

Antifungal activity of total saponins of *Convolvulus arvensis* L from Tlemcen, Western North of Algeria

Atividade antifúngica de saponinas totais de *Convolvulus arvensis* L de Tlemcen, oeste do norte da Argélia

Actividad antifúngica de las saponinas totales de *Convolvulus arvensis* L de Tlemcen, norte occidental de Argelia

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ABSTRACT

Convolvulus arvensis L. is used in traditional medicine in Algeria. It has powerful benefits for diseases of the digestive system and the skin. It also, know to have a potent role in plants protection. This research aims to study the antifungal potential of crude saponins of *C. arvensis* L. leaves from the Tlemcen region. The results showed that *C. arvensis* L is rich in various secondary metabolites (e.g., sterols, tannins, alkaloids, terpenoids, saponins, and steroids). Otherwise, findings reveal that the in vitro antifungal abilities of total saponins were more potent at 0.04 mg/mL for all molds tested. *A. flavus* was more sensitive to the effect of saponins (45.30%); *in contrario*, *P. expansum* was more resistant (26.57%). Furthermore, the antifungal effect of saponins depended on the fungal strains, the dose of saponins, and the incubation time. Based on these findings, *C. arvensis* L crude saponins had exhibited a significant antifungal impact which further potential biological activities should be explored.

Keywords: antifungal activity, *Convolvulus arvensis* L, food spoiling, total saponins.

RESUMO

A *Convolvulus arvensis* L. é usada na medicina tradicional da Argélia. Ela tem benefícios poderosos para doenças do sistema digestivo e da pele. Além disso, sabe-se que tem um papel importante na proteção das plantas. Esta pesquisa tem como objetivo estudar o potencial antifúngico das saponinas brutas das folhas de *C. arvensis* L. da região de Tlemcen. Os resultados mostraram que a *C. arvensis* L. é rica em vários metabólitos secundários (por exemplo, esteróis, taninos, alcaloides, terpenoides, saponinas e esteroides). Por outro lado, os resultados revelam que as habilidades antifúngicas in vitro das saponinas totais foram mais potentes a 0,04 mg/mL para todos os fungos testados. O *A. flavus* foi mais sensível ao efeito das saponinas (45,30%); em contrapartida, o *P. expansum* foi mais resistente (26,57%). Além disso, o efeito antifúngico das saponinas dependeu das cepas de fungos, da dose de saponinas e do tempo de incubação. Com base nessas descobertas, as saponinas brutas de *C. arvensis* L apresentaram um impacto antifúngico significativo, e outras atividades biológicas em potencial devem ser exploradas.

Palavras-chave: atividade antifúngica, *Convolvulus arvensis* L, deterioração de alimentos, saponinas totais.

RESUMEN

Convolvulus arvensis L. se utiliza en la medicina tradicional de Argelia. Tiene poderosos beneficios para las enfermedades del aparato digestivo y la piel. También se sabe que desempeña un potente papel en la protección de las plantas. El objetivo de esta investigación es estudiar el potencial antifúngico de las saponinas crudas de las hojas de *C. arvensis* L. de la región de Tlemcen. Los resultados mostraron que *C. arvensis* L. es rica en diversos metabolitos secundarios (por ejemplo, esteroides, taninos, alcaloides, terpenoides, saponinas y esteroides). Por lo demás, los resultados revelan que las capacidades antifúngicas in vitro de las saponinas totales fueron más potentes a 0,04 mg/mL para todos los mohos probados. *A. flavus* fue más sensible al efecto de las saponinas (45,30%); por el contrario, *P. expansum* fue más resistente (26,57%). Además, el efecto antifúngico de las saponinas dependía de las cepas fúngicas, la dosis de saponinas y el tiempo de incubación. Sobre la base de estos resultados, las saponinas crudas de *C. arvensis* L han mostrado un impacto antifúngico significativo, por lo que deberían explorarse otras posibles actividades biológicas.

Palabras clave: actividad antifúngica, *Convolvulus arvensis* L, deterioro de alimentos, saponinas totales.

1 INTRODUCTION

Phytotherapy is a traditional medicine that uses different parts of plants. It is considered by several researchers effective and potentially less dangerous than chemical medications (Awaad *et al.*, 2011). During centuries, humans have used plants in a variety of contexts, including healing, cosmetics, cooking and food preservation. Furthermore, plants produce a large variety of secondary metabolites which to protect itself against abiotic and biotic stresses (Ozay & Mammadov, 2022). Among these plants, *C. arvensis* L take a leader place on traditional medicine. It belongs to the *Convolvulaceae*, which contains a wide range of species (85 genera and 2800 species).

Over the world people, have used *C. arvensis* L to treat constipation, diabetes, skin infections and asthma. Data suggested that crude extracts of *C. arvensis* had anti-inflammatory, antioxidant, antibacterial and antifungal abilities (Benmerache *et al.*, 2013; Rachitha *et al.*, 2018). These properties can be attributed to the richness of *C. arvensis* L in many bioactive metabolites, including phenolics, alkaloids, saponins and sterols. Saponins, also called phytoanticipins are present constitutively in plants and seem to be implicated in plant disease resistance because of their eminent plant protection against phytopathogenic organisms and abiotic stresses (Barile *et al.*, 2007). They are synthesized by various plants families : *Asparagaceae* , *Amaryllidaceae*, *Dioscoreaceae*, *Smilacaceae*, *Liliaceae*, *Costaceae* , *Melanthiaceae*, *Plantaginaceae*, *Zygophyllaceae* , *Fabaceae*, *Asteraceae*, and *Solanaceae* (Faizal & Geelen, 2013). Although, considerable research has been conducted on the biological activity of the phenolics and flavonoids of *C. arvensis* leaves (Azman *et al.*, 2015; Rachitha *et al.*, 2018; Gupta & Fernandes, 2019), the antifungal feature of the saponins are poorly reported. For that, saponins have gained significant attention due to their amphiphilic

properties which allow them to exert huge biological activities. This study, is designed to elucidate the antifungal potency of the crude saponins of *C. arvensis* leaves against many fungal species.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

C. arvensis L leaves were collected from Beni Mester Mountain (Altitude: 697 m, Latitude: 34.8667, Longitude: -1.41667 34° 52' 0" North, 1° 25' 0" west), Tlemcen province, Algeria. *C. arvensis* L was identified in the laboratory of botany at the agronomy department of Tlemcen University. Then, plant samples were dried at room temperature, grinded by hand, and stored in a closed sterile container until they were used.

2.2 PHYTOCHEMICAL SCREENING

The phytochemical analysis of the various extracts was determined using the standard procedures developed by Nemlin & Brunel (1995). 20 g of *C. arvensis* leaf powder was macerated thrice with 60 mL of diethyl-ether during 15 mn each. After that, the extract was filtered and concentrated with a Rotary Evaporator. Subsequently, the marc was macerated twice in 60 mL of methanol according to the method described previously. However, aqueous extract was prepared using 5 g of plant powder. This amount was macerated with 50 mL of distilled water during 15 minutes under stirring. The extract was filtered and labelled as an aqueous extract.

2.2.1 Saponin screening

In a boiled water bath, 2 g of *C. arvensis* leaves powder were decocted in 100 mL of distilled water for 30 minutes. Then, the obtained extract, was filtrated and adjusted to 100 mL with distilled water. Moreover, the extract divided into ten tubes of varying sizes (1 mL, 2 mL, 3 mL, 4 mL, 5 mL,...10 mL). To generate a persistent foam, the final amount of each tube was completed to 10 mL of distilled water and shaken for 15 seconds. The formation of persistence foam after 15 minutes indicated the presence of saponin (Kapoor *et al.*, 1969; Benmehdi *et al.*, 2012).

2.2.2 Tannin Screening

2 drops of 2% FeCl₃ reagent were added to 5 mL of each extract. After stirring, the presence of gallic tannins is known by the formation of a blue-black colour and precipitate, while the presence of catechic tannins is indicated by the formation of a blue-green colour and precipitate (Segeliman *et al.*, 1969 ; Vijayakumari *et al.*, 2011).

2.2.3 Flavonoids screening

A small amount of magnesium ribbon was added to each extract (5 mL). Then, 1 mL of iso-amylic alcohol, and a drop of concentrated hydrochloric acid was added. Flavones are represented by their colours ranging from orange to red, and flavonols by their colours ranging from red to crimson. However, flavonones are characterised by their colours ranging from crimson to magenta (Vijayakumari *et al.*, 2011; Benmehdi *et al.*, 2012).

2.2.4 Free alkaloids screening

10 ml of *C arvensis* extracts were evaporated to dryness. Then, each extract's residue was dissolved in 1.5 mL of 2% HCl. After that, the mixture was treated with a few drops of Mayer's and/or Wagner's reagent. Turbidity and the production of a creamy white precipitate suggest the presence of free alkaloids (Harborne, 1978; Benmehdi *et al.*, 2012).

2.2.5 Anthracenosides screening

10 mL of methanolic extract and 5 mL of 10% HCl were combined. After 30 minutes of refluxing, and cooling, the mixture was extracted in triplicate with 10 mL of diethyl ether. After evaporation, 2 mL of boiling water and a few drops of 10% NH₄OH were added to the residue. The presence of anthracenosides is indicated by the appearance of a reddish-orange colour (Benmehdi *et al.*, 2012. Berroukeche *et al.*, 2024).

2.2.6 Anthocyanins screening

A few drops of ammonia were added to 5 mL of aqueous extract. The formation of blue colour indicates the presence of anthocyanins (Mamyrbékova-Békro, 2008).

2.2.7 Coumarins screening

5 mL of the diethyl ether extract was evaporated to dryness. Then, 1 mL of hot distilled water was used to dissolve the residue. After cooling, the extract was separated into two parts. The first part, was used as a control, and the second one was used as a test tube which 0.5 mL of 25% NH₄OH was added. The presence of coumarins is indicated by the emission of fluorescence light under UV excitation (λ_{max} = 365 nm) (Benmehdi *et al.*, 2012).

2.2.8 Sterols and triterpenes screening

2.2.8.1 For sterols

2 mL of acetic anhydride and H_2SO_4 were added to 0.5 mL of methanol extract. When steroids were present, the colouring shifted from purple to blue or green.

2.2.8.2 For terpenoids

5 mL of each extract was diluted with 2 mL of chloroform. Then, 3 mL of saturated H_2SO_4 was added carefully. The presence of terpenoids is indicated by the formation of reddish-brown colour.

2.2.9 Anthraquinones screening

2.2.9.1 Free anthraquinones

Free anthraquinones were extracted by boiling 0.5 g of *C. arvensis* powder in 5 mL of chloroform for 3 minutes. After that, the filtrate was shaken with 5 mL of chloroform. Then, 1 mL of 10% NH_4OH (or KOH) was added to 1 mL of chloroform extract. The presence of free anthraquinones is indicated by the formation of red colour (Rizk, 1982).

2.2.9.2 Anthraquinones O-heterosids

O-heterosids were revealed as following: the chloroformic residue was combined with 10 mL of distilled water and 1 mL of HCl . Then, the mixture was boiled in a water bath for 15 minutes. After cooling, 5 mL of chloroform was added to 5 mL of the mixture. Then, 1 mL of dilute NH_4OH was used to form the organic layer. The dark red colour formation indicates the presence of O-heterosid anthraquinones.

2.2.9.3 Anthraquinones C- heterosids

C-heterosids were determined from the chloroformic residue. This latter, was mixed with 10 mL of distilled water and 1 mL of 10% FeCl_3 . After 30 minutes of incubation in a water bath, 5 mL of chloroform was added. Then the chloroformic layer was mixed with 1 mL of dilute NH_4OH . The presence of genins C- heterosids is indicated by the appearance of moderate dark redish colour.

2.2.10 Free quinones screening

A few drops of NaOH 1/10 were added to the diethyl ether extract. The existence of free quinones was established when the aqueous layer became yellow, red, or purple (Rizk, 1982).

2.2.11 Reducing compounds screening

1 mL of Fehling's solutions was added to 1 mL of water extract. The formation of burgundy red precipitate after heating indicates the existence of reducing chemicals (Okwu, 2005; Benmehdi *et al.*, 2012).

2.2.12 Starch screening

The starch reagent was applied to the aqueous extract. The presence of starch was indicated by a blue-purple colour (Benmehdi *et al.*, 2012).

2.2.13 Emodols screening

Dry etheric extract was dilute by 1 mL of a 25% ammonia solution. The presence of emodols is indicated by the formation of cherish-red solution (Benmehdi *et al.*, 2012).

2.3 SELECTIVE EXTRACTION AND QUANTIFICATION OF SAPONINS

Selective extraction of saponins was used according to Edeoga *et al.* (2005) method. A total of 10 g of grounded leaves of *C arvensis L.* were immersed in 100 mL of a hydro-alcoholic solution (20% ethanol, v/v) at 55°C for 4 hours with stirring. This step was carried out twice. Then, the concentrate solution was evaporated to 40 mL after filtering it was subjected to a series of liquid-liquid extractions. First of all, 20 mL of diethyl ether was added and shaken strongly. The purifying procedure was carried out once more. After that, the separated aqueous phase was extracted three times with 40 mL of n-butanol. The n-butanol extracts were washed twice with 20 mL of a 5% NaCl, the samples were dried in the oven at 60°C until they reached a constant weight. The saponin yield was calculated as a percentage by the following equation (Rajhi *et al.*, 2021):

$$Yield (\%) = \frac{m_0}{m_1} \times 100 \quad (1)$$

where:

m_0 : represent the mass in grams of the evaporated dry residue.

m_1 : represent the mass in grams of the initial dry plant matter.

2.4 ANTIFUNGAL ACTIVITY OF SAPONOSIDS

2.4.1 Fungal materiel

The Research Laboratory of Valorisation of Plant Resource and Food Security in Semi-Arid Areas of Tahri Mohamed University of Bechar, Algeria, provided the fungal strains *Aspergillus*

flavus MTTC 2799, *Aspergillus ochraceus* CECT 2092, *Penicillium expansum*, and *Fusarium oxysporum*. Before usage, the tested strains were stored in PDA_{ac} medium at 4°C.

2.4.2 Antifungal activity evaluation

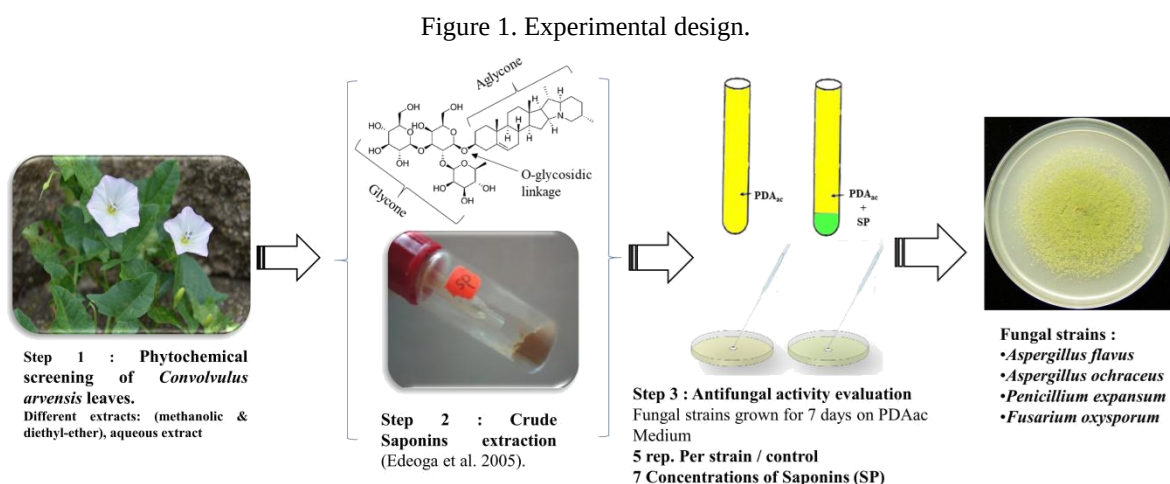
The agar diffusion method was used to calculate the percentage of mycelial inhibition. The center of Petri dishes, were filled with PDA_{ac} additionned to different concentration of saponins (0.002, 0.003, 0.005, 0.01, 0.02, 0.04 and 0.1 mg/ml). Then, an aliquot of 10µl of conidial solution (10⁴ spore/mL) was inoculated for each saponin dose. After that, Petri dishes were incubated at 25±2°C for 7 days. Each sample and control were repeated five times. The antifungal index was calculated as a percentage according to Wang *et al.* (2012) formula :

$$Ic (\%) = [(Dc - Dt)/Dc] \times 100 \quad (2)$$

Where:

D_c and D_t are the diameters of the fungal colony growth in control and test Petri dish (mm) respectively.

The **Figure 1.** illustrates the experimental steps.



Source: Designed by the authors (2024).

2.5 DATA ANALYSIS

Results are presented as mean (n = 5) ± standard deviation (SD). The data analysis was performed by SPSS Statistics 22 software. The data was subjected to analysis of variance (ANOVA) followed by Dunnett's test. A probability of p-value < 0.05 was considered as significant.

3 RESULTS

3.1 PHYTOCHEMICAL SCREENING

According to phytochemical analysis, *C.arvensis L.* is rich in tannins, alkaloids, saponins, sterols, terpenoids, steroids, O-heteroside, C-heteroside, and reducing compounds (Table 1).

Table 1. Phytochemical screening of extracts from *Convolvulus arvensis L.*

Phytochemical compounds	Diethyl-ether extract	Methanol extract	Water extract
Alkaloïds	+	+	+
Flavonoïds	-	-	-
Tannins	-	-	+
Anthracenosides	-	-	-
Anthracyanosides	-	-	-
Saponins	-	+	+
Coumarins	-	-	-
Sterols	+	-	-
Terpenoids	-	-	-
Anthraquinone	-	-	-
O-heterosid	+	+	+
C-heterosid	+	+	+
Free quinone	-	-	-
Reducing compounds	-	-	+
Starch	-	-	-
Emodols	-	-	-

Source: Prepared by the authors (2024): (+): present; (-): absent.

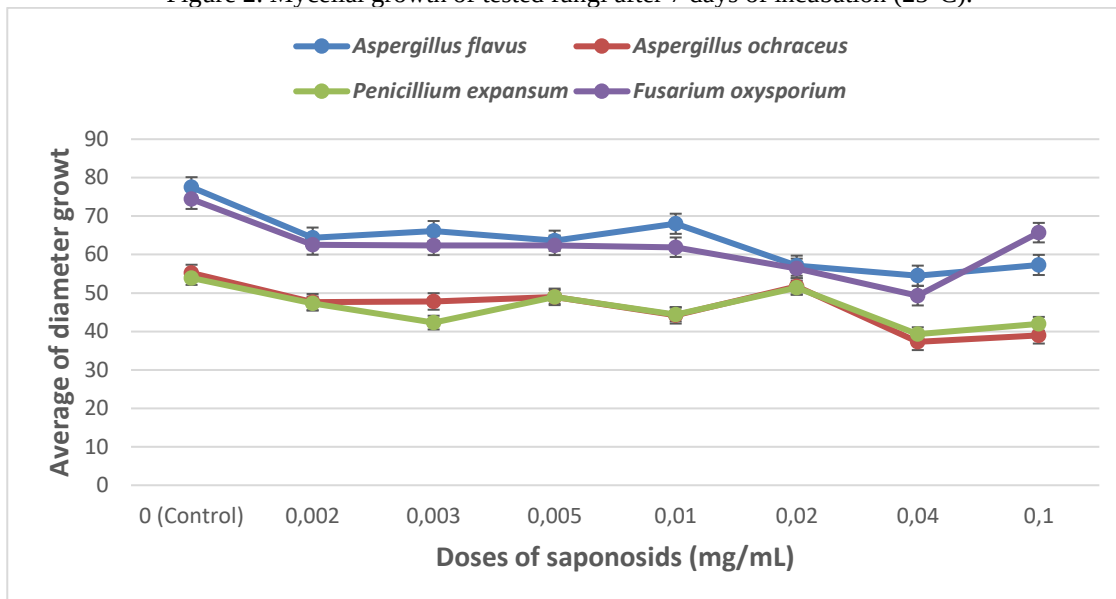
3.2 SAPONOSIDS DETERMINATION

The persistent emulsion upon one centimeter indicates the presence of saponosids. Indeed, qualitative results obtained reveal that the leaves of *C.arvensis L.* are rich in saponinss. The percentage yield of saponins extractable obtained from aqueous extract was 7%. They appear as a yellow powder.

3.3 ANTIFUNGAL ACTIVITY

Antifungal activities of *C.arvensis L.* saponins leaves have been investigated against four fungal pathogens. The first descriptive analysis results (Figure 2) revealed that the antifungal activity of saponins powder is clearly discriminating. It appeared two pairs of strains based on their reactions: *Aspergillus flavus*–*Fusarium oxysporium* and *Aspergillus ochraceus*–*Penicillium expansum*. The first pair is slightly more sensitive (31.5% on average) in comparison to the second pair (29.5% on average). On the other hand, each strain, had responded differently to saponins concentrations.

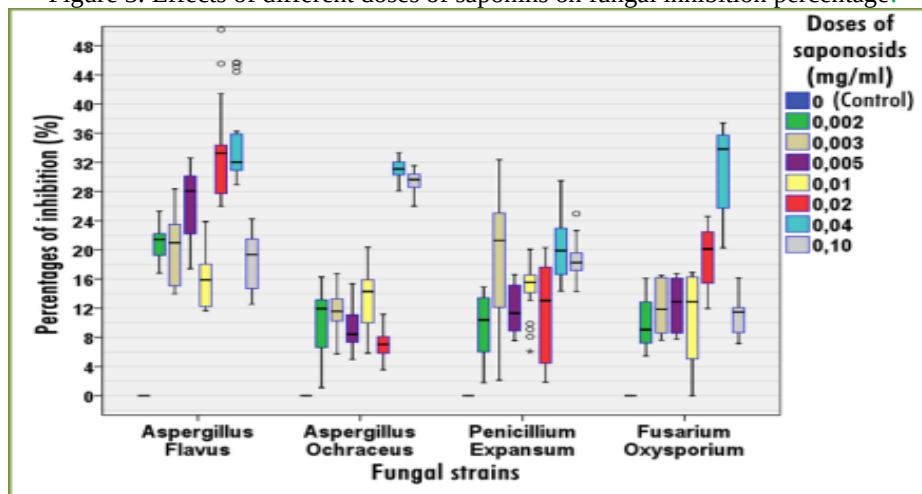
Figure 2. Mycelial growth of tested fungi after 7 days of incubation (25°C).



Source: Prepared by the authors (2024)

Figure 3 depict fungal-inhibition percentages of saponins at different doses. The result showed that *A. flavus* had higher medians of growth inhibition, in comparison with other fungal studied species. It was more sensitive to saponins at 0.04 mg/mL and 0.02 mg/mL (45.30% and 43.47% respectively) after 5 days of incubation. After that, the percentage of inhibition decreased progressively, to 29.68% (0.04mg/mL) and to 26.27% (0.02mg/mL) after 7 days of incubation. In addition, *F. oxysporium* was more sensitive to saponins after 5 days of incubation with 36.47%. However, *A. ochraceus* was significantly sensitive after 7 days of incubation with 32.45% of inhibition at 0.04 mg/mL. In parallel *P. expansum* was more resistant (26.97%) to the studied extract compared with other studied fungi at the same concentration. To sum up results revealed that crude saponins at 0.04 mg/mL were effective against all tested molds.

Figure 3. Effects of different doses of saponins on fungal inhibition percentage.



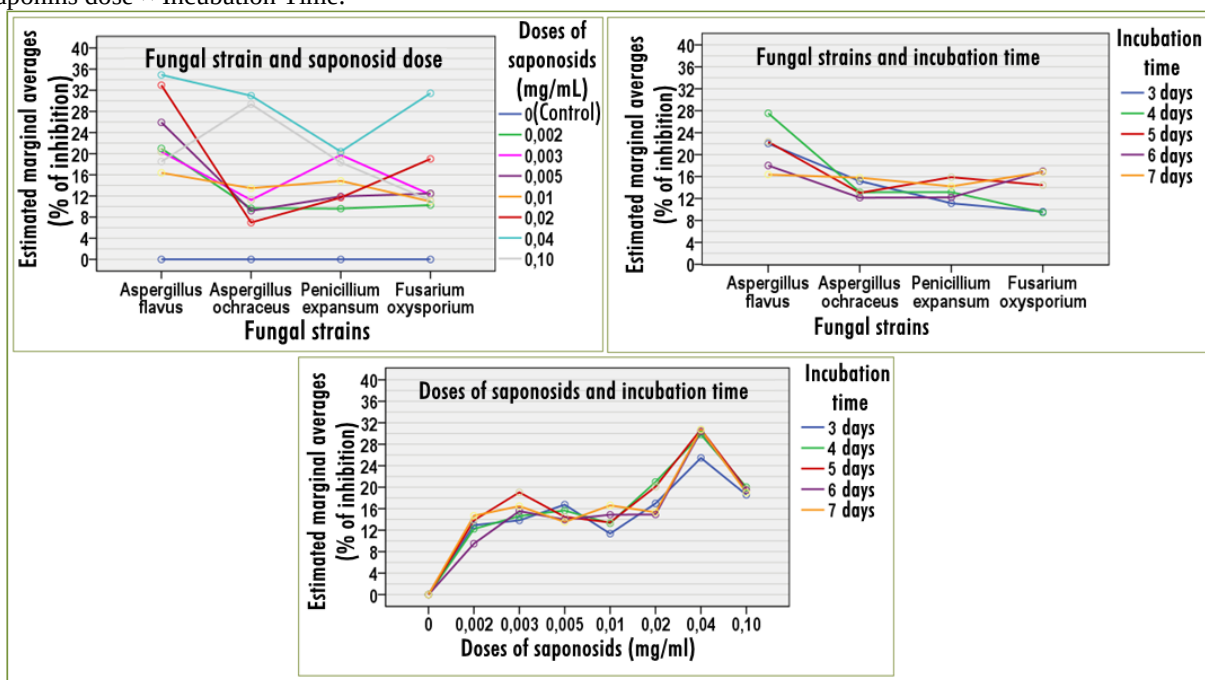
Source: Prepared by the authors (2024)

The purpose of the factorial ANOVA test was to investigate the significance of the differences found above, particularly those between the means of the inhibition percentages of the four studied fungi, compared to the controls, and the combined effect of three cross-factors (fungi strain-dose of the saponins-incubation time) on the inhibition indices. This test will allow us to study the effect of each factor apart from each other, but also to detect combinations between the three factors that would give an average of the rates of inhibition percentages in one class, different from the other classes.

The null hypothesis demonstrated that all the tested fungi, regardless of the dose of saponins used and/or the incubation time, they had an equivalent percentage of inhibitions. Considering the effect of the fungus species, the ANOVA results showed a significant differences between the four fungi tested ($F_{(2,62)}=2272.142$; $P_{\text{value}}=0.000$). For that, these results suggest that the percentage of inhibition varies significantly between fungi species without taking on consideration the effects of saponins doses and incubation time. Furthermore, the effect of the extract dose is highly significant ($F_{(2,02)}=5055,11$; $P_{\text{value}}=0,000$). In addition, according to the incubation time factor, the differences between inhibition averages are very significant ($F_{(2,38)}=75.95$; $P_{\text{value}}=0.000$). Thus, the percentages of inhibition vary significantly between incubation times when the effects of the other two factors were ignored.

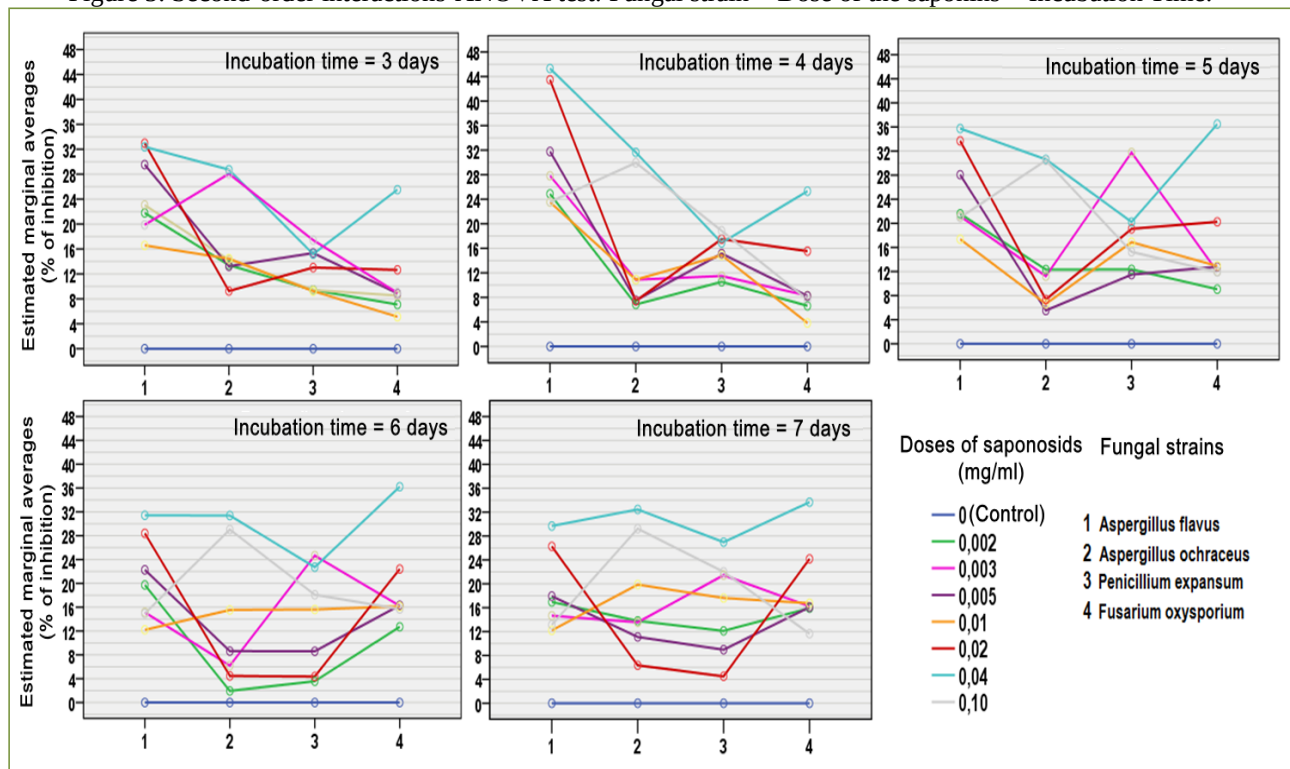
On the other hand, data, showed that the variability of the inhibition percentages depended also to the factor of interaction. In first-order interaction, results (**Figure 4**) were very significant between the fungus strain and the dose of the saponins ($F_{(1,59)}=599,89$; $P=0,000$), the fungus strain and the incubation time ($F_{(1,77)}=366.24$; $P=0.000$) and between saponins dose and the incubation Time ($F_{(1,59)}=47.04$; $P=0.000$). Similarly, in second-order interaction, the demonstrated result was very significant between the three factors ($F_{(1,37)}=40,01$; $P=0,000$). Consequently, the growth of each fungal strain depends on the dose of saponins and the time of incubation (**Figure 5**).

Figure 4. First-order interactions-ANOVA test : Fungal strain $\times$ Dose of the saponins; Fungal strain $\times$ Incubation Time; Saponins dose $\times$ Incubation Time.



Source: Prepared by the authors (2024)

Figure 5. Second-order interactions-ANOVA test: Fungal strain $\times$ Dose of the saponins $\times$ Incubation Time.



Source: Prepared by authors (2024)

The obtained results revealed that at alpha (α) = 0.05, it was clear that the dose of 0.04 mg/mL had a higher significant average (29.42711*), suggesting that, the potent antifungal effect of saponins was at 0.04 mg/mL.

4 DISCUSSION

It is usually, known that plants are very rich in secondary metabolites and they are dotted with tremendous benefits. Among these activities the antimicrobial properties are the most investigated (Anyasor *et al.*, 2010; Al-Rifai *et al.*, 2017; Bourhia *et al.*, 2019; Porte *et al.*, 2022). In the current study, the phytochemical investigation demonstrates that *C. arvensis* L leaves were mainly rich in saponins, phenolic and aromatic compounds, while the flavonoids were absent in the three extracts according to the used method. Al-Rifai *et al.*, (2017) observed that flavonoids were present in the ethanolic and *n*-Butanol extract, but absent in diethyl ether and chloroformic fraction. Moreover, Ozay & Mammadov (2019), had confirmed the presence of flavonoid, total phenolic and saponin of different solvent extracts (acetone, methanol, and petroleum) of *Convolvulus phrygius* Bornm with spectrophotometric methods. They added that the highest concentration of flavonoids was observed in the methanol extract. Previous studies had isolated flavonoids, alkaloids, coumarins, saponins, sterols, and tannins from several plants of the genus *Convolvulus* L. Accordingly, the positive or the negative determination of phytochemicals metabolites depend on the nature of the organic solvent and the extraction method (Todd *et al.*, 1995 ; Menemen *et al.*, 2002). For a long time, scientists had considered that saponins are an interesting phytochemical component used in biological control against pathogenic microorganisms (Hassan *et al.*, 2013; Khorasani Esmaeili *et al.*, 2015; Ozay & Mammadov, 2019). This study explored the antifungal effect of saponins extracted from the leaves of *C. arvensis*. In fact, results highlight that the leaves of *C. arvensis* are very rich in saponins. This finding is in accordance with many previous studies (Abdul Jalill *et al.*, 2014; Khan *et al.*, 2015, 2017; Ullah *et al.*, 2018; Ozay & Mammadov, 2019; Salim *et al.*, 2022; Ozay & Mammadov, 2022; Salamatullah, 2022).

Results analysis suggested that saponins had the highest biological effects which *F. graminearum* was the most susceptible strain, followed by *A. flavus*, and *A. niger*. However, *A. parasiticus* and *A. alliaceus* were more resistant.

There are no reports that had been available treated the antifungal activity of saponins crude from *C. arvensis*, For that, the obtained results from the antifungal activity of saponins will be compared with other medicinal or non-medicinal plants. Saponins from a large number of plants had demonstrated an effective antifungal potency (Hostettmann & Marstton, 1995; Tanaka *et al.*, 1996; Obsourn, 1996; Wina *et al.*, 2005 ; Tava & Avato, 2006). Several literature had studied the antifungal activities of saponins from *Medicago* species. They observed that *Trichoderma viride*, *Aspergillus niger*, *Alternaria solani*, *Fusarium spp*, *Phytophthora drechsleri*, *Phoma sp.* *Rhizoctonia solani*, and *Verticillium alboatrum* were sensitive to the presence of saponins in the medium (Zimmer *et al.*, 1967; Nonaka, 1986; Levy *et al.*, 1986, 1989; Olezek *et al.*, 1990; Gruiz, 1996;

Jurzysta & Waller, 1996; Jurzysta & Bialy, 1999). Moreover, the significant effect of saponins was obtained from the roots part of *Medicago* sp. (Saniewska *et al.* 2001, 2003, 2005). As stated by Teshima *et al.* (2013), the best antifungal effect of crude saponins was deduced from the basal plate of Shallot (*Allium cepa* L. Aggregatum Group) against *Alternaria* spp, *Botrytis* spp, *Colletotrichum* spp, *Curvularia lunata*, *Epicoccum nigrum*, *Fusarium* spp, *Magnaporthe* spp, *Sclerium cepivorum* et *Thanatephorus cucumeris*. The percentage of inhibition was arranged from 10% (*Botrytis cinerea*) to 90% (*Magnaporthe grisea*). It's crucial to keep on mind that saponins can vary in their precise chemical structures and features from a plant to another, just like other phytochemicals (Sikarwar *et al.*, 2023).

The variability observed in the chemical structures of saponins poses challenges in accurately determining their mode of action. Although, the precise mechanism by which saponins exert their effects on fungal species remains uncharacterized and unclear, various hypothesized pathways are proposed : (i) The capacity of saponin aglycone-sugar structure to form complex with the pathogen sterols in the cell membrane, leading to form pores and consequently loss of membrane integrity (Sikarwar *et al.*, 2023). Coleman *et al.* (2010) attest that various molecular mechanisms has been suggested for saponins antimicrobial abilities, including membrane lipid re-arrangement, pores, and cell lysis. In addition, saponins can fluidize the lipid layers of biological membrane increasing their permeability. This hypothesis was supported by Liu *et al.* in 2019. (ii) The production of ROS (Reactive Oxygen Species). Porte *et al.* (2022) declared that certain steroidal saponins can generate excessive ROS and increase the membrane permeability. Legault and co-workers have demonstrated that bidesmosidic betulinic saponin induce ROS production and decrease membrane potential, resulting to apoptosis and DNA fragmentation (Juang & Liang, 2020). (iii) The ability of saponins to inhibit fungal enzymes synthesis and interfering with the formation of fungal cell walls (Barile *et al.*, 2007). However several fungi are able to develop the resistance mechanism by producing enzymes that degrade saponins into non-toxic molecules (Curir *et al.*, 2006).

5 CONCLUSION

To sum-up, the current study show that the saponins of leaves of *C. arvensis* could be suggested as a potent natural antifungal agent. These findings can serve as fundamental studies for academics and society, highlighting various expected investigations in the fields of pharmacology, medicine, and agro-industry. Moreover, saponins may be used as a bioactive agent for the treatment of fungal infections.

Therefore, this study present some limits : (i) Saponins exhibit great structural variability, hence we were unable to precisely identify the specific type of molecule responsible for the antifungal effect ; (ii) the ability of resistant fungus to decrease the repressive effect of saponins ; (iii) The difficulties in precisely identifying the biological mechanism of action of saponins.

Due to the above-mentioned reasons, further works should be carried out : (i) Use the chromatographic tool to reveal the chemical nature of saponins ; (ii) Evaluate the antifungal activity of the triterpene and Steroid glycosides saponins (iii) Elucidate the precise mechanism behind the antifungal activities of saponins ; (iv) Investigate the antioxidant, anti-proleferative, antibacterial and anti-inflammatory potentiality of saponins molecules of *Convolvuls arvensis L.*

CONFLICT OF INTEREST

The authors of this article declare that there are no potential conflicts of interest. This manuscript is submitted on behalf of all authors.

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