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Study of volatile compounds of *Pyrus mamorensis* Trab. a characteristic plant of Mamora forest (north-western Morocco)

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Abstract

Pyrus mamorensis Trab. commonly called “wild pear” was considered for long time as an endemic of the Mamora forest of Morocco (north-west). Although lack of protection and it is threatened with extinction in its range, this tree is of great interest due to its ecological and medicinal properties. This study focused on evaluation of volatiles in *Pyrus mamorensis* for its valorisation as a natural resource. So, the volatile compounds from different parts (leaves, stems, fruits, and flowers) were investigated after extraction by cold maceration in ethyl ether and analyzed by gas chromatography coupled to the mass spectrometry (GC - MS). Thirty one (31) compounds were identified and quantified including fifteen (15) terpenes, seven (7) alcans, two (2) aldehydes, four (4) esters and three (3) allyl alkoxybenzen derivatives. Estragol was the dominant component of the plant and that was only detected in leaves (83,09 %), while hexadecane (36,92 %) and allyl hexanoate (29,39 %) were mainly detected in the stems. The fruits showed relatively high levels (20,59 %) of benzyl butanoate, whereas in the flowers, limonene is the most abundant constituent (30,12 %). The analysis of the relative rates of different classes of volatile compounds, revealed the diversity of these compounds in *P. mamorensis* compared to two cultivars of edible species : *P. communis* L. et *P. pyrifolia*. The significant difference in composition in this case could be due to the wild character of P. mamorensis.

Keywords: *Pyrus mamorensis; wild pear; volatile compounds; Mamora; Gas Chromatography-Mass Spectrometry (GC-SM).*

1. Introduction

P. mamorensis, commonly called “wild pear”, is a calcifuges tree of the Mamora forest located on the Atlantic frontage of north-western Morocco, where does not form large stands. This species belongs to the subfamily Maloideae in the family Rosaceae, one of the most common in the moderate and

subtropical areas of the northern hemisphere. It was long considered an endemic species of the forest of Mamora in Morocco until the issue that it would be a subspecies or variety was raised. The taxon is apparently named as a synonym for *Pyrus bourgaeana* in some databases [1-2-3]. So, *P. bourgaeana* is described in the literature as Iberian wild pear [4]. However, some other indexed databases still cite this taxon as a separate species, since its first description in 1915 by Trabut as a Moroccan pear [5]. This has encouraged us to continue our research on this tree which has no conservation plan despite its rarity [6] and it is threatened by various human activities and pathogenic fungi attacks [7-8-9]. Recently, some works suggested implementing conservation strategy preserving the genetic resources of the wild pear of north-western Morocco [10]. According to our investigations, researches on this species are rare and there are no phytochemical studies available. Some of our studies [11-12] included a phytochemical screening of the extracts from different parts of the tree providing a synoptic description of principal classes of secondary metabolites such as flavonoids, tannins, saponins, terpenes and coumarins. Another study revealed significant antifungal effects of some natural extracts of *P. mamorensis* [13]. So, the purpose of this work is to investigate the volatile compounds from various parts of *P. mamorensis* to provide more complete information on this plant for its eventual valorisation and preservation.

2. Material and methods

2.1 Samples preparation

Various parts of *P. mamorensis* trees including stems, leaves, flowers and fruits, were sampled *in situ* from the Mamora forest, then dried in the dark at ambient temperature (24-26˚C) in a ventilated room in the laboratory. To facilitate drying action, samples were regularly turned.

2.2 Extraction

Ten grams (10g) of each dried sample were crushed in an electric grinder, then macerated in 50 ml of ethyl ether in hermetically closed bottles and agitated for one week at ambient temperature (24-26 ˚C). The extracts were filtered and concentrated by evaporation of solvent under a hood until obtain aliquots of about 3 to 5 ml.

3.2 GC-MS Analysis

The analysis of the volatile substances of *P. mamorensis* was carried out by gas chromatography coupled with mass spectrometry (GC-MS, standard Hewlett Packard 5941). The chromatograph, was equipped with a silica capillary tube (25m x 0,20 mm internal diameter) and furnished with polydimethylsiloxane $(C_2H_6OSi)_n$ standard DB 5. It uses helium as carrier gas with 0,6 ml.min^{-1}flow. The injector was 220°C and the detector 240°C with temperature programming between 50°C (for 3 min) and 50°C- 250°C at a rate of 3°C min^{-1}. The recording of the spectra was done with a quadripole detector and ionization carried out by electronic impact under a potential 70 eV. The volatile compounds were identified by the spectrum of mass and the relative index of Kovats *IK* [14-15].

3. Results and discussion

Thirty one (31) volatile compounds belonging to various classes were detected in different analyzed parts of *P. mamorensis* including oxygenated monoterpenes, unoxygenated monoterpenes,

unoxygenated sesquiterpenes, alkanes, aldehydes, esters and allyl alkoxybenzene derivatives (Table 1). The volatile profiles and rates varied largely among the analyzed parts of the plant. In the leaves the major constituent is estragol (81,04%); stems are rather characterized by hexadecane and allyl hexanoate with respectively levels of 36.92% and 29,39 % and fruits had relatively high rates of benzyl butanoate (20,59%) and hexadecane (11,43%), whereas the flowers are rich in limonene and hexadecane with respective rates of 32,11% and 18,88%.

These results showed that the estragol is the most dominant of all identified compounds of the plant. It is characteristic of the leaves of *P.mamorensis*, representing 81,04 % of the total of the identified compounds, adding thereto some other compounds which appear to be specific to the leaves and that are not detected in any of the analyzed organs of the plant, namely : linalool, bornyl acetate, methyleugenol, hexyl benzoate, hexenyle benzoate. However, no compound of the unoxygenated monoterpenes class is detected in the leaves. In the flowers, the specific constituents are: nonane, sabinene, β-pinene (non-oxygenated monoterpenes) δ- terpinene, anethol and δ-cardinene and the stem is rather marked by : myrcene (monoterpenes), allyl hexanoate, dodecane and decanal, while the fruits are characterized by nonanal, undecane, thymol, tetradecane and benzyl butanoate.

The camphor is the only common compound to all organs of *P. mamorensis*, showing rates with a decreasing gradient from major amounts detected in the stem and the leaves to the flowers which contain least amounts. The volatiles were grouped according to their chemical class including terpenes (unoxygenated monoterpenes, oxygenated monoterpenes and unoxygenated sesquiterpenes), alkanes, allyl alkoxybenzene derivatives, esters and aldehydes, compared to those from two *Pyrus* species : *P. communis L* (Bartlet var) and *P. pyrifolia* (Zaobaimi var). The composition of the analyzed parts was represented in table 2. The relative contents of the different class of volatiles were dependent on the organs. Then, the terpenes and alkanes are detected in all analyzed organs, with an abundance of terpenes in the flowers and alkanes in the stems and fruits. Esters are rather more present at the stems and fruit are almost absent in the flowers. As the aldehydes, they were detected only in the stems and fruits with lower rates, unlike the allyl alkoxybenzene derivatives, which estragol is the major compound; they are abundantly present in the sheets, while they are weakly represented in the flowers.

In the other hand, comparing the volatile contents in the fruits of *P. mamorensis* and those of two edible species *P. communis* L (Bartlet) and *P. pyrifolia* (Zaobaimi) we noted that the two edible species are relatively less rich in volatiles and showed a variable composition of detected compounds compared to *P. mamorensis*. Indeed, four classes of volatiles among five analyzed (terpenes, alkanes, aldehydes and esters) were detected in the *P. mamorensis* fruit with relatively less variable rate.

While the composition of fruit of *P. communis L* (Bartlet) only three classes of volatile compounds : the class of esters which is majority (96%) and aldehydes and terpenes that are poorly represented. As for *P. pyrifolia* (Zaobaimi) whose fruits are much less rich, the results showed only two classes among the analyzed compounds: the majority class aldehydes (89.19%) and esters showing a low rate.

This difference in the volatile composition between *P. mamorensis* and the two edible species of *Pyrus* could be attributed to its domestic character. In fact, these two species are widely cultivated in Europe and America (*P. communis*) and Asia (*P. peryfolia*) and are recognized by the high quality of their edible fruits and economically appreciated through odor and appearance characteristics.

Li et al. [16] affirmed that aroma is an important fruit sensory factor that is significantly influenced by the volatile compounds present in the fruit, and it determines their consumability.

Table 1: Volatile compounds of stems, leaves, fruits and flowers of *P. mamorensis* extracted by ethyl ether and analyzed by GC-MS. The percentages were expressed compared to the peaks areas.

Volatile Compounds	***P.mamorensis***				
	IK	***Leaf (%)***	***Stem (%)***	***Fruit (%)***	***Flower (%)***
Unoxygenated monoterpenes					
Sabinene	**971**	nd	nd	nd	**1,2**
β –pinene	**976**	nd	nd	nd	**0,80**
Myrcene	**991**	nd	**1,17**	nd	Nd
ρ –cymene	**1024**	nd	**2,04**	**4,48**	Nd
Limonene	**1029**	nd	**6,65**	**11,04**	**30,12**
δ –terpinene	**1058**	nd	nd	nd	**1,2**
Oxygenated monoterpenes					
1-8 cineole	**1032**	**7,13**	nd	nd	Nd
Linalool	**1098**	**1,01**	nd	nd	Nd
Camphor	**1145**	**1,36**	**1,47**	**5,54**	**9,62**
Thymol	**1287**	nd	nd	**7,19**	Nd
Bornyl Acetate	**1290**	**0,58**	nd	nd	nd
Unoxygenated sesquiterpenes					
δ cardinene	**1421**	nd	nd	nd	**5,63**
Isocaryophyllene	**1431**	nd	nd	nd	**14,86**
β –caryophyllene	**1441**	**1,35**	**4,25**	nd	**8,84**
β –farnesene	**1451**	**1,34**	nd	nd	Nd
Alkanes					
Nonane	**900**	nd	nd	nd	**3,2**
Undecane	**1100**	nd	nd	**2,63**	Nd
Dodecane	**1200**	nd	**3,61**	nd	Nd
Tridecane	**1300**	nd	**2,23**	**8,62**	Nd
Tetradecane	**1400**	nd	nd	**9,89**	Nd
Pentadecane	**1500**	**1,14**	**10,18**	**11,03**	nd
Hexadecane	**1600**	nd	**36,92**	**11,43**	**18,88**
Aldehydes					
Nonanal	**1079**	nd	nd	**4,56**	Nd
Decanal	**1204**	nd	**2,09**	nd	Nd
Allyl alkoxybenzene derivatives					
Estragol	**1190**	**<u>81,04</u>**	nd	nd	Nd
Anethol	**1290**	nd	nd	nd	**5,65**
Methy eugenol	**1390**	**3,26**	nd	nd	Nd
Esters					
Allyl hexanoate	**1080**	nd	**29,39**	nd	Nd
Benzyl butanoate	**1335**	nd	nd	**23,59**	Nd
E-3 hexenyl benzoate	**1534**	**0,94**	nd	nd	Nd
Hexyl benzoate	**1576**	**0,85**	nd	nd	Nd
Total (%) :		**100**	**100**	**100**	**100**

IK : indicates *index of Kovats ;* **nd** : not detected

(*) : Rates calculated considering other classes of compounds not detected in *P. mamorensis* ; **nd** : not detected

Indeed, the green and herbaceous flavor notes of fruits of *Pyrus* are attributed to aldehydes action [17]. Moreover, aldehydes and esters are considered among the principle volatile components (PVCs) which determine the contribution of each compound in the total aroma of the cultivar [16]. knowing that PVCs are those compounds that occur in high quantities of at least three times the concentration of any other constituents [18], this would explain the results obtained for the fruits of the wild pear *P. mamorensis*, according to which the contents of these compounds (esters, aldehydes and terpenes) do not show significant discrepancies, Perhaps because the fruits of the wild pear are not edible and therefore are not subject to the same maturation process as their counterparts from the edible cultivars.

To date, in our knowledge, our study on volatile compounds of *P. mamorensis* is the first of its kind; it revealed its richness in volatiles (31 in total) belonging to various classes, often considered to be essential oils that are widely used in cosmetics, aromatherapy and pest control and plant protection. Some of the monoterpenes of the flowers could play a role in attraction of pollinators and could improve pollination of the plant [19]. These include limonene which is used as a disinfectant; beta-pinene, as an anti-inflammatory drug; and nonanal, as a sedative and anti-infection drug [20-21]. Terpinenes could have sedatives, expectorants, antiviral, anti-inflammatory and anti-stress virtues. Other proprieties such as anti-depressive, antispasmodic, antiviral and memory stimulating actions have been attributed to anethol, estragol, camphor and cineole. In fact, some studies noted that 1-8 cineole and other oxygenated monoterpenes had several effects on many pest insects, it acts by blocking the synthesis of juvenile hormone or inhibiting acetylcholinesterase activity by occupying the active site and it could also inhibit the development of insect eggs, larvae and/or nymphs [22-23]. On the other hand, many authors showed that pinene can have an antimicrobial (insecticidal, fungicidal and/or bactericidal) capacity [24-25-26-22-27-28]. Wilson et al. [29] affirmed the usefulness of volatile compounds in integrated pest management by reporting the fungicidal effects efficiency of limonene, cineole, β -myrcene, α -pinene, β -pinene and camphor of ripe fruit of *Prunus persica* and *Pyrus communis* against *Botrytis cinerea*. Similar effects of camphor and cineol on *Fusarium* [30] and those of camphor and anethole on *Aspergillus* [31] have been reported. Otherwise, some bactericidal effects on *Staphylococcus* of limonene and linalool were also demonstrated [32].

4. Conclusion

The wild pear tree, like the other wild and/or endemic plants, is a good pollinator of cultivated varieties. It constitutes an essential irreplaceable genetic resource for breeding new resistant fruit varieties to diseases or as rootstocks. Our findings revealed that *P. mamorensis* is rich in volatile substances known for their interesting medicinal and cosmetic virtues still to be explored, hence the originality of this study. Indeed, these promising results are being supplemented with further investigations particularly on essential oils of *P. mamorensis* in order to study their potential uses as an important component of the integrated pest management system and in aromatherapy.

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References

[1] http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?30532

[2] http://eol.org/pages/233767/names

[3] http://www.eu-nomen.eu/portal/taxon.php?GUID=9EDF2603-F79C-4159-9C35-EF30E752FC98#

[4] J. Fernández-Haeger and D. Jordano-Barbudo, *Forests*, *5*, 1304-1330; doi :10.3390/f5061304 (2014).

[5] W. A. Taylor, US department.of griculture, bureau of Plant Industry, Office of for;eign seed and plant introduction (1918) 123 pp.

[6] M. Fennane, M. Ibn Tattou, F. M. Raimondo, B. Valdés, *Herbarium Mediterraneum Panormitanum*, Bocconea 8 (1998) 243 pp.

[7] K. Yamni, , N. Dohou, A. Outkoumit, A. Ouazzani Touhami, A. Douira, *Phytopathologia Mediterranea* 45 (2006) 40-42.

[8] Z. Sellal, J. Dahmani, R. Benkirane, A. Ouazzani Touhami, A. Douira, *Revue Marocaine de Protection des Plantes,* 3 (2012) 71-86.

[9] Z Sellal, J. Dahmani, R. Benkirane, A. Ouazzani Touhami, A. Douira, *Atlas Journal of Biology,* 2 (2) (2013)125–129.

[10] A. Ait Said, A. Oukabli, F. Gaboun, M. H. Simard, C. El Modafar, *Genetic Resources & Crop Evolution*, 60 (2013) 927-938.

[11] N.Heimeur, L. M. Idrissi Hassani, M. A. Serghini, *Reviews in biology and biotechnology*, 3(1) (2004) 37-42.

[12] N. Heimeur, L. M. Idrissi Hassani, M. A. Serghini, *Annales de la recherche forestière au Maroc*, 37 (2006 a) 1-9.

[13] N. Heimeur, L. M. Idrissi Hassani, M. A. Serghini, *Moroccan Society of Biochemistry MSB, Proceeding of International Congress of Biochemistry,* Agadir. Morocco. (2006 b) May 9-12.

[14] V. Pacakova, L. Pelt, New York: Wiley & Sons, (1992) 2087 p.

[15] S. Kirsten, W. Gert, K. B. Dinesh, F. Oliver, *BMC Bioinformatics*, 12 (2011) 321 p.

[16] G. Li, H. Jia, R. Wu, S. Hussain, Y. Teng, *Afr. J. Agric. Res,* 7(34) (2012) 4761-4770.

[17] J. M. L. Nicolas, A. J. A. Sevilla, A. A. C. Barrachina, F. G. Carmona, *J. Agric. Food Chem*., 57 (2009) 9668-9675.

[18] S. S. Pandit, H. G. Chidley, R. S. Kulkarni, K. H. Pujari, A. P. Giri, V. S. Gupta. Chem. 114 (2009) 363-372.

[19] A. K. Borg-Karlson., C. R. Unelius, I. Valterova, L. A. Nilsson, *Phytochemistry*, 41 (6) (1996) 1477 -1483.

[20] J. P. Willem, *Edt. Dauphin*, ISBN 2-7163-1206-0. (2002) 311p.

[21] E. J. Bowles, J. Brunet,. « Guide des huiles essentielles. *Paris : Le Courrier du Livre*, (2004).160 p.

[22] C. H. Obeng-Ofori, J. B. Reichmuth, A. W. Hassanali, *Applied Entomology,* 121 (1997) 237-243.

[23] M. Safaei Khorram, S. Jafarnia, S. Khosroshahi, 51 (3) (2011) 225 p.

[24] K. Kambu, N. D. Phanzu, C. Coune, J. N. Wauters, L. Angenot, *Plantes Medicinales et Phytotherapie*, 16 (2) (1982) 34 - 38.

[25] D. Bamba, J. M. Bessiere, C. Marion, Y. Pelissier, I. Fouraste, *Planta Medica*, 59(2) (1993) 184 - 185.

[26] W. E. Taylor, B. Vickery, *Agricultural Sciences*, 7 (1995) 61-62.
[27] A. O.Oyedeji, O. Ekundayo, O. N. Olawore, B. A. Adeniyi, W. A. Kenig, *Fitoterapia*, 70 (5) (1999) 526 - 528.
[28] K. Cimanga, K. Kambu, L. Tona, S. Apers, T. De Bruyne, N. Hermans, J. Totte, L. Pieters, A. J. Vlietinck, *Ethnopharmacology*, 79 (2) (2002) 213 - 220.
[29] C. L.Wilson, J. M. Solar, A. El Ghaouth, M. E. Wilsniewski, *Plant disease*, 81 (2) (1997) 204-210.
[30] D. Pitarokili, , O. Tzakou, A. Loukis, C. Harvala, *Journal of. Agricultural and. Food Chemistry*, 51 (2003) 3294–3301.
[31] D. N. Mimica, , S. Kujundzic, M. Sokovic, M. Couladis, *Phytotherapy. Research*, 17 (2003) 368–371.
[32] A. Sonboli, , F. Eftekhar, M. Yousefzadi, M. R. Kanani, *Z. Naturforsch*, 60 (2005) 30–34.