# Polyphenols, antioxidant activity and mode of action of antimicrobial compounds of *Dittrichia viscosa* extracts

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In order to promote the spontaneous plant *Dittrichia viscosa* known by its use in traditional medicine, the content of phenolic compound in the aqueous and ethanolic extracts was determined according to the colorimetric method based on the Folin-Ciocalteu reagent, and the antioxidant activity which was evaluated by two methods: DPPH and FRAP. For antimicrobial activity, the microtiter microplate method was applied to the yeast *Candida albicans* and to two bacteria *Staphylococcus aureus* and *Escherichia coli*. Regarding the mode of action of the extracts of tested germs, an evaluation of the nucleic acids released during the lysis of the treated cells was carried by the spectrophotometric assay at wave length 260 nm. The results revealed that the extracts have an interesting content of polyphenols, which is higher in the ethanolic extract. Antioxidant and antimicrobial activities are higher for the alcoholic extract than the decoction. The MIC for *E. coli* is 0.28 ± 0.05 mg / ml, 2.290 ± 0.20 mg / ml for *Staphylococcus aureus* and 33.33 ± 0.33 mg / ml for *C. albicans*. The alcoholic extract and decocted of *D. viscosa* act respectively by damaging the bacterial and yeast wall.

**Key words:** *Dittrichia viscosa*, total polyphenols, antioxidant activity, antimicrobial activity, mode of action.

## 1. Introduction

Medicinal plants are used in traditional medicine for the treatment of different types of infections. Among the species known in Morocco especially in the province of Sidi Kacem *Dittrichia viscosa*. It used by the population of this region against respiratory, cutaneous and digestive infections (Ennacerie *et al*. 2017a).

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*D. viscosa* known by the name Inule (in French) and Magramane (in Arabic), belongs to the Asteraceae family. It is a perennial herb, characterized by glandular hairs, producing a sticky resin at the origin of the name "viscosa", covering the entire plant, thus conferring its strong typical odor (Araniti *et al*. 2017). Its long clusters characterize this speciewith yellow flowers forming a pyramidal inflorescence. Its fruit is composed of hairy achenes (Bensegueni-Tounsi 2001). As for its distribution, it grows on wet meadows, salty soils and the edges of streams (Bensegueni-Tounsi 2001).Its use in traditional medicine is highly regarded as an antibacterial, antifungal, anti-inflammatory, antipyretic and analgesic agent (Wollenweber et al. 1991). In addition, different scientific studies have demonstrated its antibacterial, antifungal and antioxidant properties (Rhimi *et al*. 2017; Albano *et al*. 2012). These virtues are due to the richness of the plant by phytochemicals known for their biological activity in pharmacology. Among its constituents highlighted flavonoids, sesquiterpenic acids, triterpenes esters (Benayache *et al*. 1991), terpenoids and sesquiterpenic lactones (Cohen *et al*. 2006).

Indeed, among the universal medical problems that occupy the minds of a variety of researchers are the oxidative stress and microbial infections. As for the oxidation, the aggression that causes the free radicals causes anomaly on different levels of the cells: membranes, lipids, lipoproteins and DNA. These cellular or tissue abnormalities are associated with aging, atherosclerosis, carcinogenesis, cardiovascular disease and Alzheimer's disease (Albano *et al*. 2012). Regarding microbial infections, their frequency has increased significantly in recent decades.

In addition, antifungals and antibiotics most used clinically have limits of use despite their development because of their cost, toxicity or effectiveness, as they are responsible for the appearance of resistant strains after frequent use (Supreetha *et al*. 2011).

For these reasons, finding new products is a challenge. Recently, natural products have attracted a growing interest in solving this problem. This interest is due to their availability, the reduction of their side effects or their toxicity, as well as their rapid biodegradability compared to available antibiotics and preservatives. The challenge was therefore to develop effective strategies for the treatment of bacterial and fungal infections as well as for the prevention or reduction of oxidation-related diseases using plant extracts.

In this context, the objective of this study is to quantify the polyphenol content in the two ethanolic and aqueous extracts of the aerial part of the *D. viscosa* plant. To evaluate their antioxidant activities and their antibacterial power against *Staphylococcus aureus* and

*Escherichia coli*, and antifungal against *Candida albicans* as well as to highlight the action of these extracts on the integrity of the cell wall and cell membranes.

## 2. Materials and methods

### Plant material

The harvesting of *D. viscosa* leafy stems was done manually in May 2016, from the edges of Oued Rdoum to the city of Sidi Kacem (Latitude: 34.221 °, Longitude: -5.707) in Morocco.

The species has been identified at the Laboratory of Plant Biotechnology and Molecular Biology of the Faculty of Science of Moulay Ismail University, Meknes (Ennacerie *et al*. 2017a). This plant material was sorted and dried in the shade at room temperature. After drying, electric grinding is performed to obtain a fine powder that would be used for the preparation of the extracts.

### Biological material

Bacterial strains: *Staphylococcus aureus* and *Escherichia coli*.

Yeast: *Candida albicans*.

The three germs were clinically isolated from the pathological products of the medical analysis laboratory of public hospital Mohammed V located in the city of Meknes in Morocco. Their susceptibility to antibiotics was realized, to mount their acquired multiresistance and consequently the planned failure to anti-biotherapy treatment.

### Preparation methods for *D. viscosa* extracts

An ethanol extract and a 10% decoction were prepared, with 95% ethanol and water, from the leafy stem powder of the plant and the, according to the procedure described by Ennacerie *et al*. (Ennacerie *et al*. 2017b). After removal of the solvents used, and obtaining a dry residue for each extract. A solubilization in water or in dimethyl-sulfoxide (DMSO), of decoction and ethanol extract respectively is carried out for each dry residue. The reagents are obtained from Sigma-Aldrich.

It is reported that the extracts are stored in dark bottles at 4 ° C.

### Determination of total polyphenols

In order to determine the content of phenolic compounds in the aqueous and ethanolic extracts of the leafy stems of *D. viscosa*, the colorimetric method based on the Folin-Ciocalteu reagent (Sigma) described by Boizot and Charpentierin 2006, is followed. The measurements repeated 3 times and Gallic acid used as a standard for tracing the calibration curve. With reference to

the latter, the concentration of total phenolic compounds expressed in mg equivalent of Gallic acid per g (mg EGA / g).

**Antioxidant activity**

***Iron (III) Iron (III) Reduction Method***

Antioxidant activity was determined by the ability of the extract to reduce ferric iron ($Fe^{3+}$) to ferrous iron ($Fe^{2+}$), which provides a blue stain complex readable by the spectrophotometer (Spectrophotometer UV-2005). The measurement of this activity was carried out according to the protocol described by Koné *et al*. (Koné *et al*. 2004), whose positive control is represented by ascorbic acid (Sigma-Aldrich).

$$\textbf{Reducing power of iron (\%)} = [1 - (A_1 / A_0)] \times 100.$$

- $\mathbf{A_0}$: Absorbance of the negative control;
- $\mathbf{A_1}$: Absorbance of the extract

***DPPH method***

In the presence of free radical scavengers, the violet colored 2-diphenyl-1-picryl-hydrazyl (DPPH) (Sigma-Aldrich) is reduced to 2.2 yellow diphenyl-1-picryl hydrazine.

This scavenging activity of the DPPH radical was measured according to the protocol described by Lopes-Lutz *et al*. (Lopes-Lutz *et al*. 2008).

The results can be expressed as anti-free radical activity or percent free radical inhibition (I %) using the following formula:

$$\textbf{I\% = [1 - (Abs Sample / Abs Negative Control)] x 100}$$

- **Abs Sample**: Absorbance of the sample;
- **Abs Negative control**: Absorbance of the negative control

The results obtained for each test compound are expressed relative to those obtained for ascorbic acid taken as reference. The 50% inhibition concentration ($IC_{50}$) was calculated from the percent inhibition graph as a function of the concentration of the test product.

**Antimicrobial activity**

***Preparation of the inoculum***

The inoculum was prepared from an microbial culture of 18 to 24 hour, grown on a solid medium, nutrient agar for bacteria and Sabouraud agar for the yeast tested, previously incubated at 37 ° C. Colonies of the same morphology were suspended in sterile physiological saline of 9% NaCl to have a density equivalent to 0.5 Mc Farland standard (Anon 2015).

***Determination of the minimum inhibitory concentration (MIC)***

The evaluation of the MIC of the bacteria and of the yeast is carried out according to the microtiter technique on microplates following the protocol described by Ennacerie *et al*. (Ennacerie *et al*. 2017b) for the antibacterial activity and that of Thirach *et al*. (Thirach *et al*. 2003) concerning antifungal activity. The number of repetitions is three times for each test.

***Determination of the minimum bactericidal and fungicidal (MBC) and (MFC) concentration***

Using a loop calibrated at 2µl, the contents of the wells, in which no growth was observed, were collected and seeded on an agar (Muller Hinton, Sabouraud), starting with the well of the MIC. The lowest concentration of extracts tested, which resulted in absence of colonies, was recorded as MBC or MFC (Thirach *et al*. 2003); (Ennacerie *et al*. 2017b).

**Measurement of optical density of cytoplasmic absorbing components at 260 nm evacuated by treated germs**

***Preparation of washed germs***

From a stock of *Staphylococcus aureus* and *E. coli* bacteria, stored at -30 °C in glycerol (20%), and a 24hours agar culture of *C. albicans*, a washed microbial suspension is prepared following the method described by Rhayour *et al*. (Rhayour *et al*. 2003).

***Operating mode***

The measurement the release of the components of the absorbent cellular contents at 260 nm, from the germs treated with the two extracts was established for 4 different concentrations, 0.25, 1, 4 and 10 mg / ml according to the protocol described by Rhayour *et al*. (Rhayour *et al*. 2003).

## 3. Results and discussion

**Determination of polyphenols in *D. viscosa* extracts**

**Table 1.** Content of *D. viscosa* extracts of total polyphenols (mg EGA / g dry matter)

| Extracts | Total polyphenols (mg Equivalent of Gallic Acid / g dry matter) |
|---|---|
| **Ethanolic extract** | 69.40 |
| **Decoction 10%** | 24.64 |

The results in **Table 1** show that the alcoholic extract is richer in total polyphenols compared to the decoction. The comparison of the contents of the polyphenol of *D. viscosa* extracts with those of other studies has shown, on the one hand, that of the aqueous extract can be moderately important than that of the methanolic extract which are respectively 57.3 and 43.9 mg. EGA / g dry weight (Alali *et al*. 2007). On the other hand, the concentration of these compounds differs according to the collection zone and the part of the plant used. In fact, the leafy stems of *D. viscosa* collected from Portugal by Albano and his colleagues (Albano *et al*. 2012) are 14.3 mg / ml. Salim *et al*. (Salim *et al*. 2017) and Side Larbi *et al*. (Side Larbi *et al*. 2016) found interesting levels of total phenols in Palestinian and Algerian *D. viscosa* stems of 97.6 and 174.51 mg EGA / g dry extract, respectively. While, Chahmi et al. (Chahmi *et al*. 2015) found a higher level of about 274 mg EGA / g in the whole plant of *D. viscosa* harvested from Safrou in Morocco.

Hence, these results remain relative and are only an estimate of the polyphenol content. Because, firstly, the Folin-Ciocalteu reagent has a major disadvantage to the colorimetric assay because of its high sensitivity. It can cause a reduction of all the hydroxyl groups present in the solution and not only those of the phenolic compounds.

Since it also acts as a reducer of certain sugars and proteins, which sometimes gives rise to an increase in phenolic content (Side Larbi *et al*. 2016). Secondly, the difference in the parts of the plant studied, giving a different distribution in secondary metabolites, may be related to climatic conditions (high temperature, solar exposure, drought, salinity). These factors are responsible for the stimulation of the biosynthesis of secondary metabolites such as polyphenols (Fadili *et al*. 2017). Thirdly, the difference in standard used for the determination of polyphenols, or the different treatment methods, are probably responsible for this difference in values. However, in general, the Moroccan *D. viscosa* plant has remarkably interesting polyphenol content.

**Antioxidant activity of *D. viscosa* extracts**

Evaluation of the antioxidant activity of the two extracts of the *D. viscosa* plant by the FRAP method revealed that ascorbic acid had the highest antioxidant activity at low concentration relative to both extracts. Therefore, that at 0.5 mg / ml the absorbance reached 1.829 for the reference and 0.55 for the two extracts. However, at 1 mg / ml ascorbic acid reached the plateau, which is not the case for the extracts tested. However, from 4 mg / ml, the absorbance of the two alcoholic and aqueous extracts of the leafy stems of *D. viscosa* have a very interesting antioxidant activity, which exceeds that of ascorbic acid **(Fig. 1).**

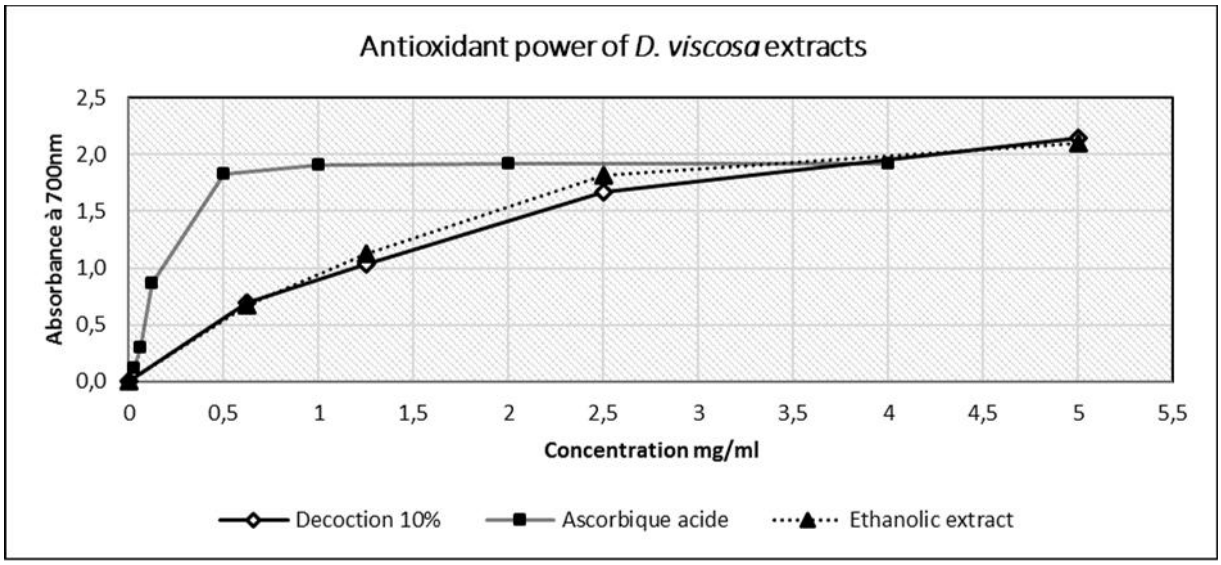


**Figure 1.** Antioxidant power of *D. viscosa* extracts as a function of the concentration expressed in mg / ml

It also appears that the antioxidant power is almost equal for the two extracts, with a small positive difference for the ethanolic extract.

The result found for the 10% decoction is in agreement with that found by Albano *et al.* (Albano *et al.* 2012), who worked on the aerial part of *D. viscosa* collected from Portugal, whose $IC_{50}$ was 1.011 mg / ml, which confirms this activity. As Alali *et al.* (Alali *et al.* 2007) evaluated the antiradical power of the two aqueous and methanolic extracts of this species collected from Jordan, they found an important effect for the aqueous extract as for the alcohol extract respectively 269.8 and 247.8 µmol TE / g dry weight.

The second method for evaluating the antioxidant capacity of the two extracts of this plant is DPPH, the results of which are presented in **Table 2,** grouping the $IC_{50}$ values of each product.

**Table 2.** $IC_{50}$ extracts of *D. viscosa*

| **Products** | **$IC_{50}$ (mg/ml)** |
|---|---|
| **Ascorbic acid** | 3,55 |
| **Ethanolic extract** | 0,09 |
| **Decoction 10%** | 0,19 |

The results show that the ethanolic extract has the strongest antiradical activity with an $IC_{50}$ of 0.09 mg / ml, which is almost double that of the decoction whose $IC_{50}$ is 0.19 mg / ml. As for

the reference, which is ascorbic acid, it has the lowest activity. Therefore, this plant is described as a stronger antioxidant activity compared to that of ascorbic acid.

This results confirm those found by the FRAP method. Similarly, the ethanolic extract had a more pronounced antioxidant effect. Similarly, Chahmi *et al*. (Chahmi *et al*. 2015) confirmed this result for the thanolic extract of *D. viscosa* harvested from Taounat in morocco. This antiradical power can be explained, on the one hand, by the high content of polyphenol revealed in the alcoholic extract especially. On the other hand, by the richness of all the aerial part of *D. viscosa* in phenolic compounds especially the flavonoids (Salim *et al*. 2017).
In addition, several previous studies have reported the positive correlation between total phenols and free radical scavenging (Alali *et al*. 2007).

**Antimicrobial activity of *D. viscosa* extracts**

***Bacterial resistance profile***

The resistance profile of the two bacteria tested against antibiotics revealed a resistance to some antibiotics prescribed for antibiotic therapy (**Table 3**). *E. coli*, which was sensitive to amoxicillin (AMX), decreased its sensitivity over time (Moukrad *et al*. 2012) and acquired multiple resistance. It became resistant to the tested antibiotics belonging to Beta-lactams, Aminoglycosides, Sulfamides and Glycopeptides. *Staphylococcus aureus* also selected for this study is resistant to the antibiotics belonging to Fluoroquinolones, Sulfamide and Macrolide.

**Table 3.** Antibiotic resistance profile of tested bacteria

| Disc of antibiotic | Disc content µg | *Staphylococcus aureus* | *E. coli* |
|---|---|---|---|
| **Amoxicilline** | 25 | - | Resistant |
| **Amoxicilline + Acideclavulanique** | 20/10 | - | Resistant |
| **Ceftriaxone (C3G)** | 30 | Sensitive | - |
| **Ceftazidime (C3G)** | 30 | - | Sensitive |
| **Oxacilline** | 5 | Resistant | - |
| **Gentamycine** | 15 | Sensitive | Resistant |
| **Ciprofloxacine** | 5 | Resistant | Sensitive |
| **Ofloxacine** | 5 | Resistant | Sensitive |
| **Sulphamethoxazole + Trimethoprime** | 1,25/23,75 | Resistant | Resistant |
| **Doxycycline** | 30 | Sensitive | Resistant |
| **Erythromycine** | 15 | Resistant | - |

***Antibacterial activity***

The evaluation of the antibacterial effect of the two aqueous and ethanolic extracts, prepared from the leafy stems of *D. viscosa*, on the two strains tested **(Table 4)** shows that the MICs are 0.28 ± 0.05 and 2.29 ± 0.05 mg / ml for the alcoholic extract respectively for *E. coli* and *Staphylococcus aureus*. In addition, 33.33 ± 0.00 mg / ml for the decoction for the two bacteria mentioned above. It should be noted that Gram-negative is more vulnerable to alcoholic extract compared to Gram positive and that the alcoholic extract has a broad-spectrum bactericidal activity and that the decoction is rather bacteriostatic **(Table 4).**

**Table 4.** Antibacterial Activity Evaluated by Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) mg / ml of extracts of leafy stems of *D. viscosa*

| | | ***E. coli*** | ***Staphylococcus aureus*** |
|---|---|---|---|
| **Ethanolic extract** | **MIC(mg/ml)** | 0,28 ±0,05 | 2,290±0.20 |
| | **MBC (mg/ml)** | 1,198±0,07 | 3,935±0,912 |
| **Decoction 10%** | **MIC (mg/ml)** | 33.33±0.00 | 33.33±0.00 |
| | **MBC (mg/ml)** | - | - |

The comparison of the results of the antibacterial activity with those of other studies shows that the ethanolic extract of the leaf stem of Moroccan *D. viscosa* has a strong inhibition on *E. coli* compared to that collected from Palestine whose MIC is 10 mg/ ml (Salim *et al*. 2017). On the other hand a concordance was found between our results and those found by Rhimi *et al*. (Rhimi *et al*. 2017), showing a more pronounced efficiency of the ethanolic extract on Gram-negative bacteria.

The sensitivity of the latter is related to the characteristics of their membranes and their permeability to bioactive molecules. However, the work done by Laghrifi *et al*. (Laghrifi *et al*. 2013) on *D. viscosa* collected from Ain Taoujtat in Morocco, revealed a bactericidal effect on all tested germs (*Staphylococcus aureus, E. coli, Klebsiella pneumoniae, Salmonella paratyphi, Proteus vulgaris* ...). This observed difference between the results of the two different extracts of the same plant or the same extract of the same species but coming from two different regions, can be explained on the one hand, by the variation of the chemical composition of the extracts, related to the type of the solvent used and the extraction technique. On the other hand, the effect of edaphic and climatic factors as well as the drying

technique. It is also reported that despite the low richness of active molecules or their low levels, their synergy may be responsible for a strong antibacterial activity (Rhayour *et al.* 2003).

***Antifungal activity of D. viscosa extracts***

The ethanolic extracts has an antifungal activity on yeast *C. albicans*, the MIC and MFC are of the order of 33.33 ± 0.33 mg / ml compared to the decoction which has a MIC of 66.66 ± 1.89 and it is non-fungicide (**Table 5**).

**Table 5**. Antifungal activity evaluated by Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) mg / ml of extracts of *D. viscosa*

| Extract | MIC (mg/ml) | MFC (mg/ml) | CMF/CMI |
|---|---|---|---|
| **Ethanolic extract** | 33.33±0.33 | 33.33±0.33 | 1 |
| **Decoction 10%** | 66.66±1.89 | - | - |

The comparison of antifungal results with those of Harti *et al.* (Harti *et al*., 2019), which evaluated the antifungal effect of the ethanolic extract of *D. viscosa* leaves of Morocco on *C. albicans*, confirms our result. They found a MIC of 125 µg / ml. Likewise, Ali-Shtayeh and Abu Ghdeib (Ali-Shtayeh and Abu Ghdeib, 1999), show that they recorded lower MICs for aqueous extracts prepared from the whole plant harvested from Palestine. They vary from 5 to 20 µg / ml, on three dermatophytes (*Microsporum canis, Trichophyton mentagrophytes and Trichophyton violaceum).* Regarding the type of antifungal effect, Bensegueni-Tounsi (Bensegueni-Tounsi 2001) revealed a fungistatic action against *C. albicans*. While Maoz and Neeman (Maoz and Neeman 1998), they showed in their work testing the aqueous extract that it has a fungicidal effect.

The difference in the antifungal potential of the extracts from the different studies can be attributed to the variation in the nature and / or concentration of the chemical inhibitors present in each species. As well as their relative solubility in water or in another solvent namely that methanol-water is a polar solvent that can extract alkaloids, flavonoids, glycosides, tannins, salts, which are known by their biological activity (Bensegueni-Tounsi 2001).

In conclusion, it is found that there is a positive correlation between the polyphenol content and the antimicrobial activity and that the alcoholic extract is richer and more active than the

decoction. This correlation confirms the biological properties that characterize phenols in general.

**Study of the mode of action of antibacterial compounds of *D. viscosa* extracts**

***Treatment of bacteria and estimation of cytosolic constituents released in the supernatant***

With the aim of highlighting the action of the aqueous and alcoholic extracts of *D. viscosa*, on the two bacterial strains *Staphylococcus aureus* and *E. coli* of which they are sensitive. Quantification of cell lysis by measuring the absorbent cellular content at 260 nm, after treatment with increasing concentrations of the extracts, is shown in **Figures 2A and B**.

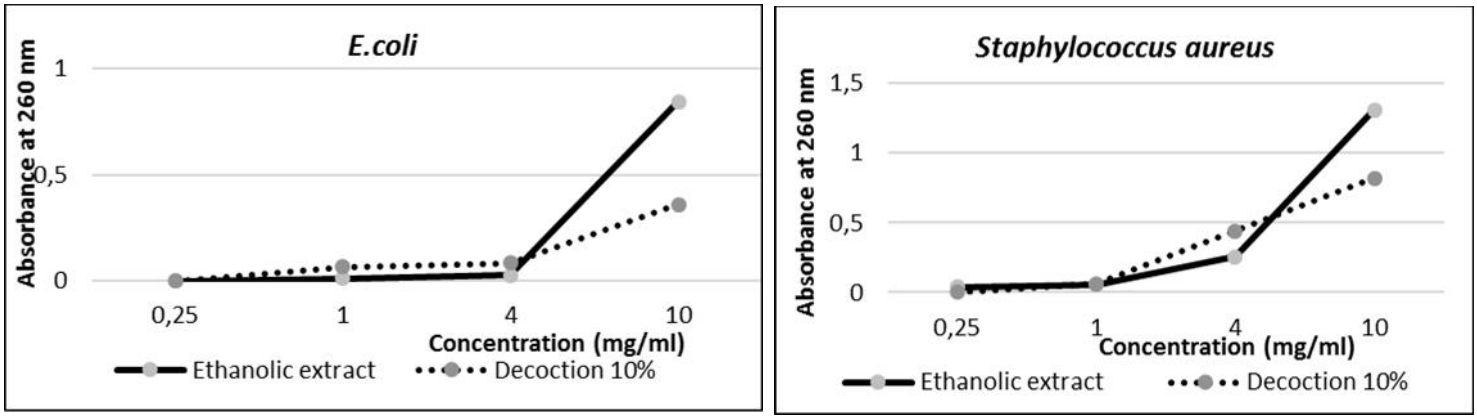


**Figure 2.** Absorbance of the 260 nm cellular content of *E. coli* (A) and *Staphylococcus aureus* (B) under the effect of different concentrations of *D. viscosa* extracts

The results shown in **Figure 2A** revealedhat lysis of *E. coli* bacterial cells starts from the 4mg/ml concentration for both types of extracts. However, at a concentration of 10 mg / ml, a strong lysis especially for the ethanolic extract was observed. Concerning the *Staphylococcus aureus* strain (**Figure 2B**), the release of its cellular content is generally comparable for the two types of extract and begins at lower concentrations than in the previous case. However, at 10 mg / ml, the lysis of the bacteria is more important for the alcoholic extract.

Taking into account the MIC values of the two bacteria tested *E. coli* and *Staphylococcus aureus*, we observe a higher efficiency of the ethanol extract in Gram-negative bacteria than that observed in Gram Positive. We can therefore suggest that the wall structure of both types of bacteria is involved in the difference in lysis concentration for both types of cells.

The abundance of lipids in the negative Gram and the existence of an outer membrane, in addition to the peptidoglycan layer, in their wall seem to be at the origin of a concentration of the higher extracts inducing their lysis. It is also logical to explain the regressive relationship

between the MIC and the lysis concentration of gram-negative bacteria by the presence of other action sites sensitive to the active principles of the extracts of this plant in parallel with the wall as another target (Rhayour *et al*. 2003).

Through this test, we have tried to highlight the output of the cytoplasmic components with an absorbance at 260 nm, which are generally relate to the bacterial DNA, these nucleic acids can escape from the cell only during a perforation of the bacterial wall, following a destruction of its structure and an attack of its integrity.

Referring to the bibliographic data, the difference in the mode of action of the extracts on the bacteria depends on two main parameters. Firstly, the chemical composition of the extract that also depends on several factors. Secondly, the bacterial species that has structural and morphological characteristics, which distinguish. Indeed, previous studies have shown that phenols are classify among the components acting on the permeability barrier of the cytoplasmic membrane. These phenols cause leaks of various substances, such as ions, ATP, nucleic acids and amino acids such as glutamate … (Lambert *et al*. 2001). As a result, leakage of nucleic acids and proteins could lead to protein synthesis and DNA-based material disturbance, as well as inhibition of bacterial growth.

***Treatment of C. albicans and estimation of cytosolic constituents released in the supernatant***

Cell lysis of *C. albicans* yeast treated with increasing concentrations of the aqueous and alcoholic extract of *D. viscosa* is quantified by measuring the absorbance at 260 nm of the supernatant separated from the cell pellet **(Figure 3).**

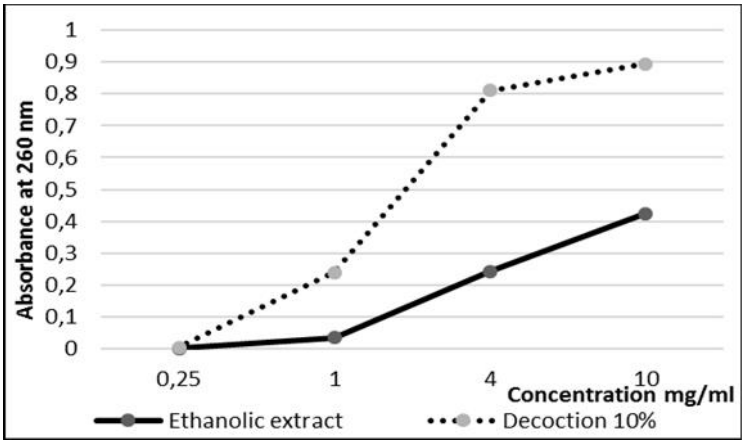


**Figure 3.** Absorbance at 260 nm of the cellular content evacuated from *C. albicans* under the effect of different concentrations of *D. viscosa* extracts

Concerning the *D. viscosa* plant, its two extracts caused cell lysis in *C. albicans* with lower concentrations than those observed in bacteria and a more remarkable lytic activity for the decoction. In fact, the decoction at 10% affects the yeast membrane more than the ethanolic extract.

Taking into account the results found and referring to the antifungal activity, it appears that the alcoholic extract has a lower MIC. This behavior of the particular *Candida albicans* membrane is most likely related to the different nature of bacterial and fungal membrane envelope composition, as well as that of the wall of this yeast during its different growth phases (Goyal and Khuller 1992; Mishra *et al*. 1992).

The cell wall of *Candida albicans* acts as an impermeable membrane and maintains morphological characteristics. It is an essential component for many biological and pathogenic aspects of yeast. This external cellular structure allows the first physical interactions between the microorganism and its environment. In addition, other yeast components such as nucleic acids and proteins, which reside throughout the interior of the cell and cytoplasm, are the key structural components.

In view of this primordial role played by the wall, the researchers were interested in the stability of this subcellular structure and which is one of the targets chosen in this study for the evaluation of the antifungal effect of the extracts.

The results of this study showed that both extracts caused lysis of the yeast and release of its cellular content. Regarding the studies that treat the mode of action of extracts on fungi and yeasts, they remain limited, most of the time who is the targets of essential oils and their major components. However, in general, the antifungal activities revealed in medicinal plants are due to the composition of secondary metabolites of their extracts. Indeed, flavonoids, alkaloids, tannins, phenols, saponins, glucosides, terpenes and anthraquinones are the main secondary metabolites involved in the antifungal activities of plant extracts (Kuete and Efferth 2010). These compounds act on microorganisms by various mechanisms. Tannins bind to proline-rich proteins and interfere with protein synthesis in yeast (Shimada 2006). While flavonoids that are hydroxylated phenols are synthesize in the plant in response to microbial infection, they act by forming insoluble complexes with the cell wall proteins, weakening it (Marjorie 1999). As for saponins, they cause the lysis of certain membrane and plasma proteins (Zablotowicz *et al*. 1996). For steroids, they associate with membrane lipids and cause lysis of liposomes (Raquel 2007).

Similarly, referring to the results of pre-established phytochemical studies of *D. viscosa* plant, we note the richness of this species in secondary metabolites targeting the structure of the membrane and the wall such as saponins, flavonoids, triterpenes and steroids (Salim *et al*. 2017) hence the interesting activity of this species. Willem confirmed the lytic effect of flavonoids causing disruption of cell membranes and lysis of pathogens, by evaluating grapefruit seed extract rich in bioflavonoids (mainly naringine) (Willem 2014).

At the end of this study, the antibacterial and antifungal activity of the extracts studied, attributes to this plant a very broad antioxidant and antimicrobial therapeutic effect.

## 4. Conclusion

*Dittrichia viscosa* is a medicinal plant with an impressive traditional antibio-therapeutic use due to its antioxidant, antimicrobial and antifungal activities and its high content of polyphenols. However, a toxicity study will be essential to ensure patient safety during treatments prescribed by traditional healers.

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