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Identification of *Artemisia campestris* L. subsp. *glutinosa* (Besser) Batt. from Oriental Morocco based on its morphological traits and essential oil profile

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Abstract
Artemisia campestris L. (*Asteraceae*) is a perennial herb, commonly known as field wormwood and widespread in Asia, North America, Europe and North Africa. This plant consists of many subtaxa differentiated on the basis of floral data. However, the subspecies that prevails in Morocco is the subsp. glutinosa (Besser) Batt. that spans a wide geographical distribution, mainly in the Anti-, High- and the Middle Atlas of Morocco, in the Rif, along the coast of the Mediterranean and widely common in high plateaus of Eastern Morocco. Previous studies exhibited that *A.campestris* L. from different origins is represented by serval essential oil-chemotypes. In this perspective, this work was elaborated to characterize *A.campestris* L. subsp. *glutinosa* from Eastern Morocco focusing on the morphological features and essential oil-based chemotyping. The microscopic observations of *A.campestris* L. subsp. *glutinosa* specimen showed that this plant is distinguished by a heterogamous and ovoid capitula surrounded by an involucre of bracts and constituted by peripheral functionally male flowers and central female flowers, organized on convex receptacle. Moreover, the GC-MS analysis of the essential oil extracted from aerial part of *A.campestris* L. subsp. glutinosa confirmed our previous study (submitted manuscript) and revealed a new chemotype characterized by spathulenol as a major compound. Morphological and chemical criteria represent a key role for the identification of *A.campestris* L. subsp. *glutinosa*. Nonetheless, additional descriptors could be useful to optimize this botanical and chemical determination especially to determine the intraspecific variabilities in the different populations of Moroccan *A.campestris* L. subsp. *glutinosa*, like cytological and molecular markers.

Keywords: Artemisia campestris L., capitula, morphology, essential oil, spathulenol, chemotype.

1. Introduction
An authentic botanical identification of plants is one of the most recommended criteria that may guarantee the success of herbal drug studies. The classical concept of botanical plant identification is mainly referring to the utilization of external characters or morphology of the organism for its identification as a species [1]. In fact, the first classification step of the plant material could be based on morphology, since the morphological characters are the most obvious. However, in the essential oil-containing plants, the morphological features remain insufficient to characterize the various aspects of the studied taxon, which imply the chemical profiling as an additional descriptor for the intraspecific determination. The genus *Artemisia* L. is one of the major polymorphic genera of the Asteraceae family, and the classical classification of this group is mainly based on floral characters variations [2]. Also, *Artemisia* L. is characterized by a wide range of essential oil chemical variability and biological effects, which is associated with different geographical origins of the specimens [3].

In Morocco, many studies have been undertaken, focusing on the chemical identification and biological activities of species of the genus *Artemisia* L., and the plant *Artemisia herba alba* was the species mostly studied among all the genus. The essential oil of *A. herba alba* was found to be rich in camphor, chrysanthenone, α-terpin-7-al, α- and β-thujone [4,5]. Also, this plant has expressed a wide brand of biological potentials, and both essential oil and aqueous extract of *A. herba alba* were reported to exert many biological activities as antioxidant, [6,7], insecticidal [8-11], antilashmanial [12], antimicrobial [13,5], antifungal [14] and antiproliferative activities [15,16], besides the relaxant effect on aorta and jejunum contractions [10,11]. *Artemisia absinthium* growing in Morocco is another Asteraceae plant which was studied by Moroccan teams; in fact, the essential oil was found to be rich in β-thujone and chamazulene and exerting remarkable effect as antibacterial [17] and insecticidal [9]. Many other studies have been reported on two Moroccan endemic Asteraceae plants; it's about: *Artemisia mesatlantica* and *Artemisia ifranensis.* The Moroccan *Artemisia mesatlantica* was abundant in β–thujone, camphene, camphor, 1,8-cineole and α-thujone [18,19] and characterized by an antimicrobial and anticandida efficacies [20,21,18], while *Artemisia ifranensis* was recently studied for its antibacterial effect [22]. The species *Artemisia campestris* L. known as field wormwood and called "Allal" in Moroccan community, is a plant belonging to *Asteraceae* family, that can be segregated into six subspecies distinguished by morphological characters and which can be listed as: (a) subsp. *campestris* L., (b) subsp. *glutinosa* (Gay ex Besser) Batt., (c) subsp. *maritima* Arcangeli, (d) subsp. *alpina* (DC.) Arcangeli; (e) Subsp. bottanica A. N. Lundstrōm ex Kindb, and (f) Subsp. borealis (Pallas) Hall & Clements [23]. However, it has been pointed out that the subspecies occurring in Morocco is an *A.campestris* L. subsp. *glutinosa* (Besser) Batt [24,25], that grows in the stony lands for pastures, in the mountain and piedmont regions, and spans a wide geographical distribution, from the Anti-Atlas, crossing the High Atlas up to the Middle Atlas of Morocco, it exists also in the region of Rif, along the Coastal Region of the Mediterranean and widely spread in the high plateaus of Oriental Morocco [26]. According to the bibliography, there is a very rare scientific research about *A.campestris* L. occurring in Morocco. Although, a recent ethnobotanical survey inventoried this plant as part of the flora of Eastern Morocco [27]. On the other hand, this plant is highly rich in essential oils and have been the subject of numerous chemical studies which showed a wide variability in the volatile composition leading to the determination of many chemotypes mainly depending on different geographical localities, and which are: β-pinene chemotype in Algeria, Tunisa and Italy, γ-terpinene and capillene chemotype in France, caryophyllene oxide and germacrene D chemotype in Lithuania, β-pinene and cadin-4 en 7-ol chemotype in Portugal, α-pinene chemotype in Southern Ural, 1,2-dehydro-acenaphthylene and tremetone chemotype in Turkey, (Z)-falcarinol chemotype in Poland and spathulenol chemotype in Iran and Serbia. [28-52]. In the optic to give a general insight about this plant, we conducted this study with the aim to verify the morphological traits previously cited in the literature, and to confirm a new chemotype of *A. campestris* L. subsp. *glutinosa* occurring in Eastern Morocco.

2. Materials and methods

2.1. Plant material

Aerial part of *A. campestris* L. subsp. *glutinosa* was collected in 2014 at the flowering stage in Figuig (oriental Morocco), situated in a desert area near the frontier border between Morocco and Algeria. The species was identified by Pr. Atika Mihamou from biology department, and a voucher specimen was deposited in the herbarium of the Faculty of Sciences, University Mohamed First (Oujda, Morocco) under the number HUMPOM-151.

2.2. Morphological analysis

The aerial parts of plant were prepared according to usual and standard techniques for herbarium material conservation. The observations were done on fresh collected samples of the plant. Capitulum and peripheral flower were identified under binocular magnifier (Nikon, Japan), while, ray flower was analyzed under optic microscope (Olympus, Japan; Objective: 4x). Photographs were taken using digital camera (Canon EOS 700D, Japan).

2.3. Preparation of A.campestris L .essential oil (AcEO)

About 200g of the air-dried plant were used for the extraction of the essential oil; the stems were thrown and the rest of the plant (leaves and flowers) was subjected to the hydrodistillation for 3 hours by using *Dean stark* apparatus. The obtained oil was yellowish with characteristic odour and yielded 0.4% (w/w). AcEO was then separated from the distillate and stored in sealed glass vial, in darkness at 4°C until the time of analysis.

2.4. Gas-Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis was performed on gas chromatograph coupled with mass spectrometry Shimadzu QP 2010 fitted with an injector at 250 °C (Splitless mode) and equipped with capillary column coated with 5% phenyl-methylsiloxane (30 m length × 0.25 mm internal diameter × 0.25 µm of film thickness). The column pressure was set to 100 KPa. The oven temperature was held at 50°C for 2 min, and then programmed to 160°C (3 min) at a rate of 10°C/min to 250°C (2 min) at a rate of 30°C/min. Helium was used as the carrier gas at a flow rate of 1.69 ml/min. The mass spectra (MS) were operated in electron impact mode (70 eV), and the MS data were acquired in scan mode. The peaks were quantified by calculating the percentage of the peak area of each component by comparison to the sum of the peaks of other compounds. The identification of the components was performed on the basis of chromatographic retention times and by comparison of the recorded spectra with computed data library NIST 147.

3. Results and discussion

This work is intended to contribute to the characterization of *A.campestris* L. subsp. glutinosa from Eastern Morocco, mainly by giving highlights about its morphological traits and its essential oil chemical composition.
It has been reported that the most widely characteristics used in the taxonomical identification of the genus *Artemisia* L. are floral and capitular morphology and gender [2,53]. However, the morphology of leaves is not considered as a taxonomically important character since it presents wide variability within the same population at each species [54]. In this optic, we focused our study on the morphological traits of capitulum morphological details. As described by Tutin et al. (1976), *A. campestris* L. consists altogether of six subtaxa, with subsp. *campestris* L.subsp. *glutinosa* (Gay ex Besser) Batt., subsp. *maritima* Arcangeli, subsp. *alpina* (DC.) Arcangeli, subsp. *bottanica* A. N. Lundstrōm ex Kindb, and subsp. *borealis* (Pallas) Hall & Clements, which can be distinguished based on some differences in terms of certain morphological characteristics, including the wide and viscosity of capitula, the presence or absence of capitula peduncle, the fleshiness of leaves and the glabrescence of stems and leaves [23]. Nevertheless, both of *A.campestris* L. subsp. *eu-campestris* Briq. & Cav. and *A.campestris* L. subsp. *glutinosa* (Gay ex Besser) Batt. were claimed to be the subspecies that exist in the desert region of Southern Algeria [55], which seems to be geographically near the Eastern region of Morocco, and hence, imply the possibility that the subspecies studied in our report may be one of those two subspecies. Otherwise, it has been clearly determined that the subspecies occurring in Morocco is an *A. campestris* L. subsp. *glutinosa* [25,24], that occurs in the stony lands for pastures, in the mountain and piedmont regions, and prefers typical climates of arid, cold semi-arid and sub-humid regions; also, it has been recently reported by Fennane et al. (2015) that the said plant spans a wide geographical distribution from the Anti-Atlas, crossing the High Atlas up to the Middle Atlas of Morocco, in the region of Rif, along the Coastal Region of the Mediterranean and widely spread in the high plateau of Oriental Morocco [26]. According to our observations on the field, the flowering of the plant begun in mid-August and continued up to the end of November, and as shown in figure 1, the inflorescence is an ovoid capitulum growing on short peduncle. The capitula are very small (2-2.5mm wide) and heterogamous constituted of both fertile and sterile flowers; each capitulum is surrounded by an involucral glabrous bracts organized in several rows. The flowers (20 to 28 flowers by capitulum) are organized on convex and glabrous receptacle. The disk flowers are tubular, yellowish, lacking calyx, with 5 fused petals, and 5 fused stamens; these flowers are sterile and functionally male with abortive ovaries, while the ray flower are fertile, female and pistillate, with the presence of bi-lobe stigma and voluminous ovary. Our results obtained from morphological application of *A. campestris* L. subsp. *glutinosa* are consistent with those previously reported on the basis of morphology [23,25,55-57,26].
The chemical composition of essential oils from *Artemisia* genus has been extensively studied in several species, and expressed a large fluctuation in their volatile components **[3]**. In Morocco many studies conducted on *Artemisia* L. essential oil composition showed a diversified content of volatile components. Though, the overall of the essential oils of this genus were in majority constituted by α/β-thujone and camphor. Several studies carried out on *Artemisia herba alba* essential oil allowed the carachetrization of the most preponderants chemicals: α- thujone (65.26-20.3%), camphor (60.8-12.62%), verbenol (21.83%), 1,8-cineole (20.37-7.08%), farnesene epoxide (17.08%), bisabolone oxide (17.55-10.27%), β-thujone (14.5-6.14%), myrcene (7.3%),

camphene (7.2%), filifolone (7.04%) [9,58,59,4,60,61,15,8]. Furthermore, another comparative study revealed a large variability of the volatile profile during different phenological stages and showed that the oil extracted from a sample collected on the flowering period was predominated by the chrysanthenone and camphor while that obtained from the plant on the seeding period was mainly represented by the camphor and the α-terpin-7-al [5]. For the endemic *Artemisia mesatlantica,* the major components were: β-thujone (62.05-33.9%), camphor (14.4-7.5%), camphene (7.48%), 1,8-cineole (6.9%) and sabinene (6%) [18,19,21]. Similarly, the *Artemisia arborescens* seems to have a close chemical composition, mainly represented by the major terpenes: β-thujone (40.1%), camphor (20.1%) and myrcene (7.3%) [59].

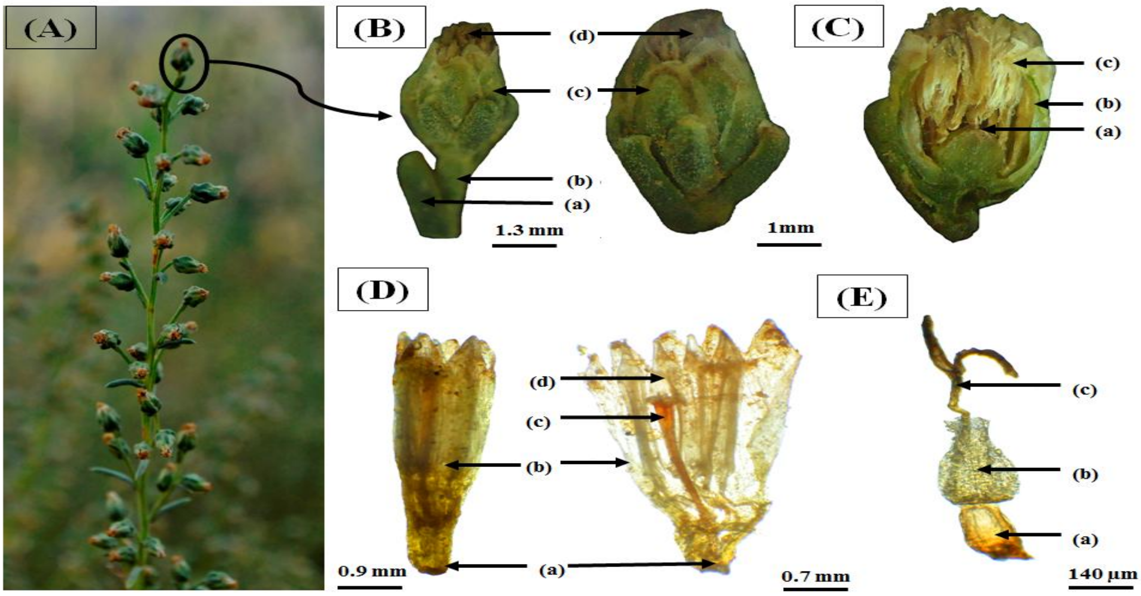

Figure 1. Morphological traits of *Artemisia campestris* L. subsp. *glutinosa* (Gay ex Besser) Batt. (**A**) General shape (**B**): Capitula; (B-a) Leaf; (B-b) Peduncle; (B-c) Involucre of bracts; (B-d) Flowers. (**C**): Longitudinal section of capitula. (C-a) Convex receptacle; (C-b) Bracts ; (C-c) Flower. (**D**) Male flower (tubular flower); (D-a) abortive ovary; (D-b) 5 fused petals; (D-c) style and stigma; (D-d) 5 fused stamens. (**E**) Female flower; (E-a) functional ovary; (E-b) style; (E-D) bi-lobe stigma**.**

For *Artemisia absinthium*, the essential oil expressed the major volatiles: β-thujone (35.6%) and chamazulene, (3.1%) [17]. Taking into consideration these data, there is a scarcity of information concerning the species *A. campestris* L., which has been reported as a species occurring in Eastern Morocco [27]. In fact, our previous work showed for the first time the existence of new chemotype of the plant specific to the Eastern region of Morocco, containing spathulenol as prominent component (Submitted manuscript). Hence, this study has been performed in the perspective to confirm this finding, taking into account that the plant was collected in a different time and analysed with a different method. Therefore, the results assert our previous data and demonstrate the preservation of the chemical content of the essential oil of *A. campestris* L. subsp. *glutinosa* growing in Eastern Morocco, which revealed the presence of the main compounds (Table 1 and Figure 2): spathulenol (13.13%), β-pinene (11.92%), p-cymene (9.43%), α-pinene (7.26%) and β-eudesmol (4.68%), and proved the existence of a new Moroccan *A. campestris* L. subsp. *glutinosa* chemotype (spathulenol chemotype). Compared to other studies, the composition of essential oil of *A. campestris* L. growing in different countries showed that this plant displays an intraspecific variation in the terpenic composition, which is dependent upon the phenological stage, plant part, geographic location, chemotype or subspecies. Consequently, *A.campestris* L. essential oil exhibited various chemotypes, mainly correlated to the place of growing of the plant and the subspecies studied. For *A.campestris* L. belonging to the South-eastern part of Europe and Western area of Asia, the essential oils defined various chemotypes. From Iran, the oils extracted from leaves, stems and flowers were predominated by spathulenol (15.8-29.2%), *α*-pinene (23-29.2%), *β*-pinene (4.5-12.6%) and bicyclogermacrene (9.1-12%) [28], while the volatile compounds of the essential oil from the aerial part of *A. campestris* L. from Turkey were mainly: 1,2-dehydro-acenaphthylene (20.71%), tremetone (15.83%), capillin (10.38%) and spathulenol (6.47%) [29]. The volatile oils from the aerial parts of *A. campestris* L. collected in Southern Ural contained *α*-pinene (41%), *β*-pinene (29.7%) and limonene (6.4%) [30], whereas, the Serbian *A. campestris* L. essential oil yielded weaker terpenes, and was only represented by the major terpenes: spathulenol (9.2%) and β-pinene (9.1%) [31].

Table 1. Retention times and percentages of the chemical components of essential oil *extracted from aerial parts of Artemisia campestris* L. subsp. *glutinosa* (Gay ex Besser) Batt. (Comparison of the recorded spectra with computed data library NIST 147)

Components	Retention time (min)	Percentage (%)
α-Pinene	4.82	7.26
ß-Pinene	6.13	11.92
p-Cymene	6.90	9.43
δ-Limonene	6.52	3.20
γ-Terpinene	7.12	0.82
Linalyl alcohol	8.02	0.76
Trans-pinocarveol	9.29	1.32
4-Terpineol	9.66	1.42
α-Terpineol	9.99	1.64
p-Cymene-8-ol	10.275	1.24
Myrtenal	10.51	1.09
Copaene	11.83	1.10
δ-Cadinol	13.92	1.31
α-Curcumene	14.24	1.02
δ-Cadinene	15.16	2.92
Trans-Nerolidol	15.83	1.37
Citronellyl propionate	15.67	1.48
Spathulenol	16.55	13.13
Carotol	16.64	2.40
Trans-Longipinocarveol	16.98	2.89
γ-Cadinene	17.11	1.10
ß-Eudesmol	17.37	4.68
7-Methyl-4-(1-methyl-ethylidene) bicycle[5.3.1] undec-1-en-8-ol	17.83	3.91

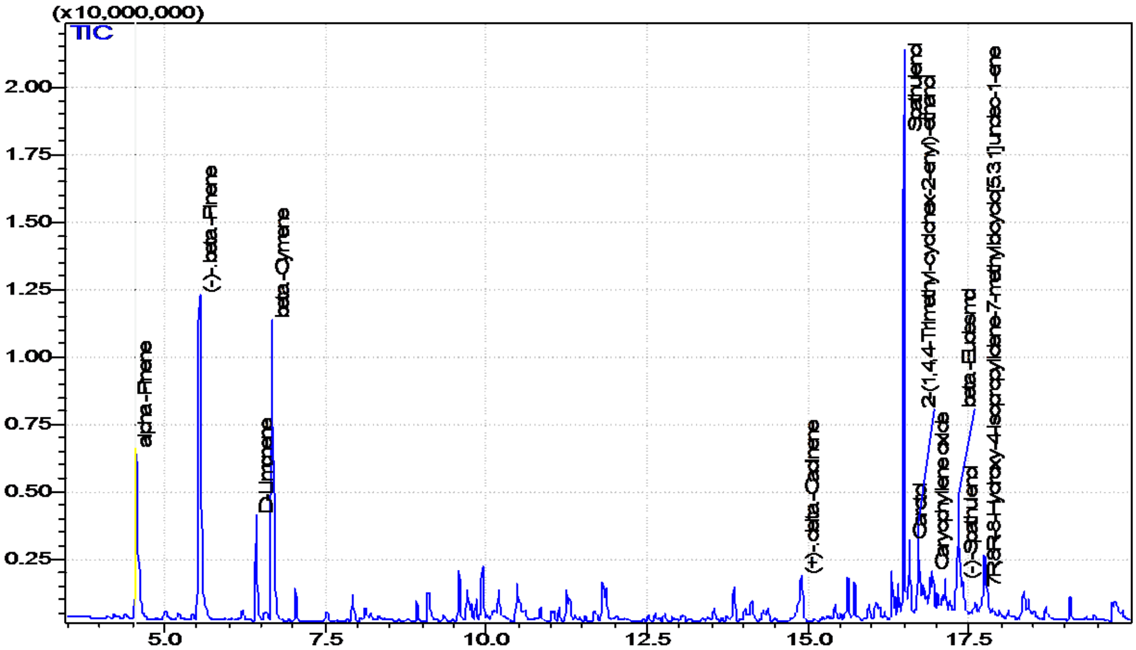

Figure 2. GC-MS Chromatogram of *Artemisia campestris* L. subsp. *glutinosa* (Gay ex Besser) Batt.essential oil

On the other hand, *A. campestris* L. originally from Mediterranean region have been reported. Hence, the major components of essential oil from *A.campestris* L. aerial parts (leaves and fruits) occurring in Algeria have been identified as *β*-pinene (6.1-25.6%), *α*-terpenyl acetate (18.8%), *α*-pinene (9.9-19.9%), β-myrcene (16.2-17.34%), sabinene (17%), germacrene D (7.1-10.6%), *trans-β*-ocimene (12.61%) *(Z, E)*-farnesol (10.3%), camphor (9.2%), β-cymene (8.15%), limonene (5.9%) and camphor (5.85%). [32-35,52,62,63]. Also, the essential oil composition of *A. campestris* L. leaves, occurring in different regions of Tunisia, at different vegetation periods afforded variable volatile content, for which the main components were: *ß*-pinene (24-49.8%), *p*-cymene (2.3-22.3%), *α*-pinene (4.1-12.5%), camphor (10.3%), spathulenol (1.2-10%), *γ*-muurolene (0.5-9.6%) and limonene (4.9-9.3%) [36-41]. In another report, the essential oil of the Tunisian *A. campestris* L. subsp. glutinosa was found to contain mainly β-pinene (41.1%) and p-cymene (9.9%) [42]. However, the seasonal chemical profile of essential oils hydrodistillated from flowers of *A. campestris* L. subsp. glutinosa originated from different regions of Italy revealed the existence of the main components: *β*-pinene (6.9-57.2%), germacrene D (5.9-28.6%), bicyclogermacrene (3.9- 14.5%), myrcene (3.8- 11.2%) and *α*-pinene (5.3-9.2%) [43]. The terpenic profiling was quite different for *A. campestris* subsp. glutinosa from France, which exhibited a similar composition of the volatiles extracted over different phenological stages of the plant, with such as components: γ-terpinene (20.8-46.5%), capillene (8.9-33.1%), 1-phenyl-2,4-pentadiyne (16.2-29.7%) and spathulenol (11.3%) [44]. Another study reported the occurrence of capillene (49.1%), γ-terpinene (23%) and 1-phenylpenta-2,4-diyne (18.1%) as the main volatiles of the aerial part of French *A. campestris* L. subsp. glutinosa [45]. Oils of aerial part of *A. campestris* L. subsp. campestris collected from numerous regions of Lithuania at the flowering stage were characterized by the major terpenics: caryophyllene oxide (3.7-38.8%), germacrene D (3.8-31.2%), *γ*-curcumene (4-14.8%), *β*-pinene (3.9-13.8%), *α*-pinene (4-11.4%) and humulene epoxide II (3.7-11.7%) [47,46]. The essential oil of *A. campestris* L. subsp. campestris from Poland investigated on different organs (inflorescences, flowers, stems, roots and flowers) and over five ontogenesis phases, was mainly dominated by (Z)-falcarinol (19-38.8%), germacrene D (9.7-28%) and *γ*-humulene (4-8.2%) [64,48]. The oil from aerial part of *A. campestris* L. subsp. borealis collected from Italy appeared to be containing caryophyllene oxide (18.2%) as the main component, followed by *α*-pinene (15.3%), *β*-pinene (9.8%) and spathulenol (9.3%) [49]. Yet, the subspecies *A. campestris* L. subsp. maritima from Portugal allowed the detection of the major components: *β*-pinene (17.8%) and cadin-4-en-7-ol (16.4%) and γ-terpinene (8.7%) [50,51].

Conclusion

The morphological criteria determined in this study matches with the species *A.campestris* L. subsp. *subsp. glutinosa (Gay ex Besser) Batt.* as described in the litterature, which is mainly distinguished by the heterogamous capitular infloresence constituted of both disck male flowers and ray female flowers. In addition, the volatile composition analysis of Moroccan *A. campestris* L. subsp. *glutinosa* allowed, to confirm the determination of the new Moroccan chemotype of this plant charcterized by spathulenol as the major component. According to these findings, it can be concluded that traditional identification of plants based on descriptive morphological characteristics is widely used in almost all Flora. Besides, the chemical fingerprinting are useful keys for systematic classification. However, this identification should be further investigated based on the molecular markers, cytological determination and hitological analysis.

Moreover, additional studies dealing with the identification of the subspecies of *A.campestris* L. subsp. *glutinosa* growing in Eastern Morocco could be undertaken in the aim to contribute to the valorization of this plant, and to determine the level of conservation of the chemical profile upon the edaphic, pedological and climatic conditions that predominate in this region. As perspective, this work will be considerd as the first part, that would be further completed by another study, by focusing on the separation of the essential oil constituents and determination of their structure.

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