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Antibacterial, antifungal and antioxidant activity of total polyphenols of *Withania frutescens.L*

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ABSTRACT

Our objective in this work is evaluated the antibacterial, antifungal and antioxidant activity of the phytochymic compounds of the roots and leaves of a species *Withania frutescens*. In the first part, the phenolic compound is determinate by the Folin-Ciocalteau reaction, the richness of the roots in polyphenols (53.33 ± 1.20 mg EGA/g Extract) is six times higher than that of the leaves. The antioxidant test is evaluated by four methods: DPPH test, reducing power test (FRAP), total antioxidant capacity (CAT) and the ß-carotene discoloration test. The IC-50 values of the DPPH test of the studied parts are of the order of 0.36 µg/ml and 6.63 µg/ml, which showed a lower anti-free radical activity than that of BHT (0.12 µg/ml). The results obtained by the FRAP method revealed a low reducing power of iron for two extracts (EC-50 of 0.45%) compared to Quercetine (EC-50 of 0.03%). The compounds of root and leaf extracts have a significant total antioxidant capacity, respectively 477.65 ± 37.60 and 317.03 ± 46.64 mg EAA/g Extract. In the β-carotene discoloration test, extracts from the aerial and underground parts showed antioxidant activity of 57% followed by (36%), respectively. The evaluation of the antibacterial activity of in vitro extracts against microorganisms is carried out by two methods: disc diffusion and microdilution. The results show that the extracts exert an intermediate inhibitory effect (inhibition diameter between 8 and 15 mm, the smallest MIC obtained is 2.80 mg/ml) on all strains tested. The antifungal activity was estimated by determining the growth inhibition rate of the fungus tested. Indeed, the compounds studied exhibit a good antifungal effect since the minimum inhibitory concentration (MIC) of 4.5 mg/ml for root extract and 9 mg/ml for leaf extract.

1. Introduction

Superior plants are synthesized by complex metabolic pathways, many compounds that they use for various adaptive functions, to respond to the biotic and abiotic stresses that these plants may experience. They contain a diversity of phytochemical compounds (Coumarins, terpenes, polyphenols, tannins, alkaloids, mucilages…) [1], with very different molecular properties and biological activities (Antimicrobial, antioxidant…). Plants are an important reservoir of bioactive molecules, which is why the application of scientific research to seek new naturally occurring drugs must involve the inventory of plants and the systematic evaluation of their biological activity. It is with this in mind that this work aims to contribute to the enhancement of the natural plant heritage in order to develop new products with high added value. To achieve this objective, we have focused on medicinal plants in the arid and semi-arid zone whose therapeutic properties have been proven by the use in traditional medicine by the indigenous population. Our objective is therefore to try to establish links between the qualities and possibilities granted to plants used by indigenous populations and the scientific realities revealed by biological evaluation. The choice of Withania frutescens was guided by traditional use (against poisoning, gastric ulceration…), known by the indigenous population and the wealth of secondary metabolites (polyphenols, tannins…) [2]. We will also present general information on plant extracts and the main biological activities mentioned in this study will be discussed, in this case antibacterial, antifungal and antioxidant activities, as well as

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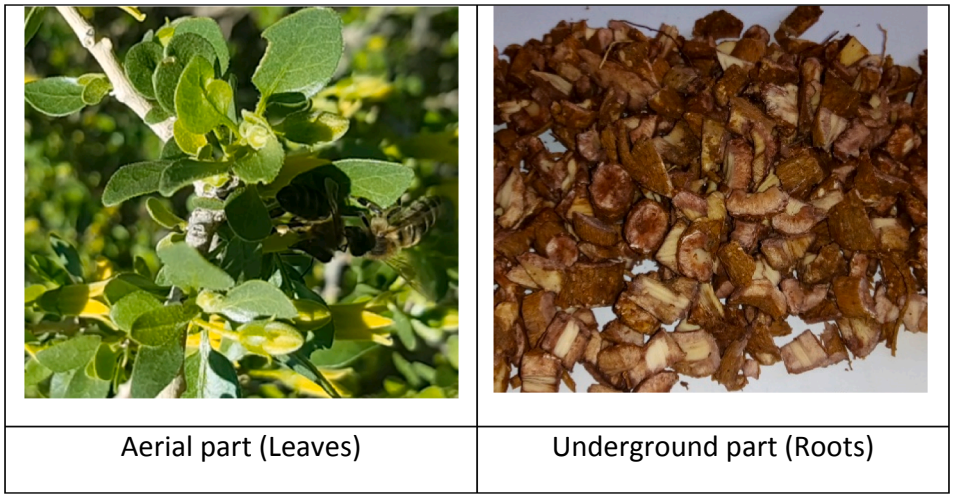

Fig. 1. The studied parts of the selected plant; Withania frutescens.

complete our study on this plant [3].

2. Material and methods

2.1. Plant material

The plant material selected in this work includes the underground and aerial parts of *Withania frutescens.L*, the roots and leaves were collected from a shrub in the province of Sefrou, Morocco. For the sampling period, spring extends from March to April (2018), as this is the season when development and flowering are at their peak, Fig. 1. After harvesting, we clean the plant material by washing it with water to ensure proper conservation of the plant. It was dried at 35 °C, stored in a sheltered place for a few days before being crushed by an electric crusher and stored in boxes. Hydro-ethanolic extracts were prepared by the method of maceration of vegetable powder at room temperature using 70% ethanol and 30% distilled water at a rate of 10% (mass/volume) for 24 h.

2.2. Determination of total polyphenols

500 µl of Folin reagent (1/10) is added to 100 µl of diluted sample. After 4 min, 400 µl sodium carbonate (75 mg/ml) is added to the reaction medium. After 2 h of incubation at laboratory temperature, the optics density is measured by the spectrophotometer at 760 nm. The concentration of total polyphenols is calculated by the equation of the calibration range established with gallic acid, it is expressed in milligram gallic acid equivalent of one milligram extract (mg EAG/mg E) [4].

2.3. Antioxidant activity

The antioxidant potency of two extracts tested was evaluated in vitro using four tests, the DPPH test, reducing potency test (FRAP), total antioxidant potency test (CAT) and the ß-carotene discoloration test

2.3.1. Scavenging of the free radical DPPH

The DPPH test is performed using the method described by Bekta.S in 2005 [5]. Where 100 µl of each methanol solution of the tested extracts are mixed with 750 µl DPPH in methanol (0.004%). After incubation at laboratory temperature for 30 min, the optics density is measured at 517 nm. For the negative control the sample is replaced by methanol. The percentage inhibition of DPPH is determined by the following equation:

$$PI(\%) = (A_0 - A/A_0) * 100$$

- PI: Percentage of inhibition.
- A_0: The optics density of the free radical (DPPH) solution in the absence of the extract (negative control).
- A: Absorbance of the free radical (DPPH) solution in the presence of the extract.

2.3.2. Reducing power test

This test was performed using the Moattar method [6]; 500 µl of phosphate buffer (0.2 M; pH = 6.6) and 500 µl of potassium ferricyanide [$K_3Fe(CN)_6$] (1%) are added to 100 µl of different concentrations of the samples prepared in methanol. After incubation for 20 min in the water bath at 50 °C; 500 µl of a 10% aqueous solution of TCA, 100 µl FeCl3 (0.1%) and 0.5 ml of distilled water are added to the reaction medium. Then the absorbance is determined at 700 nm against a blank containing all the reagents in the absence of the tested sample. The results are expressed in effective concentration at 50% (EC-50) which reflects the antioxidant concentration required to obtain an absorbance of 0.5 nm.

2.3.3. Total antioxidant capacity test

25 µl of every extract studied was mixed in one ml of liquid reactive solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After incubation in a temperature of 95 °C for 90 min, the optical density was measured by the spectrophotometer at 695 nm with a blank containing 25 µl of methanol instead of the extract [7]. The antioxidant capacity was expressed in milligrams of ascorbic acid equivalent per gram of extracts (Mg EAA/g extracts) from an ascorbic acid standard curve.

2.3.4. Test de décoloration de ß-carotène :

One ml liquid solution of ß-carotene in chloroform (1 mg/5 ml) was introduced into a vial, which contains 0.01 ml of linoleic acid and 100 ml of Tween 80. The chloroform was evaporated at 45 °C for 5 min. 25 ml of oxygenated water (H_2O_2) was added to the residue [8]. To 2.5 ml of the previous mixture, 100 µl of each of the sample diluted in methanol were added to tubes. After incubation of the tubes in a water at 50 °C for 120 min, the optical density was measured in 470 nm. The determination of the percentage of antioxidant activity was measured by the equation [9]:

$$AA\% = (A_E/A_{BHT}) * 100$$

- AA%: percentage of antioxidant activity.
- A_E: absorbance after 120 min with negative control.
- A_{BHT}: the absorbance after 120 min of BHT.

A blank containing the stock solution without β-carotene was used to bring the spectrophotometer to zero. The percentage of antioxidant activity was compared with the BHT standard which was used under the same conditions as the extracts.

2.4. Antibacterial activity

To test the antibacterial activity of our extracts, five strains were chosen, namely Staphylococcus aureus, Escherichia coli, Acinetobacter baumanii, Klebsiella pneumoniae and Streptococcus pneumoniae, which were provided by the microbiology analysis laboratory of the Centre Hospitalier of Fez-Morocco. These bacteria are often responsible for nosocomial infections, which are a major public health problem. The emergence of multi-resistant bacteria in these bacteria is one of the main causes of failure in healthcare treatments. The different strains were preserved under nutrient agar (NG) and the study of the sensitivity of bacteria in solid media to antibiotics as well as extracts was conducted on Mueller-Hinton agar (MHB). The antibiotics used in our study are: kanamycin, Oxacillin, Ceftizoxime, and Streptomycin, at a load of 10 μg/disk. We chose these antibiotics because they have a wide spectrum of action, allowing us to determine the sensitivity of the entire range of bacteria studied to these antibiotics. Both root and leaf extracts are tested on the previously selected bacterial strains, on the one hand in the raw state (for the disc method) and on the other hand in the diluted state in 0.2% agar (for the determination of the MIC). The bacterial strains are enriched in a tube containing 9 ml (MHB) at 37 °C for 18–24 h. Using a platinum loop, a drop of the culture is sown on a petri dish containing GN, with incubation at 37 °C for 18–24 h. From these pure cultures, the bacterial suspension (inoculum) is prepared: Three to five well isolated and perfectly identical colonies, the platinum loop is discharged into 10 ml of 0.9% NaCl sterile physiological water. After homogenization of the bacterial suspension using a vortex, standardization of the optical density at 0.5 Mc Farland is performed by spectrophotometer set to a wavelength of 625 nm. The optical density obtained must be between 0.08 and 0.1 nm, which corresponds to a concentration of 10^7 to 10^8 CFU/ml according to Mc Farland.

2.4.1. Evaluation of antibacterial activity

The technique used is that of disk diffusion in agar medium or disk method [10]. It is a method of qualitative in vitro evaluation of the antibacterial power of extracts. For this purpose, front discs with a diameter of 0.6 cm prepared from Whatman paper number 1 were impregnated with extract and deposited on the surface of a agar medium previously sown on the surface with the aid of a bacterial suspension. After incubation, the effect of the antimicrobial agent on the target is assessed by measuring the diameter of the free area around the disc in millimetres. Depending on the diameter of the inhibit zone, the strains studied can be classified as susceptible (S) or resistant (R) strains. For each of the discs deposited, the inhibition diameter is calculated by a graduated rule and the classification of strains sensitive to the extracts studied is based on the diameter of the inhibition zone (DI) and according to the following criteria:

- Not sensitive (−) for DI is less than 8 mm
- Sensitive (+) for 9 ≤ DI ≤ 14 mm
- Very sensitive (++) for 15 ≤ DI ≤ 19 mm
- Extremely sensitive (++++) for DI is higher than or equal to 20 mm

2.4.2. Minimum inhibitory concentration (MIC)

The MICs of Withania frutescens extracts against the bacterial strains tested are determined by the microdilution method [11]: 200 μl of the extract stock solution (1 mg/ml) are added to the wells in the first column, and the remaining wells are filled with 100 μl MHB and then 100 μl of each dilution is transferred to the 10 consecutive wells. Finally, 50 μl of the previously prepared inoculum is added to the microplate wells except for the first column wells (with one strain per microplate line). The final column containing 100 μl of MHB and 50 μl of inoculum is considered a negative control with a final volume of 150 μl in each well. After incubation at 37 °C for 18 h, bacterial growth is detected by the presence of a white "pellet" in the wells and as an indication of the growth of microorganisms with 10 μl TTC (5 mg/ml) is added to the wells before incubation in 37 °C for 30 min. The development of a colour indicates the presence of biologically active organisms (viable bacteria).

2.5. Antifungal activity

The fungal strain used is *Fusarium sp*. provided by the Biotechnology Laboratory Faculty of Sciences Fez-Morocco. The methodology used to evaluate the antifungal effect of the two extracts from the aerial part and the underground part of the species studied is the direct contact method that allows the antifungal activity of the extracts studied to be detected. Each extract tested is incorporated at varying concentrations into the agar culture medium. The results give the MIC, which is defined as the low concentration of extracts at which fungal growth is not observed with the naked eye.

2.5.1. Preparation of the fungal suspension

The spores from the new cultures (5-day cultures) of the strain are recovered after scraping the surface of the petri plates, in a volume of 3 ml of sterile physiological water. From this suspension, the spores are counted using a Mallassez cell and a microscope. Then the optical density (OD) of the fungal suspension is measured by a spectrophotometer at 630 nm, in order to determine the concentration of the spore suspension at 10^7 spores/ml. An optical density of 0.04 is estimated to correspond to a concentration of 10^7 spores/ml.

2.5.2. Preparation of culture medium containing extract concentrations

Considering the immiscibility of the extracts studied, the emulsion of these two extracts was made by Agar 0.2% to obtain the homogeneity of the compounds dispersed in the culture medium [12]. Media of different concentrations (v/v) of

1/100; 1/200; 1/400; 1/800; 1/1600; 1/3200

in extracts with Agar 0.2%, are incorporated into the potato dextrose agar medium, after autoclaving at 121 °C, during 20 min.

2.5.3. Seeding and incubation of petri plates

Using a propette, 10 μl as an inoculum spot in each medium except for the negative control which contains only the medium alone. The Petri boxes were incubated at a temperature of 27 °C for 6 days. For each concentration, the replicates are prepared three times in the same way.

2.5.4. Parameters studied for the evaluation of mycelial growth

In this study, the influence of the two plant extracts on mycelial growth was compared in order to estimate the evolution of mycelial growth that is performed daily, by measuring the diameter of the mycelial colony of the fungus. This reading is always made in comparison with those of the controls, which are conducted under the same test conditions.

2.5.4.1. Inhibition rate (TI %). Growth inhibition rates in relation to the control are calculated according to the following formula:

$$TI(\%) = 5[(dc - dE)/dc] * 100$$

- TI (%) = Inhibition rate expressed as a percentage.
- dC = diameter of the colony in Petri dishes - initial disc diameter (mm).
- dE = diameter of the colony in the petri dishes in the medium

containing samples - initial diameter of the disc (mm).
- ddi = initial diameter of the disc used (06 mm).

2.5.4.2. Minimum inhibitory concentrations (MIC). MIC is the lowest concentration of extracts for which growth visible to the naked eye on the solid medium is not observed [13]. It therefore measures a fungistatic effect and does not provide information on the state of the fungus population, in particular not allowing us to specify whether it has been killed in part or totally or whether it has only stopped multiplying. Petri dishes containing concentrations and showing the absence of mycelial growth have been selected to determine the minimum inhibitory concentration.

2.5.4.3. Mycelial growth speed (MGS). According to the method of Cahagnier [14] the rate of mycelial growth of each concentration is determined by the formula:

$$VC = Di1/T1 + (Di2 - Di1)/T2 + (Di3 - Di2)/T3 + \cdots + (Din - Din - 1)/Tn$$

- D = Diameter of the daily growth area (mm).
- Te = Incubation time (Day).

3. Results and discussion

3.1. Total polyphenol

Polyphenols were estimated by the Folin-Ciocalteu reagent, and the results obtained are in milligram gallic acid equivalent per gram of extract (mg EGA/g E), using the following equation of the linear regression of the calibration curve plotted for gallic acid: $Y = 11.325X + 0.0209$ and $R^2 = 0.9948$. The results show that the average total phenol content of the root extract is (53.33 ± 1.20 mg EGA/g extract) while that of the leaf extract is (12.04 ± 0.61 mg EGA/g extract). We recorded relatively high yields of crude extracts compared to those found by Alam [15] at different parts (leaves, roots and fruits) of the species Withania soumnifera, which shows the richness of the parts of the plant Withania frutescens in total polyphenols. The variability of polyphenolic content varies qualitatively and quantitatively from one plant to another, is probably due to climatic and environmental conditions (geographical area, drought, disease attack…), genetic make-up, harvesting period and stage of plant development, as well as the extraction technique and method and quantification can influence phenolic compounds [16,17].

3.2. Antioxidant activity

3.2.1. Scavenging of the free radical DPPH

The antioxidant activity of the root extract and foliar extract was tested by the trapping capacity of the free radical DPPH. The results obtained in the test to measure the percentage inhibition of the DPPH radical and the IC-50 are shown in Fig. 2. The CI-50 is related to the antioxidant capacity of a phytochemical compound, because it expresses the need for the antioxidant content necessary to reduce 50% of the concentration of the free radical.

Based on the results obtained, it is noted that the percentage of free radical inhibition for extracts is lower than that of BHT for all concentrations used. The anti-free radical activity of the root extract (IC-50 = 0.36 µg/ml) is higher compared to the other extract studied (IC-50 = 6.63 µg/ml), the latter remains lower compared to BHT (IC-50 = 0.12 µg/ml), whereas that of the root extract is closer to the antioxidant activity of BHT. The studies that were done by El bouzidi [18], on the same parts that we selected in this species, reported values higher than our results, which confirms that our extracts have an important antioxidant power.

3.2.2. Reducing power

According to these results in Fig. 3, the two extracts have significantly lower antioxidant activities than the reference (Quercitin). Thus, the effective concentration at 50% of the extracts studied seems to be the same. Metal ions are necessary for the functioning of cellular biochemical and physiological processes, but in some cases and when their mechanism of action is not well controlled, these same ions can cause lipid peroxidation, oxidative stress or tissue injury [19]. For the FRAP method, the revelation of the reducing power is based on the shift from the yellow colour of potassium ferrocyanide to a greenish blue colour whose intensity depends on the reducing power of each sample. The latter depends essentially on the quantity of gearboxes present in the test medium. This is reflected in the increase in absorbance which is measured at 700 nm.

The presence of phytochemical compounds in plant extracts reduces the Fe3+/ferricyanide complex to a ferrous. To this end, it can evaluate Fe2+ by calculating and monitoring the evolution of the blue color density in the reaction medium at 700 nm [20]. Many publications have shown that antioxidant activities have linked with the reducing power of the phytochemical components of certain plant species. The presence of hydroxyl compounds in the phytochemical compounds of plant extracts gives them a reducing power and they can be used as electron donors. For this reason, antioxidants are considered as oxidant reducers and inactivators. The plant extract autoxidation mechanism depends on certain parameters such as temperature, metal ion and polyphenol concentration, pH and the presence of complexing agents [21].

3.2.3. Total antioxidant capacity (TAC)

The standard used is ascorbic acid at different concentrations and the expression of total antioxidant capacity is in milligrams of ascorbic acid equivalent per gram of sample. (Mg EAA/g EXT). The antioxidant power rate of the extracts studied was obtained from an ascorbic acid calibration curve, Fig. 4.

The results show that the two extracts have different antioxidant activities, Table 1. The extract from the underground part of the plant studied has the best total antioxidant capacity of about 477.65 ± 37.60 mg/g, while the extract from the leaves has an activity of about 317.03 ± 46.61 mg/g.

3.2.4. Discoloration test of β-carotene

Decomposition of fatty acids is one of the causes of deterioration of some foods. The use of natural preservatives is important in the food industry, especially in inhibiting the oxidation of fatty acids by. In this test, β-carotene is used to inhibit the oxidation of linoleic acid.

We note that the estimate of β-carotene exists of the two extracts, BHT and negative control gradually decreases, this decrease is important at the beginning while after 100 min becomes very low. The decrease in negative control OD is the most important followed by leaf extract, root extract and finally BHT. This change in absorbance at different time intervals shows that our extracts appear to be effective as an oxidation inhibitor of linoleic acid (Fig. 5).

These results (Fig. 6) showed that the extracts studied and BHT inhibit the oxidation of linoleic acid compared to the negative control. It should be noted that oxidation inhibition was less important for leaves (57%) than roots (63%), so the antioxidant activity of both extracts remains less important than the antioxidant BHT used as a reference. The antioxidant test of the extracts is confirmed with the four methods used. The research in the bibliography found very large differences in views on the correlation of phenolic content with antioxidant potency, those who have pushed scientific research further to the molecular structure of phytochemical compounds to know the reaction mechanism of certain phytochemical compounds precisely the phenolic content. Some studies have shown a good correlation between IC50s and polyphenol and flavonoid content, while other studies have not found this correlation [22,23]. Moreover, antioxidant activity is positively correlated with the chemical structure of phytochemicals.

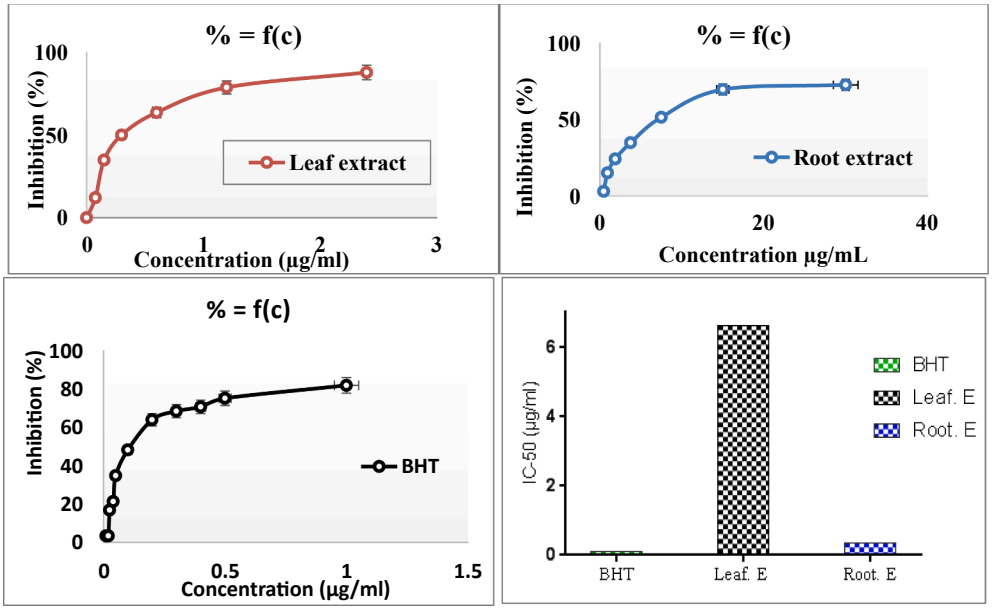

Fig. 2. Results of antioxidant power by the DPPH method for the two extracts studied and BHT.

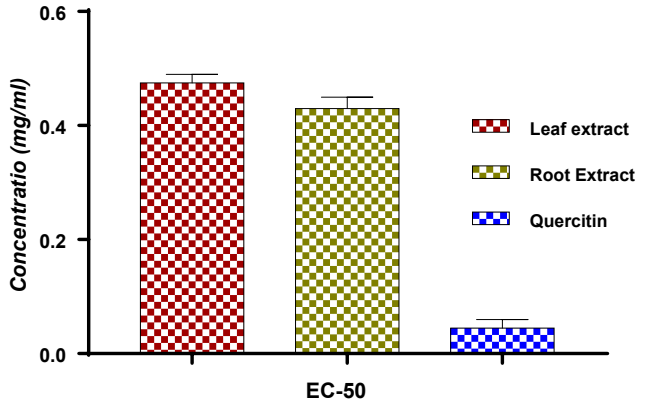

Fig. 3. Results in 50% effective concentration (EC-50) of extracts and quercitin using the FRAP method.

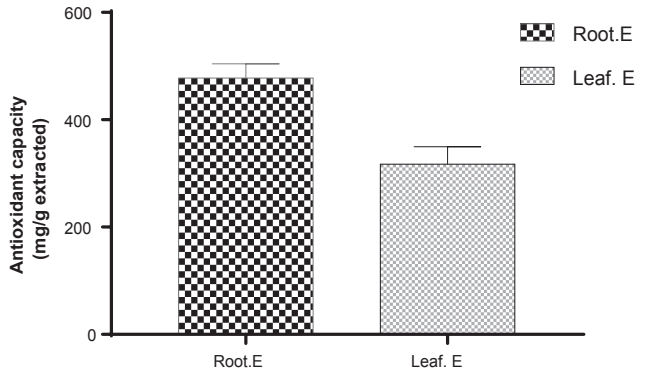

Fig. 4. The total antioxidant capacity of the two extracts.

Table 1
Results of total antioxidant activity.

	Total antioxidant capacity (mg/g extracted)
Root extract	477.65 ± 37.60
Leaf extract	317.03 ± 46.61

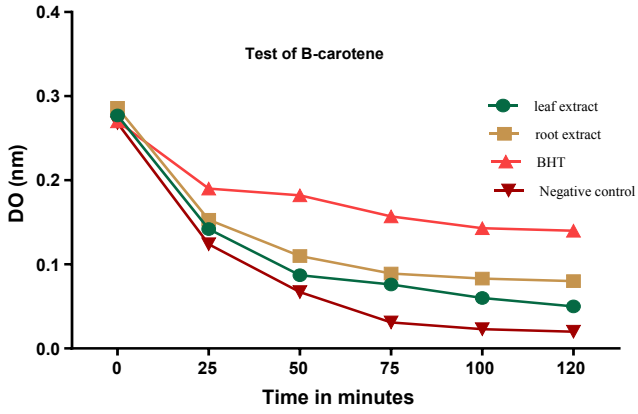

Fig. 5. Absorbance of β - carotene in the presence of the extracts studied, BHT and negative control.

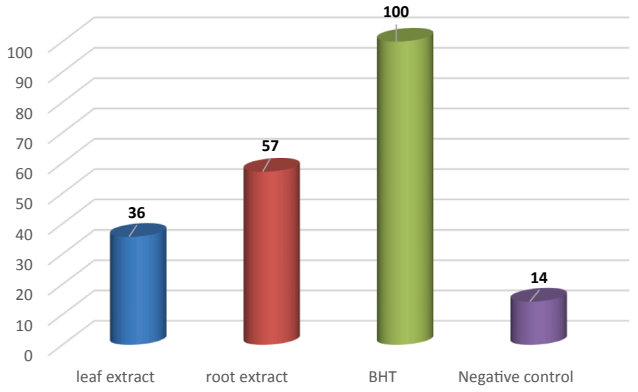

Fig. 6. Relative antioxidant activity of extracts, BHT and negative control.

Generally, Polyphenols have a high number of hydroxyl complex have high antioxidant activity because of their ability to give more free sites on molecules to trap free radicals [24,25]. This may partly explain why anti-free radical activity is dependent on the number, position and nature of substituents on rings B and C (hydroxyl, metaxyl, glycosylated groups) and the degree of polymerization. Thus, the antioxidant effect

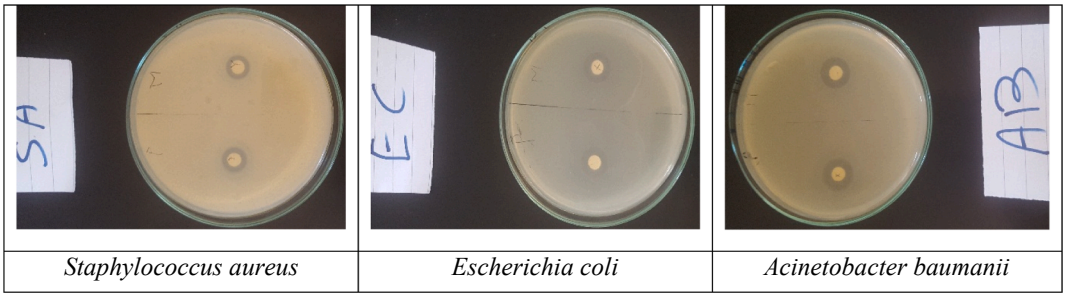

Fig. 7. Antibacterial activity of extracts studied by the solid medium disc method.

does not only depend on the dose but also on the structure [26].

3.3. Antibacterial activity

3.3.1. Qualitative evaluation by the disc method

The qualitative test of the antibacterial activity of the root and leaf samples against the bacteria tested was performed by the solid medium disc method, Fig. 7. The zones of inhibition of extracts and antibiotics are grouped in Table 2.

According to the values recorded in Table 2, the extracts have moderate antibacterial activity against all strains studied (diameter of the inhibition zones between 8 and 14 mm). The extract from the underground parts was more or less effective compared to the extract from the leaves. All strains were found to be sensitive to antibiotics tested with various diameters, with the exception of two strains, Acinetobacter baumanii and Streptococcus pneumoniae, which are shown to be resistant to Oxacillin.

3.3.2. Determination of MIC by microdilution in liquid media

The minimal inhibitory concentration of the extracts studied is in agreement with the results obtained in the preliminary test by the aromatogram method, i.e. the larger the diameter around the disc, the more interesting the MIC is and this is the case for A. baumanii which gives a MIC of 2.8 mg/ml for root extract, Fig. 8.

Extracts are evaluated with Gram-positive and other Gram-negative bacterial strains. The majority of studies on the action of phytochemicals against food decomposition organisms and foodborne pathogenic microorganisms agree that, in general, phytochemicals are more effective against bacterial strains that are Gram-positive than Gram-negative strains [27]. The low sensitivity of Gram-negative microorganisms to antibacterial agents could be explained by the fact that they have an outer membrane on a cell wall, which reduces the passage of hydrophobic molecules through its lipopolysaccharide coating. In addition, due to their lipophilic nature, some phytochemical compounds can easily cross cell walls and the cytoplasmic membrane, causing structural disorders of polysaccharides, fatty acids and phospholipids as well as their permeability [28].

3.4. Antifungal activity

3.4.1. Assessment of mycelial growth

At first, the mycelial growth of the fungal strain tested was normal (control), which is not in the presence of both extracts. This parameter changes over time and during incubation.

The assessment of the diameter of mycelial growth is shown in Fig. 9. It should be noted that the degree of efficacy of the extracts on mycelial growth of the fungal strain is observed up to a concentration of 2.3 for the underground part and 4.5 mg/ml for the aerial part. Beyond these two concentrations, no growth was observed. On the other hand, the lower concentrations than those mentioned above have a slight effect on the two samples studied compared to the control.

3.4.2. Mycelial growth kinetics

Mycelial growth of the fungal strain began after the first day of incubation in the concentrations of 0.2815–0.5625 and 1.125 mg/ml, which is marked by their controls, Fig. 10. For the concentration 2.25 mg/ml, the strain showed growth after four days, which explains why the extract has a fungistatic effect. Concerning the concentrations of 9 and 4.5 mg/ml, no growth is reported. According to Fig. 11, mycelial growth of the fungal strain in concentrations of 0.28125–0.5625 and 1.125 mg/ml is close to control and was observed from day one. For concentrations of 2.25 mg/ml and 4.5 mg/ml, slight growth is found only after 2 and 3 days, respectively. For the 9 mg/ml concentration, there is total inhibition of the strain.

3.4.3. Inhibition rate

The inhibition rate of the two extracts studied is shown in Fig. 12. The latter shows that our samples have a different inhibitory activity, whose fungicidal effect (total inhibition 100%) for the root extract was marked by the application of a concentration of 4.5 mg/ml, while the fungicidal effect shown by the leaf extract reaches a concentration of 9 mg/ml. Based on these results (Fig. 12): the minimum inhibitory concentration of the root extract is 4.5 mg/ml and that of the other extract is 9 mg/ml.

Table 2
Qualitative evaluation of the antibacterial activity of root and leaf extracts.

	Muting zone diameter (mm)					
Bacterial strains	Root extract	Leaf extract	Antibiotics			
			OX	S	K	ZOX
E. coli	13.5 ± 0.7	11.0 ± 1.4	29.5 ± 2.1	16.0 ± 1.4	17.5 ± 0.7	13.0 ± 1.4
S. aureus	13.0 ± 1.4	13.5 ± 0.7	24.0 ± 1.4	20.0 ± 1.4	17.0 ± 1.4	14.5 ± 0.7
A. baumanii	12.0 ± 1.4	9.5 ± 0.7	0	16.0 ± 1.4	15.0 ± 1.4	20.0 ± 1.4
S. pneumoniae	11.5 ± 0.7	10.5 ± 2.1	0	19.5 ± 0.7	15.0 ± 1.4	21.0 ± 1.4
K. pneumoniae	11.5 ± 0.7	12.0 ± 1.4	30 ± 1.4	21.5 ± 2.1	16.5 ± 0.7	12.5 ± 0.7

kanamycin (K); Oxacillin (OX); Ceftizoxime (ZOX); Streptomycin (S).

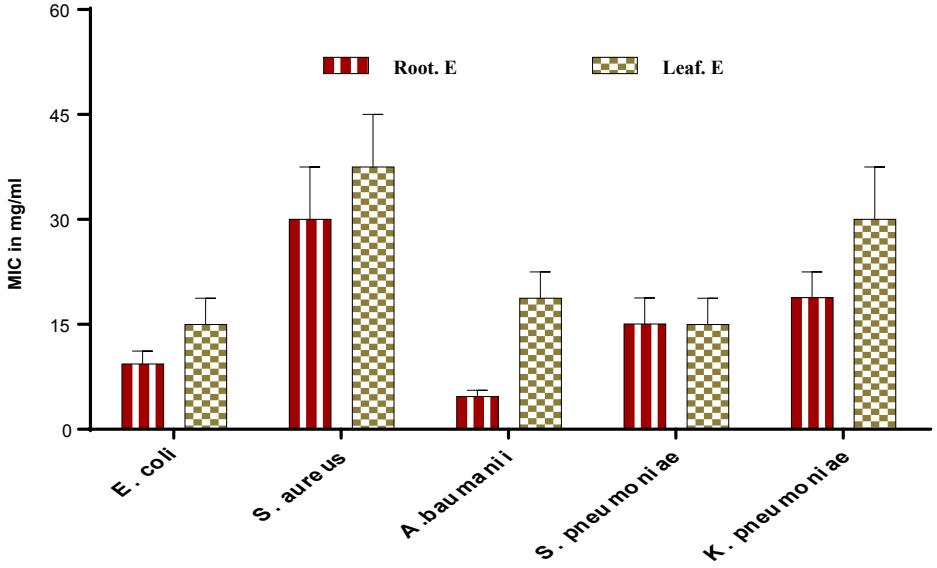

Fig. 8. Results of antibacterial activity by the microdilution method.

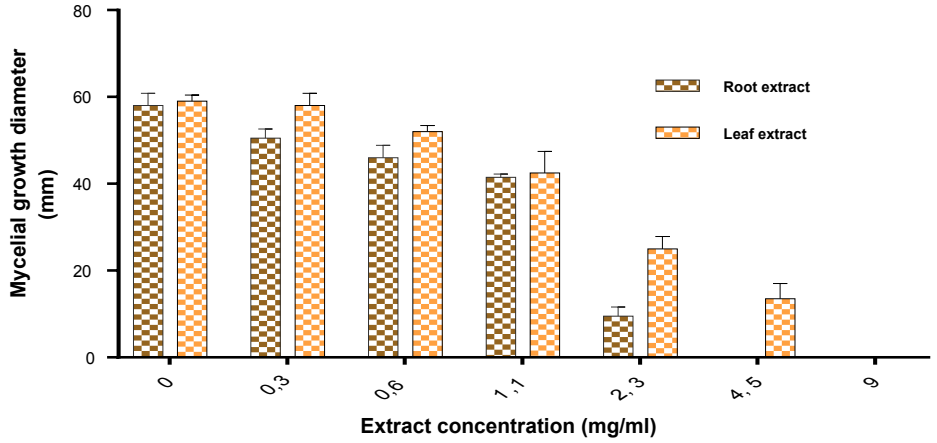

Fig. 9. Effect of extracts on the growth of the tested strain.

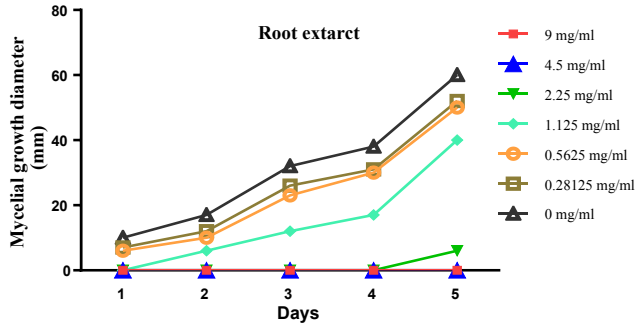

Fig. 10. Kinetics of strain growth as a function of time and root extract concentration.

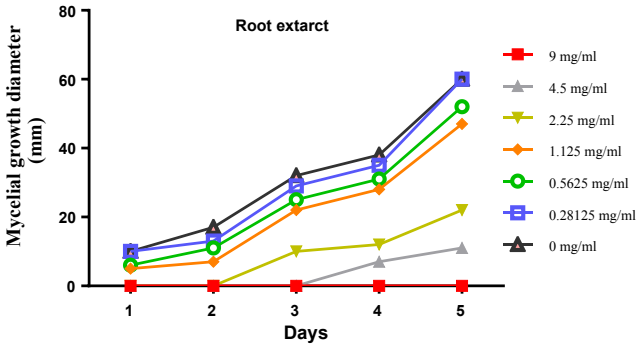

Fig. 11. Kinetics of strain growth as a function of time and concentration of extract from the aerial part.

3.4.4. Speed of mycelial growth

For the effect of the two extracts studied on mycelial growth rate, we notice that the highest growth rate is expressed by the control and that the rate is decreased by increasing the concentration of the extracts, Fig. 14. The effect of the aerial sample on mycelial growth rate is greater than in the presence of the other root extract, Fig. 13.

It appears that there are plant species that could be further developed in the control of many fungal strains responsible for different plant pathogenic forms [29]. The efficacy of the extracts studied in vitro could be given a vision of the richness of Withania frutescens in biologically active compounds, which need purification after extraction and identification to know the molecular structure responsible for the antimicrobial effect and the synergistic effect of these compounds. The development of a molecule with an antifungal activity is linked to the ultrastructure of the three barriers of the fungal cell: the chitinous wall, membrane ergosterols and genetic material [30] and the antifungal molecules themselves can cause resistance to the fungal strain [31].

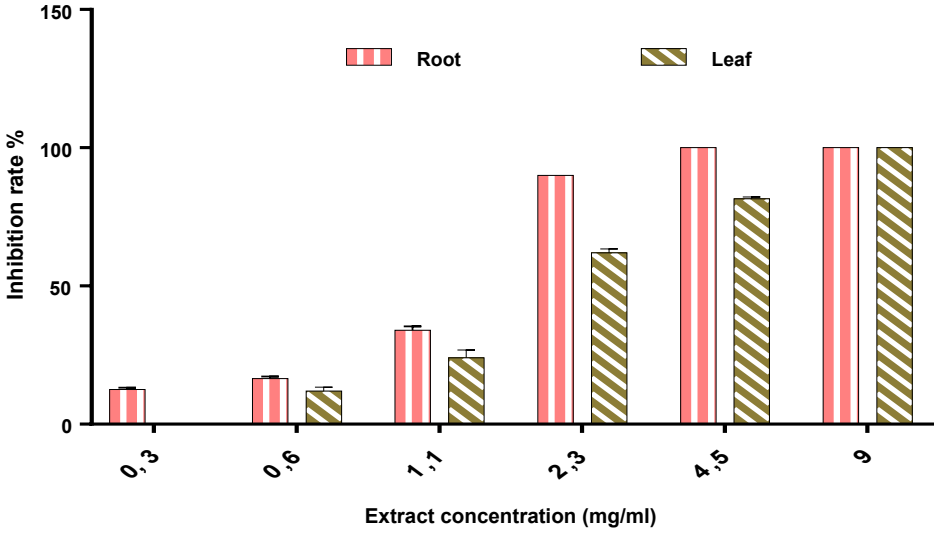

Fig. 12. Inhibition rate of the strain as a function of extract concentration.

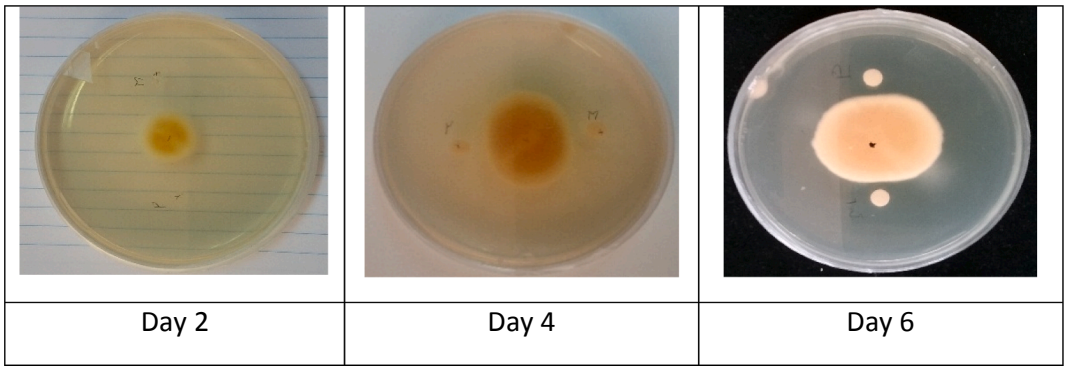

Fig. 13. Antifungal activity and mycelial growth of the samples studied.

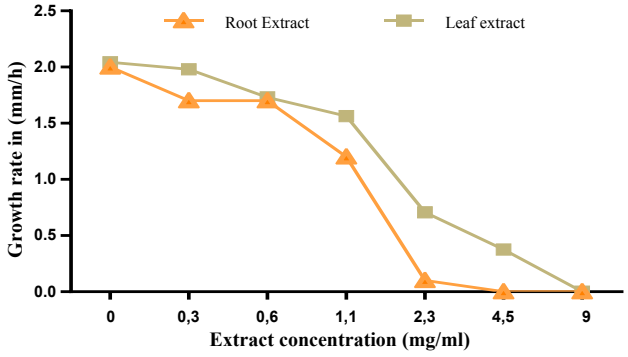

Fig. 14. Rate of mycelial growth under the effect of different concentrations of each extract.

4. Conclusion

The results of this in vitro study are only a first step in the search for biologically active natural substances. This study reveals that the extract from the leaves and roots of *W. frutescens* was active against the microbiological strains tested, so the plant can be used in areas related to the treatment of microbial infections, food preservation and biological control of certain plant diseases in the cereal field. These preliminary studies can be supplemented by other more in-depth studies: purification and molecular identification to determine the chemical structure responsible for a biological effect, as well as other in vivo and in situ tests.

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