# CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF VOLATILE FRACTION OF THE PEEL OF *Maclura pomifera* FRUIT GROWING IN TUNISIA

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**ABSTRACT:** The volatile compounds from *Maclura pomifera* (Osage orange fruit) peel have been studied by GC-FID and GC–MS. Volatiles fraction proportion was estimated to 60 mg/kg. Identified compounds consist on aliphatics (52.6%), monoterpenes along with sesquiterpenes (27.3%), phenylpropane derivatives along with related compounds (10.0%) and other compounds (10.6%). Among the 60 identified compounds, 1-hexanol (13.5%), (*Z*)-Hex-3-en-1-ol (10.2%), α-cadinol (10.1%), Tetradecanoic acid (8.4%), β-eudesmol (8.3%) and Megastigma-4,6(*Z*),8(*E*)triene (5.3%) were the majors. Essential oil was tested for antibacterial activity against five strains. The oil showed a moderate antibacterial effect against *Bacillus subtilis*, *Staphylosccus aureus*, *Enterobacter faecalis* and *Echerchia coli*.

**Key words:** antibacterial activity, GC-MS, hydrodistillation, *Maclura pomifera*, retention indice, volatile fraction.

**RESUME :** Les composés volatiles de l'écorce de l'Oranger des Osages (fruit d'Osage orange) ont été étudiés par GC et GC-MS. La fraction volatile s'est élevée à 60 mg / kg. Les composés ainsi identifiés comprennent des constituants aliphatiques (52,6%), les monoterpènes avec sesquiterpènes (27,3%), les dérivés phenylpropane ainsi que de composés apparentés (10,0%) et autres composés (10,6%). Au total 60 composés ont été identifiés, avec le 1-hexanol (13,5%), (*Z*) acétate, 3-Hexen-1-ol (10,2%), α -cadinol (10,1%), acide tétradécanoïque (8,4%), β-eudesmol (8,3%) et megastigma-4, 6(*Z*),8(*E*)triène (5,3%) étaient les principaux composés. L'huile essentielle a été testée pour une activité antibactérienne contre cinq souches. L'huile a montré un potentiel moyen de l'effet anti-bactérien contre *Bacillus subtilis*, *Staphylosccus aureus*, *Enterobacter faecalis* et *Echerchia E. coli*.

**Mots clés :** activité antimicrobienne, fraction volatile, GC-MS, hydrodistillation, indice de rétention, *Maclura pomifera*.

## 1. INTRODUCTION

*Maclura pomifera* (Rafin.) Schneider (syn. *Maclura aurantiaca* Nutt pomiferum Ioxylon Raf. *Toxylon pomiferum* Raf.ex Sarg) is a member of the Moraceae family. This tree is known as Osage orange, horse apple, mock orange or hedge apple [1, 2]. Planted in USA, it originates from southern Oklahoma and northern Texas [3]. It was introduced in Tunisia as ornamental tree. The isolated compounds from different parts of the Osage orange tree belongs to different chemical classes such as flavonoids, xanthones, triterpenes and stilbenes [4-6]. The phytochemical studies of this fruit focused on isoflavonoids (ojasin and pomiferin), prenylated isoflavones (scandenone and auriculasin) and lectin [7-9]. Lipid fraction extracted from seeds is also studied [10]. Several

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biological activities are determined for this fruit such as antioxidant, antimicrobial and cholinesterase inhibitor activity [11].

In the last decades, special attention was paid to volatile compounds and their contribution to fruit aroma [12, 13]. However, only two works were concerned with volatile fraction of Osage orange tree from American and Croatian origins [14, 15]. Sesquiterpenoids well represent among volatile compounds of this fruit [14, 12] notably: elemol, α-copaene, α-cubebene, δ-cadinene, β-elemene, β-caryophyllene, α-ylangene (*Z,E*)-farnesol and hexyl hexanoate [14]. Currently, unavailable chemical profile of the Osage orange growing in Tunisia. As part of our research, we studied the volatile fraction of the fruit peel. The aim of our research is to characterize the volatile compounds by gas chromatography and gas chromatography-mass spectrometry and evaluate antibacterial activity of Tunisian fruit peel.

## 2. EXPERIMENTAL

### 2.1. Plant material

Plant Fruits collected from abandoned plantations that are for several years in Belvedere garden (Tunis, Tunisia). The mature fruits (harvest of November) resulting from only one tree, have approximately 0.5 g of weight and 8 cm of diameter. External part of fruits (Peel or exocarp) were removed, rinsed with water and kneaded until a smooth paste.

### 2.2. Extraction of volatiles compounds

Fresh material (1000 g) is introduced into the half-filled flask with distilled water. The mixture is heated to boiling using a heating mantle. Vapor was cooled by cold water circulatory through the condenser. After four hours, heating is stopped. This step is followed by liquid-liquid extraction with methylene chloride $CH_2Cl_2$. extraction was repeated three times (30 mL x 3). The solvent was dried over anhydrous sodium sulphate and the volatile fraction was concentrated by spontaneous evaporation of the solvent to obtain viscous yellow liquid. The volatile fraction was stored in sealed vials protected from the light at −20 °C before analyses. The volatile fraction was isolated in a yield of (0.006%) (w/w).

### 2.3. GC-FID and GC-MS analysis

GC-FID analysis of the volatile components was carried out (Agilent 6890N, California, USA). Using tow different columns a HP-5 capillary column (5% phenylmethylpolysiloxane, 30 m, 0.25mm i.d.; 0.25 µm film thickness) and a HP-Innowax column (polyethylene glycol column, 0.25mm internal diameter, 30m length and 0.25 µm film in thickness) were used with the following temperature program: 1 min at 50 °C, and up at a rate of 2 °C/min to 310 °C, held for 2 min; for HP-Innowax column 1 min at 40°C and up at a rate of 2°C/min to 240 °C. Injector temperature, 270 °C; carrier gas, helium (1.0 ml/min); 1 µl of volatile fraction was injected with split ratio1:100. GC-MS analysis was performed using an Agilent 6890 GC-MS system operating in the EI mode at 70 eV, using two different columns a HP-5MS capillary column (5% phenylmethylpolysiloxane, 30 m, 0.25 mm i.d., 0.25 µm film thickness) and a HP-Innowax polyethylene glycol column (0.25mm internal diameter, 30m length and 0.25 µm film in thickness), The temperature program was identical to that used previously for FID detection. Helium; flow rate: 1.0 ml/min; injector and transfer line temperatures: 250 °C; injection volume: 1 µl; split ratio: 1:100; acquisition mass range: 50–550 amu.

### 2.4. Identification and quantification of volatile components

The compounds identity is assigned by comparison of their retention indices (RIs) on both HP5 and Innowax columns and from their mass spectra. The RIs are calculated using a C8–C30 hydrocarbons standard and compared with those of literature data [16, 17]. Acquired mass spectra were based on the NIST05 and WILEY7 mass spectra databases. The relative amounts of individual components are calculated on the basis of GC peak areas.

### 2.5. Antibacterial activity tests

The antibacterial activity of essential oil was screened by agar disk diffusion assay against five human pathogenic bacteria, including Gram-positive *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* clinical strains (INSAT), Gram-negative bacteria *Echerchia coli* ATCC 8739, *Salmonella typhimurium*

ATCC 14028 and *Enterobacter faecalis* ATCC 29212. The bacterial strains were first grown on nutrient agar at 37°C for 24 h. One or several well isolated colonies of the indicator bacteria were transferred into a sterile saline solution and adjusted to the 0.5 McFarland turbidity standards. A sterile 6 mm diameter filter disk (Whitman paper) was placed on the infusion agar seeded with bacteria, and 15 µl of essential oil was dropped onto each paper disc. The treated Petri dishes were kept at 4 °C for 1 h, and incubated at 37 °C for 24 h. The antibacterial activity was assessed by measuring of growth inhibition zone surrounding these disks. Standard disks of ampicillin served as positive antibiotic controls.

## 3. RESULT AND DISCUSSION

Chemical composition of the volatile fraction from peel fruit is reported in Table 1. Sixty components were identified in this volatile fraction. The major constituent of this fraction were hexan-1-ol (13.5%) as the major compound, (*Z*)-Hex-3-en-1-ol (10.2%), α-cadinol (10.1%), Tetradecanoic acid (8.4%), β-eudesmol (8.3%) and Megastigma-4,6(*Z*),8(*E*)triene (5.3%). Other components were dominant *p*-cresol (5.0%), *E*,*Z*-Nona3,6-dien-1-ol (4.7%) and (-)-Caryophyllene oxide (2.5%). The oil was characterized by large amounts of aliphatic compounds and sesquiterpenes which occurred in relatively high amounts (52.6% and 27.3%, respectively).

Megastigma-4,6(*Z*),8(*E*)triene is one of four isomers from β-inone derivatives who is generated from β-carotene degradation [18].

Regarding this composition, our study shows partially similar results to those obtained with Osage orange fruit of Croatian origin extracted by hydrodistillation in a Clevenger-type apparatus for 3 h. A mixture of pentane: ether (1:1) was used as the collection liquid, and the oil was stored at −20 °C until analysed. In this study 43 compounds were identified, including elemol (19.2%) as the major component, trans-caryophyllene (5.6%), β-selinene (3.8%), γ-selinene (3.5%) and δ-cadinene (2.6%), aliphatic alcohols, such as 1-dodecanol (9.7%), 1-hexanol (4.4%) and 1-decanol (3.0%) [15]. The steam distillate of American fruit contained (*Z*,*E*)-farnesol (13.6%) as the major component, accompanied by elemol (7.3%), other sesquiterpenes and aliphatic compounds, in total 22 compounds [14].

*p*-Cresol was the major component among the phenylpropane derivatives and related compounds; while α-cadinol, β-eudesmol and (-)-Caryophyllene oxide were dominant among the terpene compounds. Some compounds found in the fruit peel essential oil were not found in the fruit of American or Croatian origin. Observed differences in chemical composition of essential oils depend mainly on climatic, seasonal and geographic conditions [19].

In vitro antimicrobial activities of the essential oil of *Maclura pomifera* against two Gram-positive and three Gram-negative bacteria are reported in Table 2. Generally Gram-positive bacteria are more susceptible to essential oils action compared to Gram-negative ones [20, 21]. In the five different tested strains, the highest activities were recorded against *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter faecalis* and *Escherichia coli*. In contrast, *Salmonella typhimurium* was found to be less sensitive. Tested essential oil was generally much less active than ampicillin used as positive control. The antimicrobial activity of essential oils is mainly based on their chemical composition, and the particular nature of their major volatile compounds [22].Aliphatic alcohols were reported to possess strong to moderate activities several bacteria. The position of the alcohol functional group was found to affect molecular properties of the component. Essential oils rich in 1,8-cineole demonstrated activity against Gram-positive and Gram-negative bacteria [23]. This activity is also probably associated with the pure monoterpene hydrocarbons [24. The fruit peel volatile fraction contains major aliphatic alcohols such as 1-hexanol, Z-hex-3-en-1-ol acetate, (*E*,*Z*)-3,6-Nonadien-1-ol, and other compounds responsible of antimicrobial activity like (-)-Caryophyllene oxide, 1,8-cineole.

In future, it will be suitable to expand antimicrobial activity to a wide range of bacteria strains. Moreover, we expect improvement in yield by proceeding to a whole fruit essential oil extraction this may allow other biological activities testing.

**Table I:** The composition of volatiles compounds from the bark of *Maclura pomifera*

| No. | Compound | Ia | Ib | % | Identification[c] |
|---|---|---|---|---|---|
| Aliphatic compounds | | | | | |
| 1 | 2-Methyl pentan-3-ol | 801 | / | 2.2 | Ia, MS |
| 2 | Ethyl but-2-enoate | 839 | 1037 | 0.5 | Ia, Ib, MS |
| 3 | (*Z*)-Hex-3-en-1-ol | 856 | 1378 | 0.2 | Ia, Ib, MS |
| 4 | 1-Hexanol | 866 | 1362 | 13.5 | Ia, Ib, MS |
| 5 | 3-Methylbutyl acetate | 876 | 1149 | 0.1 | Ia, Ib, MS |
| 6 | Hexanoic acid | 999 | 1878 | tr | Ia, Ib, MS |
| 7 | *Z*-hex-3-en-1-ol acetate | 1007 | 1336 | 10.2 | Ia, Ib, MS |
| 8 | *E*-Hex-2-en-1-ol acetate | 1016 | 1392 | 0.2 | Ia, Ib, MS |
| 9 | Octan-1-ol | 1069 | 1520 | 2.4 | Ia, Ib, MS |
| 10 | Nonanal | 1106 | 1386 | 0.1 | Ia, Ib, MS |
| 11 | *Z*-non-3-en-1-ol | 1128 | 1707 | tr | Ia, Ib, MS |
| 12 | *E*,*Z*-Nona3,6-dien-1-ol | 1155 | 1748 | 4.7 | Ia, Ib, MS |
| 13 | Trans, Cis-Nona-2,6-dien-1-ol | 1167 | / | 0.3 | Ia, MS |
| 14 | 3-Hexenyl Hexanoate | 1385 | 1646 | 1.6 | Ia, Ib, MS |
| 15 | Hexyl Hexanoate | 1387 | 1620 | 1.2 | Ia, Ib, MS |
| 16 | Tetradecanoic acid | 1763 | 2714 | 8.4 | Ia, Ib, MS |
| 17 | *n*-Hexadecanoic acid | 1973 | / | 1.0 | Ia, MS |
| 18 | Ethyl hexadecanoate | 1996 | / | tr | Ia, MS |
| 19 | 3,6,6-Trimethyl-norpinan-2-ol | 2089 | 2785 | 2.8 | Ia, Ib, MS |
| 20 | Ethyl linoleate | 2162 | 2548 | 1.3 | Ia, Ib, MS |
| 21 | *Z*,*Z*,*Z*- ethyl-octadecatrien-9,12,15-oate | 2166 | 2599 | 0.7 | Ia, Ib, MS |
| 22 | Heptadecyl octanoate | 2167 | / | 0.3 | Ia, MS |
| 23 | Heptacosane | 2707 | 2693 | tr | Ia, Ib, MS |
| 24 | Squalene | 2822 | 2818 | 0.5 | Ia, Ib, MS |
| 25 | Nonacosane | 2900 | / | 0.4 | Ia, MS |
| Phenylpropane derivatives and related compounds | | | | | |
| 26 | Phenol | 993 | 2053 | 0.1 | Ia, Ib, MS |
| 27 | Benzyl Alcohol | 1039 | 1882 | 0.2 | Ia, Ib, MS |
| 28 | p-Cresol | 1075 | 2134 | 5.0 | Ia, Ib, MS |
| 29 | Phenylethyl Alcohol | 1117 | 1969 | 1.5 | Ia, Ib, MS |
| 30 | Ethyl-Phen4-ol | 1178 | 2196 | tr | Ia, Ib, MS |
| 31 | Estragole | 1195 | 1675 | 0.6 | Ia, Ib, MS |
| 32 | Phenyl-2-ethyl Acetic acid | 1256 | 1864 | 1.3 | Ia, Ib, MS |
| 33 | Eugenol | 1353 | / | 1.0 | Ia, MS |
| 34 | Methyl eugenol | 1406 | 2077 | tr | Ia, Ib, MS |
| 35 | 4-Methyl-Benzaldehyde | 1080 | 1657 | 0.3 | Ia, Ib, MS |
| Monoterpenes, diterpenes and sesquiterpenes | | | | | |
| 36 | 1,8-Cineol | 1031 | 1219 | 0.9 | Ia, Ib, MS |
| 37 | (+)-Fenchone | 1085 | 1396 | 0.4 | Ia, Ib, MS |
| 38 | α-Cubebene | 1349 | 1463 | tr | Ia, Ib, MS |
| 39 | α-Caryophyllene | 1454 | 1670 | 0.1 | Ia, Ib, MS |
| 40 | D-Germacrene | 1479 | 1708 | 0.8 | Ia, Ib, MS |

**Table I:** The composition of volatiles compounds from the bark of *Maclura pomifera* (continued)

| No. | Compound | Ia | Ib | % | Identification[c] |
|---|---|---|---|---|---|
| 41 | (+)-Epi-bicyclosesquiphellandrene | 1484 | 1720 | 0.1 | Ia, Ib, MS |
| 42 | α-Selinene | 1495 | 1730 | 0.2 | Ia, Ib, MS |
| 43 | Ermophilene | 1504 | 1738 | tr | Ia, Ib, MS |
| 44 | δ-Cadinene | 1525 | 1771 | 0.4 | Ia, Ib, MS |
| 45 | Elemol | 1553 | 2077 | 0.3 | Ia, Ib, MS |
| 46 | (-)-Caryophyllene oxide | 1584 | 1969 | 2.5 | Ia, Ib, MS |
| 47 | Viridiflorol | 1588 | 2066 | 0.3 | Ia, Ib, MS |
| 48 | (+-)-5-Epi-Neointermedeol | 1611 | 2256 | 1.2 | Ia, Ib, MS |
| 49 | γ-Eudesmol | 1632 | 2171 | 0.9 | Ia, Ib, MS |
| 50 | β-eudesmol | 1651 | 2215 | 8.3 | Ia, Ib, MS |
| 51 | α-cadinol | 1652 | 2221 | 10.1 | Ia, Ib, MS |
| 52 | Farnesol | 1713 | 2358 | 0.2 | Ia, Ib, MS |
| 53 | Phytol | 2107 | 2608 | 0.6 | Ia, Ib, MS |
| Furan molecules | | | | | |
| 54 | Furfural | 829 | 1471 | 1.8 | Ia, Ib, MS |
| 55 | Dihydro-5-pentyl-2(3H)-Furanone | 1365.5 | 2007 | 0.1 | Ia, Ib, MS |
| Azulene molecules | | | | | |
| 56 | Guaiol* | 1600 | 2106 | 0.9 | Ia, Ib, MS |
| 57 | 1-(1,3a,4 ,5,6,7-hexahydro-4-hydroxy-3,8-dimethyl-5-azulenyl) Ethanone | 1684 | 2603 | 0.7 | Ia, Ib, MS |
| Others compounds | | | | | |
| 58 | *p*-Mentha-1,5-dien-8-ol | 1165 | 1670 | 1.5 | Ia, Ib, MS |
| 59 | Megastigma-4,6(*Z*),8(*E*)triene** | 1544 | 1951 | 5.3 | Ia, Ib, MS |
| 60 | 3(4-*tert*-butylphenoxy)-butan-2-one | 1547 | 1869 | 0.3 | Ia, Ib, MS |

Ia, retention indices on HP-5 column; Ib, retention indices on HP-innowax column; MS, mass spectral data; /, not detected on this column; tr, traces (<0.1%).

*the guaiol nucleus is 2-[(3S,5R,8S)-3,8-dimethyl-1,2,3,4,5,6,7,8-octahydroazulen-5-yl]propan-2-ol

**The megastigma-4,6,8-triene nucleus is l-(but-2-enylidene)-2,6,6-trimethylcyclohex-2-ene The semi-trivial nomenclature is used here to facilitate stereochemical descriptors [18]

[c] Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those reported from literature [16, 17] and NIST05

**TableII:** Inhibitory effect of volatile fraction from the peel of Osage orange on five human pathogen bacteria, compared to that of positive standard (Ampicillin)

| Bacterial strains | Diameter of growth inhibition (mm) | |
|---|---|---|
| | Ampicillin | Volatile fraction of Osage orange |
| *Staphylococcus aureus* ATCC 6538 | 40 | 11 |
| *Bacillus subtilis* | 34 | 14 |
| *Escherichia coli* ATCC 8739 | 18 | 7 |
| *Salmonella typhimurium* ATCC 14028 | 20 | 0 |
| *Enterobacter faecalis* ATCC 29212 | 24 | 9 |

## 4. CONCLUSION

The Analysis by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) demonstrated that *M. pomifera* fruit peels essential oil contained mainly aliphatic and sesquiterpenes compounds with exceptionally rich content of aliphatic alcohols. The volatile fraction of fruit peel has a moderate potential to inhibit the growth of tested bacteria.

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