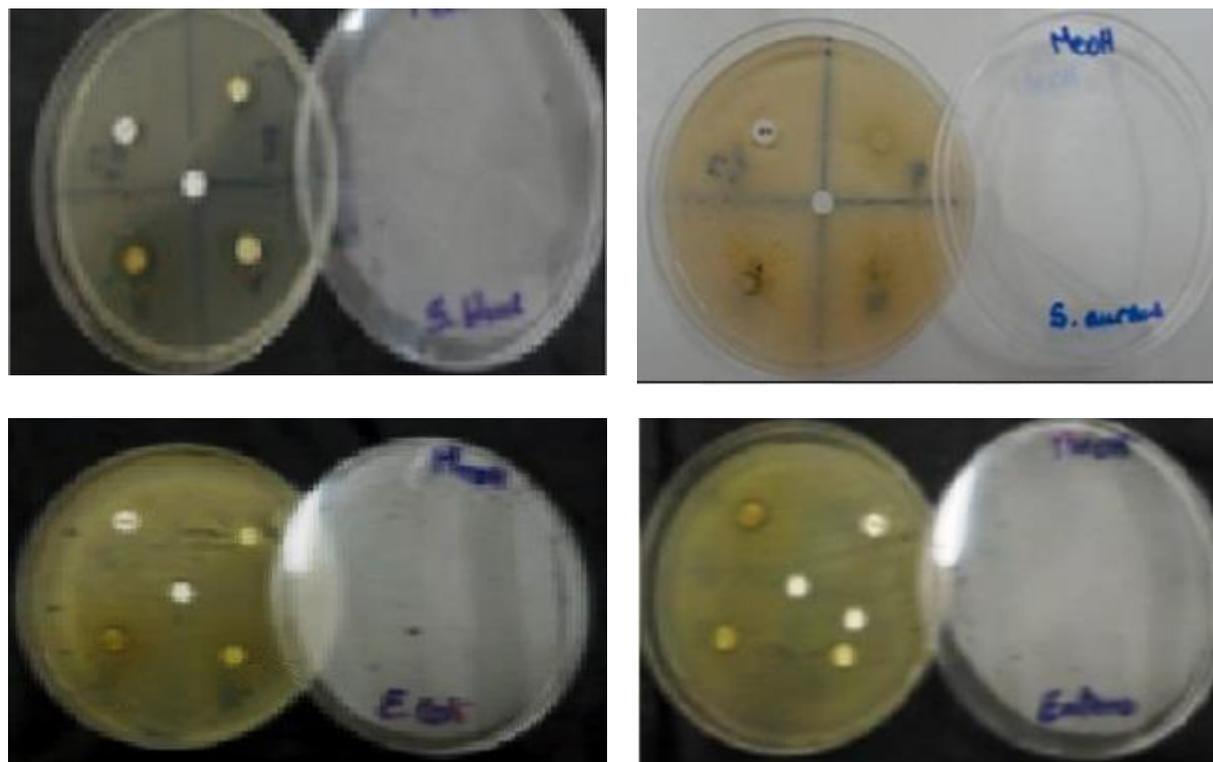
(Karamian and Ghasemlou, 2013) showed higher levels of polyphenols compared to the results of the present study. The variation in total phenolic content between species of the same genus could be due to various intrinsic factors (genetic potential of the individual species for the biosynthesis of polyphenols) and extrinsic factors (environment, stage of maturation and storage period) (Nicoletti *et al.*, 2015).

The antioxidant activity observed in all the tested extracts obtained from the species *S. inflata* can be attributed to the presence of several types of secondary metabolites, known for their antioxidant potential. The comparison of the obtained result with previous studies showed that some species of the genus *Silene* (*S. Compacta*, *S. guntensis*, *S. swertiifolia*, *S. spergulfolia* and *S. gynodioca*) were less active as scavengers agents against DPPH free radical (Boğa, 2017; Karamian and Ghasemlou, 2013; Mamadaliyeva *et al.*, 2011). While other species of the same genus (*S. vulgaris*, *S. arenarioides* and *S. alba*) revealed a high ability to scavenge free radicals related to their high polyphenolic content (Golea *et al.*, 2017; Taskin and Bitis, 2013; Morales *et al.*, 2012).



**Fig. 5.** Antibacterial activity of EtOAc and PE extracts of the plant *Silene inflata* Sm. against four bacterial strains.

The crude extracts: petroleum ether (EP) and ethyl acetate (AcOEt) did not display any antibacterial effects against all the tested strains (*Enterococcus* sp, *Staphylococcus albus*, *Staphylococcus aureus* and *E. coli*).



**Fig. 6.** Antibacterial activity of MeOH extract from *Silene inflata* Sm. against four bacterial strains.

The methanolic extract showed a sensitivity only to the clinical *Staphylococcus albus* with a value of MIC at 0.5 mg/ml.

The optimal effectiveness of crude extracts may not be due to the main active compounds, but to the combined action (synergy) of various secondary metabolites. Indeed, the evaluation of the antimicrobial properties of *Silene multifida* revealed that crude extracts were more active and potent than their fractions (Ertürk *et al.*, 2006).

The antibacterial activity of the methanolic extract which only affects the strain *S. albus* and not *S. aureus* within the same genus (*staphylococcus*), reflects the specificity of secondary metabolites present in the methanolic extract. This specificity has great importance since the bacteria (*Staphylococcus albus*) constitute the main cause of nosocomial infections and considered as the most

multiresistant strains to antibiotics (De Leon and Wenzel, 1984). These results indicate that the methanolic extract of *Silene inflata* is active only against the Gram-positive strain. Indeed, previous studies on methanolic extracts of the same genus, such as *S. swertiifolia*, *S. ginodioca* and *S. spergulifolia*, showed antibacterial activity only against gram-positive bacteria (Karamian and Ghasemlou, 2013).

The absence of the antibacterial activity in the tested extracts (PE and EtOAc) could be explained by the nature and the amount of the bioactive compounds with antibacterial properties, which could be totally absent or present in a very small amount in these extracts. According to Balouiri and his collaborators (2016), the method adopted for the extraction

procedure and the nature of the used solvents influence the antibacterial activity of the phenolic compounds. Indeed, the addition of DMSO to plant extracts decreases their antibacterial activities (Ciobanu *et al.*, 2012). In addition, the absence of the antibacterial activity of these extracts could be due to the loss of sensitive compounds during the grinding or the conservation of the vegetal material (Azwanida, 2015).

The choice of techniques, conditions and tools used to perform the test would be another factor affecting the obtained results. According to Jorgensen and Turnidge (2015), the diffusion method from wells on agar is more suitable for studying the activity of aqueous and organic extracts of plants than the diffusion method in agar medium.

The absence of the antibacterial activity of the tested extracts *in vitro* would not condition their antibacterial activity *in vivo*. Indeed, some secondary metabolites are active only after their metabolization and in this case, they are inactive *in vitro* but active *in vivo* (Tan and Lim, 2015). However, previous studies on the antibacterial activity of *Silene* species indicated that essential oils could be more active than crude extracts. The essential oils of *Silene armeria* L possess a wide range spectrum of fungicidal activity (Bajpai *et al.*, 2008) and that non polar extract are more active than polar one, according to Mamadaliyeva and his collaborators (2010) chloroform extracts which is rich in lipids, triglycerides, free fatty acids and sterols prepared from the species *S. brachuica*, *S. viridiflora*, and *S. wallichiana* exhibited a strong antimicrobial activities against various bacterial strains (Mamadaliyeva *et al.*, 2010).

## CONCLUSION

The present study reported the biological evaluation of the species *Silene inflata* Sm. growing in Algeria. The phytochemical screening of the crude extracts (PE, EtOAc and MeOH)

indicates the presence of various classes of secondary metabolites known for their antibacterial and antioxidant proprieties. Strong to moderate phenolic contents and antioxidant activities were observed in all the crude extracts. The organic extracts (petroleum ether and ethyl acetate) of *S. inflata* did not display any antibacterial effects on all the bacterial strains, while the methanolic extract revealed an antibacterial effect only against the clinical strain *Staphylococcus albus*. Furthermore, it can be concluded that *S. inflata* extracts could be used as a good source of alternative natural products helpful in preventing or slowing the progression oxidative and infectious diseases.

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## CONFLICT OF INTEREST

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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