INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

PHYTOCHEMICAL SCREENING AND ANTIFUNGAL ACTIVITY OF SAPONIN EXTRACT FROM ALGERIAN *SILENE HOGGARIENSIS* QUEZEL

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Article Received on: 23/09/14 Revised on: 13/10/14 Approved for publication: 17/10/14

DOI: 10.7897/2230-8407.0510152

ABSTRACT
This study aims to investigate the presence of active phytochemical compounds of *Silene hoggariensis* Quezel and to assess the antifungal activity of saponin extracts. Plant sample was collected from Adrar department (south Algeria), the phytochemical screening was conducted on the arial part by three solvents. The saponin extracts were screened for antifungal activity against *Aspergillus flavus, Aspergillus ochraceus, Fusarium oxysporium and Penicillium expansum* using two methods: inhibition of radial growth on solid medium (PDAa) and inhibition of biomass on liquid medium (PDB). The phytochemical analysis revealed the presence of various secondary metabolites of therapeutically importance. The major phytochemicals found were saponins (3.2%), tannin (0.08%) and alkaloids (0.01%). The *in vitro* antifungal assay of the saponins exhibit antifungal potency against fungal strains tested with varied effectiveness. Higher antifungal inhibition was observed on *Fusarium oxysporium* (IAF: 40.69%), and *Penicillium expansum* (IAF: 36.20%). Less antifungal effect of saponins was observed for *Aspergillus ochraceus and Aspergillus flavus.* The results provided evidence that the studied saponin extract might indeed be potential sources of natural antifungal agents.

Keywords: *Silene hoggariensis Quezel* –phytochemical screening - saponin-antifungal activity - fungi

INTRODUCTION

The pathogenic fungi are real inducers of infections causing a serious threat to public health. The emergence of these fungal infections over the last few years is due to the susceptibility of the population to opportunistic infections caused by immune-suppression associated to malnutrition, balanced nutrition or inadequate supply [1]. These problems are also the result of an aquise resistance to antibiotics and toxicity caused by antifungal drugs particularity during prolonged treatment [2]. Today, many drugs made using natural or synthetic products are available to treat fungal infections, but are ineffective. In addition to the development of resistance in fungi against most of these drugs is increasingly reported in more. In addition to the presence of side effects associated with this medication enhances the evidence to find new drugs safe and non-toxic [3].

Plants produce a great deal of secondary metabolites many of them with antifungal activity [4]. Similarly, traditional medicine has made use of many different plant extracts for treatment of fungal infection and many of these have been tested in vitro antifungal activity [5].

Development of natural resources inevitably involves the identification of these plants and heritage of traditional medicinal practices acquired by the population over time [6]. In this context, we conducted an ethno-botanical survey of medicinal plants in three areas of Adrar department (Gourara, Touat and Tidikelt) south of Algeria. The aim of this study is to investigate the antifungal efficacy and phytochemical compounds present and active in *Silene Hoggariensis* Quezel against fungal strains namely: *Aspergillus flavus, Aspergillus ochraceus, Fusarium oxysporium and Penicillium expansum.*

MATERIALS AND METHODS

Plant material

The aerial parts of *Silene Hoggariensis* Quezel plant were collected from Adrar department located at southern Algeria in February 2013. The levy was made from three districts: Gourara, Touat and Tidikelt. These areas are characterized by a continental climate with Saharan varying degrees, a very hot and long season and a very cold and short season. The plant sample was identified with the help of herbalists in the region. The plant parts were cut into small pieces and shade dried at room temperature (20°C) for two weeks, finely powdered plant materials were stored in airtight polythene bags protected from sunlight until use.

Extraction

Using the protocol of Nemlin and Brunel [7], 20g each of the powdered leaves of the plants were macerated three times with 60 ml of diethyl ether for ten minutes. The extracts were filtered using Whatman filter paper and concentrated to 25ml. the filtrates were labeled appropriately as diethyl ether extract. The marc of each part was then macerated in methanol using the same above protocol. The obtained extracts were labeled as methanol extract. Another 5g of 15 minutes, the extracts were filtered through Whatman's filter paper and labeled as methanol extract.

Phytochemical screening

The phytochemical analysis was carried out respectively on the three obtained extracts diethyl ether extract, methanol extract and water extract using standard procedures to identify the constituents as described by Sofowara [8], Trease and Evans [9], and Harborne [10, 11]. Into a 250 mL three-necked flask equipped with a drooping funnel, a sealed stirrer unit a

double surface condenser were placed 3 g of fatty acids and 25 mL of methanol and Added slowly a solution of concentrate sulfuric acid (1 mL) with vigorous stirring. The mixture was refluxed for about 2 h. The reaction mixture was allowed to reach room temperature and to stand for 2 h. After cooling, the mixture was poured onto 300 g of crushed ice. The aqueous layer was then extracted with chloroform. The organic layer was dried on anhydrous sodium sulfate. The solvent was distilled off yielded 1.12 g of methyl esters residue [12, 13].

Test for alkaloids

Alkaloids salts: the aqueous extract of the plant (25 mL) was stirred with 15 mL of 10 % HCl on a steam bath for 30 minutes. The mixture was extracted then three times with diethyl ether. 1 mL of the aqueous layer was treated with two drops of Wagner's reagent. Formation of brownish precipitate was regarded as evidence for the presence of salts alkaloids in the extract.

20 ml of the ethanolic solution were evaporated to dryness. 5 ml of 2N HCl were added to the residue and then heated in a water bath. The mixture was filtered and then the filtrate was divided into two equal parts. The first was treated with few drops of Mayer's reagent and the second with Wagner's reagent. The test is positive if a turbidity + yellowish-white precipitate are obtained in the Mayer's reagent tube and turbidity + brown precipitate are present in the Wagner's reagent one [14].

Free Alkaloids: 10 mL of organic layer (diethyl ether) was evaporated to dryness. The residue was then dissolved in 1.5 mL of HCl 2 % and treated with two drops of Mayer's reagent. Turbidity and formation of creamy white precipitate was regarded as evidence for the presence of free alkaloids in the extract [15] and all results were compared with blanks.

10ml of the etheric solution were evaporated to dryness using a rotavapor. The residue was then dissolved in 1.5 mL of HCl 2 % and treated with two drops of Mayer's reagent. The formation of a yellowish white precipitate indicates the presence of the Alkaloid bases [15].

Test for flavonoids

5 ml of the methanolic extract were treated with a few drops of concentrated HCl and 0.5 g of magnesium turnings. The mixture was left on for few minutes until the end of the reaction. The presence of Flavonoids is confirmed if a pink, red or purple color develops in the course of 3 minutes [10, 14, 15].

Test for saponins

2 g of powdered plant and 100 ml of water were placed in a 250 ml graduated flask, the mixture was heated to gentle boiling (decoction) for half an hour. The mixture is then filtered. After cooling, the volume of filtrate was adjusted to 100 ml of water. In ten test tubes, the following volumes of the mother solution (1, 2 ... 10 mL) were introduced. The final volume was then readjusted to 10 mL with distilled water. Each test tube was shaken vigorously in a horizontal position for 15 seconds. After standing for 15 minutes in a vertical position, the height of the persistent foam of each test tube was recorded. The formation of persistent foam after 15 minutes confirms the presence of saponins[12].

Test for steroids

2 ml of acetic anhydride was added to 0.5 g methanol extract of each sample with 2 mL H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids

5 ml of the etheric extract were mixed with 2 ml of chloroform and 3 ml of concentrated sulfuric acid. The appearance of a red - brown coloring layer interface indicates the presence of triterpene glycosides.

Test for tannins

The water extract of the crude dry powder of each organ was treated with 2 drops of 2 % $FeCl_3$ reagent. Blue dark color and precipitate indicated the presence of hydrolysables tannins [13]. To search Condensed tannins, to 5 mL of each extract (5 %) was added 5 mL of concentrated HCl. The mixture was boiled for 15 minutes and filtered hot using a filter paper and collected in a beaker. Formation of red precipitate soluble in isoamylic alcohol indicated the presence of condensed tannins.

Test for anthracenosides

This test was performed on the methanolic extract. First, 15 ml of 10 % HCl were added to 25 mL of the methanol extract, and then refluxed for 30 minutes. After cooling, the mixture was extracted three times with diethyl ether to obtain two phases, aqueous and etheric. 8 ml of the etheric phase were evaporated to dryness. The residue was treated with 2 ml of warm water a few drops of 10% NH_4OH. The appearance of an orange -red color designates a positive reaction.

Test for coumarins

5 ml of the methanolic extract were evaporated to dryness. The residue was then treated with 1 - 2ml of hot water. The volume was divided in two. 0.5 ml was treated with NH_4OH (10 %) and the other served as a control. The appearance of an intense UV fluorescence (λ = 265nm or 365nm) indicates the presence of Coumarins.

Test for anthraquinones

0.5 g of the part plant was boiled with 10 mL of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 mL of chloroform. The chloroform layer was pipette into another test tube and 1 mL of dilute ammonia was added. The resulting solution was observed for color changes (delicate rose pink color showed the presence of anthraquinones).

Test for reducing compounds

To 1 mL of the methanol concentrate was added 2 ml of distilled water. Fehling's solutions (A and B), 1 mL each were added, followed by heating in a test tube on a water bath. A brick red precipitate denotes the presence of reducing compounds.

Test for starch

5 mL of the aqueous extract was treated with the starch reagent. The appearance of a purplish blue color indicates the presence of starch. Starch reagent: 1.2 g of I2 and 2.5 g KI dissolved in 500 ml of distilled water.

Test for emodols

Adding the dry etheric extract to 25 % ammonia solution a cherish-red solution indicated the presence of emodols (aglycones of anthracenosides in oxidized form).

Free quinones

Few drops of NaOH 0.1 N were added to 10 ml of the etheric extract. The presence of free quinones is confirmed if the aqueous phase turns yellow, red or purple [16].

Fatty acids

14 ml of the etheric solution were evaporated to dryness using a Rotavapor. The residue thus obtained was dissolved in ethanol. The ethanolic solution is then concentrated to dryness. The residue was then saponified by adding 10 ml of 2N KOH. The mixture was then extracted with diethyl ether. After evaporation to dryness, obtaining a greasy residue indicates the presence of Fatty Acids.

Anthocyanosids

The aqueous phase obtained previously in the anthracenosids test was treated with NaOH, if there is a color change to different pH; it indicates the presence of anthocyanosids. The appearance of a red color at pH < 3 and Blue 4 to 6, characterize the presence of anthocyanosids [17].

Fungal materials and confirmation of testing strain

Fungal strains (*A. flavus* MTTC 2799, A. *ochraceus* CECT 2092, *Penicillium expansum sp* and *Fusarium oxysporum sp*) were procured from biology laboratory of Bechar University. The strains were regularly maintained by sub culturing on PDA acidified at 4°C.

***In vitro* evaluation of saponin antifungal activity of *Silene Hoggariensis* Quezel**

Silene Hoggariensis Quezel saponin extract were used to their antifungal potency. Antifungal activity was demonstrated by two widely used methods namely determination of percent mycelial inhibition by growth radial technique on solid medium which consist in placing simultaneously in test tubes 15 mL of PDAa (Potatoes Dextrose Agar) added of different volumes of saponin. After agitation, the selected solutions were transferred into dishes which were inoculated by the respective spore solution of each tested fungal strain (Agar solution at 0.2%+5% Tween 80 and spores of fungal strains). The dishes were incubated for 7 days at 25±2 °C. Mycelial radial growth was measured from the third day of incubation [18,19]. The inhibition percentage of mycelial growth was calculated using the following formula: (PIg= ((DT-D)/DT)*100 where DT is mean diameter of mycelial growth in control and D is mean diameter of mycelial growth in treatment [20].

From the dishes used for the evaluation of the radial growth on solid medium for each fungal strain tested (dishes incubated at 25°C for 10 days) were taken four washers 5mm diameter and transposed into tubes containing 9ml of physiological salt solution and 5% Tween 80 (few drops of). The extent of sporulation was measured for all the fungal strains tested. Spore count was performed using a Malassez cell. The percentage of sporulation inhibition was calculated as given below:

Is (%) =No-Nc/No X 100

Is(%): percentage of sporulation inhibition; **No**: Average number of spores in control; **Nc**: Average number of spores in presence of saponin

The antifungal potential was also tested by the determination of percent mycelial inhibition by biomass technique on liquid medium using bottles in which we put different volumes of saponosid:10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µL and complete to 50 mL by the PDBa medium (Potato Dextrose Broth acidified). After inoculation (30µl of spore suspension), the bottles were incubated at (25 ± 2 ° C) for 14 days [21, 22]. After filtration, the filter paper was dried at 60 ° C for 24 hours [23]. The weight of the biomass formed (P) is determined by the formula described by Imtiaj and Lee (2007) [24] ($P = P_1 - P_0$) where P_0: Weight of filter paper and P_1: Weight of filter paper and fungal biomass formed after drying.

Statistical analysis

All experiments were repeated at least five times. Ms Excel 2007 was used to determine means inhibition.

RESULTS

Phytochemical screening

Phytochemical screening was performed on the arial parts of *Silene Hoggariensis* Quezel using three different reagents. The results of qualitative tests summarized on Table 1, indicated that saponins, flavonoids, alkaloids, sterols, terpenoides, tannins, coumarines, fatty acids and volatile oils were present. In contrast, the other secondary metabolites like flavonoids, anthraquinones and anthrcyanosides were not detected.

Selective extractions have shown that saponin, alkaloids and tannins are the major groups of chemical compounds present in the studied plant. In fact, the plant material content from 100 g revealed that saponins (3.2%) are the most common chemical group. They are followed by tannins (0.8%) against alkaloids present only 0.01%. Table (2).

Antifungal activity

The results of the *in vitro* screening of antifungal activity of the saponin study by the inhibition of radial growth on solid medium depicted on table (3), revealed that the saponin extracts possess potential antifungal activity. Indeed, the saponin extract tested at 1/150 concentration showed the highest antifungal index for all the fungal strains tested. This activity was more pronounced on *Fusarium oxysporum* IAF = 40.69 ± 0, 48%. Less antifungal activity was revealed against *Penicillium expansum*, *A.ochraceus* and *A.flavus*. Antifungal activity indices obtained were respectively 36, 2± 0, 13%, 16, 91± 0.77% and 12, 54± 0, 37%.

Extent of sporulation

Results obtained from extent of sporulation revealed that the saponin showed different and variable inhibitory effect on sporulation at all concentrations tested. The highest sporulation inhibition effect was detected on1/150 for all tested strains. This inhibition was more pronounced on *F.oxysporum* Is=73, 68%. The different extents of sporulation enregistred for the other strains were 67, 41 %; 49, 18 % and 42, 86 % respectively for *Penicillium expansum*, A.*ochraceus* and *A.flavus*. (Figure 1)

The antifungal activity potency recorded from the evaluation of biomass weight on liquid medium showed different and variable antifungal index. The saponin extract tested at concentrations of 1/500, 1/250 and 1/150 appeared to be active against the fungal strains tested. The results depicted on table 4 showed that *F.oxysporum* and *A.flavus* biomass growth weight were considerably reduced (*Fusarium oxysporum* IAF $_{[1/500]}$ = 55.47±0, 10% - *Aspergillus flavus* IAF $_{[1/250]}$ = 42.52±0, 16%). In contrast, saponin fractions were less effective on the other fungal strains (*Penicillium*

expansum IAF $_{[1/375]}$ = 32.44±0, 19%, A.*ochraceus* IAF $_{[1/500]}$ = 27.27±0, 11%). (Table 4)

DISCUSSION

The plant world is full of resources and virtues or man draws not only food but also a reservoir of active substances which often provide a benefit to the organism sometimes affected by insidious disorders [28].

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. Plants contain numerous biologically active compounds, many of these have been shown to exhibit antimicrobial properties and therefore they were in use as antimicrobial drugs in traditional medicine. [27] Knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information be of value in disclosing new resources of such chemical substances [28]. According to a report of world health organization more than 80% of word's population depends on traditional medicine for their primary health care needs [27].

Plants represent indeed huge reservoir potential compounds attributed to secondary metabolites that have the advantage of being a very large variety of chemical structure and having a very wide range of biological activities [29]. These bioactive components are said to be responsible for the antimicrobial effects of plants extracts in vitro. They are grouped as flavonoids, alkaloids, glycosids, saponnins, tannins, terpenoids, carbohydrates and sterols [28].

Phytoconstituent saponins are steroidal glycosides generally associated with plant defense but also have wide range of biological properties [30]. These include deterrence to insects antifungal properties, anti-inflammatory and anticancer properties. The assigned objective to this study is to detect the phytochemical compounds of *Silene Hoggariensis* Quezel and to evaluate the *in vitro* antifungal potency of saponins extracted from the aerial parts of these widely used plants in local tradionnal medicine.

The first part of the study was devoted to qualitative phytochemical screening of the aerial part of *Silene Hoggariensis* Quezel. The phytochemicals occurring in various solvents extracts were analyzed qualitatively. Several solvents with different polarity have been used starting with the idea that the polarity is an essential parameter of the solubility of the constituents in the used solvent. The findings indicated that alkaloids, saponins, sterols, tannins, coumarines, fatty acids and essential oils were present in the aerial plant parts extracts under study. However, flavonoids, anthraquinones, anthracenosides were not detected in all extracts tested. These findings are in agreement with the studies of [30, 31]. The major phytochemicals found were saponnins, tannins and alkaloids. These compounds are known for their biological properties including antimicrobial activity antifungal properties, anti-inflammatory and anti cancer properties.

The outcomes of selective extractions showed that the saponins, tannins and alkaloids are the most present phytoconstituent in our plant. These findings justified the use of *Silene Hoggariensis* Quezel by local traditional medicinal practitioners for treatment of numerous diseases.

In the second part of the study, antifungal potency of saponin was assessed using two methods: the radial growth on solid medium and the evaluation of the biomass on liquid medium. The antifungal activity was carried out on the basis of the fact that the saponnin are responsible for the antifungal properties according [32, 33]. Four toxigenic fungal strains namely *Aspergillus flavus, Aspergillus ochraceus, Fusarium oxysporium* and *Penicillium expansum* were selected to evaluate the *in vitro* antifungal efficacy.

The findings of the antifungal activity assessing using the two methods revealed that the saponin fractions exhibited potential antifungal activity and inhibit the growth of the different fungal strains tested on all concentrations tested. These results are in analogy with literature data which confirm the antifungal potency of the saponins extract from plants. [28, 27, 34].

Inhibition of radial growth on solid medium was proportional to the concentration of saponin used in the medium. The optimal antifungal power of the saponnin was observed on *Fusarium oxysporium* and *Penicillium expansum*. Our results agree with those of Sandhu et *al*. who found that the saponins are endowed with antifungal power. However less inhibition was detected on the *Aspergillus* strains. This difference may be due to the differences of the cell wall composition of these fungi according to [35]. Furthermore, the extent of sporulation inhibition was greater against *Fusarium oxysporium* and *Penicillium expansum.*

As regards the antifungal potency recorded on tested strains conducted by the quantification of biomass on liquid medium, less and variabe index inhibition were noticed. Similar findings were reported by Amrouche et al [36]; Ozdemir et *al*. (2012) [37] and Elsemra et *al*. (2012) [38]. Fluctuation effect can be assigned to the used method.

Table 1: Phytochemicals detection extracts of *Silene hoggariensis* Quezel

Phytochemicals	Results
Reducing sugar	-
Anthocyanosides	-
Starch	-
Coumarines	+
Emodols	-
Essential oils	+
Fatty acids	+
Alcaloids (salts)	+
Flavonoids	-
Anthracenosides	-
Tanins	+
Saponosids	+
Sterols/Terpenes	+
Anthraquinone	-
Quinone libre	+
Flavonosides	+

(+): Present (-): Absent

Table 2: **Extractions screening of alkaloids, tannins and saponins**

Phytochemicals	Mass of plant material (g)	Mass of phytochemical	Yields (%)
Alcaloides	100g	0.01	0.01
Tannins	100g	0.08	0.08
Saponosids	100g	3.2	3.2

Table 3: *In vitro* antifungal index of saponins extracted from areal parts of *Silene Hoggariensis* Quezel (Growth radial technique on solid medium) (P<0.05)

	1/1500	1/750	1/500	1/375	1/300	1/250	1/214	1/187	1/167	1/150
FOA	18.48± 0.50	21.48± 0.83	22.44± 0.02	27.04± 0.93	27.18± 0.75	32.86± 0.70	34.51± 0.24	32.12± 0.70	36.99± 0.99	**40.44± 0.48**
PE	18.48± 0.50	18.97± 0.78	18.97± 0.78	26.85± 0.93	27.1± 0.75	32.7±0.67	34.35± 0.24	32.01± 0.43	32.9± 0.56	**36.2± 0.13**
AO	16.01± 0.48	16.2± 0.37	17.38± 0.87	14.22± 0.65	13.76± 0.38	15.6± 0.66	15.86± 0.75	16.72± 0.52	**16.91± 0.77**	16.55± 0.66
AF	6.56± 0.93	11.08± 0.73	13.42± 0.78	6.44± 0.11	3.25± 0.44	8.33± 0.61	10.64± 0.06	12.54± 0.37	12.54± 0.37	12.54± 0.37

Table 4: ***In vitro* antifungal index of saponins extracted from aerial parts of *Silene Hoggariensis* Quezel (Biomass technique on liquid medium). (P<0.05)**

	1/1500	1/750	1/500	1/375	1/300	1/250	1/214	1/187	1/167	1/150
FOA	15.41±0.14	26.43±0.13	**55.47±0.10**	15.36±0.12	15.22±0.13	11.10±0.15	15.52±0.35	25.41±0.15	16.64±0.10	15.23±0.16
PE	12.43±0.12	17.58±0.16	21.44±0.16	**32.44±0.19**	10.61±0.19	12.60±0.16	14.49±0.13	15.73±0.17	13.61±0.13	18.54±0.19
AO	22.45±0.13	13.54±0.16	**27.27±0.11**	4.89±0.35	13.45±0.17	22.47±0.17	14.46±0.18	9.95±0.39	10.24±0.11	13.60±0.16
AF	15.13±0.44	14.41±0.14	25.35±0.16	10.49±0.15	17.45±0.14	**42.52±0.16**	11.39±0.15	14.24±0.10	13.39±0.10	14.13±0.21

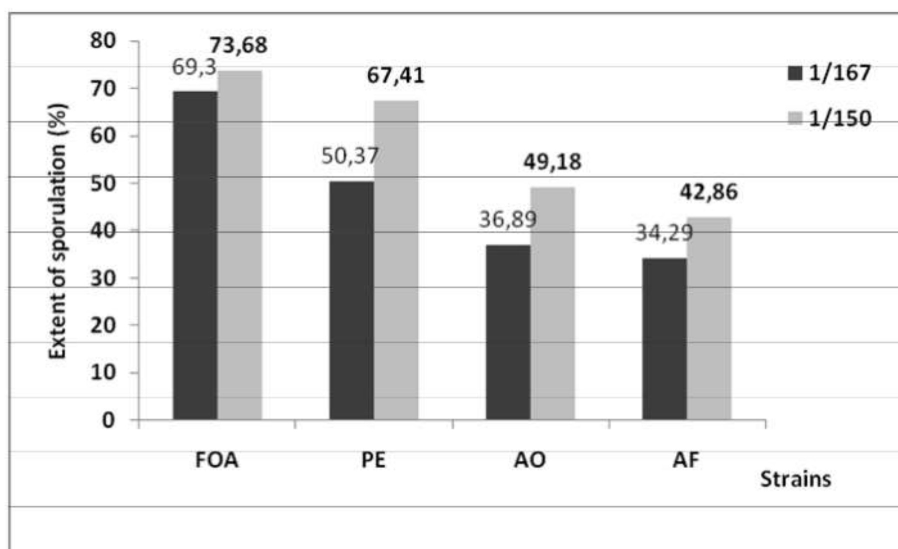

Figure 1: Extent of sporulation of the saponins on the tested fungi

CONCLUSION

In light of the preliminary interesting findings it could be concluded that the saponins extract from aerial parts of *Silene Hoggariensis* Quezel showed interesting antifungal potency against pathogenic fungal strains and provide evidence for traditional medicinal use. Further studies are needed in order to purify the bioactive compound for industrial formulation for the treatment of infectious diseases.

ACKNOWLEDGMENTS

Authors are thankful to the pedagogical biology laboratories of the University of Bechar for providing all facilities to carry out this study.

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Cite this article as:
Abdelillah Amrouche, Houcine Benmehdi, Daoudi Chabane sari, Saliha Bouras, Nacera Cherfaoui, Karima Hoggari. Phytochemical screening and antifungal activity of saponin extract from Algerian *Silene hoggariensis* Quezel. Int. Res. J. Pharm. 2014; 5(10):751-756 http://dx.doi.org/10.7897/2230-8407.0510152

Source of support: Nil, Conflict of interest: None Declared