Phytochemical Analysis and Antifungal Activity of Anvillea Radiata

Mebarki Lakhdar, Kaid Harche Meriem, Benlarbi Larbi, Rahmani Amina and Sarhani Aicha

Laboratory of Production, Vegetal and Microbial Valorization, Department of Biotechnology, Sciences Faculty, University of Sciences and Technology Mohamed Boudiaf, P.O. Box. 1505, El M’n’naouer, Oran, 31000, Algeria

Laboratory of valorization of vegetal Resource and Food Security in Semi Arid Areas, South West of Algeria, BP 417, University of Bechar, Algeria

Laboratory of valorization of vegetal Resource and Food Security in Semi Arid Areas, South West of Algeria, BP 417, University of Bechar, Algeria

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Abstract: Recently, natural products have been evaluated as sources of antimicrobial agents with efficacies against a variety of microorganisms. This study described the phytochemical screening and the antifungal activity of Anvillea radiata. Phytochemical analysis revealed the presence of some chemical groups such as Volatile oils, Fatty acids, Tannins, Flavonoids, Anthracénosides, Emodols, Saponins, Free quinones, Anthraquinones, Alkaloids, Sterols and triterpenes. The antifungal activity of flavonoid extracts and cell-wall polysaccharide extracts derived from the flower and leaves of A. radiata were tested against plant pathogenic fungi Fusarium oxysporum f. sp. albedinis (Foa) (causing vascular wilt of date palm) by agar well diffusion method. The results indicate that flavonoid extracts had the strongest inhibitory effects on spore germination and on soil population density of Foa. Highly methylated pectins (HMP) from flowers produce the greatest inhibitory effect on mycelial growth. The sporulation was strongly inhibited using cellulose-based agar of leaves. Thus, it can be concluded that the use of A. radiata extracts could be considered as an antifungal available to develop novel types of natural fungicides and to control several plant pathogenic fungi.

Key words: Antifungal activity · Anvillea radiata · Flavonoids extracts · Fusarium oxysporum f. sp. albedinis · Phytochemical screening · Polysaccharide extracts

INTRODUCTION

Since ancient times, people have been exploring the nature particularly plants in search of new drugs [1, 2]. Medicinal plants represent a rich source of antimicrobial agents and they are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [3, 4]. Drugs from plants can be derived from barks, leaves, flowers, roots, fruits, seeds [5]. In addition, knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [6]. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated [7]. In this context, Anvillea radiata is a plant in Asteraceae family that grows in northern Africa and particularly in the two Maghreb countries, Morocco and Algeria. It is widely used in traditional medicine for the treatment of dysentery, gastric-intestinal disorders [8], chest cold and has been reported to have hypoglycemic activity [9]. Although so far there has been no study on antimicrobial effects and phytochemical constituents of this species.

Hence, the main goal of this report was studying on the qualitative phytochemical analysis of Anvillea radiata, of South-western region of Algeria and on the antifungal effects of its polysaccharide extracts and flavonoid extracts against Foa, the causal agent of vascular wilt of date palm called "Bayoud disease".
MATERIALS AND METHODS

Plant Material: At flowering stage, Fresh parts (leaves and flowers) of *Anvillea radiata* were collected from the area of Bechar (Southwest of Algeria). The harvested parts were shade dried. After drying, the plant materials were ground well using mechanical blender into fine powder and transferred into airtight containers for future use.

Plant Pathogenic Fungi: One isolate of Foa, was used in this study. The fungus was isolated from rachis of infected date palm in infested palm grove in the region of Bechar, Algeria. This fungal species was maintained on potato dextrose agar acidified (PDAa) and stored at 5°C for further study.

Phytochemical Analysis: Phytochemical tests were performed on different extracts prepared from the dried plant materials and ground, using four solvents of different polarities: water, ethanol, diethyl ether and petroleum ether. Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described by various authors [10, 11].

Preparation of Plant Extracts:
Preparation of Flavonoids Extract: A large number of flavonoid extraction methods have been developed in the past few years [12]. In this study flavonoid extracts were prepared as described by Lee et al. [13]. 50 g of plant powder (leaves and flowers of *Anvillea radiata*) were heated separately at 90°C under reflux in a mixture of distilled water/ethanol (250 ml/250 ml) for 4 h and the extract was filtered through a filter paper. The aqueous-ethanolic phase was evaporated until eliminating ethanol and was extracted with 100 ml of n-butanol, then was acidified to pH = 3 with 10% HCl and then the n-butanol phase was dried. The dry residue was extracted three times with a mixture of distilled water/ethyl acetate (100 ml/100 ml) for one hour and the organic phase was basified to pH = 9 with NaOH. After 15 minutes of rest, the organic phase (represents flavonoids extract) was dried before being weighed and recovered in 1% ethanol for biological tests.

Preparation of the Cell-Wall Polysaccharides Extracts: According to Harche et al. [14] the extraction of cell-wall polysaccharides requires a preliminary operation consists in the preparation of the parietal residue. For this, each of the powdered plant materials (30 g) was put in an erlenmeyer flask containing a mixture of methanol and chloroform (1:1, v/v) and was stirred for 14 h under a fume hood in order to remove the lipid soluble, tannins and other cytoplasmic constituents. The operation was repeated twice. After filtration, the residue was placed in 95% ethanol for 2 h with stirring to remove traces of chloroform and then was incubated in boiling 95% ethanol for 2 h to get better elimination of chloroform traces. The residue was then dried in an oven at 60°C for 48 h [15].

Extraction of Cellulose and Hemicelluloses: Cellulose and hemicelluloses were obtained by putting 5 g of parietal residue in an erlenmeyer flask containing 100 ml of 4% NaOH and were stirred for 14 h. After filtration, the residue was rinsed with distilled water and then with acetone, then the residue was dried in an oven at 60°C for 14 h before being weighed. The resulting part represents the cellulosic fraction [16].

The two filtrates obtained were neutralized by pure acetic acid and then precipitated in ethanol (1:3, v/v) for 14 h. After centrifugation at 3600 g at room temperature for 30 min, the pellet was washed with distilled water and then with acetone. Next, it was dried in an oven at 60°C for 14 h and finally weighed. This part represents the hemicellulosic fraction [16].

Extraction of HMP: HMP extracts were prepared by putting 5 g of parietal residue in an erlenmeyer flask containing 100 ml of distilled water and stirred for 14 h at room temperature and were put to boiling under reflux twice for two hours. After filtration, the filtrate was concentrated in a rotary evaporator. Then it was precipitated in cold acetone (1:2, v/v) for 14 h. After centrifugation at 3600 g at room temperature for 30 min, the pellet was rinsed with distilled water and with alcohol and then the residue was dried in an oven at 50°C and was weighed. This part represents the HMP [17].

Antifungal Activity Assay: Before being added to the PDAa medium, hemicelluloses, HMP and flavonoids were diluted in NaCl solution (5 mM), sterile distilled water and in 1% ethanol respectively. A range of concentrations (0.25; 0.5; 1; 2 and 4 mg/ml of culture medium) of the extract were used. However, cellulose extracts were intended to reconstruct cellulose-based media (With the following composition : NaNO₂, 2 g; K₂HPO₄, 1
Spore Germination Assay: Spore germination assay was performed as described by Maouni et al. [19]: 0.1 ml of a spore suspension (10^5 spores/ml) of Foa, prepared in sterile distilled water (counting using a Malasséz cell) was spread on the Petri dishes containing PDA media with hemicellulose and HMP extracts (separately) at different concentrations and on the cellulose-based media. The Petri dishes were incubated at 25°C for 24 h. The counting of spores germinated or ungerminated was determined under microscope on a total of 200 spores. A spore is considered germinated if the germ tube length is greater than its diameter.

Mycelial Growth Assay: Petri dishes containing PDA media with extracts (hemicelluloses, HMP and flavonoids in separated experiments) at different concentrations were inoculated with a mycelial disc (6 mm diameter) obtained from a pure culture of Foa. The Petri dishes containing cellulose-based medium were inoculated in the same way. The Petri dishes were incubated at 25°C for 7 days. The diameter of the Foa colony was obtained by calculating the average of two perpendicular diameters [20].

Sporulation Assay: All colonies used to evaluate mycelial growth and incubated for 10 days at 25°C were used to study the effect of the extracts on sporulation. Sporulation assay was performed as described by Maouni et al. [19]. Control tests were performed under the same conditions in absence of extracts. The percentage of inhibition was calculated using the formula of Amadioha [21], as shown below: Inhibition (%) = (C - T). 100 / C.

Where C and T represent Foa germination (radial growth or sporulation) in control and treated plates, respectively.

Fusarium Soil Population Assay: Foa was incorporated into soil (previously sifted and sterilized) at an inoculum density of 10^6 spores/g of soil. Aliquots (10 g) of the infested soil were introduced aseptically into sterilized tubes and treated by 1, 5 and 10% (w/w) of cellulose, hemicellulose, HMP and flavonoids in separated experiments. Population density of Foa was determined using dilution plate techniques at 0 (before soil treatment), 1, 3, 7, 14 and 21 days after soil treatment [22]. 0.5 g of soil (sample unit) from each tube (replication) was placed in 5 ml of distilled water. 0.1 ml from the appropriate dilution (depending on the treatment) was pipetted onto the surface of Petri plates containing PDA media. Plates were then incubated at 25°C for 24 to 48 h. Colony forming units (CFU) were counted in Petri dishes and then reported per gram of soil. CFU were then transformed to log 10 (CFU/g of soil) [23]. Control tests (infested soil with no medicinal plant treatment) were performed under the same conditions.

RESULTS

Phytochemical analysis: The phytochemical screening of Anvillea radiata showed the presence of medically active compounds in this plant (Table 1).

From the table, it could be seen that, Volatile oils, Fatty acids, Tannins, Flavonoids, Anthracénosides, Emodols, Saponins, Free quinones were present in both organs studied. Anthraquinones, Sterols and triterpenes were absent in the leaves. Alkaloids were absent only from the flowers. But Starch and Reducing compounds were absent in the leaves and also in the flowers.

Antifungal Activity: On spore germination, the evaluation of the effectiveness of the extracts tested was based on the calculated percentages of spore germination (200 spores counted represent a percentage of 100%). The percentage of spore germination in control treatments was equal to 83.44% (Table 2). Comparing with the control test, spore germination was stimulated by the polysaccharide extracts at all the tested concentrations (with spore germination percentages ranging from 99.66% to 100%). By contrast, flavonoid extracts inhibited spore germination at all dosages. The strongest inhibitory activity was observed with floral flavonoid (48.66%) at high concentration. Cellulose-based media were also capable of inhibiting the spore germination (The inhibition rate has reached 16% and 46% on cellulose-based medium of leaves and flowers respectively).

For mycelial growth fungal strain colony radius in control treatments was equal to 45.9 mm (Table 3). From the concentration of 0.5 mg/ml all the incorporated extracts (hemicellulose, HMP and flavonoid) showed inhibitory effect with varying degrees of inhibition rate. An increase in antifungal activity was observed with increase in concentration. Comparing with
Table 1: Phytochemical constituent of *Anvillea radiata*

<table>
<thead>
<tr>
<th>Phytochemical Constituent</th>
<th>Starch compounds</th>
<th>Reducing sugars</th>
<th>Volatile fatty acids</th>
<th>Alkaloids</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Anthraquinones</th>
<th>Anthocyanosides</th>
<th>Sterols and triterpenes</th>
<th>Quinones</th>
<th>Anthraquinones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flowers</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2: Effect of *Anvillea radiata* extracts on spore germination of *Foa*

<table>
<thead>
<tr>
<th>Plant organ</th>
<th>Extracts</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>Flavonoid</td>
<td>69.66</td>
<td>59.66</td>
<td>61.66</td>
<td>45.83</td>
<td>83.44</td>
<td>83.44</td>
</tr>
<tr>
<td></td>
<td>hemicellulose</td>
<td>99.83</td>
<td>99.83</td>
<td>99.33</td>
<td>98.16</td>
<td>96.16</td>
<td>83.44</td>
</tr>
<tr>
<td></td>
<td>HMP</td>
<td>100</td>
<td>99.16</td>
<td>100</td>
<td>99.66</td>
<td>96.16</td>
<td>83.44</td>
</tr>
<tr>
<td>Flowers</td>
<td>Flavonoid</td>
<td>61.5</td>
<td>49.33</td>
<td>55.83</td>
<td>42.83</td>
<td>83.44</td>
<td>83.44</td>
</tr>
<tr>
<td></td>
<td>hemicellulose</td>
<td>97.33</td>
<td>100</td>
<td>98.33</td>
<td>100</td>
<td>99.83</td>
<td>83.44</td>
</tr>
<tr>
<td></td>
<td>HMP</td>
<td>98.6</td>
<td>99.83</td>
<td>100</td>
<td>99.66</td>
<td>97.5</td>
<td>83.44</td>
</tr>
</tbody>
</table>

Table 3: Effect of *Anvillea radiata* extracts on mycelial growth of *Foa*

<table>
<thead>
<tr>
<th>Plant organ</th>
<th>Extracts</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>Flavonoid</td>
<td>4.95</td>
<td>4.58</td>
<td>4.51</td>
<td>4.35</td>
<td>3.88</td>
</tr>
<tr>
<td></td>
<td>hemicellulose</td>
<td>4.45</td>
<td>4.37</td>
<td>4.05</td>
<td>4.07</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>HMP</td>
<td>4.63</td>
<td>4.56</td>
<td>4.4</td>
<td>4.03</td>
<td>3.77</td>
</tr>
<tr>
<td>Flowers</td>
<td>Flavonoid</td>
<td>4.63</td>
<td>4.56</td>
<td>4.4</td>
<td>4.03</td>
<td>3.77</td>
</tr>
<tr>
<td></td>
<td>hemicellulose</td>
<td>3.75</td>
<td>4.52</td>
<td>3.9</td>
<td>3.82</td>
<td>3.47</td>
</tr>
<tr>
<td></td>
<td>HMP</td>
<td>3.8</td>
<td>3.6</td>
<td>3.81</td>
<td>3.65</td>
<td>3.18</td>
</tr>
</tbody>
</table>

Table 4: Effect of *Anvillea radiata* extracts on sporulation of *Foa*

<table>
<thead>
<tr>
<th>Plant organ</th>
<th>Extracts</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>Flavonoid</td>
<td>0.243</td>
<td>0.286</td>
<td>0.857</td>
<td>16.55</td>
<td>19.91</td>
</tr>
<tr>
<td></td>
<td>hemicellulose</td>
<td>36.94</td>
<td>15.77</td>
<td>18.53</td>
<td>18.99</td>
<td>39.24</td>
</tr>
<tr>
<td></td>
<td>HMP</td>
<td>-11.67</td>
<td>0.090</td>
<td>0.353</td>
<td>24.37</td>
<td>31.59</td>
</tr>
<tr>
<td>Flowers</td>
<td>Flavonoid</td>
<td>36.80</td>
<td>0.21</td>
<td>0.353</td>
<td>24.22</td>
<td>31.59</td>
</tr>
<tr>
<td></td>
<td>hemicellulose</td>
<td>17.15</td>
<td>13.80</td>
<td>0.21</td>
<td>26.82</td>
<td>39.70</td>
</tr>
<tr>
<td></td>
<td>HMP</td>
<td>-07.14</td>
<td>-05.53</td>
<td>-11.90</td>
<td>11.63</td>
<td>28.20</td>
</tr>
</tbody>
</table>

Table 5: Soil population densities of *Foa* 21 days after soil treatment with *Anvillea radiata* extracts

<table>
<thead>
<tr>
<th>Plant organ</th>
<th>Extracts</th>
<th>1%</th>
<th>5%</th>
<th>10%</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>Flavonoid</td>
<td>4.3</td>
<td>4.47</td>
<td>5.44</td>
<td>5.57</td>
</tr>
<tr>
<td></td>
<td>hemicellulose</td>
<td>5.43</td>
<td>6.12</td>
<td>5.14</td>
<td>5.57</td>
</tr>
<tr>
<td></td>
<td>HMP</td>
<td>5.34</td>
<td>5.36</td>
<td>6.31</td>
<td>5.57</td>
</tr>
<tr>
<td></td>
<td>Cellulose</td>
<td>5.53</td>
<td>4.3</td>
<td>5.61</td>
<td>5.57</td>
</tr>
<tr>
<td>Flowers</td>
<td>Flavonoid</td>
<td>4.3</td>
<td>4.47</td>
<td>5.44</td>
<td>5.57</td>
</tr>
<tr>
<td></td>
<td>hemicellulose</td>
<td>5.44</td>
<td>5.07</td>
<td>4.9</td>
<td>5.57</td>
</tr>
<tr>
<td></td>
<td>HMP</td>
<td>5.71</td>
<td>5.2</td>
<td>5.38</td>
<td>5.57</td>
</tr>
<tr>
<td></td>
<td>Cellulose</td>
<td>5.71</td>
<td>5.76</td>
<td>5.71</td>
<td>5.57</td>
</tr>
</tbody>
</table>
the other extracts, HMP from flowers exhibited the most interesting inhibition on mycelial growth (30.71% at the highest concentration). On cellulose-based agar the mycelial hyphae elongation of Foa was occurred in a similar way as on PDAa without addings. But the significant difference is that the cellulose-based media have developed a very weakly dense mycelium compared to the PDAa with no addings.

Inhibitory but also stimulatory effect of conidial production by the tested compounds was found (Table 4).

In particular, flavonoid and hemicellulose from both organs inhibited sporulation of Foa at all dosages (the dose increase is not necessarily accompanied by better efficiency) while sporulation of the pathogen was only inhibited at the intermediate and high dosages of HMP from both organs. The highest sporulation inhibition was obtained with hemicellulose of flowers (39.70% at 4 mg/ml). In addition, cellulose-based media also greatly inhibited production of conidia of Foa (The inhibition percentage has reached 80% and 77% on the cellulose-based agar of leaves and flowers respectively).

Twenty-one days after soil treatment with 1%, flavonoid from both organs reduced population densities of Foa by 22.80%, compared with the nontreated control soil (Table 5).

However, the treatment with the other extracts has no significant effect. No significant change in the case of the treatment with 5% of Anvillea radiata extracts. The population of Foa in the soil treated with 10% of medicinal plants powders was not significantly less than in the untreated soil after 21 days.

DISCUSSION

Phytochemical screening conducted on the Anvillea radiata extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [24]. Analysis of the plant extracts revealed the presence of phytochemicals such as tannins, flavonoids, saponins, Volatile oils, Sterols, Anthracénosides, Emodols, triterpenes and alkaloids. The presence of these bioactive compounds indicates the medicinal value of this plant. Natural phytochemicals derived from fruits, vegetables and herbs have been reported to possess a wide range of biological effects, including antioxidant, antimicrobial and anti-inflammatory actions [25].

Various extract of medicinal plants have shown inhibitory effects against phytopathogenic fungi in vitro [26]. In this study, the biological tests showed that flavonoids exhibited appreciable inhibitory effect against Foa in all tests. Previous studies indicate that flavonoids of several plant extracts showed antifungal activities [27-29]. The other roles attributed to them were to promote physiological survival of the plant by protecting them from fungal pathogens (Galeotti et al., 2008) [30]. According to Huang and Chung [31], phenolic compounds caused swelling of hyphal tips, plasma seeping around hyphae, leaking of plasma, cell wall distortion, abnormal branching or fusion of hyphae and consequently wrinkling of hyphae surface.

Our results showed that polysaccharide extracts have presented an inhibitory effect both on the mycelial growth and sporulation neatly superior to that on the spore germination. The antifungal activity of polysaccharide extracts was also confirmed experimentally by Ballance et al. [32]. The antimicrobial properties of natural polysaccharides are based on their chemical structure where the presence of a highly reactive carbonyl group was detected. The carbonyl group is able to bond primary amines to produce a stable combination of polysaccharides with proteins (glycolconjugates). Bonding of exoenzymes of saprogenic microorganisms by reactive polysaccharides is likely the reason for their antimicrobial activity [33].

CONCLUSION

The results revealed the presence of medicinally important constituents in the plant studied. In this study, the antifungal activity of cell-wall polysaccharide extracts and flavonoid extracts of Anvillea radiata against Foa was determined. Flavonoid extracts showed the strongest inhibitory effects on spore germination and on soil population density of Foa. HMP from flowers had the greatest inhibitory effect on mycelial growth. The sporulation was strongly inhibited using cellulose-based agar of leaves. The traditional medicine practice is recommended strongly for this plant as well as it is suggested that further investigation should be carried out to isolate, purify and characterize the active constituents responsible for the activity of this plant.

REFERENCES


