An abstract of the thesis of

Meaad Fhad Alenazi for Master's of Science in Botany presented on The Department of Biological Sciences- Emporia State University.

Title: Comparison of morphological and anatomical characteristics among *Artemisia* species *(Artemisia campestris*, *Artemisia carruthii*, *Artemisia dracunculus*, *Artemisia filifolia* and *Artemisia ludoviciana)* in Kansas.

Thesis Chair: ____

Approved: ____________________________________ (Thesis Advisor Signature)

Artemisia species are members of a wide spread and diverse genus belonging to the family Asteraceae. Kansas has five different species of *Artemisia*. These include *Artemisia campestris* (field sagewort), *Artemisia carruthii* (sagewort), *Artemisia dracunculus* (tarragon), *Artemisia filifolia* (Louisiana wormwood) and *Artemisia ludoviciana* (sand sage). Most of the *Artemisia* species present in Kansas cover more than half of the state. In this study, I focus on a comparative analysis between the five species of *Artemisia* that are found in Kansas and the outgroup plant, *Antennaria neglecta*, based on the morphological and anatomic characteristics of roots, stems, leaves and inflorescences, including flowers, and use these differences to construct a phylogenetic tree. I collected my samples from the field and from the ESU and KANU herbaria. In the morphological study, I measured the parts of plant using a vernier caliper. For the anatomical study, I perpared fresh-fixed and refreshed samples and embedded the samples in paraplast, sectioned at 10 µm, and stained with safranin and fast green. There were no differences between the sections that from fresh sample of plant or dry samples from herbarium; I used fresh and dry sample of *Artemisia ludoviciana* to demonstrate that. I used 46 characteristics to construct a phylogenetic

tree. I compared my tree to the molecular tree published by Watson et al. (2002). The two trees were congruent except *Artemisia carruhii* that was not included in the molecular tree, and I added it in my tree. I found that *Artemisia ludoviciana* and *Artemisia filifolia* are in the same clade as are *Artemisia compestris* and *Artemisia dracunculus*. *Artemisia carruthii* show a common ancestor with the *Artemisia compestris* and *Artemisia dracunculus* clade, and this clade and the *Artemisia ludoviciana* and *Artemisia filifolia* clade show a common ancestor closely related to the outgroup *Antennaria neglecta*.

Keywords: *Artemisia campestris, Artemisia carruthii, Artemisia dracunculus, Artemisia filifolia, Artemisia ludoviciana,* morphological characteristics, anatomical characteristics, Molecular and morphology phylogenetics, *Antennaria neglecta*.

Comparison of morphological and anatomical characteristics among

Artemisia species *(Artemisia campestris, Artemisia carruthii, Artemisia dracunculus, Artemisia filifolia* and *Artemisia ludoviciana)* in Kansas.

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Acknoeledgments

All praise to Allah, today I fold the days' tiredness and the errand summing up between the cover of this humble work, and my deepest thanks to:

The Spring that never stops giving, who weaves my happiness with strings from her merciful heart... to my mother.

Whom he taught me to promote life stairs wisely and patiently… to my dearest father.

Whom he strives to bless comfort and welfare and never stints what he owns to push me in the success way…to my spouse, Zaid.

My happiness, my children… to Yara, Dana, Abdullah and Haneen.

Whose love flows in my veins, and my heart always remembers them… to my brothers and sisters.

Those who taught me letters of gold and words of jewel of the utmost and sweetest sentences in the whole knowledge. Who reworded to me their knowledge simply and from their thoughts made a lighthouse guides me through the knowledge and success path … to my honored professors, Dr.Sundberg, Dr.Mckenzie and Dr. Koerner.

Table of Contens

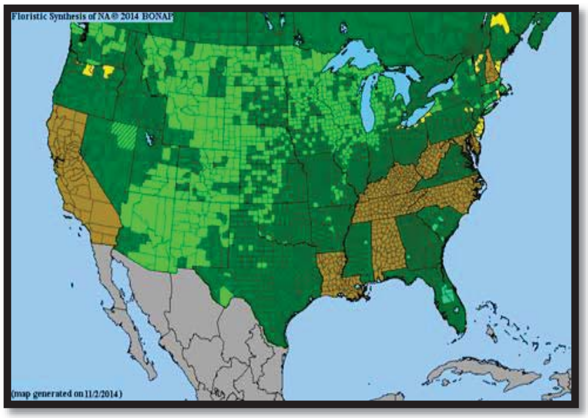
Introduction

The study of plants is crucial to our understanding of biodiversity, particularly the important branch of botany which deals with plant classification and puts plants in different taxa according to the similarities and differences. Traditionally the characteristics of flowers were the basis for the classification of Angiosperms (Scutt, Theissen and Ferrandiz, 2007).

Previous taxonomic studies relied on easy to observe morphological characteristics, but today many different techniques and approaches such as anatomy, chromatography, cytotaxonomy and palynology have helped to refine our understanding of plant species and contribute to taxonomic studies. Anatomical characteristics are now considered as important as morphological characteristics. The study of precise structures (xylem, epidermis, cuticle, trichomes and stomata) is complementary to morphological characteristics. My study will use both anatomical and morphological approaches to examine the relationships of Kansas species of *Artemisia*.

Native plants are plants indigenous to a given area in geologic time and include plants that have developed for many years in an area. Some native plants have adapted to very limited unusual environments, very harsh climates or exceptional soil conditions. In these cases, some species exist only within a very limited range (endemism) while others can live in diverse areas or by adaptation to different surroundings. There are many native genera in Kansas, and one of them is *Artemisia*. Kansas has five species of *Artemisia*: *Artemisia campestris*, *Artemisia carrthii*, *Artemisia dracunculus*, *Artemisia filifolia* and *Artemisia ludoviciana*.

Artemisia is one of the largest genera of the plant kingdom with about 500 species worldwide (Demirci, Demirci and Baser, 2005). The genus is classified in the tribe Anthemideae within the family Asteraceae. *Artemisia* species are distributed throughout North America. Worldwide they are mainly distributed in temperate areas of mid to high latitudes of the northern hemisphere, colonizing arid and semiarid environments. There are only a few representatives in the southern hemisphere. Central Asia is its center of diversification while the Mediterranean region and North-West America are two secondary speciation areas. Only a few species grow in Africa and Europe (Hayat et al. 2009, Garcia, Garnatje, McArthur, Pellicer, Sanderson, and Valles. 2011). Of the five Kansas species, *Artemisia campestris* (Figure1A) occurs in Oregon, Ohio, Pennsylvania and Vermont oddly, and it is native in east and central south of USA except Louisiana, Alabama, Tennessee, North Carolina, Kentucky, West Virginia and New Hampshire. In the center of USA, *Artemisia campestris* grows but it is not rare, and it is native in the west of USA, except California. It is native also across the border in neighboring Canada. *Artemisia campestris* is questionably present in the northeast of Nevada and Utah. *Artemisia carrthii* (Figure 1B) is native in Texas, Oklahoma, Kansas, Missouri, Nebraska, and some areas of Nevada. It is present, but not rare in New Mexico, Colorado, Utah and Arizona. In the east of USA, *Artemisia carrthii* is native in Michigan, Indiana and New York. It is not present in the rest of the states and north of the border in Canada. *Artemisia dracunculus* (Figure 1C) is distributed from central to western United States, but it is not present in the east of USA except New York and Massachusetts. *Artemisia filifolia* (Figure 1D) is native and not rare in the central west of USA, but it is not present in the eastern USA except New York, and it is not present in the border with Canada. *Artemisia ludoviciana* (Figure 1E) is spread across the eastern states, except West Virginia. In Kansas, *Artemisia campestris* spread in two thirds of the area while *Artemisia carrthii*, *Artemisia dracunculus* and *Artemisia filifolia* are concentrated in western Kansas and spread across half of the state, and *Artemisia ludoviciana* is distributed throughout Kansas (Haddock, 2007, 2016).



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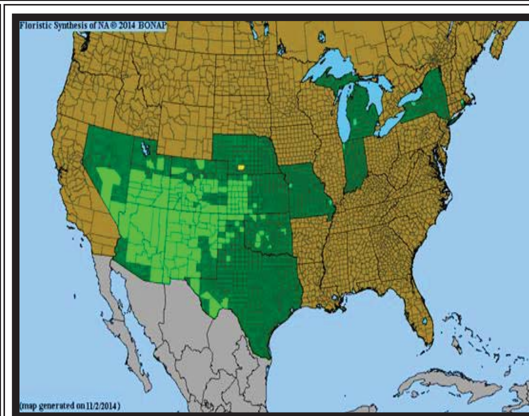

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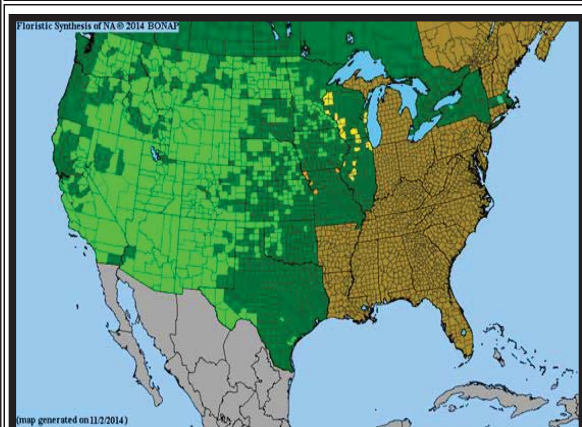

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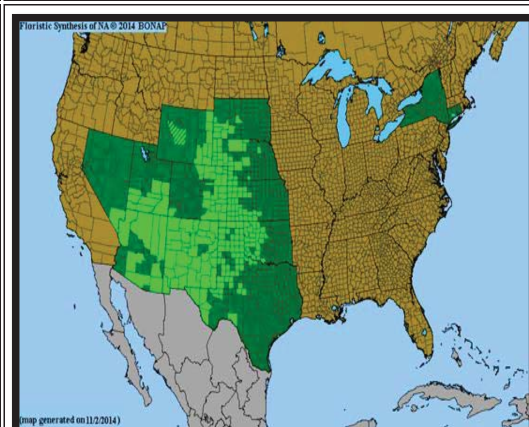

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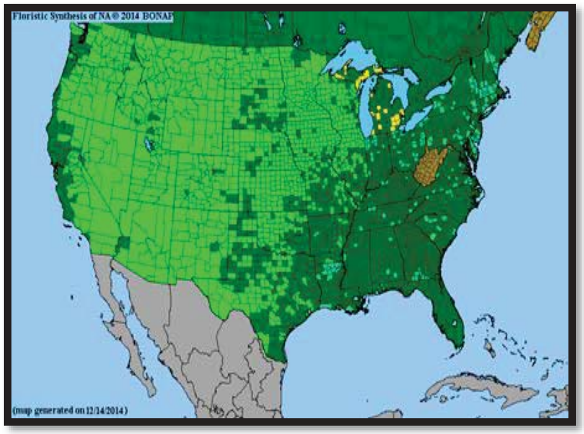
 E	Yellow=species present in state and exotic. Dark green= species present in state and native. Light green=species present and not rare. Linear green= questionable presence (cross-hatched). Brown =species not present in state.

Figure 1: The distribution of Artemisia species in USA. A- *Artemisia campestris,* B- *Artemisia carrthii*, C- *Artemisia dracunculus,* D- *Artemisia filifolia* and E- *Artemisia ludoviciana.*
http://bonap.net/Napa/TaxonMaps/Genus/County/Artemisia

<u>The economic and medical significance of *Artemisia* species</u>

The five Kansas species are characterized as perennial herbs or shrubs. The leaves, alternately arranged on the stem, subtend small flower heads in an inflorescence along the distal part (towards the top). All the species of *Artemisia* are known to produce scented oils and mostly utilized to produce the pharmaceuticals products due to their biological and chemical diversity (Abad, Bedoye, Apaza and Bermejo, 2012). The plants of this genus have several applications like the extraction of volatile oils and production of anti-biotic, anti-viral, anti- fungal, anti-bacterial and anti- malaria compounds (Abad et al. 2012). In addition, there are reports of anti-cancer, anti-pyretic, analgesic, anti-inflammatory, anti-oxidant, hepatoprotective, anti-spasmodic, anti-coagulants, anti-ulcer, anti-anginal, anti-septice and immunostimulating effects (Bianca, Miron and Lungu, 2015). Some species of *Artemisia* are ecologically and economically significant. For instance, some are used as vermifuges, as well as insecticides while others are grown for ornamental purposes and soil stabilizers in disturb habitats (Hayat, Khan, Ashraf and Jabeen, 2009). They are also used as culinary herbs or as flavorings (Watson et al. 2002). The negative side of *Artemisia* species is production of aromatic oil which may cause allergies in humans (Watson, Bates, Evans, Unwin and Estes, 2002), and some *Artemisia* species may be toxic (Valles &McArthur, 2001).

Morphological characteristics of *Artemisia* species

The morphological features of an organism, the size, shape and the structure of plant parts, are important in traditional taxonomic classification. *Artemisia* is a taxonomically complex genus because some species have diverse morphological structures, and others closely resemblance each other, so these characteristics make it quite difficult to correctly identify a sample without detailed morphological review (Hayat et al. 2009).

Artemisia species have hairy bodies that are strongly aromatic. The roots of *Artemisia* are either taproots, a stout vertical root continuing the main axis of the plant downward, or rhizomatous (modified stems), a root- like stem usually horizontal, underground and perennial, bearing buds or shoots and adventitious roots. Leaf morphology is an important classification characteristic because of the variation in size, shape and texture. Most species have pinnatifid, entire, or lobed leaves, and leaf blades are linear, lanceolate, or eliptic, and palmately or pinnately veined. (Haddock, 2016). Sometimes lobes develop at the bottom of the leaf, suggesting the existence of stipules (Ferreira and Janick, 1995). The average size of *Artemisia* species leaves is between 0.5 cm -12.5 cm long and 0.1cm- 4.5 cm wide (Haddock, 2016). The inflorescence is a capitulum having the shape of a paniculate-raceme with the presence of herbaceous involucral bracts. All the five species are wind pollinated and heterogamous with disciform captula bearing pisillate ray florets, and perfect disk florets except *Artemisia dracunculus* and *Artemisia campestri*, which have staminate disk florets (Watson et al. 2002).

The color of the corolla is usually yellow or in rare occurrences green or brown. The cypselas fruits, a small and dry one seeded fruit, are obovoid and brown (Hayat et.al. 2009).

Anatomical characteristics of *Artemisia* species

Some characteristic anatomical features of *Artemisia* are nonglandular hairs, medulary canals, secretory cavities and clustered crystals (Noorbakhsh, Ghahreman and Attar, 2008). In stems, vessels of xylem are arranged into short and long types. All vessel perforations are simple, and all inter-vessel pits are round and arranged in alternating position (Schweingruber, Borner and Schulze, 2013). Many species of *Artemisia* contain dark-staining substances in vessels, and have thin to thick walled fibers (Schweingruber, Borner and Schulze, 2013). Phloem has straight radial rows of paranchema cells and sieve tubes and a small secretory duct (Invanescu, Miron and Lungu, 2015). There are large secretory ducts (Invanescu, Miron and Lungu, 2015) in the cortex, and secretory cells are very thin walled (Schweingruber, Borner and Schulze, 2013).

The ecological characteristic of *Artemisia* species

Artemisia species can grow in moist soil, but most prefer a well-drained or sandy soil with a pH of neutral to slightly alkaline (6.8-7.7). They are somewhat drought tolerant. *Artemisia* species require sun full to partial shade. *Artemisia* is considered as an indicator of steppe climate with moderate precipitation (Hayat et al. 2009).

Artemisia campestris inhabits pastures, prairies, roadsides, waste places and open sandy sites while *Artemisia carrthii* lives in mixed-grass and shortgrass prairies

(Haddock, 2016). *Artemisia dracunculus* inhabits in sandy to gravelly mixed-grass and shortgrass prairies, and *Artemisia filifolia* lives in pastures and prairies, and open, sandy soil while *Artemisia ludoviciana* inhabits in open prairies, open woods, disturbed sites, and roadsides (Haddock, 2016).

Artemisia dracunculus is a fire-adapted species, and it is top-killed by low-intensity fire (Anonymous, 2017). It can reestablish quickly from surviving rhizomes. *Artemisia ludoviciana* may sprout from rhizomes following fire, increasing stem density and percent covering after burning (Anonymous, 2017). The information regarding the fire adaption on *Artemisia campestris* is lacking, but it is described in early postfire communities suggesting rapid recolonization through vegetative sprouting, germination of on-site seed, or movement of seed from off-site sources (Anonymous, 2017). *Artemisia filifolia* sprouts after top-kill by fire. Postfire seedling establishment has not been documented, but fire kills *Artemisia filifolia* and abundant seedlings are produced after a fire (Anonymous, 2017). There is no information about fire adaption of *Artemisia carruthii*.

Molecular and morphology phylogenetics

Molecular phylogenetics is the branch of phylogeny that analyses hereditary molecular differences, mainly in DNA sequences, to gain information on a plant's evolutionary relationships. The result of a molecular phylogenetic analysis is expressed in a phylogenetic tree in the same way that morphological phylogenetic analyzes based on morphological characteristics.

The history of phylogenetics has depended primarily upon morphological data, but molecular data, protein and DNA sequences have been increasingly used to investigate the phylogeny and divergence times of extant organisms (Pisani, Benton and Wilkinson, 2007). Few attempts have been made to examine the degrees of conflict and consensus between these techniques (Hillis, 1987).

The greatest advantage of molecular data is the extent of the data set, and the set of morphological data with a genetic basis is a small subset of molecular information because all heritable information is encoded in DNA (Hillis, 1987). For comparative data to be useful for phylogenetic reconstruction, the characters must represent heritable variation, and the environmental influences on the phenotype must be sorted from genetic variation. Environment seems to have little influence on phenotype for some groups, but the effects of it are great for others (Hillis, 1987). Thus, biomolecular data are confounded less by environmental influences than morphological data (Hillis, 1987).

One of the important advantages of morphological over molecular approaches to systematics is much greater applicability of the former approach to extensive collections of preserved specimens in museums. Most molecular methods require fresh or cryopreserved material (Hillis, 1987). In addition, paleontology always has been primarily a morphological endeavor, and a low percentage of biomolecules are preserved in fossils. A few molecular methods have been applied with considerable success to well- preserved fossil specimens, but relatively little molecular information has been obtained from extinct species (Hillis, 1987). Most morphological data can be collected with minimal expenditures on supplies and equipment, but molecular laboratories require tens of thousands of dollars to establish and maintain (Hillis, 1987).

Pisani, Benton & Wilkinson (2007) showed that comparing trees can increase confidence (congruence) or demonstrate that at least one tree is incongruent because there are greater differences between than within the morphological and molecular partitions.

<u>Molecular phylogeny of *Artemisia* species</u>

The ITS (internal transcribed spacers) of nuclear ribosomal DNA has been used for studying and analyzing, the phylogenetic relationships among several *Artemisia* species (Kornkven, Watson and Estes, 1998). The tree they produced was rooted with *Artemisia dracunculus* as the outgroup (Kornkven, Watson and Estes, 1998). *Artemisia dracunculus* and *Artemisia filifolia* are strongly supported in monophyly, but *Artemisia ludoviciana* is weakly supported in monophyly (Kornkven, Watson and Estes, 1998).

Watson et al. (2002) produced a phylogenetic tree with two main subgeneric clades: 1) *Artemisia* subg. *Artermisia)* that includes most species including *Artemisia ludoviciana* and *Artemisia filifolia*, and 2) species of Artemisia subg. Dracunculus including *Artemisia dracunculus* and *Artemisia campestris* (Watson et al. 2002). They did not examine *Artemisia carrthii*. The tree they produced was rooted with *Ajania pacifica, Arctanthemum arcticum, Dendranthema intricatum, Elachanthemum intricatum, Kascharia komarovii, Stilnolepis centiflora, Leucanthemella serotine, Nipponanthemum nipponicum, Cymbopaappus adenosolen, Pentzia dentata* and *Oncosiphon grandiflorum*, and they are from Anthemidea, tribe of the Asteraceae.

<u>Outgroup plant</u>

Antennaria is genus of around 40 species and belongs to the family of the Asteraceae and the tribe Gnaphalieae (Bayer, 1996). I chose this because *Antennaria* is found in Kansas and is in a sister tribe, Gnaphaliinae, to the Anthemideae which contains *Artemesia*. *Antennaria* is widely distributed in temperature to arctic regions of the northern hemisphere (Bayer, 1987). *Antennaria neglecta* is herbaceous perennial, and it is native in the north, east of USA and across the border in Canada. It is not present in the south and western USA. It is concentrated in eastern of Kansas (Haddock, 2007). *Antennaria neglecta* is found in dry prairies, pastures, old fields, and open wooded slopes (Haddock, 2007).

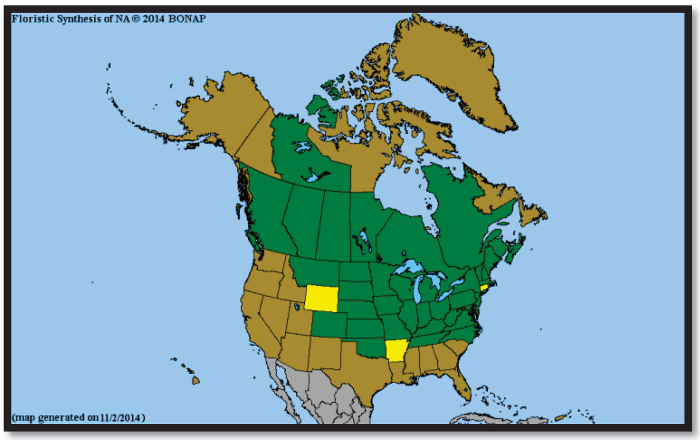


Figure 2: the distribution of *Antennaria neglecta* in USA.

http://bonap.net/Napa/TaxonMaps/Genus/State/Antennaria

Antennaria neglecta prefers clay, sandy or gravelly soil with pH (5.5-7.5). *Antennaria neglecta* requires sun full to partial shade, and it is not fire adapted.

Antennaria neglecta has erect and white-woolly stems (Haddock, 2007). Leaves are simple with size 2.5 cm- 6.5 long and 0.6 cm-2 cm wide (Haddock, 2007). Their margins are entire, and blades are sessile, linear, or curled. The inflorescences are cyme-like (Haddock, 2007). Male and female flowers occur on separate plants, and the ray florets are absent and disk florets are white (Haddock, 2007)

The xylem in the stems of *Antennaria* species is semi- ring porous, and a row of phelloids is present on outside of the phloem (Schweingruber, Borner and Schulze, 2013).

The three questions in this study:

1-Are the *Artemisia* species similar or different in morphological and anatomical characteristics?

2-Do the herbarium specimens have the same size of cells and tissue as that in the fresh samples?

3-Does the morphological and anatomical phylogenetic tree match with the molecular phylogenetic tree?

Material and Method

Collecting the samples and information

I collected *Artemisia ludoviciana* from Ross Natural History Reservation in Lyon County in September 2016. I also used the fresh samples for *Artemisia campestris* (Ellsworth Country, C.C. Freeman, 2017), and *Artemisia dracunculus* (Ellsworth Country, Ks- C.C. Freeman- 2017) collected by Dr. Freeman of the University of Kansas.

For morphological study, I prepared herbarium specimens and used standard techniques (Maden, 2004). Also, I used specimens from ESU Herbarium for *Artemisia filiolia, Artemisia ludoviciana, Artemisia carruthii* and *Antennaria neglecta* samples, and I used the specimens from KANU Herbarium for *Artemisia campestris* and *Artemisia dracunculus* samples (Table 1). I measured the length and width of leaves using a vernier caliper. Under a microscope at a 40x magnification, I measured the length and width of flowers with a metric ruler.

For morphological characteristics, I focused on habit and growth habit, ascending stem, branched stems, order of branches, trichomes in stem, length/width ratio of leaves, margin types, blade types, trichomes in leaves, inflorescence types, flower color, length and width of flowers, type of flowers (pistillate, staminate and perfect), and trichomes in flowers.

Table 1-Collection data from for specimens used (all samples in each species used as one sample in the study).

Species	Collection data	Herbarium voucher	Fresh materials
Artemisia campestris	Colorado. Pueblo, CO-C.C. -Freeman & R.L. Hartman-1998 Washington County, CO-S. -Stephens-1972	KANU00322964 KANU00121199	Collector C.C. Freean-2017
Artemisia carruthii	-Antrim: beach area at EIk Rapids; Traverse Bay- J.S. Wilson-1964 -Hamilton CO, KS-C.A. Morse-2007	022069 022072	
Artemisia dracunculus	-Emmons County, ND- S. Stephens-1972 -Hyde County, SD- S. Stephens-1972	KANU00121426 KANU00121428	Collector C.C. Freean-2017
Artemisia filiolia	-Morgan Co-J.S. Wilson-1963	022098	
Artemisia ludoviciana	Disturbed meadow- D. Birkholz--1966	022129	Collector M.F. Alenazi-2016
Antennaria neglecta	Cherokee Co, Ks-J.S. Wilson--1961	021869	

For the anatomical study, I fixed fresh samples of *Artemisia ludoviciana*, *Artemisia campestris* and *Artemisia dracunculus* in Formalin Acetic Acid Alcohol(FAA) (Berlyn & Miksche, 1976). Herbarium samples were refreshed by putting them in water for 8-10 hours in the oven at 60° C, followed by ammonium hydroxide overnight at 60° C. After that, samples were washed three times in distilled water, each two hours, and put into FAA -killing and fixation step (Venning, 1954)

Both fresh-fixed and refreshed samples were dehydrated in Tertiary Butyl Alcohol (TBA) series (Berlyn & Miksche, 1976). From TBA samples were transited to half of paraffin oil and half of TBA in an oven at 60° C overnight, followed by three changes two hours each by Paraplast™ in an oven at 60° C (Berlyn and Miksche, 1976).

I embedded the samples in Paraplast™ and sectioned at 10 µm. The steps followed for staining samples that I used are as follows: put slides in 1% safranin (5 g safranin in 500 ml 50% Ethyl Alcohol) for 12 hours, washed slides by water until colorless, dehydrate in 30% ETOH, 50% ETOH, 70% ETOH and 95% ETOH for two minutes in each concentration, put slides in fast green 0.05%(0.25 g fast green in 500ml 95% Ethyl Alcohol) for two minutes, then put them in absolute alcohol two times for two minutes in each time, finally put slides in xylene for three times (the first time for five seconds and the second and third times for ten minutes) (Berlyn and Miksche, 1976). Permanent slides were in mounted Permount ™ (Berlyn and Miksche, 1976).

I examined the slides using a Nikon Eclipse E600 microscope and recorded digital images with a Zeiss Axiocam ERc 5s camera. I used Image J (Image J) to analyze cell form, size and shape for leaf, stem and root tissues. For root, stem and leaf tissues, I sampled 10 cells to measure the average cell size.

In the anatomical study, I focused on leaf characters (the size of epidermis, the length of stomata, the quantity of stomata in a certain area, the size of guard cells (width and height), the size of xylem and phloem in the main vein (width and height), the size of palisade and spongy mesophyll cells), stems and roots tissues (the size of epidermis, cortex, xylem and phloem), and flowers (the shapes, type and size of pollen, pollen wall thickness, pollen apertures, surface arnamentation, sizes of floral parts-disk floral and ray floral, and how many florets).

To examine pollen structure, I put the flowers in a porcelain sieve and crushed them with added absolute ethyl alcohol. Then I placed the solution in centrifuge tubes and centrifuged (Clay Adams, CAT.NO.0131) for 2-3 minutes. I pipetted a small amount of the pellet onto a clean slide and added a drop of 100% alcohol (ABS) and allowed it to evaporate. I repeated the alcohol wash two more times. Then I added a drop of Basic Fuchsin (1% in 95%ETOH) and allowed it to stand for a few seconds. I followed with 3 washes with ABS. I added a drop of xylene and immediately a drop of immersion oil before placing the coverglass. I used 30 pollen grains for measuring the average of size of pollen, and I examined them under microscope on 400x.

To examine stomata structure, I took the leaves from the herbarium specimens and refreshed them by putting in water and placing in the 60° C oven for 8-10 hours. After the leaves dryed, I put a layer of nail polish on a small area and allowed it to dry. Then I put clear sticky tape on it and removed the tape. I placed the tape on a slide and examined it under the microscope on 400x; I measured 20 stomata for calculating the average of size(areas).

To examine floret structure, I crushed (pressed) the flowers on a slide with a drop of water and added a coverglass. I used 5 flowers for measuring the average of length of disk florets and ray floret, and I examined under a microscope on 20x.

Constructing a Data Matrix.

The condition of each character for each species was organized into a data table. Character states from the table were later assigned numerical values to create a data matrix table. Plesiomorphic state of the outgroup were assigned the value zero, and apomorphies in each subsequent species were assigned sequential whole numbers. One is a score for the first apomorphic state, so all taxa sharing in this state must score one. Two is a score of the second apomorphic state, and the state continues to third, forth, etc if that is necessary. For example, the most possible character states with five species plus the outgroup is 6, so the score will be 0-5 (Brooks, Caira, Platt and Pritchard, 1985). I used *Antennaria neglecta* as outgroup in my phylogenetic tree.

Constructing a Cladogram

A cladogram must have a root, the origin from which the branches of the tree grow. The outgroup is used to root in the cladogram. Taxa are added sequentially, but in random order, to the root to provide the most parsimonious tree. I used the simple Wagner Neighborhood method to manually construct the tree (Brooks et al. 1985). First, I connected the outgroup, first and second taxa from the data matrix, and in parentheses listed the character state scores in order from the data matrix. The three closest taxa in the growing tree are called a Wagner Neighborhood, and the node is the tree joined at a single point. The character state of the node is determined from the values of the neighbored taxa with a majority or median value. The fourth taxon is then added in the cladogram, in all three possible positions; between the root and the node; between the node and second taxon or the node and the third taxon. Then each of the three possible trees are constructed, and the new node characteristics of each one is compared. The most parsimonious tree is chosen, and the process is repeated to add each additional taxon (Brooks et al. 1985).

Results

A- Morphological characteristics results

(A-1)- Habit and growth habit

The species studied in this research are herbaceous or shrubs, and all of them are perennial. *Artemisia campestris*, *A. carruthii, A. dracunculus, A. ludoviciana* and *Antennaria neglecta* are perennial herbs, but *Artemisia filiolia* is a perennial shrub.

Growth habits of the species were rhizomatous (producing rhizomes that are horizontal underground stem; root stock), stoloniferous (producing stolons that are elongate, horizontal stem creeping along the ground and rooting at the nodes or at the tip and giving rise to a new plant), or bunch type (taproot that is a root system with a main root axis and smaller branches). *Artemisia campestris* (Figure3A) and *A. dracunculus* (Figure3C) form a taproot, and *A. carruthii* (Figure3B) *A. filiolia* (Figure 3D) and *A. ludoviciana* (Figure 3D) are rhizomatous. However, *Antennaria neglecta* (Figure 3F) is stoloniferous.

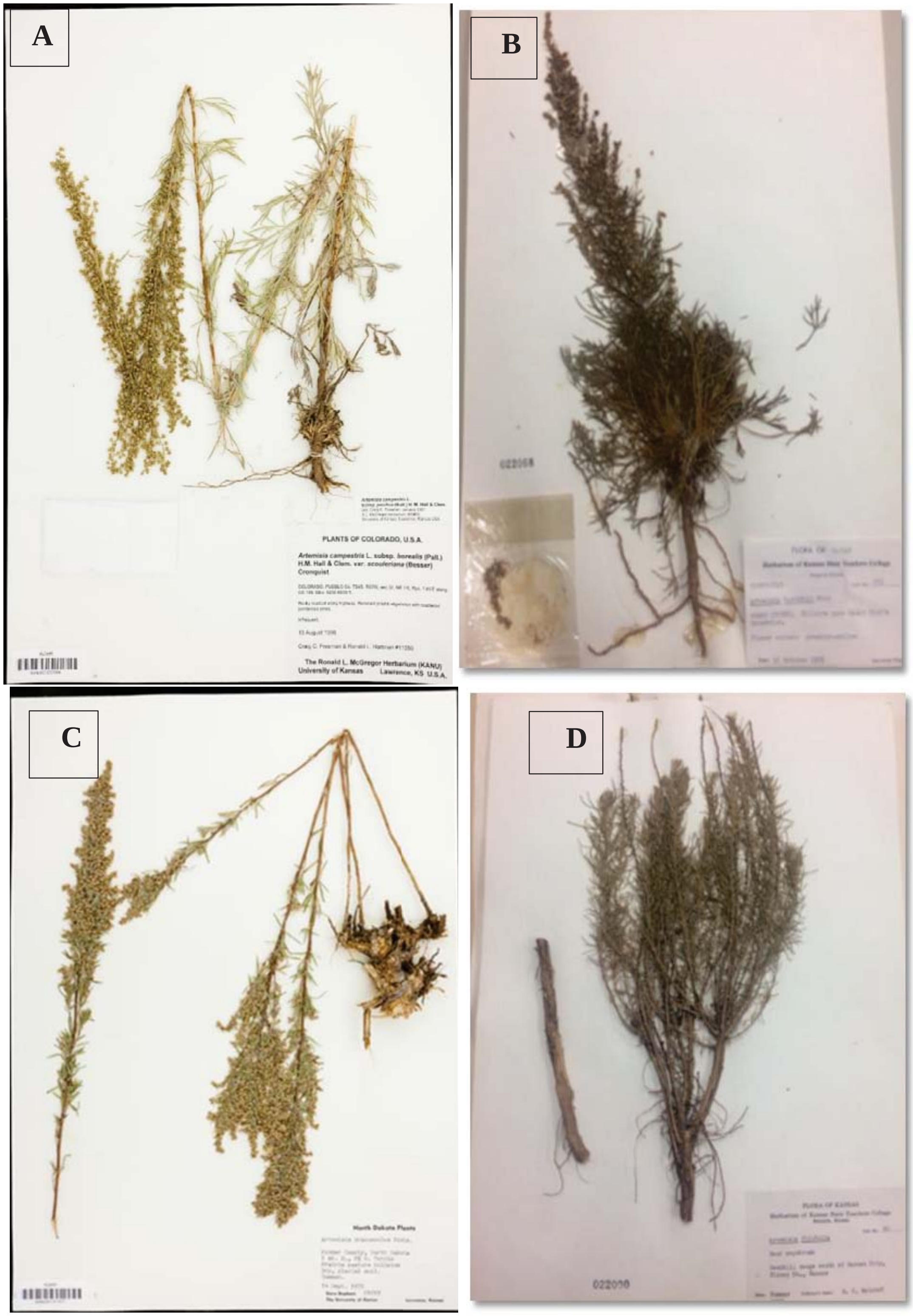

A
PLANTS OF COLORADO, U.S.A.
Artemisia campestris L. subsp. borealis (Pall.) H.M. Hall & Clem. var. scouleriana (Besser) Cronquist
The Ronald L. McGregor Herbarium (KANU)
University of Kansas Lawrence, KS U.S.A.
B
C
North Dakota Plants
D

Figure 3: The growth habit of *Artemisia* species and outplant group. A- *Artemisia campestris,* B- *Artemisia carrthii*, C- *Artemisia dracunculus,* D- *Artemisia filifolia* , E- *Artemisia ludoviciana* and F- *Antennaria neglecta*.(ESU and KANU herbarium).

(A-2)- Stems

Artemisia campestris (figure3A) has simple branches and erect brown stems, and *A. carruthii* (Figure3B) has a simple branch ascending greenish gray stems. Also, *A. dracunculus* (Figure3C) has a simple branch with erect brown stems, and *A. filifolia* (Figure3D) has a woody, much branched, erect brownish stems. *A. ludoviciana* (Figure3E) has a simple branch erect greenish gray stems, and *Antennaria neglecta* (Figure 3F) has a simple branch with an erect whiteish green stem.

There are many types of trichomes that cover the stem in these species such as glabrate, tomentose, glabrous and sericeous. Stems of *Artemisia campestris* (Figure 4A) are covered with glabrate (nearly bald and becoming glabrous with age). Stems of *A. carruthii* (Figure 4B), *A. ludoviciana* (Figure 4E) and *Antennaria neglecta* (Figure 4F) are covered with tomentose trichomes that are short, matted, soft, wooly hairs. Stems of *Artemisia dracunculus* (Figure 4C) are glabrous (hairless), and stems of *A.*

filifolia (Figure 4D) are usually covered with sericeous trichomes (silky, long, soft, slender, somewhat appressed hairs).

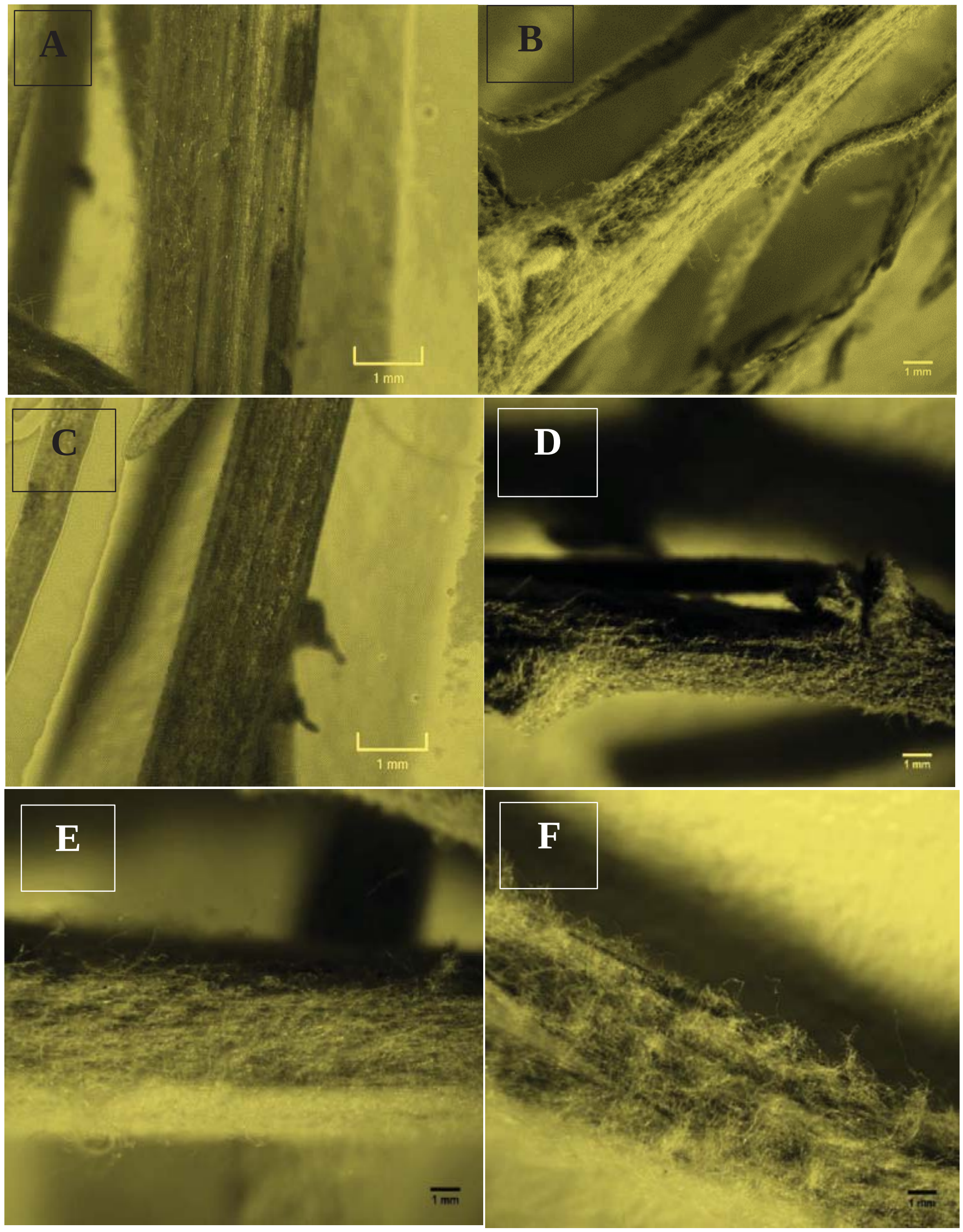
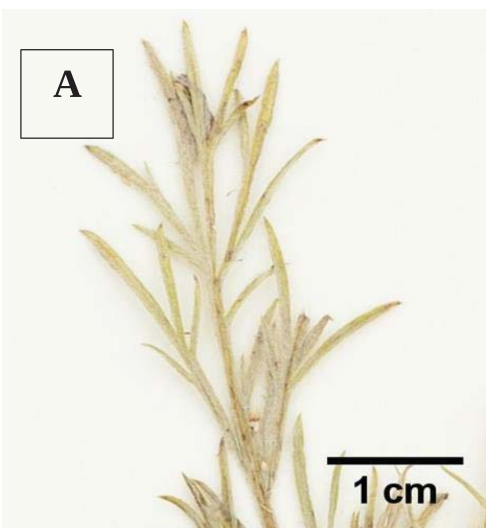


Figure 4: the types of trichomes in stems. A- *Artemisia campestris*, B- *Artemisia carrthii*, C- *Artemisia dracunculus*, D- *Artemisia filifolia*, E- *Artemisia ludoviciana* and F- *Antennaria neglecta*.

(A-3)- Leaves

The leaves are characterized by blade, margin, size, and the types of trichomes. All the species have alternate leaf arrangements, except *Antennaria neglecta* has a basal rosette and alternate leaf arrangements on the erect stem. The blade has many types, including lanceolate, pinnatifid, and linear. Lanceolate blade is a leaf much longer than wide with the widest point below the middle, and this type is found in *A.dracunculus* (Figure 5C) and *A. ludoviciana* (Figure 5E). *Antennaria neglecta* (Figure 5F) has a lanceolate to spatulate blade. Pinnatifid blade leaf is pinnately cleft or lobed half the distance or more to the midrib but not reaching the midrib, and this type is found in *Artemisia carruthii* (Figure 5B) and *Artemisia campestris* (Figure 5A). Linear blade is a leaf much longer then wide with parallel sides, and this type found in *A. filifolia* (Figure 5D).

All species have entire leaf margins except *Artemisia campestris* and *Artemisia carruthii*, which have 3 lobed leaf margins. There is variation in the length to width ratio of leaves of these species. The length to width ratio of *Artemisia campestris* is 2, and the length to width ratio of *A. carruthii* is 1.4. The length to width ratio of *A. dracunculus* is 11, and the length to width ratio of *A. filifolia* is 11.7. The length to width ratio of *A. ludoviciana* is 6, and the length to width ratio of *Antennaria neglecta* is 5.



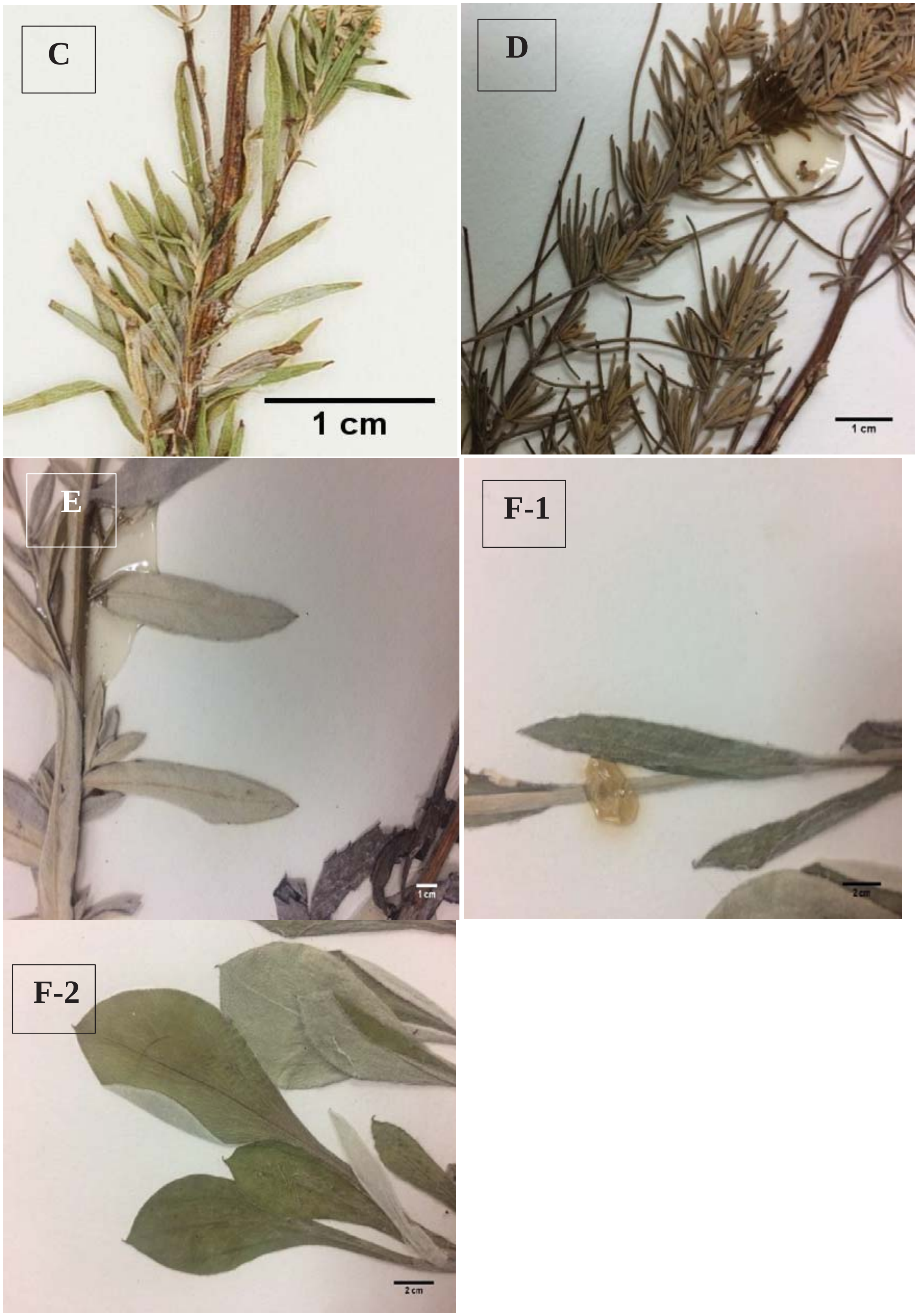
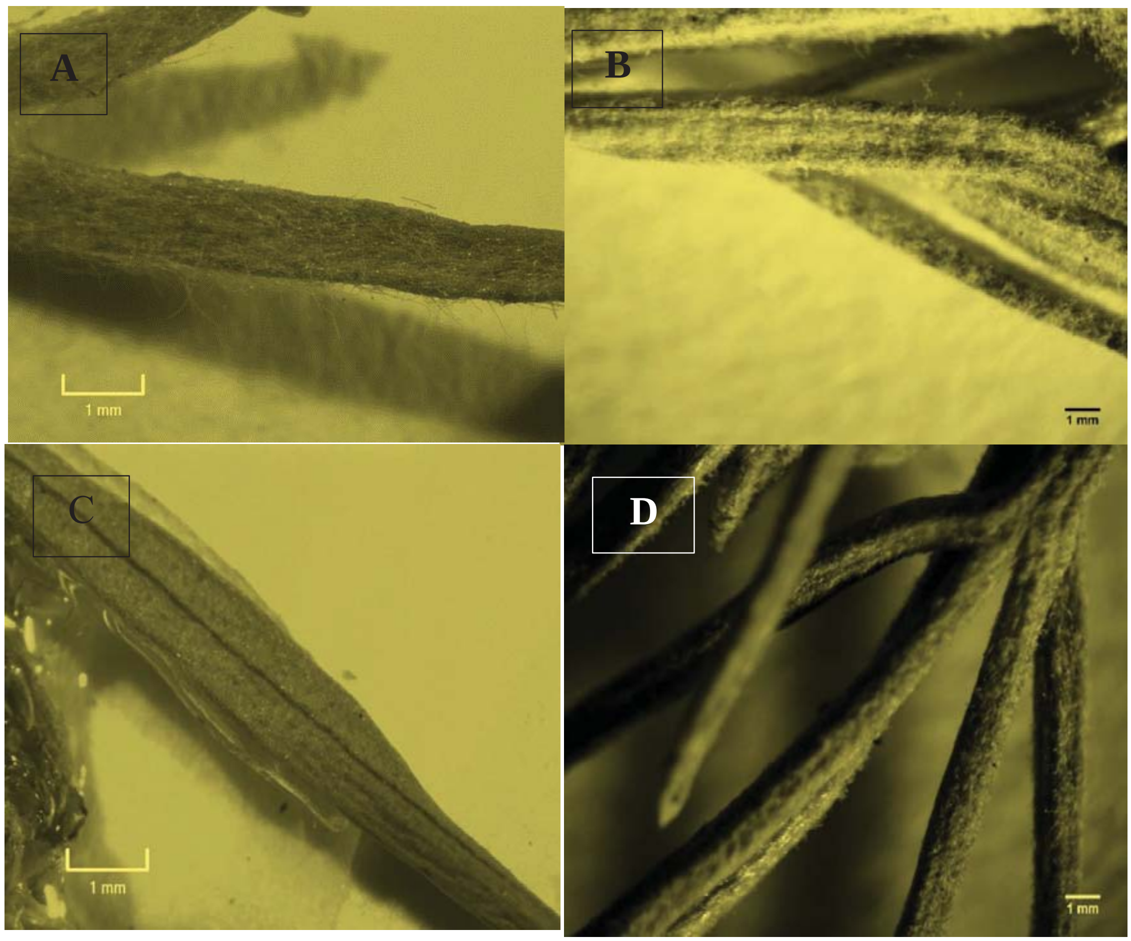


Figure 5: The blade and margin types of leaves. A- *Artemisia campestris*, B- *Artemisia carrthii*, C- *Artemisia dracunculus,* D- *Artemisia filifolia* , E- *Artemisia ludoviciana* and F- *Antennaria neglecta*. (1-upper leaves, 2- basal leaves).

There are many type of trichomes that distinguish these species. A glabrate surface is common in *Artemisia campestris* leaves (Figure 6A) and *A. filifolia* (Figure 6D) leaves, and the tomentose trichomes are common on the surface of *A. carruthii* (Figure 6B), *A. ludoviciana* (Figure 6E) and *Antennaria neglecta* (Figure 6F) leaves. The surface of *Artemisia dracunculus* leaves is glabrous (Figure 6C).



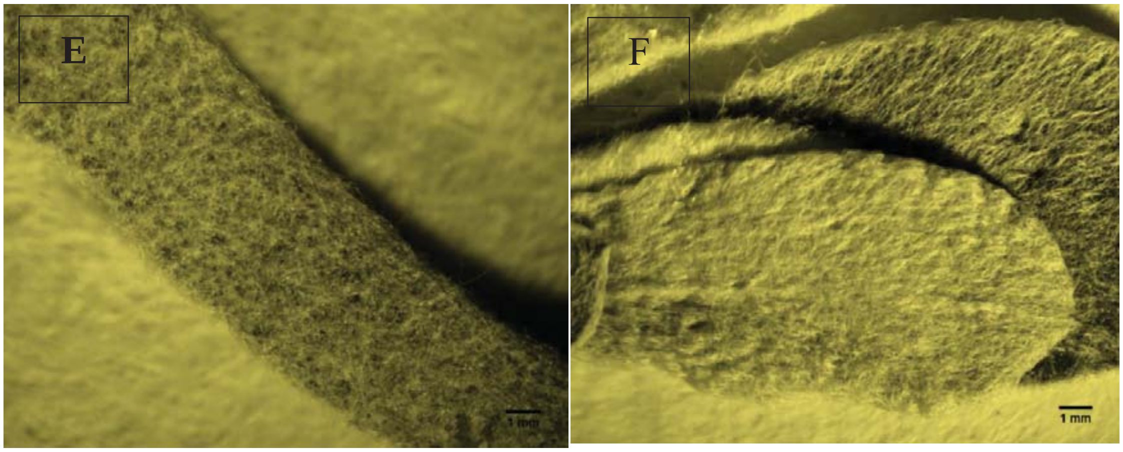


Figure 6: the types of trichomes in leaves. A- *Artemisia campestris,* B- *Artemisia carrthii*, C- *Artemisia dracunculus,* D- *Artemisia filifolia* , E- *Artemisia ludoviciana* and F- *Antennaria neglecta*.

(A-4)- Inflorescences

All the species of *Artemisia* have a paniculate inflorescence that is a branched, racemose with flowers maturing from the bottom upwards. The species have discoid heads. The inflorescence in *Artemisia campestris* (Figure 7A) has the heads in arrays about 2-34 cm long, and *A. carruthii* (Figure 7B) has the heads in arrays about 5-15 cm long. The heads in *A. dracunculus* (Figure 7C) are in arrays about 9.5-35 cm long, and *A. filifolia* (Figure 7D) has the heads in arrays about 6-15 cm long. *A. ludoviciana* (Figure 7E) has the heads in arrays about 10-36 cm long.

The trichomes of *Artemisia campestris* (Figure 8A), *A. carruthii* (Figure 8B) and *A.dracunculus* (Figure 8C) are glabrous, and tomentose surface covers heads of *A. filifolia* (Figure8D) and *A.ludoviciana* (Figure 8E).

Antennaria neglecta (Figure 7F) has cyme inflorescence- determinate inflorescence, paniculate in which the terminal flower blooms first- with few heads that are around 1-6, and it is discoid head, and tomentose surface covers heads of *Antennaria neglecta* (Figure 8F).

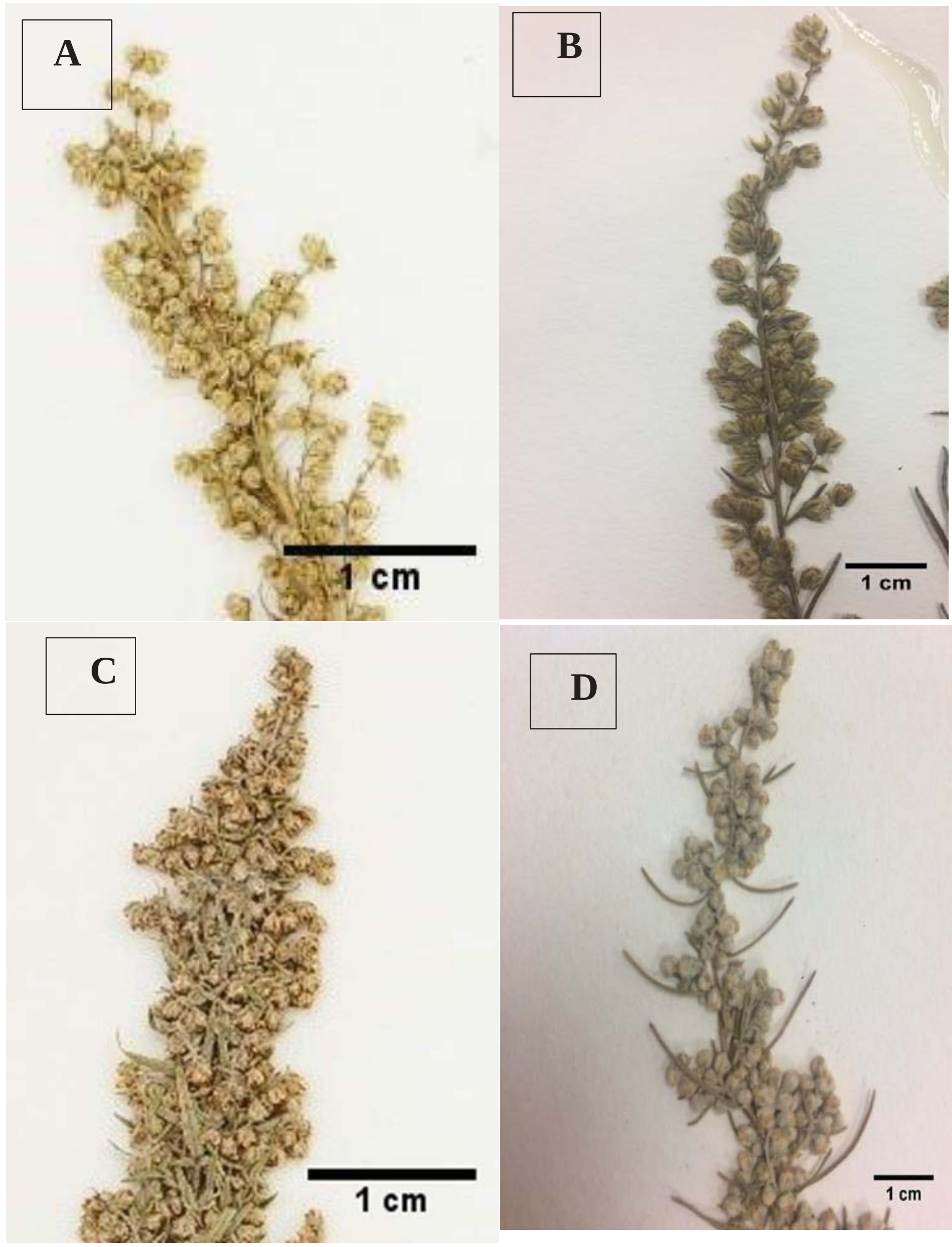

A
1 cm
B
1 cm
C
1 cm
D
1 cm

Figure 7: the types of inflorescences. A- *Artemisia campestris,* B- *Artemisia carrthii*, C- *Artemisia dracunculus,* D- *Artemisia filifolia*, E- *Artemisia ludoviciana* and F- *Antennaria neglecta*.

(A- 5)- flowers

Artemisia campestris has yellow flowers and they are 0.3 cm long × 0.2 cm wide. The average number of disk florets is 20 and the average number of ray florets is 12. *A.carruthii has* yellow flowers, and they are 0.3 cm long × 0.2 cm wide. The average number of disk florets is 16 and the average number of ray florets is 3. *A. dracunculus* has also yellow flowers, and they are 0.2 cm long × 0.2 cm wide. The average number of disk florets is 12 and the average number of ray florets is 15. *A. filifolia* has whitish yellow flowers, and they are 0.1 cm long × 0.1 cm wide. The average number of disk florets is 4, and the average number of ray florets is 2. *A.ludoviciana* has also whitish yellow flowers, and they are 0.2 cm long × 0.1 cm wide. The average number of disk florets is 25, and the average number of ray florets is 8.

Antennaria neglecta has white flowers, and male and female flowers on separate plants. Male flowers are 1.4 cm long × 1.3 cm wide, and they are purplish brown anthers with 17-47 stamens. Female flowers are 1.2 cm long × 1 cm wide, and they have 27-49 carpels. They do not have ray florets.

The disk floret in *Artemisia campestris, Artemisia dracunculus* and *Artemisia filiolia* is staminate and *Artemisia carruthii* and *Artemisia ludoviciana* have perfect florets. All ray florets of *Artemisia* species are pistillate.

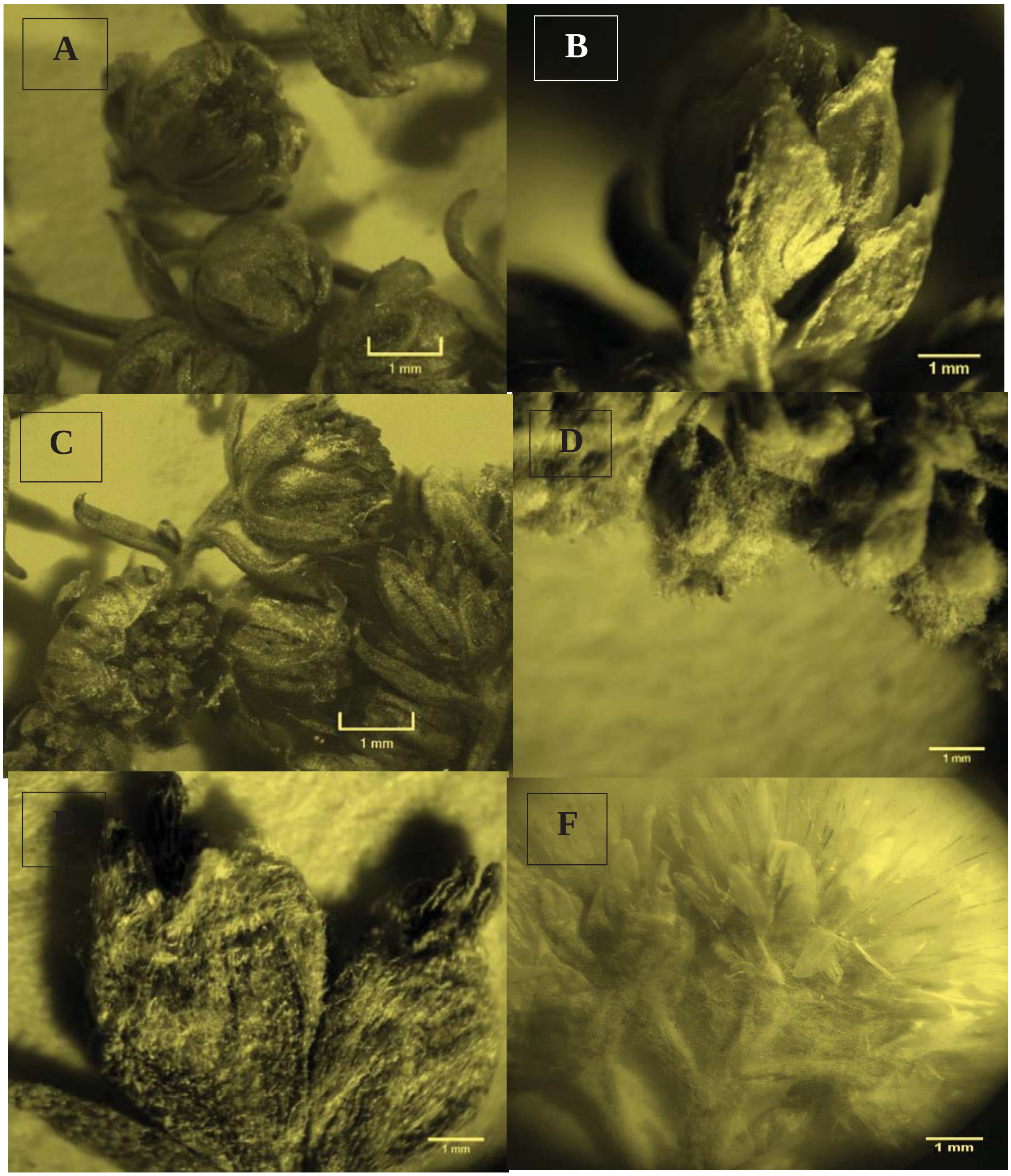


Figure 8: the types of trichomes in flowers. A- *Artemisia campestris*, B- *Artemisia carrthii*, C- *Artemisia dracunculus,* D- *Artemisia filifolia*, E- *Artemisia ludoviciana* and F- *Antennaria neglecta*.

B-Anatomical characteristics comparing between *Artemisia ludoviciana* (fresh samples) and the specimens from herbarium

I examined 10 cells from each sample in every tissue (roots, stems and leaves) to find if had different sizes or the drying plants effect in the tissues, and I used 2 sample t-test for analyzing if have different or not by calculating p value.

(B-1)- Root

In root tissues, there are no significant differences between fresh samples and dry samples that from herbarium (Table2).

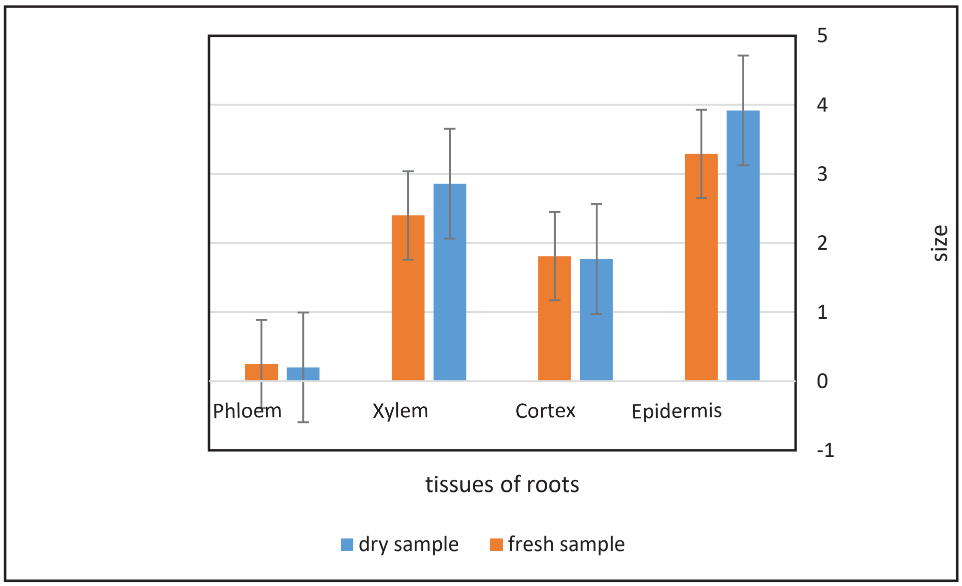


Figure 9: comparative between dry and fresh samples of *Artemisia ludoviciana*

(B-2)- Stems

In stem tissues, there are no significant differences between fresh samples and dry samples that from herbarium (Table2).

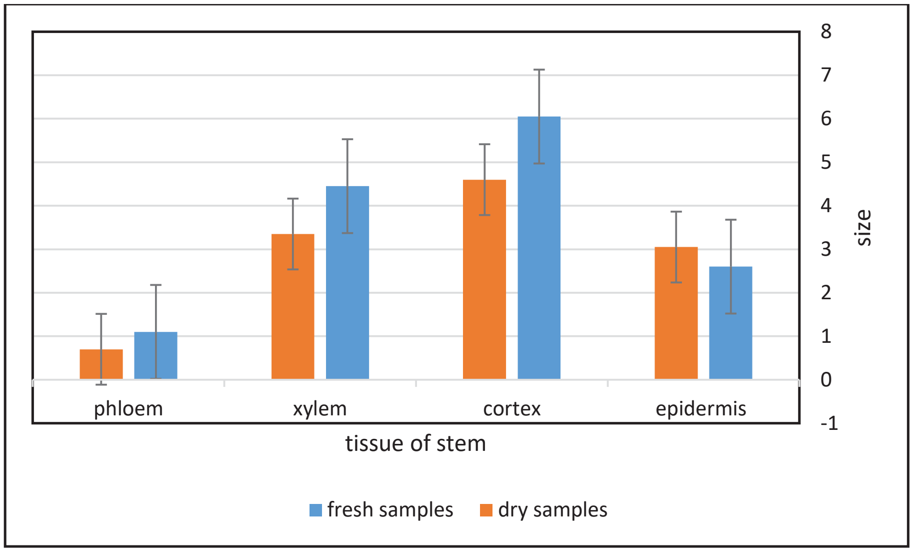


Figure 10: comparative between dry and fresh samples of *Artemisia ludoviciana*

<u>(B-3)- Leaves</u>

In leaf tissues, there are no significant differences between fresh samples and dry samples that from herbarium (Table2).

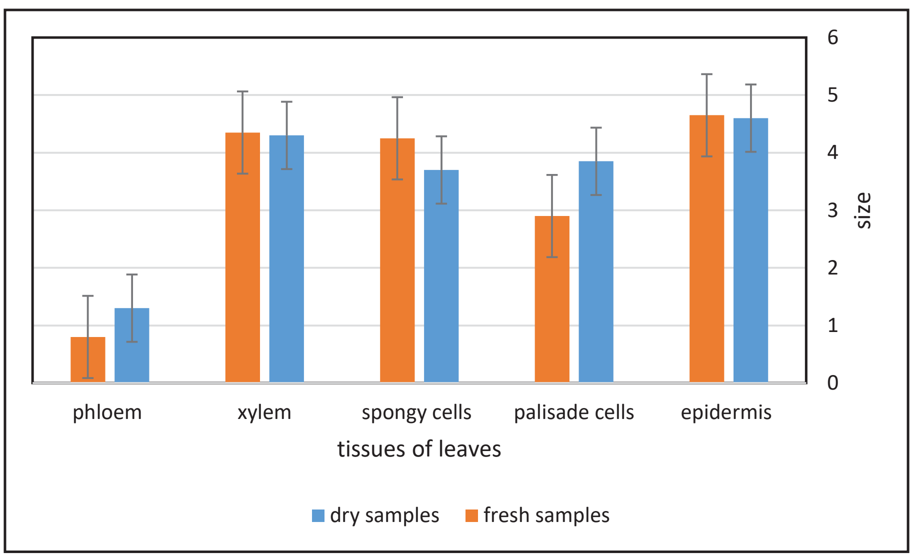


Figure 11: comparative between dry and fresh samples of *Artemisia ludoviciana*

Table 2: the t value and p value for tissue types

Tissue type	t-value	p-value
Roots-epidermis cells	-2.059	0.054
Cortex	0.122	0.905
Vessels	-0.945	0.357
sieve tubes	1.367	0.188
Stems-epidermis cells	1.445	0.170
Cortex	1.636	0.119
Vessels	-1.004	0.329
sieve tubes	-2.005	0.068
Leaves-epidermis cells	-0.040	0.969
palisade mesophyll cells	1.070	0.299
spongy mesophyll cells	-1.125	0.276
Vessels	0.020	0.984
sieve tubes	2.001	0.063

C- Anatomical characteristics results

(C-1)- Roots

I used 10 cells for measuring the average of cell areas, and I examined them under 200x.

(C-1-1) Epidermis

The largest average size of epidermis in roots of *Artemisia* species is *Artemisia ludoviciana* (16.45±0.20 µm^2) (Figure16B), and the smallest average size of epidermis is *A.dracunculus* (4.40 ±0.09 µm^2)(Figure 14B). The average size of epidermis of *Artemisia* species are *A. filifolia* (7.80 ±0.34 µm^2) (Figure 15B), *A. carruthii* (6.25±0.30 µm^2) (Figure13B) and *A. campestris* (5.90±0.15µm^2) (Figure 12B). The average size of epidermis of *Antennaria neglecta* is 20.60±0.54 µm^2 (Figure 17A).

(C-1-2) Cortex

The sequence of average size of cortex in roots from larger to smaller is *Artemisia filifolia* (6±0.09 µm^2) (Figure 15B), *A. carruthii* (5.55±0.11 µm^2) (Figure13B), *A.ludoviciana* (2.55±0.06 µm^2) (Figure 16B), *A.campestris* (1.90±0.04 µm^2) (Figure 12B), and *A. dracunculus* (0.25±0.01 µm^2) (Figure 14B). The average size of cortex of *Antennaria neglecta* is 5.60±0.22 µm^2 (Figure 17A).

(C-1-3)- Xylem (Vessels)

The largest average size of vessels in roots of *Artemisia* species is *Artemisia carruthii* (46±0.92 µm^2) (Figure 16A), and the smallest average size of vessels is *A. filifolia* (10±0.35µm^2) (Figure 15A). Other average size of vessels of *Artemisia* species are *A. dracunculus* (22.20±0.42 µm^2) (Figure 14A), *A. campestris* (15.85±0.77 µm^2) (Figure 12C), and *A. ludoviciana* (11.50±0.15 µm^2) (Figure16A). The average size of vessels of *Antennaria neglecta* is 3.50±0.14 µm^2 (Figure 17B).

(C-1-4) -Phloem (Sieve tubes)

The sequence of average size of sieve tubes in roots from larger to smaller is *Artemisia carruthii* (6.10±0.15 µm^2) (Figure13D), *A. campestris* (5.45±0.2µm^2) (Figure 12A), *A. dracunculus* (3.60 ±0.09 µm^2) (Figure 14B), *A. filifolia* (1.55±0.4µm^2) (Figure 15C) and *A. ludoviciana* (1.25±0.02 µm^2) (Figure16B).The average size of sieve tubes of *Antennaria neglecta* is 1.45±0.02 µm^2 (Figure 17A).

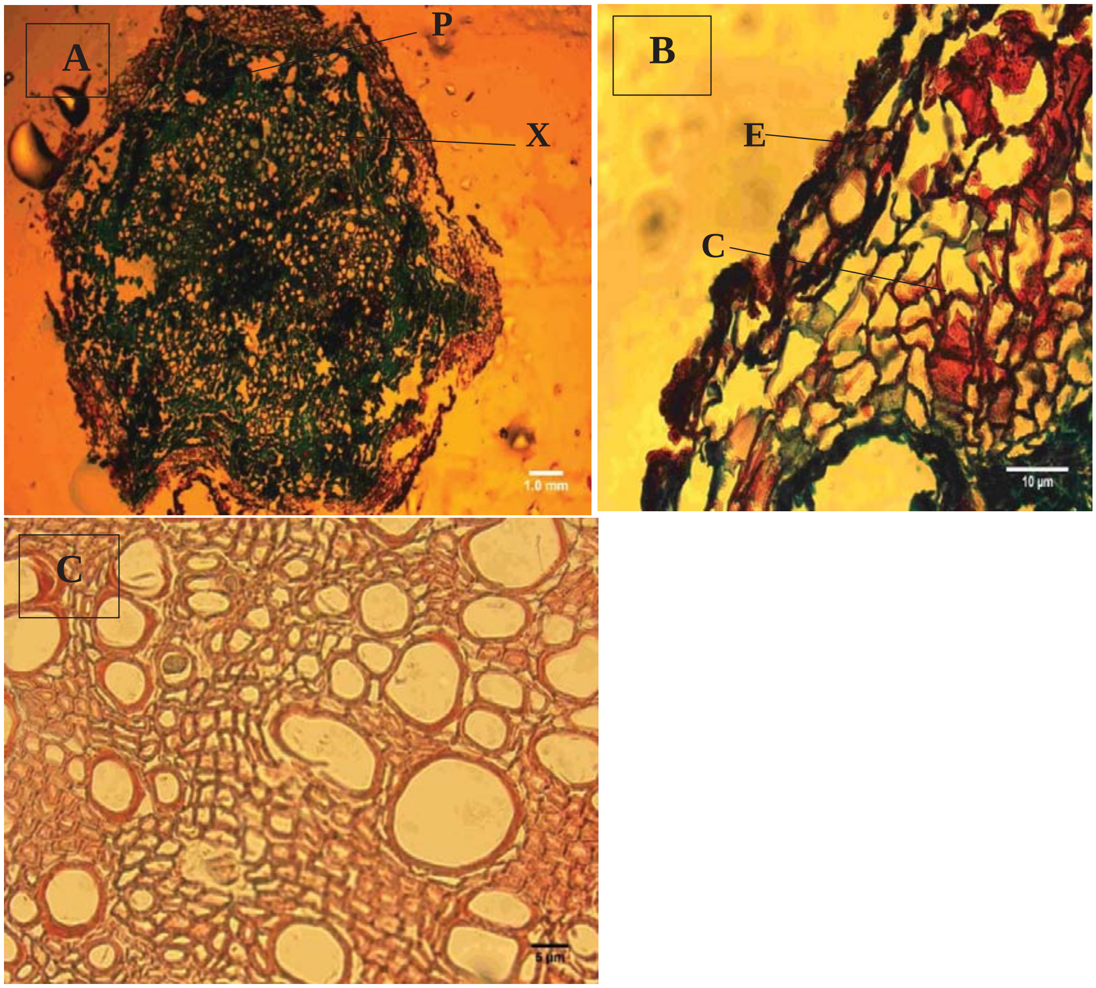
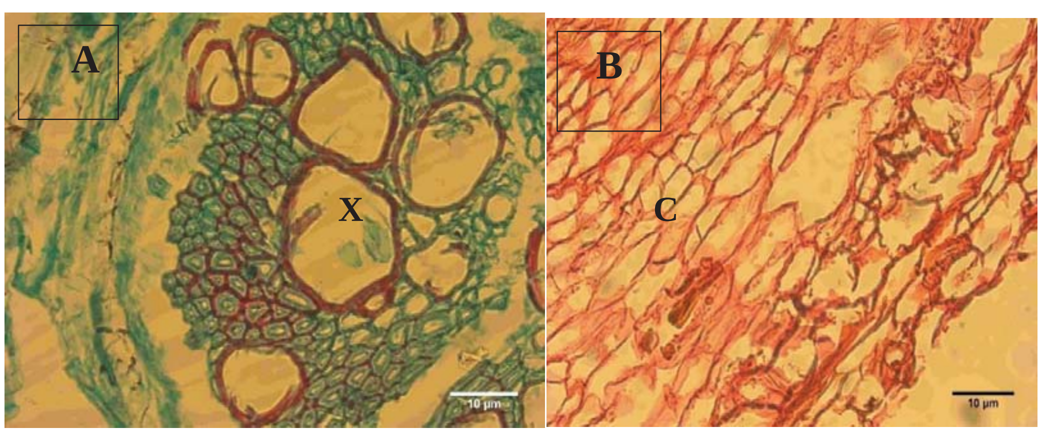


Figure 12: The root section of *Artemisia campestris.* A- cross section B-epidermis and cortex, C-xylem.



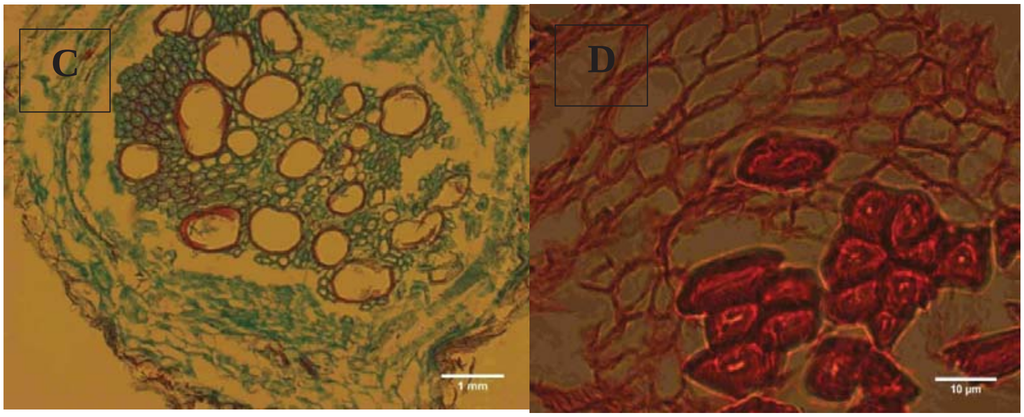


Figure 13: The root section of *Artemisia carruthii*. A-xylem, B- epidermis and cortex, C- whole section, D- phloem

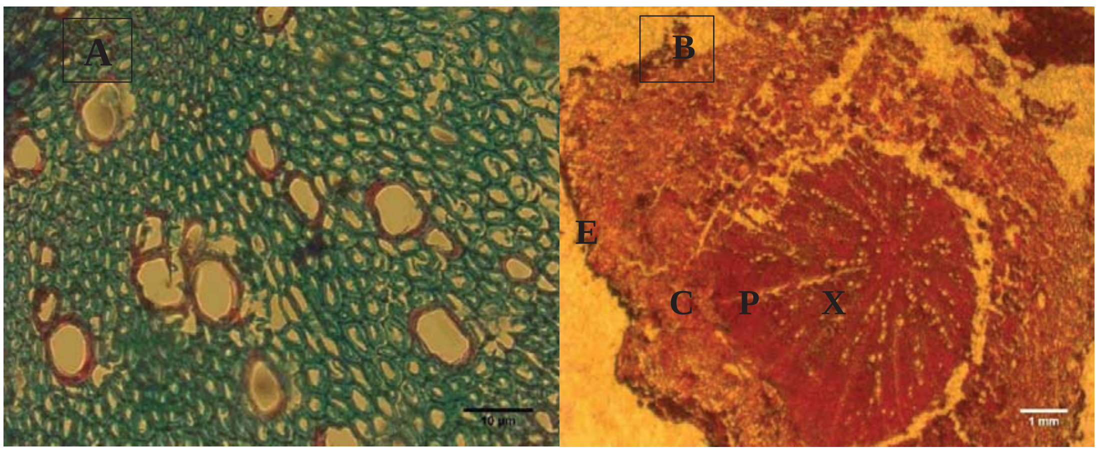
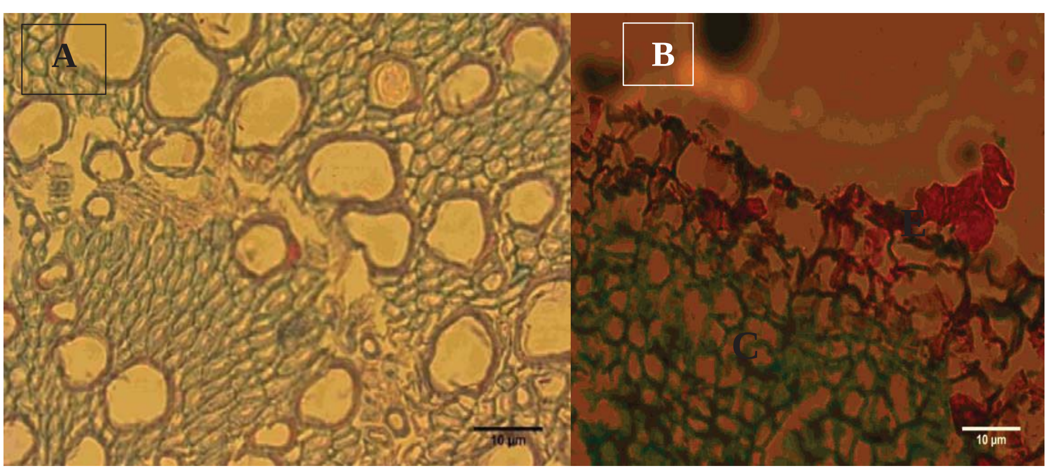


Figure 14: The root sections of *Artemisia dracunculus.* A- Xylem, B- Section of root (E) epidermis, (C) cortex, (P) phloem, (X) xylem.



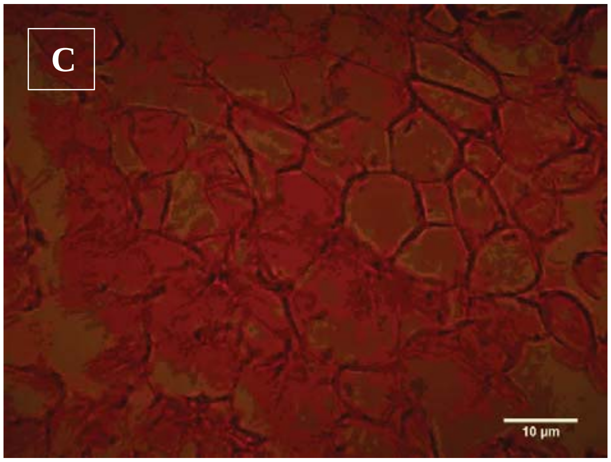


Figure 15: The section root of *Artemisia filifolia.* A-Xylem, B- epidermis and cortex, C- phloem

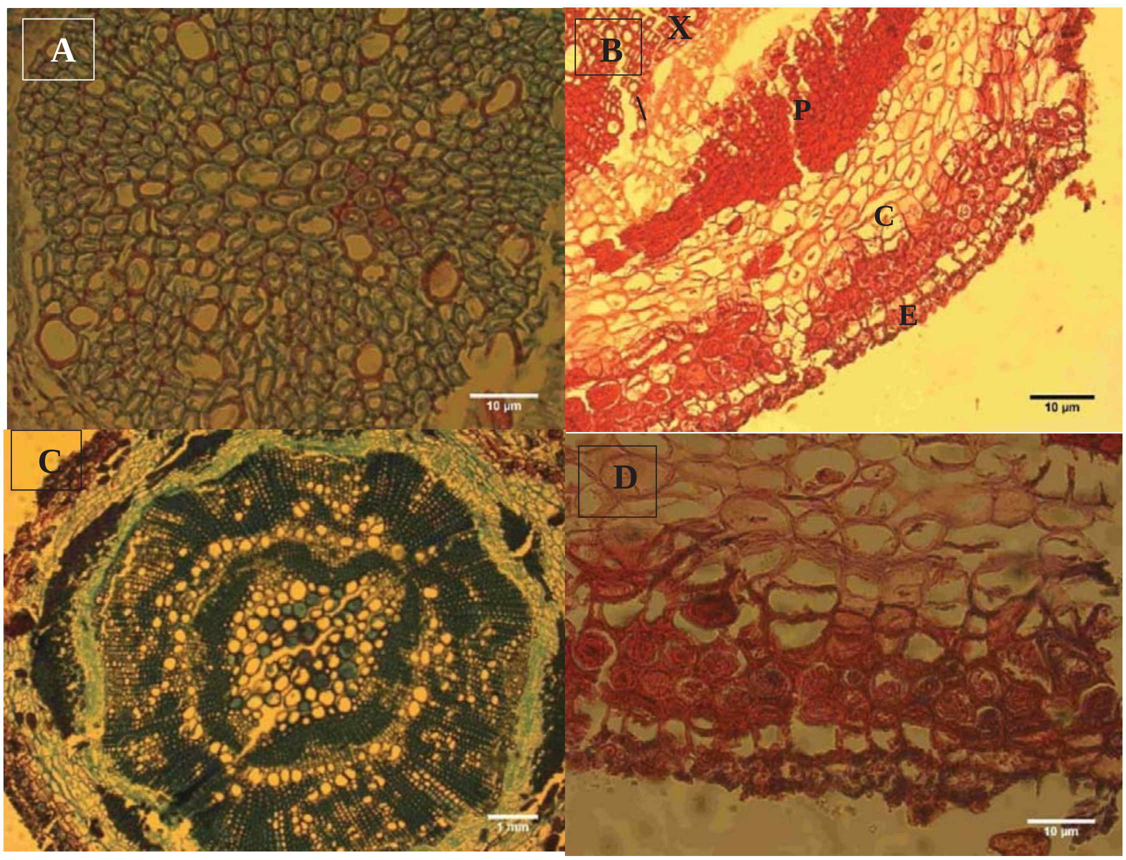


Figure 16: The root section of *Artemisia ludoviciana*. A- Xylem, B- epidermis, cortex, xylem, phloem. C- whole section, D-epidermis and cortex.

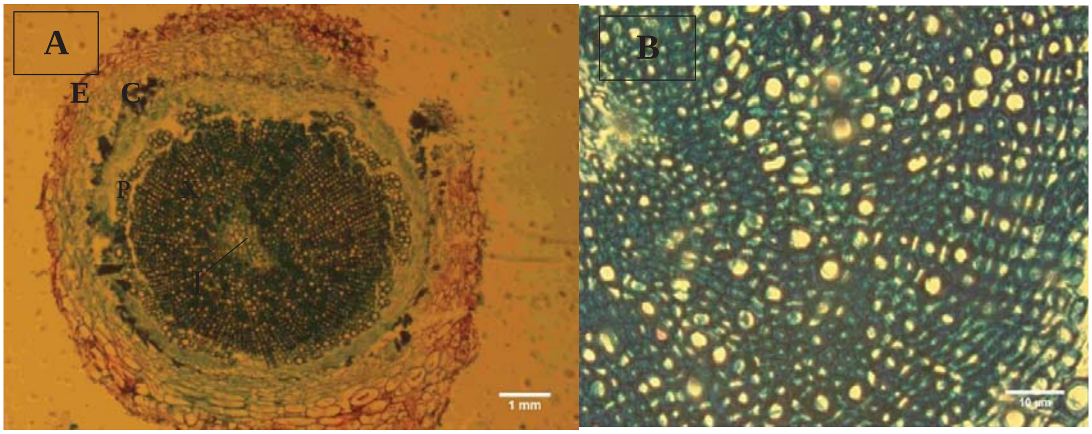


Figure 17: The root section of *Antennaria neglecta*. A-the whole section (epidermis, cortex, xylem, phloem, pith), B-Xylem.

(C-2)-Stems

I used 10 cells for measuring the average of cells' area, and I examined them under 200x.

(C-2-1) Epidermis

The largest average size of epidermis in stem in *Artemisia* species is *Artemisia dracunculus* (4.40 ±0.13 μm^2) (Figure 20), and the smallest average size of epidermis is *A. filifolia* (2.20±0.04 μm^2) (Figure21). Other average size of epidermis of *Artemisia* species are *A. carruthii* (3.50±0.06 μm^2) (Figure19), *A. campestris* (3.25±0.03 μm^2) (Figure 18B) and *A. ludoviciana* (2.60±0.03 μm^2) (figure 22). The average size of epidermis of *Antennaria neglecta* is 4.05±0.05 μm^2 (Figure 23A).

(C-2-2)- Cortex

The sequence of average size of cortex in stems from larger to smaller is *Artemisia filifolia* (6.35 ±0.18 μm^2) (Figure21), *A. ludoviciana* (6.05±0.01 μm^2) (Figure22), *A. dracunculus* (3.65±0.20 μm^2) (Figure20), *A. campestris* (2.65±0.05 μm^2) (Figure18B) and *A. carruthii* (1.40±0.04 μm^2) (Figure19). The average size of cortex of *Antennaria neglecta* is 15.50±0.33 μm^2 (Figure 23A).

(C-2-3)- Xylem(Vessels)

The largest average size of vessels in the stem in *Artemisia* species is *Artemisia campestris* (4.10±0.16 µm^2) (Figure 18A), and the smallest average size of vessels is *A. dracunculus* (0.90±0.03 µm^2) (Figure20). Other average size of vessels of *Artemisia* species are *A. carruthii* (2.90±0.06 µm^2) (Figure19), *A. ludoviciana* (2.45±0.12 µm^2) (Figure22) and *A. filifolia* (1.40± 0.04µm^2) (Figure21). The average size of vessels of *Antennaria neglecta* is 4±0.06 µm^2 (Figure 23B).

(C-2-4)- Phloem (Sieve tubes)

The sequence of average size of sieve tubes in stems from larger to smaller is *Artemisia ludoviciana* (0.65±0.01 µm^2) (Figure 22), *A. filifolia* (0.29±011 µm^2) (Figure21), *A. campestris* (0.25 ±0.01 µm^2) (Figure18B), *A. dracunculus* (0.10±0.01 µm^2) (Figure20) and *A. carruthii* (0.05±0.01 µm^2) (Figure19). The average size of sieve tubes of *Antennaria neglecta* is 2.05±0.04 µm^2 (Figure 23B).

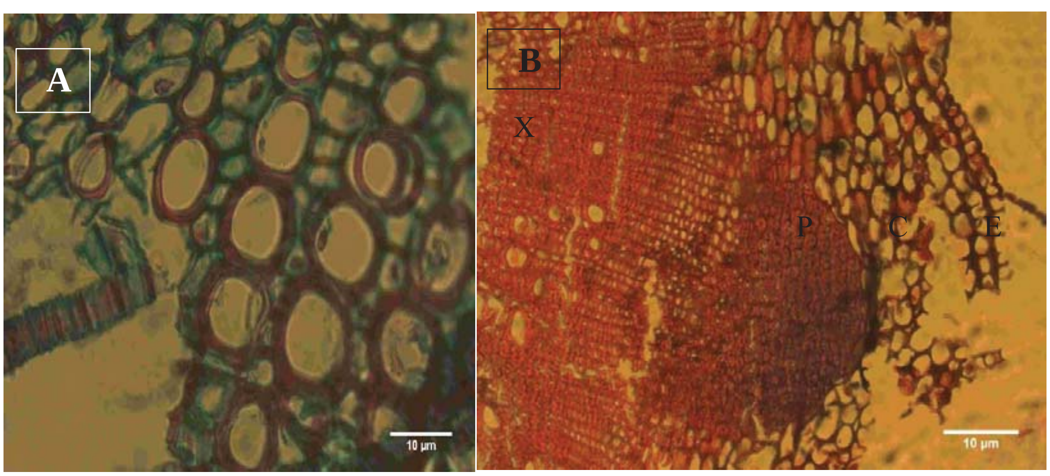


Figure 18: The stems section of *Artemisia campestris*. A- xylem, B- epidermis, cortex, xylem, phloem.

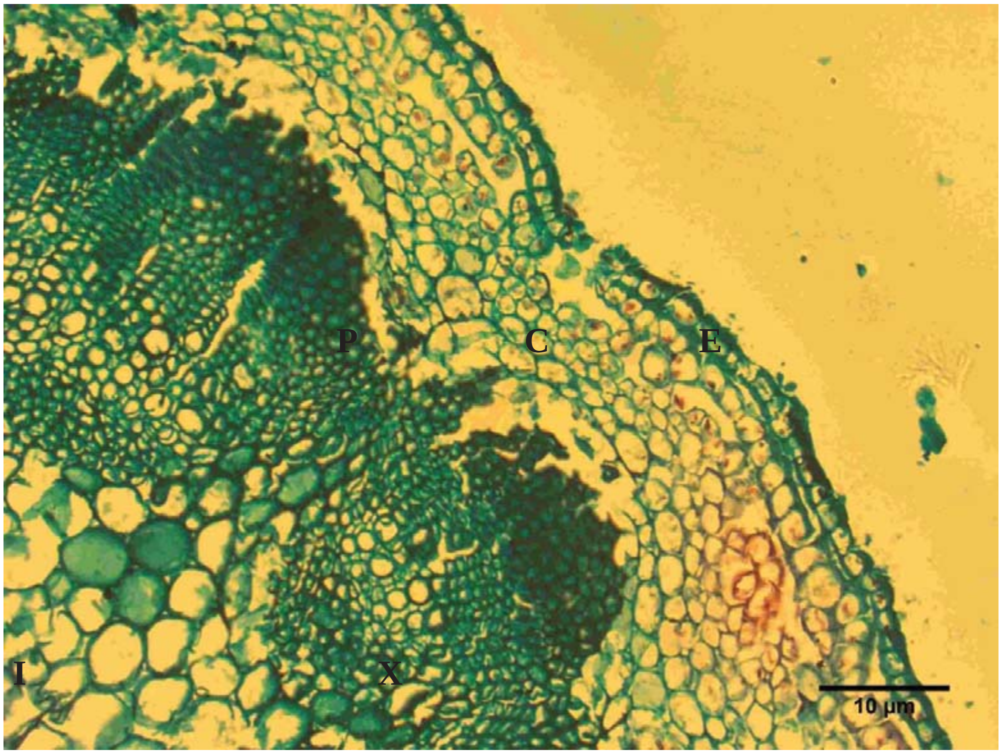


Figure 19: The stem section of *Artemisia carruthii* (epidermis, cortex, xylem, phloem, pith)

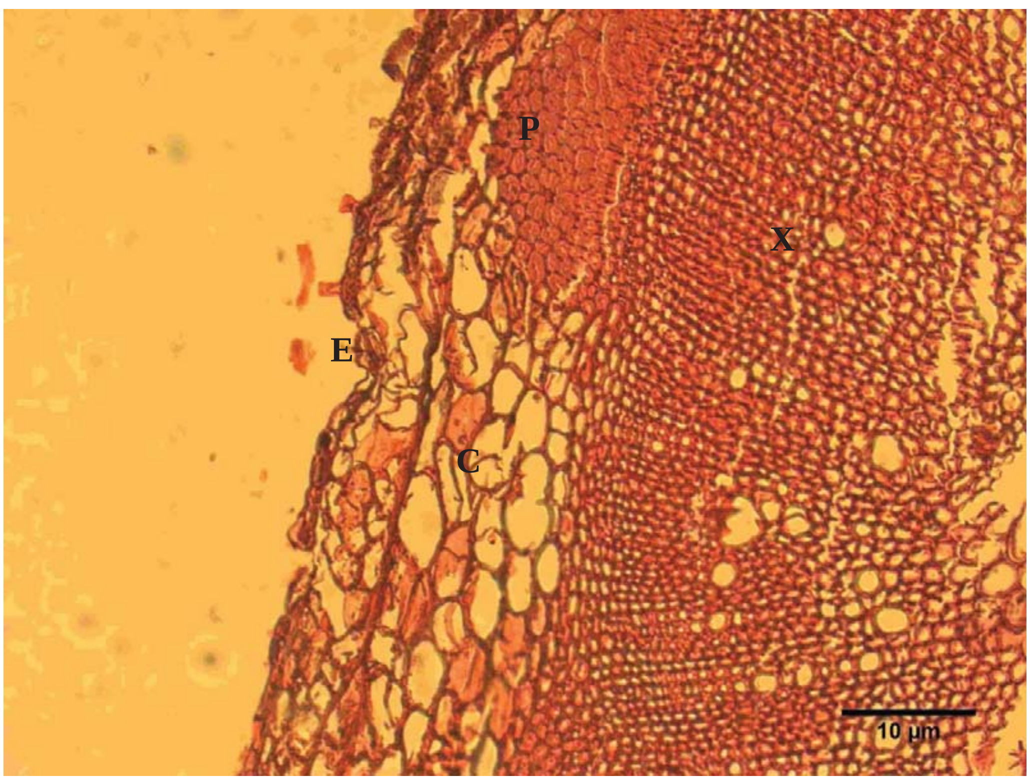


Figure 20: The stem section of *Artemisia dracunculus* (epidermis, cortex, xylem, phloem).

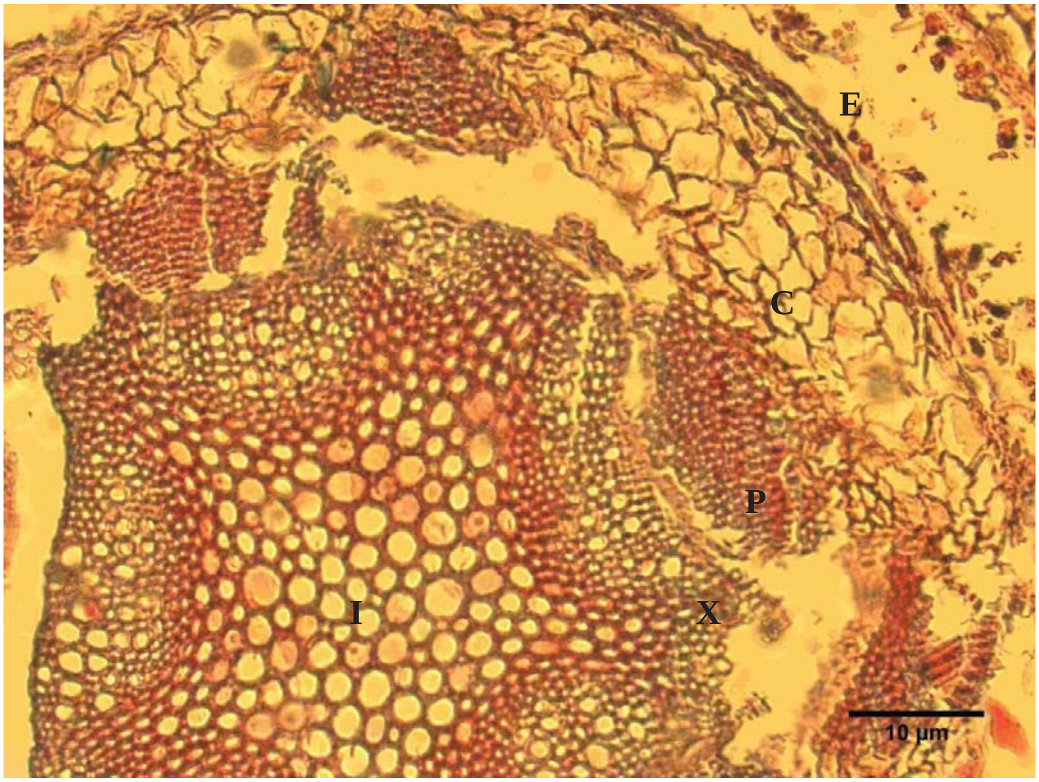


Figure 21: The stem section of *Artemisia filifolia.* (epidermis, cortex, xylem, phloem, pith).

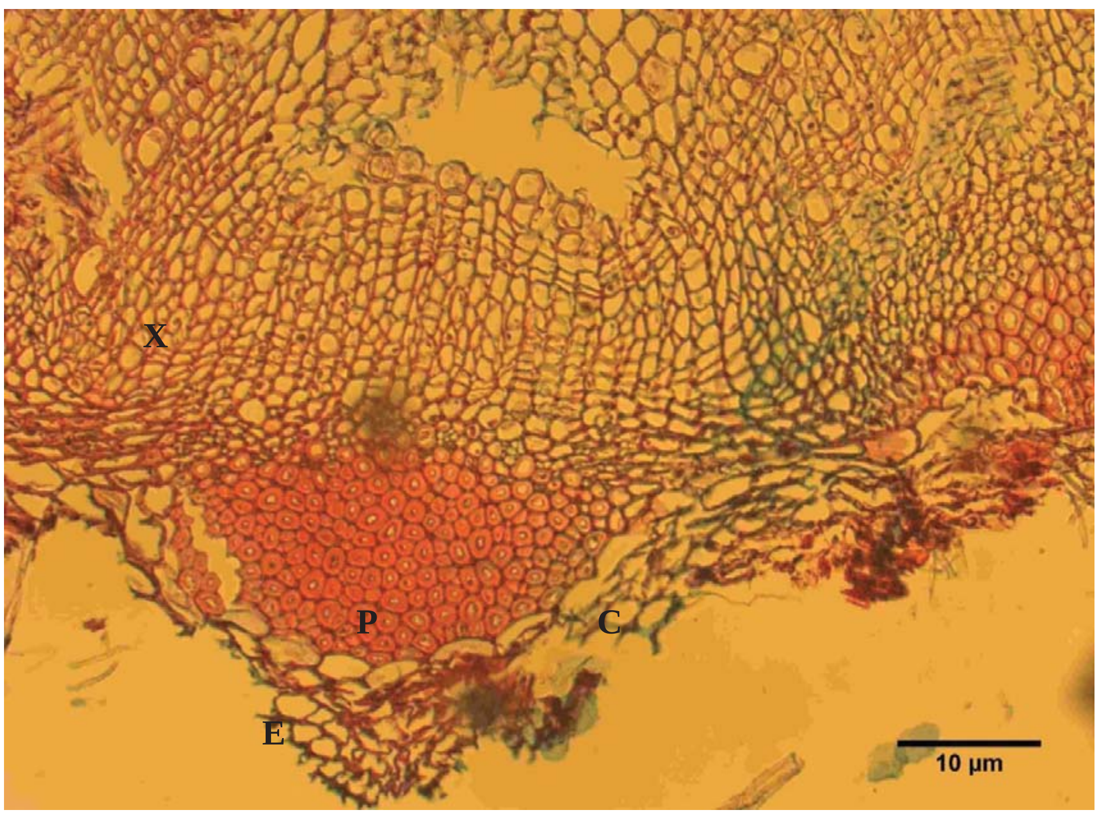


Figure 22: The stem section of *Artemisia ludoviciana* (epidermis, cortex, xylem, phloem)

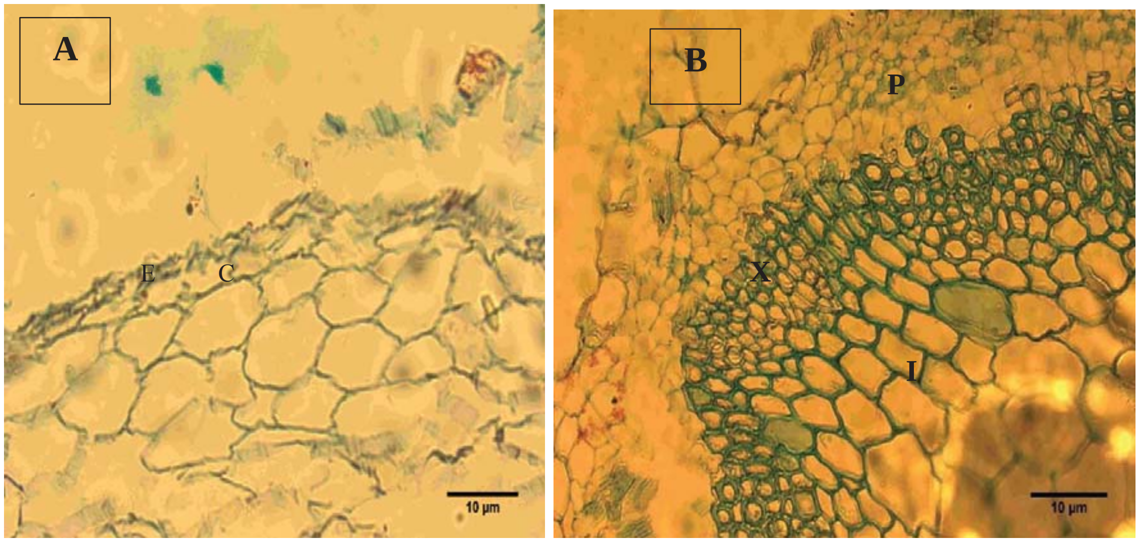


Figure 23: The stem section of *Antennaria neglecta* (A-epidermis and cortex, B- xylem, phloem and pith).

(C-3) Leaves

I used 10 cells for measuring the average of cells' area, and I examined them under 400x.

(C-3-1)- Epidermis

The sequence of average size of epidermis in leaves from larger to smaller is *Artemisia carruthii* (7.95±0.11 μm^2) (Figure 25A), *A. filifolia* (6.05±0.09 μm^2) (Figure 27), *A. ludoviciana* (4.65±0.31 μm^2) (Figure28B), *A. dracunculus* (3.60±0.05 μm^2) (Figure26) and *A. campestris* (2.40±0.05 μm^2) (Figure 24). The average size of epidermis of *Antennaria neglecta* is 4.20±0.11 μm^2 (Figure 29B).

(C-3-2)- Palisade mesophyll cells

The largest average size of palisade mesophyll cells in leaves in *Artemisia* species is *Artemisia ludoviciana* (2.90±0.06 μm^2) (Figure 28B), and the smallest average size of palisade mesophyll cells is *A. filifolia* (1±0.03 μm^2) (Figure 27). Other average size of palisade mesophyll cells of *Artemisia* species are *A. carruthii* (2.65±0.06 μm^2) (Figure 25B), *A. campestris* (2.05±0.04 μm^2) (Figure 24), and *A. dracunculus* (1.90±0.04 μm^2) (Figure 26). The average size of palisade mesophyll of *Antennaria neglecta* is 8.10±0.20 μm^2 (Figure 29B).

(C-3-3)- Spongy mesophyll cells

The sequence of average size of spongy mesophyll cells in leaves from larger to smaller is *Artemisia campestris* (4.55±0.05 μm^2) (Figure24), *A. ludoviciana* (3.70±0.08 μm^2) (Figure28B), *A. carruthii* (3.40±0.11 μm^2) (Figure25B), *A. dracunculus* (1.25±0.02 μm^2) (Figure26) and *A. filifolia* (0.65±0.02 μm^2) (Figure 27). The average size of spongy mesophyll cells of *Antennaria neglecta* is 4.75±0.10 μm^2(Figure 29B).

(C-3-4)- Xylem(Vessels)

The largest average size of vessels in leaves in *Artemisia* species is *Artemisia ludoviciana* (4.35±0.09μm^2) (Figure 28A), and the smallest average size of vessels is *Artemisia filifolia* (1.10 ±0.05 μm^2) (Figure 27). Other average size of vessels of *Artemisia* species are *A. campestris* (3.95±0.06 μm^2) (Figure 24), *A. carruthii* (3±0.07 μm^2) (Figure 25B) and *A. dracunculus* (1.70±0.02 μm^2) (Figure 26). The average size of vessels of *Antennaria neglecta* is 2.20±0.05 μm^2 (Figure 29A).

(C-3-5)- Phloem (Sieve tubes)

The sequence of average size of sieve tubes in leaves from larger to smaller is *Artemisia ludoviciana* (2.45±0.06μm^2) (Figure 28A), *A. campestris* (2.28±0.01 μm^2) (Figure 24), *A. dracunculus* (1.15±0.03 μm^2) (Figure 26), *A. carruthii* (1.05±0.03

µm^2) (Figure 25B) and *A. filfiolia* (0.35±0.01 µm^2) (Figure 27). The average size of sieve tubes of *Antennaria neglecta* is 0.90 ±0.05 µm^2(Figure 29A).

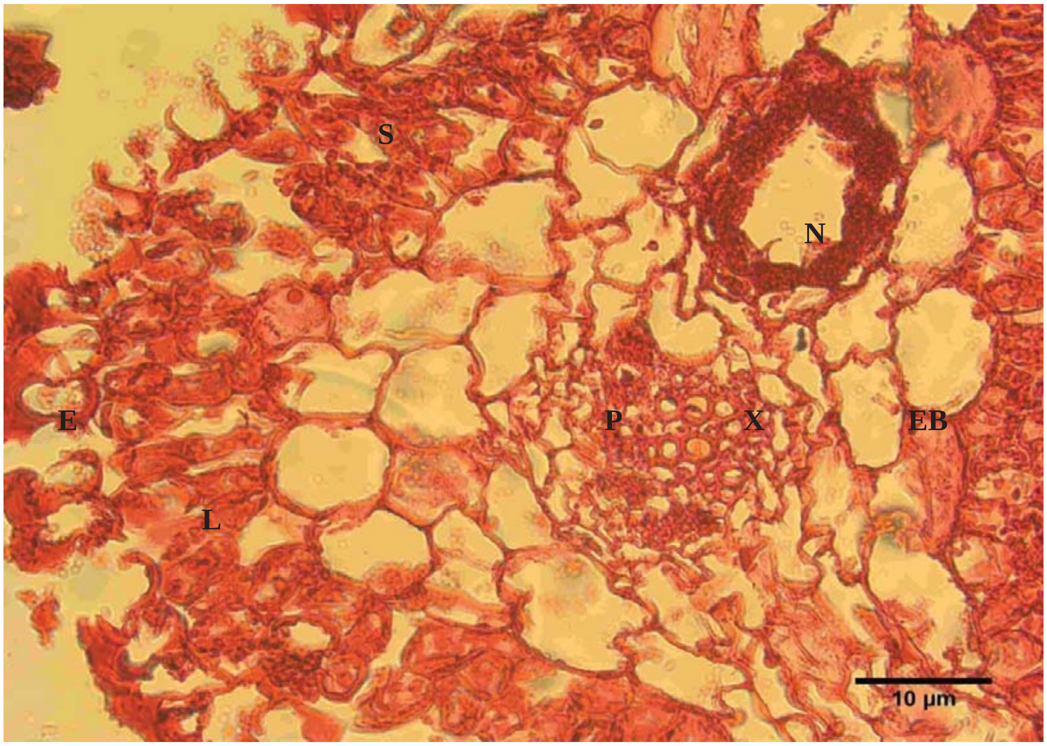


Figure 24: The leaf section of *Artemisia campestris* (epidermis, xylem, spongy mesophyll cells(s), palisade mesophyll cells (L), Canals (N), extension bundles (EB).

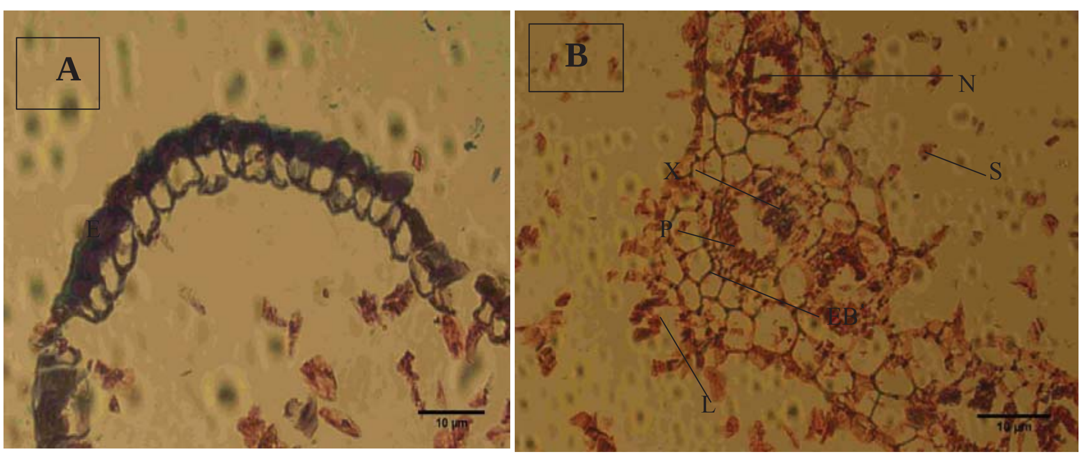


Figure 25: The leaf section of *Artemisia carruthii* (A- epidermis, B- xylem, spongy mesophyll cells(s), palisade mesophyll cells (L), Canals (N), extension bundles (EB).

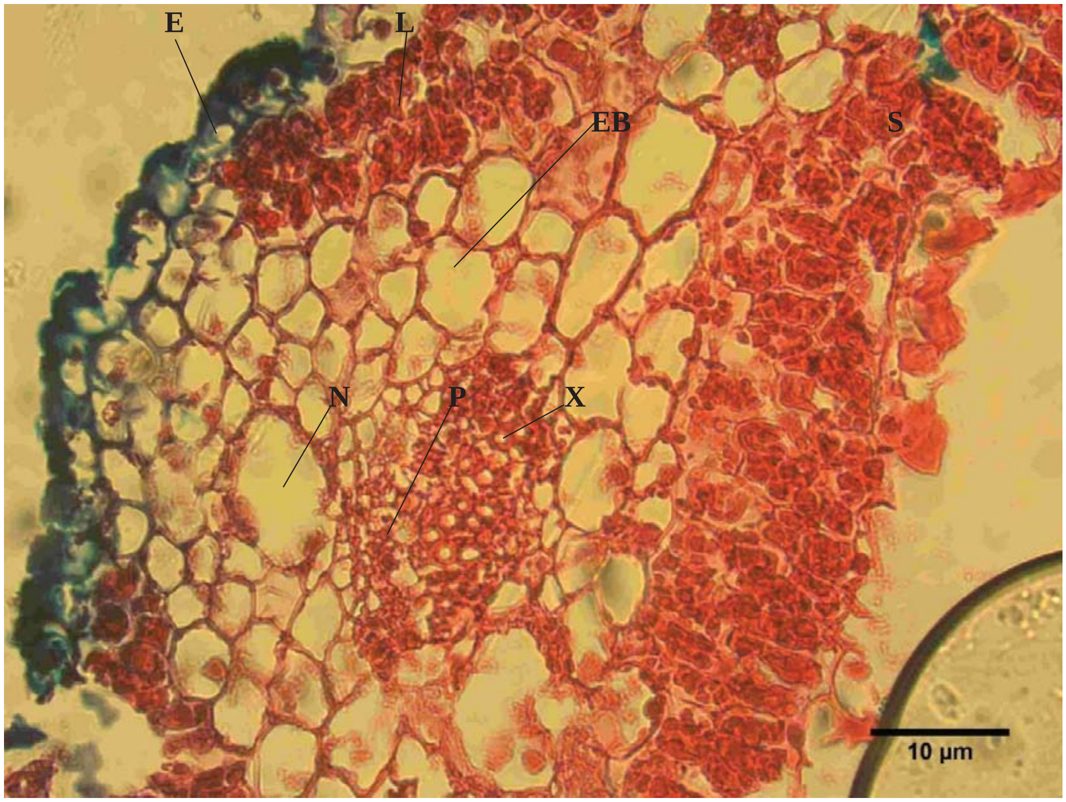


Figure 26: The leaf section of *Artemisia dracunculus* (epidermis, xylem, spongy mesophyll cells(s), palisade mesophyll cells (L), Canals (N), extension bundles (EB).

Figure 27: The leaf section of *Artemisia filifolia***.** (epidermis, xylem, spongy mesophyll cells(s), palisade mesophyll cells (L) extension bundles (EB).

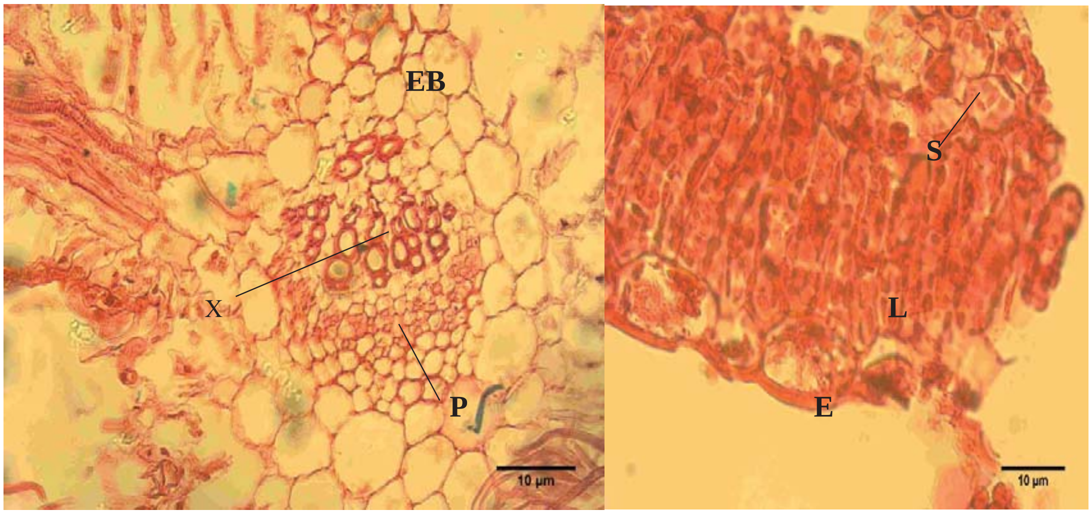


Figure 28: The leaf section of *Artemisia ludoviciana*. A- xylem, phloem, extension bundles (EB), B- epidermis, spongy mesophyll cells(s), palisade mesophyll cells (L).

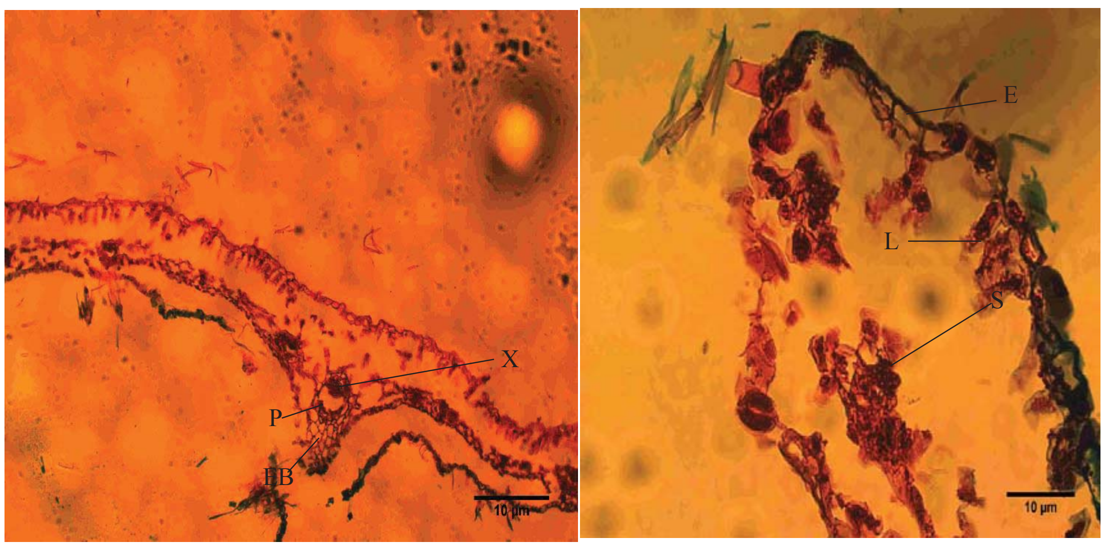


Figure 29: The leaf section of *Antennaria neglecta*. A- xylem, phloem, extension bundles (EB), B- epidermis, spongy mesophyll cells(s), palisade mesophyll cells (L).

(C-3-6)- Stomata

I used 20 cells for measuring the average of stomata size, and I examined them under 400x.

(C-3-6-1)- The length of stomata

The largest average length of stomata in leaves in *Artemisia* species is *Artemisia dracunculus* (9.80±0.01 µm) (Figure 30C), and the smallest average length of stomata is *A. filifolia* (0.50±0.05 µm) (Figure 30D). Other average length of stomata of *Artemisia* species are *A. campestris* (9.05±0.09 µm) (Figure 30A), *A. ludoviciana* (3±0.05 µm) (Figure 30E), *A. carruthii* (1.45±0.13 µm) (Figure 30B). The average length of stomata of *Antennaria neglecta* is 2.20±0.03 µm (Figure 30F).

(C-3-6-2)- The size of guard cells

The sequence of average width of guard cells in leaves from larger to smaller is *Artemisia campestris* (5±0.05 µm) (Figure 30A), *A. dracunculus* (4.80±0.03 µm) (Figure 30C), *A. carruthii* (2.50±0.05 µm) (Figure 30B), *A. ludoviciana* (2.15±0.02 µm) (Figure 30E) and *A. filifolia* (1.10±0.01 µm) (Figure 30D). The average width of guard cells of *Antennaria neglecta* is 1.30±0.11 µm (Figure 30F).

The largest average length of guard cells in leaves in *Artemisia* species is *Artemisia campestris* (16.10±0.07 µm) (Figure 30A), and the smallest average length of guard cells is *A. filfiolia* (4.25±0.05 µm) (Figure 30D). Other average length of guard cells of *Artemisia* species are: *A. dracunculus* (15±0.01 µm) (Figure 30C), *A. carruthii* (8.60±0.03 µm) (Figure 30B), *A. ludoviciana* (7.25±0.04 µm) (Figure 30E). The average width of guard cells of *Antennaria neglecta* is 4.60±0.05 µm (Figure 30F).

(C-3-6-3)- Density of stomata

I measured the density data three times and calculated the average of stomata density. The sequence of average quantity of stomata in 25 mµ ×25 mµ (400x) from more to less is: *Artemisia dracunculus* (39 stomata), *Artemisia campestris* (32 stomata), *Artemisia carruthii* (27 stomata), *Artemisia filiolia* (13 stomata) and *Artemisia ludoviciana* (11 stomata). The average quantity of stomata of *Antennaria neglecta* is 25 stomata in 25 mμ ×25 mμ (400x).

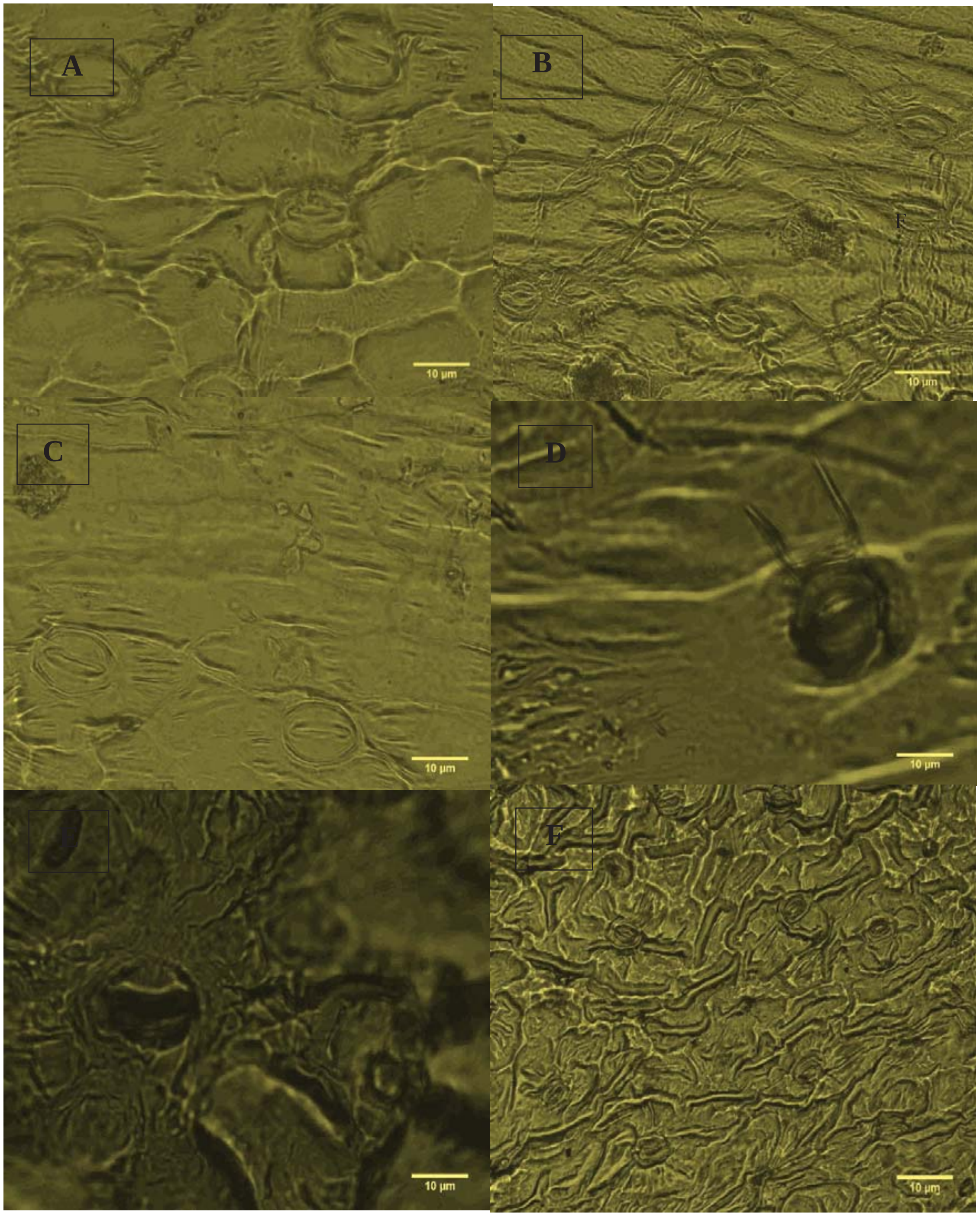


Figure 30`: The stomata of species. A- *Artemisia campestris,* B- *Artemisia carrthii*, C- *Artemisia dracunculus,* D- *Artemisia filifolia* , E- *Artemisia ludoviciana* and F- *Antennaria neglecta*.

<u>(C-3-7)- Canals and extension bundles presenting</u>

All Artemisia species and *Antennaria neglecta* have extension bundles. Canals are present in *Artemisia campestris* (Figure 24), *Artemisia carruthii* (Figure 25B) and *Artemisia dracunculus* (Figure 26), but they absent in *Artemisia filiolia*, *Artemisia ludoviciana* and *Antennaria neglecta*.

<u>(C-4)- Flowers</u>

I used 5 flowers for measuring the average of length of disk florets and ray floret, and I examined them under 20x.

(C-4-1)-Disk florets

The sequence of average length of disk florets in flowers from larger to smaller is *Artemisia carruthii* (Figure 32A) and *A. ludoviciana* (1.3 mm) (Figure 35A), *A. campestris* (1.2 mm) (Figure 31A), *A. dracunculus* (1.1 mm) (Figure 33A) and *A. filifolia* (0.7mm) (Figure 34A). The average length of male disk florets of *Antennaria neglecta* is 14 mm (Figure 36 B), and female disk florets is 11 mm (Figure 36 A).

(C-4-2)-Ray florets

The largest average length of ray florets in flowers in *Artemisia* species is *Artemisia carruthii* (2.3 mm) (Figure 32B), and the smallest average length of ray florets is *A. filifolia* (0.8 mm) (Figure 34B). Other average length of ray florets of *Artemisia* species are *A. campestris* (2.1 mm) (Figure 31B), *A. ludoviciana* (1.7 mm) (Figure 35B) and *A. dracunculus* (1.6 mm) (Figure 33B). *Antennaria neglecta* does not have ray florets.

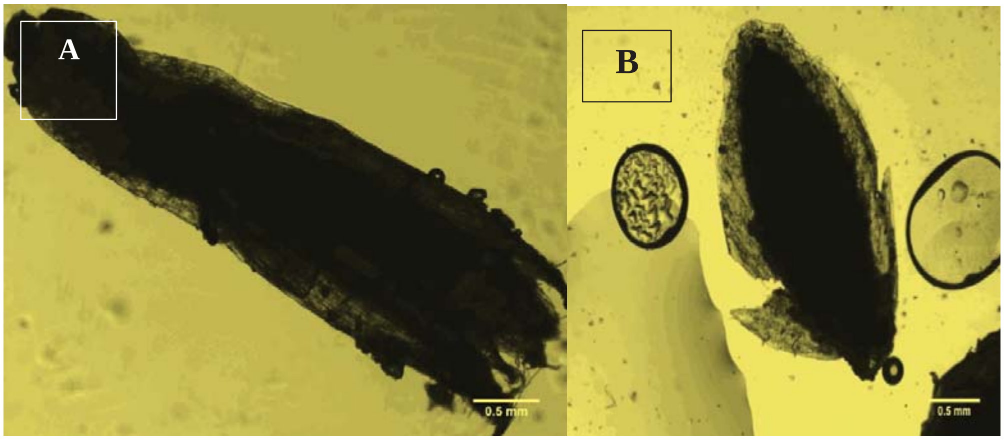


Figure 31: The florets of *Artemisia campestris.* A- disk floret, B- ray floret

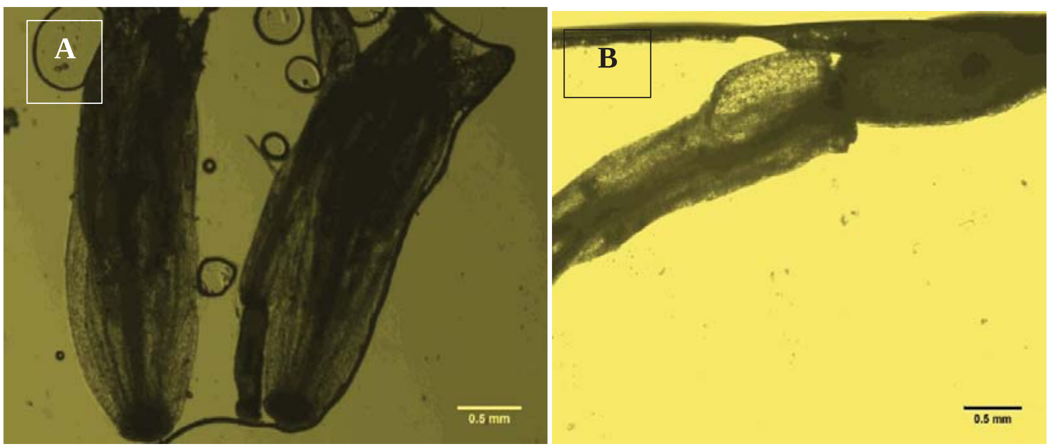


Figure 32: The florets of *Artemisia carruthii* A- disk floret, B- ray floret.

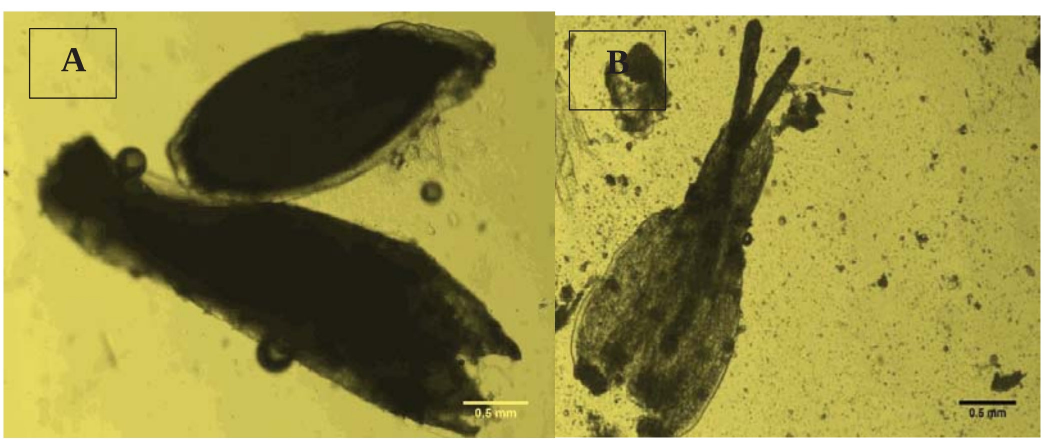


Figure 33: The florets of *Artemisia dracunculus* A- disk floret, B- ray floret.

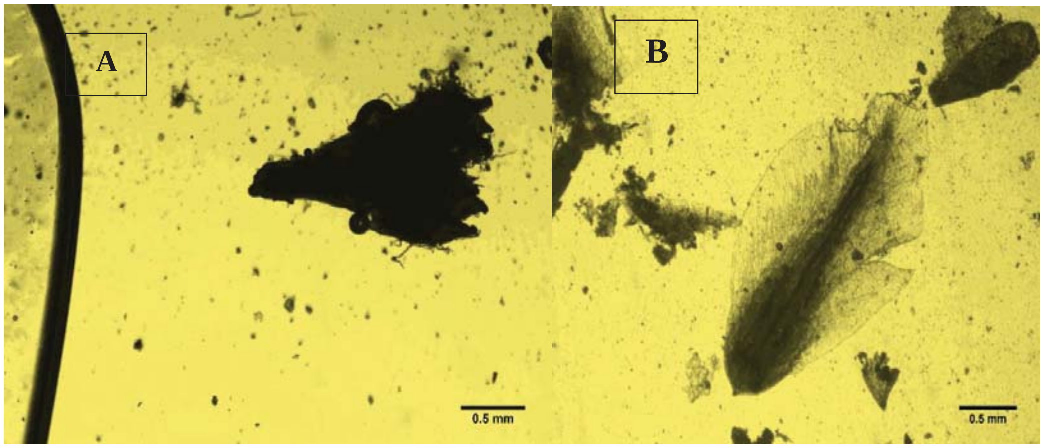


Figure 34: The florets of *Artemisia filifolia* A- disk floret, B- ray floret.

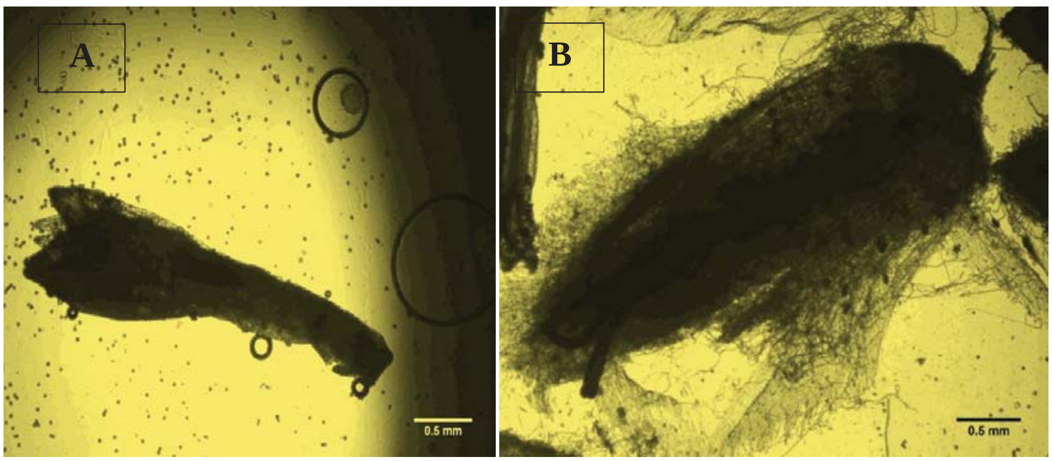


Figure 35: The florets of *Artemisia ludoviciana* A- disk floret, B- ray floret.

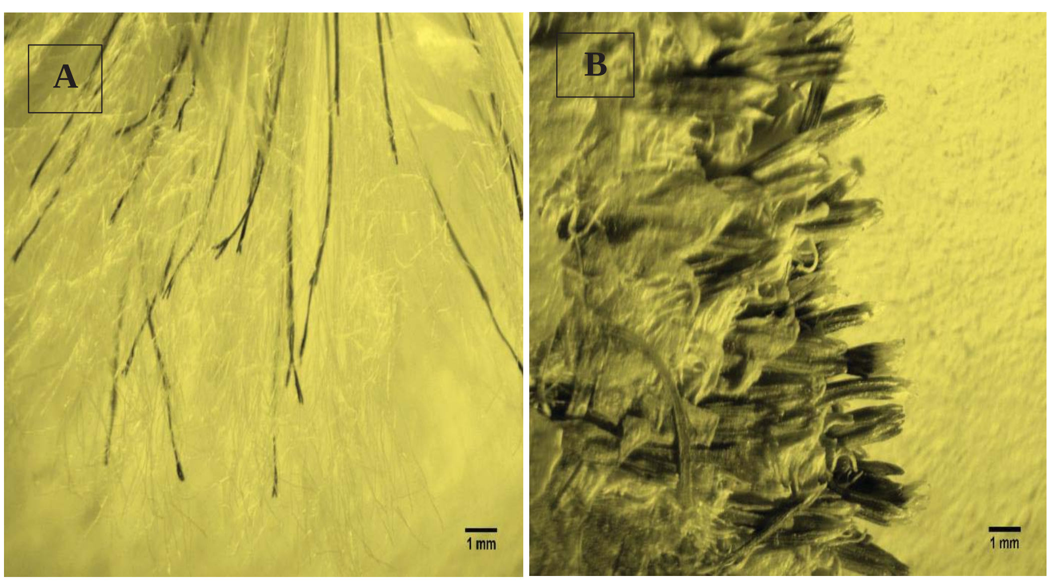


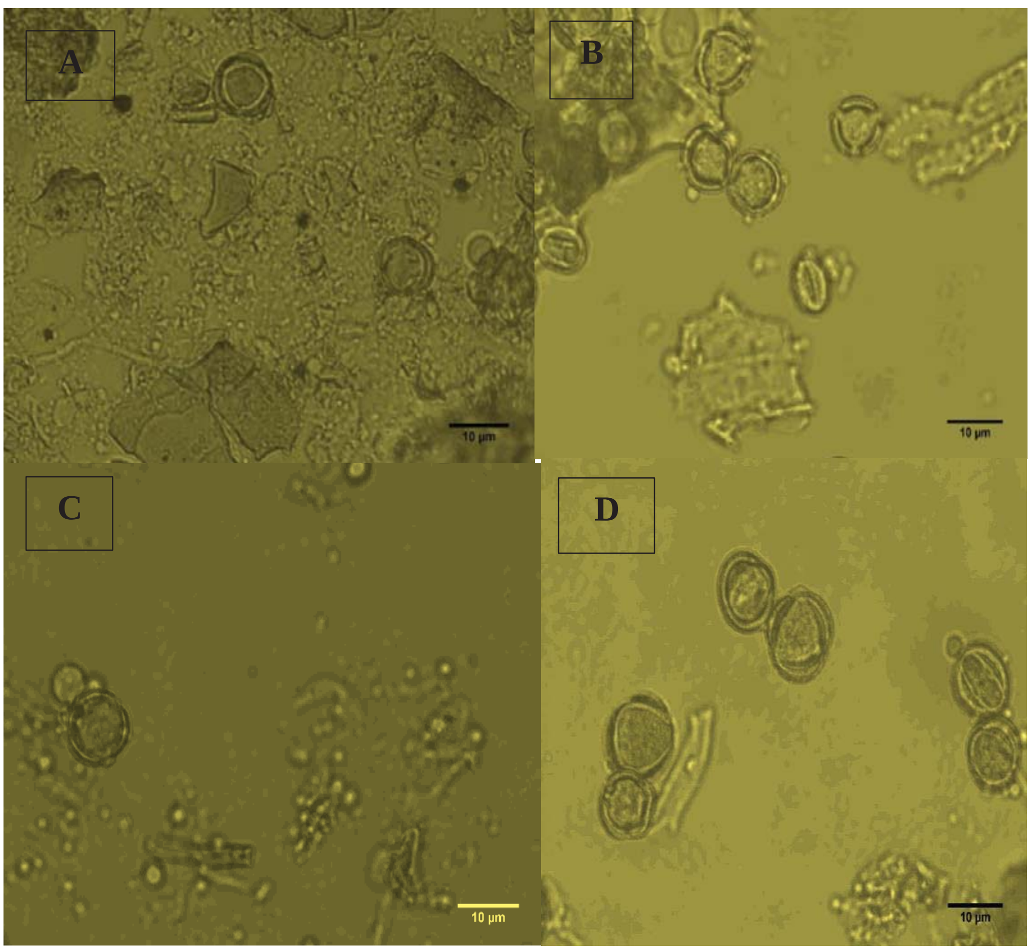
Figure 36: The disk florets of *Antennaria neglecta* A- female disk floret, B- male disk floret.

(C-4-3) Pollen

I used 30 pollen for measuring the average of size of pollen, and I examined in 400x.

(C-4-3-1)-The size(area) of pollen

The sequence of average size of pollen from larger to smaller is *Artemisia ludoviciana* (16.10±0.14 μm^2) (Figure 37E), *A. filifolia* (15.20±0.15 μm^2) (Figure 37D), *A. dracunculus* (15.10±0.12 μm^2) (Figure 37C), *A. carruthii* (12.85±0.11 μm^2) (Figure 37B) and *A. campestris* (11.05±0.11 μm^2) (Figure 37A). The average size of pollen of *Antennaria neglecta* is 14.85±0.15 μm^2 (Figure 37F).



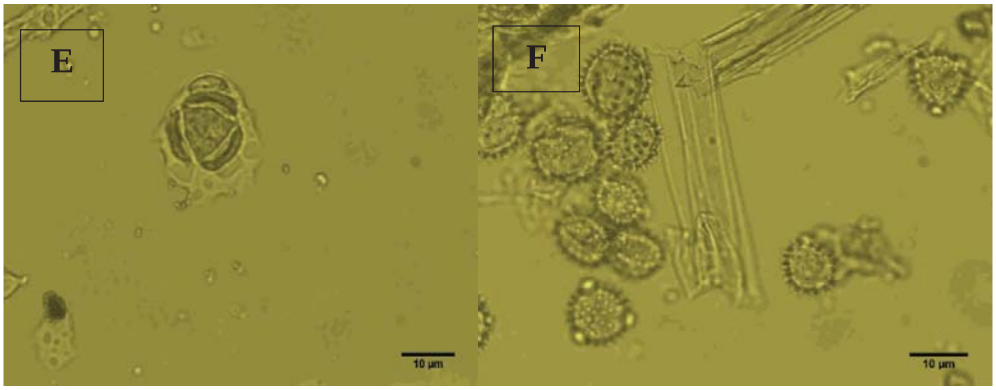


Figure 37: The pollen of the species. A- *Artemisia campestris,* B- *Artemisia carrthii*, C- *Artemisia dracunculus,* D- *Artemisia filifolia* , E- *Artemisia ludoviciana* and F- *Antennaria neglecta*

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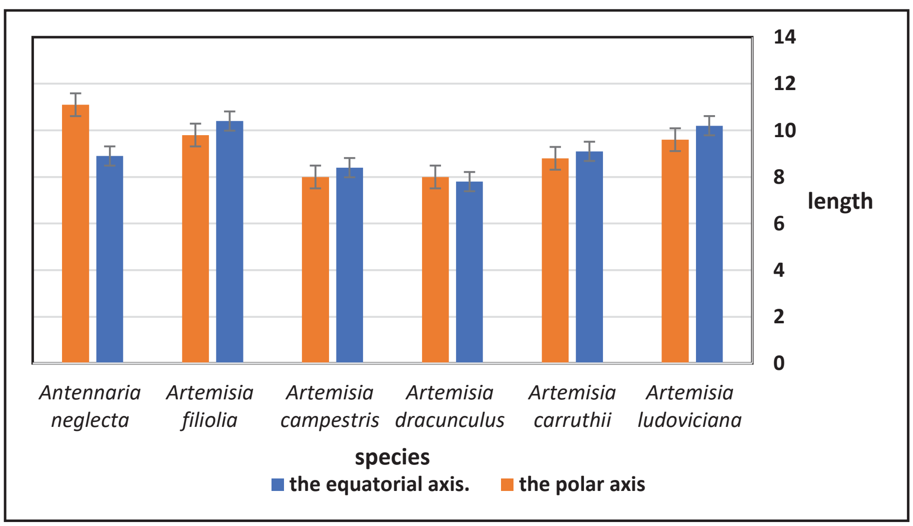
(C-4-3-2)- Type and shape of pollen

All species that I studied in this research have tricolporate type. The shape is determined by the ratio between length of the polar axis and length of the equatorial axis. *Artemisia ludoviciana* (9.6\10.2), *Artemisia campestris* (8 \8.4) and *Artemisia filiolia* (9.8\10.4) have oblate spheroidal shapes. *Artemisia dracunculus* (8\7.8) and *Artemisia carruthii* (8.8\9.1) have spheroidal shapes. *Antennaria neglecta* (11.1\8.9)



has a prolate spheroidal shape (Figure 38).

Figure 38: The variation between polar length and equatorial length.

(C-4-3-3)- Pollen wall thickness

The largest average wall thickness of pollen in *Artemisia* species is *Artemisia ludoviciana* (2.53 µm) (Figure 37E), and the smallest average wall thickness is *A. dracunculus* (0.86 µm) (Figure 37C). Other average wall thickness of *Artemisia* species are *A. filifolia* (1.84 µm) (Figure 37D), *A. carruthii* (1.77 µm) (Figure 37B) and *A. campestris* (1.27 µm) (Figure 37A). The larger average wall thickness of pollen in *Antennaria neglecta* is 0.56 µm (Figure 37F).

(B-4-3-4)-Pollen apertures

The sequence of average length of diameter of pollen apertures from larger to smaller is *Artemisia ludoviciana* (2.21 µm) (Figure 37E), *A. dracunculus* (2.06 µm) (Figure 37C), *A. filifolia* (1.97 µm) (Figure 37D), *A. campestris* (1.91 µm) (Figure 37A) and *A. carruthii* (1.16 µm) (Figure 37B). The average length of diameter of pollen apertures of *Antennaria neglecta* is 1.50 µm (Figure 37F).

(C-4-3-5)- Surface ornamentation

All *Artemisia* species have perforate surface ornamentation, and *Antennaria neglecta* has echinate surface ornamentation.

(C-5-1)-Table A

Table 3- raw data used in phylogenetic analysis of *Artemisia* species. Characters and character states are described in table.

chracters	*Antennaria neglecta*	*Artemisia.ludoviciana*	*A.filifolia*	*A.carruhii*	*A.campestris*	*A.dracunculus*
habit	Herb	Herb	shrub	Herb	Herb	Herb
growth habit	stoloniferous	rhizomatous	rhizomatous	rhizomatous	taproot	taproot
asecrding stem	no	no	no	yes	no	no
order of branched	simple	simple	much	simple	simple	simple
trichomes in stem	tomentose	tomentose	sericeous	tomentose	globarate	glabrous
arrangement of leaves	basal and alternate	alternate	alternate	alternate	alternate	alternate
bland types	lanceolate	lanceolate	linaer	pinnatifid	lanceolate	pinnatifid

margin types	entire	entire	entire	3-lobed	3-lobed	entire
length to width ratio of leaves	5	6	11.7	1.4	2	11
trichomes in leaves	tomentose	tomentose	glabrate	tomentose	glabrate	glabrous
inflorescence type	cyme	paniculate	paniculate	paniculate	paniculate	paniculate
heads arrays	1-6 heads	10-36 cm	6-15 cm	5-15 cm	2-34 cm	9.5-35 cm
flower color	white	white	white	yellow	yellow	yellow
head long	M (1.4 cm)-F(1.2cm)	0.2 cm	0.1cm	0.3 cm	0.3 cm	0.2 cm
head wide	M (1.3 cm)- F(1cm)	0.1 cm	0.1 cm	0.2 cm	0.2 cm	0.2 cm
number of disk florets	17-47 stamens, 27-49 carpels.	25	4	16	20	12
number of ray florets	do not have	8	2	3	12	15
flower type	Separate flowers	bisexual	bisexual	bisexual	bisexual	bisexual
disk florets type	separate	perfect	staminate	perfect	staminate	staminate
ray florets type	do not have	pistillate	pistillate	pistillate	pistillate	pistillate
trichome in heads	tomentous	tomentous	tomentous	glabrous	glabrous	glabrous
area of epidermis (root)	20.60 µm2	16.45 µm2	7.80 µm2	6.25 µm2	5.90 µm2	4.40 µm2
area of cortex (root)	5.60 µm2	2.55 µm2	6 µm2	5.55 µm2	1.90 µm2	0.25 µm2
area of vessels (root)	3.50 µm2	11.50 µm2	10 µm2	46 µm2	15.85 µm2	22.20 µm2
area of sieve tubes (root)	1.45 µm2	1.25 µm2	1.55 µm2	6.10 µm2	5.45 µm2	3.60 µm2
area of epidermis (stem)	4.05 µm2	2.60 µm2	2.20 µm2	3.50 µm2	3.25 µm2	4.40 µm2
area of cortex (stem)	15.50 µm2	6.05 µm2	6.35 µm2	1.40 µm2	2.65 µm2	3.65 µm2
area of vessels (stem)	4 µm2	2.45 µm2	1.40 µm2	2.90 µm2	4.10 µm2	0.90 µm2
area of sieve tubes (stem)	2.05 µm2	0.65 µm2	0.29 µm2	0.05 µm2	0.25 µm2	0.10 µm2
area of epidermis (leaf)	4.20 µm2	4.65 µm2	6.05 µm2	7.95 µm2	2.40 µm2	3.60 µm2
area of palisade mesophyll cells	8.10 µm2	2.90 µm2	1 µm2	2.65 µm2	2.05 µm2	1.90 µm2
area of spongy mesophyll cells	4.75 µm2	3.70 µm2	0.65 µm2	3.40 µm2	4.55 µm2	1.25 µm2
area of vessels (leaf)	2.20 µm2	4.35 µm2	1.10 µm2	3 µm2	3.95 µm2	1.70 µm2
area of sieve tubes (leaf)	0.90 µm2	2.45 µm2	0.35 µm2	1.05 µm2	2.28 µm2	1.15 µm2
length of stomata	2.20 µm	3 µm	0.50 µm	1.45 µm	9.05 µm	9.80 µm
width of guard cells	1.30 µm	2.15 µm	1.10 µm	2.50 µm	5 µm	4.80 µm
length of guard cells	4.60 µm	7.25 µm	4.25 µm	8.60 µm	16.10 µm	15 µm
Density of stomata	25	11	13	27	32	39
Canals presenting	no	no	no	yes	yes	yes
length of disk florets	M(14mm)- F(11mm)	1.3 mm	0.7 mm	1.3 mm	1.2 mm	1.1 mm
length of ray florets	do not have	1.7 mm	0.8 mm	2.3 mm	2.1 mm	1.6 mm
pollen area	14.85 µm2	16.10 µm2	15.20 µm2	12.85 µm2	11.05 µm2	15.10 µm2
shape of pollen	prolate spheroidal	oblate spheroidal	oblate spheroidal	spheroidal	oblate spheroidal	spheroidal
Pollen wall thickness	0.56 µm	2.53 µm	1.84 µm	1.77 µm	1.27 µm	0.86 µm

Pollen apertures	1.50 µm	2.21 µm	1.97 µm	1.16 µm	1.91 µm	2.06 µm
Surface ornamentation	echinate	perforate	perforate	perforate	perforate	perforate

(C-5-2)- Table B

Table 4-character state matrix used in phylogenetic analysis of *Artemisia* species. Characters and character states are described in table.

chracters	***Antennaria neglecta***	***Artemisia ludovician***	***A.filifolia***	***A.carruhii***	***A.campestris***	***A.dracunculus***
habit	0	0	1	0	0	0
growth habit	0	1	1	1	2	2
asecrding stem	0	0	0	1	0	0
order of branched	0	0	1	0	0	0
trichomes in stem	0	0	2	0	3	1
arrangement of leaves	0	1	1	1	1	1
bland types	0	0	2	1	0	1
margin types	0	0	0	1	1	0
length to width ratio of leaves	0	0	2	1	1	2
trichomes in leaves	0	0	1	0	1	2
inflorescence type	0	1	1	1	1	1
heads arrays	0	1	2	2	1	1
flower color	0	0	0	1	1	1
head long	0	2	3	1	1	2
head wide	0	3	3	2	2	2
number of disk florets	0	2	4	3	2	3
number of ray florets	0	1	4	4	2	2
flower type	0	1	1	1	1	1
disk florets type	0	2	1	2	1	1
ray florets type	0	1	1	1	1	1
trichome in heads	0	0	0	1	1	1
size of epidermis (root)	0	0	1	1	2	2
size of cortex (root)	0	1	0	0	2	3
size of vessels (root)	0	1	0	3	1	2
size of sieve tubes (root)	0	0	1	3	3	2
size of epidermis (stem)	0	2	2	1	1	0
size of cortex (stem)	0	1	1	3	2	2
size of vessels (stem)	0	1	2	1	0	2
size of sieve tubes (stem)	0	1	2	3	2	2
size of epidermis (leaf)	0	0	1	0	2	0
size of palisade mesophyll cells	0	1	3	1	2	3
size of spongy mesophyll cells	0	1	3	2	0	3
size of vessels (leaf)	0	2	3	1	1	0
size of sieve tubes (leaf)	0	2	0	1	2	1
length of stomata	0	2	1	0	3	3
width of guard cells	0	1	0	1	2	2
length of guard cells	0	1	0	1	2	2
Density of stomata	0	1	1	0	2	2

Canals presenting	0	0	0	1	1	1
length of disk florets	0	1	2	1	1	1
length of ray florets	0	2	1	3	3	2
pollen size	0	3	1	2	2	1
shape of pollen	0	2	2	1	2	1
Pollen wall thickness	0	3	2	1	1	0
Pollen apertures	0	1	0	2	0	1
Surface ornamentation	0	1	1	1	1	1

(C-5-3)-Table C

Table5- characters and character states of *Artemisia* species for phylogenetic analysis. The number in brackets represent the codes of character state

characters	**Character states**
habit	Herb (0), Shrub (1).
growth habit	Stoloniferous (0), Rhizome (1), taproot (2)
asecrding stem	Yes (0), no (1).
order of branched	simple (0), much (1).
trichomes in stem	tomentose(0), glabrous (1), sericeous(2), glabrate(3).
arrangement of leaves	basal (0), alternate (1).
bland types	Lanceolate (0), pinnatifid (1), linear (2).
margin types	Entire (0), lobed (1).
length to width ratio of leaves	4-6 (0), 1-3 (1), 7-12 (2).
trichomes in leaves	Tomentose (0), Gloabrate (1), Glabrous (2).
inflorescence type	Panicle (0), cyme (1).
heads arrays	5 cm≥ (0), 15 cm ≥ (1), 36 cm ≥ (2)
flower color	White (0), Yellow (1).
head long	14-12 mm (0), 3-4 mm (1), 2-1.1 mm (2), 1-0.1 mm (3).
head wide	13-10 mm (0), 4-3 mm (1), 2-1 mm (2)
number of disk florets	≥40 (0), 39-30 (1), 29-20 (2), 19-10 (3), 9-1 (4).
number of ray florets	0 (0), 6-10 (1), 11-15 (2), 16-20 (3), 1-5 (4).
flower type	Separate flowers (0), bisexual (1).
disk florets type	Separate flowers (0), male (1), perfect (2).
ray florets type	absent (0), female (1).
trichome in heads	Tomentose (0), Glabrous (1).

size of epidermis (root)	20-15 mμ (0), 9-6 mμ (1), 5.9-4 mμ (2).
size of cortex (root)	6-5.50 mμ (0), 3- 2.50 mμ (1), 2- 1.50 mμ (2), 0.5- 0.25 mμ (3).
size of vessels (root)	1-10 mμ (0), 11-20 mμ (1), 21-30 mμ (2), 41-50 mμ (3).
size of sieve tubes (root)	1-1.50 mμ (0), 1.51-3 mμ (1), 3.1-5 mμ (2), 5.1-6.1 mμ (2)
size of epidermis (stem)	4-5 mμ (0), 3.9-3 mμ (1), 2.9-2 mμ (2).
size of cortex (stem)	20-15 mμ (0), 7-6 mμ (1), 4-2 mμ (2), 1.50-1 mμ (3).
size of vessels (stem)	4.50- 4 mμ (0), 3-2 mμ (1), 1.90-0.50 mμ (2).
size of sieve tubes (stem)	2-1 mμ (0), 0.90- 0.50 mμ (1), 0.40- 0.10 mμ (2), 0.09-0.01 mμ (3).
size of epidermis (leaf)	5-3 mμ (0), 6-7 mμ (1), 2.90-2 mμ (2).
size of palisade mesophyll cells	9-8 mμ (0), 3-2.50 mμ (1), 2.40-2 mμ (2), 1.90-1 mμ (3).
size of spongy mesophyll cells	5-4.50 mμ (0), 4.40-3.50 mμ (1), 3.40-2 mμ (2), 1.9-0.5 mμ (3).
size of vessels (leaf)	2-2.50 mμ (0), 3-4 mμ (1), 4.1-5 mμ (2), 1.9-1 mμ (3).
size of sieve tubes (leaf)	0.1-0.9 mμ (0), 1-1.5 mμ (1), 2-3 mμ (3).
length of stomata	1-2.5mμ (0), 0.1-0.5 mμ (1), 3-5 mμ (2), 9-10 mμ (3).
width of guard cells	1-2 mμ (0), 2.1-3 mμ (1), 4-5 mμ (2).
length of guard cells	4-5 mμ (0), 6-8 mμ (1), 15-16.1 mμ (2).
Density of stomata	29-20 (0), 19-10 (1), 40-30 (2).
Canals presenting	absent (0), present (1).
length of disk florets	15-10 mm (0), 1.5- 1 mm (1), 0.9-0.5 mm (2).
length of ray florets	absent (0), 0.6-1 mm (1), 1.1-2 mm (2), 2.1-2.5 mm (3).
pollen size	14-13 mμ (0), 15.9-15 mμ (1), 12-11 mμ (2), 17-16 mμ (3).
shape of pollen	prolate (0), spherical (1), oblate (2).
Pollen wall thickness	0.5-1 mμ (0), 1.1-1.5 mμ (1), 1.6-2 mμ (2), 2-3 mμ (3).
Pollen apertures	1.9-1.5 mμ (0)- 2-2.5 mμ (1), 1.4-1.1 mμ (2).
Surface ornamentation	echinate (0), perforate (1).

(C-5-4)- Phylogeny tree

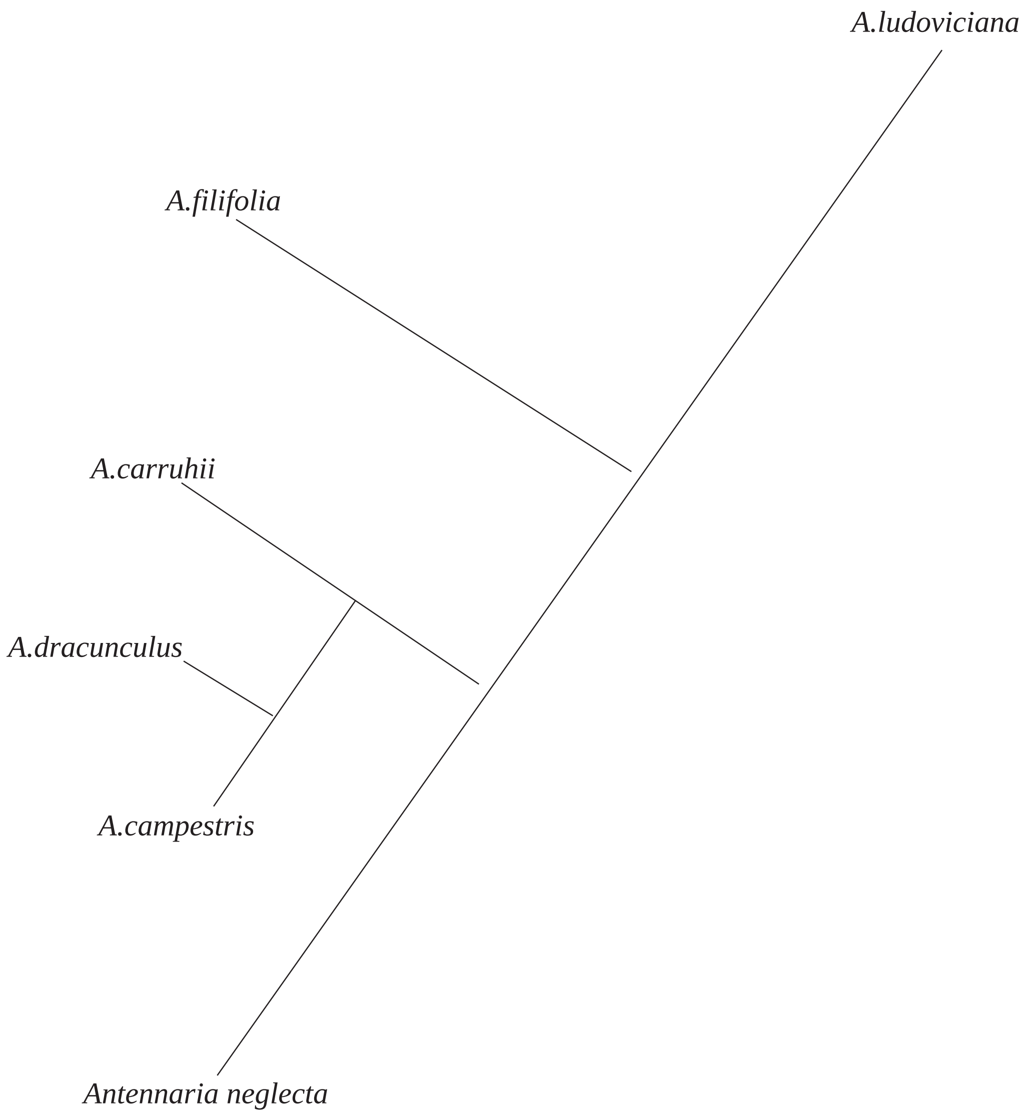


Figure 38- The phylogeny tree of *Artemisia* species and out group plant, *Antennaria neglecta*.

Discussion

A-Comparing between fresh samples and the specimens from herbarium

I did not find any statistically significant differences in measuring between the fresh samples and dry samples by analyzing tissues from roots, stems and leaves of *Artemisia ludoviciana* in the anatomical study. These results suggest that working with dry herbarium samples an adequate substitute for collecting fresh samples for all species.

B- Morphological and Anatomical Studies (systematic relationship)

Growth habits of the species of *Artemisia* are either rhizomatous or taproots, however, the growth habit in outgroup plant, *Antennaria neglecta*, is stoloniferous. *Artemisia filifolia* is characterized as a shrub with much branched stems that are unlike other plants in this study. Trichomes are considered as distinctive property for these species. They are found in stems, leaves and flowers. Tomentose surface found in *Antennaria neglecta, Artemisia carruthii* and *A.ludoviciana* in their stems, leaves and flowers, and this surface coverd also the flower of *A. filifolia.* Glabrate surface coverd the stems and leaves of *Artemisia campestris* and leaves of *A. filifolia.* Sericeous is found only on stems of *A. filifolia.* Gloabous surface found in stems, leaves and flowers of *Artemisia dracunculus* that means not hairs in these parts, and it found in flowers of *A. carruthii* and *A. campestris.*

Blade and margin types are important taxonomic characteristics for leaves, and I can be distinguished these species by the type of these characteristics.*, A. dracunculus, A.ludoviciana* and *Antennaria neglecta* have lanceolate blades, *A. carruthii* and *Artemisia campestris* have pinnatifid blade, and *A. filifolia* has linear blade. Leaf margins do not vary considerably, and most have entire margins except *Artemisia campestris* and *A. carruthii* that have 3-lobed margins. *Antennaria neglecta* differs from *Artemisia* species in arrangement of leaves in stems. *Artemisia* species are alternate all along the stem and the outgroup plant is basal, a whorl of basal leaves, with a different pattern, alternate, on the elongate distal part of the stem. The length to width ratio of the leaves varies from 1.4 to11.7. This character helped to nest the species with different clades on my tree.

The inflorescence that distinguishes *Artemisia* species is a panicle while cyme inflorescence is found in the outgroup. This character is to differentiate between *Artemisia* species and the outgroup. Also, the numbers of disk and ray florets differ in these plants, so the high number of disk florets in *Artemisia campestris*, and the low number of disk florets in *A. filifolia*. In ray florets, the high number in *Artemisia dracunculus*. However, the low number of disk and ray florets in *Artemisia filifolia*, and other species the disk and ray number are between 20-12 in disk florets and 12-3 in ray florets. The important differences between *Artemisia* species and outgroup plant is absence of ray florets in outgroup plant. The length and width of flowers vary from one *Artemisia* species to each other, so the length is between 3-1 mm and width 1-2 mm, and *Antennaria neglecta* flowers are bigger than other *Artemisia* species.

In this study, I found that the disk florets in *Artemisia campestris, A. dracunculus* and *A. filifolia* are staminate, and *A.carruthii* and *A. ludoviciana* have perfect florets. This matches with Watson et al. (2002) study except *A. filifolia* where he said that its disk florets are perfect. I did not see any female parts in disk florets of this species. This maybe because the disk florets are so small. All ray floret of *Artemisia* species are pistillate, and this matches with Watson et al. (2002) and Hayat et.al. (2009).

The sizes of tissues as epidermis, cortex, vessels and sieve tubes differ in roots, stems and leaves. Canal presence is an important characteristic to distinguish some leaves from others; they are found in three species of *Artemisia*: *A.carruthii*, *Artemisia campestris* and *A. dracunculus*, so that makes these leaves distinguished from other leaves. The length of disk and ray florets vary between *Artemisia* species and the length of disk florets is between 1.3 -0.7 mm and of ray florets between 2.3-0.8 mm. Also, stomata length and size of guard cells are varied among these species.

The shapes of pollen are ether prolate, spherical or oblate, and the surface ornamentation are perforate or echinate. The sizes of pollen, pollen wall thickness and pollen apertures differ between the species that are studied, and these characteristics are useful in taxonomic study.

C-Comparative morphological and anatomical phylogeny with molecular phylogeny

A primary goal of this study was to compare a tree based on morphological and anatomical characteristics with already published molecular tree. In addition, I will be adding a new species, from Kansas, to the phylogeny of the genus. The morphological and anatomical phylogenetic tree of *Artemisia* species shows the relationship between them in relation to the outgroup plant, *Antennaria neglecta*. In the phylogenetic tree, *Artemisia ludoviciana*, *Artemisia filifolia* and *Antennaria neglecta*, and are related node1. *Artemisia carruhii* and *Antennaria neglecta* related in node 2. *Artemisia carruhii* is sister to *Artemisia campestris*, and they are related in node 3. Also, *Artemisia campestris* is sister for *Artemisia dracunculus*, and they are related in node close 4(Figure 38).

The all outgroup plants (*Ajania pacifica, Arctanthemum arcticum, Dendranthema intricatum, Elachanthemum intricatum, Kascharia komarovii, Stilnolepis centiflora, Leucanthemella serotine, Nipponanthemum nipponicum, Cymbopaappus adenosolen, Pentzia dentata* and *Oncosiphon grandiflorum*) used by Waston et al. belong to Anthemidea tribe, and my out group, *Antennaria neglecta*, belong to Gnaphaliea tribe. Anthemidea and Gnaphaliea tribes are most closely related (Jose, Funk and Funk, 2002).

Morphological and anatomical tree matches with recent molecular tree that is studied by Watson et al. 2002 (Figure 39). *Artemisia carruhii* did not have any information in molecular tree, but in my tree, it has place in it, and it is close to the outgroup and branched from it to the *Artemisia* subg. *Dracunculus*, *Artemisia campestris* and *Artemisia dracunculus*. Thus, *Artemisia carruhii* is more closed to *Artemisia* subg. *Dracunculus* than *Artemisia* subg. *Artemisia* that are *Artemisia ludoviciana* and *Artemisia filifolia* (Figure 40).

That is match with Pisani, Benton & Wilkinson (2007) study, and they showed that comparing trees can increase confidence (congruence) between morphological and molecular trees.

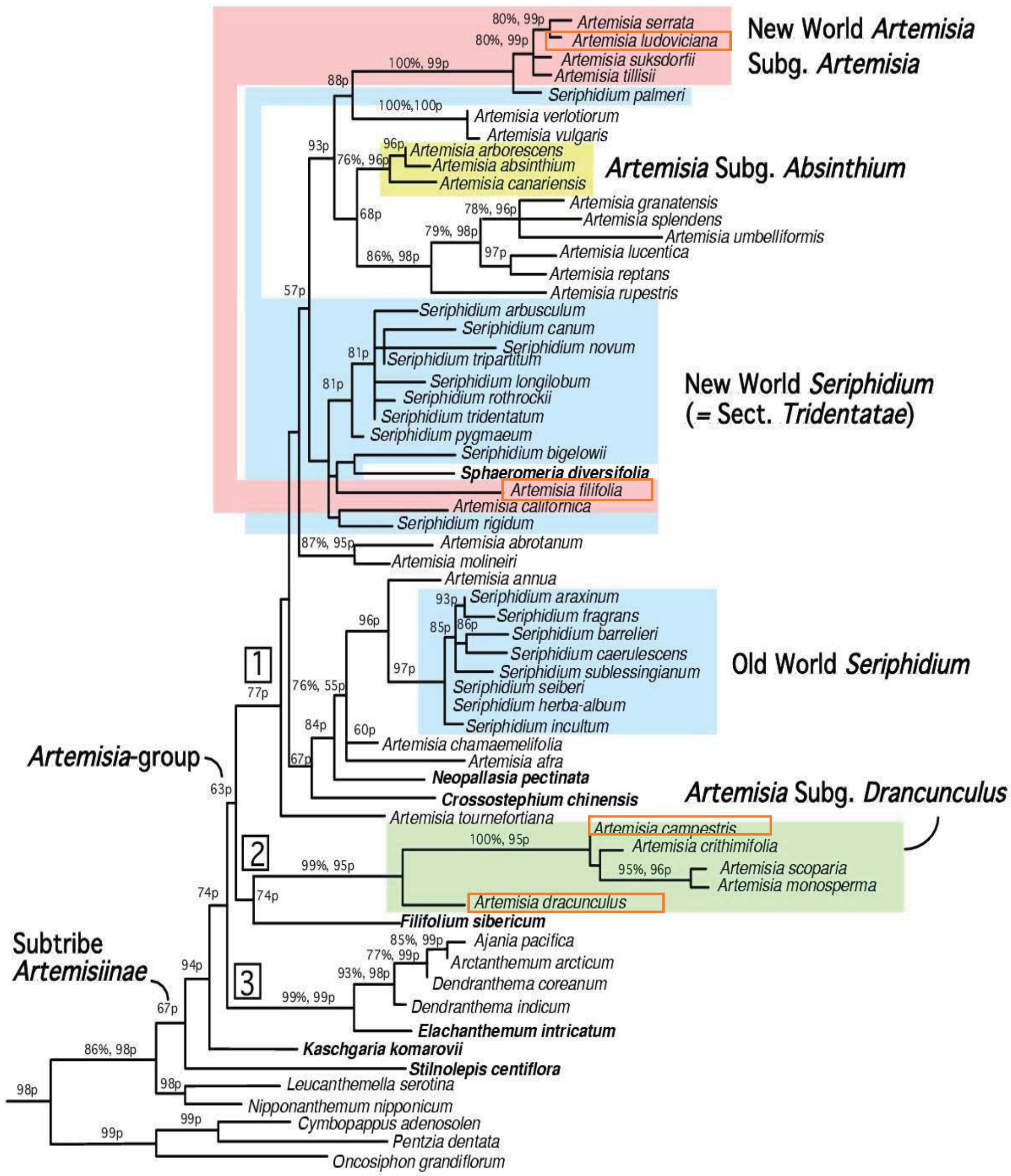


figure 39: Molecular phylogeny among *Artemisia* species (Watson et al. 2002).

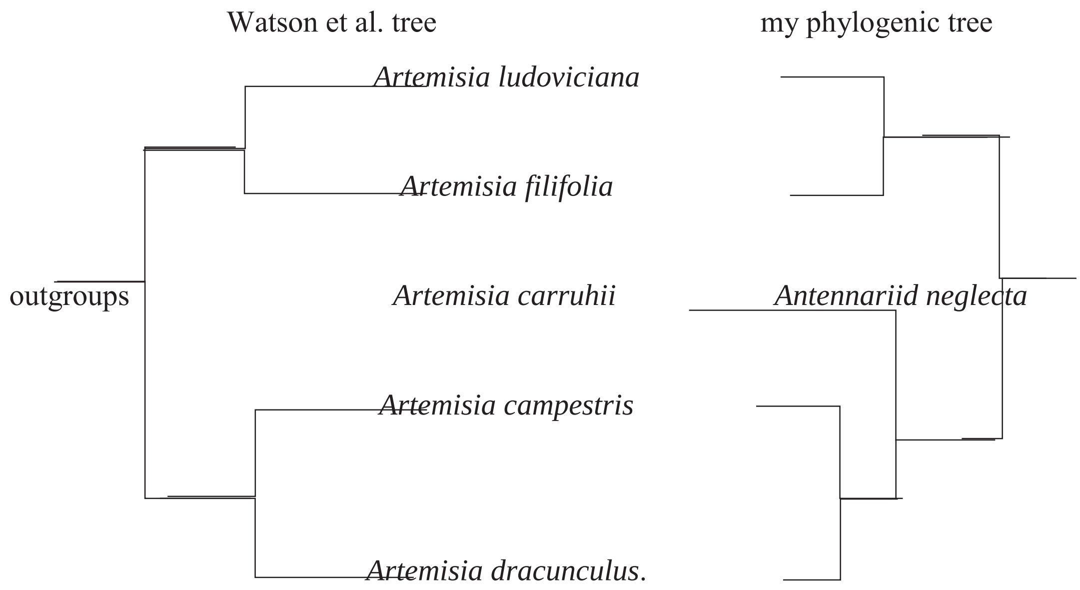


Figure 40: Phylogenetic trees for Watson et al. tree (left) and my phylogenetic tree (right), that showed the matching between molecular tree and morphological and anatomical tree.

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