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Antifungal and antioxidant activities of some aromatic and medicinal plants from the southwest of Morocco

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ABSTRACT

The purpose of this study was to assess levels of phenols and flavonoids and determine the antioxidant potential of 36 aromatic and medicinal plants from the southwest of Morocco. The antifungal activity against the growth of Penicillium digitatum, the causal agent of citrus green mold, was studied in 11 species that showed high antioxidant power. The analysis of total phenols showed that leaves of Pistacia atlantica and Periploca laevigata contained 63.73 µg cafeic acid equivalent (CAE)/mg of dry weight (DW). The resin of P. atlantica and the whole plant Cistus villosus content was 60.93 µg CAE/mg DW. Ceratonia siliqua, Pistacia lentiscus also had high levels of total phenols; 56.80 and 54.80 µg CAE/mg DW respectively. The flavonoids content ranged from 1.41µg rutine equivalent (RE)/mg DW in extract of Senecio antheuphorbium to 31.77 mg RE/mg DW in that of Rhamnus alaternus. The antioxidant activity of plant extracts was above 80% for P. atlantica, C. villosus, Rumex thyrsoides, Vitis vinifera, Rhus tripartita, Rhus pentaphylla and P. lentiscus. Five plants (C. villosus, Ononis natrix, Rosa canina, P. atlantica and Lawsonia inermis) showed strong antifungal activity that could be related to their antioxidant activity.

Key words: Antifungal activity-Antioxidant activity- Flavonoids-Phenols-Plant extract

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INTRODUCTION

Morocco has a varied climate and geo-morphological characteristics favoring the development of a rich and diverse flora [1]. Indeed, Morocco represents a reservoir of plant species in mediterranean area [2] and consequently a wide variety of secondary metabolites such as phenolic compounds, flavonoids, terpenes and alkaloids.

Phenolic compounds have multiple biological activities including antifungal and antioxidant effects [3, 4, 5]. Thus, extracts of plants rich in polyphenols are of growing interest in food and pharmaceutical industries. In fact, phenols delay lipid oxidation and improve the quality and nutritional value of food. In addition, because of their antifungal effects, they can be used in post-harvest against fungi development in the food industry [6].

In Morocco, the green mold caused by the fungus *Penicillium digitatum* is a limiting factor for the packaging, transport and distribution of citrus. This disease causes significant economic losses for the country which is one of the leading exporters of citrus in the world. The objective of this work was to evaluate the antifungal activity against *P digitatum*, and the antioxidant effect in relation to the content of polyphenols and flavonoids in 36 medicinal and aromatic plants of the southwest of Morocco.

EXPERIMENTAL SECTION

1. Methanol extraction

Medicinal and aromatic plants were harvested in the spring, around two villages (Asgherkiss and Oulben) located at 140 km from Agadir city in the southwest of Morocco. Plants were first identified [7], then dried at 40 ° C and ground to a fine powder. The extraction was carried out for aerial parts of the plant, leaves, flowers or seeds as available. For the phytochemical analysis and the antioxidant activity, 50 mg of fine powder of each sample were extracted with 1 ml of methanol-water solvent (80/20; v / v), whereas for the test of the antifungal activity the extraction was performed with 1 ml of methanol-water solvent (50/50; v / v). The methanol content of extracts was removed by evaporation. After sonication for 15 min and centrifugation at 12 000 rpm for 10 min at room temperature, the supernatant was recovered and kept at 4 ° C.

2. Determination of total phenols content [8]

To 25 µl of plant extract were added 110 µl of Folin – Ciocalteu solution. The mixture was stirred for 3 minutes and then 200 µl of sodium carbonate (Na_2CO_3) and 1.9 ml of distilled water were added. After incubation for 30 min at 60 ° C in a water bath in the dark, the optical density was measured at 750 nm (IC 6400 visible spectrophotometer). The calibration range was made using cafeic acid. The results were expressed in terms of µg cafeic acid equivalent (CAE)/ mg of dry weight (DW).

3. Determination of total flavonoids content [9]

To 600 µl of plant extract were added 300 µl of $AlCl_3$. The mixture was incubated for 30 min at room temperature. Then, the absorbance was measured at 430 nm. Rutine was used as standard. The concentrations were expressed in terms of µg rutine equivalent/mg of dry weight (µgRE/mg of DW).

4. Determination of antioxidant activity

The evaluation of the antioxidant activity was carried both by free radical scavenging method and Ferric reducing antioxidant power.

4.1 Scavenging method

The antiradical power of substances was measured by the decrease of absorption of DPPH (1,1-Diphenyl-2-picrylhydrazyl) according to the method of Shimada et al. [10]. To 950 µl of a methanol solution of DPPH (0.1 mM) were added 50 µl of the plant extract. After 30 min, the absorbance of the mixture was measured at 517 nm. The ability to scavenge DPPH radical was calculated using the following formula:

$$\%\ \text{inhibition of DPPH} = \frac{(A_c - A_s)}{A_c} X100$$

A_c : absorbance of control
A_s : absorbance of test sample

4.2 FRAP method [11]

It is based on reduction of ferric tripyridyltriazine (Fe3 – TPTZ) to ferrous complex tripyridyltriazine (Fe2 – TPTZ) by an antioxidant in acidic pH. The ferrous Fe (II) complex -TPTZ develops a blue color which absorbs at 593 nm. The methodology of Benzie and Strain [12] was used. FRAP mixture consists of a) Solution 1: 10 parts of an acetate buffer solution (300 mM) at pH 3.6; b) Solution 2: 1 volume of a solution of TPTZ (tripyridyl triazine); c) Solution 3: 1 volumes of a solution of FeCl3 6H2O (20mM).

To 2 ml of the FRAP mixture, were added 10 µl of the plant extract. After incubation of 15 min at room temperature, the absorbance was measured at 593 nm. The calibration range was prepared with trolox having a standardized antioxidant activity. Results are expressed as µmol of trolox equivalent antioxidant capacity (µmol TEAC)/mg of DW.

5. Assay of antifungal activity

The antifungal power of plant extracts were evaluated for species with high antioxidant activity. *P. digitatum* was isolated from a moldy orange on a culture medium containing dextrose agar and potato (PDA). The culture medium was autoclaved and distributed in sterile Erlenmeyer flasks (40 ml of culture medium per flask); 200 µl or 1 ml of plant extract were added to achieve final concentrations of 0.625 mg/mL or 1.25 mg/ml. The culture medium was divided into four sterile Petri dishes. Discs of the preculture of *P. digitatum* are placed in the center of the Petri dish. The dishes were then incubated at 25 ° C for one week. The antifungal activity of plant extracts was estimated by

measuring the radial growth of mycelia formed from the fungal disc compared to the control prepared with sterile distilled water. The inhibition rate was deduced from the following relationship:

$$\% \text{ inhibition} = \frac{(R_c - R_s)}{R_c} \text{x} 100$$

Rc: radius of the mycelia growth of the control
Rs: radius of the mycelia growth in the presence of the plant extract

6. Statistical analysis
Results are given as mean ± standard deviation (SD) of 4 replicates for the antifungal test and 2 repetitions for the content of polyphenols, flavonoids, and antioxidant activity. An analysis of variance (ANOVA) was used for comparison of means using the Statistica software v. 6. A difference was considered statistically significant when $P < 0.05$ by using Newman & Keuls test.

RESULTS AND DISCUSSION

Table 1 shows the content of polyphenols and flavonoids of studied species. The highest content of total phenols was recorded in the leaves of *Pistacia atlantica* and those of *Periploca laevigata* with 63.73 µg/mg DW. The resin of *P. atlantica* and the whole plant of *Cistus villosus* contained 60.93 µg/mg DW. *Ceratonia siliqua*, *Pistacia lentiscus* also had high levels of total phenols, with 56.80 and 54.80 µg/mg DW respectively. The lowest levels were recorded in seeds of *Trigonella graecum foecum*, the whole plant of *Carum carvi*, the whole plant of *Diplotaxus catholica* and leaves of *Senecio anteuphorbium*. In the literature, P. atlantica was reported as species rich in phenols [13, 14]. However, Benhammou et al. [15] recorded a lower phenol content in the species of Tlemcen region (Algeria) and Peksel et al. [16] noted a higher rate for the region of Istanbul (Turkey).

For other species, the phenol content varied with authors and the study area. Thus the richness of *P. laevigata* of southern Morocco was reported by Hajji et al. [17]. For other plants, the values we obtained were higher than those noted in other regions of the mediterranean area. This is the case of *C. villosus* of Tunisia [18], *C. siliqua* of Spain [19], and *P. lentiscus* of Algeria [15]. Indeed the content of polyphenols depends on several factors such as the harvest period, the extraction technique, the assay method used and the studied ecotype [13, 15, 16, 17, 18, 19].

Regarding flavonoids, our results showed high values comprised between 8 and 13 µg/mg DW for *Rhus tripartita*, *Thymus saturieodes*, *P. lentiscus*, *C. villosus* and *Rosa canina*, except *Rhamnus alaternus* that contained 31.77 µg/mg DW. Other plants contents were between 2 and 5 mg/g DW. The lowest value was recorded for *S. anteuphorbium* (1.41 µg/mg MS). These results were consistent with those reported on *P. atlantica*, *C. siliqua* and *R. canina* from the south of Morocco [20]. Furthermore, we notice that the resin and leaves of *P. atlantica* showed a low rate of flavonoids (2 and 3.25 µg/mg DW, respectively) despite their richness in polyphenols.

The antioxidant activity of plant extracts is reported in Table 2. Extracts from *P. atlantica* (leaves and resin), *C. villosus*, *Rumex thyrsoides*, *Vitis vinifera*, *R. tripartita*, *Rhus pentaphylla* and *P. lentiscus* showed the higher values (80 %) according to the DPPH method. This high antioxidant power was confirmed by FRAP method except extracts from *C. villosus* and *R. thyrsoides* (FRAP under 100 µmol TEAC/mg DW). Weaker antioxidant activities were recorded in extracts of *T. graecum foecum*, *Papaver rhoeas* and *Marrubium vulgare* (DPPH less than 65%, FRAP less than 70 µmol TEAC/mg of DW). These results showed that the antioxidant activity was greater in species with high levels of polyphenols. Thus, linear regression yielded a correlation factor of 0.52 and 0.81for DPPH and FRAP test respectively (Figures 1). These results are in agreement with the literature reporting that the antioxidant activity depends on the content of polyphenols [21, 22].

The 2 species of *Pistachia* exhibited high antioxidant power. These results agree with the literature data which reported high antioxidant activity of *Pistachia* species from Algeria [23, 24]. Furthermore, our results showed that the antioxidant activity was close among similar species. This could be explained by their relative contents in polyphenols. This applies to both species of the genus of *Pistacia*, *Rhus*, *Thymus* and *Lavendula*.

Other authors related the antioxidant activity to the presence, among others, of various compounds as polyphenols, flavonoids and tannins [25, 26, 27, 28]. The antioxidant power of the phenolic compounds is due to their ability to chelate metals, and their capacity as donors of hydrogen and electron allowing eliminating free radicals [26]. The chemical nature of polyphenols is also involved in anti-oxidant processes. Thus, the molecules with hydroxyl group (OH) have a greater antioxidant effect than substituted forms. In contrast, methyl groups (CH3) and oxy-methyls (-

OCH3) decrease the antioxidant power [29]. This antioxidant capacity of plant extracts and polyphenols they contain, allows them to be recommended for use in the areas of food and drugs preservation [30].

Table 1. Phenols and flavonoids contents in different plants parts

Family	Species	Used parts	Polyphenols (µg/mg DW)	Flavonoids (µg/mg DW)
Anacardiaceae	*Pistacia atlantica*	Leaves	63.73±1.04	3.25±0.11
	Pistacia atlantica	Resin	60.93±1.23	2.00±0.21
	Pistacia lentiscus	Leaves	54.80±1.04	9.89±0.65
	Rhus pentaphylla	Leaves	39.33±0.66	7.45±0.53
	Rhus tripartita	Leaves	32.80±0.47	10.86±0.76
Apiaceae	*Carum carvi*	Areal parts	1.84±0.04	3.58±0.21
	Carum carvi	Fruits	5.89±0.02	2.50±0.13
Apocynaceae	*Nerium oleander*	Leaves + Flowers	10.11±0.02	5.13±0.42
Asclepiadaceae	*Periploca laevigata*	Leaves	63.73±1.04	5.56±0.23
Asteraceae	*Clandanthus arabicus*	Areal parts	4.61±0.00	3.53±0.10
	Launea arborescens	Areal parts	12.08±0.02	4.69±0.17
	Pulicaria glandulosa	Areal parts	26.73±0.42	3.47±0.34
	Senecio anteuphorbium	Areal parts	1.55±0.04	1.41±0.06
Brassicaceae	*Diplotaxus catholica*	Areal parts	1.59±0.02	3.04±0.29
Cactaceae	*Opuntia ficus indica*	Flowers	5.93±0.08	7.12±0.38
Chenopodiaceae	*Chenopodium ambrosioides*	Areal parts	2.96±0.02	3.96±0.34
Cistaceae	*Cistus villosus*	Areal parts	60.93±1.23	9.84±0.99
Cupressaceae	*Juniperus oxycedrus*	Leaves	21.80±0.28	4.95±0.23
Euphorbiaceae	*Ricinus communis*	Leaves	11.75±0.07	4.30±0.57
Fabaceae	*Ceratonia siliqua*	Leaves	56.80±1.23	8.75±0.57
	Ononis natrix	Areal parts	8.52±0.05	8.09±0.30
	Trigonella foecum graecum	Seeds	0.48±0.03	2.30±0.23
Lamiaceae	*Lavandula dentata*	Areal parts	13.16±0.10	4.13±0.19
	Lavandula multifida	Areal parts	29.87±0.57	5.51±0.19
	Lavandula stoechas	Areal parts	15.13±0.14	4.30±0.42
	Marrubium vulgare	Areal parts	5.61±0 .00	2.24±0.13
	Thymus leptobotrys	Leaves	22.20±0.38	3.31±0.34
	Thymus saturieodes	Leaves	22.20±0.33	13.04±0.95
Lythraceae	*Lawsonia inermis*	Leaves	27.47±0.52	8.06±0.76
Moraceae	*Ficus carica*	Leaves	6.99±0.03	5.38±0.25
Oleaceae	*Olea europea subsp maroccana*	Leaves	18.87±0.19	6.72±0.49
Papaveraceae	*Papaver rhoeas*	Fruits	0.65±0.03	5.47±0.19
Polygonaceae	*Rumex thyrsoides*	Areal parts	6.51±0.00	3.35±0.34
Rhamnaceae	*Rhamnus alaternus*	Leaves	22.07±0.38	31.77±2.28
Rosaceae	*Rosa canina*	Leaves	38.27±0.66	8.63±0.30
Solanaceae	*Withania frutescens*	Leaves	12.69±0.09	5.44±0.29
Verbinaceae	*Vitex agnus-castus*	Leaves	18.00±0.19	8.55±0.68
Vitaceae	*Vitis vinifera*	Leaves	35.33±0.028	7.98±0.65

The antifungal effect of the 11 species tested is shown in Figure 2. It varied depending on the species and the concentration used. Indeed, the majority of plant extracts selected for their antioxidant effect and their high content in polyphenols, exhibited a strong antifungal activity at a concentration of 1.25 mg/ml. However, this was not the case for *R. tripartita* and *P. leavigata* that had antifungal activity comparable to that of *S. anteuphorbium* (9% inhibition about) taken as a control because of insufficiency of polyphenols and low antioxidant power. The extract of *P. lentiscus* was an exception because it was rich in polyphenols, but had almost no antifungal activity (0.95%). The highest values are recorded for the extract of *C. villosus* (57.14%), followed by *R. canina*, *O. natrix*, *P. atlantica* (45.71%) and *L. inermis* (36.19%). These plants showed an antioxidant activity greater than 75%. Previous works demonstrated the effectiveness of extracts of *C. villosus* and *O. natrix* on the mycelia growth of *P. digitatum* [31, 32]. Similarly, Talibi et al. [6] reported a high inhibitory effect of the extract of *P. atlantica* on mycelia growth of *Geotrichum candidum*.

For the extract of *C. siliqua*, it showed no inhibitory effect on the growth of *P. digitatum* at a concentration of 1.25 mg/ml despite its high total phenols and flavonoids contents, and antioxidant capacity. This is consistent with another work undertaken on the germination of *Penicillium italicum* [33].

Inhibition of mycelia growth is attributed to polyphenols which are capable of forming complexes with enzymes like kinases [35]. Other mechanisms of inhibition are based on the depletion of iron by chelation [36]. Incidentally, flavonoids are capable of chelating certain metals and therefore inhibit the reactions of Fenton and Haber-Weiss, which are important sources of active oxygen radicals [37, 38].

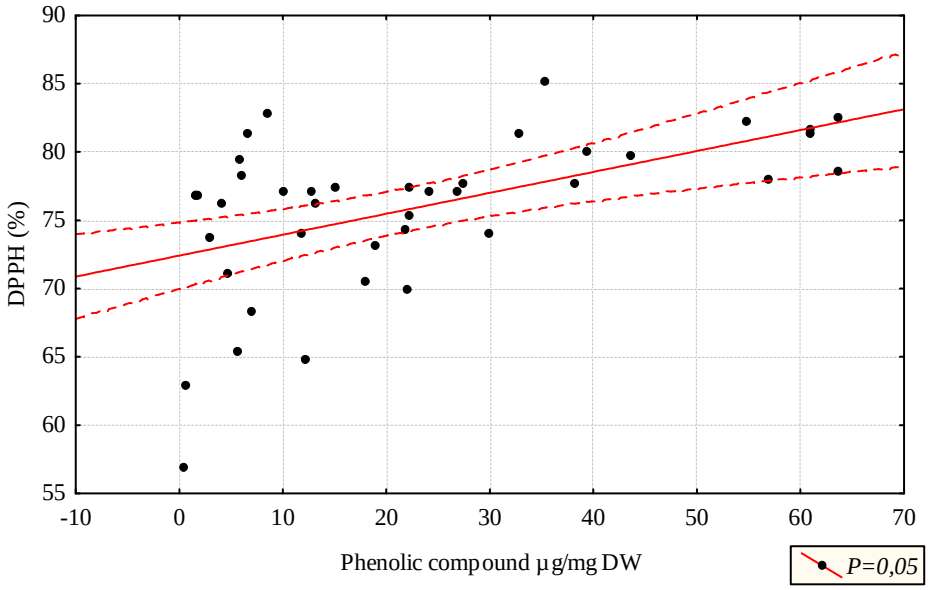
Table 2. Antioxidant activity of plant extracts

Family	Species	DPPH (%)	FRAP (µmole/g DW)
Anacardiaceae	*Pistacia atlantica*	81.33±0.24	1010.75±6.36
	Pistacia atlantica	81.33±0.24	1010.75±6.36
	Pistacia lentiscus	82.23±0.28	909.37±4.58
	Rhus pentaphylla	80.12±0.24	932.41±5.15
	Rhus tripartita	81.33±0.17	846.39±3.61
Apiaceae	*Carum carvi*	76.81±0.42	45.47±0.93
	Carum carvi	79.52±0.10	107.53±1.54
Apocynaceae	*Nerium oleander*	77.11±0.33	143.63±1.54
Asclepiadaceae	*Periploca laevigata*	78.61±0.20	841.78±3.00
Asteraceae	*Clandanthus arabicus*	71.08±0.40	74.35±1.13
	Launea arborescens	64.76±0.38	301.08±1.50
	Pulicaria glandulosa	77.11±0.27	565.28±3.35
	Senecio anteuphorbium	76.81±0.17	39.78±0.78
Brassicaceae	*Diplotaxus catholica*	76.81±0.28	31.95±1.56
Cactaceae	*Opuntia ficus indica*	78.31±0.16	66.36±0.45
Chenopodiaceae	*Chenopodium ambrosioides*	73.80±0.28	57.14±0.35
Cistaceae	*Cistus villosus*	81.63±0.42	83.41±0.57
Cupressaceae	*Juniperus oxycedrus*	74.40±0.21	599.08±3.08
Euphorbiaceae	*Ricinus communis*	74.10±0.57	144.09±1.75
Fabaceae	*Ceratonia siliqua*	78.01±0.23	854.07±3.18
	Ononis natrix	82.83±0.13	95.39±1.98
	Trigonella foecum graecum	56.93±0.21	24.42±0.33
Lamiaceae	*Lavandula dentata*	0.35 ± 76.20	486.94± 3.42
	Lavandula multifida	74.10±0.14	325.65±3.20
	Lavandula stoechas	77.41±0.13	436.25±1.63
	Marrubium vulgare	65.36±0.27	66.67±1.73
	Thymus leptobotrys	77.41±0.33	513.06±1.78
	Thymus saturieodes	75.30±0.17	150.08±1.22
Lythraceae	*Lawsonia inermis*	77.71±0.17	479.26±2.97
Moraceae	*Ficus carica*	68.37±0.35	121.35±1.84
Oleaceae	*Olea europea subsp maroccana*	73.19±0.24	612.90±3.51
Papaveraceae	*Papaver rhoeas*	62.95±0.24	33.18±0.41
Polygonaceae	*Rumex thyrsoides*	81.33±0.25	94.16±0.78
Rhamnaceae	*Rhamnus alaternus*	69.88±0.30	344.09±1.44
Rosaceae	*Rosa canina*	77.71±0.33	788.02±3.62
Solanaceae	*Withania frutescens*	77.11±0.31	342.55±3.32
Verbinaceae	*Vitex agnus-castus*	70.48±0.20	347.16±0.88
Vitaceae	*Vitis vinifera*	85.24±0.30	474.65±1.60

A: Phenolic compound µg/mg DW vs. DPPH (%)

Y = 72,408 + 0,15324 X

Correlation: r = 0,52



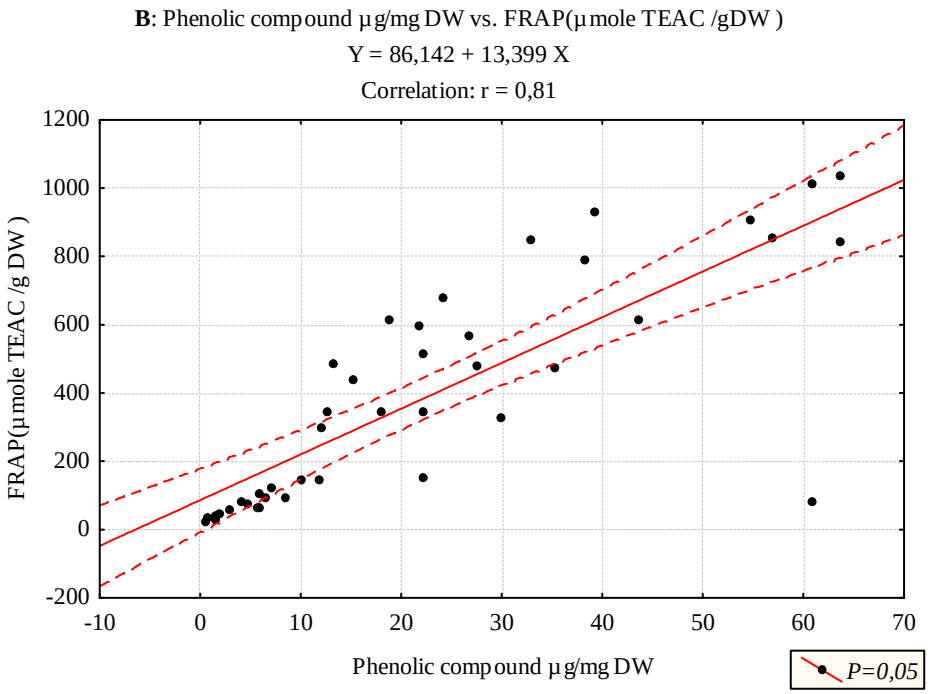


Figure 2. Correlation between phenolic content and DPPH inhibition (A), phenolic content and FRAP (B)

In addition, the inhibition rate obtained in our study was less than 60%. This could be explained by the concentration of our extracts which was lower than that used by other authors [6, 33, 34]. Our results also showed that for the concentration of 0.625 mg/ml (figure 2), some plant extracts promoted mycelia growth of *P. digitatum*. This was the case of *S. anteuphorbium*, *L. inermis R. alaternus*, *R. tripartita* and *P. laevigata*. Indeed, the plant extracts contain a mixture of diverse compounds. It is possible that in the same extract, compounds have inhibitory activity while others promote mycelia growth. For this reason, it would be better to conduct the study of the antifungal effect on fractions or purified products.

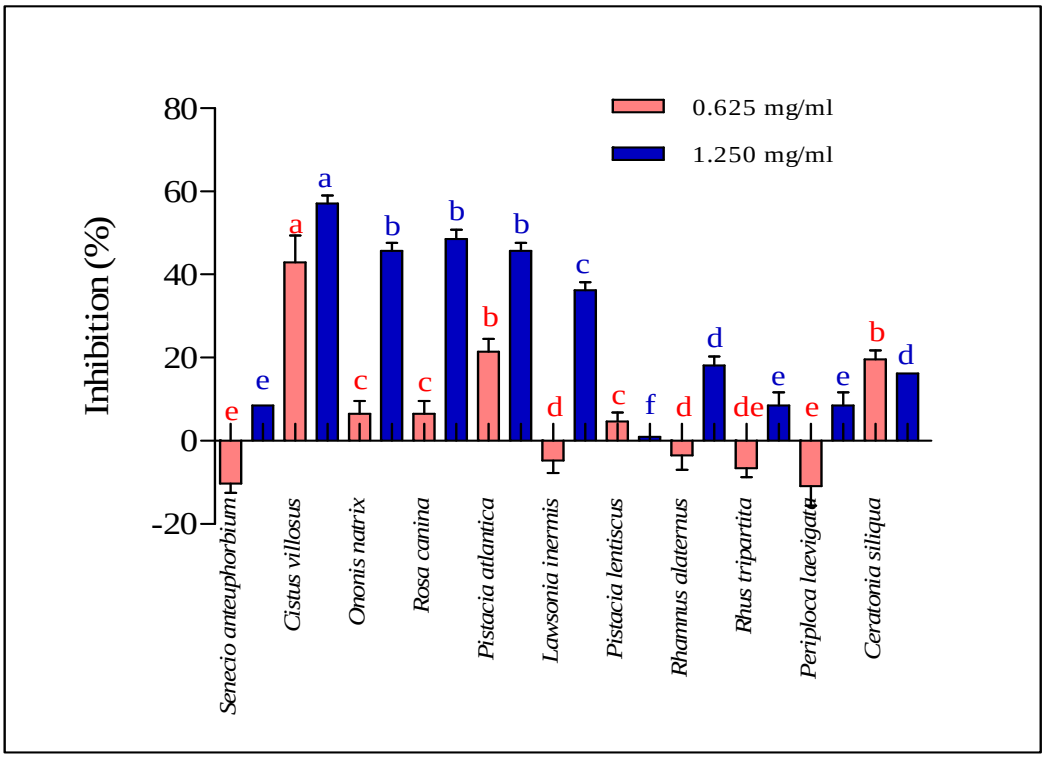


Figure 2. Antifungal effect of plants extracts against *Penicillium digitatum*

For each concentration, values followed by the same letter are not significantly different at *P=0.05.*

CONCLUSION

Five aromatic and medicinal plants showed significant antifungal activity. This was *C. villosus, O. natrix, R. canina, P. Atlantica* and *L. Inermis.* These plants also have a high content of polyphenols and a strong antioxidant effect. Thus, extracts of these plants, especially that of *P. atlantica*, could be considered as a potential alternative to the use of antifungal chemicals that have a detrimental effect on the environment and public health.

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