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Evaluation of Antibacterial and Antifungal Capacities of Two Species of the Asteraceae Family *Centaurea Papposa blue* and *Hypochaeris Laevigata*

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ABSTRACT:

Hypochaeris and *Centaurea* are endemic plants belonging to the Asteraceae family, localized in the eastern Algeria. The objective of this study was the evaluation of the antibacterial and antifungal activity of their dichloromethane, AcOEt and *n*-butanol extracts using the method of diffusion by discs on five strains with Gram- and Gram + and the method of wells on three phytopathogenic fungal strains. The results revealed that the bacterial strains are very sensitive to the dichloromethane extract of *H. Laevigata* and *C. Papposa blue* with significant inhibition zones, with the fungal strains being sensitive to *n*-butanol of *H. Laevigata* and *C. Papposa blue* dichloromethane extracts. The richness of these two species in several secondary metabolite classes could give them an important therapeutic and medicinal value. The presented results could provide an overview of the antibacterial and antifungal potential of extracts from these plants.

Keywords: *Hypochaeris Laevigata, Centaurea Papposa blue*, antibacterial activity, antifungal activity.

1. Introduction

For a long time, medicinal plants and their preparations constituted the only source of medicines, and nature diversified by these inhabitants is considered a large plant manufacturing plant, these very diversified in turn for the healing of our diseases [1]. Preparation, standardization, and quality control of herbal medicinal plants in Africa including Algeria are essential to ensure the quality and consistency of the traditional medicinal plant products [2]. In fact, there are hundreds of thousands of plants on Earth, of which tens of thousands of species have medicinal properties [3]. The use of plants for therapeutic

purposes has been mentioned in ancient Arabic, Chinese, Egyptian, Hindu literature, etc. The therapeutic power of plants was known experimentally by our ancestors. Thus, plants constitute an interesting source of new compounds in the search for biologically active molecules [4].

Algeria, with its very big area, its different bioclimatic and ecological regions, as well as its remarkable specific diversity, ranks moderately with those known for their taxonomic, ecosystemic, landscape, and cultural diversity [5]. Thanks to its privileged geographical location in the Mediterranean, it forms a mixture of wild plant species, which have medicinal properties, including species of *Centaurea* and *Hypochaeris* genera those well known by its uses in traditional medicine and on their great richness and diversity in secondary metabolites, mainly phenolic compounds, therapeutics, and lignans, making them an essential source of active ingredients [6-10].

Our work was aimed to evaluate the antibacterial and antifungal activity of the *n*-butanol, ethyl acetate, and dichloromethane extracts from two Algerian medicinal plants belonging to two different genera, namely the species *Centaurea papposa blue* and *Hypochaeris laevigata*.

2. Materials and methods

2.1. Plant Material

Hypochaeris laevigata var. *hipponensis*) and *Centaurea papposa blue* (Coss.) Greuter were collected from Annaba region, in northeastern Algeria in May 2021. The plant materials were confirmed and deposited in the herbarium of the laboratory of functional ecology and environment, Larbi Ben M'hidi University.

2.2. Preparation of samples

The aerial parts (stems, leaves, and flowers) of *Centaurea papposa blue* and *Hypochaeris laevigata* were macerated for 24 hours on a magnetic stirrer at a constant stirring speed of 200 rpm, at room temperature, in a methanol/water mixture (70/30, *v/v*) that is brought to a boil. The suspension is then filtered through Whatman N^{o} 1 paper. The extraction process is repeated three times with fresh solvent. The solvent is removed from the filtrate by rotary evaporation under low pressure at 40°C (Rota Vapor, Büchi R-200, Germany). The obtained crude extracts is mixed with boiling distilled water (*v/v*) and left at room temperature for 24 hours. After filtration, the obtained crude extracts undergone three successive extractions using dichloromethane, ethyl acetate, and *n*-butanol. The crude extracts were initially mixed with dichloromethane for 24 hours. The mixture is allowed to settle, and the upper organic phase is collected. The residual aqueous phase of the dichloromethane is subjected to another extraction with ethyl acetate (1 hour), following the same steps as the first extraction. The residual aqueous phase from the ethyl acetate extraction is subjected to another extraction with *n*-butanol (24 hours), following the same steps as the previous extractions. After evaporation and complete removal of the solvents, the three fractions are stored at a temperature of 4°C until use.

2.3. Antibacterial activity

Method of diffusion on the disk: The antibacterial activity of extracts of dichloromethane, *n*-butanol, and ethyl acetate is realized *in vitro* by the method of diffusion on the disk in the middle of the wire using the protocol described by [11]. The principle of this method will result in a paper disc that impregnates the excess of different concentrations, placing it directly on the gel, with a uniform texture with the tester. The growth is at a distance from the disc due to the high sensitivity of the outside world. The limit of the inhibition zone is detected at the end and agreed upon when the growth bacteria begin [12]. Interpretation of the exhibition zone will help in the operation of the day at the table, where the germs are classified as sensible, intermediate, or resistant [13]. Revitalization of the skin using the daily nutritional supplement for 24 hours. The materials (test tubes, the discs in Wattman paper, the nutritional gel, the drops, and the micropipettes) were sterilized first using a pressure cooker (minute-long cocotte). The culture used is Muller-Hinton. The solution is filled with complete dissolution in a Bain.

2.3.1. Preparation of dilutions

The plant extracts obtained were dissolved in dimethyl sulfoxide (DMSO) to prepare the different concentrations with successive dilutions, knowing that the concentration of the mother solution of each extract is 100 mg/ml.

2.3.2. Preparation of the inoculums and seeding

The bacterial strains were cultured in nutrient broths. Their density must be equivalent to 0.5 McFarland. The inoculums can therefore be adjusted, either with culture if it is too weak or with sterile physiological water if it is too strong. The bacterial suspensions were spread on the surface of the M.H. agar using swabs. The discs impregnated with the extracts (10 µl) are gently placed on the surface of the inoculated agar using sterile forceps, as well as the discs impregnated with DMSO (negative controls). The Petri dishes are incubated for 24 hours at 37°C. The experiment is repeated twice for each extract and for each bacterial species. The reading was done by measuring the diameters of the inhibition halos around the discs. The results are expressed by the diameter of the inhibition zone. Non-sensitive (-) or resistant: diameter less than 8 mm; Sensitive (+): diameter between 9 and 14 mm; Very sensitive (++): diameter between 15 and 19 mm; extremely sensitive (+++): diameter more than 20 mm.

2.4. Antifungal activity

The antifungal activities of the different plant extracts were evaluated on the growth of phytopathogenic fungi. The Fusarium isolates were grown on PDA medium (Potato Dextrose Agar) to be reactivated and incubated for 3 days at 27°C in the dark. The material (test tubes, Wattman paper discs, and nutrient agar, eppendorfs, and micropipette tips) was first sterilized using a pressure cooker. The choice of an adequate culture medium is essential for the proper development of the pathogen; in our experiment we used the PDA medium because it ensures good culture conditions for fusarium. A volume of 150 µl of DMSO solution for each concentration of dry extract was added to 20 ml of previously prepared PDA medium. Similarly, 150 µl of DMSO was added to 20 ml of PDA medium and was considered a positive control. The mixture (PDA + extract) / (PDA + DMSO (control)) was poured into Petri dishes, which were left to

dry at room temperature. To prepare the different concentrations used in the antifungal test, a dilution series of 140 mg/ml, 70 mg/ml, and 35 mg/ml of the N.E. but/EAC/Dich extracts was carried out with 150 µl of DMSO.

To prepare the different concentrations used in the antifungal test, a dilution series of 140 mg/ml, 70 mg/ml, and 35 mg/ml is carried out in order to obtain a final concentration range in the wells between 140 and 35 mg/ml for the EBut/EAc/Dich extracts, each concentration of extract prepared with 150 µl of DMSO in test tubes, then the contents were shaken to be homogenized and then put in contact with 20 ml of PDA medium. Seeding the surface of the PDA culture medium with the solution containing the three fungal strains (CER, CUL, OXY) after cooling and solidification of the culture medium, mycelial wells of 08 mm diameter of a young colony of fungal strains are placed in the Petri dish on the semi-solid and sterile PDA medium containing the previously sterilized extract + DMSO (4 wells per dish). It should be noted that each concentration was replicated 3 times. The Petri dishes were incubated at 37°C for 24 hours. The mycelial growth of the phytopathogenic agent is measured at the millimeter scale, and the results were expressed in the average diameter of the fungal colonies.

3. Results and discussions

3.1. Evaluation of antibacterial activity

3.1.1. Bacterial strains

Table 1. Classification of Gram + / Gram - bacterial strains.

Gram + bacteria	**Gram - bacteria**
Bacillus	*Escherichia coli*
Staphylococcus	*Pseudomonas*
	Proteus

3.1.2. Antibacterial activity of concentrated extracts of *H. Laevigata*

Concerning concentrated extracts, inhibition zones are observed. The results noted are the averages of the sets of diameters of the same test. The diameters of the inhibition zones (mm) obtained are represented in the Table 2.

Table 2. Antibacterial activity of dichloromethane extract from *H. laevigata* with inhibition zones measured in mm.

Dichloromethane extract	***E. coli***	***Staphylococcus***	***Proteus***	***Bacillus***	***Pseudomonas***
SM	15.5	14	11	12.5	11
½	12.5	7	7.5	12	10.5
¼	11.5	6	7	8.5	9

Table 3. Antibacterial activity of *n*-butanol extract from *H. laevigata* with inhibition zones measured in mm.

***n*-Butanol extract**	***E. coli***	***Staphylococcus***	***Proteus***	***Bacillus***	***Pseudomonas***
SM	8	6	7.5	10	7
½	7.5	6	8.5	8.5	6.5
¼	6	6	6.5	10	6.5

Table 4. Antibacterial activity of ethyl acetate extract from *H. laevigata* with inhibition zones measured in mm.

AcOEt extract	***E. coli***	***Staphylococcus***	***Proteus***	***Bacillus***	***Pseudomonas***
SM	8.5	11	11	12	12
½	7	7	9.5	8.5	10.5
¼	6.5	6.5	7.5	6.5	9

Concerning the concentrated extracts, inhibition zones are observed, indicating that all the concentrated extracts have an antibacterial activity. The inhibition diameters obtained are between 6.0 and 15.5 mm. The extracts are active to different degrees on all the bacteria tested. The largest inhibition zone is that of the dichloromethane extract from *H. laevigata* towards the bacterial strain *E. coli* with a diameter of 15.5 mm. The smallest inhibition zone is that of *n*-butanol extract from *H. laevigata* towards the Staphylococci strain with 6.0 mm in diameter.

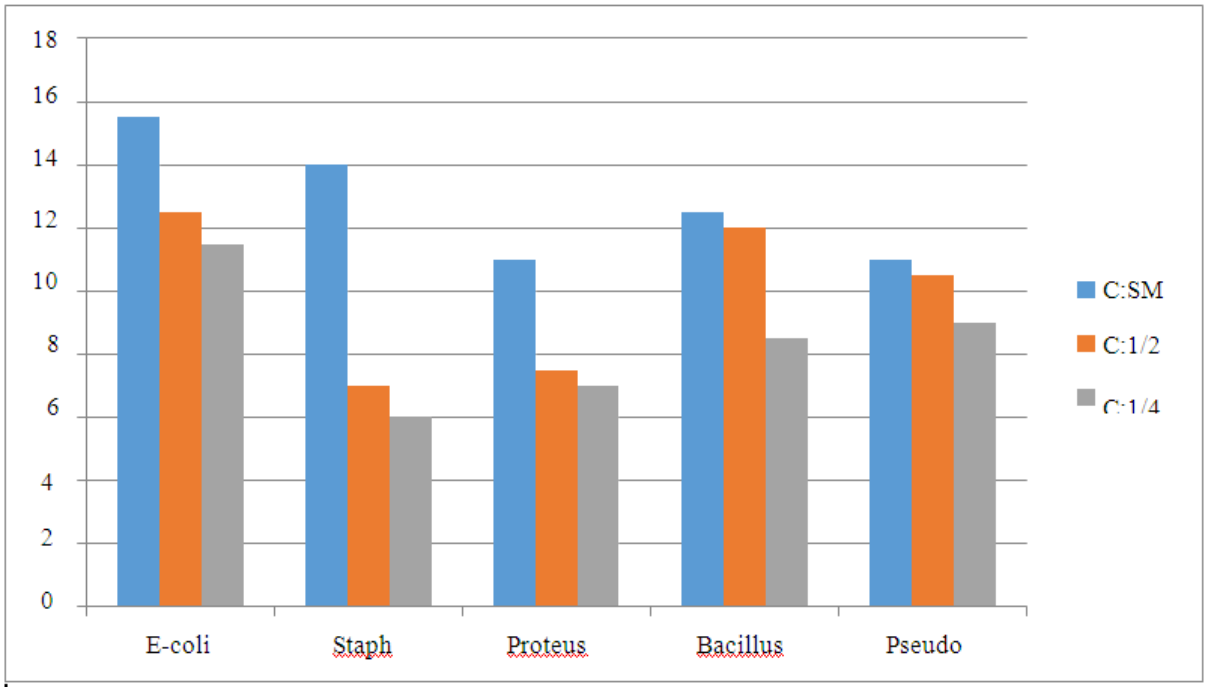

Figure 1. Antibacterial activity of the dichloromethane extract from *H. laevigata*

The results concerning the non-concentrated or diluted extracts (1/2 and 1/4) show that the latter have an effect on the tested strains *E. coli*, *Bacillus*, and *Pseudomonas*. A better antibacterial effect is observed on the *E. coli* strains with the initial concentration (SM) of the extract, which presents the maximum inhibition effect (15.5 mm). The other dilutions of the same extract for the same strain have an activity because the diameter is greater than 8 mm, the threshold from which we can speak of an antibacterial activity.

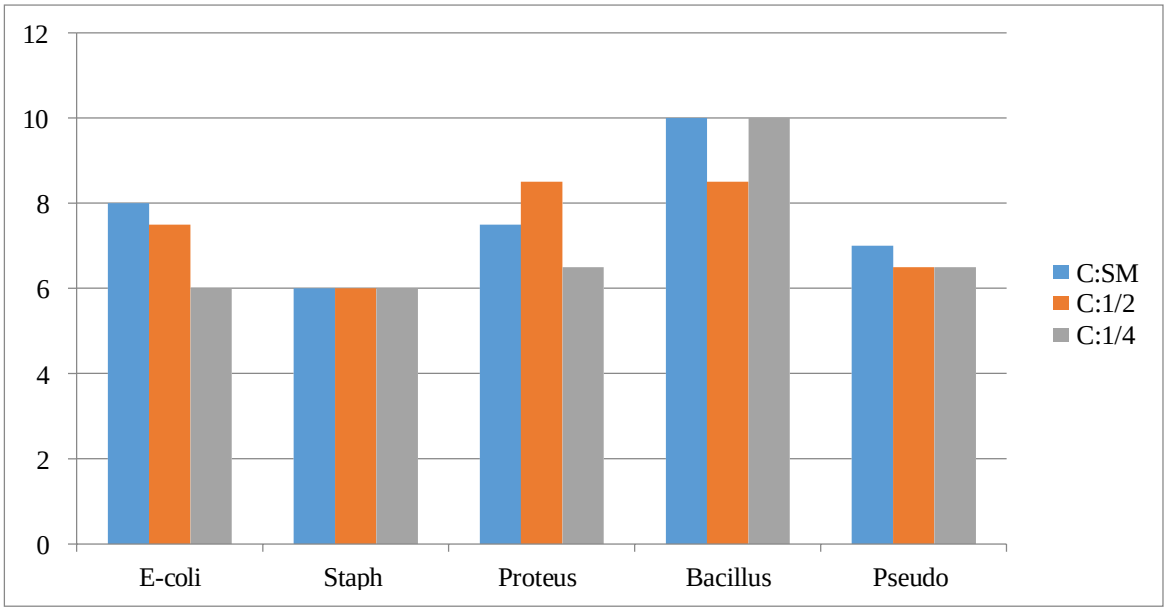

Figure 2. Antibacterial activity of the *n*-butanol extract from *H. laevigata*.

The results concerning the non-concentrated or diluted extracts (1/2 and 1/4) show that the latter have no antibacterial effect against the strains tested. An antibacterial effect is observed against the E. coli and Bacillus strains tested with the initial concentration (SM). Also, an antibacterial effect is positive with the ½ dilution against two strains (Proteus, Bacillus).

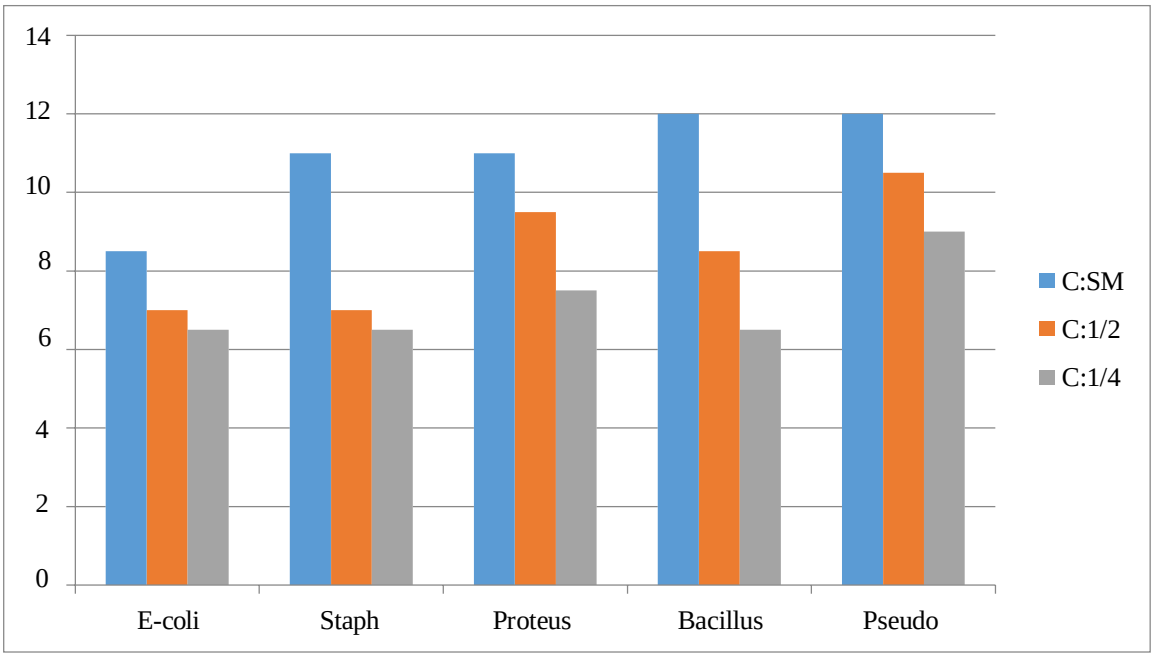

Figure 3. Antibacterial activity of AcOEt extract from *H. laevigata.*

The results concerning the non-concentrated or diluted extracts (1/2, 1/4) show that the latter have no effect on the tested strains *E. coli*, Staph, Proteus, and Bacillus. An antibacterial effect is observed against all strains with the initial concentration (SM) of the extract (≥ 8 mm). Also, an antibacterial effect is positive with the 1/2 dilution against the strains *Proteus, Bacillus,* and *Pseudo* with the 1/4 dilution.

Table 5. Diameter of the inhibition zones (mm) of the dichloromethane extract of *H. laevigata*

	Bacterial strains	**Dichloromethane extract (mm)**
Gram+	*Staphylococcus*	14
	Bacillus	12.5
Gram -	*Escherichiacoli*	15.5
	Proteus	11
	Pseudomonas	11

According to the above table, the bacterial strains tested were found to be sensitive to highly sensitive to dichloromethane extract. This clearly shows that this extract exerts antibacterial activity on all five strains studied, *Bacillus, Proteus,* and *Pseudomonas,* with an inhibition zone of 12.5 mm, 11 mm, and 11 mm, respectively, and Escherichia coli and Staphylococcus were found to be highly sensitive to the same extract, with an inhibition diameter of 15.5 mm and 14 mm, respectively. This can be explained by the fact that the antibacterial activity of *H. laevigata* extract is due to the different chemical agents present in this extract, including lipid compounds and pigments, which are classified as very active antibiotic compounds [14]. Other researchers have shown that the antibacterial activity is due to the nature of the Gram- or Gram+ bacteria, which is linked to the differentiation in the membrane structure of these bacteria and also to the extraction method (the variation in the extraction yield can therefore be explained by the difference in solubility of the chemical compounds in the extraction solvent, to their degree of polymerization). The appearance of an inhibition zone around the paper disk impregnated with the crude extract studied reflects the bacteriostatic action. The diameter of the inhibition zone differs from one bacterium to another and from one extract to another. As reported in the literature, we considered that an extract has a bacteriostatic action if its inhibition diameter is greater than 8 mm [15].

3.1.3. Antibacterial activity of *C. Papposa blue* concentrated extracts

Regarding the concentrated extracts, inhibition zones is observed. The results noted are the averages of the sets of diameters of the same test.

Table 6. Antibacterial activity of *C. Papposa blue* dichloromethane extract with the inhibition zones measured in mm.

Dichloromethane extract	***E. coli***	***Staphylococcus***	***Proteus***	***Bacillus***	***Pseudomonas***
SM	12	11.5	11.5	12.5	15
½	6	7.5	9	8.5	11.5
¼	6	7.5	8	8.5	8

Table 7: Antibacterial activity of *n*-butanol extract *from C. papposa blue* with the inhibition zones measured in mm.

***n*-Butanol extract**	***E. coli***	***Staphylococcus***	***Proteus***	***Bacillus***	***Pseudomonas***
SM	8.5	7.5	10	7.5	9.5
½	7	7	8	6.5	8
¼	6.5	7	6	6.5	6.5

Table 8. Antibacterial activity of *C. papposa blue* AcOEt extract with the inhibition zones measured in mm.

AcOEt extract	***E. coli***	***Staphylococcus***	***Proteus***	***Bacillus***	***Pseudomonas***
SM	7	11	15.5	15.5	14
½	6	6	12	8.5	11.5
¼	6.5	6	10	9	11.5

The inhibition diameters obtained are between 7.0 and 15.5 mm. The extracts are active to different degrees on all the bacteria tested. The largest inhibition zone is that of the ethyl acetate extract *C. papposa blue* towards the two bacterial strains, *Proteus* and *Bacillus*, with a diameter of 15.5 mm. The smallest inhibition zone is that of the AcOEt extract from *C. papposa blue* towards the *E. coli* strain with a diameter of 7.0 mm.

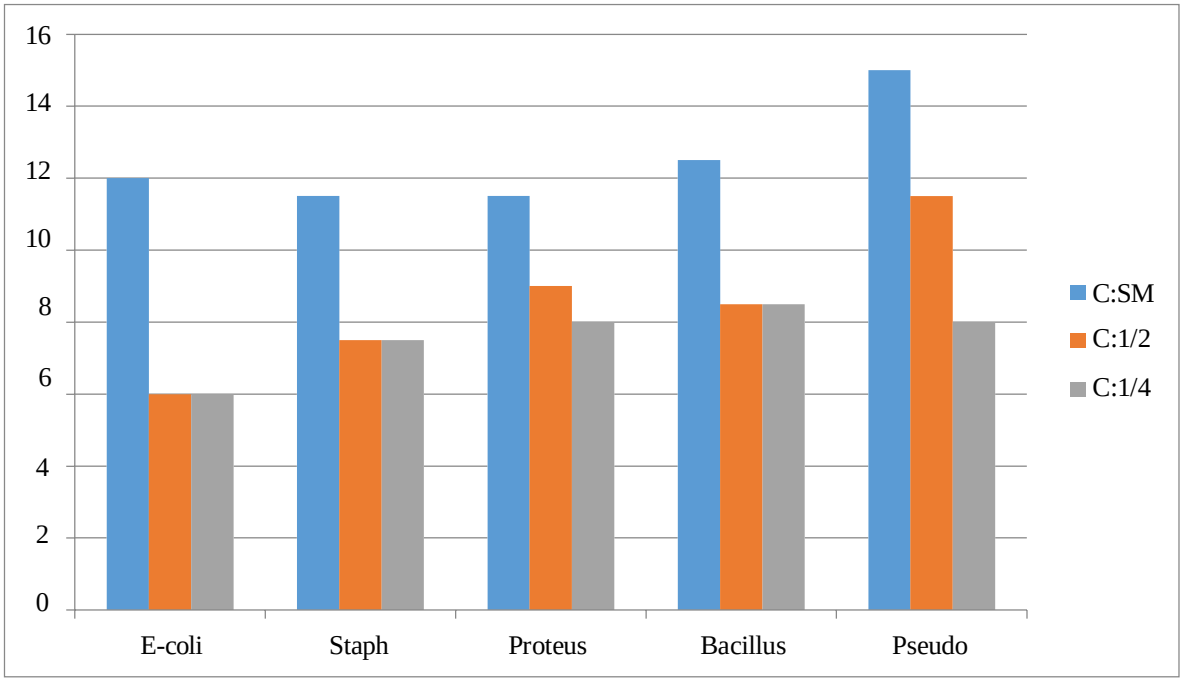

Figure 4. Antibacterial activity of dichloromethane extract from *C. papposa blue.*

The results concerning the non-concentrated or diluted extracts (1/2 and 1/4) show that the latter have an effect on the tested strains, *Proteus, Bacillus*, and *Pseudomonas*. An antibacterial effect is observed on all strains with the initial concentration (SM) of the extract (≥ 8 mm). The antibacterial activity of the n-butanol extract of *C. papposa blue* is shown in figure 5.

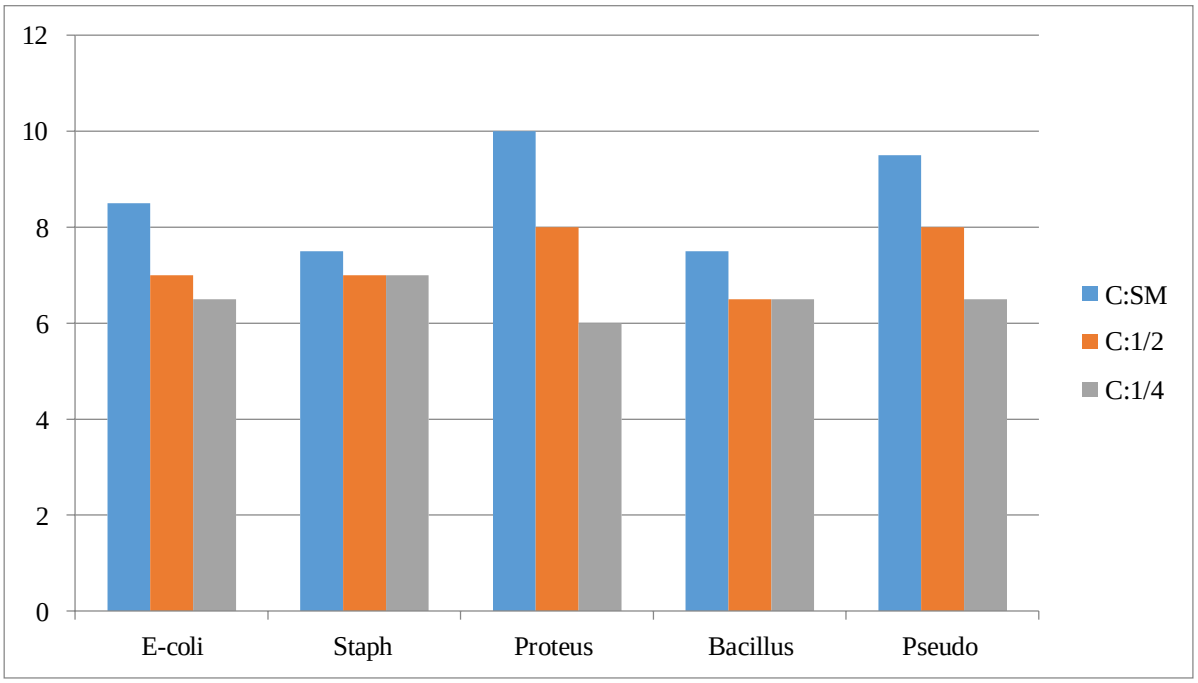

Figure 5. Antibacterial activity of *n*-butanol extract from *C. Papposa blue*.

The results for the non-concentrated or diluted extracts (1/2 and 1/4) show that all strains have no antibacterial effect against the strains tested. An antibacterial effect is observed against the

E. coli, *Proteus*, and *Pseudomonas* strains tested with the initial concentration (SM). Also, an antibacterial effect is positive with the 1/2 dilution against two strains (*Proteus* and *Pseudomonas*).

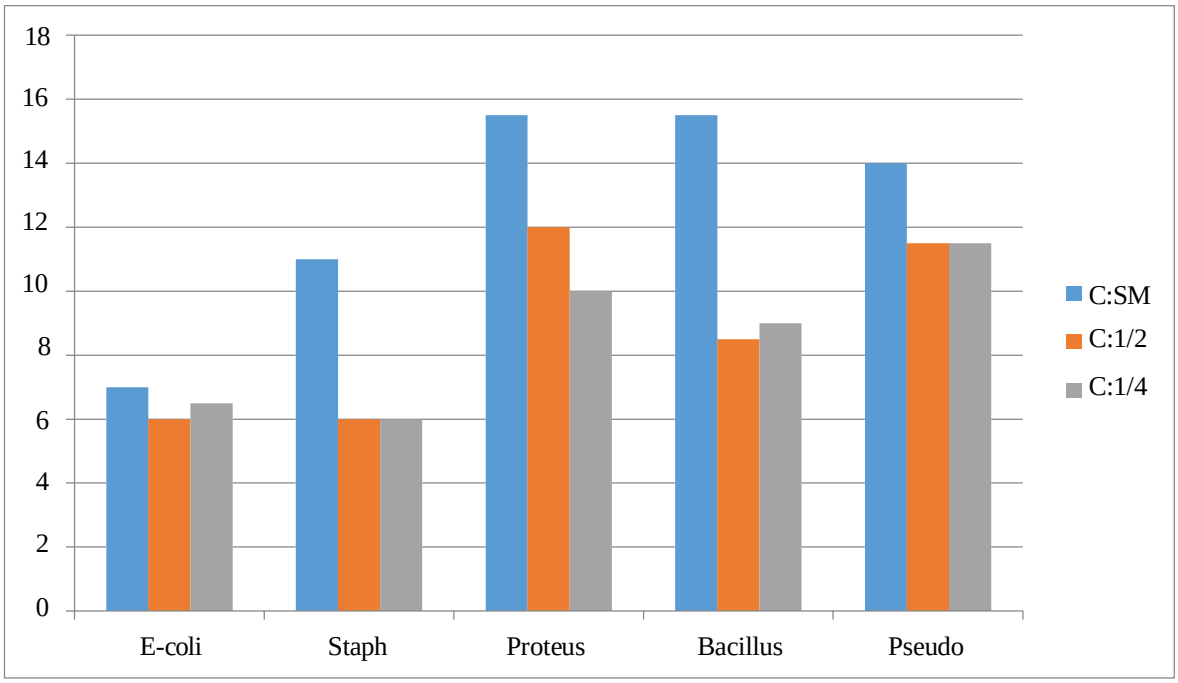

Figure 6. Antibacterial activity of *C. Papposa blue* AcOEt extract.

The results concerning the non-concentrated or diluted extracts (1/2 and 1/4) show that the latter have an effect on the tested strains, Proteus, Bacillus, and Pseudomonas. A better antibacterial effect is observed on the *Proteus* and *Bacillus* strains with the initial concentration (SM) of the extract, which presents the maximum inhibition effect (15.5 mm). The other dilutions of the same extract for the same strain have an activity because the diameter is greater than 8 mm. Threshold from which we can speak of an antibacterial activity.

Table 9. Inhibition zones (mm) of *C. Papposa blue* AcOEt extract.

	Bacterial strains	**AcOEt extract (mm)**
Gram+	*Staphylococcus*	11
	Bacillus	15.5
Gram -	*Escherichiacoli*	7
	Proteus	15.5
	Pseudomonas	14

According to the table above and the figures below, the bacterial strains tested appeared to be sensitive to very sensitive to the ethyl acetate extract. This clearly shows that this extract exerts antibacterial activity on the five strains studied, *Staphylococcus* and *Pseudomonas*, with an inhibition zone of 14 mm, respectively. *Bacillus* and *Proteus* were shown to be very sensitive to the same extract, with an inhibition diameter of 15.5 mm. This can be explained by the fact

that the antibacterial activity of the *C. Papposa blue* plant extract is due to the different chemical agents present in this extract, including flavonoids, tannins, and triterpenes, mainly saponins. Other researchers have shown that the antibacterial activity is due to the nature of Gram - or Gram + bacteria, which is linked to the differentiation in the membrane structure of these bacteria and also to the extraction method (the variation in extraction yield can therefore be explained by the difference in solubility of chemical compounds in the extraction solvent, their degree of polymerization, and the concentration of the active ingredient). The appearance of an inhibition zone around the paper disk impregnated with the crude extract studied reflects the bacteriostatic action. The diameter of the inhibition zone differs from one bacterium to another and from one extract to another. As reported in the literature, we considered that an extract has a bacteriostatic action if its inhibition diameter is greater than 8 mm [16].

3.2. Antifungal activity

The antifungal activity of aromatic compounds seems to be linked to the presence of certain chemical functions. Indeed, the major antifungal compounds in plants can belong to different classes of secondary metabolites, such as phenolic acids, quinones, flavonoids, tannins, coumarins, terpenoids, alkaloids, saponins, lectins, or polypeptides. In this study, we attempted to compare the influence of *n*-butanol and dichloromethane extracts on the mycelial growth of pathogenic fungi of the genus *Fusarium* in order to estimate the evolution of mycelial growth by measuring the diameter of the mycelial colony of the fungus. The reading was made by measuring the diameters of the inhibition halos around the wells. Concerning the concentrated extracts, inhibition zones are observed. The results noted are the averages of the sets of diameters of the same test.

3.2.1. Antifungal activity of *H. laevigata* concentrated extracts

The diameters of the inhibition zones (mm) obtained are shown in the following tables:

Table 10. Antifungal activity of dichloromethane extract from *H. laevigata*.

***n*-Butanol extract**	***F. culmorum***	***F. oxysporum***	***F. cerealis***
SM	16.3	18.6	20.3
½	12.6	11	16.3
¼	10.3	8	11

Table 11. Antifungal activity of *n*-butanol extract from *H. laevigata*

***n*-Butanol extract**	***F. culmorum***	***F. oxysporum***	***F. cerealis***
SM	15.6	14	14.6
½	13	12.3	11.6
¼	11.6	10	8.6

Concerning the concentrated extracts, inhibition zones are observed, indicating that all the concentrated extracts have antifungal activity. The inhibition diameters obtained are between 14 and 20.3 mm. The extracts are active to different degrees on all the fungi tested. The largest inhibition zone is that of the dichloromethane extract of *H. laevigata towards the fungus F. cerealis* with a diameter of 20.3 mm. The smallest inhibition zone is that of *n*-butanol extract of *H. laevigata* towards the *fungus F. oxysporum* with a diameter of 14 mm.

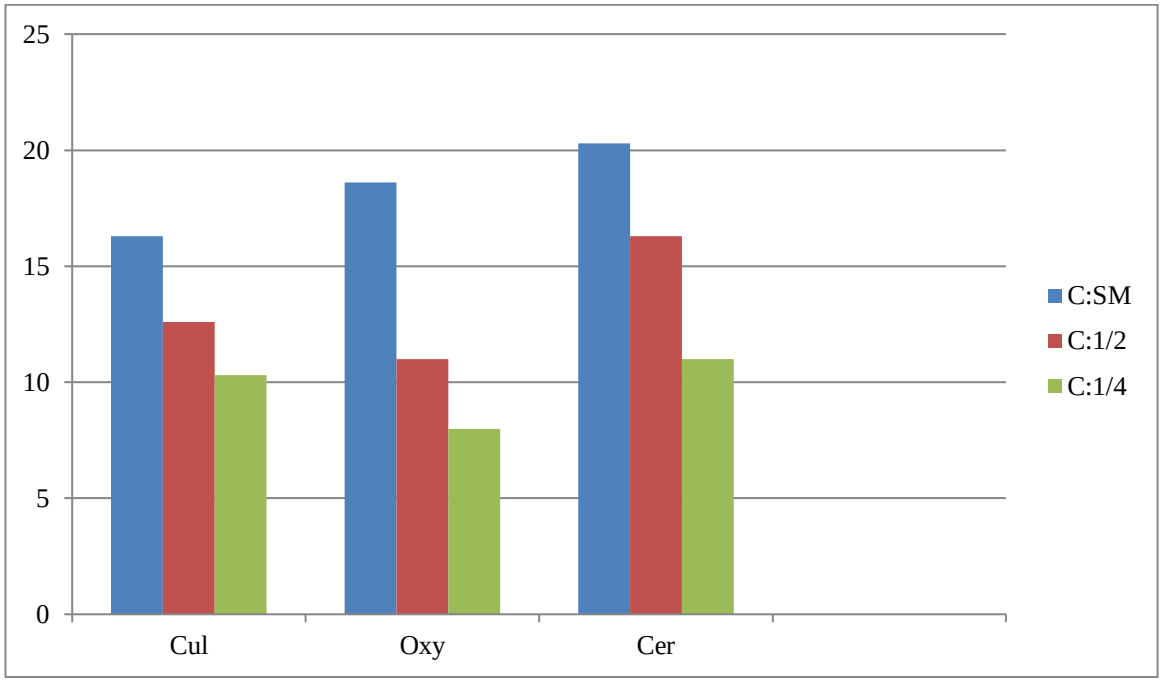

Figure 7. Antifungal activity of dichloromethane extract from *H. Laevigata.*

The results concerning the non-concentrated or diluted extracts (1/2 and 1/4) show that the latter have an effect against the tested fungi *F. culmorum. F. cerealis.* A better antifungal effect is observed against the fungus *F. cerealis* with the initial concentration (SM) of the extract, which presents the maximum inhibition effect (20.3 mm). The other dilutions of the same extract for the same fungus have an activity because the diameter is greater than 8 mm, the threshold from which we can speak of an antifungal activity. Also, an antifungal effect is positive with the ½ dilution against the fungus *F. oxysporum*.

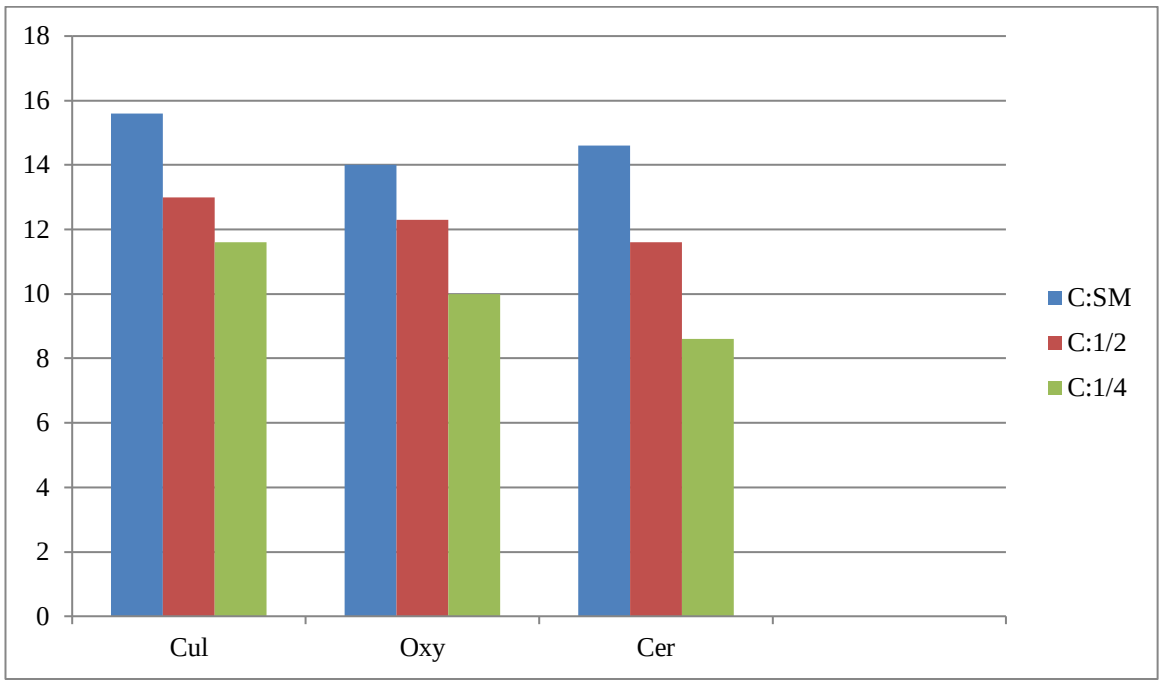

Figure 8. Antifungal activity of *n*-butanol extract of *H. laevigata.*

The results for the non-concentrated or diluted extracts (1/2 and 1/4) show that all fungi have an antifungal effect. An antifungal effect is observed against the fungi *F. culmorum*. *F. cerealis* and *F. oxysporum were* tested with the initial concentration (SM). It is noted that the best results are obtained by the N-butanol extract on the three fungal strains tested. The mycelial growth of the fungal strain tested *F. culmorum* increases according to the concentration (140 mg/ml, 75 mg/ml, and 35 mg/ml). From the results, we noted that the concentration of 140 mg/ml gives the highest inhibition on growth (15.6 mm); that of the control is equal to 8 mm. This differs from the other concentrations (75 mg/ml, 35 mg/ml), which showed a slight effect on mycelial growth (13 mm, 11.6 mm, respectively).

3.2.2. Antifungal activity of *C. papposa blue* concentrated extracts

The diameters of the inhibition zones (mm) obtained are shown in the following tables:

Table 12. Antifungal activity of dichloromethane extract from *C. papposa blue*.

Dochloromethane extract	*F. culmorum*	*F. oxysporum*	*F. cerealis*
SM	14.3	10	8
½	14.6	13.3	10
¼	16.3	13.6	12.3

Table 13. Antifungal activity of *n*-butanol extract from *C. papposa blue*.

n-Butanol extract	*F. culmorum*	*F. oxysporum*	*F. cerealis*
SM	13	12	10
½	15.6	14.6	11
¼	13.6	11.6	8.5

The inhibition diameters obtained are between 8.0 and 14.3 mm; the extracts are active to different degrees on all the fungi tested. The largest inhibition zone is that of the dichloromethane extract of *C. Papposa blue* against the fungus *F. culmorum* with a diameter of 14.3 mm. The smallest inhibition zone is that of the same extract of *C. papposa blue* against the fungus *F. cerealis* with 8.0 mm in diameter.

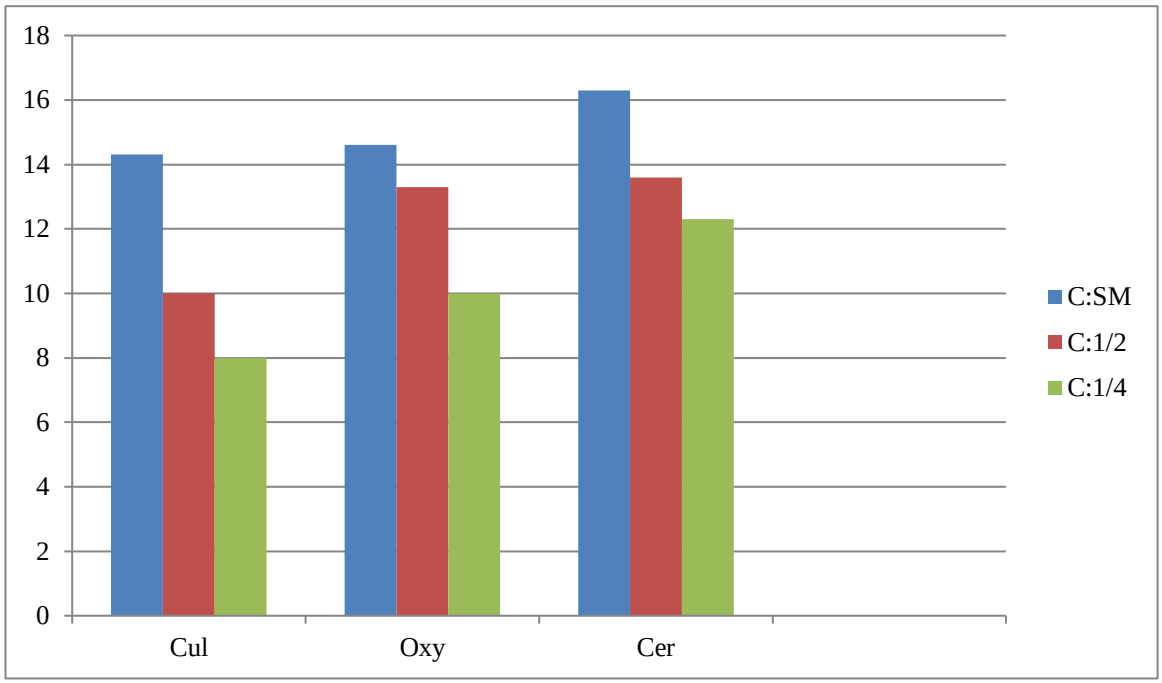

Figure 9. Antifungal activity of dichloromethane extract from *C. papposa blue*.

The results for the non-concentrated or diluted extracts (1/2 and 1/4) show that all dilutions have an antifungal effect. An antifungal effect is observed against the fungi *F. culmorum and F. oxysporum* tested with the initial concentration (SM) as well as a maximum inhibition effect against the fungus *F. cerealis* (16.3 mm).

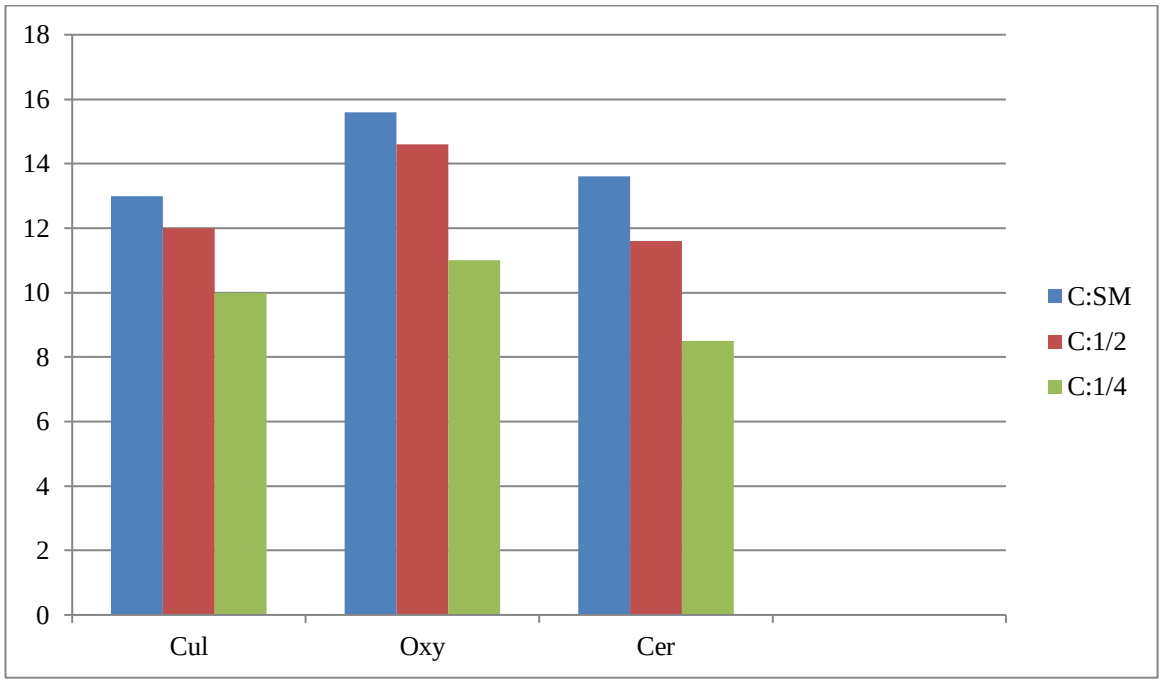

Figure 10. Antifungal activity of *n*-butanol extract from *C. papposa blue*.

The results concerning the non-concentrated or diluted extracts (1/2 and 1/4) show that all dilutions have an antifungal effect. An antifungal effect is observed against the fungi *F. culmorum, F. oxysporum, and F. cerealis* tested with the initial concentration (SM). It is noted that the best results are obtained by the

dichloromethane extract on the three fungal strains tested; the mycelial growth of the fungal strain tested *F. oxysporum* increases according to the concentration (35, 75, and 140 mg/ml). From the results, we noted that the concentration of 140 mg/ml gives the highest inhibition on growth (14 mm); that of the control is equal to 8 mm. This differs from the other concentrations (75 and 35 mg/ml), which showed a slight effect on mycelial growth (12.3 and 10 mm, respectively).

4. Conclusion

Algeria boasts a diverse botanical history that includes thousands of therapeutic plants. The goal of scientific study is to promote these plants as natural sources of bioactive compounds. Five Gram-positive and Gram-negative bacteria were used in a study to test the antibacterial and antifungal qualities of dichloromethane, *n*-butanol, and ethyl acetate extracts. All examined strains were bacteriostatically affected by the dichloromethane of *H. laevigata* and the ethyl acetate extract of *C. papposa blue*, according to the results. The well technique was used to assess the antifungal activity of dichloromethane and n-butanol against the pathogenic fungus *F. culmorum, F. oxysporum,* and *F. cerealis*. According to the findings, fungi were inhibited by the dichloromethane extract from *C. papposa blue* and the *n*-butanol extract of *H. laevigata*. Indeed, this study is preliminary and does not represent the true mechanism of action of these plants. To create more tolerable natural remedies for infectious disorders, more research is required to enhance antibiotics made from these plant extracts, look into their anti-inflammatory and anticancer properties, and assess their *in vivo* toxicity.

5. References

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