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Anti-fungal properties of 43 plant species against *Alternaria solani* and *Botrytis cinerea*

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Investigation of plants containing natural anti-microbial metabolites for plant protection has been identified as a desirable method of disease control. Crude methanolic extracts of 43 plant species belonging to 27 families, which most of them are medicinal plants, mostly collected from the west of Iran were screened for anti-fungal activity against two economically important phytopathogenic fungi, *Alternaria solani* and *Botrytis cinerea* during 2010–2012. Bioassay of the extracts was conducted by agar diffusion method on agar plate cultures with five replications. Among all the 43 plant methanolic extracts, mycelia growth of *A. solani* and *B. cinerea* was reduced by 28 (65%) and 30 (70%) plant extracts when compared to the control, respectively. The strongest extracts with more than 50% inhibition against *A. solani* were *Elaeagnus angustifolia*, *Dodonaea viscosa*, *Haplophyllum perforatum* and inflorescence of *Allium hirtifolium*, respectively. Leaves of *A. hirtifolium*, *H. perforatum*, inflorescence of *A. hirtifolium* and *D. viscosa* showed highest inhibitory effect ($\geq 50\%$) against *B. cinerea*. Moreover, complete inhibition of leaves of *A. hirtifolium* against *B. cinerea* was due to their fungistatic activity. The results of this experiment and high number of plants with anti-fungal activity showed that the flora in the west of Iran could be regarded as a rich source of plants with anti-fungal activity. Therefore, further screening of other plant species, identifying active fractions or metabolites and *in vivo* application of active extracts are warranted.

Keywords: agar diffusion; *Allium hirtifolium*; Anti-fungal activity; medicinal plant; methanolic extract

1. Introduction

Plant diseases worldwide are responsible for about 14% crop losses (Agrios 2005) and 20% for major foods and cash crops (Oerke et al. 1994). As compared to other plant parasites, fungi cause the greatest impact with regard to diseases and crop production losses. The most popular method of protecting the plants against the fungal attack is the use of chemical fungicides. However, they are not considered as a long-term solution due to the concerns associated with pesticides application such as problems of public health, environmental pollution, reduction in crop quality, toxic effect on non-target organisms and causing resistance in pest and disease agents (Rai & Carpinella 2006). Natural products seem to be a viable solution to the environmental problems caused by the synthetic pesticides and many researchers are trying to identify the effective natural

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products to replace the synthetic pesticides (Kim et al. 2005). Investigation of plants containing natural anti-microbial metabolites for plant protection has been identified as a desirable method of disease control (Kim et al. 2002; Rai & Carpinella 2006). Plant metabolites and plant-based pesticides appear to be one of the better alternatives in plant disease management, as they are known to have minimal harmful impact on the environment and danger to human in contrast to the synthetic pesticides (Varma & Dubey 1999). Therefore, considerable research to search for biocides that are environmentally safe and easily biodegradable have been carried out during last two decades (Tegegne et al. 2008).

Iran is divided to 31 provinces. Kermanshah and Hamadan, with a vast range of climatic conditions and rich plant variation are located in the west of the country. Therefore, it is expected to find significant variation in plants and secondary metabolites with anti-fungal activity. Iranian plants have been screened previously for anti-microbial activity (Sardari et al. 1998; Bazzaz & Haririzadeh 2003; Shahidi Bonjar et al. 2004), but with a focus on activity against agents of diseases in human. There have been no comprehensive screening studies for activity of Iranian plants against the phytopathogenic fungi.

In this study, two destructive phytopathogenic fungi, *Alternaria solani* (Ellis & G. Martin) L.R. Jones & Grout and *Botrytis cinerea* Pers. were considered to test the anti-fungal activity of plant species. Early blight is one of the main diseases of tomatoes, potatoes and eggplants all over the world. The disease is caused by the species of *Alternaria* and the most destructive and important species is *A. solani*. In humid areas with frequent rains followed by hot and dry weather, the disease inflicts considerable reductions in yield (Yazici et al. 2011). In Iran, the amount of tomato infection in Jiroft and Kahnouj has been reported between 60 and 90% (Shahbazi et al. 2011). There are some methods such as fungicides application, resistant varieties, biological elements and crop rotation to control this disease (Neeraj & Verma 2010). Grey mould caused by *B. cinerea* is an economically important disease worldwide (Takagaki et al. 2010). *B. cinerea* is a fungal pathogen that causes grey mould in many fruit, vegetable and ornamental crops (Vinale et al. 2009). It can infect flowers and green parts of tissues of plants that belong to Cucurbitaceae, Solanaceae and different vegetables.

Given the effect of the plant species origin and genetic diversity on chemical composition, studies screening for novel anti-fungal compounds in plants from different parts of the world are needed. Therefore, regarding the importance of screening plant crude extracts as first step of the project and the importance of bioactive crude extracts as eco-friendly agents, 43 randomly collected plant species in Kermanshah and Hamadan were collected, screened and assessed for anti-fungal activity against *A. solani* and *B. cinerea*.

2. Materials and methods

Forty-three plant species from 27 families were collected from the various parts of the provinces of Kermanshah and Hamadan in western Iran except *Adiantum capillus veneris* L. which was collected from Behshahr located in Mazandaran (Table 1) during 2010–2012. As a part of a wider screening programme, plants were randomly collected to increase the chance of finding plants with bioactive extracts. The plants were identified by Razi University, College of Agriculture at Herbarium and the scientific names were checked in the International Plant Names Index (<http://www.ipni.org/ipni/plantnamesearchpage.do>). Each sample was cleaned, air-dried in the shade and ground to a fine powder with a coffee grinder. Two economically important phytopathogenic

Table 1. *In vitro* screening for anti-fungal activity of plant extracts. Each one was calculated from five replicates.

Plant	Family	Location	Part used	Plant pathogen	
				<i>Alternaria solani</i>	<i>Botrytis cinerea</i>
<i>Adiantum capillus veneris</i> L.	Adiantaceae	Behshahr	Total	1.60 ± 0.49*	10.76 ± 1.52
<i>Allium hirtifolium</i> Boiss.	Alliaceae	Tuiserkan	Leaf	21.42 ± 2.77	100.00 ± 0.00
<i>Allium hirtifolium</i> Boiss.	Alliaceae	Tuiserkan	Stem	6.89 ± 5.51	49.15 ± 1.15
<i>Allium hirtifolium</i> Boiss.	Alliaceae	Tuiserkan	Inflorescence	50.92 ± 5.87	73.73 ± 3.20
<i>Amaranthus blitoides</i>	Amaranthaceae	Kermanshah	Total	8.86 ± 1.98	13.08 ± 2.23
<i>Artedia squamata</i> L.	Apiaceae	Sarpole zahab	Total	13.14 ± 1.13	17.99 ± 1.05
<i>Jorygium</i> sp.	Apiaceae	Sarpole zahab	Shoot	-2.86 ± 0.00	15.46 ± 1.37
<i>Johrenia aromatica</i> Rech.f.	Apiaceae	Kerend gharb	Shoot	7.90 ± 2.73	0.68 ± 0.93
<i>Anthemis altissima</i>	Asteraceae	Sarpole zahab	Total	22.68 ± 0.70	23.91 ± 2.70
<i>Centaurea</i> sp.	Asteraceae	Javanrood	Total	4.80 ± 1.09	-6.08 ± 2.40
<i>Centaurea iberica</i> Sennen and Elias	Asteraceae	Sarpole zahab	Total	20.61 ± 0.56	15.37 ± 1.38
<i>Centaurea solstitialis</i> L.	Asteraceae	Kermanshah	Total	37.03 ± 0.49	35.28 ± 2.52
<i>Chardinia orientalis</i> (L.) Kuntze	Asteraceae	Tuiserkan	Total	-2.63 ± 0.34	0.12 ± 1.72
<i>Cichorium intybus</i> L.	Asteraceae	Kermanshah	Total	13.06 ± 2.35	3.64 ± 1.96
<i>Cousinia stenocephala</i> Boiss.	Asteraceae	Kerend gharb	Total	40.86 ± 1.18	48.88 ± 3.30
<i>Senecio vulgaris</i> L.	Asteraceae	Kermanshah	Total	ND	15.31 ± 2.21
<i>Bongardia chrysogonum</i>	Berberidaceae	Palangan	Total	-4.92 ± 0.72	3.92 ± 5.11
<i>Trichodesma zeylanicum</i> R.Br.	Boraginaceae	Tuiserkan	Shoot	-3.09 ± 0.00	-2.75 ± 0.34
<i>Conringia orientalis</i>	Brassicaceae	Kermanshah	Total	-16.43 ± 0.00	9.54 ± 0.47
<i>Crabe orientalis</i> L.	Brassicaceae	Bide sorkh	Total	ND	-7.62 ± 0.99
<i>Cerastium dichotomum</i> L.	Caryophyllaceae	Kermanshah	Total	-20.46 ± 1.63	-1.06 ± 2.70
<i>Minuartia meyeri</i>	Caryophyllaceae	Kermanshah	Total	-5.34 ± 2.83	4.94 ± 0.90
<i>Iaccaria pyramidata</i> Medik.	Caryophyllaceae	Sarpole zahab	Total	6.91 ± 5.19	4.12 ± 1.49
<i>Perocephalus canus</i>	Dipsacaceae	Tuiserkan	Total	-37.40 ± 0.00	-0.98 ± 3.18
<i>Elaeagnus angustifolia</i> L.	Elaeagnaceae	Homail	Flower	61.25 ± 1.46	7.46 ± 3.71
<i>Ricinus communis</i> L.	Euphorbiaceae	Kermanshah	Stem + leaf	13.48 ± 8.30	13.87 ± 2.87
<i>Onobrychis sativa</i> Lam.	Fabaceae	Kermanshah	Total	-20.95 ± 10.25	-8.73 ± 1.86
<i>Sophora alopecuroides</i> L.	Fabaceae	Kermanshah	Total	8.86 ± 4.59	-2.69 ± 3.62
<i>Nepeta crispa</i> Willd.	Lamiaceae	Tuiserkan	Shoot	16.03 ± 1.66	14.64 ± 2.03

(Continued)

Table 1. (Continued).

Plant	Family	Location	Part used	Plant pathogen	
				<i>Alternaria solani</i>	<i>Botrytis cinerea</i>
<i>Vitex pseudonegundo</i>	Lamiaceae	Sarpole zahab	Leaf + inflorescence	31.85 ± 15.00	44.18 ± 4.74
<i>Allium eriophyllum</i> Boiss.	Liliaceae	Sarpole zahab	Leaf	-9.46 ± 0.99	ND
<i>Allium noeanum</i> Reut.	Liliaceae	Sarpole zahab	Leaf	47.31 ± 4.96	10.25 ± 1.46
<i>Fumaria officinalis</i> L.	Papaveraceae	Kermanshah	Total	19.21 ± 9.17	10.38 ± 2.81
<i>Pinus eldarica</i> Medw.	Pinaceae	Kermanshah	Leaf	-12.67 ± 0.52	6.28 ± 0.94
<i>Plantago lanceolata</i> L.	Plantaginaceae	Sarpole zahab	Total	-5.17 ± 0.85	-6.96 ± 1.48
<i>Acantholimon</i> sp.	Plumbaginaceae	Tuiserkan	Total	15.81 ± 1.93	-2.06 ± 0.78
<i>Rheum rhabarbarum</i> L.	Polygonaceae	Kermanshah	Shoot	-4.55 ± 3.42	11.52 ± 1.35
<i>Portulaca oleracea</i> L.	Portulacaceae	Sarpole zahab	Total	-46.55 ± 0.61	0.11 ± 0.33
<i>Delphinium comptocarpum</i>	Ranunculaceae	Kermanshah	Total	13.74 ± 2.79	12.54 ± 1.49
<i>Prunus amygdalus</i> var. <i>amara</i>	Rosaceae	Tuiserkan	Leaf	-37.40 ± 0.00	-14.74 ± 0.45
<i>Citrus grandis</i>	Rutaceae	Market	Fruit	22.59 ± 0.57	9.36 ± 1.96
<i>Haplrophyllum perforatum</i> (M.B.) Kar. & Kir.	Rutaceae	Tuiserkan	Total	55.44 ± 1.33	76.32 ± 1.48
<i>Dodonaea viscosa</i> Jacq.	Sapindaceae	Ghaser shirin	Leaf + flower	57.26 ± 1.56	58.07 ± 1.14
<i>Bellardia</i> sp.	Scrophulariaceae	Tuiserkan	Total	6.85 ± 3.63	-9.58 ± 0.49
<i>Tribulus terrestris</i> L.	Zygophyllaceae	Kermanshah	Total	23.78 ± 5.93	11.52 ± 3.10

Notes: *Percentage of inhibition ± standard error, n = 5; ND, not done.

fungi, *A. solani* and *B. cinerea* were provided by Plant Pathology Laboratory, Campus of Agriculture and Natural Resources, Razi University.

The powdered plant materials were extracted at room temperature using methanol. Methanolic extracts were obtained as described by Bahraminejad et al. (2008). Briefly, 5 g of ground sample was extracted with 100 ml methanol for 24 h by shaking at 300 rpm. Then, 30 ml distilled water was added to 70 ml of the methanolic extract and lipids were removed with 100 ml n-Hexane mixed at 250 rpm for 2 h. Methanolic phase was concentrated using a rotary evaporator. Finally, the residues were dissolved in 45% methanol in distilled and sterilised water and a sample of the extract at a concentration of 100 mg/ml was provided.

In agar diffusion method, initially the required amount of extract was solubilised in one ml 50% methanol and was shaken well by a vortex for preparing 2000 ppm concentrations. The potato dextrose agar medium was sterilised at 121 °C for 20 min and 1 atmosphere pressure. The prepared extract was added to culture medium when the temperature of the medium decreased to about 40 °C. The culture media loaded by 1 ml 50% methanol was considered as a control. The culture media immediately was poured into plates. A 6 mm diameter plug of 7 day fungal colonies was placed at the centre of the plates. Plates were incubated at 25 ± 4 °C and diameter of colony was measured until the control plates or one of the treatments was covered by the fungus mycelia completely. The experiments were performed in five replicates. Percentage of inhibition of growth for each fungus was calculated based on conventional formula (Sarkar et al. 2003).

$$IP = [(C - T)/C] \times 100$$

IP = percentage of mycelia growth inhibition; *C* = mean diameter (mm) of the control; *T* = mean diameter (mm) of tested concentration.

3. Results and discussion

The present study tested the anti-fungal activity of crude methanolic extracts of 43 plants species belonging to 27 families against *A. solani* and *B. cinerea*. The data from Table 1, Figures 1 and 2 revealed that mycelia growth of both the fungi was affected by the tested extracts. Among all the 43 plant methanolic extracts, mycelia growth of *A. solani* and *B. cinerea* was reduced by 28 (65%) and 30 (70%) plant extracts when compared to the control, respectively. Four extracts showed very low inhibitory or stimulatory effect (≤1%) on the growth of *B. cinerea*. The strongest extracts with more than 50% inhibition against *A. solani* were *Elaeagnus angustifolia* L., *Dodonaea viscosa* Jacq., *Haplophyllum perforatum* (MB.) Kar. & Kir. and inflorescence of *Allium hirtifolium* Boiss., respectively. Whereas, the most stimulating extract for this fungi was *Portulaca oleracea* with 47% stimulatory effect. Leaves of *A. hirtifolium*, *H. perforatum*, inflorescence of *A. hirtifolium* and *D. viscosa* (≥50%) showed highest inhibitory effect against *B. cinerea*. As shown in Table 1, *Prunus amygdalus* var. *amara* stimulated the mycelia growth of the fungus (about 15%).

Results indicated that the growth of both the fungi was reduced more than 50% when they exposed to the extracts of *H. perforatum*, inflorescence of *A. hirtifolium* and *D. viscosa*. It means that these plant species probably have broad spectrum of anti-fungal activity which needs further research on their effect against more fungi. The data in Table 1 indicated that different tested plant parts of *A. hirtifolium* inhibited the growth of both the fungi at different level of inhibition and this inhibition against *B. cinerea* was more than *A. solani*. These findings could help to conclude that the amount and

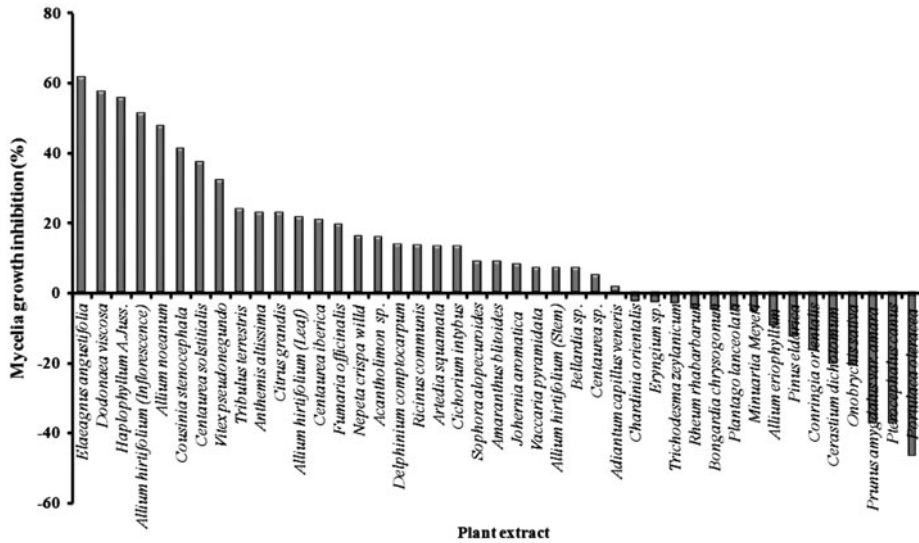


Figure 1. Anti-fungal activities of methanolic extracts of 43 plant species against *A. solani*.

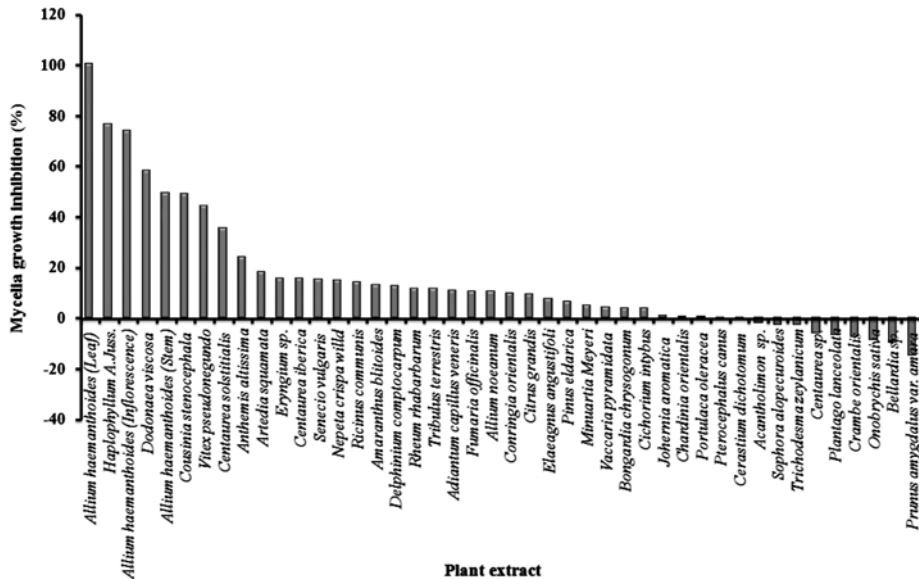


Figure 2. Anti-fungal activities of methanolic extracts of 43 plant species against *B. cinerea*.

perhaps kinds of anti-fungal compounds were varied in different plant parts. Regarding 100% inhibition of leaves of *A. hirtifolium* against *B. cinerea*, the fungicidal or fungistatic activity of this plant extract was tested. Results of this experiment showed that the observed activity is fungistatic because mycelia growth was observed when the inhibited plug was cultured in a new medium without extract.

The biological activity of significant plant species in this research was previously documented. The Persian shallot (*A. hirtifolium*) as one of the strongest plant species against both the fungi, is an annual herbaceous plant that belongs to Alliaceae which

grows wildy in the Zagros Mountains, Iran. There are more than 500 species in the genus of *Allium* (Fateh et al. 2010). In *Allium* plants, different biological active compounds such as, alliin, allicin, allicipin, saponins, steroids, flavones, fistulosin, polyphenol carboxylic acids and ajoene were reported (Carotenuto et al. 1999; Phay et al. 1999; Barile et al. 2007; Zill-e-Huma et al. 2009). Bagiu et al. (2012) showed that allicin and S-methyl cysteine in *A. ursinum* were responsible to the observed anti-microbial activity. Besides, it was reported that the anti-fungal activity of the flower extract of this plant species was stronger than leaf extract. This was because of higher allicin content in flower part of the plant (Parvu et al. 2011). Parvu et al. (2009) stated that alliin is an important anti-fungal active compound in hydroalcoholic extract of *A. obliquum*. The quality and quantity of the anti-microbial substances in *Allium* sp. depend on plant part used, plant species, harvest time and geographical conditions. Therefore, the anti-fungal activity observed in this study could be due to the presence of allicin, alliin, saponin, S-methyl cystein or a combination of all of them in *A. hirtifolium*.

The genus *Haplophyllum* from Rutaceae comprises about 50 species and is distributed from Africa to Eurasia. About 30 species of this perennial plant grow in Iran and 14 of them are endemic to it. *Haplophyllum* contains several quinoline alkaloids (Staerk et al. 2009). In this study, it was shown that *H. perforatum* collected from Tuiserkan contains strong anti-fungal activity. Our results are in accordance with the previous findings reported by Cantrell et al. (2005), Bahraminejad et al. (2011), Bahraminejad (2012) and Bahraminejad et al. (2012) who found that this plant has anti-fungal activity. Cantrell et al. (2005) concluded that quinoline alkaloids especially flindersine are responsible for the observed anti-fungal activity.

Dodonaea viscosa belonging to Sapindaceae is known as a medicinal plant with different biological activity. Anti-inflammatory, anti-ulcer and anti-microbial activities of the leaves of *D. viscosa* are some of its properties (Sama et al. 2008). The presence of flavonoids, terpenoids and saponins like aliarin, dodonic acid, viscosol stigmasterol, isorhamnetin, penduletin, quercetin, doviscogenin dodonosides A and B in *D. viscosa* were previously documented (Wagner et al. 1987; Khan et al. 1988). The inhibitory activity of the crude extract of *D. viscosa* was already reported against Gram-positive bacteria (Getie et al. 2003).

E. angustifolia as a tree belonging to Elaeagnaceae grown wildy in the west of Iran contains flavonoids compounds, sitosterols, cardiac glycosides, terpenoids, vitamins B and A and also vitamin K in its aqueous fruit extract (Beigom Taheri et al. 2010). The anti-bacterial activities of different parts of *Elaeagnus umbellata* were documented by Sabir et al. (2007). As flavonoids and saponins are known as anti-microbial compounds, it could be valuable to correlate the amount of isolated compounds with the observed anti-fungal activity to determine whether these compounds are responsible for the anti-fungal activities observed.

P. oleracea with high stimulating activity of the growth of *A. solani* (in this research) and belonging to the family Portulacaceae is an herbaceous plant widely distributed throughout the world. It contains vitamin C, oleoresins-I and II, saponins, tannins, saccharides, triterpenoids, α -tocopherol and glutathione (Mizutani et al. 1998). As some of the mentioned secondary metabolites are known as anti-microbial compounds, high content of these compounds could be the candidate for anti-microbial activity (Chan et al. 2000; Oh et al. 2002). Although, the anti-*Pythium* and anti-*Phytophthora* activity of the metanolic extract of *P. oleracea* was previously reported by Bahraminejad (2012), Bahraminejad et al. (2012), it was found that the extract of *P. oleracea* stimulate the growth of the *A. solani* by 47% when compared to the control.

As Chitwood (2002) stated, the results of these kinds of research could help to develop new natural fungicide and chemically synthesised derivatives. These results will also help to find out the active metabolites in active plants and subsequently used in reverse genetic engineering from metabolites to genes. The findings of this study encouraged us to continue screening more plant species for anti-fungal agents. They may form the basis of further investigation on fractionation for finding active fractions, the effect of origin of growth on the quality and quantity of active compounds, the amount of bioactive compounds in different plant parts and finally *in vivo* application of the extracts.

The conclusions drawn from this research are: (1) A high variation was found among the activity of plant extracts against *A. solani* and *B. cinerea*, (2) The flora in the west of Iran could be regarded as a rich source of plants with anti-fungal activity, (3) The extracts of *H. perforatum*, inflorescence of *A. hirtifolium* and *D. viscosa* inhibited the growth of both the fungi and (4) Leaves of *A. hirtifolium* completely inhibited the growth of *B. cinerea* and this inhibition had fungistatic effect. This plant species could be suggested for more investigation especially on the *in vivo* research.

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