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Fungicidal effect of *Acacia salicina* oils against three phytopathogenic fungi (*Aspergillus sp.*): effect of growing areas

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Abstract

This work was conducted to study the antioxidant activity, total phenol content and antifungal activity of of two fixed oils extracted from the arils and the seeds of *Acacia salicina* harvested from three different sites of Tunisia (Dissa, Zarat and INRGREF). The extraction of oils from seeds and arils of *Acacia salicina* was performed by a Soxhlet apparatus. The antioxidant activity of the oils was determined by DPPH method and the total polyphenol content was measured by the Folin-Ciocalteu method. The antifungal activity was tested against strains of *Aspergillus nidulans*, *Aspergillus oryzae* and *Aspergillus clavatus*. The results showed that the best yields of oils and the highest antioxidant activity were recorded for oils from the arils of Zarat (oil yield: 37.8%; IC50 = 1.72 mg / ml). The highest rate in polyphenols was recorded for seed oils with about 0.087 g GAE / ml. The antifungal activity against phytopathogenic tested strains *Aspergillus nidulans*, *Aspergillus oryzae* and *Aspergillus clavatus* showed that the three strains are quite sensitive to both aril and seed oils of *Acacia salicina*.

1. Introduction

Natural substances derived from plants have multiple interests put to good use in industry: in food, cosmetology and pharmacy. Among these compounds we find a large extent of the secondary metabolites which are especially illustrated in therapy. For thousands of years, human has used various resources found in its environment to treat and cure all kinds of diseases. The genus *Acacia* has 700 species, the best known are *A. cyanophylla*, *A. salicina*, *A. trobilis* and *A. nilotica*.

Acacia species are frequently used for the treatment of various diseases due to their well-known pharmacological effects. Published studies for *Acacia* included anti-inflammatory, hypoglycemic aggregation, spasmogenic, vasoconstrictor, antihypertensive effects and inhibitory effect against the hepatitis C virus [1,2,3]. *Acacia salicina* is of Australian origin. This species is recently introduced in North Africa, particularly in Tunisia; it is widely distributed in the Mediterranean region. The uses of this plant in Tunisia are rare. Several studies reported the biological properties of this plant. Boubaker et al. [4] indicate that the extracts from *A. salicina* leaves are a significant source of compounds with antimicrobial, antimutagenic and antioxidant activities. The antimutagenic, antigenotoxic, antioxidant and anti-inflammatory activities of extracts from *A. salicina* were reported in the literature [5,6,7].

The present work was carried out to valorize *Acacia salicina* through the study of the antioxidant activity, total polyphenols and antifungal activity of the fixed oils of this plant.

2. Methodology

2.1 Plant material

The work was carried out on the arils and seeds of *Acacia salicina* harvested in three sites located in North (Tunis) and South Tunisia (Dissa and Zarat). The aril was separated from the seeds, the plant material was then ground using an electric chopper.

Figure 1. Seeds and arils of *Acacaia salicina*

2.2 Oil extraction

The fixed oil was extracted using a Soxhlet apparatus using 20 g of the crushed seeds or arils. Hexane was used as the extraction solvent. The oil obtained was kept in the refrigerator until to its use.

2.3. Oil yield

The percentage of oil from arils and seeds of *Acacia salicina* was calculated according to the following formula:

$$\text{Oil yield (\%)} = (Mh/MMF) \times 100$$

Where:
R(%): Yield expressed in %.
Mh: Mass of the oil in g.
MMF: Mass of the fresh material in g.
The result is expressed in percent (%)

2.3 Antioxidant activity

The antioxidant activity of the oil samples was determined by calculating the percentage inhibition of DPPH. First, successive dilutions were made in ethanol and then 25 µl of each sample was added to 2.5 ml of DPPH in the ethanol solution (60 µM). After incubation at 27°C for 60 min, the absorbance of each solution was determined at 517 nm using a spectrophotometer [8]. The percentage of inhibition of DPPH was calculated according to the following equation:

$$IR = [(DOc – DOe) /DOc] \times 100$$

Where:

DOc is the absorbance of the control (containing 25 µl of ethanol and 2.5 ml of DPPH)

DOe is the absorbance of the DPPH containing the oil samples.

It was expressed in g/ml and compared with that of BHT [9].

2.4 Total phenols content

2.4.1. Preparation of the phenolic extract

2 g of oil were weighed and mixed with 5 ml of methanol/water (80/20, v/v) for 1 min in a vortex device. The mixture was then separated in an ultrasonic bath for 15 min at room temperature and centrifuged at 5000 rpm for 25 min. The methanol phrase was removed and stored cold and in the dark.

2.4.2. Total phenol content

The determination of total phenols was carried out adapted from Singleton and Ross [10] with the Folin-Ciocalteu reagent. 500 µl of the extracts of each sample was mixed with 100 µl of the Folin–Ciocalteu reagent (10 times diluted) and 2 ml of sodium carbonate Na2CO3. The whole is incubated at room temperature for 30 minutes and the reading is carried out against a blank using a spectrophotometer at 755 nm. From an aqueous stock solution of gallic acid, with a mass concentration of 0.5 g/l, a standard range of solutions in an aqueous medium was prepared.

100 µl of 10% folin-Ciocalteu reagent (10 times diluted in distilled water) is added. After two minutes of incubation, 2 ml of 2% Na2CO3 sodium carbonate are added. The tubes are then shaken and placed in the dark for 30 minutes at room temperature. The reading of the absorbance of each solution prepared using a UV-Visible spectrophotometer, at a wavelength of 755 nm against a blank prepared in the same way except that it does not contain gallic acid but distilled water instead of the test substance. The absorbance values of each concentration allowed us to plot the calibration curve for gallic acid.

2.5 Antifungal activity

2.5.1. Fungal strains

The test of antifungal activity of *Acacia salicina* oils was carried out against 3 fungal strains: *Aspergillus oryzae*, *Aspergillus nidulans* and *Aspergillus clavatus*. These strains were provided by the National Institute for Research in Rural Water and Forest Engineering (INRGREF).

2.5.2. Antifungal test

The culture was made on a PDA medium at the rate of 15 ml per Petri dish. The oil was added in the medium at the rate of 0.75 ml of oil per Petri dish. After cooling the medium, a disc of 5 mm in diameter of each fungal strain was placed in the center of the Petri dish while placing the mycelial surface down. The plates were incubated at 22°C for five days. The fungicidal effect was determined by calculating the growth diameter of the strain in question and comparing it to that of a negative control, i.e. an oil-free PDA medium [11]. For each antifungal test, the oils from the arils and seeds were tested in pure form, three tests for each of the three sites were carried out and the average value of the three measurements of the growth zone was taken in consideration. The results are calculated according to the method of Singh et al. (1993) while calculating the percentage inhibition (I) according to the following formula:

$$I(\%) = [(dC-dE) /dC] \times 100$$

Where:
dC: diameter of the control (mm)
dE: diameter in the presence of the tested extract (mm)

2.6 Statistical analysis

The statistical processing of the data was carried out using the SAS GLM (General Linear Models) procedure. An analysis of variance relative to the parameters studied was carried out. The most significant correlations between them are also noted. Results are presented as the mean of three replicates ± standard deviation.

3. Results and Discussion

3.1 Oil yield

The oil yield values of arils and seeds from the three sites studied (Tunis, Zarat and Dissa) are shown in Table 1.

Table1. Yield of oils from arils and seeds of *Acacia salicina* (%)

Site	Plant material	Yield (%)
Zarat	Aril	37.8^{a} ±3.25
	Seed	30.3^{b}±0.28
Dissa	Aril	34.5^{a}±2.47
	Seed	27.32^{b}±0.10
Tunis	Aril	23.15^{c}±1.06
	Seed	17.67^{d}±0.45

Values with different letters are significantly different

The results of the analysis of variance showed a highly significant variation in terms of yield between harvesting sites and between aril and seed from the same site. Without taking into consideration the plant material, the best yields were recorded by the Zarat and Dissa site. The Tunis site showed the lowest yield. When considering only the plant material, the best yields were obtained for all of the aril oils. The highest yield was achieved by the aril of Zarat with a percentage of around 37.8%, followed by that of the aril of Dissa (34.5%) and Tunis (23.15%).

Similarly for seeds, the highest yield was recorded for oils from Zarat (30.3%), followed by those from Dissa (27.32%). Tunis seed oils showed the lowest yield, resulting in a percentage of around 17.67%. These yield variations may be due to several factors including the degree of maturity of *Acacia salicina* seeds, interaction with the environment (type of climate, soil), harvest time and extraction method [12,13].

3.2 Antioxidant activity

Results of antioxidant activity of phenolic extracts from *A. salicina* oils are summarized in figure1. In comparison with BHT, the control compound which gave an IC50 of the order of 15.5 (µg/ml), it is possible to conclude that the phenolic extracts of the oils of the aril of *A. salicina* have a significant

antioxidant power. Statistical analysis showed that the IC50 values of the phenolic extracts of aril oils are lower than those recorded for the seeds ($\alpha < 0.002$). This shows that the phenolic extracts of aril oils possess a greater antioxidant activity than that of the phenolic extracts of seed oils. This result can be explained by the presence of more concentrated active ingredients in aril oils during extraction with non-polar solvents such as hexane [14].

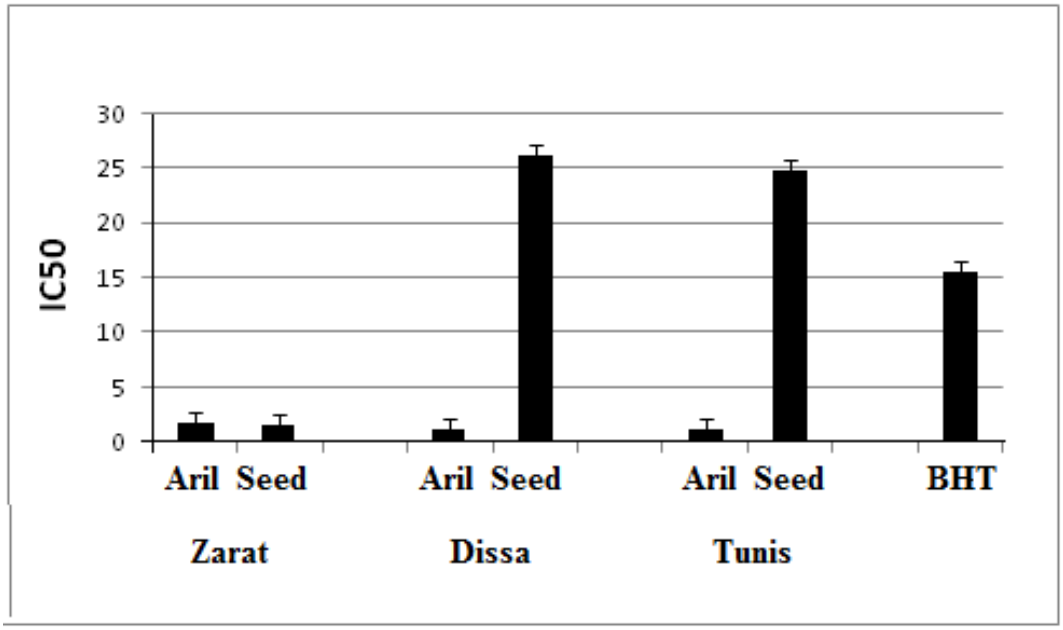

Figure 2. IC50 values (µg/ml) of phenolic extracts of the fixed oil from the aril and seeds of *Acacia salicina*

The work done by Teyeb *et al.* [15] proves that the oil richest in phenolic compounds generally shows the greatest antioxidant power. Statistical analysis revealed the presence of significant differences between seed collection sites ($p<0.001$). The lowest IC50 value, corresponding to the highest antioxidant activity, was recorded for Zarat oils (1.72 µg/ml for the aril and 1.43 µg/ml for the seeds). This difference can be attributed to the differences between the climatic and edaphic conditions in the three sites studied. Indeed, the study developed by Abu-Darwish *et al.* [16] showed that there is a large difference between oil compositions which could be attributed to the variation of climatic factors between provenances. It has been shown that climatic conditions influence the composition of oils. These variations in antioxidant power may be related to temperature. This has also been demonstrated by numerous studies claiming that temperature can modify the composition of oils as pointed out by Ravi *et al.*, and Maxwell *et al.* [17,18].

3.3 Total phenols content

Results of total phenol content of seed and aril oils of *A salicina* are summarized in Table 2. Statistical analysis showed that the phenolic extracts of the seed oils are so close in value to those of the aril, that there is no significant difference between the polyphenol content in the aril and the seeds. For the phenolic extract of aril oil, Dissa oil showed the highest polyphenol content, resulting in a content of around 0.066 g GAE/ml. The lowest levels were recorded for Zarat aril (0.05 g EAG/ml) and Tunis (0.02 g EAG/ml). Similarly, for the phenolic extracts of the fixed oils extracted from the seeds, the highest content was recorded for Dissa (0.087 g EAG/ml), followed by Zarat (0.033 g EAG/ml) and Tunis (0.038 g EAG /ml).

Table 2. Values of polyphenol content of aril and seeds oils of *A. salicin* (g EAG/ml)

Site	Aril	Seed
Dissa	0.066^{b}±0.036	0.087^{a}±0.015
Zarat	0.05^{c}±0.024	0.033^{e}±0.014
Tunis	0.02^{f}±0.002	0.038^{d}±0.004

Values with different letters are significantly different

Different factors can be at the origin of the variability of polyphenol contents in plants. Indeed, Fabbri *et al.* [19] demonstrated that variations in total phenol content can be influenced by the degree of maturity of the fruits, which does not correspond with our case study given that the harvest was made during the same period for all the sites.

A study by Ferret [20] stated that there is a relatively strong correlation between climatic and edaphic conditions and polyphenol content. In the same context, other studies have shown that within a given region, variations in phenolic compound levels are related to soil salinity [21] and the availability of nutrients and in minerals [22]. This difference can be explained by the depth of the sampling site and therefore the penetration of light radiation. Stiger et al. [23] effectively proved that polyphenols have a photoprotective role, and consequently their content tends to increase in sites that are more exposed to the sun than in those rather in the shade. The study by Cieslik *et al.* [24] on the polyphenol content noted that the climatic conditions as well as the choice of the extraction solvent represent an essential factor for this variation of the polyphenols.

3.4 Antifungal activity

The antifungal activity expressed as percentage inhibition of aril and seed oils was determined by measuring the diameters of the zones of inhibition compared to a negative control. The percentages of inhibition of the three fungal strains are illustrated by the histograms in figures 2, 3 and 4. The results obtained during this study showed that the oils of the aril exert a significant inhibitory activity on all the strains with values higher than those obtained for the oils of the seeds. Statistical analysis showed that there is a significant difference between aril and seed oils ($p < 0.001$). The difference between the antifungal activities of aril and seed oils may be the result of the different chemical composition between these two products. Work carried out by Bianchini [25] confirmed the effectiveness of aril oils compared to seed oils obtained on certain fungal strains. A study by Amri *et al.* [26] proved that the antifungal activity of a fixed oil is to be related to its chemical composition and the synergistic effects between the components of this oil. The different oils tested on the *Aspergillus nidulans* strain showed significant antifungal activity, particularly for the Zarat aril oils, which inhibited the mycelial growth of the strain. On the other hand, the lowest value was obtained for Dissa and Tunis aril oils (about 32% inhibition). The inhibition effect revealed on the *Aspergillus oryzae* strain was clearly higher for the oils of the aril in the three sites, each of which was around 30%. However, the seed oil showed the weakest antifungal activity. The weakest antifungal power was reached in the presence of the seed oil from Tunis with about 24%, followed by that of Dissa (14.92%) then of Zarat presenting a percentage inhibition of 8.93%. On the other hand, the fungal strain *Aspergillus clavatus* was more sensitive in the presence of seed oils in the three sites (about 38%). These three seed oils allowed an equally important

inhibition of the mycelial growth of this strain. The lowest antifungal power was achieved in the presence of aril oil in each of the sites, presenting an inhibition percentage of around 18%.

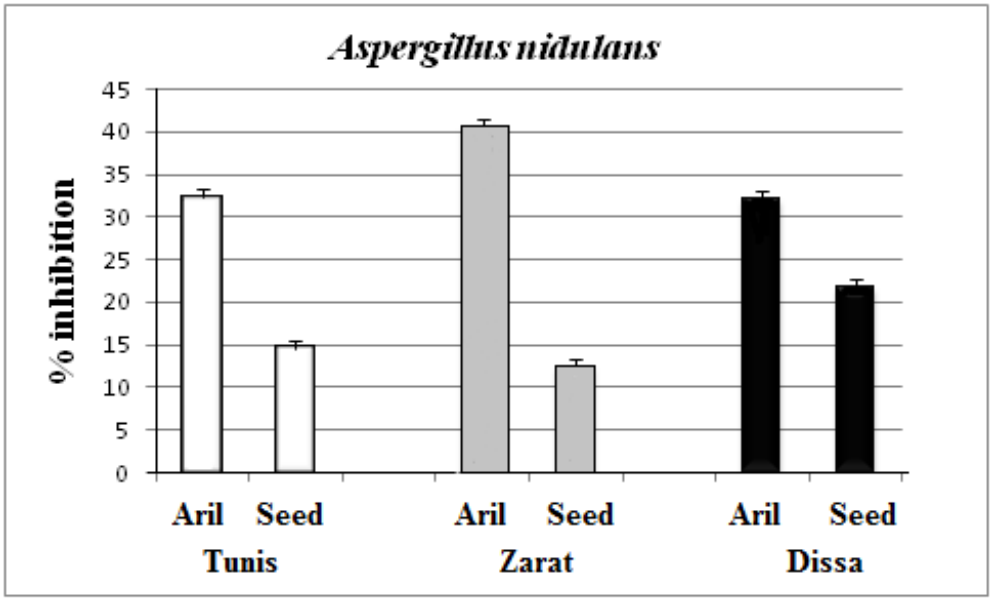

Figure 3. Antifungal activity of seed and aril oils against *Aspergillus nidulans*

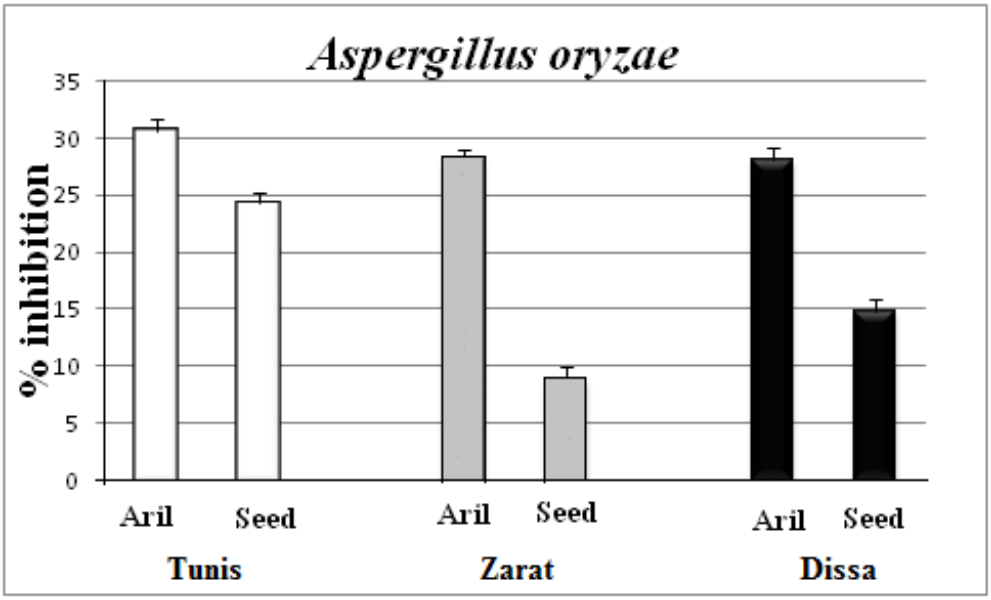

Figure 4. Inhibitory effect of aril and seed oils on the *Aspergillus oryzae* strain

The antifungal activity of *Acacia* extracts has long been studied. These extracts showed important antifungal power against different phyto pathogenic strains such as *Alternaria alterna*, *Fusarium culmorum*, *Fusarium oxysporum* and *Fusarium solani* [27]. In general, the variable antifungal effect of the oils studied may be related to their different compositions. A study by Youzbachi et al. [28] showed that the fixed oils of *Acacia salicina* contain active molecules such as flavonoids, polyphenols and coumarins. Moreover, a study made by Getachew [29] showed that among the species of *Acacia*, *A. salicina* was described as being a species rich in tannins. The families of these molecules are reported to play an important role in the inhibition of fungal growth [30].

We can conclude that the biological activity of oils depends not only on phenolic compounds but also on the presence of various secondary metabolites with antifungal effect such as steroids, saponosides and oils which have already been reported by several authors [31,32].

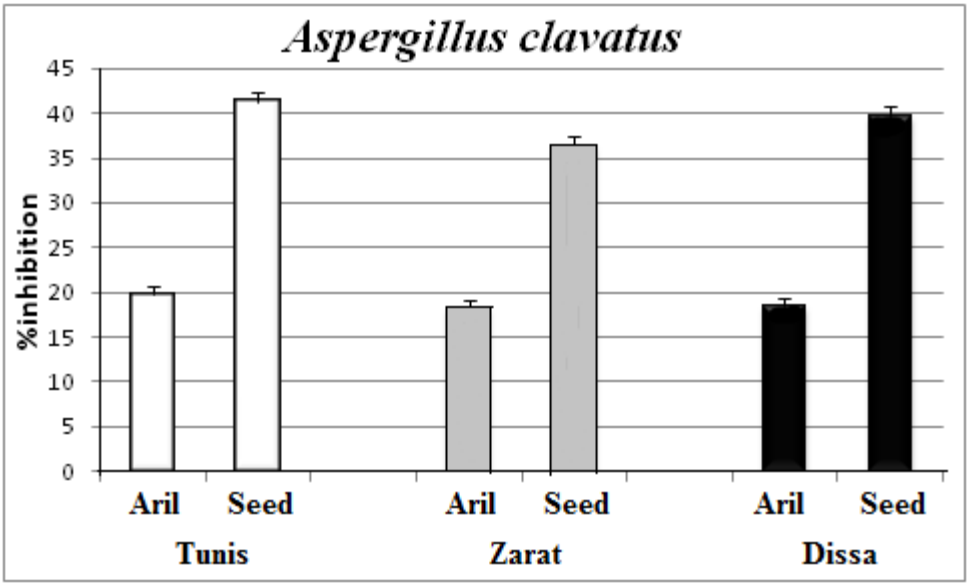

Figure 5. Inhibition effect of aril and seed oils on the *Aspergillus clavatus* strain

Conclusions

The objective of this study was to investigate the antioxidant activity, the content of total polyphenols and the antifungal activity of the fixed oils of the aril and seeds of *Acacia salicina*.

The measurement of the antioxidant power of the phenolic extracts of the fixed oils of the aril and seeds showed that these oils have an important antioxidant activity especially for the oils of the aril. In addition, this work revealed that the phenolic extracts of the two oils contain significant contents of total polyphenols which vary according to the harvest site. Likewise, these oils have shown significant antifungal power.

The results of this study support the idea that *Acacia salicina* is a promising source of natural antioxidants. This species showed potent antioxidant properties and contained significant amounts of phenolic compounds.

These results support the potential uses of *Acacia salicina* in traditional medicine and increase the chance of its use in the pharmaceutical and medicinal fields as an antioxidant and antifungal agent.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest. *Compliance with Ethical Standards:* This article does not contain any studies involving human or animal subjects.

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