DENTAL USE OF SOME LAMIACEAE SPECIES FROM MOROCCO AND PRINCIPAL COMPONENT ANALYSIS BETWEEN TOTAL POLYPHENOLIC CONTENT OBTAINED BY DIFFERENT AQUEOUS EXTRACTION

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INTRODUCTION
The family of lamiaceae is one of the largest plant families used as a framework to evaluate the occurrence of typical secondary metabolites (Wink, 2003). It belongs to the lamales order, from the asterids class (clade Euasterids I); It is the sixth-largest family of Angiosperms comprising 12 subfamilies, 16 tribes, 9 subtribes, 236 genera, and more than 7000 species (Pignatti, 1982). Also, lamiaceae is highly represented in the Mediterranean basin, where it’s represented by approximately 1000 wild species grouped in 48 genera (Valverde, 2000) and most of the species are aromatic and medicinal herbs used in traditional pharmacopoeia, against many human affections. For example, according to recent studies this family is more presented against COVID-19 (Najem et al., 2022), is also a powerful source of therapeutic agents for the regulation of metabolic dysfunction which include diabetes (Elssassala et al., 2021), in Meknes city of Morocco, it has been reported that lamiaceae species, especially Origanum compactum Benth. are commonly prescribed by local herbalists against oral diseases (Harouak et al., 2019a); this plant contain thymol which is used as antibiotic in dentistry product (Harouak et al., 2019b), is also a valuable additional agent for treating patients with periodontitis and its probable effect on the cardiovascular health (Castellino et al., 2021).

On the other hand, the efficiency of medicinal plants is due to their richness in bioactive phytochemicals compounds, among which, polyphenols are the most distributed and structurally diversified. In fact, multiple biological activities have been ascribed to polyphenols, particularly they are well-known to be excellent antioxidants (Lingué et al., 2016; Alamgir, 2017), and to possess antimutagenic, anticarcinogenic, anti-inflammatory activities and neuro- protective actions (Skendi et al., 2017). Also, polyphenols seem to be among the useful solutions for controlling the induction of dental caries (Farkash et al., 2019), and have the ability to inhibit the formation of mixed biofilms on orthodontic surfaces in dentistry (Hamada et al., 1984).

So, in this study, we chose five species from lamiaceae family, Origanum compactum Benth. (Oregano), Marrubium vulgare L. (White horehound) Thymus satureioides Coss. (Thyme), Mentha pulegium L. (Pouliot Mint) and Lavandula multifida L. (Lavender); these species are used against oral diseases in traditional medicine (Harouak et al., 2019a) and our objective is to quantify their content of major polyphenolic compounds as well as to compare three types of extraction for a profitability (extraction type-secondary metabolite).

MATERIAL AND METHODS
Plant materials, samples collection and preparation
- Marrubium vulgare L. (White horehound or Common horehound) is widespread in North Africa, where it covers vast territories valued at more than ten million hectares (Aouati and Berchi, 2015).
- Origanum vulgare Agavi. is a shrub in plas, low and medium mountains under arid, and soft and cold arid, subhumid and humid bioclimates (Fennane et al., 2007).
- Origanum compactum Benth. is an aromatic and medicinal plant known in Morocco by its vernacular names “Zaatar” and “Sahtar”. This species has long been used in the Moroccan pharmacopoeia for its multiple medicinal virtues (Bouyalya et al., 2019). It is an Ibero-Moroccan endemic, dominant in forests and matorrals in plains and low mountains of the Rif and Middle Atlas in arid, semi-arid and subhumid bioclimates (Fennane et al., 2007).
- Mentha pulegium L. (Pennyroyal) commonly named “Filyou” is a perennial, aromatic, popular and herbaceous plant, which can reach up to half a meter in height. It was extensively used as a preservative ingredient in the food industry and as a natural flavor for folk medicine (Rodrigues et al., 2013). It is frequent in humid stations of the plains, low and medium mountains, under semi-arid, subhumid and humid conditions, in non-Saharan Morocco (Fennane et al., 2007).
- Lavandula multifida L. (Lavender) is a small semi-evergreen perennial shrub native of the South-Western Europe and North Africa (Pignatti, 1982). In Morocco, its commons names are “Khzama and Kohyla” and it is widespread in plains and low mountains in arid semi-arid, subhumid bioclimates in non-Saharan Morocco (Fennane et al., 2007).
- Thymus satureioides Coss. (Thyme) is an endemic species of Algeria and Morocco commonly known as “Zaazra & Tzoukenit”; it represents large wide natural formations in the central and western High Atlas and in northern (upper eastern Rif) moroccan regions (Fennane et al., 2016).

First, aerial parts of these species were collected during the flowering session, and a code has been assigned to each of the five species (tab 1).

Table 1 Studied plants pictures by Harouak, 2019 and their informative data

<table>
<thead>
<tr>
<th>Species Name</th>
<th>Scientific Name</th>
<th>Specimen Number</th>
<th>Harvest Date</th>
<th>Harvesting Site</th>
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</thead>
<tbody>
<tr>
<td>Mentha pulegium L.</td>
<td>MICSOLE NV-MPN/14</td>
<td>23/06/2019</td>
<td>34°04'07.7'' N 5°30'42.9'' W</td>
<td></td>
</tr>
</tbody>
</table>
The plants after; were air dried at room temperature in our laboratory greenhouse during fifteen of days, then pass through a 0.5 mm sieve in a mill; our own samples were stored after, in plastic bags in a dark place, dry and cool.

**Determination of moisture content**

The moisture content is the quantity of water contained in the plant material, it is expressed as a percentage (%) and calculated by the following formula Eq. (1) (Mohammadpour et al., 2019).

\[
MC (\%) = \left(\frac{M1-M2}{M1}\right) \times 100
\]

Where: 
- \( M1 \) : weight of the sample in grams after harvesting (fresh matter); \( M2 \) : weight of the sample in grams after drying (dry matter) (Mohammadpour et al., 2019).

**Extraction of plant samples**

Using Infusion, Decoction and Soxhlet techniques for each species, three types of aqueous extracts were prepared, at the rate of 10% of each sample in distilled water, and after each extraction, the mixture, was filtered with a Wathman filter paper and aqueous extracts were prepared, at the rate of 10% of each sample in distilled water.

**Using Infusion, Decoction and Soxhlet techniques**

Infused extracts: For this preparation, the plant materials were added to boiling distilled water, and were then left under stirring for 15 min.

Decoction extracts: All samples were added to distilled water and heated under stirring with a hot plate until boiling, then the mixture was left to stand under stirring for 15 minutes.

Soxhlet extracts: The powdered samples were extracted using distilled water for 4 hours (12 cycles) in a Soxhlet apparatus.

Using Eq. (2) to evaluate the percentage of extraction yield (Alara et al., 2018).

\[
\% \text{ Yield of extracts } = \left(\frac{W_e}{W_t}\right) \times 100
\]

Where, \( W_e \) is the weight of extracts from plant sample (w) and \( W_t \) is the weight of dried plant sample.

**Statistical analysis**

In order to highlight the significant differences between extraction yields and between the three types of extraction and studied plants, a statistical study of results was carried out using GraphPad prism 8.2.263 software, by analysis of variance two ways (ANOVA), followed by Tukey test to detect the degree of significance, this significance is taken at the probability of \( p<0.05 \) for a significant difference, \( **p<0.001 \) for a highly significant difference, \( **p<0.0001 \) for a very highly significant difference (\( P \) value is calculated from \( F \) (Dfn, Dfd) the order of the two degrees of freedom matters; Dfn is the degree of freedom for the numerator and Dfd is for the denominator), a large value of \( F \) means that something is significant, while a small value of \( p \) means that all the results are significant, so that the null hypothesis can be rejected starting at \( p<0.05 \).

The diagrams representing dosed polyphenolic compound were plotted by the same software by the mean of three replicates ± standard deviation (n=3).

The principal component analysis (PCA) was carried out to highlight the different correlations between variables (dosed polyphenolic compounds) and types of extraction between individuals (studied plants); this analysis was performed by XLSTAT 2016 (18.02.01) software.

**Phytochemical Screening**

It is a qualitative analytical study, that we apply to the research of different phytochemical groups contained in the extracts of studied plants.

The crude aqueous extracts, underwent phytochemical screening in order to detect the presence (or absence) of terpenoids (Liebermann Burchard reaction), alkaloids (Dragendorff and Mayer reagent), flavonoids (cyanidine reaction), tannins (ironcloride), and reducing sugar (Fehling reagent) (Karruni et al., 2004).

**Extraction**

For saponin revelation, in a test tube containing the extract with distilled water, the mixture was stirred for 20 seconds and left to stand for 15 min, the appearance of persistent foam greater than 1 cm in height indicates the presence of saponiosides, and the foam index was calculated as follows (Jean, 2009).

**Foam index**

\( (\text{N number of the tubes in which the foam equal } 1\text{cm}). \)

**Determination of total phenolic content (TPC)**

The total phenolic content (TPC) was determined according to the procedure reported by Slinkard and Singleton using the reagent of FolinCiocalteu (Slinkard and Singleton, 1977).

In test tubes for each extract (concentration of 1 mg/ml in distilled water), 100 µl was added to 4.5 ml of distilled water, then to 100 µl of Ciocalteu Folin reagent. This mixture was left to stand for 3 min at room temperature of the laboratory, then 300 µl of NaOH (2% in water) was added. After 1h30 incubation in the dark and at room temperature, the absorbance was measured with a spectrophotometer (UV-2005) at 760 nm against a blank containing distilled water. A calibration curve was drawn under the same operating conditions using gallic acid as a standard phenolic compound (100 to 1000 µg/ml); the results were expressed as mg of Gallic Acid Equivalent (GAE)/1g of dry extract and the analysis was expressed as mean of three replicates ± standard deviation.

TPC was calculated according to Eq. (3)

\[
\text{TPC} = \left(\frac{C \times V}{m}\right)
\]

where \( C \): is the concentration of TPC from the equation of calibration curve with gallic acid (y=0.0008x-0.0145), \( R^2 = 0.9978 \) (Figure 1), \( V \): is the total volume of solvent (distilled water) used in the test (ml), and \( m \) represents the weight of the dried sample used (g) (Alara et al., 2018).

![Figure 1](image-url)
Determination of total flavonoids content (TFC)

According to the method used by Sladana et al. (2011) (Žilić et al., 2011), the total flavonoid content (TFC) was estimated. A solution composed of (500 μl) of each extract (1 mg/ml) and 75 μl of NaNO₂ (5%) was prepared. After 6 min, 150 μl of an AlCl₃ solution (10%) was added, then the whole was left to stand at room temperature for 5 min, then 500 μl of NaOH (1M) was added and the total volume was adjusted to 2.5 ml with distilled water. The absorbance was measured at 510 nm against a blank of distilled water as extraction solvent. Under the same operating conditions, a calibration curve was drawn using catechin as reference flavonoid (50 to 500 μg/ml); the results were expressed as mg of catechin Equivalent (CE)/ 1g of dry extract and the analysis was expressed as mean of three replicates ± standard deviation. TFC was calculated according to Eq. (4).

\[ TFC = \frac{C \times V}{m} \]

where \( C \) is the concentration of TFC from the equation of calibration curve with catechin (y=0.0031x+0.0517), \( R² = 0.998 \) (µgg/ml) (Figure 2), \( V \) is the total volume of solvent (distilled water) used in the test (ml), and \( m \) represents the weight of the dried sample used (g) (Alara et al., 2018).

**Figure 2 Calibration curve of catechin**

**Determination of total condensed tannins content (TCT)**

According to the method described by Broadhurst and Jones (1978), tannins condensed content using acidified vanillin was estimated. In a test tube covered with aluminum foil, 0.5 ml of each aqueous extract (1mg/ml) was added to 1.5 ml of vanillin reagent (4%, v/v, vanillin in methanol). After vortexing, 750 μl of concentrated hydrochloric acid was added. After a second vortex agitation, the mixture was left in the dark at 20 °C for 15 min. The absorbance was determined at 500 nm, against a blank of distilled water as extraction solvent. Under the same operating conditions, a calibration curve was drawn using catechin as reference condensed tannin (100 to 1000 µg/ml); the results were expressed as mg of Catechin Equivalent (CE)/ 1g of dry extract and the analysis was expressed as mean of three replicates ± standard deviation. TCT was calculated according to Eq. (5).

\[ TCT = \frac{C \times V}{m} \]

where \( C \) is the concentration of TCT from the equation of calibration curve with catechin (y=0.0003x+0.0062), \( R² = 0.999 \) (µg/ml) (Figure 3), \( V \) is the total volume of solvent (distilled water) used in the test (ml), and \( m \) represents the weight of the dried sample used (g) (Alara et al., 2018).

**Table 2 Moisture content %**

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<tr>
<td>Moisture Content (%)</td>
<td>60,54</td>
<td>28,56</td>
<td>53,98</td>
<td>65,68</td>
<td>43,19</td>
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Moisture levels ranged from 65.68% to 28.56% (tab. 2), with the highest moisture recorded in Lavandula multifida L. while the plant with the least quantity of water is Thymus satureioides Coss. while Hossain et al., 2010, reported that the moisture content of some plants lamiaceae family ranged between 71.2 and 89.6% . This difference may be due to the difference of the considered plants, especially as the family contains more than 1000 wild species (Valverde, 2000), and to the climatological difference at the harvesting time; in fact, the temperature is the only variable that determines the water concentration of air in the internal intercellular spaces and when the leaf receives radiation, its temperature tends to rise, which increases the water content of the air in the leaves (Jones, 2013).

**Figure 3 Calibration curve of Catechin**

**Determination of total anthocyanin content**

Using a differential pH method with a double buffer system as described by Lako et al. (2007), total anthocyanin was quantified. Potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M) were prepared. 0.3 ml of extract was added to 2.7 ml of the corresponding buffer. Against a blank containing distilled water, the absorbance of each solution was measured by the spectrophotometer at 510 nm and 700 nm. The total anthocyanin content was expressed as cyanidin-3-glucoside (%w/w) equivalents as follows Eq. (6) (Jakobek et al., 2007).

\[ [\text{Anthocyanin}] \text{ mg/l} = \frac{(A_510-\text{nm, pH}=0.025\text{M})-(A_510-\text{nm, pH}=4.5\text{-}3\text{Glu})}{(0.4\text{M})} \times 1000 \]

where:
- \( A_510-\text{nm, pH}=0.025\text{M} \) and \( A_510-\text{nm, pH}=4.5\text{-}3\text{Glu} \) were prepared, 0.3 ml of extract was added to 2.7 ml of the corresponding buffer.
- Against a blank containing distilled water, the absorbance of each solution was measured by the spectrophotometer at 510 nm and 700 nm.
- The final anthocyanin content was expressed as mg of cyanidin-3-glucoside equivalent CYE/100 g of dry extract; the measurements was expressed as mean of three replicates ± standard deviation.

**Nitrogen total content, pH and electric conductivity**

In order to get an idea of the soils in which the plants were growing, the amount of total nitrogen, pH and electrical conductivity were determined. Total nitrogen was determined using the Kjeldahl (PRO-NITRO S) method (Wilke, 2005).

The percentage of total nitrogen is calculated by the following formula Eq. (7):

\[ \%N = \frac{[(14.01\times(V_\text{V} - V_\text{Vo})\times M\text{MOE})\times 100]}{V_\text{Vo}} \]

where:
- \( V_\text{V} \) is Volume in ml of HCl titrating the sample, \( V_\text{Vo} \) is Volume in ml of HCl titrating the blank, \( M\text{MOL} \) is Molarity of hydrochloric acid (=0.05M), \( m \) is Mass in (g) of the sample (1g).
- Soil pH was measured at 25 °C using pH meter (WTW-inoLab series) in the supernatant suspension of soil. Electric conductivity was determined at 25 °C using conductivity meter Sension+ EC71 GLP.

**RESULTS AND DISCUSSION**

**Moisture content**

Otherwise, according to (Díaz et al., 2002), lamiaceae plants generally contain 75 to 80% water in their fresh state, and this water level must be reduced to at least 15% for their conservation.
Figure 4 Yield extractions percentage, Values sharing **p<0.001, ***p<0.0001 are significantly different, (ns) means are not significantly different (p > 0.05)

The inter- species comparison revealed that White horehound is the plant which has more yield compared to the other studied species, when the same type of extraction is considered and in the same operating conditions; as in different environments conditions plants from the same specie may have different secondary metabolite concentrations (Radušienė et al., 2012), our result logically looks mainly at the fact that the species studied are different from each other. Concerning intra-species comparison, the type of extraction which gave the highest yield was Soxhlet for 3 plants, followed by infusion for 2 plants and decoction for 1 plant. The comparison between decoction and infusion revealed that the latter can be able to give more yield.

Extraction type accounts for 23.23 of the total variance, F = 448.02, DFn = 2, DFe = 30, the P value is < 0.0001, the effect is considered very highly significant for all plants, except for Mentha pulegium L., where there is no significant difference between the extraction by infusion and decoction, while between infusion and Soxhlet or between decoction and soxhlet the difference is highly significant (p=0.0003, p=0.0002 respectively).

It has been reported that phenolic constituents of one species from the lamiaceae family (Lamium amplexicaule. L.) are higher in the extract obtained by Soxhlet than those by maceration (Ferdjoun and Belhattach, 2022), also Soxhlet or hot continuous extraction is the most efficient technique for extracting vegetable oil (Teixeira et al., 2018); it can also extract a large amount of Lawsonite with smaller quantity of solvent (Makhman et al., 2014). This extraction type is well-applied technique, because of its high performance compared to other conventional extraction methods (De Castro and García-Ayuso, 1998), it maintains a relatively high extraction temperature with heat from the distillation flask (Tandon and Rane, 2008).

It is necessary to note that the performance of any kind of extraction depends on many parameters, including extraction temperature, time, type of solvents and volume used (Chew et al., 2011; Costa et al., 2012). Based on this, our yields can be explicated by temperature factor, and we can see that high temperature can increase extraction yield, means that Soxhlet ensure more temperature (increase and stable) during extraction than decoction (increase and decrease) or infusion (decrease).

**Phytochemical Screening**

Table 3 Phytochemical screening results

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<td><strong>Tannins</strong></td>
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<tr>
<td>Catechic tannins</td>
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<td>+++</td>
<td>+++</td>
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<td><strong>Alkaloids</strong></td>
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<td><strong>Comounds</strong></td>
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<td><strong>Reducing</strong></td>
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<td><strong>Free anthersens</strong></td>
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<td>-</td>
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<td><strong>Coumarins</strong></td>
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+++: Very positive reaction; ++: Moderately positive reaction; +: Weakly positive reaction; -: Total absence

Most of the secondary metabolites were detected by phytochemical screening; all plants contain catechic tannins, flavonic compounds, sterols and triterpenes, reducing compounds and anthracenic derivatives; but only Thymus satureioides contains saponosides (foam index =333.3), and coumarins are reaveled in Thymus satureioides, Mentha pulegium and with a higher amount in Origanum compactum. On the other hand, for all species, neither alkaloids nor free antheracene were listed in the table 3.

Our results are consistent with others researchs; on different parts of Marrubium vulgare, it has been reported according to some phytochemical investigations the presence of steroids, terpenoids, flavonoids, tannins, saponins, and volatile oils (Lodhi et al., 2017). Thymus satureioides results showed the presence of saponins, sterols, triterpene, tannins and favone aglycones, and an absence of alkaloid (Kouar et al., 2019), the aerial parts of Mentha pulegium reveal the presence of gallic tannins, flavonoids, alkaloids, sterols and triterpenes and saponins (Zekri et al., 2013). Flavonoids, saponins, tannins, alkaloids, anthocyanins, terpenes and reducing compounds have been reported in some lavender species (Bachiri et al., 2016). The studies on the chemical composition of Origanum compactum extracts revealed that the plant contains phenolic acids, tannins and flavonoids (Amakran et al., 2014).

The qualitative difference of these phytochemicals compounds noticed between species of the same family can be clearly noticed in the same plant, in fact fruits can contain significantly higher levels of phytochemical diversity than leaves, even if leaves have been the principal research focus of chemical ecology of plants for many years (Whitehead et al., 2022).

In general, the presence or absence of certain secondary metabolites from one plant to another or even within the same species is due to a very complex system; in fact,
the synthesis of these compounds is affected by many factors, in particular the internal genetic circuits of development (regulated gene, enzyme) and by external environmental factors (light, temperature, water, salinity, etc.) (Li et al., 2020).

Quantitative distribution of polyphenols

Figure 5 Average concentrations of polyphenolic compounds in MV: Marrubium vulgare L., TS: Thymus satureioides Coss, MP: Mentha pulegium L., LM: Lavandula multifida L., OC: Origanum compactum Benth. (fig A: Phe=Phenols, fig B: Flav= Flavonoids, fig C: CTa=Condensed tannins, fig D: Anth=Anthocyanin; I=Infusion, D=Decoction and S=Soxhlet extracts), Values sharing same letters are not significantly different (p > 0.05) and sharing *p<0.05 **p<0.001 are significantly different (fig. D).

Total phenolic content (fig. 5A)

The interaction between studied plants and extraction type is considered extremely significant with: F = 2710.03. DFn = 4, DFd = 30, the P value is < 0.0001; also, the difference is very highly significant between plants F = 442.67. DFn = 4, DFd = 30, the P value is < 0.0001; while between the types of extraction, the content of total phenols is significant F = 6.76. DFn = 2, DFd = 30, the P value = 0.0038. The total phenol content recorded values ranging from significant (p<0.05) to extremely significant (p<0.0001) between plants and between extracts following Tukey’s multiple comparison; the plants that contain more phenols are: LM followed by OC then TS and MP; on the other hand, for MV, its phenol content is very low and the effect of extraction type is not significant.

From one species to another, the content according to the type of extraction differs; this appears normal because it should be noted that the extraction efficiency of phenolic compounds at increasing temperatures above 60°C can promote concomitant degradation of phenolic compounds (Ruenroengklin et al., 2008; Benmeziane et al., 2014). Also, many authors agree on the fact that, to improve both the solubility of the solute and the diffusion coefficient, it is necessary to increase the temperature and the extraction time; this procedure and even under less mild conditions can degrade certain quantities of phenolic compounds (Yilmaz et al., 2006; Pinelo et al., 2005).

Total flavonoids content (fig. 5B)

The interaction between plants and type of extraction is considered extremely significant with: F = 62.36. DFn = 8, DFd = 30, the P value is < 0.0001; also, the difference between plants, as well as between the types of extraction is extremely significant, respectively with: F = 2710.03. DFn = 4, DFd = 30, the P value is < 0.0001; also, the difference is very highly significant between plants F = 442.67. DFn = 4, DFd = 30, the P value is < 0.0001. The quantity of total flavonoids reveals values ranging from significant (p<0.05) to extremely significant (p<0.0001) between plants and between extracts. OC and LM contains almost the same amount of total flavonoids compared to TS and MP. MV only shows very low values, and this is in agreement with the preliminary results (phytochemical screening) for MV which only contains catechol. The most striking thing about the difference between the types of extract within each species is that this difference is not significant (p>0.05) for 2 extracts on 3. This gives us the reflection on the difference of the total flavonoid content between the three types of extraction. in fact, the Soxhlet extracts more flavonoids for OC, while decoction and Soxhlet extracts more of these compounds for LM. We can conclude that for these two plants, the flavonoids are extracted by a higher temperature and time than the flavonoids of MP and TS which degrade under these conditions. This is well explained by the fact that the degradation of flavonoids may be the reason for the decrease in total flavonoid at higher temperatures, which also depends on the structure of the particular flavonoids (Sharma et al., 2015).

Total Condensed tannins content (fig. 5C)

The interaction between plants and used extraction is considered very highly significant with: F = 356.54. DFn = 8, DFd = 30, the P value is < 0.0001; also, between plants as well as between the types of extraction, the difference is extremely significant with, respectively : F = 214.83. DFn = 4, DFd = 30, the P value is < 0.0001, and: F = 165.90. DFn = 2, DFd = 30, the P value is < 0.0001. OC, LM, TS and MP followed by MV is the order of the total condensed tannin content; respectively, the difference between the extracts is very significant...
p<0.0001) for OC (Soxhlet), LM (decoction), MV (Soxhlet). On the other hand, the difference between MP and TS contains almost the same condensed tannin content for the same type of major extract infusion and Soxhlet.

We can deduct from these results that OC, LM contain more polyphenols, followed by TS and MP, while MV contains only small values of condensed tannins; also, the type of extraction depends on the plant and if these compounds are degradable with temperature or not.

**Total Anthocyanin content (fig. 5.D)**

The interaction between plants and extraction mode is considered highly significant with: \( F = 14.25, \) \( DFn = 2, \) \( DFd = 12, \) and the P value = 0.0007; whereas, the difference is not significant neither between plants, nor between the types of extraction; respectively: \( F = 3.00, \) \( DFn = 1, \) \( DFd = 12, \) and the P value = 0.1089, and \( F = 1.08, \) \( DFn = 2, \) \( DFd = 12, \) and the P value = 0.3694. Both plants contain the same anthocyanins content from a statistical point of view by different extraction methods; the LM decoctate has a very significant difference \( p<0.001 \) compared to the TS decoctate, while extraction by infusion and Soxhlet of TS does not have a significant difference \( p>0.05 \) with same extraction mode of LM. Then this is related to the significant interaction between extraction type and plant species.

**Principal Component Analysis (PCA)**

![Figure 6 PCA Scree plot](image.png)

**Figure 6 PCA Scree plot**

![Variables (axes F1 et F2 : 88,45 %)](image.png)

**Figure 7 Correlation circle of variables (Phe: Phenols, Flav: Flavonoids, CTa: Condensed tannins, I: infusion, D: Decoction, S: Soxhlet)**

The F1 axis represents (67.27%) and F2 (21.18%) of information; On the correlation circle, the variables are well presented on the F1 and F2 plane which explains 88.45% of the variability.

CTaS and CTaI are strongly positively correlated; PheS, FlavS, PheI, Flav I are strongly positively correlated; FlavD, PheD and CTaD are also positively correlated; But CTaD and CTaI are negatively correlated.

The very positive correlation between flavonoids and total phenols can be justified by the fact that flavonoids constitute the dominant polyphenolic compounds (Harouak et al., 2022).

This correlation has been established by several works; according to Maisuthisakul et al., (2008), the content of total phenolic compounds is related to the content of flavonoids in the ethanol extracts of 28 plants.

The important correlation between extraction by infusion and by Soxhlet which is mutualized from one plant to another, gives us an idea about the nature of degradable molecules in same species with the continuity of high temperature (case of Soxhlet).

**Soil Analysis**

**Table 4 Nitrogen content, pH and electric conductivity of soils from where the studied species where harvested**

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>%N</td>
<td>1.07 ± 0.20</td>
<td>0.23 ± 0.03</td>
<td>1.31 ± 0.001</td>
<td>0.63 ± 0.13</td>
</tr>
<tr>
<td>pH</td>
<td>7.74</td>
<td>7.24</td>
<td>7.47</td>
<td>7.76</td>
</tr>
<tr>
<td>EC [µS/cm]</td>
<td>220</td>
<td>1081</td>
<td>111.8</td>
<td>206</td>
</tr>
</tbody>
</table>

Nitrogen percentage content ranged between 0.23 ± 0.02 (rich 0.15-0.25%) to 2.17 ± 0.20 (very rich >0.25%) for all soil types (Calvet and Villemin, 1986). It was reported that some plants for the production of secondary metabolites, can inhibit nitrification or mineralization of soil nitrogen (Van et al., 2007; Radersma and Smit, 2011); so, it can be inferred that as long as the soil is rich in nitrogen, the plant will be able to produce more secondary metabolites.

Except the Marrubium vulgare soil which has high salinity (0.75<EC<2.25 dS/m), all the others types are unsalty (EC<0.25 dS/m); according to Hide standards (Hide, 1954).
They are all weakly basic (pH ranged between 7.3 to 7.8) according to DIAEA/DRHA/SEEN (2008). According to several authors, soil composition and soil type may be among the determining factors in the composition of secondary metabolites (Perera et al., 2000).

In fact, the survival and growth of many plant species are severely depressed in poorly drained soils, affecting the chemical composition of volatile compounds and thus significantly reducing yields of essential oils (Lawrence et al., 1988). Soil with the three most important nutrients (nitrogen, phosphorus and potassium) generally showed an increase in oil yields, and the addition of these nutrients separately gave different results in the yield and even in the chemical composition of the oils (Figueredo et al., 1997).

We can say that slightly basic soils can be good soils for our samples, on the other hand a more saline soil can reduce polyphenolic compounds such as the case of white marburrus.

**CONCLUSION**

In this study, most of secondary metabolites were presented during the phytochemical screening; flavonoid compounds, catecholic tannins sterols and triterpenes, reducing compounds and anthraquinone derivatives and total absence of alkaloids in the six-studied species. The difference in quantitative terms was shown with origano erich rich in total phenol and flavonoids as well as condensed tannins and lavender which contains the most total anthocyanins; as the pilot plants of our results.

On the other hand, the white hoarehound was poor in total phenols and flavonoids except for a small amount of total condensed tannins. Also, the reliable type of extraction for each plant depends on the thermal nature of its compounds; which is more extracted by soxhlet apparatus in the case of origano and by decoction in the case of lavender.

**REFERENCES**


