Phytochemistry and Antioxidant Activities of *Rhus tripartitum* (Ucria) Grande Leaf and Fruit Phenolics, Essential Oils, and Fatty Acids

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Abstract

*Rhus tripartitum* (Ucria) Grande leaves and fruits were investigated for their contents in phenolic compounds, essential oils, and fatty acids. Chemical composition and antioxidant potential of these secondary metabolites were investigated using chromatographic tools and different antioxidant tests. Results displayed high amounts of phenolic compounds in leaves, concomitant with important antioxidant potentialities, probably due to their richness in phenolic acids and flavonoids as identified by reverse phase high performance liquid chromatography (RP-HPLC). Amounts of essential oils were higher in leaves. Oxygenated sesquiterpenes are exclusively synthesized by the fruits, expressing better antioxidant activities.

Keywords

*Rhus tripartitum*, phenolics, essential oils, fatty acids, terpenoids, flavonoids, bioactivity, antioxidants

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Introduction

Different antioxidant formulations are being sold as diet supplements by nutraceutical and food companies in developed nations.¹ Enhancement of the diet with naturally occurring antioxidants is one of many proposed solutions to fight the deleterious effects of reactive oxygen species (ROS).²,³ Besides, the increasing consciousness about the toxic effects of synthetic antioxidants has driven the interest of consumers and industry towards the use of natural additives in food, pharmaceutical, and cosmetic products as a replacement for synthetic chemicals.¹ This upheaval involves a constant growing requirement of raw materials and new ingredients from natural sources.¹,² In this context, plants constitute the major source of diverse bioactive compounds.³ Underutilized plant species can fulfill this demand due to their richness in phenolics, vitamins, minerals, and other bioactive compounds, but they are not exploited on a large scale.⁴

Results and Discussion

Extract yields: Fraction yields varied significantly as a function of the studied organs (Table 1). In fact, yields registered in leaf extracts were more important than their similar in the fruits (2.6% vs 0.98% dry weight (DW)). This fact may be explained by the differences in the chemical composition of the 2 organs and the affinity of the solvent to the different classes of phenols. Ethyl acetate is a medium polarity solvent, usually used to extract phenols of low to medium weight, and it is essentially known for its affinity to flavonoids and some phenolic acids.⁶ The leaves constitute the place of photosynthesis hence the need for the presence of antioxidant molecules, such as flavonoids, to cope with the deleterious effects of reactive oxygen species generated during this process. On the other side, the lowest yields of the fruit extract may be explained by the richness of this organ with polar substances with high molecular weight such as tannins, carbohydrates, and glycosylated compounds, generally abundant in fruits as they ensure their color, energetic value, and sweetness.⁷
Table 1. Phenolic Compound Contents and Antioxidant Activities of Rhus tripartitum Leaf and Fruit Extracts (in Comparison With Positive Controls).

| Samples              | Leaf       | Fruit      | Positive controls |
|----------------------|------------|------------|-------------------|
| Extract yield (% DM) | 2.6 ± 0.3  | 0.98 ± 0.1 | BHT               |
| TPC (mg GAE.g⁻¹ DW)  | 171 ± 0.9  | 39 ± 1.4   | BHA               |
| TFC (mg CE.g⁻¹ DW)  | 186 ± 8.4  | 42 ± 2.7   | AsA               |
| CTC (mg CE.g⁻¹ DW)  | 57 ± 3.8   | 14 ± 0.1   |                   |
| TAA (mg GAE.g⁻¹ DW) | 572 ± 35   | 64 ± 1     |                   |
| DPPH test (IC₅₀:µg.mL⁻¹) | 3.8 ± 0.13 | 18.8 ± 1.4 |                   |
| FRAP (EC₅₀:µg.EC.mL⁻¹) | 109 ± 4.4  | 310 ± 13.6 |                   |
| BCBT (IC₃₀:µg.mL⁻¹) | 150 ± 20   | 34 ± 4     |                   |

Means followed by the same letter in the row are not significantly different at P < .05 (means of 3 replicates).

Abbreviations: AsA: Ascorbic Acid; BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene; BCBT: β-carotene bleaching test; CTC: condensed tannins content; DPPH test: 2,2-diphenyl-1-picrylhydrazyl test; EC₅₀: effective concentration at which the absorbance was 0.5; FRAP test: ferric reducing antioxidant power; IC₅₀: inhibiting concentration of 50% of the synthetic radical DPPH; mg CE.g⁻¹ DW: milligram catechin equivalent per gram dry weight; mg GAE.g⁻¹ DW: milligram gallic acid equivalent per gram dry weight; TPC: total polyphenols content; TFC: total flavonoids content; TAA: total antioxidant activity.

**Phenolic compound contents:** Leaf phenolic amount is high (Table 1). Besides, estimation of the amounts of total flavonoids showed that these molecules are very abundant, especially in leaves. This result is perfectly in agreement with the point discussed above and dealing with the high affinity of the ethyl acetate to this class of phenolic compounds. Amounts of condensed tannin in fruits represent only a quarter of those found in leaves. This result is perfectly in agreement with the point discussed above and dealing with the high affinity of the ethyl acetate to this class of phenolic compounds.

**Antioxidant activities of R tripartitum extracts:** Total antioxidant activity (TAA) differed greatly among the studied organs of this species (Table 1). The total antioxidant activity of leaf extract is nine fold higher than that of fruit extract. Concerning the antiradical activity, leaf extracts expressed a very high capacity to quench the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, and so largely exceeded the antioxidant efficiency of the synthetic butylated hydroxytoluene (BHT) and ascorbic acid (AsA). Antiradical activity of the fruit extracts is high too, but not as much as that of the leaves.

**RP-HPLC identification of R tripartitum leaf and fruit phenolic compounds:** Seven phenolic compounds were identified in leaf extract (Table 2), with rutin and ellagic acid as major ones. Eight compounds were identified in the fruit extract. Apigenin and naringenin are dominant flavonoids in this organ. The fruit composition is enriched with a variety of flavonoids (eg rutin, myricetin, and isorhamnetin), besides the m-coumaric acid. The specific segregation of R tripartitum phenolics and related antioxidant properties is closely tied to the physiological specificity of the plant organ. The phenolic composition of R tripartitum leaves is basically constituted by phenolic acids and flavonoids (majorly represented by the rutin). As for the fruit, and apart from gallic and m-coumaric acids, the phenolic profile of this organ is exclusively belonging to the large group of flavonoids. These differences among organs’
chemical composition explain, partially, the disparity of the antioxidant activities between the studied extracts. Besides, Tili et al\textsuperscript{11} established the phenolic composition variability among localities and ripening stages in fruit methanol extracts. They found that flavone and betulinic acid are dominant phenolics in \textit{R tripartitum} fruit, regardless of the locality and maturation stage, which is completely different from the chemical composition that we found dominated by apigenin and naringenin, even for the fruits collected in the same stage (intermediate maturation stage). These differences may be explained by 2 hypotheses. The first one is resumed in intra-specific variability that may be due to specific edapho-climatic conditions among localities and/or genetic mutations. The second hypothesis is based on technical factors, mainly the extraction solvent and method. In fact, the principal objective of the solvent fractionation is to obtain a concentrated phenolic fraction using the extraction technique and of the right choice of solvent. For another side, it can be seen that there are 4 major and 25 minor compounds. However, those obtained from the fruits are a mixture of mono- and sesquiterpene hydrocarbons and, to a lesser extent, oxygenated sesquiterpenes (13.56%), which are exclusively produced by the fruits of this species, with viridiflorol as a major compound of this class. From another side, it can be seen that there are 4 major and common monoterpene hydrocarbons in both leaf and fruit essential oils is counterbalanced by the abundance of both sesquiterpene hydrocarbons and oxygenated sesquiterpenes in the fruit ones. This chemical composition seems to be a common trait in related species of this genus.\textsuperscript{13-15} The comparison of our chemical analyses to this provided by this study to Algerian ones allows us to deduce that Tunisian provenance presents different chemotypes from Algerian ones.\textsuperscript{12} The comparison of our chemical analyses to this study allows us to deduce that Tunisian provenance presents different chemotypes from Algerian ones.

**Antioxidant activities of \textit{R tripartitum} essential oils:** Apart from the iron-reducing capacity, where the differences between the 2 organs are not significant, the other tests used showed a variation of the antioxidant potential between the leaves and fruits of \textit{R tripartitum} (Table 4). The capacity of the essential
### Table 3. Percentage and Classification of Volatile Compounds of Essential Oils Obtained from Leaves and Fruits of *Rhus tripartitum*.

| Identified compounds | RI<sup>A</sup> | RI<sup>B</sup> | Identification<sup>C</sup> | Leaves % | Fruits % |
|----------------------|--------------|--------------|---------------------------|--------|---------|
| **Monoterpene hydrocarbons** |              |              |                           |        |         |
| 1 α-Pinene           | 939          | 1032         | RI, MS, Co GC             | 21.78 ± 1.45<sup>a</sup> | 6.7 ± 0.38<sup>b</sup> |
| 2 Camphene           | 951          | 1053         | RI, MS                    | 0.77 ± 0.07<sup>a</sup>  | -       |
| 3 Sabinene           | 976          | 1132         | RI, MS, Co GC             | 0.21 ± 0.02<sup>a</sup>  | -       |
| 4 β-Pinene           | 980          | 1118         | RI, MS, Co GC             | 3.89 ± 0.37<sup>a</sup>  | 1.11 ± 0.06<sup>b</sup> |
| 5 β-Myrcene          | 993          | 1174         | RI, MS                    | 1.59 ± 0.15<sup>a</sup>  | 1.04 ± 0.17<sup>b</sup> |
| 6 α-Phellandrene     | 1006         | 1206         | RI, MS, Co GC             | 0.46 ± 0.04<sup>a</sup>  | -       |
| 7 (β)-3-Carene       | 1011         | 1160         | RI, MS, Co GC             | 18.9 ± 2.05<sup>a</sup>  | 17.34 ± 1.61<sup>a</sup> |
| 8 α-Terpine          | 1018         | 1183         | RI, MS, Co GC             | 1.79 ± 0.14<sup>a</sup>  | 0.78 ± 0.11<sup>b</sup> |
| 9 e-Cymene           | 1020         | 1187         | RI, MS                    | 2.09 ± 0.2<sup>a</sup>   | -       |
| 10 p-Cymene          | 1026         | 1280         | RI, MS, Co GC             | 1.75 ± 0.19<sup>a</sup>  | -       |
| 11 α-Limonene        | 1031         | 1203         | RI, MS, Co GC             | 7.23 ± 0.78<sup>a</sup>  | 7.72 ± 1.21<sup>a</sup> |
| 12 γ-Pyronene        | 1338         | -            | RI, MS                    | 0.33 ± 0.03<sup>a</sup>  | -       |
| 13 (E)-β-Ocimene     | 1050         | 1262         | RI, MS                    | 0.35 ± 0.04<sup>a</sup>  | -       |
| 14 γ-Terpine         | 1061         | 1255         | RI, MS, Co GC             | 3.93 ± 0.32<sup>a</sup>  | 0.8 ± 0.52<sup>b</sup> |
| 15 α-Terpinolene     | 1092         | 1290         | RI, MS, Co GC             | 14.39 ± 1.86<sup>a</sup> | 11.71 ± 1.02<sup>a</sup> |
| **Total**            |              |              |                           | 77.72 ± 3.53<sup>a</sup> | 48.43 ± 3.75<sup>b</sup> |
| **Oxygenated Monoterpens** |              |              |                           |        |         |
| 16 Bornyl acetate    | 1295         | 1597         | RI, MS, Co GC             | 0.39 ± 0.03<sup>a</sup>  | -       |
| 17 Terpinen-4-ol     | 1178         | 1611         | RI, MS, Co GC             | 2.33 ± 0.02<sup>a</sup>  | -       |
| 18 β-Citronellol     | 1229         | 1772         | RI, MS                    | 1.38 ± 0.15<sup>a</sup>  | -       |
| 19 Thymol methyl ether | 1235     | 1607         | RI, MS                    | 1.55 ± 0.07<sup>a</sup>  | -       |
| 20 Geraniol          | 1258         | 1857         | RI, MS, Co GC             | 1.84 ± 0.57<sup>a</sup>  | -       |
| 21 Citronellyl formate | 1275     | -            | RI, MS                    | 0.68 ± 0.1<sup>a</sup>   | -       |
| 22 Geranyl formate   | 1298         | 1718         | RI, MS                    | 0.53 ± 0.11<sup>a</sup>  | -       |
| **Total**            |              |              |                           | 4 ± 0.27<sup>b</sup>     | 6.76 ± 0.81<sup>b</sup> |
| **Sesquiterpene hydrocarbons** |              |              |                           |        |         |
| 23 β-Cubebene        | 1388         | 1547         | RI, MS                    | 2.83 ± 0.08<sup>a</sup>  | -       |
| 24 β-Caryophyllene   | 1418         | 1612         | RI, MS, Co GC             | 0.71 ± 0.09<sup>a</sup>  | -       |
| 25 δ-Ma-liene        | 1422         | 1547         | RI, MS                    | 1.07 ± 0.1<sup>a</sup>   | -       |
| 26 Aromadendrene     | 1441         | 1628         | RI, MS                    | 4.49 ± 0.5<sup>a</sup>   | -       |
| 27 γ-Gurjunene       | 1473         | 1687         | RI, MS                    | 1.44 ± 0.18<sup>a</sup>  | -       |
| 28 Germacrene D      | 1489         | 1726         | RI, MS                    | 9.98 ± 1.51<sup>a</sup>  | -       |
| 29 Viridiflorene     | 1495         | 1695         | RI, MS                    | 2.22 ± 0.2<sup>a</sup>   | -       |
| 30 Bicyclogermacrone | 1513         | 1756         | RI, MS                    | 2.94 ± 0.6<sup>a</sup>   | -       |
| 31 β-Bisabolene      | 1510         | 1741         | RI, MS                    | 1.11 ± 0.16<sup>b</sup>  | -       |
| 32 γ-Badinene        | 1512         | 1765         | RI, MS                    | 1.93 ± 0.16<sup>a</sup>  | -       |
| 33 δ-Guaiene         | 1515         | -            | RI, MS                    | 30.66 ± 3.1<sup>a</sup>  | -       |
| 34 α-Bisabolene      | 1520         | 1746         | RI, MS                    | 16.99 ± 2.67<sup>b</sup> | -       |
| 35 δ-Cadinene        | 1530         | 1773         | RI, MS                    | 13.56 ± 0.84<sup>a</sup> | -       |
| **Total**            |              |              |                           | 1.77 ± 0.12<sup>a</sup>  | -       |
| **Oxygenated sesquiterpenes** |              |              |                           | 1.1 ± 0.16<sup>a</sup>  | -       |
| 36 Palustrol         | 1570         | 1953         | RI, MS                    | 5.46 ± 0.55<sup>a</sup>  | -       |
| 37 Spathulenol       | 1586         | 2150         | RI, MS, Co GC             | 2.39 ± 0.08<sup>a</sup>  | -       |
| 38 Viridiflorol      | 1590         | 2098         | RI, MS, Co GC             | 1.41 ± 0.01<sup>a</sup>  | -       |
| 39 Eudesm-5-en-11-ol | 1621         | -            | RI, MS                    | 0.46 ± 0.01<sup>a</sup>  | -       |
| 40 ρ-Cadinol         | 1640         | 2170         | RI, MS                    | 1.13 ± 0.04<sup>a</sup>  | -       |
| 41 Isopatulinol      | 1652         | 2223         | RI, MS                    | 13.56 ± 0.84<sup>a</sup> | -       |
| 42 β-Eudesmol        | 1653         | 2257         | RI, MS, Co GC             | 0.91 ± 0.03<sup>a</sup>  | -       |
| **Total**            |              |              |                           | 0.43 ± 0.42<sup>b</sup>  | -       |
| **Total identified** | 0.91 ± 0.03<sup>a</sup> | 0.43 ± 0.42<sup>b</sup> |             | 99.62 ± 0.63<sup>a</sup> | 99.84 ± 0.15<sup>b</sup> |

RI<sup>A</sup> and RI<sup>B</sup>: Retention indices calculated using an apolar column (HP-5) and polar column (HP-INNOWax).<sup>C</sup>: Retention indices relative to C8 to C40 n-alkanes on the (HP-INNOWax). Abbreviations: Co-GC, co-injection with authentic compound; RI: retention index; MS, mass spectrum. Values followed by the same lowercase letter in the rows did not share significant differences at 5% (Duncan test) (means of 3 replicates ± S).
the extraction of fatty acids of the best of our knowledge, this is the fashion wave invading the research world is the investigation of oxygen compounds. On the other hand, the prepotency of fruit oils over leaf ones is partitum arguing about the nutritional and industrial importance of fruits one. Oil contents are expressed as % dry weight basis.

Fatty acid composition of both organs (Table 5) is dominated by C18 fatty acids, represented by oleic, linoleic, and palmitic acids. The sum of these compounds gathered is in the range of 84.8% and 72.5% of leaf and fruit oils, respectively. Besides, good proportions of stearic acid were detected too. Previous study on R tripartitum fruit oils obtained at the intermediate maturation stage showed that they are dominated by higher percentages of palmitic acid and lower ones of oleic and linoleic acids when compared to our samples. These differences highlight the important effects of biotic and abiotic factors on the amounts and chemical composition of these molecules.

Table 4. Antioxidant Activities of Rhus tripartitum Leaf and Fruit Essential Oils.

|                  | DPHH test | FRAP test | BCBT test |
|------------------|-----------|-----------|-----------|
|                  | (IC30<sub>g</sub> mg.mL<sup>-1</sup>) | (EC30<sub>g</sub> mg.mL<sup>-1</sup>) | (IC50<sub>g</sub> mg.mL<sup>-1</sup>) |
| Leaf             | 8.3 ± 0.11<sup>a</sup> | 1.8 ± 0.9<sup>a</sup> | 4.9 ± 0.9<sup>a</sup> |
| Fruit            | 2.8 ± 0.03<sup>c</sup> | 1.65 ± 0.09<sup>a</sup> | 3.9 ± 0.4<sup>a</sup> |
| Positive control | 5.15 ± 0.64<sup>b</sup> | 1.55 ± 0.1<sup>b</sup> | 1.9 ± 0.6<sup>b</sup> |

Means followed by the same letter are not significantly different at P < 0.05 (means of three replicates).

Abbreviations: BCBT: β-carotene bleaching test; DPHH test: 2,2-diphenyl-1-picrylhydrazyl test; EC<sub>30</sub>: effective concentration at which the absorbance was 0.5; FRAP test: ferric reducing antioxidant power; IC<sub>50</sub>: inhibiting concentration of 50% of the synthetic radical DPHH.

Oils to act as antioxidant molecules thus expressed is higher than that determined in the essential oils extracted from aromatic plants. The biological activity of essential oils depends on the chemical composition and functional groups (alcohol, phenol, terpenes and ketones). Thus, the nature of the terpenic compounds and their proportions play a determining role in the antioxidant activity of the oils. This property seems to be attributed to oxygenated monoterpenes, especially phenols and aldehydes. These chemical classes are poorly represented in R tripartitum oils, which explain to some extent, their antioxidant potential. The antioxidant activity hence determined can be linked to their richness in monoterpane hydrocarbons, especially in α-pinene which acts as a scavenger of free radicals. On the other hand, the prepotency of fruit oils over leaf oils is closely related to its richness in mono- and sesquiterpene oxygen compounds.

**Oil yield and fatty acid composition:** Nowadays, the new fashion wave invading the research world is the investigation of new sources of seed oils from underexploited plants. To the best of our knowledge, this is the first work describing the extraction of fatty acids of R tripartitum leaves and the analysis of their chemical composition, in comparison with the fruits one. Oil contents are expressed as % dry weight basis (Table 5). Oil contents are almost fivefold higher in leaves than in the fruits. These results are of great importance when arguing about the nutritional and industrial importance of R tripartitum. However, Tili et al. found higher amounts in the fruits collected in the same maturation stage but from other locations. Fatty acid composition of both organs (Table 5) is dominated by C18 fatty acids, represented by oleic, linoleic, and palmitic acids. The sum of these compounds gathered is in the range of 84.8% and 72.5% of leaf and fruit oils, respectively. Besides, good proportions of stearic acid were detected too. Previous study on R tripartitum fruit oils obtained at the intermediate maturation stage showed that they are dominated by higher percentages of palmitic acid and lower ones of oleic and linoleic acids when compared to our samples. These differences highlight the important effects of biotic and abiotic factors on the amounts and chemical composition of these molecules.

A common objective of the scientists and nutritionists from all around the world is to find the balance between a secure intake, high quality, and nutritional value of natural products. Thus, the study of fatty acid profiles is a key tool in the determination of the comestible oil quality. Analysis of R tripartitum lipid composition indicated that saturated fatty acids (SFAs) part was statically the same in the studied organs, representing less than 1/5 of the identified fatty acids. However, R tripartitum leaves and fruits are characterized by the presence of high amounts of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFAs) (Table 5). Generally, the lipid storage composition represents high percentages of unsaturated fatty acids (UFAs), the most abundant of which are oleic and linoleic acids, in addition to a ratio of UFAs/SFAs exceeding 3 to ensure a longer shelf life and effective protection against lipid peroxidation and food spoilage. Proportions of UFAs are 80.95% and 78.48% in leaves and fruits, respectively, and so are four times higher than SFA levels, which are revealed by the ratios SFAs/UFAs equal to 4.5 and 4.00 in leaves and fruits, respectively (Table 5). These ratio values are higher than the usual recommended ones. The major MUFA found in these plant leaves and fruits is oleic acid. This fatty acid is widely recognized for its improving the immune system due to its anti-inflammatory

Table 5. Fatty Acid Composition (% of Total Fatty Acids), DBI, and oil Yield of Rhus tripartitum.

|                  | Leaves | Fruits |
|------------------|--------|--------|
| C08:0 (Caprylic acid) | 0.03 ± 0.01<sup>a</sup> | - |
| C12:0 (Lauric acid) | 0.04 ± 0.01<sup>b</sup> | 0.18 ± 0.02<sup>a</sup> |
| C14:0 (Mircistic acid) | 0.17 ± 0.04<sup>b</sup> | 0.44 ± 0.02<sup>a</sup> |
| C16:0 (Palmitic acid) | 13.02 ± 0.01<sup>b</sup> | 14.45 ± 0.24<sup>a</sup> |
| C16:1 (Palmitoleic acid) | 0.09 ± 0.0<sup>a</sup> | 0.023 ± 0.0<sup>b</sup> |
| C16:2 (Hexadecadienoic acid) | 0.12 ± 0.01 | 0.86 ± 0.12<sup>a</sup> |
| C18:0 (Stearic acid) | 5.30 ± 0.01<sup>a</sup> | 4.07 ± 1.63<sup>a</sup> |
| C18:1 (Oleic acid) | 43.51 ± 0.0<sup>a</sup> | 43.29 ± 2.22<sup>a</sup> |
| C18:2n6 (Linoleic acid) | 35.96 ± 0.2<sup>a</sup> | 25.15 ± 1.35 |
| C18:3n3 (α-Linolenic acid) | 0.86 ± 0.08<sup>b</sup> | 2.67 ± 0.6<sup>a</sup> |
| C18:4n3 (Stearidonic acid) | - | 0.35 ± 0.07<sup>a</sup> |
| C20:0 (Arachidic acid) | 0.36 ± 0.02<sup>a</sup> | 0.39 ± 0.03<sup>a</sup> |
| C20:1 (Gadoleic acid) | 0.29 ± 0.01<sup>b</sup> | 2.62 ± 0.54<sup>a</sup> |
| C22:0 (Behenic acid) | 0.12 ± 0.0<sup>b</sup> | 0.26 ± 0.02<sup>a</sup> |
| C22:1 (Eruccic acid) | 0.12 ± 0.08<sup>b</sup> | 1.68 ± 0.14<sup>a</sup> |
| C22:5n3 (Clupanodonic acid) | - | 0.74 ± 0.27<sup>a</sup> |
| C22:6n3 (Docosahexaenoic acid) | - | 0.87 ± 0.04<sup>a</sup> |
| SFA | 19.5 ± 0.06<sup>a</sup> | 19.78 ± 1.85<sup>a</sup> |
| MUFA | 44.01 ± 0.08<sup>b</sup> | 47.83 ± 1.9<sup>a</sup> |
| PUFAs | 36.94 ± 0.13<sup>b</sup> | 30.65 ± 2.37<sup>b</sup> |
| UFA | 80.95 ± 0.06<sup>a</sup> | 78.48 ± 3.27<sup>a</sup> |
| UFA/SFA | 4.25 ± 0.02<sup>a</sup> | 4.00 ± 0.61<sup>a</sup> |
| DBI | 1.18 ± 0.07<sup>a</sup> | 1.05 ± 0.07<sup>b</sup> |
| Oil yield (%) | 5.21 ± 0.12<sup>a</sup> | 1.07 ± 0.03<sup>b</sup> |

Values with different lowercase letters in the same row are significantly different at P < 0.05 (means of three replicates).

Abbreviations: DBI: double-bound index; MUFA: monounsaturated fatty acid; PUFAs: polyunsaturated fatty acid; SFA: saturated fatty acid; UFA: unsaturated fatty acid.
were measured as described by Sun et al. The amount of total condensed tannins are expressed as mg CE.\text{g}^{-1} \text{DW}, through the calibration curve of \( (r^2 = 0.99) \). All samples were analyzed in 3 replications.

**RP-HPLC identification of** \( R \) \textit{tripartitum} **phenolic compounds**: The identification of \( R \) \textit{tripartitum} phenolic compounds was done using Agilent 1260 HPLC system consisting of a vacuum degasser, an autosampler, and a binary pump with a maximum pressure of 600 bar (Agilent technologies), equipped with a reversed-phase C18 analytical column of 4.6 \( \times \) 100 mm and 3.5\( \mu \)m particle size (Zorbax Eclipse XDB C18). The DAD detector was set to a scanning range of 200 to 400 nm. Column temperature was maintained at 25°C. The injected sample volume was 2 \( \mu \)L and the flow rate of mobile phase was 0.4 mL.min\(^{-1}\). Mobile phase B consisted of 0.1% formic acid Milli-Q water and mobile phase A was methanol. The elution program was as follows: 0 to 5 min: 90% to 80% B; 5 to 10 min: 80% to 70% B; 10 to 15 min: 70% to 50% B; 15 to 20 min: 50% to 30% B; 20 to 25 min: 30% to 10% B; 25 to 30 min: 10% to 50% B; 30 to 35 min: 50% to 90% B. Each sample was directly injected and chromatograms were monitored at 280 nm.

**Essential oils isolation**: Triplicate samples of 100 g of leaves and fruits were subjected to hydrodistillation in 1L of deionized water using a Clevenger apparatus for 4 and 6 h, respectively. Obtained oils were dried over anhydrous sodium sulphate and stored at +4°C until tested. Essential oil yields were expressed as percent of the plant material weight used.

**Gas chromatography (GC) analysis**: GC analysis was carried out using an Agilent 6890 gas chromatograph equipped with a flame ionization detector and split–splitless injector attached to HP-INNOWax polyethylene glycol capillary column (30m \( \times \) 0.25mm; 0.25\( \mu \)m film thickness). One micro-liter of the sample (dissolved in hexane as 1/50 v/v) was injected into the system. The compounds were identified by comparing their relative retention times with those of authentic compounds injected in the same conditions and by comparing their retention index (RI) calculated with C8 to C40 Alkanes Calibration Standard (40147-U, Supelco, Germany).

**Gas chromatography/mass spectrometry (GC-MS) analysis of essential oils**: Identification of the essential oils was performed using a Hewlett Packard HP5890 series II GC-MS equipped with an HP5MS column (30 m \( \times \) 0.25 mm). Helium was used as carrier gas at 1.2 mL.min\(^{-1}\). Each sample (1\( \mu \)L) was injected in the split mode (1:20), the program used was isothermal at 70°C, followed by 50 to 240°C at a rate of 5°C.min\(^{-1}\), then held at 240°C for 10 min. The mass spectrometer was an HP 5972. The total electronic impact mode at 70 eV was used. The components were identified by comparing their relative retention times and mass spectra with the data from the library of essential oils constituents, Wiley, MassFinder, and Adams GC-MS libraries and by comparing their RI calculated with C8 to C40 Alkanes Calibration Standard (40147-U, Supelco, Germany).

**Fatty acid extraction**: Oils were obtained according to ISO method 659:1998. Powder samples (30 g) were extracted with petroleum ether in a Soxhlet apparatus for 4 h. Extracts were concentrated using a rotary evaporator under reduced pressure.
at 50°C and then oils were dried with nitrogen before storing at −20°C. Analyses were performed in triplicate.

**Preparation and GC-MS and GC-FID analysis of FAMEs:** FAMEs were prepared as described by Megdiche-Ksouri et al. The identification of FAMEs was performed using an HP-5980 Series II instrument, equipped with HP-5MS capillary column (30m × 0.25mm; 0.25µm film thickness) and split/splitless injector (220°C). The oven temperature was held at 150°C, then programmed to increase 15°C.min⁻¹ until reaching 220°C, and detained isothermally at 220°C for 5 min. The carrier gas was helium used at an initial flow rate of 1 mL.min⁻¹. Split ratio was 20:1. Injection volume was equal to 2µL. The components were identified by comparing their relative retention times and mass spectra with the data from the library of fatty acids constituents: Wiley, MassFinder, and Adams GC-MS libraries. The quantification of fatty acid methyl esters, expressed as percentages, was obtained directly from GC-MS libraries. The quantification times and mass spectra with the data from the library of fatty acids constituents: Wiley, MassFinder, and Adams GC-MS libraries.

**Split ratio was 20:1. Injection volume was equal to 2µL. The components were identified by comparing their relative retention times and mass spectra with the data from the library of fatty acids constituents: Wiley, MassFinder, and Adams GC-MS libraries.**

**FRAP assay:** The iron (III) reducing capacity was assessed as described by Oyayiu. Results are expressed as Effective Concentration at which the absorbance was 0.5 (effective concentration at which the absorbance was 0.5 [EC50] in mg.mL⁻¹) obtained from linear regression analysis.

**BCBT:** The antioxidant activity of the extracts was evaluated in terms of β-carotene bleaching as described by Koleva et al. The results are expressed as IC₅₀ (µg.mL⁻¹) which is the Inhibiting Concentration of 50% of the β-carotene initially used.

**Antioxidant Activities of R tripartitum Extracts and EOs**

**TAA:** Experiments were conducted according to Koleva et al. The antioxidant capacity was expressed as mg GAE.g⁻¹ DW. The calibration curve range was 0 to 500 µg.mL⁻¹. All samples were analyzed in triplicate.

**DPPH assay:** DPPH quenching ability was measured according to Hanato et al. All samples were analyzed in triplicate. The results are expressed as IC₅₀ (µg.mL⁻¹) which is the Inhibiting Concentration of 50% of the synthetic radical.

**FRAP assay:** The iron (III) reductive capacity was assessed as described by Oyayiu. Results are expressed as Effective Concentration at which the absorbance was 0.5 (effective concentration at which the absorbance was 0.5 [EC₅₀] in mg.mL⁻¹) obtained from linear regression analysis.

**BCBT:** The antioxidant activity of the extracts was evaluated in terms of β-carotene bleaching as described by Koleva et al. The results are expressed as IC₅₀ (µg.mL⁻¹) which is the Inhibiting Concentration of 50% of the β-carotene initially used.

**Statistical analysis**

Means were statistically compared using the STATI-CF program. A one-way analysis of variance (ANOVA) and Newman–Keuls multiple range tests were carried out to test any significant difference between samples at P < .05. Values were the means of 3 replicates.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Ethical Approval**

Ethical approval is not applicable for this article.

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**Statement of Human and Animal Rights**

This article does not contain any studies with human or animal subjects.

**Statement of Informed Consent**

There are no human subjects in this article and informed consent is not applicable.

**Trial Registration**

Not applicable, because this article does not contain any clinical trials.

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