Australian Journal of Crop Science AJCS

AJCS 17(7):591-599 (2023) ISSN:1835-2707
doi: 10.21475/ajcs.23.17.07.p3926

Effect of geographical origin on yield and secondary metabolite content of extracts of Moroccan *Juniperus thurifera*

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Abstract

The thuriferous juniper (*Juniperus thurifera*) is an important species in the Mediterranean region due to its ecological and economic values. It plays a significant role in preventing soil erosion and desertification, as well as being used in traditional medicine and as a source of essential oils for various industries. In addition, its extracts are considered as a source of bioactive compounds with various pharmacological activities, such as antioxidant, anti-inflammatory and antimicrobial effects. However in Morocco, it is considered as one of the undervalued and least preserved species. Its stands continue to be vigorously degraded. The present work aims to study the effect of the origin on the yield, the phenolic compounds, flavonoids and tannins contents and the antioxidant activity of the leaves extracts of this species. In order to accomplish this study, we carried out the extraction by soxhlet from the leaves of *J. thurifera*, coming from three geographical origins, using two solvents (hexane and ethanol). Then the dosage of different compounds (polyphenols, flavonoids and condensed tannins) and the evaluation of the antioxidant activity were carried out. The results show a significant variability between the samples coming from three different biogeographical zones, namely: the Eastern, Central and Western High Atlas in terms of yield and levels of polyphenols, flavonoids and condensed tannins. This allowed to demonstrate the effect of geographical origin and the interaction between environmental conditions and genotype on the production of secondary metabolites. In fact, we found that the Midelt population has a good yield in ethanolic extracts (9.41% ± 0.59) and hexane 6.57% ± 0.29. Furthermore, the El Haouz population is the richest in polyphenols in ethanolic extracts (191.30 ± 4.27 mg GAE /g Extract), as well as it has an important reducing potential (IC_{50} = 0.98 ± 0.05 mg/ml). These results can be exploited as bioindicators in all programs of valorization and conservation of Moroccan *Juniperus thurifera*.

Keywords: *Juniperus thurifera,* provenance, extract, yield, compound, Morocco.
Abbreviations: MAP_Medicinal and aromatic plants; CE_Catechin equivalent; DPPH_ α, α-diphenyl-β-picrylhydrazyl; IC_Concentration inhibition; ddl_ Degree of freedom; Haw_El Haouz population, Mid_Midelt population; Ang_Anergui population; PC_ principal component.

Introduction

The search for new bioactive molecules is increasingly becoming a concern for researchers, especially in the medical, agri-food and cosmetic fields. Yet, the phytochemical profile of the plants could be influenced by the environmental conditions of the provenances combined with intrinsic genetic factors (Ruiz Rodríguez et al. , 2011; 2014; Maieves et al. 2015). In fact, the expression of secondary metabolites is only a response to several factors including genetic and environmental factors, which act independently or combined (Crocoll et al., 2010; Lamamra, 2018). However, Medicinal and aromatic plants (MAP) are considered as a real deposit of these molecules due to their richness in secondary metabolites with a remarkable bioactive potential. Due to this potential, MAPs are endowed with considerable therapeutic, cosmetic and food preservation virtues (Raeiszadeh et al., 2018; Sanna et al., 2019). Among these MAPs is the thuriferous juniper (*Junipurus thurifera* L. ssp. *africana* (Maire) (Gauquelin et al., 1999; Mansouri et al., 2011).

Junipurus thurifera is a dioecious tree belonging to the Cupressaceae family. It has important ecological, socio-economic, floristic and cultural interests (Gauquelin et al., 1999; Montès et al., 2002; Bouzouita et al., 2008 ; Lafraxo et al., 2022). It is endemic to the western Mediterranean basin (Charco, 1999; Olano et al., 2008). It thrives in North Africa (Morocco and Algeria), Spain, France (Pyrenees, Alps and Corsica) and Italy (Alps) (Montès et al., 2002; Quintana et al., 2007). This resinous tree is found in semi-arid climates and is

resistant to the droughts and cold temperatures of the Mediterranean (Quintana et al., 2007).

Thuriferous juniper is original to the Tertiary (Suárez Cardona et al., 1991; Montesinos et al., 2010). Its maximum distribution was observed during the cold periods of the Pleistocene, but it continues to be reduced with progressive warming to scattered populations limited to the high mountains of the western Mediterranean basin (Terrab et al., 2008; Montesinos et al., 2010).

In Morocco, this endemic plant of North Africa covers about 31,000 ha. It is distributed in areas with semi-arid and subhumid bioclimates with cold to very cold variants in the Middle Atlas, the Eastern Anti-Atlas and the Eastern and Central High Atlas at altitudes ranging from 1800 m to 3300 m and on all types of substrates (Aafi et al., 2002). It represents a wood resource for heating, cooking and construction for local populations (Fromard and Gauquelin, 1993; Montès et al., 2002). It is also the main resource for livestock during the winter dearth period (Gauquelin et al., 1999; Montès et al., 2002).

In traditional medicine, the essential oils of *Junipurus thurifera* have been used as abortifacients and regulators of menstruation (Bellakhder, 1997), while the tar of its wood is used as a veterinary remedy (Barrero et al., 2004).

Despite all these virtues and its socio-economic and phytotherapeutic importance, this plant is less valued and subjected to anthropic pressure combined with the unfavorable conditions of climate change. Nevertheless, and to our knowledge, there is no program for its regeneration and its reintroduction in its natural area. To the best of our knowledge, phytochemical characterization and the antioxidant activity of this species have never been the subject of an exhaustive and thorough study.

In this context, our work aims to evaluate the effect of the geographical origin on the phytochemical profile and antioxidant activity of *Junipurus thurifera* leaves extracts from three regions of its natural distribution in the Moroccan High Atlas. This work, aims to establish different tracks of valorizations and conservation of natural resources of this species for its economic and socio-cultural values.

Results

Yield of extracts from Junipurus thurifera

The results obtained on the yield of extracts of *Junipurus thurifera* leaves, collected from different provenances in Morocco, show a variation between populations as well as between the solvents used (Figure 1). Indeed, the ethanolic extracts show a good yield compared to the extracts obtained with hexane. On the other hand, the population of Midelt (Mid) presents the highest yield for both extracts with values of 9.41% ±0.59 (ethanol) and 6.57% ±0.29 (hexane), followed by the population of Anergui (Ang) for the ethanolic extract with a yield of 8.18% ± 0.64 and the population of El Haouz (Haw) for the hexane extract (6.82% ± 0.32). The population from the Western High Atlas (El Haouz), records a value of 8.14% ± 0.72 for the ethanolic extract and the population of Anergui a value of 6.05% ± 0.24 for the hexane extract. The variation of the origin and the solvent used is reflected on the variation of yield in dry extracts. The one-way ANOVA (Table 1) shows a significant variation between the populations studied in terms of the yield of ethanolic extracts from the leaves of this plant, which means that the origin of the samples has an effect on the yield. However, the hexane extracts do not show significant variation.

Polyphenol content of Junipurus thurifera extracts

The level of total polyphenols in Moroccan juniper leaves shows a significant variation between the populations studied (Table 2). For ethanolic extracts, the population of El Haouz is considered the richest in polyphenols with an average rate of 191.30 ± 4.27 mg GAE /g Extract and which vary between 188.83 and 196.23 mg GAE /g Extract, followed by the Midelt population from the Eastern High Atlas with an average rate of 186.77±2.49 mg GAE /g Extract with a minimum value of 183.89 mg GAE /g Extract and a maximum value of 188.21 mg GAE /g Extract. However, the population of High Atlas Central (Anergui) presents a low level of polyphenols by a value of 73.60±1.78 mg GAE /g Extract. On the other hand, for the hexane extracts, the population of Midelt is the richest in polyphenols with a value of 58.03±11.10 mg GAE /g Extract, followed by the population of El Haouz (51.54±8.88 mg GAE /g Extract). While the population of Anergui shows low values of polyphenols in hexane extracts (35.27±2.93 mg GAE /g Extract).

Flavonoid content of Junipurus thurifera extracts

The flavonoid content of Moroccan thuriferous juniper varies significantly between the biogeographic zones studied (Table 2). In fact, the Anergui population presents higher values in terms of flavonoids in ethanolic extracts, as opposed to the values of polyphenols, with a mean level of 135.47±19.22 mg CE/g Extract, with a minimum value of 114.80 mg CE/g Extract and a maximum of 152.80 mg CE/g Extract. On the other hand, the population of El Haouz which is the richest in polyphenols, records the lowest level of flavonoids with an average value of 74.47±15.50 mg CE/g Extract. While the population of Midelt from Central High Atlas presents moderate values in flavonoids with an average rate of 100.13±8.88 mg CE/g Extract. However, in hexane extracts, the Midelt population presents high values compared to the other populations with a value of 40.63±7.32 mg CE/g Extract.

Tannin content of Junipurus thurifera extracts

The content of condensed tannins in thuriferous juniper extracts also shows a significant variability among the studied populations (Table 2). In fact, the level of these compounds varies from 155.50±5.00 mg CE/g Extract in the El Haouz population to 304.67±9.46 mg CE/g Extract for the Midelt population in ethanolic extracts. However, the population of Anergui records an average level of 273.00±27.04 mg CE/g Extract. Similarly, in hexane extracts, the Midelt population is the richest in condensed tannins with an average level of 51.33±3.82 mg CE/g Extract, while the Anergui population records a low level with a value of 18.00±2.50 mg CE/g Extract.

The One-way Analysis of Variance (ANOVA) (Table 1), shows a highly significant difference ($P<0.001$) between the populations studied for the content of flavonoids, polyphenols and condensed tannins in the ethanolic extracts. A significant difference of these compounds also observed in the hexane extracts especially in terms of tannins. However, the flavonoid and polyphenol content in the hexane extracts did not show a highly significant variation compared to the ethanolic extracts. These results show that the origin could have a significant effect on the

level of secondary metabolites in the extracts of *Junipurus thurifera* leaves, as well as the solvent used could be behind the variation in the amount of secondary metabolites of this plant.

On the other hand, the principal component analysis (PCA) using the mean values of yield and mean contents of polyphenols, flavonoids and tannins, shows a total dispersion of the studied populations (Figure 2), which come from different biogeographic zones of the Moroccan High Atlas. This confirms the results of the ANOVA and shows that the origin is the determinant of the variability of those parameters between populations. However, the Pearson correlation coefficient shows a negative relationship between polyphenols and flavonoids (R^2 = - 0.922). On the other hand, the tannin content is significantly correlated with the polyphenol content (R^2 = 0.912) of the extracts of *Junipurus thurifera* leaves.

Antioxidant activity of Junipurus thurifera extracts

The antioxidant activity of the compounds is due to their reducing potential. This activity is evaluated by the DPPH test. The antioxidant activity of thuriferous juniper extracts from the three provenances was determined by the concentrations that provide 50% inhibition (IC_{50}) in comparison to the IC_{50} of a reference antiradical, in this case ascorbic acid (Figure 3). The IC_{50} values were calculated from the regression curve of the graphs in Figure 4 and are presented in Figure 5. The results in this figure show that the ethanolic extracts show a significant antioxidant activity compared to the hexane extracts. In fact, the IC_{50} of ethanolic extracts varies from 0.98 ± 0.05 mg/ml for the El Haouz population to 1.78 ± 0.05 mg/ml for the Anergui population. On the other hand, this potential for hexane extracts varies from 10.19 ± 0.12 mg/ml (Midelt) to 12.56 ± 0.16 mg/ml (Anergui). On the other hand, these results show a significant difference between the IC_{50} values of the extracts of the three populations which are always lower than that of ascorbic acid (0.49 ± 0.02 µg/ml). In fact, the ethanolic extracts of the population of El Haouz present an important anti-radical capacity by an IC_{50} = 0.98 ± 0.05 mg/ml, followed by the population of Midelt with a value of IC_{50} = 1.43 ± 0.07 mg/ml. The population of Anergui presents a weak antiradical capacity compared to the other populations with a value of IC_{50} = 1.78 ± 0.05 mg/ml. However, the hexane extracts which present a very low anti-free radical capacity, also present a variation between the three provenances studied. Thus, the one-way ANOVA (Table 3) shows a significant variability between the populations studied ($P<0.001$) for the values of the effector concentration, for ethanolic and hexane extracts, which shows that the provenance also has an effect on this biological parameter.

Discussion

The present research was aimed at revealing the effect of the origin and its environmental conditions on the yield, the content of secondary metabolites and the antioxidant activity of Moroccan *Junipurus thurifera* extracts. In fact, the results show a significant variability between the three biogeographic zones for all the parameters studied.

The yield of ethanolic and hexane extracts of Moroccan *Junipurus thurifera* varies significantly between the studied populations. Indeed, the Midelt population is the richest in ethanolic extracts compared to the other populations. Our results corroborate with the results of previous studies (Athamena et al., 2019). These authors recorded values of 8.5% for the yield of methanolic extracts of *Junipurus thurifera*. Regarding hexane extracts, the results show high values compared to those found by Athamena et al. (2019) using the same solvent. In fact, in concordance with ethanolic extracts, the Midelt population is the richest in hexane extracts. The variability observed in the quantity of these extracts could be due to the polarity of the solvents (Athamena et al., 2019) and also to the intrinsic factors related to the specimens namely: genetics, vegetative stage and harvesting period (Bradesi et al., 1997; Jamoussi et al., 2005), as well as to the difference in eco-climatic factors of the sampling sites. In fact, the Eastern High Atlas zone (Midelt), which recorded high yield values, is characterized by a semi-arid climate with a cold variant with a low annual precipitation rate of approximately 160 mm/year and a average annual temperature of about 20°C, in addition to a skeletal substratum formed by limestone. On the other hand, the other two zones (Central and Western High Atlas) having marked medium and identical values in these extracts, are characterized by a semi-arid climate with cold variant and semi-arid with temperate variant, respectively. These areas differ from the climatic point of view by the difference in the annual rainfall received (355 mm/year and 290 mm/year, respectively), as well as in the typology of the soil which is of calcareous nature in the Anergui region and schistose in the Azzaden valley (El Haouz). These data show that environmental factors could be the cause of this variation and influence on the production of secondary metabolites (Szakiel et al., 2010; Sharma et al., 2012; Fadil et al., 2016). In the present work, the variability of environmental conditions is well observed, as long as the sampling sites belonging to different biogeographic and bioclimatic zones.

On the other hand, the contents of secondary metabolics also show variability between samples from different biogeographical zones. This means that the provenance could be at the origin of this variability. In fact, the population from the Western High Atlas (El Haouz), characterized by a moderate annual precipitation rate, is the poorest in flavonoids which are compensated by a richness in total polyphenols and condensed tannins. In contrast, the population of Central High Atlas the most rainy is the richest in flavonoids in contrast to the rate of polyphenols. In regard to tannins, we observed that the population of Eastern High Atlas is the richest in these compounds. These results could be explained by the difference between the sampling sites regarding climate (precipitation and temperature) and soil. In fact, the nature of the substrate can have an impact on the biosynthesis of secondary metabolites (Brada et al., 2007; Ghanmi et al., 2007; Mansouri et al., 2011). However, our results show high values in terms of polyphenol, flavonoid and tannin contents in methanolic extracts of *Junipurus thurifera* from Algeria (Athamena et al., 2019), as well as in other juniper genus namely: *Juniperus sibirica* from Serbia (Lesjak et al., 2011), *Juniperus oxycedrus* L. subsp. *oxycedrus* and *Juniperus* oxycedrus L. subsp. *Macrocarpa* from Turkey (Taviano et al., 2013). Other studies have shown that juniper genus twigs are richer in secondary metabolites compared to leaves (Soltani et al., 2018). However, the content of phenolic compounds or other secondary metabolites could be dependent on the polarity of the solvent used, the part of the plant subject of extraction, the

Table 1. One-way ANOVA of the effect of provenance on yield and Flavonoid, polyphenol and tannin content of Moroccan *Junipurus thurifera* extracts.

Extraction method		ddl	Average of squares	F	Significance
Ethanol	Yield	2	1.362	20.718	0.002***
	Flavonoid	2	2814.111	12.263	0.008**
	Polyphenol	2	13339.938	1445.339	0.000***
	Tannin	2	18515.583	64.803	0.000***
Hexane	Yield	2	0.333	1.5	0.296
	Flavonoid	2	301.562	5.567	0.043*
	Polyphenol	2	412.33	5.87	0.039*
	Tannin	2	867.361	73.471	0.000***

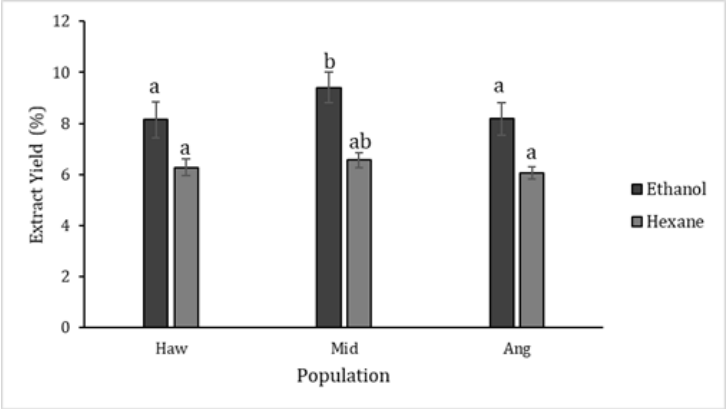

Figure 1. Yield of extracts of Junipurus thurifera leaves in Morocco

Table 2. Mean, Min, Max and Coefficient of Variation of Polyphenols, Flavonoids and Tannins in *Junipurus thurifera* extracts.

		Ethanol			Hexane		
Population		Polyphenols (mg GAE /g Extract)	Flavonoids (CE/g Extract)	Tannins (CE/g Extract)	Polyphenols (mg GAE /g Extract)	Flavonoids (CE/g Extract)	Tannins (CE/g Extract)
Haouz (Haw)	Mean Min-Max CV	191.30±4.27 (188.83-196.23) 2.17	74.47±15.50 (58.8-89.80) 17.26	155.50±5.00 (150.50-160.50) 3.22	51.54±8.88 (45.33-61.71) 17.23	37.30±10.10 (28.13-48.13) 27.09	28.83±3.82 (20.50-33.00) 13.24
Midelt (Mid)	Mean Min-Max CV	186.77±2.49 (183.89-188.21) 1.33	100.13±8.88 (90.80-108.47) 8.86	304.67±9.46 (298.00-315.50) 3.11	58.03±11.10 (51.36-70.84) 19.13	40.63±7.32 (40.63-54.38) 15.91	51.33±3.82 (48.00-55.50) 7.44
Anergui (Ang)	Mean Min-Max CV	73.60±1.78 (71.54-74.63) 2.42	135.47±19.22 (114.80-152.80) 14.18	273.00±27.04 (250.50-303.00) 9.91	35.27±2.93 (32.40-38.26) 8.31	26.05±2.60 (23.13-28.13) 9.99	18.00±2.50 (15.50-20.50) 13.89
F-value		12.263**	1445.339***	64.803***	5.567*	5.87*	73.471***

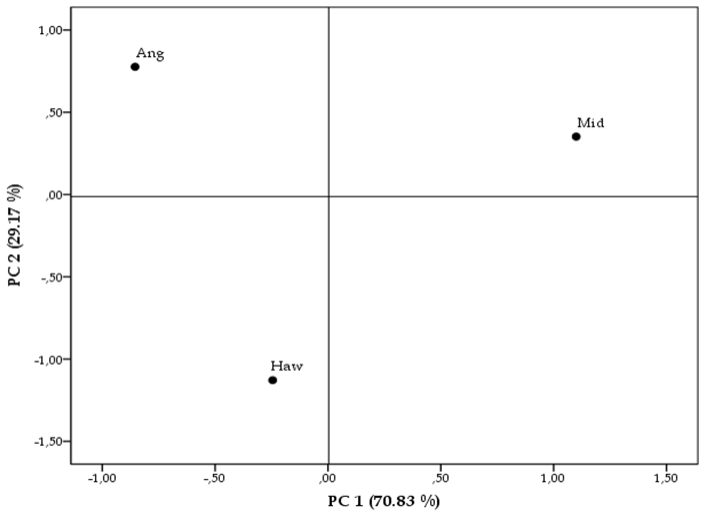

Figure 1. Component analysis of variability in yield and content of plyphenols, flavonoids of *Junipurus thurifera* in Morocco.

Table 3. One-way ANOVA of the effect of provenance on the IC_{50} of Moroccan *Junipurus thurifera* extracts.

	ddl	Average of squares	F	Signification
IC_{50} of Ethanol extract	2	1.362	20.718	0.002***
IC_{50} of Hexane extract	2	0.333	1.5	0.004***

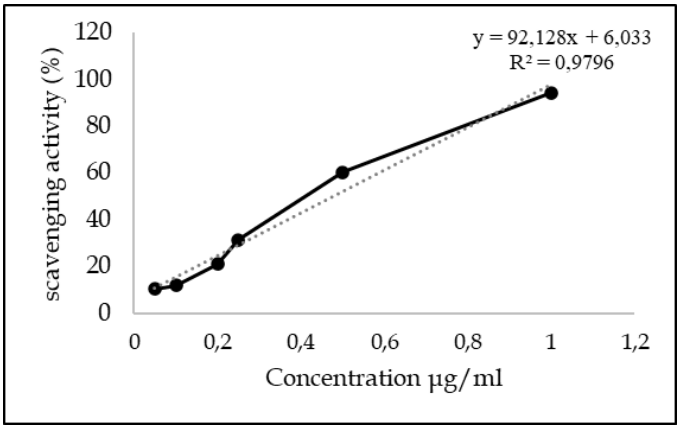

Figure 2. Free radical scavenging activity of ascorbic acid.

Table 4. Geographical and climatic characteristics of the sampling sites.

Sampling site	**Ait Ouaalou Forest (Midelt)**	**Azzaden Valley (El Haouz)**	**Iferd N'Ali – El houanet Forest (Anergui)**
Latitude Longitude	N 32°19'/W 04°33'	N 31°10'/W 07°59'	N 32°10'/W 05°57'
Altitude (m)	1412	1697	2149
Bioclimat	Semi-arid with cold variant	Semi-arid with fresh to temperate variation	Semi-arid with cold variant
Soil type	Skeletal/limestone soils	Poorly developed soils /Schist	Skeletal soils (remnants of podzolic soils) / limestone
Average annual precipitation (mm)	157	290	355
Average annual temperature (° C)	20.1	19.7	18.9

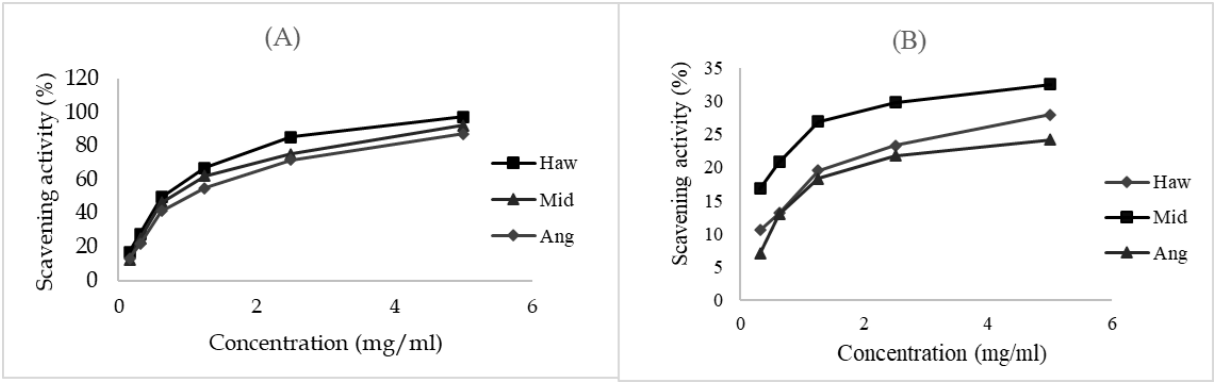

Figure 3. Free radical scavenging activity of ethanolic (A) and hexane (B) extracts of Moroccan Junipurus thurifera leaves.

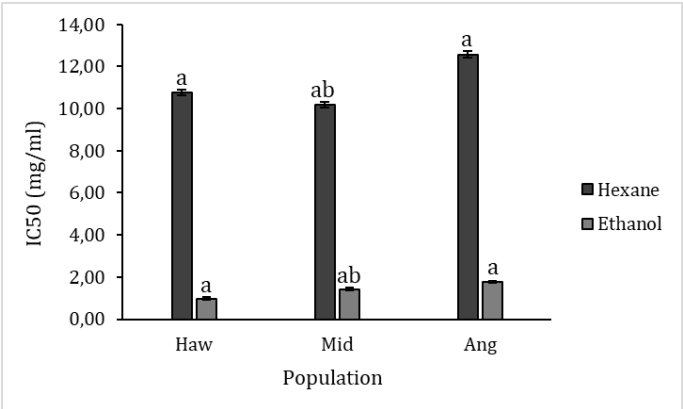

Figure 4. IC_{50} of extracts from the three *Junipurus thurifera* provenances.

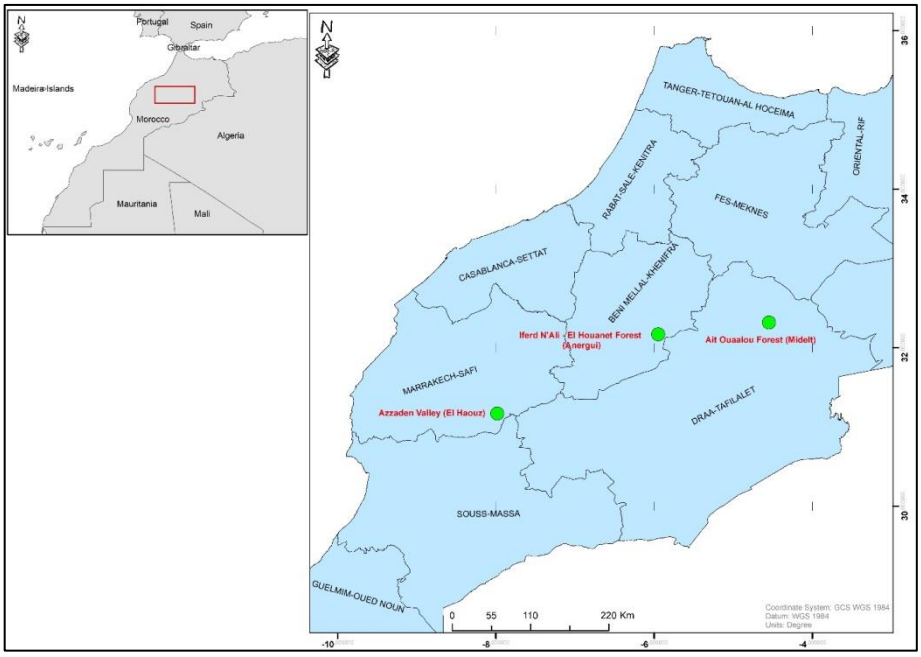

Figure 5. *Junipurus thurifera* sampling sites.

condition of the plant material and the extraction method used (Athamena et al., 2019).

In addition, the results of the ANOVA show a significant variability for the yield and secondary metabolites of the extracts of *Junipurus thurifera* needles. This means that the origin of the samples could have an effect on the biosynthesis of its compounds, which was proved by the distribution of the populations following the PCA performed based on the average values of yield, polyphenols, flavonoids and tannins. In fact, the variation between the provenances could be explained by the extreme variations in their environmental conditions, which are evident by the fact that the sites belong to biogeographic zones distinguished between them by climate (rainfall, temperature, etc.), orography, nature of the soil, etc.

The variability observed between the three areas studied in the yield of ethanolic and hexane extracts, as well as chemical compounds, could be explained by the variation of intrinsic factors of the plant (genetics, age of the plant, vegetative cycle, etc.) and extrinsic factors related to the environment of the sites (climate, edaphic factors, exposure, orography, altitude, etc.). The adaptive capacity of the plant to these factors acts on the preferential biosynthesis of chemical compounds and their content and is translated by their differential expression (Brada et al., 2007; Ghanmi et al., 2007; Mansouri et al., 2011).

As for the antioxidant potential, we used the DPPH radical scavenging test. We know that the choice of a single test is insufficient to evaluate the reducing potential of extracts in a comprehensive way (Gianni et al., 2005), but the reason why we chose a single test is that the objective of this study is to evaluate the effect of the origin and the extraction solvent on the antioxidant potential of extracts of *Junipurus thurifera*. In the light of the results obtained, we noticed a significant difference between the ethanolic extracts and those of hexane in the reduction of DPPH. Moreover, this activity is also very different between the three origins studied. In fact, the ethanolic extracts show a significant antiradical power compared to the hexane extracts. Moreover, the samples from El Haouz region, rich in polyphenols, show a high reducing potential towards DPPH in comparison to the extracts from Anergui population which present a low reducing potential that could be explained by their poverty in phenolic compounds. Indeed, several previous studies have proven a strong correlation between the reducing capacity of the extracts and these compounds (Keskes et al., 2014; Taviano et al., 2013; Lesjak et al., 2011). On the other hand, these compounds are used as scavengers of peroxide radicals and intermediate alkoxyl radicals and as chelating agents of metal ions that are of major importance for the initiation step of radical reactions, thus as anti-inflammatories (Lesjak et al., 2011). Subsequently, it is observed that the variation in antioxidant activity of the extracts from the three studied areas could be explained by their richness in phenolic compounds. By this reason, we observed that the populations rich in polyphenols present a significant reducing capacity. This means that the provenance by these characteristics, especially, geographical, climatic and environmental could be at the origin of the variation of the contents of secondary metabolites and consequently the biological potential of the extracts of plants (Mansouri et al., 2011).

Material and Methods

The present study aims to evaluate the effect of geographical origin on the phytochemical profile and antioxidant activity of juniperus thurifera leaf extracts. We focused on two extracts: ethanolic extracts and hexane extracts. In these two extracts we measured the yield of extracts and the content of secondary metabolites (polyphenols, flavonoids and condensed tannins). The extraction was carried out on leaves from three geographically distinct areas in order to explore the effect of the geographical origin through these pedoclimatic conditions on the yield, the content of secondary metabolites and the antioxidant activity of the leaves of this plant.

Plant material

Collection of thuriferous juniper samples was carried out in July 2021 through three biogeographical areas, namely; the

region of El Haouz (Azzaden valley; Western High Atlas), the region of Anergui (El Houanet Plateau; Central High Atlas) and the region of Midelt (Ait Ouaalou Forest; Eastern High Atlas). This sampling was done in such a way to cover the whole natural distribution of this species in the Moroccan High Atlas. 30 specimens were collected (each sample represents a genotype) at a rate of 10 specimens per population (Table 4, Figure 6). The collected samples were stored in paper bags in the laboratory for open air drying.

Preparation of extracts

Methanolic extracts are obtained by soxhlet by adopting the method described by Raaman (2006) with some modifications. The collected juniper leaves were dried in the open air under shade for a 7 days and then powdered. The extraction was performed for 6 hours at 60°C with 250 ml ethanol and for the same time at 45°C in the same volume of hexane. The extracts were then concentrated using a rotary evaporator under reduced pressure at 60°C for the ethanolic extracts and at 45°C for the hexane extracts. The dry extracts obtained were stored at 4°C until further use.

Determination of flavonoids

In a tube, we put 0.25 ml of the extract diluted 1/10, we add 1.25 ml of distilled water and 75µl of sodium nitrite ($NaNO_2$) at 5%. After 5 min, 150µl of aluminum chloride $AlCl_3$ (2% m/v) is added. After 5 minutes 0.5 ml of 1M sodium hydroxide (NaOH) was added, the reaction mixture was completed to 2.5ml and the solution was mixed well and left to stand for 30 minutes. The absorbances were measured immediately at a wavelength of 510 nm (Chang et al., 2002).
The contents of total flavonoids in each extract are calculated with reference to the calibration range regression equation, established with the standard Rutin at different concentrations (50, 100, 150, 200, 250, 300, 350, 400 µg/ml) and under the same conditions and steps of the assay.

Determination of polyphenols

A volume of 0.3 ml of each extract (1mg/ml) diluted 1/5 is added to 2.5 ml of Folin-Ciocalteu reagent (10 times diluted in water). Then, 2 ml of sodium carbonate $Na2CO_3$ (7.5%, w/v) is added to promote an alkaline medium to start the redox reaction. The mixture is then incubated in a water bath at 50 °C for 5 minutes. The intensity of the blue coloration produced was measured using a spectrophotometer at 760 nm wavelength (Deepika et al., 2018).
Quantification of total polyphenols was done based on a calibration curve performed by a standard (Gallic acid) at different concentrations (0.02, 0.04, 0.06, 0.08, 0.1 mg/ml) under the same conditions as the extracts and the results are expressed as milligram of gallic acid equivalent per 1 gram of extract (mg GAE / 1 g Extract).

Determination of tannins

The analysis of condensed tannins was performed according to the method of Broadhurst and Jones (1978) slightly modified. 50µl of each of the extracts (1 mg/ml) was mixed with 1.5 ml of 4% vanillin, followed by 1.5 ml of 8% HCl hydrochloric acid were added. After the solution is mixed well and left for 20 min in the dark at an ambient temperature. The absorbances were measured at 500 nm. Catechin tannin contents were calculated from the linear regression equation of the calibration curve established with catechin at concentrations of (50, 100, 200, 300, 400, 500, 600, 800, 1000 µg/ml) and were expressed as milligram equivalents of catechin per gram of extract (mg CE/g of extract).

Antioxidant activity of Junipurus thurifera extracts

The antioxidant activity is estimated by the DPPH free radical scavenging assay adopted by Brand-williams et al. (1995). A 0.1 mM methanolic solution of DPPH was prepared, 2.7 ml of DPPH solution (0.1 mM) was mixed with 0.3 ml of different concentrations of *Junipurus thurifera* leaves extracts, prepared by cascade dilution (1/2) starting with an initial concentration of 5 mg/ml. After 30 min of incubation at ambient temperature in the dark, the absorbance is measured at 517nm. The results obtained from the measurement of the anti-free radical activity of DPPH were expressed in relation to those obtained by the measurement of the activity of Ascorbic acid, the reference antioxidant, at different concentrations (0, 50, 100, 200, 250, 500, and 1000 µg/ml) carried out under the same conditions. The free radical scavenging activity of the extracts was expressed as a percentage according to the formula:

$$IP = \frac{(\mathrm{A(DPPH)} - \mathrm{A(sample)})}{A(DPPH)}$$

$A_{(DPPH)}$: Absorbance of control (Methanol + DPPH) ;
$A_{(sample)}$: Absorbance of sample (Extract + DPPH)

The 50% inhibitory concentration (IC_{50}) was determined from the percentages of DPPH reduction. The IC_{50} is expressed in µg/ml and is compared with that of the standards.

Conclusion

We conclude that the Moroccan *Junipurus thurifera* is rich in phenolic compounds, flavonoids and tannins. Thus, the extracts of the needles of the aforementioned plant present an important antioxidant activity especially the ethanolic extracts. On the other hand, the yield of extracts and the content of polyphenols, flavonoids and tannins of this plant depend on the geographical, bioclimatic and edaphic conditions of each sampling site. The results obtained on the extracts of thuriferous juniper are competitive with the different extracts of other species of the genus *Juniperus*. This allows us to foresee programs for the valorisation of this species through applications in the food, pharmaceutical and cosmetic fields. Thus to rationalize its exploitation with the aim of conserving its resources on the one hand. On the other hand, it is of necessity to establish programs of its reforestation and its reintroduction in its natural environment. Moreover, it is necessary to start other analyses of biochemical, chemical and biological characterization of this plant, as well as to widen the area of study in order to have a general vision on its benefits for a sustainable socio-economic valorization.

Acknowledgements

We would like to express our gratitude to all the members of Environmental, Ecological and Agro-Industrial Engineering Laboratory for their collaboration and help in the completion of this work.

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