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Comparative Study of Bioactive Compounds and Antioxidant Activity of *Schinus terebinthifolius* RADDI Fruits and Leaves Essential Oils

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Abstract: ***This study focused on the physico-chemical characterization of Schinus terebinthifolius RADDI leaves and seeds essential oils and the evaluation of their antioxidant activity. Several volatiles components were identified by GC-MS. The major compound detected in leaves and fruits essential oils, was bicyclogermacrene (35.58 % and 23.56 %, respectively). Schinus terebinthifolius fruits essential oil showed lower total phenolic and flavonoids content but higher antiradical activity than the leaves one. This antioxidant activity characterizing Schinus terebinthifolius essential oils was high enough to consider the plant as a new and natural source of antioxidants.***

Keywords: *Schinus terebinthifolius*, essential oil, GC-MS, total phenolic content, antioxidant activity.

1. Introduction

Schinus terebinthifolius Raddi (Anacardiaceae) is a perennial tree indigenous to the coast of Brazil, and has been introduced into other South American countries, parts of Central America, Bermuda, the Bahama Islands, the West Indies, Florida, Southern Arizona, California, Hawaii, Mediterranean Europe, North Africa, Southern Asia and South Africa [1-3]. It is known by a variety of common names including “aroeira-vermelha”, “aroeira-pimenteira”, Brazilian pepper, Christmas-berry, pink-pepper, poivre rose [1, 4-5]. Many medicinal properties have been attributed to this plant, such as antioxidant [6-8] wound-healing [9] antitumor [10] and antimicrobial activities [11, 12].

In addition, *S. terebinthifolius* has been used to treat sexually transmitted diseases, uterine inflammation, urinary tract infections, skin ulcers and gastroduodenal disorders [13]. Bacchi [14] reports the use of aroeira species in the treatment of skin, mucous membrane injuries, ulcers and infections of the respiratory, digestive and genitourinary systems. Extracts of Brazilian pepper tree were shown to be the most effective of a number aromatic and medicinal plant species in suppressing several important pathogenic bacteria [15]. The extract of stem bark is used as an anti-inflammatory and to heal over or cicatrize wounds [16]. The crushed, dried leaves are applied as antiseptic poultices upon skin ulcers. Relief from bronchitis and other respiratory ailments is treated by leaf infusions. Interestingly, the juice of macerated roots is considered effective in treating ganglionic tumors [13]. The fruits are used for colds, fungal and bacterial infections. In addition, the berries of *S. terebinthifolius* are rich in essential oil, which imparts a peppery flavor, and are used as a food seasoning that is highly sought after and of significant economic value. These fruits are also used in syrups, vinegar, and beverages in Peru as well as in Chilean wines. In some countries, dried and ground berries are used as a pepper substitute or as an adulterant of black pepper (*Piper nigrum*). They have also been used in the perfume industry [17]. In Tunisia, *S. terebinthifolius* have been introduced as ornamental specie at the end of the 1900s by the Frensh colonizers. Its successful introduction in a non-native range is attributed to its high drought and heat tolerance, great potential to compete for nutritive resources and light, high growth rate and prolific seed production, as well as, their phytotoxic activities [18]. Most previous studies concerned with the chemical composition of *S. terebinthifolius* essential oil were focused on fruits, and little is known about the leaf and fruits oil constituents and biological activities. Therefore, the present study was intended at identifying the physic-chemical characterization of tunisian *S. terebintifolius* leaves and fruits essential oil and determination of its antioxidant activities.

2. Materials and Methods

2.1. Plant material

Leaves and fruits from *S. terebinthifolius* were randomely collected from plants growing from the northern Tunisia). After collection, samples were spread over a plane surface then were dried in open air and in the shade for two weeks. After drying, samples were finely ground by a grinder "Laboratory Blender" type and stored in paper bags at 4 ° C until their use.

2.2. Physico-chemical study of *S. terebinthifolius*

2.2.1. Dry material

The determination of leaves and fruits dry matter was carried out according to the standard FAO Food Codex Methods.

$$DM\ (\%) = ((P_0-P_1) / P_0) \times 100$$

where P_0 is the initial mass of leaves or fruits and P_1 is the mass after drying.

2.2.2. Density at 20 ° C:

Essential oil density at 20 °C represents the ratio of the mass to a volume of essential oil at 20 °C using the mass of the same volume of distilled water at the same temperature.
D_{20} relative density is obtained using the following equation:

$$D_{20} = (m_0-m_2) / (m_1-m_0)$$

where m_0 is the mass of the empty test tube (g), $m_1$is the mass of the test tube containing water (g) and m_2 is mass the test tube containing the essential oil (g)

2.2.3. Acid Value

The acid value (AV) of *S. terebinthifolius* leaves and fruits essential oil was calculated according to the standard ISO 1242-1973. AV number of milligrams of potassium hydroxide required to neutralize the free acids contained in 1 g of the essential oil.

2.2.4. Ester Value

The ester value (EV) was calculated according to the standard ISO 709-1980. EV is the number of milligrams of potassium hydroxide required to neutralize the acids liberated by the hydrolysis of esters present in 1 g of the essential oil.

2.2.5. Saponification Value

The saponification index was calculated according the standard ISO 3657. An appropriate amount of sample is weighed into a round-bottom flask, 25 mL ethanolic c(KOH) = 0.5 mol/L is added. After the addition of a magnetic stirring bar, the reflux condenser is attached and the solution is heated up and boiled gently for 60 minutes. To the saponified and cooled-down sample solution 40 mL ethanol is added and the solution is titrated with c(HCl) = 0.5 mol/L until after the first equivalence point. In between measurements, the electrode membrane is rehydrated for 1 min in deionized water. A blank determination is performed the same way as the sample analysis, including sample preparation.

$$\text{Saponification value (mg / g)} = (\text{BLl} - \text{EPl}) \times TF \times Cl \times K_1 / SIZE$$

where EP_1 is the titration volume (mL), BLl is the Blank level (25.029mL), TF is the Reagent (HCl) factor (1.006), Cl is the concentration conversion coefficient (28.05 mg/mL) (Potassium hydroxide in Eq.:56.11×0.5), K_1 is the Unit conversion coefficient (1) and SIZE is the Sample size (g).

2.3. Total Phenolic Content

Total phenolic content was assayed using the Folin–Ciocalteu reagent, following Singleton's method slightly modified by Dewanto et al. [19]. These molecules are able to reduce, in an alkaline medium, the Folin - Ciocalteu reagent (mixture of phosphotungstic acid and phosphomolybdic acid) of tungsten oxide and molybdenum, leading to a characteristic blue color .The intensity of the color is correlated positively with the amount of polyphenols present in the extract. 125 µL of the diluted extract was mixed with 500 µL of distilled water and 125 µL of Folin - Ciocalteu reagent. After vigorous stirring of the mixture, followed by 3 minutes of rest, 1250 µL of CO_3Na_2 (7 %) were added. Finally, the obtained mixture was adjusted with distilled water to 3 mL. After standing for 90 minutes in the dark, the absorbance reading was made at a wavelength of 760 nm. The standard range is prepared with gallic acid at concentrations ranging from 20 to 200 mg.L^{-1} (R^2 = 0.99). Total phenolic content was expressed in mg of Gallic acid equivalent per gram of dry mass (mg GAE/g DM).

2.4. Total Flavonoids Content

The total flavonoid content was measured according to Dewanto et al. [19]. A total of 250 µL of the sample appropriately diluted was mixed with 75 µL of 5% $NaNO_2$ (sodium nitrite). After 6 min, 150 µL of 10% aluminum chloride ($AlCl_3$) and 500 µL of 1 M NaOH were added to the mixture. Finally, the mixture was adjusted to 2.5 mL with distilled water. The absorbance versus prepared blank was read at 510 nm. The reference range was prepared with catechin to increasing concentrations ranging from 50 to 500 mg.L^{-1} (R^2 = 0.98). Total Flavonoid content was expressed in mg equivalent of catechin per gram of dry mass.

2.5. Antioxidant activity evaluation

The antioxidant activity was evaluated according to the method of Hanato et al. [20], using the 2,2-diphenyl-1-picrylhydrazyle (α,α-diphenyl-β-picrylhydrazyle) or radical DPPH. It is a synthetic radical presenting, when oxidized, an intense violet color. The reduction of this molecule by receiving protons from antioxidant substances induced the disappearance of the violet color whose degradation is a function of the wealth of the extract these molecules can trap this radical.

An aliquot of 1 ml of the extract at different concentrations was added to 250 µL of a DPPH solution (0.2 mM in methanol). The mixture still for 30 min in the dark for incubation, then the absorbance was measured at 517 nm with a spectrophotometer against a control (no sample). The antiradical activity was expressed as IC_{50} (µg/mL), the concentration required to cause a 50% DPPH inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\% = (DO_{control} - DO_{sample} / DO_{control}) * 100$$

where $DO_{control}$ is the absorbance of the control at 30 min, and DO_{sample} is the absorbance of the sample at 30 min. All samples were analyzed in triplicate.

3. Results and Discussion

3.1. Physico-chemical study of *S. terebinthifolius* essential oils

The dry matter content of *S. terebinthifolius* leaves and fruits was of 79.89 % and 84.65%, respectively. Hydrodistillation

of different plant parts did not yield the same production and gave rise to different amounts of essential oil, with average yields of 5.03 % (w/w on dry weight basis) in fruits and 1.48% in leaves. The extraction yield of *S. terebinthifolius* dried leaves and fruits essential oil was relatively high compared with certain plants which are industrially run as source of essential oil [21] such as peppermint (0,5-1 %), neroli (0,5-1 %), pink (rose) (0,1-0,35 %), , menthe (0.5-1%), Laurel (0.1-0.35%) [21] and *Tetraclinis* (0.22%) [22]. These values were nearly similar to those obtained by Ennigrou et al. [23] who mentioned that the hydrodistillation of *S. terebinthifolius* dried leaves yielded 1.06%. Our results are in agreement with those of Barbosa et al. [24] and Souza et al. [25] who reported that the amount of fruits EO corresponded to 4.65% and 4.87% dry weight. In contrast, Affonso et al. [26] found that *S. terebinthifolius* fruits EO yielded of about 2.6% (w/w) of dry weight. The difference between leaves and fruits EO yield could be due to the fact that essential oil accumulation is organ-dependent.

Various physicochemical indexes were determined after the essential oil extraction from fruits and leaves. No data are available concerning physicochemical properties of *S. terebinthifolius* leaves and fruits EOs. Here, for the first time density, acid, ester and saponification values of EO were measured. Results are resumed in the Table 1.

Table 1: Physicochemical properties of *S. terebinthifolius* leaves and fruits essential oils

	Acid Value	*Ester Value*	*Saponification Value*	*Density*
Fruits EO	1.5	29.9	33.2	0.84
Leaves EO	5	23.35	28.35	0.89

According to the results presented in Table 1, it worth noting that the ester and saponification values characterizing the fruits EO were higher (29.9 and 33.2 mg (KOH) / g of EO, respectively) than those observed in the leaves EO (23.35, 28.35 mg (KOH) / g of EO, respectively). However, the acid value of the fruits EO was lower (1.5 mg (KOH) / g of EO) than that of leaves EO (5 mg KOH / g EO). Fruits and leaves EO were characterized by quite similar values of 0.84 and 0.89, respectively. These differences are mainly due to the chemical composition of leaves and fruits.

3.2. Essential oils composition of *S. terebinthifolius* fruits and leaves

The essential oil extraction of *S. terebinthifolius* was conducted by steam distillation. Volatile compounds of *S. terebinthifolius* leaves and fruits, their retention indexes and percentages, are listed in Table 2. All the constituents were arranged in order of their elution on the HP column. 23 compounds were identified in the leaves EO representing 97.99 % of the total EO while 22 compounds were detected in the fruits EO forming 93.88 % of the total EO (Table 2).

Table 2: Essential oil composition (%) of *S. terebinthifolius* leaves and fruits

N°	Volatils compounds	RI	Fruits	Leaves
1	α -thujene	930	0.26	0.54
2	α -pinene	939	7.37	9.63
3	sabinene	975	0.2	0.77
4	β-pinene	979	0.47	0.53
5	α -phellandrene	1003	14.06	11.69
6	*m*-cymene	1024	-	3.07
7	*p*-cymene	1025	2.1	3.31
8	β-phellandrene	1030	1.63	--
9	terpinolene	1089	0.56	0.48
10	carvacrol	1299	0.09	0.51
11	α -elemene	1338	1.37	5.6
12	α -copaene	1377	0.24	4.54
13	β-elemene	1391	7.32	--
14	β-caryophyllene	1419	2.07	--
15	aromadendrene	1441	0.3	0.46
16	α -humulene	1455	0.21	0.42
14	β-camigrene	--	--	1.6
17	allo-aromadendrene	1470	0.8	9.47
18	germacrene D	1485	7.41	--
19	**bicyclogermacrene**	**1500**	**35.58**	**23.56**
	elemol	1514	-	3.96
20	γ-cadinene	1523	0.51	0.4
21	Germacrene B	1561	--	13.71
22	spatulenol	1578	8.3	0.55
23	globulol	1585	1.66	--
24	viridiflorol	1593	0.85	1.2
25	γ-cadinol	1640	--	1.46
26	α –eudesmol	1654	0.52	0.53

RI: retention index; (-): not identified

As can be seen, both qualitative and quantitative differences were observed between the analyzed oils. Monoterpenes hydrocarbons were found to be the main chemical classe in both plant parts accounting for 82.46 % of the fruits EO and 89.78 % of the leaves EO. *S. terebinthifolius* fruits EO, was distinguishable from that of leaves EO by the abundance of sesquiterpenoid hydrocarbons which account 11.42 % of fruits EO against 4.25 % of leaves EO. Our results are similar to those of Affonso et al. [26] who revealed that the leaves and reddish fruits are rich in essential oil with high concentrations of monoterpenes along with some sesquiterpene hydrocarbons. Whatever the part of plant, monoterpenes hydrocarbons were dominated by bicyclogermacrene (35.58 % fruits against 23.56 % leaves). It is also important to notice that α - phellandrene (14.6 %), spatulenol (8.3 %), germacrene D 7.41% and α - pinene (7.37 %) were the most abundant compounds in the fruits EO. Whereas, germacrene B (13.71 %), α -phellandrene

(11.69%), α – pinene (9.63 %) and allo-aromadendrene (9.47 %) were among the major components of the leaves.

Different results were reported by [27, 28] who mentioned that GC-MS analysis of most leaves EO samples originating from India revealed α-pinene (15.01-51.82%) as the major component. Also, Silva et al. [29] studied the EO from leaf of the Brazilian pepper tree and revealed different results highlighting that the main components were *p*-cymen-7-ol (22.5%), 9-epi-(E)-cariophyllene (10.1%), carvone (7.5%) and verbenone (7.4%). Moreover, Bendaoued et al. [30] mentioned that, among 62 compounds identified in *S. terebinthifolius* fruits EO, a marked quantity of γ--cadinene (18.04%) was identified and the main constituents were α-phellandrene (34.38%), β--phellandrene (10.61%), α -terpineol (5.60%), *p*-cymene 7.34% and α -pinene (6.49%).

The essential oils from leaves, flowers and fruits of *S.terebinthifolius* from different locations have been previously investigated and some variation on their chemical composition have been observed by (Ibrahim et al., 2004; Singh et al., 1998; Malik et al., 1994). Moreover, a number of studies with leaf´s essential oil of plants collected at different regions of the globe have shown distinct chemotypes by GC/MS analyses, and prevalence of distinct chemical compounds. For example, α -pinene (51,82%) in Indian plants, α-phellandrene (24,2%) in Egypt plants, limonene (17,7%) and *p*-cymene (15,7%) in Reunion Island plants [29].

3.3. Total Phenolic and Flavonoids Content of *Schinus terebinthifolius* Essential oils

Table 3 summarizes the results from the quantitative determination of the phenols and flavonoids of *S. terebinthifolius* fruits and leaves essential oils. Total phenolic content was determined as gallic acid equivalents in milligrams per gram essential oil (mg GAE/g EO) while total flavonoids content was calculated as myricetin equivalents in milligrams per gram essential oil (mg MYR/g EO).

Schinus terebinthifolius fruits essential oil showed a polyphenol content of 16 µg / mL GAE which was lower than that found for leaves essential oil (40 µg / mL GAE). On the other hand, our findings indicated that the flavonoids content of fruits was about 13 µg / ml while that of leaves, was of 28 µg / ml.

To the best of our knowledge this is the only comparative study between *Schinus terebinthifolius* leaves and fruits essential oil phenolics. Several studies showed that phenolics exhibit a wide range of biological effects including antibacterial, antiinflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic, and vasodilatory actions [31, 32]

Table 3: Total Phenolic and Flavonoids Contents of *S. terebinthifolius* Essentail Oils

Samples	*Total Phenolic Content (mg/mL)*	*Total Flavonoids Content (mg/mL)*
Fruits EO	0.016	0.013
Leaves EO	0.04	0.028

3.4. Antioxidant activity of *Schinus terebinthifolius* Essential oils

DPPH can be used to determine the free radical scavenging activity as it forms a stable molecule on accepting an electron or hydrogen atom [33]. There was a reduction in the concentration of DPPH due to the scavenging effect of the essential oil. Essential oils and standard antioxidants reduced DPPH to yellow coloured product in a concentration dependent manner. Free radical scavenging properties of *S. terebinthifolius* essential oils are presented in Table 4. Leaves and fruits essential oils showed higher IC_{50} value (96 µg/mL and 82 µg/mL, respectively) than that of the standard antioxidant BHT (IC_{50} = 30 µg/mL) indicating a low antioxidant capacity. Fruits essential oil had higher antriradical activity than leaves one.

Table 4: Antioxidant Activity of *S. terebinthifolius* Essentail Oils

Samples	*IC_{50} (µg/mL)*
BHT	30
Fruits EO	82
Leaves EO	96

To the best of our knowledge this is the only comparative study between *Schinus terebinthifolius* leaves and fruits essential oil antiradical activity. However, Bendaoued et al. [30] confirmed that the *S. terebinthifolius* essential oil possess antiradical activity.

This antiradical activity may be related to the presence of polyphenols in *Schinus terebinthifolius* Raddi leaves and fruits essential oil. In fact, phenolic phytochemicals are thought to promote optimum health partly via their antioxidant and free radical scavenging effects thereby protecting cellular components against free radical induced damage. But due to their diverse chemical structures, they are likely to possess different antioxidant capacities [34]. In addition, according to Tepe et al. [35], monoterpenes act as radical scavenging agents.

4. Conclusion

The objective of this work is to provide more useful information on the species *Schinus terebinthifolius* Raddi. Despite the richness of this plant on compounds with high added value, it is exploited neither commercially nor industrially. For that reason, a comparative study between leaves and fruits essential oils, was established. *Schinus terebinthifolius* fruits essential oil showed lower total phenolic and flavonoids content than the leaves one but higher antiradical activity. Bicyclogermacrene was the major volatile compound of both samples.

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