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Chemical characterization of oils from *Acacia salicina* seed and aril

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Abstract
This work was carried out on two samples of *Acacia salicina* oils extracted from arils and seeds by a soxhlet apparatus. The antioxidant activity was determined by the DPPH method and the total polyphenol content was measured by the Folin-Ciocalteu method. Gas phase chromatography revealed the fatty acids contained in these two oils.
Oil yields were estimated at 47.06% for arils and 10.9% for seeds.
The measurement of the antioxidant activity revealed that the two oils have an interesting antioxidant power. The highest activity was recorded for aril oil (0.15 10-3 µg/ml). The total contents of polyphenols were estimated at 0.10 g GAE/ml for the oil of the arils, 0.10 g GAE/ml and an average content of 0.044 g GAE/ml for the oil of the seeds. The analysis of the fatty acid composition showed that both the seed and aril oils are rich in the same fatty acids which are the linoleic acid, oleic acid and linoleic acid and palmitic acid.

Keywords: *Acacia salicina*, seed, aril, oil, fatty acids, antioxidants

Introduction
For a very long time, man has been practicing herbal medicine without realizing it. Indeed, many medicinal plants have been used and their uses have developed over time.
Nowadays, the use of plants or plant extracts is practiced all over the world, particularly by rural populations but also by the pharmaceutical industry (Nasri *et al*. 2013) [12].
In Tunisia, this practice is widespread, but each region has its plant "remedies" and these remedies are inherited from generation to generation. *Acacia salicina* is used in Tunisia for the treatment of several diseases, such as the treatment of inflammatory diseases, to treat cancer and as an activator of human fertility. In southern Tunisia infusions prepared from the fresh or dried leaves are taken orally, or, alternatively, the chopped fresh leaves are applied directly to inflamed wounds (Maslin *et al*., 2003) [10].
Several studies reported that *Acacia* is used in traditional medicine for their antibacterian, antioxidant, anti-inflammatory, antispasmodic, anti-arythmique and astringent proprieties (El Abbouyi *et al*., 2004) [7]. In the literature, and to the best of our knowledge.
The present study aims to shed light on the chemical composition of *A. salicina* seeds and aril from Tunisia in an attempt to highlight the possibility of using Acacia seeds as a raw material source of oil in industrial products.

Material and methods
1. Plant material
The work was carried out on *Acacia salicina* seeds collected in the National Institute for Research in Rural Water and Forest Engineering (INRGREF) during the month of June. The aril was separated from the seeds, the plant material was then ground using an electric chopper.

2. Oil extraction
Oils were extracted from seeds and aril using Soxhlet apparatus according to the methodology described in AOAC (2000) [1]. 20 g of plant powder was placed in a Soxhlet apparatus and petroleum ether is used as a solvent.

3. Oil yield
The percentage of aril and seed oil of *A. salicina* was calculated by using the following formula:

$$R (\%) = Mh/MMF \times 100$$

R (%): Yield expressed in %.

Mh: Mass in grams of the oil.
MMF: Mass in grams of fresh plant material.

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 (Brand-Williams *et al*. 1995) [2].
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 (Cuendet *et al*., 1997) [4].

Total phenols content
1. Preparation of the phenolic extract
2 g of oil was weighed and mixed with 5 ml of methanol/water (80/20, v/v) for 1 min in a vortex apparatus. 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 phase was removed and stored cold and dark.

2. Total phenols
The determination of total phenols was carried out adapted from Singleton and Ross (1965) [14] with the commercial 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 of the Schimadzu 1601 type, 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.

3. Fatty Acid Composition Analyzes
3.1 Methylation of Total Fatty Acids
100 µl of each oil sample were saponified in the presence of 2 ml of methanolic NaOH (0.5 M) in a water bath brought to 65°C for 15 min. The transmethylation was carried out in the presence of BF3 (14%) at 65° C. for 5 min. The addition of 10 ml of distilled water followed by petroleum ether leads to saponification.
The recovered organic phase is subjected to vacuum evaporation. Hexane is used as a solvent for the methyl esters before the injection procedure to perform gas chromatography (Metcalfe *et al*. 1966).

3.2 Identification of Fatty Acids
The CPG apparatus used is equipped with a capillary column of the HP5 MS type, 30 m in length and 320 µm in internal diameter; the thickness of the film is 0.25 µm. The temperature of the injector is 250°C. The carrier gas is nitrogen, supplied at a flow rate of 11 ml/min, the injection mode is the 20:1:1 Split mode and the temperature program is 150°C (0 min) - 5°C /min - 260°C.

4. 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.
Results are presented as the mean of three replicates ± standard deviation.

Results and Discussion
1. Oil Yield
Results showed that the highest yield was reached by the arils with a percentage of the order of 47.06% ±1.244 while the seeds gave a yield of the order of 10.9 %. ±1.354

2. Antioxidant Activity

To overcome the influence of the concentration, in the majority of studies, reactivity is estimated by the median Inhibitory Concentration IC50 of the antioxidant, which corresponds to a 50% reduction in activity (absorbance) of DPPH in the reaction medium.

The antiradical activity of the oils of *A. salicina*; Aril oil and seed oil as measured by determination of Median Inhibitory Concentration are shown in Table 1.

In comparison with BHT, the control compound which gave an IC50 of 15.5µg/ml, we can conclude that the oils extracted from the two organs of *A. salicina* have significant antioxidant power. We also note that the oil from the aril has a greater antioxidant power than that of the seeds since the inhibition concentration of aril oil (0.15 10^{-3}µg/ml) is significantly lower than that of the seeds (0.04866 µg/ml).

The antioxidant activity determined for the oils tested is probably related to the presence, according to different levels, of natural antioxidants. The term antioxidants is used to characterize a set of substances or compounds, of diverse nature, whose common characteristic is to be able to oppose or control the accumulation of free radicals at the cellular level. This property allows them to act directly or indirectly as a defense against active oxygen species. They oppose the oxidation mechanisms of certain molecules (Diplock, 1991) [6].

According to the literature, the best known antioxidants are β-carotene (provitamin A), ascorbic acid (vitamin C), tocopherol (vitamin E) and phenolic compounds. Indeed, most synthetic or naturally occurring antioxidants have Hydroxyphenolic groups in their structures and the antioxidant properties are attributed in part to the ability of these natural compounds to scavenge free radicals such as hydroxyl radicals (OH•) and superoxides (O2•) (Rice-Evans, 1995; Bartosz, 2003) [13, 3].

Several studies have demonstrated the important nutritional value of antioxidants. They discussed the correlation between nutritional antioxidants and chronic disease. These compounds are known in the prevention of cancer, atherosclerosis and coronary and cardiovascular diseases (Descheemaeker, 2004) [5].

Given the complexity of oxidation processes and the diverse nature of antioxidants, with both hydrophilic and hydrophobic components, there is no one universal method by which antioxidant activity can be measured quantitatively in any way. quite precise. Most often, it is necessary to combine the responses of different and complementary tests to have an indication of the antioxidant capacity of the sample to be tested (Tabart *et al*., 2009; Hua *et al*., 2008) [15, 8].

Table 1: IC50 values (in µg/ml) of aril and seed oils of *A. salicina*

Plant material	IC50
Seed	0,04866±0,00351
Aril	0,15 10^{-3} ±0,0007
BHT	15,5

3. Total Phenols Content

The values of the total polyphenol content are summarized in Table 2 and are expressed in g of gallic acid equivalent per ml of oil (g GAE/ml).

It was demonstrated that the aril oil has a phenols content of the order of 0.1006g GAE/ml which is higher than that of the seed oil which is equal to a value of 0.0446g GAE/ml. Several studies have highlighted the presence of a correlation between the antioxidant activity and the content of total polyphenols for a given compound (Kiselova *et al*., 2006) [9] for the present work this corresponds perfectly to the results obtained in fact the oil of aril of *A. salicina* which has the highest antioxidant power presents the highest content of total polyphenols.

Table 2: Values of polyphenol content of *Acacia salicina* aril and seed oils (g GAE/ml)

Plant material	Total phenols content
Seed	0.0446±0.0064
Aril	0.1006±0.0085

Table 3. The main fatty acids of *A salicina* seed and aril oil

Acides gras	Temps de rétention	Aril oil	Seed oil
Acide palmitique C16:0	5,036	8,46±0,135	6,83±0.524
Acide palmitoléique C16:1	5,308	0.16±0,05	0,14±0,121
Acide stéarique C18:0	7,855	2,216±0,023	2,36±0,141
Acide oléique C18:1	8,261	22,67±0,068	23,07±0,150
Acide linoléique C18:2	9,299	64,67±0,252	65,19±0,208
Acide linolénique C18 3n3	10,731	0,18±0,001	0,29±0,128
Acide arachidique C20:0	13,123	1,01±0,06	1,27±0,09
Acide gadoléique C20:1	13,714	0,58±0,07	0,76±0,11

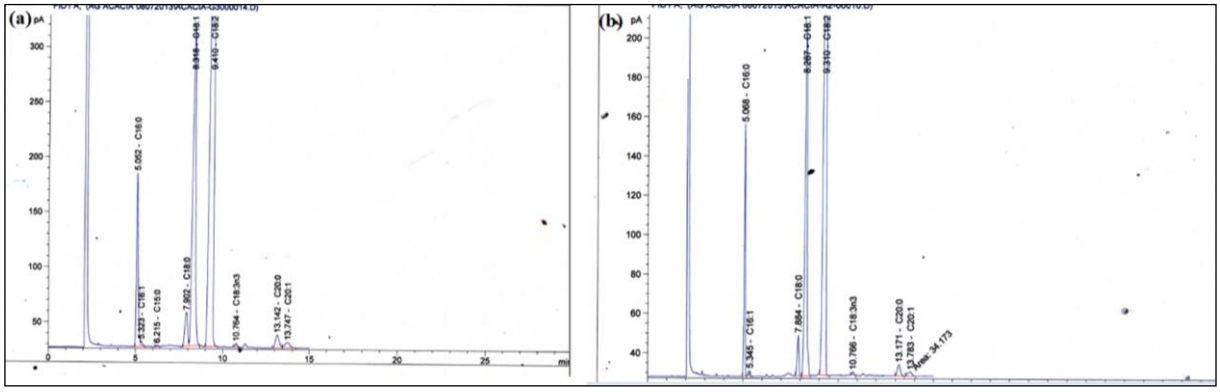

Fig 1: Chromatogram of fatty acids in *Acacia salicina* seed and aril oils

4. Fatty Acid Composition

Analysis of the fatty acid composition of samples of aril and seed oils of *A. salicina* showed that the two oils studied contain 8 main fatty acids.

Analysis by gas chromatography of seed oils and arils of *A. salicina* showed that both oils contain the same three main fatty acids which are linoleic acid, oleic acid and palmitic acid.

Linoleic acid is the major fatty acid in the two oils studied. It has a content of around 64.67% for the oil from the arils and 65.19% for that extracted from the seeds.

Oleic acid is present with a percentage of 22.67% for the oil of the arils and 22.92% for that of the seeds. Palmitic acid has 8.163% of the fatty acid composition of aril oil and 6.836% of seed oil.

We note that unlike other oils that we know (olive, palm, soybean, sunflower) the dominant fatty acid in these two oils is linoleic acid. For olive oil, for example, there is a clear predominance of monounsaturated oleic acid.

Polyunsaturated acids are of great biological importance, as they cannot be biosynthesized by humans, and therefore must be provided through the diet. Therefore the two oils obtained from *A. salicina* can be considered as an interesting food source of these essential fatty acids.

Conclusions

Through this work, it was found that the two oils analyzed have significant antioxidant power and an interesting polyphenol content. The fatty acid composition analysis results showed that these two oils are rich in unsaturated fatty acids. These properties give an important nutritional value to these two *Acacia* oils and increase the chance of their use in the food or pharmaceutical field.

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