

## Original Research Article

### Phytochemical investigation and quantification of bioactive compounds of *Rhamnus alaternus* (L.)

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#### Abstract

**Purpose:** In the context of the valorization of medicinal plants in the region of Taza-Morocco, the present study is focused on a phytochemical study of the *Rhamnus alaternus* plant in view of its use in traditional medicine and its beneficial health properties.

**Methods:** The phytochemical research is carried out through aqueous and organic extraction processes in two modalities (hot and cold), characterization and quantification of its bioactive compounds.

**Results:** The results of the phytochemical study revealed that the decocted and methanolic extract had the highest yields, with percentages of around 42.9% and 37.5% respectively. The leaves of this plant are rich in flavonoids, tannins, saponins, sterols, anthracenosides and quinones. The decocted had the highest averages for phenolic compounds among the aqueous extracts, with values of  $21.67 \pm 0.10$   $\mu\text{g GAE/mg E}$  for polyphenols,  $58.40 \pm 0.33$   $\mu\text{g QE/mg E}$  for flavonoids and  $5.43 \pm 0.01$   $\mu\text{g CE/mg E}$  for tannins. While for organic extracts, the highest concentrations of polyphenols were obtained by ethyl acetate extract with  $59.23 \pm 0.72$   $\mu\text{g GAE/mg E}$ , those of flavonoids and tannins by chloroformic extract with respectively:  $470.79 \pm 1.70$   $\mu\text{g QE/mg E}$  and  $51.75 \pm 0.14$   $\mu\text{g CE/mg E}$ .

**Conclusion:** *Rhamnus alaternus* is an attractive plant to study, given its wealth of bioactive compounds involved in interesting biological activities.

**Keywords:** *Rhamnus alaternus*, phytochemical study, screening, assay, bioactive compounds, aqueous extracts, organic extracts.

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## INTRODUCTION

The biological diversity of medicinal plants in Morocco constitutes a natural heritage and an exceptional wealth, thanks to a wide variation in species, this heritage is the result of our ancestors' careful cultivation and preservation of resources for future generations.<sup>1-2</sup> Indeed, Morocco has significant plant variability, with around 743 taxa belonging to 101 families and 371 genera. Among these taxa, 40 are endemic medicinal plants.<sup>3</sup> This biodiversity is supported by a group of parks that are distributed throughout Morocco, mainly in mountainous areas.<sup>4</sup> These parks have been a source of many medicinal plants that can be useful in the treatment of diseases through traditional practices that have been transmitted over generations, the two parks, Talasemtane National Park and Tazekka National Park, are particularly rich in medicinal plants.<sup>5-6</sup>

The domain of phytotherapy has undergone a very interesting evolution in view of the large number of studies that have highlighted the therapeutic power of medicinal plants thanks to the active principles they contain, the therapeutic effect of medicinal plants can be curative, preventive, complementary, detoxifying, etc.<sup>7-8-9-10</sup> It is an ancestral practice that is widely used in Morocco.<sup>11-12-13-14-15-16</sup> Morocco is very rich in biodiversity in terms of medicinal plants, and this botanical diversity is favored by its geographical position and varied climate, this wealth can be a source for the local population to develop their socio-economic level.<sup>17-18-19</sup> Tazekka National Park is located in the Middle Eastern Atlas Mountains, 21 km from the city of Taza, Morocco and is a source of plant species as well as a socio-economic source for the population of the Taza city.<sup>20</sup> The presence of around 600 plant species gives it a floristic biodiversity.<sup>21</sup> This wealth of medicinal plants offers opportunities and arouses the curiosity of scientists to seek out new species and conduct studies on them in order to seek natural remedies to cure certain infections and obtain new bioactive molecules for pharmaceutical purposes.

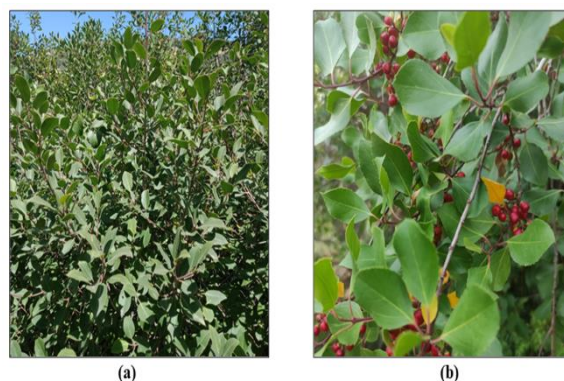
*Rhamnus alaternus* is a Mediterranean plant species belonging to the Rhamnaceae family.<sup>22</sup> This plant is widely used in traditional medicine as a digestive, diuretic, hypotensive, laxative, purgative, hepatoprotective and also to treat dermatological complications.<sup>23-24</sup> Numerous studies have highlighted this species' wealth of bioactive molecules such as polyphenols, flavonoids,<sup>25</sup> coumarins and anthraquinones.<sup>26-27</sup> These biological compounds confer antioxidant, antigenotoxic,<sup>28-29</sup> antihyperlipidemic,<sup>30</sup> genoprotective, antimutagenic and antihyperglycemic properties on this plant.<sup>31-32</sup>

The present study focuses on a phytochemical study of the leaves of the plant *Rhamnus alaternus* harvested in the vicinity of the city of Taza, Morocco, through the preparation of several aqueous and organic extracts using solvents of different polarity and various hot and cold extraction modalities, phytochemical screening and quantification of bioactive compounds in its various prepared extracts.

## MATERIAL AND METHOD

### Plant material

The plant material is represented by the leaves of the *Rhamnus alaternus* plant collected in April 2022 near the center of the rural commune of Bni Lent on the Douar Bab El Harcha road, located 33 km west of Taza, GPS coordinates: 34°19.821'N 004°13.178'W (Figure 1). Botanical identification of the plant *Rhamnus alaternus* L. was carried out by Professor Abdelilah Rahou of the Faculty of Sciences, Moulay Ismail University, Meknes, Morocco. A reference specimen has been placed in the herbarium, with the code RA- 2022/04.



**Figure 1:** (a) and (b) *Rhamnus alaternus* (Photo taken in the harvesting area, Douar Bab El Harcha road near the center of the rural commune of Bni Lent, 33 km from Taza-Morocco, GPS coordinates 34°19.821'N 004°13.178'W).

### Phytochemical study

#### Preparation of aqueous and organic extracts

In this study, we prepared aqueous extracts using three methods (decoction, infusion and maceration), while for organic extracts we used four organic solvents of different polarity (methanol, ethyl acetate, chloroform and petroleum ether) and two extraction methods (hot and cold). The procedure of aqueous and organic extractions followed for the preparation of extracts is indicated in previous works of our laboratory.<sup>33-34-35-36-37-38-39</sup>

## Phytochemical screening

Phytochemical screening tests involve qualitative characterization procedures to identify the various families of chemical compounds (alkaloids, flavonoids, saponins, sterols, quinones, anthraquinones, anthracenosides and tannins) present in plant material, aqueous and organic extracts of plant leaves. The protocols followed in these methods are based on the precipitation or staining of substances by reagents specific to each chemical family. The method used to perform this screening is provided in our previous work.<sup>15-35-38-40</sup>

## Quantification of phenolic compounds

The results of the qualitative analysis of secondary metabolites in plant leaves led us to quantify three compounds: total polyphenols, total flavonoids and catechic tannins, following the protocol described in our previous work.<sup>15-35-38-41</sup>

## Statistical analysis

Experimental data are expressed as mean  $\pm$  standard error (SEM). The ANOVA variance test is used as the basis for statistical analysis, which is then followed by Tukey's test, using GraphPad Prism 5 software. At a  $p \leq 0.05$  value, the difference is considered statistically significant.

## RESULT AND DISCUSSION

### Extraction yield

Aqueous and organic extractions were carried out in two ways: hot and cold. Hot aqueous extractions were performed by decoction and infusion, while cold extraction was made by maceration. Hot organic extractions are performed by Soxhlet with four organic solvents of different polarity: methanol, ethyl acetate, chloroform and petroleum ether, while cold organic extraction is performed by methanolic maceration. Yields for aqueous and organic extractions of *Rhamnus alaternus* leaves are shown in Table 1.

Among the aqueous extractions performed, decoction recorded the highest yield, followed by infusion and maceration with percentages of  $37.5 \pm 0.37$ ,  $30.5 \pm 0.36$  and  $25 \pm 0.57\%$  respectively, with a statistically significant difference between the three aqueous extracts. For organic extractions, methanolic extract had the highest yield ( $42.9 \pm 0.55\%$ ), followed respectively by methanolic macerate extract ( $15.6 \pm 0.37\%$ ), ethyl acetate extract ( $6.5 \pm 0.30\%$ ) and

chloroformic extract ( $4.8 \pm 0.17\%$ ). Petroleum ether extract came last, with the lowest yield ( $2.2 \pm 0.15\%$ ). Comparative analysis of organic extracts showed that all extracts were significantly different from each other, with the exception of two extracts: ethyl acetate and chloroformic, which showed a statistically insignificant difference (Table 1).

**Table 1:** Yield of aqueous and organic extractions from the leaves of the plant *Rhamnus alaternus* L.

	Extracts	Yield in %
Aqueous extracts	Decocted	$37.5 \pm 0.37^a$
	Infused	$30.5 \pm 0.36^b$
	Macerated	$25 \pm 0.57^c$
Organic extracts	Methanolic extract	$42.9 \pm 0.55^d$
	Methanolic macerate	$15.6 \pm 0.37^e$
	Ethyl acetate extract	$6.5 \pm 0.30^f$
	Chloroformic extract	$4.8 \pm 0.17^f$
	Petroleum ether extract	$2.2 \pm 0.15^g$

Note: The values given in Table 1 represent the mean of three replicates  $\pm$  mean standard error. The difference was examined as statistically significant at  $p < 0.05$ . In the same column, values with a different letter indicate a statistically significant difference ( $p < 0.05$ ) between them, while values with the same letter indicate that the difference is not statistically significant. Values in bold highlight the most important results obtained.

These results demonstrate the richness of the leaves of the *Rhamnus alaternus* plant in polar constituents, since the highest yields were recorded with the most polar solvents (water and methanol). The difference in yields between organic and aqueous extracts could be due to the extraction capacity of each solvent, which can extract well-defined families of secondary metabolites found in the plant studied. We therefore note that increasing the polarity of the solvent induces a significant improvement in extraction yield. In addition to the variation in solvent polarity, the difference in yields can also be linked to the extraction modality (hot or cold); this variation is very noticeable when comparing decocted with aqueous macerate and methanolic extract with methanolic macerate extract, in addition to other parameters such as sample composition, temperature and extraction time.

A study conducted by Herzi et al.<sup>42</sup> in 2013 showed that the extraction time required for the solvent to penetrate the plant material and extract all the natural substances directly affects extraction yield. This parameter also depends on the type of solvent and the plant material and its structure. This study also confirms that the ability to extract natural products from medicinal

plants using organic solvents is linked to the structure of the raw material. Indeed, after grinding, particle size decreases and therefore specific surface area becomes larger, which facilitates solvent penetration inside the particles.<sup>42</sup>

The same species, *R. alaternus*, was the subject of an Algerian study which found that the yields of both extraction modalities, methanolic maceration and aqueous maceration, were lower than those found in our study, which were in the order of 14.48% and 14.20% respectively.<sup>43</sup>

Comparison of our results with those of a Tunisian study on aqueous and organic extracts of *Allium roseum*, a North African endemic species very common in southern Tunisia, using the same solvents and the same protocol as we followed for extract preparation shows that for both *Allium roseum* and *Rhamnus alaternus*, decocted and methanolic extract are the extracts with the highest yields.<sup>44</sup>

The results of a study carried out by Lachkar et al.<sup>38</sup> on the aerial part of *Haloxylon scoparium* harvested in an area close to where our plant is harvested: the Taddart area 42.1 Km from the town of Taza, Morocco, are consistent with our results, since the best yield among aqueous extracts is recorded by the decocted (16%) and by the methanolic extract (14.35%) among organic extracts.<sup>38</sup> Similarly, in a study carried out by Senhaji and his collaborators in 2020 on the aerial part of *Anabasis aretioides*, the best yield was recorded by hot extraction and the most polar solvents (decoction and methanolic extraction by soxhlet).<sup>37</sup> Similar results were obtained for the extraction of active ingredients from *Peganum harmala* seeds, with yields of around 12.02% for decoction and 11% for Soxhlet methanolic extraction.<sup>45</sup> Also, another study on *Atractylis gummifera* by Bouabid and his collaborators revealed that hot extraction for both extraction modalities (aqueous and organic) recorded the best yields.<sup>33</sup>

### Phytochemical screening

The qualitative analysis carried out on the powder plant material and the various aqueous and organic extracts prepared from it are presented in Table 2.

The experimental results listed in Table 2 demonstrate the diversity of secondary metabolite groups present in *Rhamnus alaternus* leaves. The molecules identified revealed a varied presence, differing between plant material, aqueous extracts (decocted, infused and macerated) and organic extracts (methanolic, methanolic macerate, ethyl acetate, chloroformic and petroleum ether). In fact, the results showed that the main components - flavonoids, catechic tannins, saponins, sterols, anthracenosides and quinones - are

found in large quantities in the plant material, although anthraquinones are only moderately abundant.

We note that the three aqueous extracts include the majority of secondary metabolites (flavonoids, catechic tannins, saponins and sterols) in significant quantities, although levels of other metabolites in these extracts vary from high to low. For the organic extracts, we find that the presence of the molecules highlighted in the four organic extracts: methanolic, methanolic macerate, ethyl acetate and chloroformic is generally medium-high, while for the petroleum ether extract the presence of these secondary metabolite's ranges from medium to low. The leaves of this plant stand out for the absence of alkaloids and gallic tannins in the plant material and in all the extracts prepared (Table 2). These results suggest the use of *Rhamnus alaternus* as a therapeutic plant, since they highlight its richness in a number of chemical compounds known to have interesting biological actions, and the absence of potentially toxic components such as alkaloids.

An Algerian study carried out on the same species, *R. alaternus*, showed the richness of the leaves of this plant in various chemical compounds with a varied presence between high, medium and low, due to the fact that quinones and saponosides are present with high content, flavonoids and gallic tannins are present with medium content, while alkaloids and sterols are absent.<sup>46</sup> Another study, again in Algeria, revealed a moderate presence of flavonoids, a low presence of tannins and anthraquinones and a total absence of sterols.<sup>47</sup> Work carried out on this plant has mentioned its richness in flavonoids and anthraquinones. Research carried out by Kosalec et al.<sup>27</sup> in 2013 allowed the quantification of chrysophanols, which are the most abundant anthraquinones in the extracts studied from *R. alaternus*.<sup>27</sup> In addition, three other types of flavonoids, including kaempferol 3O-isorhamninoside (K3O-ir), rhamnocitrin 3O-isorhamninoside (R3O-ir) and rhamnetin 3O-isorhamninoside, were extracted from *Rhamnus alaternus* leaves.<sup>28-48</sup>

The work of Lachkar and his collaborators in 2022, carried out under the same experimental conditions on the leaves of *Chamaerops humilis* harvested in the same region, revealed the presence of several chemical compounds, notably catechic tannins, sterols, flavonoids and free quinones, with variation between the plant powder and the various extracts tested. In addition to the total absence of alkaloids, anthracenosides and anthraquinones,<sup>15</sup> the same results are found in the study carried out on *Atractylis gummifera* roots by Bouabid et al. in 2018.<sup>33</sup> In contrast, another study carried out on *Peganum harmala* seeds by revealed the plant's richness in alkaloids and flavonoids.<sup>45</sup>

**Table 2:** Phytochemical screening of the plant *Rhamnus alaternus* L.

Plant powder and aqueous and organic extracts	Flavonoids	Catechic tannins	Gallitannins	Saponins	Sterols	Alkaloids	Anthraquinones	Anthracenoses	Free quinones
Plant leaf powder	+++	+++	-	+++	+++	-	++	+++	+++
Aqueous extracts	Decocted	+++	+++	-	+++	+++	-	+	+++
	Infused	+++	+++	-	+++	+++	-	+	+++
	Macerated	+++	+++	-	+++	+++	-	++	++
Organic extracts	Methanolic extract	+++	++	-	+	+++	-	++	+++
	Methanolic macerate	+++	++	-	+	+++	-	+	+++
	Ethyl acetate extract	+++	+++	-	-	+++	-	++	+++
	Chloroformic extract	+++	+++	-	-	+++	-	+++	++
	Petroleum ether extract	+++	++	-	-	++	-	++	+

Note: (+): Presence ; (++) : Average presence ; (+++): Strong presence ; (-): Absence

**Table 3:** Content of total polyphenols, total flavonoids and catechic tannins in aqueous and organic extracts from the leaves of *Rhamnus alaternus* L.

	Extracts	Total polyphenols ( $\mu\text{g GAE/mg E}$ )	Total flavonoids ( $\mu\text{g QE/mg E}$ )	Catechic tannins ( $\mu\text{g CE/mg E}$ )
Aqueous extracts	Decocted	<b>21.67 <math>\pm</math> 0.10<sup>a</sup></b>	<b>58.40 <math>\pm</math> 0.33<sup>a</sup></b>	<b>5.43 <math>\pm</math> 0.01<sup>a</sup></b>
	Infused	17.81 $\pm$ 0.09 <sup>b</sup>	24.91 $\pm$ 0.11 <sup>b</sup>	5.08 $\pm$ 0.17 <sup>a</sup>
	Macerated	12.38 $\pm$ 0.05 <sup>c</sup>	20.66 $\pm$ 0.27 <sup>b</sup>	4.46 $\pm$ 0.02 <sup>a</sup>
Organic extracts	Methanolic	50.35 $\pm$ 0.41 <sup>d</sup>	235.51 $\pm$ 0.60 <sup>c</sup>	19.44 $\pm$ 0.80 <sup>b,e</sup>
	Methanolic	46.96 $\pm$ 0.04 <sup>e</sup>	200.84 $\pm$ 0.26 <sup>d</sup>	17.86 $\pm$ 0.47 <sup>b</sup>
	Ethyl acetate	<b>59.23 <math>\pm</math> 0.72<sup>f</sup></b>	413.80 $\pm$ 0.72 <sup>e</sup>	30.19 $\pm$ 0.66 <sup>c</sup>
	Chloroformic	47.97 $\pm$ 0.41 <sup>e</sup>	<b>470.79 <math>\pm</math> 1.70<sup>f</sup></b>	<b>51.75 <math>\pm</math> 0.14<sup>d</sup></b>
	Petroleum ether	18.71 $\pm$ 0.23 <sup>b</sup>	289.31 $\pm$ 0.22 <sup>j</sup>	20.69 $\pm$ 0.59 <sup>e</sup>

Note: The values given in Table 3 represent the mean of three replicates  $\pm$  mean standard error. The difference was examined as statistically significant at  $p < 0.05$ . In the same column, values with a different letter indicate a statistically significant difference ( $p < 0.05$ ) between them, while values with the same letter indicate that the difference is not statistically significant. Values in bold highlight the most important results obtained.

According to another study conducted on a plant belonging to the Oleaceae family, the results showed the presence of flavonoids, tannins, sterols and saponins in the leaves of the *Olea europaea* plant, while tests for alkaloids and anthraquinones were negative.<sup>49</sup>

Our results showed a variation in secondary metabolite abundance between plant material, aqueous extracts and organic extracts. Comparison of our study with others on the same species or other species in the same

region showed a very high variation. This difference may be due to the intervention of several factors such as geographical position, climatic conditions, soil composition, genetic factors, experimental conditions, harvesting season, chemical nature of plant molecules, extraction modality, part of the plant studied (leaves, roots, etc.).<sup>50-51</sup>

### Quantification of phenolic compounds

Quantification of the various families of secondary metabolites (total polyphenols, total flavonoids and catechic tannins) in aqueous and organic extracts of *Rhamnus alaternus* leaves was carried out based on the results of the qualitative analysis. Thus, a quantitative analysis of the three phenolic metabolites was necessary to determine the content of each compound in the different extracts. The results obtained are shown in Table 3.

Total polyphenol contents in aqueous extracts were  $21.67 \pm 0.10$ ,  $17.81 \pm 0.09$  and  $12.38 \pm 0.05$   $\mu\text{g GAE/mg E}$  respectively for decocted, infused and macerated, with a significant difference between the three extracts. Compared with the aqueous extracts, the total polyphenol contents of the organic extracts were higher. Ethyl acetate extract had the highest content, with a value of  $59.23 \pm 0.72$  g GAE/mg E and a statistically significant difference with all other extracts. Methanolic extract comes in second with a content of  $50.35 \pm 0.41$   $\mu\text{g GAE/mg E}$ , this value is statistically significant with all aqueous and organic extracts. While the two extracts, methanolic macerate and chloroformic, are statistically insignificant with values of  $46.96 \pm 0.04$  and  $47.97 \pm 0.41$   $\mu\text{g GAE/mg E}$  respectively. Petroleum ether extract ranks last among the organic extracts with a concentration of  $18.71 \pm 0.23$   $\mu\text{g GAE/mg E}$ . This value is not significant with that of the infused (Table 3).

For flavonoids, the decocted had the highest content among the aqueous extracts, with a value of  $58.40 \pm 0.33$   $\mu\text{g QE/mg E}$ , this concentration is much higher than the other extracts. With values of  $24.91 \pm 0.11$  and  $20.66 \pm 0.27$   $\mu\text{g EQ/mg E}$  respectively, infused and macerated occupy second and third place. Comparing infused and macerated, we can say that these two extracts are statistically non-significant. The highest flavonoid concentration for organic extracts was found in chloroformic extract, followed by ethyl acetate extract, petroleum ether extract, methanolic extract and finally methanolic macerate, with contents that were  $470.79 \pm 1.70$ ,  $413.80 \pm 0.72$ ,  $289.31 \pm 0.22$ ,  $235.51 \pm 0.60$  and  $200.84 \pm 0.26$   $\mu\text{g QE/mg E}$  respectively. All organic extracts show a statistically significant difference from each other (Table 3).

The results of catechic tannin quantification presented in Table 3 show that tannin contents vary considerably between extracts. For aqueous extracts, the decocted has the highest tannin concentration ( $5.43 \pm 0.01$   $\mu\text{g CE/mg E}$ ), followed by the infused, which has a content close to that of the decocted ( $5.08 \pm 0.17$   $\mu\text{g CE/mg E}$ ), and finally the macerated, which has the lowest content ( $4.46 \pm 0.02$   $\mu\text{g CE/mg E}$ ). A statistically insignificant difference was observed

between the three aqueous extracts. For the organic extracts, the chloroform extract contains the highest tannin levels with a rate of  $51.75 \pm 0.14$   $\mu\text{g CE/mg E}$ , followed by the ethyl acetate extract, which has a content of  $30.19 \pm 0.66$   $\mu\text{g CE/mg E}$ , with a significant difference between these two extracts. The petroleum ether extract contains a tannin content of  $20.69 \pm 0.59$   $\mu\text{g CE/mg E}$ , this extract does not differ statistically with the methanolic extract which has a tannin concentration of  $19.44 \pm 0.80$   $\mu\text{g CE/mg E}$ . The hot-prepared methanolic extract shows a statistically non-significant difference with the cold-prepared methanolic extract which contains a content of  $17.86 \pm 0.47$   $\mu\text{g CE/mg E}$  (table 3).

The results obtained in this work indicated that the contents of total polyphenols, total flavonoids and catechic tannins in *Rhamnus alaternus* leaves are strongly affected by the different solvents used. Thus, the various aqueous and organic extracts showed a statistically significant difference in total phenolic compound content depending on the extraction procedures and solvent polarity used. The variable distribution of secondary metabolites is strongly linked to the plant's growth stage and harvesting period, and may be related to environmental extremes such as temperature, drying conditions, part of the plant used, extraction technique, sun exposure, drought, salinity, pH, genetic factors, etc. All these factors stimulate the biosynthesis of phenolic compounds such as flavonoids.<sup>52-53-54-55</sup>

A study carried out in Tunisia by Ben Ammar et al.<sup>47</sup> in 2008 showed that the methanolic extract of *R. alaternus* plant leaves was rich in polyphenols, flavonoids and tannins, with contents of  $113 \pm 8$   $\mu\text{g GAE/mg E}$ ,  $178 \pm 14$   $\mu\text{g QE/mg E}$  and  $730 \pm 21$   $\mu\text{g EAT/mg E}$  respectively.<sup>47</sup> Another study demonstrated the richness of the plant's leaves in phenolic compounds and flavonoids, with contents of  $38.4 \pm 1.56$  mg EAG/g E and  $33.6 \pm 1.50$  mg EQ/g E respectively.<sup>27</sup> A Tunisian study on the same species revealed the richness of organic extracts with the phenolic compounds studied, with the methanolic extract containing contents of  $13.8 \pm 0.71$   $\mu\text{g GAE/mg E}$ ,  $28.3 \pm 0.94$   $\mu\text{g QE/mg E}$  and  $14.72 \pm 0.38$   $\mu\text{g EAT/mg E}$  respectively for polyphenols, flavonoids and tannins. For ethyl acetate extract, contents are moderately high compared with methanolic extract, with  $25 \pm 0.61$   $\mu\text{g GAE/mg E}$ ,  $33.8 \pm 1.39$   $\mu\text{g QE/mg E}$  and  $16 \pm 0.48$   $\mu\text{g EAT/mg E}$  respectively for polyphenols, flavonoids and tannins. Both chloroformic and petroleum ether extracts contained low levels of  $8 \pm 0.84$  and  $0.62 \pm 0.07$   $\mu\text{g GAE/mg E}$  respectively for polyphenols. For flavonoids, the contents were  $15.4 \pm 0.57$  and  $7.9 \pm 0.51$   $\mu\text{g QE/mg E}$  respectively. For tannins, the leaves contained low

concentrations of  $0.06 \pm 0.02$  and  $0.01 \pm 0.00$   $\mu\text{g}$  EAT/mg E respectively.<sup>23</sup>

Determination of the polyphenol, flavonoid and tannin contents of the *Marrubium vulgare* species during the vegetation and flowering periods showed a significant difference between the total polyphenol, flavonoid and tannin contents of the different organs collected during the two periods. These variations are mainly related to the season and environmental conditions of the environment such as the collection site and the climate of the region.<sup>56-57</sup>

Following the same experimental approach, a series of studies were carried out on plants from the same region. On the first, quantification of phenolic compounds in aqueous and organic extracts from the aerial part of the *Anabasis aretioides* plant showed that the decocted and ethyl acetate extracts contained the highest concentrations of total polyphenols, with contents of the order of  $1.78 \pm 0.003$  and  $46.79 \pm 0.75$   $\mu\text{g}$  GAE/mg E respectively, while the highest tannin concentrations were recorded by the infused and ethyl acetate organic extract with contents equal to  $0.70 \pm 0.03$  and  $46.46 \pm 0.67$   $\mu\text{g}$  CE/mg E respectively.<sup>37</sup> On the other side, the study conducted by Lachkar et al.<sup>38</sup> in 2021 on the *Haloxylon scoparium* plant revealed that the decocted and methanolic extract recorded the highest total polyphenol contents with values of  $6.83 \pm 0.04$  and  $161.65 \pm 1.52$   $\mu\text{g}$  GAE/mg E respectively. The most distinguished flavonoid concentrations were obtained by the decocted and methanolic macerate with  $306.59 \pm 4.35$  and  $641.03 \pm 7.8$   $\mu\text{g}$  QE/mg E respectively. Catechic tannin levels were highest in the decocted and ethyl acetate extracts, with  $3.78 \pm 0.35$  and  $23.69 \pm 0.6$   $\mu\text{g}$  CE/mg E respectively.<sup>38</sup> A further study of the aqueous and organic extracts of *Peganum harmala* seeds revealed that the decocted and methanolic macerate recorded the highest levels of total polyphenols, with concentrations of  $15.02 \pm 0.16$  and  $94.37 \pm 0.62$  mg GAE/mg E while the most remarkable flavonoid contents were obtained by decocted and ethyl acetate extract with concentrations equal to  $17.40 \pm 1.26$  and  $366.13 \pm 1.88$  mg QE/mg E respectively.<sup>45</sup>

## CONCLUSION

The present study on *Rhamnus alaternus* (L.) included extraction, phytochemical characterization and quantification of its phenolic compounds. Results showed that the best yields were obtained by hot extraction: decoction and methanolic extraction with soxhlet. Screening showed that the leaves contained tannins, flavonoids, anthraquinones and saponins, and quantitative analysis illustrated that the decoction had

the highest concentrations of phenolic compounds, while for organic extracts it was the ethyl acetate extract that had the most remarkable contents of total polyphenols, while the most distinguishable contents of flavonoids and tannins were recorded by the chloroform extract. The richness of secondary metabolites of *R. alaternus* encourages us to continue scientific investigations of this plant. Thus, we are currently carrying out *in vitro* and *in vivo* evaluations of the biological activities of the different aqueous and organic extracts. Likewise, toxicity tests are also necessary for safe use of the plant.

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## Conflict of interest

The authors declare no conflict of interest.

## Authors Declaration

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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