Morphological, anatomical, palynological phytochemical, and molecular characterization of *Achillea millefolium* (Asteraceae) in western desert of Iraq

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Abstract

In this study, morpho-anatomical features, Phytochemicals, palynology, and molecular analysis of *Achillea millefolium* growing in the western desert of Iraq were investigated. The general morphology of different plant parts (stem, leaf, bract, florets, fruit, and pollen grains) was studied. In this study, new anatomical information on this plant species like the type and frequency of both stomata and trichomes in both vegetative and reproductive parts of the plant and the type of mesophyll were reported. The total content of phenols, flavonoids, alkaloids, glycosides, terpenoids, and saponins was also included. In this study molecular analysis was conduces to identify the genetic polymorphism of A. *millefolium* to assess the phylogenetic distribution of this based on its 18S ribosomal sequence, subsequently, a comprehensive phylogenetic tree was constructed in the observed variants for these sequences to reveal the accurate phylogenetic distance alongside other relative sequences. Our results confirmed the identity of the investigated plant sample as sequence reactions. Two ribosomal variations were identified in the investigated plant sample of A. millefolium, which were 54A>G and 55-56GA, the constructed phylogenetic tree revealed that these ribosomal variations were only a minor deviation within the identified clad of A. *millefolium* from there, the present tree provided an inclusive tool for the guaranteed identity of the investigated sample of A. *millefolium*.

Keywords: *A. millefolium*, morphology, polyploidization, western desert of Iraq

Introduction

The genus *Achillea* (known as yarrow) of the family Asteraceae is one of the most polyphyletic genera and difficult genus due to the vast variation in morphology, pollen properties, polyploidization, and chromosome numbers (Bancheva *et al*., 2014) [8]. It includes more than 100-140 species and occurs in Europe and Asia with few species in northern Africa and northern America (Saeidnia *et al*., 2011, Applequist *et al*., 2011, Shah *et al*., 2015, Grytsyk *et al*., 2016, Koyuncu, 2020) [22, 7, 24, 14, 10]. In Iraq, this genus is represented by 10 species, including A. millefolium (Ghazanfer *et al*., 2019) [13]. In traditional medicine, this species is used as a diaphoretic, astringent, tonic, mild aromatic, and as a wound healing herb. (Saeidnia *et al*., 2011, Shah *et al*., 2015) [22, 24]. In modern medicine, A. millefolium showed antibacterial activity, free radical scavenging activity, anti-tyrosinase, anti-inflammatory potential as well as cytotoxicity against human skin cells (Saeidnia *et al*., 2011, Shah *et al*., 2015, Strzepek-Gomolka *et al*., 2021) [22, 24, 28]. Plant of this species is rich source active substances (flavonoids, essential oils, phenolic compounds, tannins, etc). Plants of this species are a rich source of active substances (flavonoids, essential oils, phenolic compounds, tannins, etc.) which are considered as valuable secondary metabolites for the food and pharmaceutical industries (Saeidnia *et al*., 2011, Shah *et al*., 2015, Al-Abbasi, 2019) [22, 24, 4]. *A. millefolium* and related species form a polyploid complex and to data, there was no single acceptable taxonomy of *A. millefolium* due to hybridization and intraspecific variability (Shah *et al*., 2015) [24]. Because of this, most plants of the genus *Achillea* are referred to as yarrow and there is no accepted identification of individual *Achillea* species (Shah *et al*., 2015) [24]. Field studies related to wild plants like *Achillea* species in western parts of Iraq are very limited despite their importance at the global level. Therefore, this study presents morphological, anatomical, palynological, chemical, and molecular characteristics of *A. millefolium* growing in the western desert of Iraq.

Material and Methods

Plant Samples at the flowering stage were collected from Haditha province west of Al- Ramdi city during May - July 2020. Samples were dried, identified according to Ghazanfer *et al* (2019) [13] and confirmed by Iraqi National Herbarium. Identified samples were deposited in the Department of Biology, College of Education for Pure Sciences, Tikrit University, Iraq. Morphological Features of different plant parts (stems, leaves, bracts, inflorescence and fruits) were reported. Handmade cross sections of fresh leaf and stem were prepared. Safranin and iodin in potassium iodide (IKI) were used separately in microscopy to distinguish plant tissues. Epidermis characteristics (including Ordinary, Stomata, and trichomes) were studied using stripped-off epidermis method mentioned in Al-Khesraji and Aziz (1990) [5]. Pollen grains were studied according to Erdtman (1971) [11] and Stanski *et al*,

(2021) [27] All micrographs were taken by using light microscope (Olympus) and Sony digital camera. Phytochemical test was carried out to ensure the presence or absence of the active compound classes in the studied plant as described by (Harborn, 1964, Raudone *et al*., 2022) [15, 20]. Total active compounds were determined according to Laouini and Ouahrani (2017) [18] for phenolics, Sofiane *et al* (2017) [26] for total flavonoids, Trease, (2002) [30] for total alkaloids, Tofighi, Ghazi, (2016) [29] for total glycosides, Abdelkader, *et al*. (2014) [1] for total tannins and Ezeabara *et al* (2014) [12] for total Saponins. For the molecular phylogenetic analysis**.**

1.1. Genomic DNA extraction

The genomic DNA of the isolated plant sample was extracted using the Genaid Kit according to the manufacturer's instructions (Geneaid Biotech, Taiwan). The concentration and purity of DNA were measured by a nanodrop (BioDrop µLITE, BioDrop co., UK), while the DNA integrity was checked by a standard 0.8% (w/v) agarose gel electrophoresis that is pre-stained with a higher concentration of ethidium bromide (0.7 µg/ml) in TAE (40 mM Tris-acetate; 2 mM EDTA, pH 8.3) buffer, using a 1 kb ladder as a molecular weight marker (Cat # D-1040, Bioneer, Daejeon, South Korea). The isolated plant genomic DNA was used as a template for the subsequent PCR experiments.

1.2. PCR

One fragment was selected for amplification, which partially covered the 18S rRNA sequences that were amplified in this study. The sequence of the forward primer was 5′-ATGCGCTCCTTCCCTTAACT-3′, and the sequence of the reverse primer was 5′-AACATCCTTGGCAAATGCTT-3′. The lyophilized primers were purchased from Bioneer (Bioneer, Daejeon, South Korea). The PCR reaction was performed using *AccuPower* PCR premix (Cat # K-2012, Bioneer, Daejeon, South Korea). Each 20µl of PCR premix contained 1 U of Top DNA polymerase, 250 µM of dNTPs, 10 mM of Tris-HCl (pH 9.0), 30 mM of KCl, and 1.5 mM of $MgCl_2$. The reaction mixture was completed with 10 pmol of each primer and 50 ng of genomic DNA. The following program was applied to the PCR thermocycler (MyGenieTM 96/384 Thermal Block, Bioneer, Daejeon, South Korea). The amplification was begun by initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94˚C, annealing at 57°C, and elongation at 72˚C, and was finalized with a final extension at 72°C for 10 min. Amplification was verified by electrophoresis on an ethidium bromide (0.5 mg/ml) pre-stained 1.5% (w/v) agarose gel in 1× TBE buffer (2 mM of EDTA, 90 mM of Tris-Borate, pH 8.3), using a 100-bp ladder (Cat # D-1010, Bioneer, Daejeon, South Korea) as a molecular weight marker. It was made sure that both PCR bands are specific and consisted of only one clean and sharp band before being submitted to subsequent sequencing reactions.

1.3. DNA Sequencing of PCR amplicons

The resolved PCR amplicons were commercially sequenced from forward termini, according to instruction manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI sequence files were further analyzed, ensuring that the annotation and variations are not because of PCR or sequencing artifacts. By comparing the observed nucleic acid sequences of plant samples with the retrieved reference sequences of the plant database, the virtual positions, and other details of the retrieved PCR fragments were identified.

1.4. Interpretation of sequencing data

The sequencing results of the PCR products were edited, aligned, and analyzed as long as with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The observed nucleic acids were numbered in PCR amplicons as well as in their corresponding positions within the referring genome. Each detected variant within the targeted ribosomal sequences was annotated by SnapGene Viewer ver. 4.0.4 (https://www.snapgene.com). Subsequently, the observed variations were submitted to NCBI, and a unique GenBank accession number was obtained for each investigated isolate.

1.5. Comprehensive phylogenetic tree construction

A specific comprehensive tree was constructed in this study according to the cladogram construction described by (Sarhan *et al*. 2019) [23]. The observed variants were compared with their neighbor homologous reference sequences using the NCBI-BLASTn server (Zhang *et al*. 2000) [31]. Based on the Clustal omega suit (Sievers and Higgins, 2014) [25], multiple sequence alignments were made for the retrieved nucleic acid sequences. Subsequently, an inclusive tree was built by the neighbor-joining method and visualized using the iTOL suit to generate a cladogram of clades construction (Letunic and Bork, 2019) [19]. The observed variants as well as their corresponding reference sequences were incorporated in the constructed comprehensive cladogram. The sequences of each classified phylogenetic species in the comprehensive tree were annotated accordingly.

Results

Habit & duration

Perennial herb, aromatic, with a slight spread in the study area, on rather pasture, dry clay soil. flowering period March-June.

Morphological features

The stem

Ascending, obtuse-angled,30-45cm tall, white, densely woolly-pilose (Fig.1a, Fig.2a, Table1).

The leaf

Cauline, alternate, divided, 1-3 x 0.3-0.5cm, basal leaves linear to lanceolate, including petiole imbricate, toothed woolly (Fig1b, c, Fig2c, d, Table.2).

Inflorescence

Capitulum, corymb broad, yellow 0.7 x 0.6 cm, Phyllaries

oblong to lanceolate, acute, margin narrow, brownish 0.2-0.5 x 0.1-0.2cm, two type of florets, Ray florets and Disc florets. (Fig1d. Fig2e, f, Table3).

Ray floret

Unisexual, zygomorphic, sterile 4 x 4 mm, calyx absent, three corolla gamopetalous with serrated end, each lobe denotes a petal, covered by a few short stalked glandular hairs.

Gynoecium

One pistil, bicarpous, syncarpous, unilocular, ovary inferior 1.2 x 0.5mm single ovule, 2875-2950 x 750-800µm, with simple cylindrical style 1675-1750 x 125-175µm, stigma bifid lobed, 750-825 x 150-175µm ending by very short papillae structure (Fig 1e, Fig 2g, Table 3).

Disc floret

Actinomorphic, incomplete, bisexual 4 x 0.7mm, five yellow corolla, synpetalous at base, covered by short stalked glandular hairs with high density. Androecium, five stamen fertile**,** synogenesioun**,** epipetalous, anther dithecous, stamenoid cylinder, 250-1325 x 350-400µm, filament basically, free filament 1300-1350µm. Gynoecium, One pistil, bicarpous, syncarpous, unilocular, ovary inferior,1 x 0.5 mm, single ovule, 1180-1200 x 380-360µm, cylindrical style 2000-2050 x 125-175µm, short stigma, bifid 700-750 x 100-150µm (Fig.1, h,i,g,k. Fig.2,h, Table3).

Fruit

Achene 1.8 x 9 mm, oval- elliptic, smooth glabrous, brown, dry, indehiscent, covered by a thin layer. narrowly winged at margins, thick in middle It is narrow at the hilum end. (Fig1,l-Table. 3).

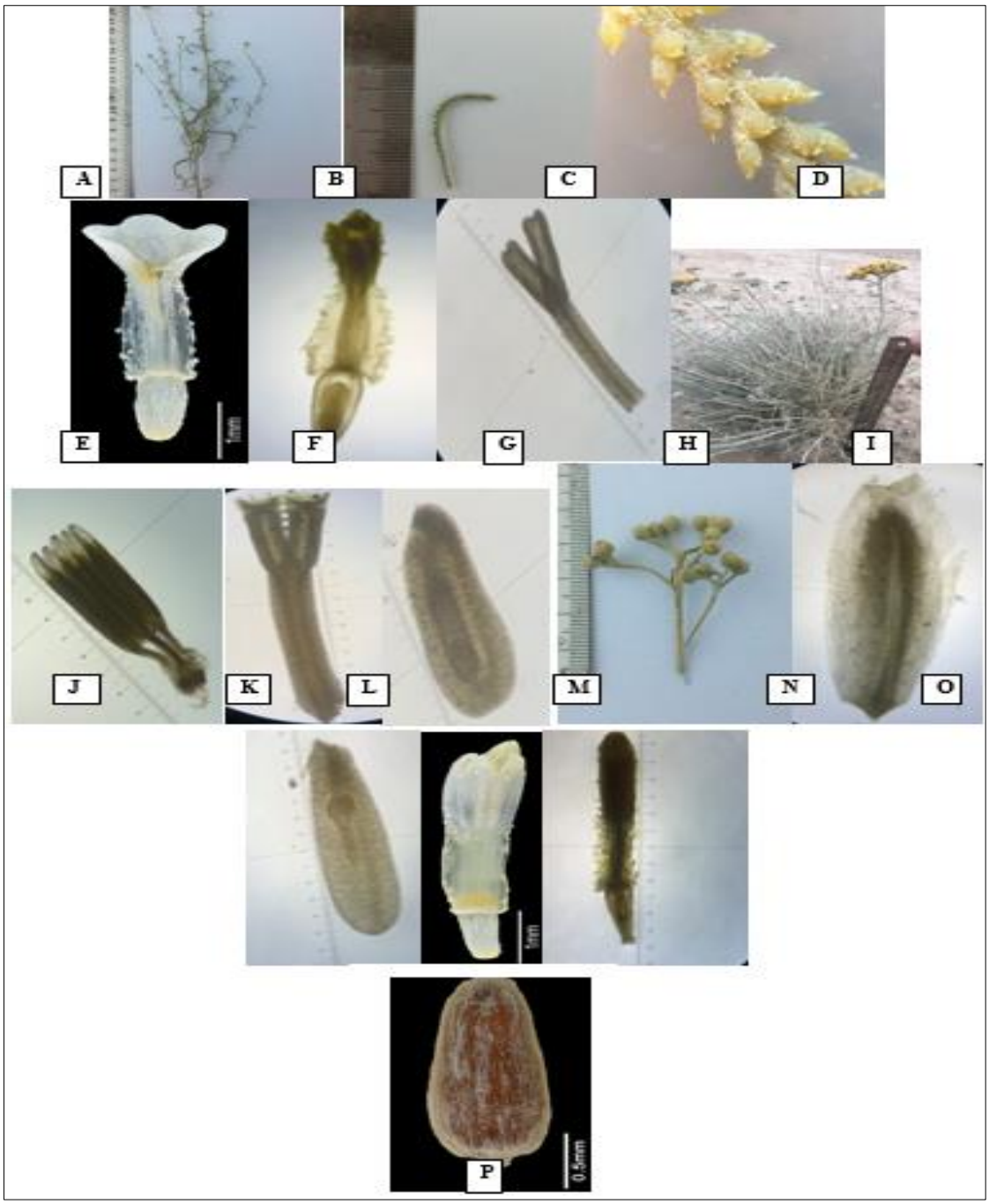

Fig 1: *A. millefolium* morphology. A, in nature; B, in lab; C, D, Leaf; E, Inflorescence; F, bract; G, H, Ray floret; I (style and stigma); J, ovule; K, L Disc floret; M, stamen; N (style and stigma); O, ovule; P, fruit

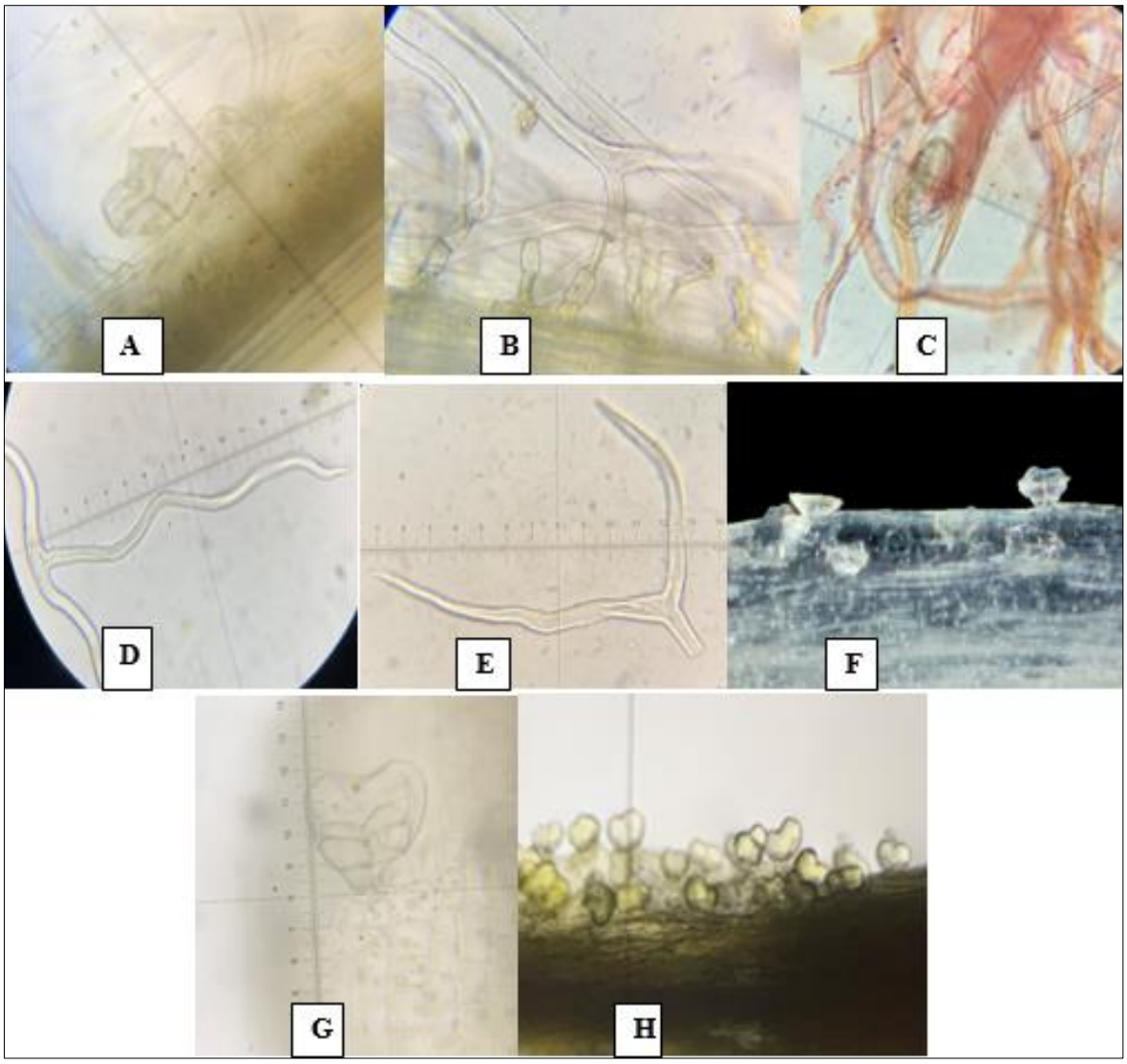

Fig 2: *A. millefolium* trichomes (A, B) stem glandular and non-glandular hairs; (C, D) leaf glandular and non-glandular hairs; E, bract non-glandular hair; F, G. Ray glandular hairs; H, Disc glandular hairs

Anatomical feature

Anatomical description based on cross-section and stripped off the epidermis.

The stem

Dermal Tissue System (DTS)

Uniseriate epidermis, polygonal cells, 50-70µm thick, 62.5-125 x 25-30 µm, stomata anomocytic 27.5-30 x 20-22.5 µm, stomatal density (21) stomata\mm^2. Trichomes (sessile Glandular hairs, 92.5-100x 50-75µm, density (10.5) hairs\mm, multi-seriate branched non-glandular hairs, 450-625 x 20-25, density (136.8) hairs\mm (Fig.2, Fig.3).

Ground Tissue System (GTS)

Includes cortex and pith, cortex 100-120 thick composed of lamellar, angular collenchyma at the corner, separated by wavy chlorenchyma tissue, ended by one layered endodermis, pith with ordinary parenchyma cells, solid wide,700-706 µm.

Vascular Tissue System (VTS)

One ring of 14-17 bundles with varying size.

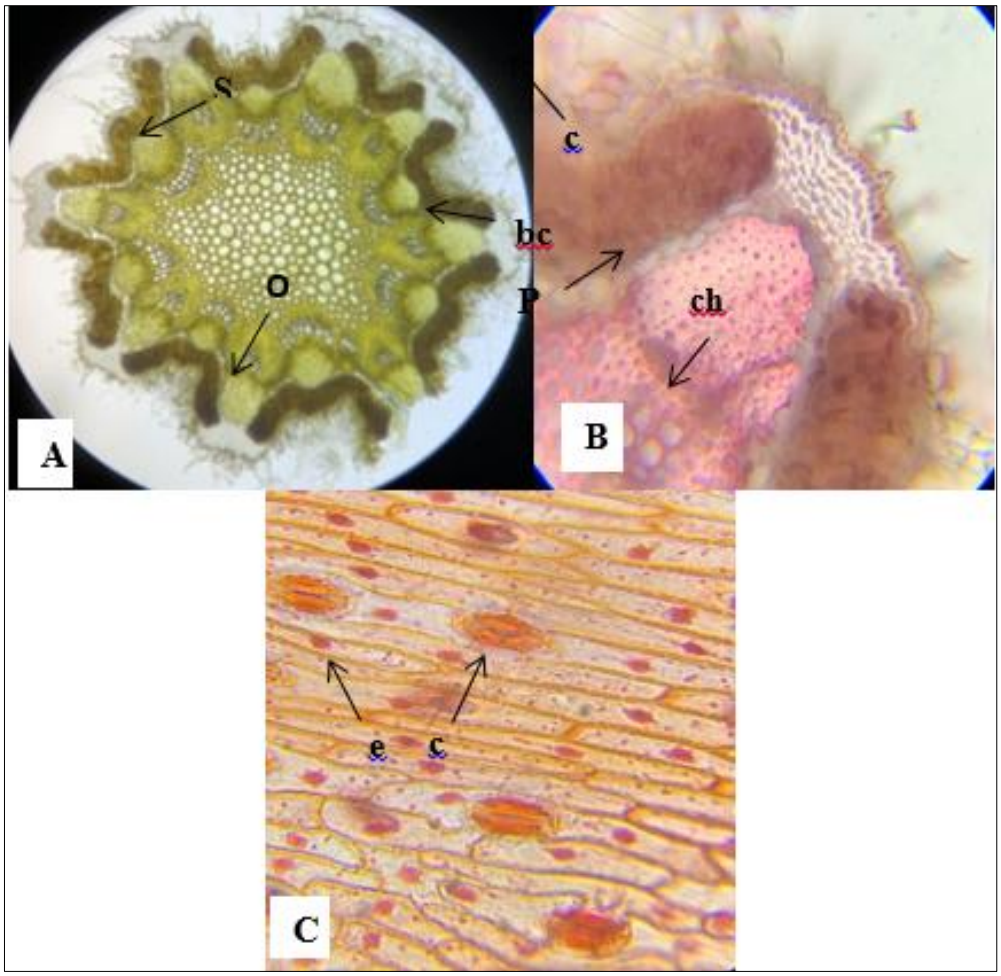

Fig 3: *A. mellifolium* stem, A, Striped epidermis (o): ordinary epidermal cells (s): stomata. B, C, cross section, (c) Collenchyma (ch) chlorenchyma, (P) pith, (bc) Bundle cap.(e) Endodermis

The leaf

DTS

Adaxial, uniseriate, 25-30µm thick, ordinary epidermal with undulate walls, 50-75 x 20-40 µm, stomata anomocytic, 37.5-42.5 x 25-30 µm, density (65.8) stomata\mm^2. Sessile glandular hairs 75-112 x 50-62.5µm, density (12.6) hairs\mm^2, multi-seriate branched non-glandular hairs 150-187.5 x 17.5-25µm, density (31.6) hairs\mm^2, abaxial, 17.5-22.5µm thick, ordinary epidermal with undulate walls, 70-80 x 17.5-25 µm, stomata anomocytic, 27.5-42.5 x 25-27.5 µm, density (82) stomata\mm^2, sessile glandular hairs 75-100 x 62.5-87.5 hair\mm^2, density (8.7) hairs\mm^2, multi-seriate branched non-glandular hairs 400-1000 x 20-30µm, density (28.4) hairs\mm^2 (Fig.2, Fig.4).

GTS

Isolateral, multi-layer of palisade parenchyma, 225-305.5µm thick for one side, spongy parenchyma absent.

VTS

Scattered closed collateral vascular bundles in the blade wings, Midrib region 700-703µm thick, composed of collenchyma under adaxial epidermis, ground ordinary parenchyma and three vascular bundles of varied size (one large bundle in the center and two small bundles on either side).

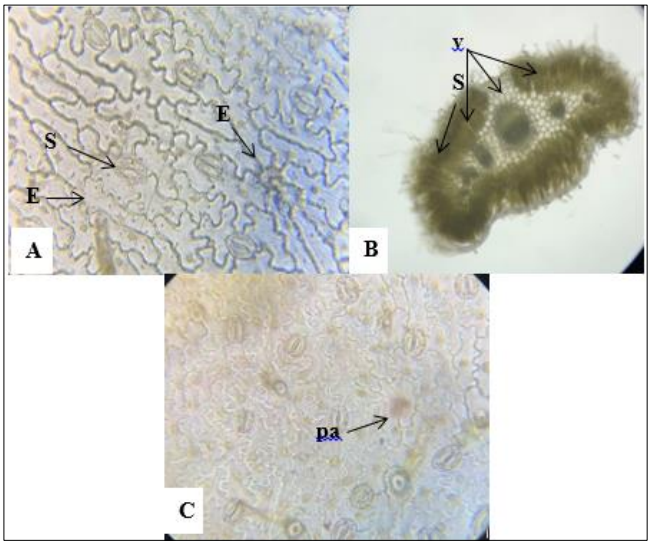

Fig 4: *A. mellifolium* leaf. A (adaxial), B (abaxial, (s), stoma (e), epidermal cell. C, Leaf cross section, (pa), palisad paraenchyma, (v), vascular bundle

Palynology

Spheroidal prolate, tricolporate echinate, spikes wide, polar view 25-30 µm, equatorial view 20-25µm**.** P\E 1.3, (they represented medium size). wall 7.5-10µm thick (Fig. 5).

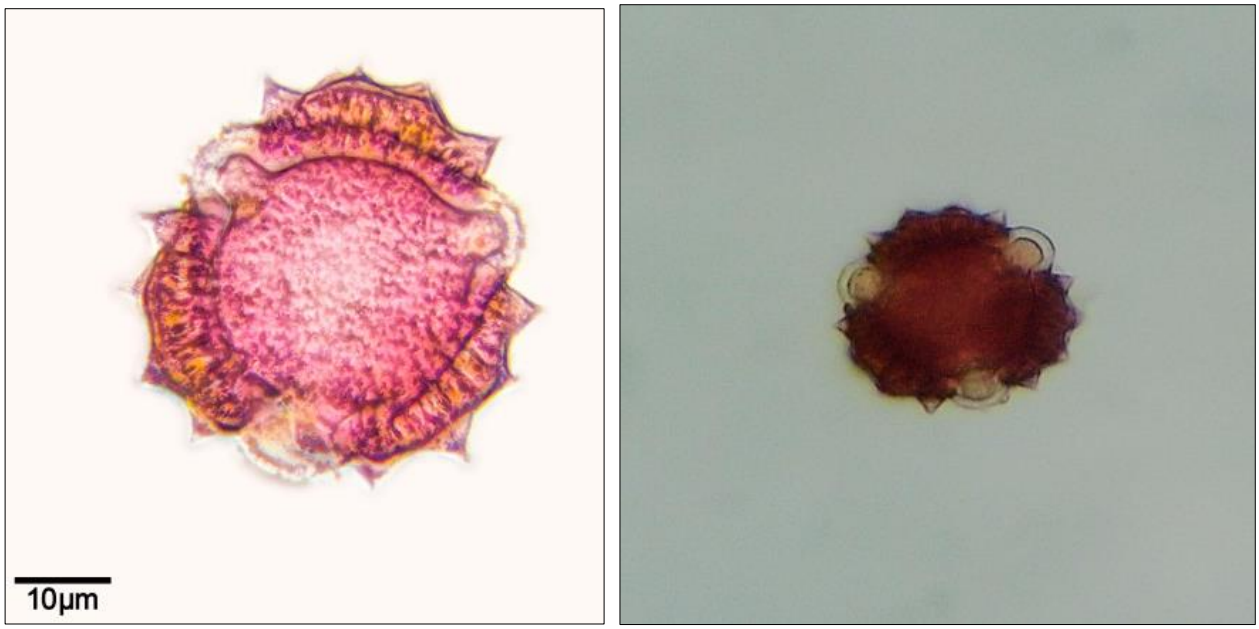

Fig 5: *A. mellifolium* pollen grain

Phytochemical contents

Table 5 shows the presence of active compound and their total contents in *A. millefolium*. Total phenolics and total flavonoids are 9.25mg\gm and 2.98 mg\gm respectively. Total contents of Alkaloids, glycosides, tannins and saponins are 6.30%, 4.00%, 1.25% and 0.36% respectively (Table 1).

Table 1: *A. mellifolium* total phytochemical contents

Chemicals	mg\gm		%			
	Total phenolic content	Total flavonoid content	Total alkaloid content	Total glycoside content	Total tannin content	Total saponins content
Test	+	+	+	+	+	+
content	9.25	2.98	6.30	4.00	1.25	0.36

Analysis genetic

Within this locus sample was included, which showed the approximate lengths of the utilized ribosomal fragment in this study. The sequencing reactions indicated the confirmed identity of the amplified products by performing NCBI blastn. After positioning the amplified fragments within the rRNA sequences, the details of the sequence were shown within the amplified sequence. The NCBI BLASTn engine showed a high sequence similarity between the sequenced samples of *Achillea millefolium* sequences. The NCBI BLASTn engine indicated the presence of 98% of homology with these expected targets that covered the specified portions of the rRNA gene sequences. The investigated sample showed homology with the GenBank accession number KT179658.1 that belonged to this species.

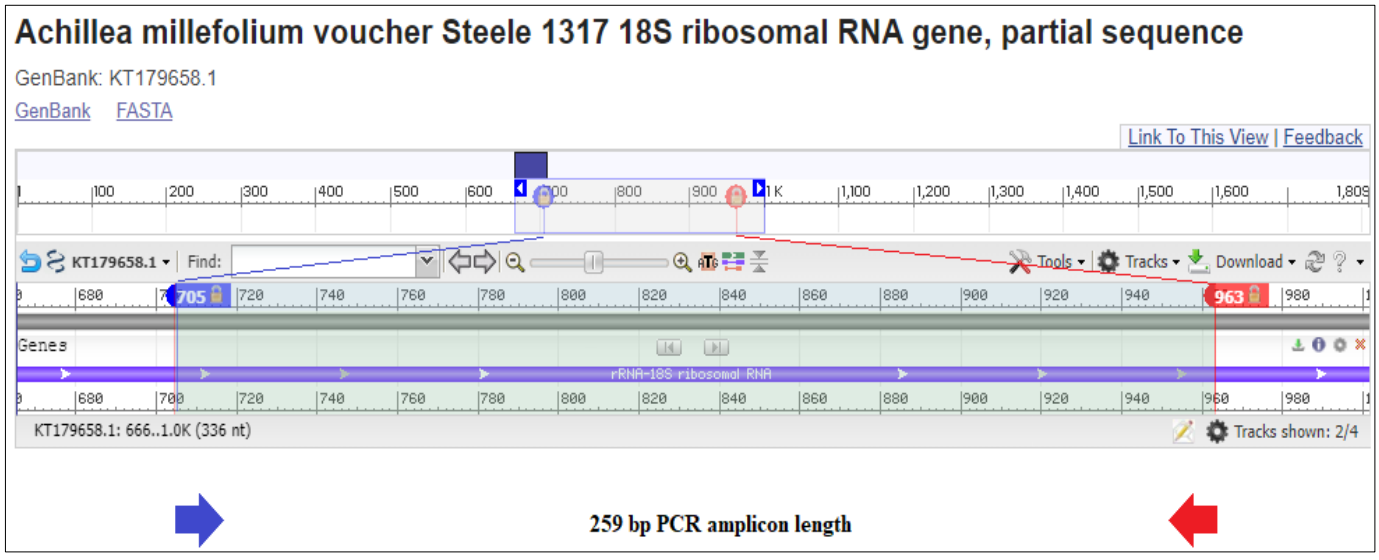

Fig 6: The exact position of the retrieved amplified fragments covered a portion of the 18S rRNA gene within the genomic sequence The blue arrow refers to the starting point of this amplicon while the red arrow refers to its endpoint

By comparing the observed DNA sequence of the currently investigated sample with the retrieved DNA sequences, the exact positions and other details of the retrieved PCR fragments were identified.

Table 2: The positions and lengths of the amplified fragments that were used to amplify a portion of the 18S rRNA gene within *Achillea millefolium* genomic DNA sequences

Taxa	Reference locus sequences (5′ - 3′)	Length
Achillea millefolium	>KT179658.1:538-966 *Achillea millefolium* voucher Steele 1317 18S ribosomal RNA gene, partial sequence ATGCGCTCCTGGCCTTAATTGGCTGGGTCGTGCCTCCGGCGCTGTTACTTTGAAGAAATTAGAGTG CTCAAAGCAAGCCTACGCTCTGTATACATTAGCATGGGATAACATCATAGGATTTCGGTCCTATT ACGTTGGCCTTCGGGATCGGAGTAATGATTAACAGGGACAGTCGGGGGCATTCGTATTTCATAGT CAGAGGTGAAATTCTTGGATTTATGAAAGACGAACAACTGCGAAAGCATTTGCCAAGGATGTT	259 bp

The retrieved sequence of ribosomal sample was aligned with their corresponding referring sequence (Fig. 2). This sequence was prepared by positioning our investigated sample with the most relative sequences deposited in the NCBI database (GenBank acc. no. KT179658.1) (Fig.7).

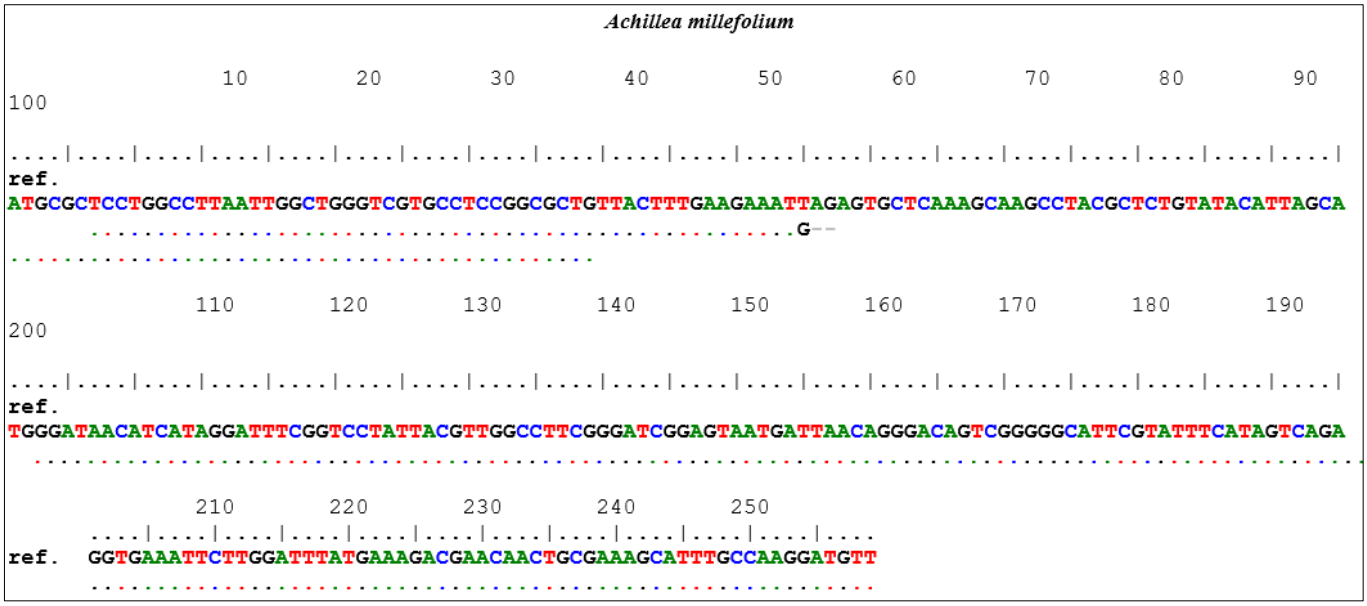

Fig 7: Nucleic acid sequence alignment of sample with the most relevant deposited genomic sequences of the species reference sequences

It was found that the sample exhibited two ribosomal variations compared with its corresponding sequences of *Achillea millefolium* (GenBank acc. no. KT179658.1). These observed variations were attributed to two variants; one nucleic acid substitution of (54A>G), and one deletion (55-56GAdel) (Fig. 8).

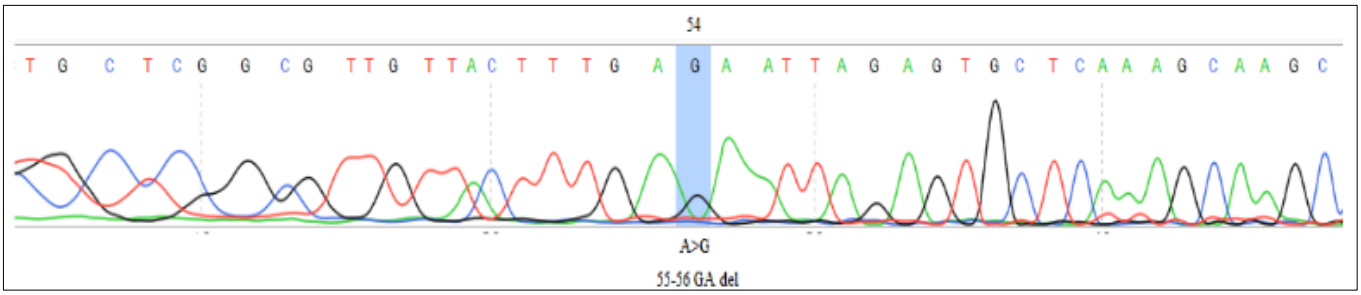

Fig 8: The chromatogram of the nucleic acid substitution observed in the currently investigated plant sample of *Achillea millefolium* in comparison with its reference sequences (GenBank acc. no. KT179658.1). The clear peaks of each nucleotide refer to the strict contamination-free technical parameters followed to validate each variant in the present samples. The symbol ">" refers to substitution mutation, and "del" refers to deletion mutation

A comprehensive phylogenetic tree was generated, which was based on the investigated 18S ribosomal nucleic acid sequences in the analyzed plant sample. Along with the other deposited DNA sequences**.**

This phylogenetic tree contained our screened plant samples aligned with their highly related sequences in a neighbour-joining mode. In the currently constructed tree, the total number of aligned nucleic acid sequences was twenty-seven sequences. This comprehensive tree entailed the presence of three different species, which represent the only incorporated nucleic acid sequences within the currently constructed tree. These three incorporated sequences were *Achillea millefolium*, *Lactuca sativa*, and *Solanum lycopersicum* were incorporated in three distinct phylogenetic positions. Based on the analyzed

genetic sequences, our 18S rRNA sequences were clustered into three major phylogenetic clades, which entailed a wide range of diversity of these plant sequences in terms of our analyzed 18S rRNA sequences. The current constructed tree was represented in two cladograms, which were kinked to generate a traditional rectangular form and curved to generate an unrooted cladogram (Fig.9, A, B). In each form, a particular phylogenetic distribution of the incorporated sequences was notified.

One of the incorporated clades was represented by the *Achillea millefolium* clade, in which the currently investigated S1 sample was incorporated. Due to the low number of deposited samples in the NCBI, only three relative sequences were found to be suited near the sample, namely GenBank acc. no. MT610918.1, KT179658.1, and MT937104.1 respectively. These sequences of *Achillea millefolium* were found to be respectively deposited from Brazil, USA, and Denmark. This may lead to the assessment of European and multi-American origins of the observed *Achillea millefolium* sequences in the current study. Unfortunately, no further sequences were found to be deposited in the NCBI to get a more comprehensive. Therefore, the phylogenetic role of the observed sample variations could not be easily identified. Nevertheless, the constructed phylogenetic tree revealed that both ribosomal variations of 54A>G and 55-56GA were only a minor deviation within the identified clade of *Achillea millefolium*, in which our investigated sample of S1 was incorporated. Interestingly, distinct phylogenetic distances were respectively found between the *Achillea millefolium* clade with *Lactuca sativa* and *Solanum lycopersicum* clades. Due to the small phylogenetic distances observed in the constructed tree (0.001), the presence of a high percentage of ribosomal homology between these three species was confirmed. Though S1 exerted two ribosomal variations, its position did not deviate within the *Achillea millefolium* clade. Thus, the genetic variations observed in the sample were only a minor variation within the *Achillea millefolium* clade (phylogenetic scale 0.001). Thus, the presence of 54A>G and 55-56GA was the only minor variation within this clade.

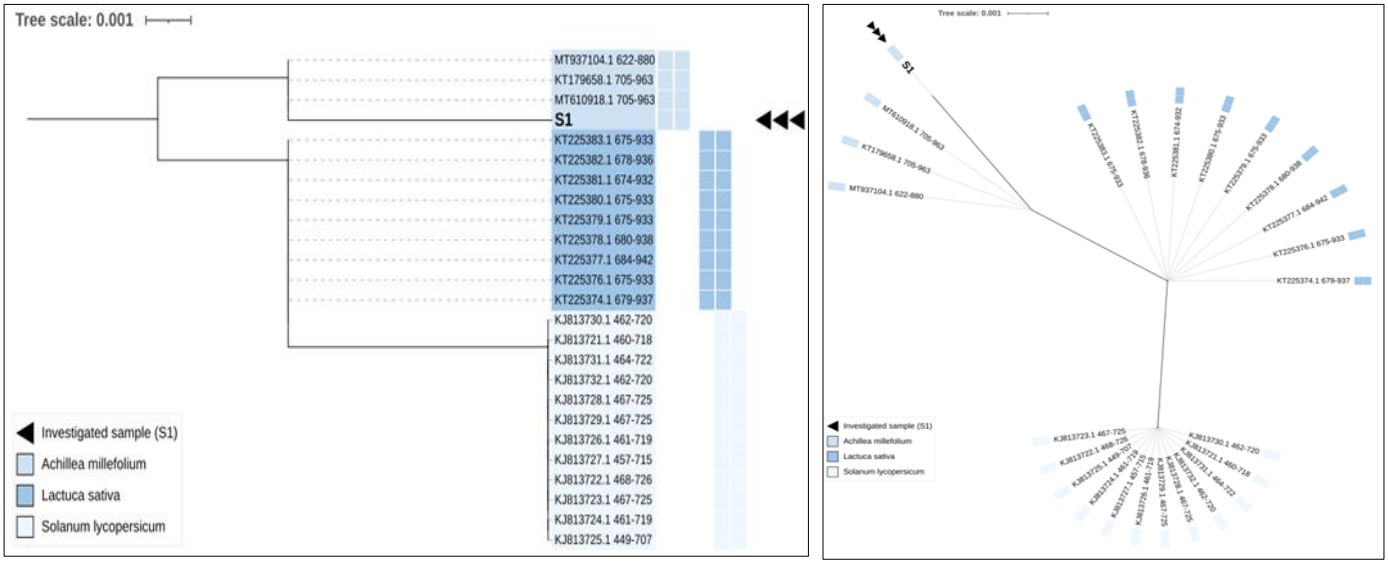

Fig 9: The comprehensive rectangular (in-branch A) and unrooted (in-branch B) phylogenetic tree of the 18S rRNA sequences within the genomic sequences of *Achillea millefolium.* The variable colors refer to the variable grouping of the analyzed variants, within their Genbank deposited sequences. The number "0.001" at the top left portion of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms

Discussion

This study provides detailed information on the morphology, anatomy, palynology and phytochemicals of *A. millefolium*. Identification of this species was confirmed by molecular analysis. In addition to the general description of the different plant parts, the study reported new morphological and anatomical properties of the investigated species including the presence of the branched multicellular covering hairs (non-glandular hairs) in different vegetative parts (stem, leaf and bracts) and the presence of two types of multicellular glandular hairs, the sessile one in the stem and the stalked one in both ray and disc florets. The vegetative parts showed the presence of glandular hairs mixed with the non-glandular hairs, while the florets showed only the presence of the non-glandular hairs. Ciccarelli *et al*. (2007) [9] mentioned that trichomes micromorphology was useful in the systematics of Asteraceae and glandular hairs in this family may be widely distributed both on the vegetative and the floral parts. This study reported for the first time the stomatal frequency and trichome frequency of the studied vegetative and reproductive parts of *A. millefolium*. The cross-section of the stem consists of a uniseriate epidermis with glandular and non-glandular hairs followed by a cortex including collenchyma in the corners followed by wavy chlorenchyma and single-layer endodermis. The stem vascular cylinder consists of several closed collateral bundles covered by a sclerenchymatous sheath. The wide parenchymatous pith occupies the center of the section. In general, this stem description is in agreement with previous studies carried out on the same species (Ilham *et al*., 2022) [16]. The cross section of the leaf in the studied species composed

of uniseriate upper and lower epidermis with both multicellular glandular and non-glandular hairs and anomocytic stomata. Unlike other *Achillea* species, such as *A. tracica*, *A. Phrygia* and *A. thracica* (Akcin and Akcin 2010, Rogova *et al*. 2015) [2, 21]. the mesophyll in the studied species is undifferentiated (i.e., of isolateral type) with no spongy layer. This type of mesophyll was also reported in *A. millefolium* by Ilham *et al*. (2022) [16]. The leaf vascular bundles are of the closed collateral type which is not consistent with the study of Ilham *et al*. (2022) [16] on the same species. Palynological characters obtained in the present study were generally similar to those reported for other *Achillea* taxa (Akyalçin *et al*., 2011). Concerning the analysis of active contents, *A. millefolium* showed the presence of different classes of secondary metabolites (phenolics, alkaloids, glycosides, tannins and saponins) which are considered as valuable compounds for the food and pharmaceutical industries (Saeidnia *et al*., 2011, Strzępek-Gomółka *et al*., 2021) [22, 28].

For confirmation of the studied species, the phylogenetic tree of *Achillea millefolium* was constructed based on the 18S r RNA, the phylogenetic tree revealed that DNA sample of the *A. millefolium* is highly significant matched to the NCBI sample KT179658.1, confirming the correct identity of the studied species. In addition, the species exhibited two ribosomal variations compared with its corresponding sequences of *A. millefolium* (GenBank acc. no. KT179658.1). These observed variations were attributed to two variants; one nucleic acid substitution of (54A>G), and one deletion (55-56GAdel) Due to the low number of deposited samples in the NCBI, only three relative sequences were found to be suited near the sample, namely GenBank acc. no. MT937104.1, KT179658.1, and MT610918.1 respectively, these sequences of *A. millefolium* were found to be respectively deposited in Denmark, USA, and Brazil. This may lead to the assessment of European and multi-American origins of the observed *A. millefolium* sequences in the current study. Unfortunately, no further sequences were found to be deposited in the NCBI to get a more comprehensive view of the investigated *A. millefolium* sample. Therefore, the phylogenetic role of the observed variations in this species could not be easily identified. It was found that the sample exhibited two ribosomal variations compared with its corresponding sequences of *A. millefolium* (GenBank acc. no. KT179658.1).

In addition, RNA extraction from plant tissue often causes most problem such as unsuccessful removal secondary metabolites during extraction such as phenolic compounds in aromatic and medicinal plants. However, in our study we successfully extracted RNA without the secondary metabolites effect on extraction process**.**

This study will contribute to support the studies that will be carried out in the future aimed at distinguishing between species belonging to *Achillea* genus and to find the genetic and evolutionary relationship between members of this genus in particular and members of Asteraceae in general.

Conclusion

This study presents for the first time from Iraq a detailed morphological, anatomical, palynological, phytochemical and molecular characterization of *A. millefolium* growing in western part of the country. The study reported new information on the anatomy of this species like the presence of branched multicellular non- glandular hairs, the presence of two types of glandular hairs (sessile and stalked) and the isolateral mesophyll. Stomatal frequency and trichomes frequency of the different plant parts were reported. Pollen morphology and phytochemicals of the studied species were also reported. Barcoding of *A. millefolium* and reconstruction of the phylogenetic tree were included.

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