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Distribution Mapping and chemical composition of *Tetraclinis articulata* (Vahl.) Masters in the Site of Biological and Ecological Interest of Kharouba (Central Plateau, Morocco)

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- ✓ *Tetraclinis articulata* ;
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Abstract

The main object of this work is to elaborate a distribution mapping of the *tetraclinis* stands in the SBEI of Kharouba and explore the opportunities of its valorization to ensure its sustainable and rational exploitation. The results of *Tetraclinis articulata* mapping using the supervised classification of high resolution multispectral satellite image showed that this specie is spread out over the great majority of the SBEI Kharouba's territory. The study of gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS) of essential oils (Eos) allowed us to identify 5 major components from the sawdust of *Tetraclinis articulata* and 5 major components from the leaves. These components are highly sought after in the international market and are used in various fields: agri-food sector, cosmetic industry and pharmacology.

1. Introduction

Compared to other Mediterranean countries and due to its geographical position between the Atlantic Ocean to the West and the Mediterranean Sea to the North, Morocco is characterized by high vascular plant diversity with an estimated 600 plant species are qualified as medicinal and aromatic plants in the country [1]. Distinguished by its floristic richness, the Site of Biological and Ecological Interest (SBEI) of Kharouba is home to over 52 medicinal plants [2]. The most prevalent specie in the SBEI of Kharouba is *Tetraclinis articulata*. This medicinal plant has vital contributions to the soil protection, the wildlife preservation and the air purification. *Tetraclinis articulata* is also valued for its wood and various products, namely; timber, wood tar, firewood, charcoal and sandarac gum. These diverse products are the origin of several types of craft and commercial activities that are considered by numerous households in this area as an important source of income. Even the potential of SBEI in terms of *Tetraclinis articulata* is important, this medicinal face an important insufficiency in terms of its valorization, which may be related to a lack of accurate data on its distribution, its potential, its productivity of essential oils (Eos), its chemistry, itsecology and often even on its properties and its intended usages.

In this context, the main goal of this work is to evaluate and diagnose the *Tetraclinis articulata*'s current situation in the SBEI of kharouba and explore the opportunities for its valorization to ensure a sustainable and a rational exploitation of this natural resource.

This work's main objectives are to:

- Develop a distribution mapping of *Tetraclinis* in the SBEI of Kharouba;
- Determine its Eos productivity and their various chemical components.

1. Materials and methods

2.1. Study area

The study area is positioned between the latitudes 33° 30' 39.567"N and 33° 36' 19.969" N and the longitudes 5° 48' 19.314"E and 5° 55' 39.317"E (Figure 1). Covering an area of 6300 hectares, the SBEI of Kharouba is

located in the Moroccan Central Plateau and is considered a priority one site [3]. This area is distinguished by its rugged and mountainous nature, multitude expositions and various slopes. Ordovician rocks are quite abundant in this region; we note mostly the presence of diverse schists including slate, sandstone and quartzite. As for the soil, it is rather skeletal.

The local climate is marked by an average annual rainfall that varies between 450 mm and 600 mm. As for the average annual temperature; it ranges from 1 ºC to 34 ºC. The SBEI bioclimate is subhumid and semi-arid with temperate and cool variants [3].

Regarding the vegetation of this SBEI, *Tetraclinis* stands thrive in low altitude and in warm zones, while the Quercus rotundifolia is abundant in the relatively humid environments.

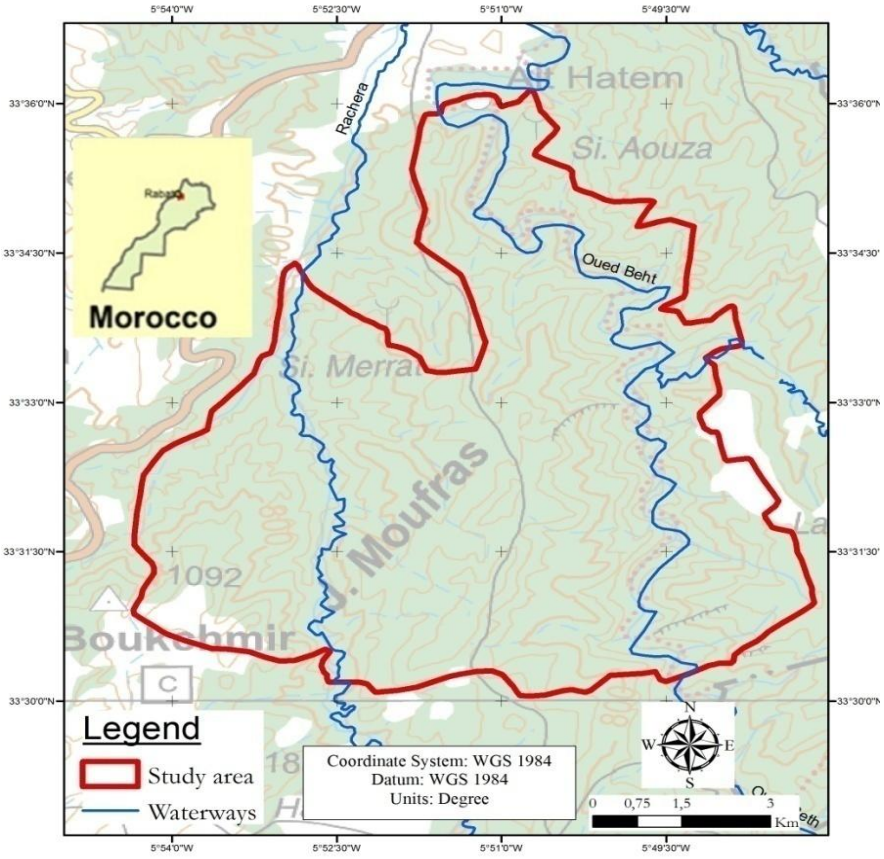

Figure 1: Kharouba SBEI location Map

2.2. Tetraclinis articulata mapping

The *Tetraclinis articulata* mapping was performed based on supervised classification of high resolution multispectral satellite image (Resolution: 2.5 m). This classification was conducted through 145 floristic surveys covering all of the *tetraclinis* stands in the SBEI.

The chosen approach is based on multispectral classification techniques. The multispectral image processing in this study is shown in the diagram of the figure processing n°2.

The multispectral image used in this work is of type SPOT, that was acquired in July 2008. It was chosen, mainly for its availability and visual quality. It is composed of four bands (green, red, near infrared and mid infrared) and a panchromatic with 2.5 m spatial resolution.

2.3. Extraction, analysis and characterization of Tetraclinis articulata essential oils

Plant material: *Tetraclinis articulata* was collected in May 2014, from SBEI of Kharouba. The sawdust and the leaves were prepared in laboratory. The Eos was extracted by hydrodistillation of sawdust and leaves in an apparatus according to European Pharmacopoeia (1996) [4]. For distillation, we separately used 200 g of sawdust and 200 g of leaves. The average oil contents were calculated and expressed in ml/100g (v/w). The oil, obtained from both wood and leaves was analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS).

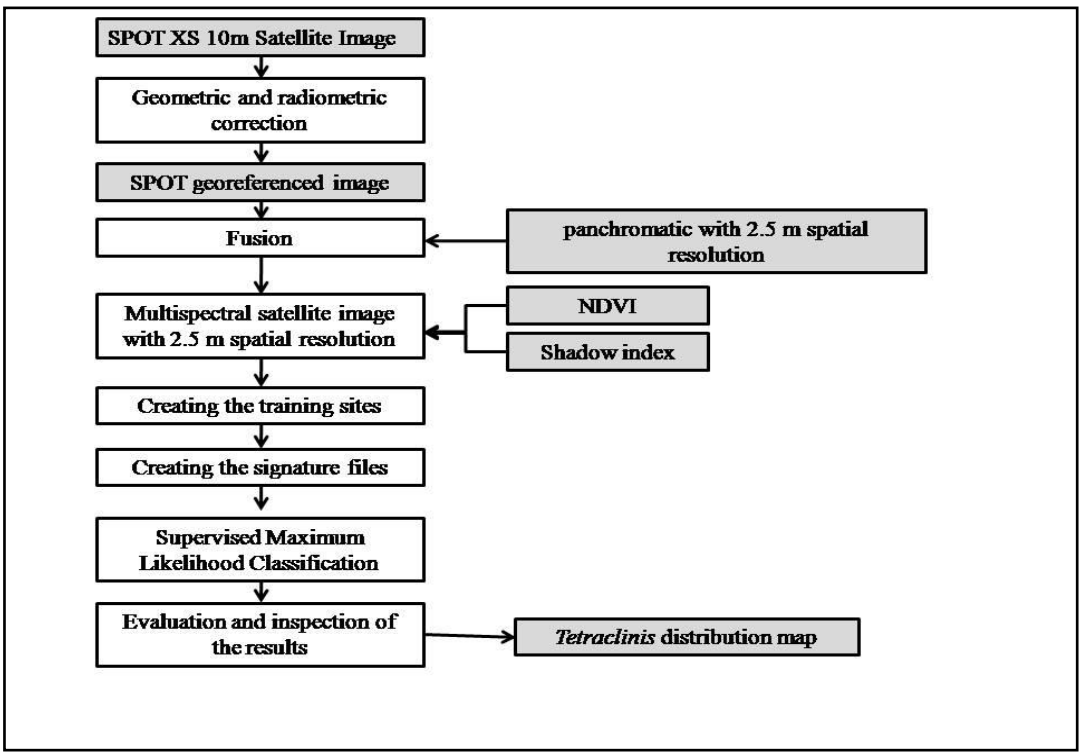

Figure 2: Diagrammatic description of satellite imagery processing

The components were identified by confirming their identities mass spectrometry (Library of NIST98 Spectra). The percentage contents of the essential oils were computed from the gas chromatography peak areas. The qualitative analysis was based on the comparison of the retention indices and mass spectra with the corresponding data in library and literature [5], and co-injection of the standard compounds.

2. Results and discussions

3.1. Tetraclinis articulata mapping

The supervised classification of high resolution Multispectral satellite image (2.5 m) allowed us to make Tetraclinis distribution map with a good cartographic accuracy, exceeding 80% (Table 1 and Figure 3).

The obtained map shows that *tetraclinis* with different density classes is the most widespread species in the studied region, occupying about 77% of the total SBEI area. However, the majority of these stands are characterised by low density. This situation shows that the *Tetraclinis articulata* is in alarming state due to the strong anthropogenic pressure.

Table 1: Stands distribution in Kharouba SBEI

Stand types	**Area (ha)**	**%**
Empty spaces	479	8
Matorral of low shrubs	517	8
Low density of *Tetraclinis articulata*	2126	35
Middle density of *Tetraclinis articulata*	1663	27
High density of *Tetraclinis articulata*	747	12
Quercus rotundifolia with middle density	455	7
Mixed stands of *Tetraclinis articulata* and *Quercus rotundifloia*	173	3

The most beautiful *tetraclinis* stands are mainly observed on altitudes ranging from 500 to 1000 m, in warm areas and on skeletal and eroded soils [6].

Quercus rotundifolia occupies an area of about 455 ha, representing 7% of the total forest massif. This stratum is located essentially on high altitudes that range between 950 m and 1200 m, on wet slopes in North and West expositions.

The matorral of low shrubs represented at SBEI consist mostly of *Olea europaea var oleaster, Pistacia lentiscus, Phillyrea latifolia, Rhus pentaphylla* and *Cistus albidus*. These species thrive over an area of 517 ha,

or 8% of the SBEI area. These formations play an important role in the conservation of soils and their protection against water erosion, especially as the terrain is rugged and the soil is very fragile. The empty spaces cover about 479 ha, or 8% of the total surface area of SBEI.

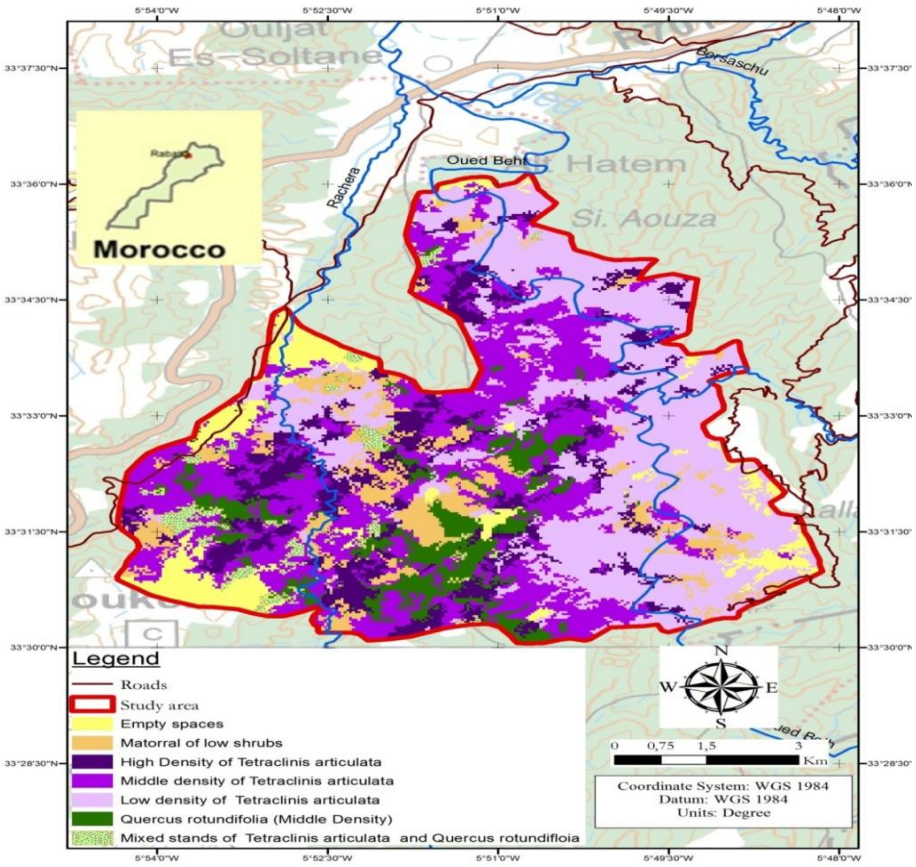

Figure 3: Stands distribution in Kharouba SBEI

3.2. Yields and chemical composition of essential oils

The average Eos yield from sawdust of *Tetraclinis articulata* is 1.6 % and from leaves is 0.2 % (Table 2). These are relatively high percentages compared to some of the plants used industrially as a source of Eos [7]. 22 components have been identified from wood and leaves respectively. The major components of the Eos from sawdust of *Tetraclinis articulata*, were: α-Acorenol (20.9%), Cedrol (17.9%), Totarol (8.8%), α-Cedrene (8.7%) and β-Acorenol (7.4%). In leaves oil, the main components were: Bornyl acetate (30,6%), Camphor (18.6%), α –Pinene (16.8%), Limonene(5.7%) and Borneol (4.7%) (Table 2 and Figure 3).

Table 2: Chemical composition of wood and leaves of *Tetraclinis articulata* Eos from Kharouba SBEI

Wood (sawdust)		**Leaves**	
Components	**Percentages (%)**	**Components**	**Percentages (%)**
α-Cedrene	8.7	α -Pinene	16.8
Cedrol	17.9	Limonene	5.7
α-Acorenol	20.9	Camphor	18.6
β-Acorenol	7.4	Borneol	4.7
Totarol	8.8	Bornyl acetate	30.6
oil Yield	1.63%	0.22%	

According to the bibliography, the *Tetraclinis articulata*'s Eos has strong antibacterial and antifungal activities [8, 9, 10]. The results obtained suggest the use of *Tetraclinis articulata* Eos in various fields such agri-food sector, cosmetic industry and pharmacology, instead of using synthetic antioxidants and anti-inflammatories. Similarly, Farah A. et *al* (2013) and Zrira S. et *al* (2011) [11, 12] noted the presence of Carvacrol in the

Tetraclinis articulata wood's Eos. This component has high antimicrobial activity, and is an ingredient that has high international demands [11].

2.3. Comparison between the sawdust of Tetraclinis articulata and the leaves Eos

The physicochemical properties of the Eos from the sawdust of *Tetraclinis articulata* are different from those of the Eos of the leaves. The difference between the two oils can be explained by the fact that the sawdust's Eos are denser than the ones from the leaves. In fact, the Eos extracted from the leaves have an acidity index lower than those derived from the sawdust of *Tetraclinis articulata* indicating that they are less rich in acids [13].

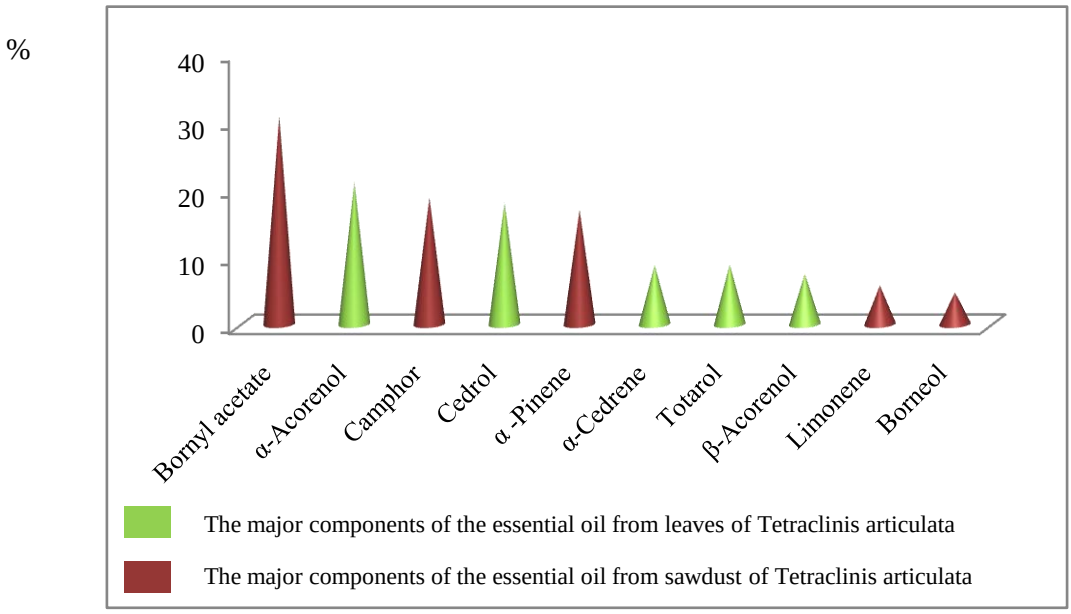

Figure 4: the major components of the Eos from leaves and sawdust of *Tetraclinis articulata*

3.4. The comparison between the Tetraclinis articulata Eos *of Kharouba SBEI with those of other region*

Comparing the previous reports for the wood oil of *Tetraclinis articulata* obtained from other region, they show that the yield differs depending on the localities [11, 12, 14] and the seasonal variation [14].

The comparison between the compositions of the *Tetraclinis articulata* wood's Eos of Kharouba SBEI with those of other region shows some resemblance with most of the components of the ones of the Central Plateau, yet it seems to be divergent from those of the region of Essaouira [12].

The comparison between the compositions of the *Tetraclinis articulata* leaves' Eos of Kharouba SBEI with those of Algeria [15] and Tunisia [14] exhibits some resemblance between most of components but with different percentages.

Conclusion

This study aims to establish a distribution mapping of *Tetraclinis* stands in the SBEI of Kharouba and explore the opportunities for its valorization. *Tetraclinis articulata* mapping results, using the supervised classification of high resolution multispectral satellite image, revealed that this specie is spread out over the great majority of the SBEI Kharouba's territory, and that it grows mainly on skeletal and eroded soils, on warm expositions and in altitudes ranging from 200 to 1000 m.

The study of gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS) of Eos allowed us to identify 22 components from wood and leaves respectively. The major components of the Eos from sawdust of *Tetraclinis articulata*, are: α-Acorenol (20.9%), Cedrol (17.9%), Totarol (8.8%), α-Cedrene (8.7%) and β-Acorenol (7.4%). In leaves oil, the main components were: Bornyl acetate (30.6%), Camphor (18.6%), α –Pinene (16.8%), Limonene(5.7%) and Borneol (4.7%).

The Eos components of *Tetraclinis articulata* are much in demand in the market and can be safely used in the agri-food sector, the cosmetic industry and pharmacology.

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