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Evaluation of the Anatomical Characteristics of Fruits, Seeds and their Active Chemical Content of Some Species Growing in Western Iraq

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ABSTRACT

Plant classification and species-level differentiation are influenced by the morphological traits and dissection of fruits and seeds over the course of evolution. This study described the structure of fruits and seeds of six species of plants growing in western Iraq. These species were *Callistemon viminalis*, *Alcea rosea*, *Alhagi maurorum*, *Amaranthus blitum*, *Amaranthus retroflexus* and *Amaranthus spinosus*. The study of the sections in the fruit and seed coats revealed a difference in the thickness of the constituent layers. The findings indicated that this was one of the distinctive and beneficial traits to support the other traits utilized to separate the various taxonomic levels. The active chemical contents were also investigated, and the species showed variety in their compound composition. Some of them were distinguished by thin, loose parenchyma tissue with numerous secretory ducts, like *Callistemon viminalis*.

Key words: Anatomical, chemical content, fruits and seeds, western Iraq

INTRODUCTION

Anatomical characteristics were used to classify plants since more than a hundred years ago, but they were limited to Dicotyledons only. These characteristics were considered differential criteria by which species and genera were separated. Phenotypic and anatomical studies were conducted on wild dicotyledonous plant species of the compound family Asteraceae, the borage family Boraginaceae, the Convolvulaceae family, and the Euphorbiaceae family (Kocyigit *et al.*, 2015; Zamani *et al.*, 2017).

In a study conducted by Eroglu *et al.* (2022) on the morphological and anatomical characteristics of the seeds of 11 species of *Hyacinthella* of the Asparagaceae family, variations were shown in the shape of the seeds, including triangular and circular. The size of the seeds ranged from 1.51 to 3.06 mm in length and from 1.06 to 2.04 mm in width, and heterogeneity in the pattern of surface decoration of the seed coat and the structure of the pedicel. Through this, it was possible to divide the species into groups. Morphological and anatomical study of the seeds of some wild plant species of dicotyledons growing in Jabal Safeen in Erbil Governorate (seeds of four plant species belonging to four genera belonging to four plant families, namely, the Papilionaceae family, Labiatae, the Crucifera and Cistaceae) were studied in terms of shape, colour, location of the navel, the nature of the surface decoration, the thickness of the seed coat and the number of layers of the seed coat.

Al-Dabbagh (2022) conducted a comparative anatomical study on 22 species of the genus *Trifolium* L. wild growing in all geographical regions in Iraq. Al-Janabi (2021) indicated that the fruits and seeds of *Eriobotry japonica* varied in shape, size and number of seeds each fruit from one place to another in the governorate of Anbar. This was attributed to the influence of the environment and its genetic makeup. A morphological and anatomical study of the seeds of seven species of the genus *Cardamine* in Turkey showed that the most common type was reticulate. Also, it confirmed that the shapes and thickness of the outer epidermis, the inner epidermis, and the presence of the epidermis were essential characteristics that revealed the relationships between the studied species. In addition, a key is presented to identify the studied species based on the characteristics of the seeds (Karaismailogu, 2022), and the results confirmed that these characteristics had a high taxonomic value (Ahmad-Mokhtar *et al.*, 2021). An anatomical taxonomic key was established to separate the species depending on these variations. Primary metabolites and secondary metabolites have an important role in the continuation of the basic physiological processes critical for the plant's survival, including amino acids, fats, proteins, sugars and others (Jan *et al.*, 2021). The plant does not need these compounds but producing these compounds helps the plant to eliminate unacceptable conditions, such as exposure to diseases caused by microorganisms (Ugboko *et al.*, 2020). These compounds provide a storehouse for important elements, like carbon and nitrogen. It's supplied to the plant when needed and is a source of hormones, plant pigments, chlorophyll and vitamins, and it works to protect the plant from the damage of ultraviolet radiation (Dias, 2020). On the other hand, it plays a vital role because it is a medically and physiologically effective substance (used to treat many diseases), including saponins, alkaloids, glycosides, resins, coumarins and terpenes, which are the basis for the composition of plant essential oils in addition to phenols (Stevenson *et al.*, 2017). In addition to being, the main source of medical drugs and active substances that are used in the preparation of medicines or used as raw materials to produce chemical compounds these are involved in the manufacture of a number of medications (Singh and Dwivedi, 2018). The recent study aimed at evaluating the anatomical and phenotypic differences of the seeds and fruits of some species growing in western Iraq and studying the qualitative content of the active compounds.

MATERIALS AND METHODS

Soft samples were collected directly from various areas in Anbar Governorate, western Iraq, during 16 field trips to different regions in the spring, summer and fall of 2021-2022. Plant samples containing ripe fruits were collected to obtain seeds and photographed using a Canon camera. A recording of information accompanied 4500. Each collection was placed on a paper card (Label) containing form on which the model number, place and date of the collection were indicated. It also recorded some information about plant characteristics required in diagnosis (Salih *et al.*, 2021). The plant samples were studied in detail for all parts of the plant using a compound and dissecting microscope. The families of the samples were diagnosed based on several sources, including Iraqi flora books. The samples were diagnosed in the vegetable herbarium in Baghdad. The plants were pressed using the plant press directly to obtain dry samples that retained their characteristics. The alcoholic extract was prepared with a weight of 25 g of powdered fruits and seeds of plants in 250 ml of ethanol alcohol, a concentration of 75%, and placed in a soxolite device at a temperature of 40°C for 6 h. It was kept for qualitative estimations.

RESULTS AND DISCUSSION

The highest thickness of the exocarp layer was 15.7 in *A. spinosis* of Amaranthaceae family (Table 1). The lowest thickness was 5.5 micrometers in *A. blitum*. As for the mesocarp layer, the highest thickness was 35.5 micrometers in *C. viminalis*. Moreover, the lowest thickness was 11.1 micrometers in *A. maurorum*. In contrast, the endocarp layer was thicker in *A. spinosis*, which amounted to 137.6 micrometers. It was less dense in *A. rosea,* which amounted to 11.5 micrometers. The seed coat's thickness and the endosperm layer's thickness were thicker in *A. retroflex*, which amounted to 30.5 and 57.2 micrometers, respectively. They were less dense in *A. blitum*.

Alcea rosea

The fruit was granular box (can) type, with a yellowish-white colour (Table 2), grainy walls, and tiny seeds, similar to a horseshoe, with a black colour (Plate 1). It consisted of three layers. The outer layer, exocarp, was comprised of the cortex and was responsible for forming a hard outer shell of the fruit. Its wall usually included several cellular layers of parenchyma tissue that eventually got thicker from the inside. A waxy cuticle layer usually surrounded the layer and consisted of several stomata.

These stomata were closed in many cases when the fruit was ripe. The inner region in some species was particularly rich in essential oils. Its thickness was 38.4 μm, referred to as the mesocarp, the middle layer

Table 1. Estimation of the thickness of the fruiting coat, seed coat and endosperm layers in the studied species

Plant species	Endosperm thickness	Testa thickness	Endocarp thickness	Mesocarp thickness	Exocarp thickness
Alcea rosea	67.9-71.5 (70.4)	27.6-35.3 (26.5)	7.4-12.3 (11.5)	32.2-36.1 (33.5)	35.9-40.1 (38.4)
Alhagi maurorum	74.6-79.1 (77.5)	8.4-12.3 (10.5)	23.4-26.7 (25.5)	10.5-13.4 (11.1)	10.2-14.3 (13.5)
Amaranthus blitum	25.3-30.1 (28.4)	3.1-5.2 (4.5)	14.5-22.3 (20.6)	7.4-15.6 (12.4)	3.4-6.6 (5.5)
Amaranthus retroflexus	54.5-59.6 (57.2)	27.7-33.1 (30.5)	37.4-44.1 (43.6)	7.9-12.3 (11.7)	4.2-7.2 (6.3)
Amaranthus spinosus	40.2-73.1 (39.4)	20.1-26.7 (25.3)	133.8-139.4 (137.6)	20.1-26.5 (25.5)	11.6-17.4 (15.7)
Callistemon viminalis	82.0-88.5 (83.4)	12.1-16.3 (14.5)	37.5-39.4 (38.1)	30.1-36.5 (35.5)	14.2-17.4 (15.5)

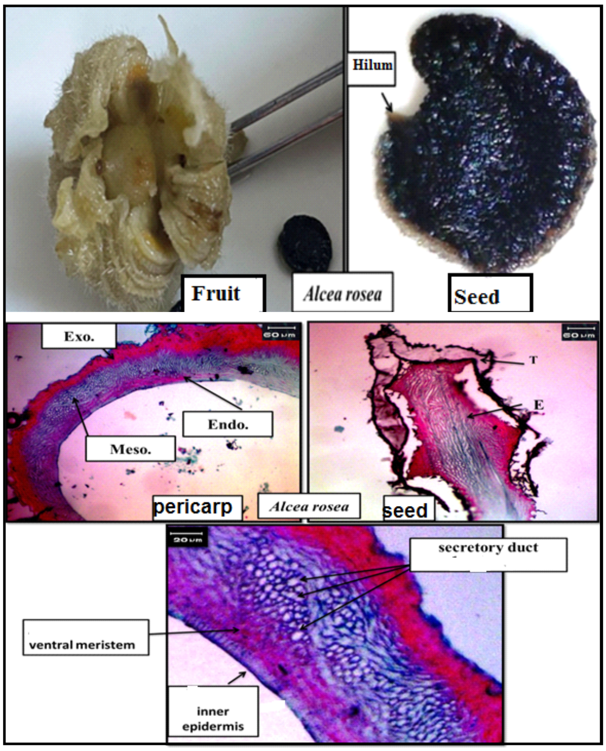

Plate 1. A cross-section of the seed coat and the fruit cover *A. rosea* where Exo–Exocarp, Meso–Mesocarp, Endo–Endocarp, E–Endosperm and T–Testa, 10X.

of the fruit's skin, and therefore found between the exocarp and the endocarp. Usually, the thickest part of the fruit was the part that was traditionally eaten in the fruit (most of the edible part was the mesocarp) and was a component of the species *A. rosea*. It consisted of loose tissue that contained many secretory ducts, its thickness was 33.5 micrometers, and the endocarp was the innermost layer of the fruit's skin. It was known to surround and protect the seeds, forming an inner epidermis layer, and to the top, it created a ventral meristem. Its thickness was 11.5 micrometers. The transverse section of the seed was usually irregular in shape, consisting of the seed coat or the so-called testa. The outer shell was usually not very developed and showed two layers of cells. The outer epidermis of the outer surface showed large cells, and the innermost layer of the inner surface had cells that elongated radially. And inside was the endosperm, composed of loose parenchymal tissue that stored food and basic materials and contained many secretory ducts that nourished the embryo (Plate 1).

Alhagi maurorum

The results showed that the fruit was of the watercress type with a light brown colour, its wall was rough, the seeds were tiny in size,

Table 2. Morphological characteristics of the fruits of the studied species

Plant species	Filament shapes	Surface decoration	Fruit colour	The shape of the fruit
Alcea rosea	Filament free	Granular	Yellowish white	Can
Alhagi maurorum	Filament free	Rough wall	Light brown	Legume
Amaranthus blitum	Filament free	Grainy and coarse	White to green	Clustered
Amaranthus retroflexus	Filament free	Smooth	Light greenish brown	Can
Amaranthus spinosis	Single-row, soft, single-celled bristles	Rough wall	Light greenish brown	Can
Callistemon viminalis	Filament free	Smooth	Dark brown	Disc tray

reniform in shape, light brown, and its wall was smooth (Plate 2). In contrast, the cross-section of the fruiting wall was oval, consisting of three layers. The outer layer was the exocarp, composed of the outer layer of the cortex, which was responsible for forming a hard outer shell of the fruit. Its wall was usually composed of the epidermis layer. It was covered with a waxy layer of cuticle. It consisted of a few stomata, and to the interior of the epidermis was a thin layer of parenchyma tissue, 13.5 micrometers thick, and the middle layer was mesocarp. It comprised loose tissue containing several secretory ducts, and its thickness was 11.1 micrometers. The endocarp layer was the innermost layer of the fruit peel. It was composed of the inner epidermis layer, and a very thin ventral meristem was at the top of it.

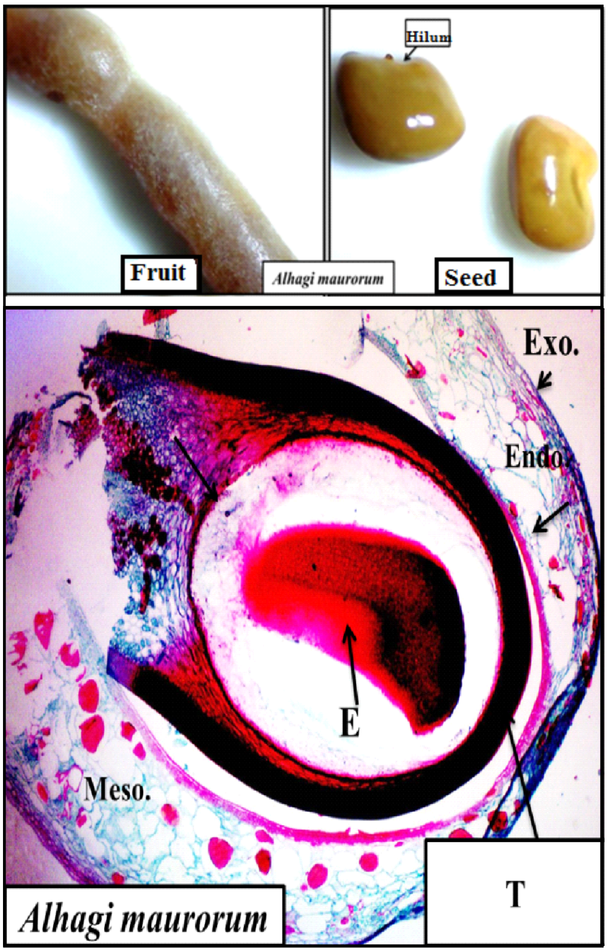

Plate 2. Cross section of the seed coat and the fruit cover *Alhagi maurorum*, where Exo–Exocarp, Meso–Mesocarp, Endo–Endocarp, E–Endosperm and T–Testa, 10X.

Its thickness was 25.5 micrometers. The cross-section of the seeds was oval, consisting of the seed coat or the so-called testa. The outer cover showed two layers of cells. The outer epidermis of the outer cover showed large cells. The deeper layer of the inner cover showed that the cells were oval with gradual thickness. To the inside laid the endosperm, which comprised loose parenchymal tissue, that stored food and basic substances that nourished the fetus (Plate 2).

Amaranthus blitum

The morphological results showed that the fruit was clustered, and each fruit had a white colour to very light green (Table 2). Its wall was grainy and rough. The seed was disc-shaped, brown in colour, covered with a light white, grainy and rough cover (Plate 3), and the cross-section in the fruit wall was polygonal in shape. It consisted of three layers. The Exocarp was composed of the outer layer of the peel and was responsible for forming a hard outer shell of the fruit. Its wall usually comprised the epidermis layer, which was covered with a waxy cuticle layer and consisted of a few stomata. Also, the inside of the epidermis was a thin layer of parenchyma (loose tissue) with thickness of 5.5 micrometers. The mesocarp layer consisted of thin, loose parenchyma tissue with 12.4 micrometers thickness. The endocarp layer was the innermost layer of the fruit peel, composed of the inner epidermis layer, and to the top of it was the ventral meristem, the thickness of which was 20.6 in. A micrometer and the cross-section of the seed were usually oval, consisting of the seed coat or the testa, and the outer cover consisting of two layers of cells. The outer epidermis showed large cells, and the innermost layer of the inner cover had oval-shaped cells, and to the inside laid the endosperm was consisting of tissue. The parenchyma stored food and nutrients for the fetus (Plate 3).

Amaranthus retroflexus

The morphological results showed that the fruit was a box or a capsule with a light greenish-brown colour, the seeds were dark brown, small and ovoid in shape, and its wall was smooth and had a smooth and glossy

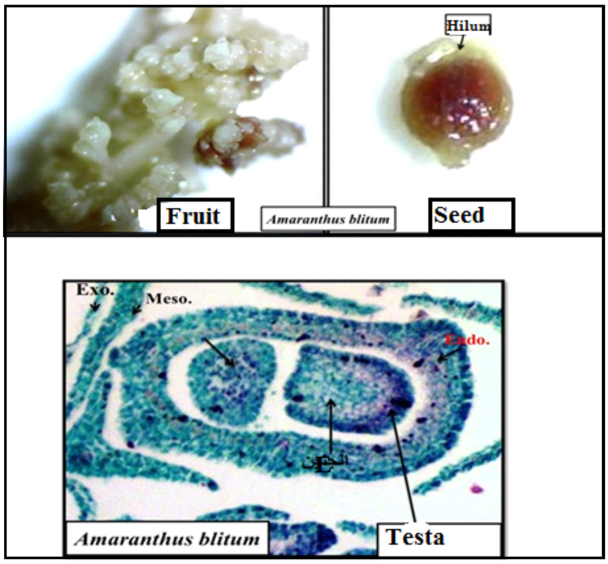

Plate 3. Section of the seed coat and fruit coat of *A. blitum*; where, Exo–Exocarp, Meso–Mesocarp and Endo–Endocarp, 10X.

appearance (Plate 4). As for the cross-section in the fruiting wall, it was ovoid, consisting of three layers. The outer layer was the Exocarp. It was a skinny epidermis layer covered with a waxy layer of cuticles composed of a few stomata. To the inside of the epidermis was a thin layer of loose layer (parenchymal tissue) with a thickness of 6.3 µm. The middle layer of the mesocarp was composed of thin, loose parenchymal tissue with a thickness of 11.7 µm. The endocarp layer was the innermost layer of the fruit peel, formed of the inner epidermis layer, and to the top of it was the loose ventral meristem. Its thickness was 43.6 µm, and the cross-section of the seeds was usually oval, consisting of the seed coat testa, and the outer epidermis was composed of two layers of cells. The outer epidermis showed large-sized cells, and the innermost layer of the inner covering had oval-shaped cells. The inside laid the endosperm, composed of loose parenchymal tissue that stored food and essential substances that nourished the embryo and contained many secretory ducts (Plate 4).

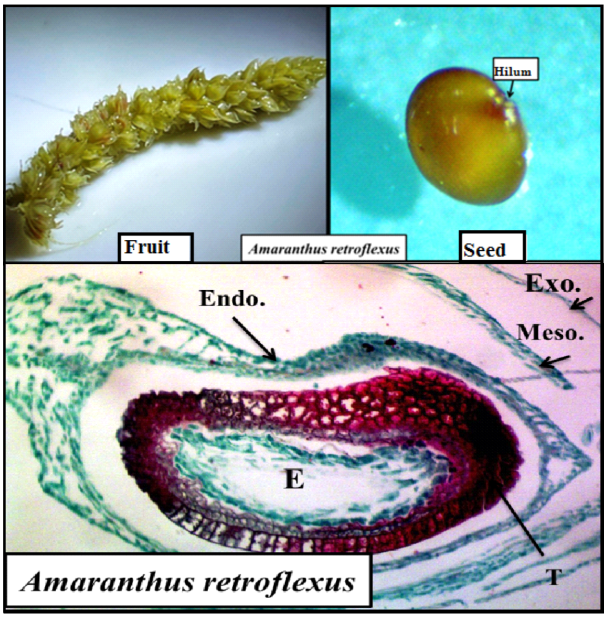

Plate 4. A cross-section of the seed coat and the fruit envelope, *A. retroflex*, where, Exo–Exocarp, Meso–Mesocarp, Endo–Endocarp, E–Endosperm and T–Testa, 10X.

Amaranthus spinosus

The results showed that the fruit was of the box type, with a light greenish-brown colour. Its wall was rough and hairy, the seeds were tiny in size, spherical in shape, with a black colour, and with a smooth border (Plate 5). As for the transverse section in the fruiting wall, it was ovoid, fragile, and composed of three layers. The outer layer, the exocarp, was skinny, consisting of the epidermis layer, which was covered with a waxy cuticle layer and consisted of a few stomata. On the epidermis's inside was a thin layer of loose parenchyma tissue, amounting to a thickness of 15.7 micrometers. The middle layer was the mesocarp, composed of loose, thin parenchymal tissue. Its thickness was 25.5 micrometers. The endocarp layer was the innermost layer

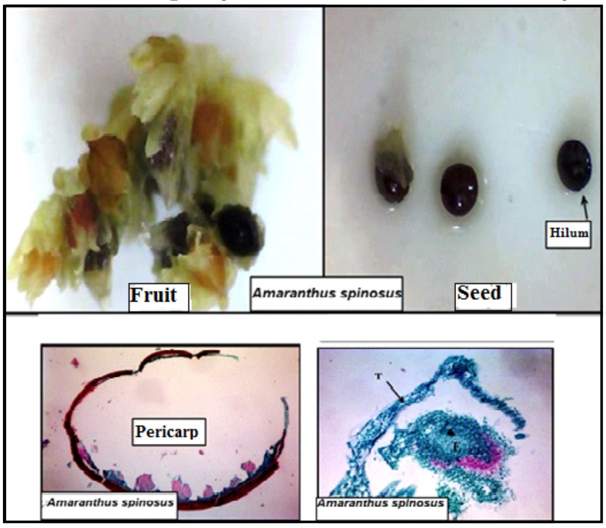

Plate 5. A cross-section of the seed coat and the fruit envelope of *A. spinosus*, where, T–Testa and E–Endosperm, 10X.

of the fruit peel, composed of the inner epidermis layer, and to the top of it was composed of the loose ventral meristem. Its thickness was 137.6 micrometers. The section of the transverse seed coat was usually oval, consisting of the seed coat or the so-called testa, and the outer cover consisted of two layers of cells. The outer epidermis showed large cells, and the inner cover's innermost layer had oval-shaped cells. To the inside laid the endosperm consisting of loose parenchymal tissue, a storehouse of food and basic materials that fed the fetus (Plate 5).

Callistemon viminalis

The fruit was found to be of the watercress type with a light brown colour. Its wall was rough, and the seeds were small, reniform in shape, and light brown, with a smooth wall (Plate 6). In contrast, the cross-section of the fruiting wall was oval and consisted of three layers. The outer layer was the exocarp, composed of the outer layer of the cortex, which was responsible for forming a hard outer shell of the fruit. Its wall was usually composed of the epidermis layer, covered with a waxy cuticle layer and consisting of a few stomata. A thin layer of parenchyma tissue, 13.5 micrometers thick, was present on the interior of the epidermis. The middle layer was the mesocarp, comprising loose tissue containing several secretory ducts, with a thickness of 11.1 micrometers. The endocarp layer was the innermost layer of the fruit peel, composed of the inner epidermis layer, with a very thin ventral meristem at the top of it.

The thickness was 25.5 micrometers, the cross-section of the seeds was oval, consisting of the seed coat or the so-called testa. The outer cover showed two layers of cells, and the outer epidermis of the outer cover showed large cells. The deeper layer of the inner cover showed that the cells were oval with gradual thickness. The inside laid the endosperm, which comprised loose parenchymal tissue that stored food and basic substances that nourished the fetus (Plate 6).

The study of sections in the seed coat and the fruit coat (Plates 1 to 6) showed a difference in the thickness of the constituent layers. These were distinctive and useful features to support other characteristics to differentiate between taxonomic taxa.

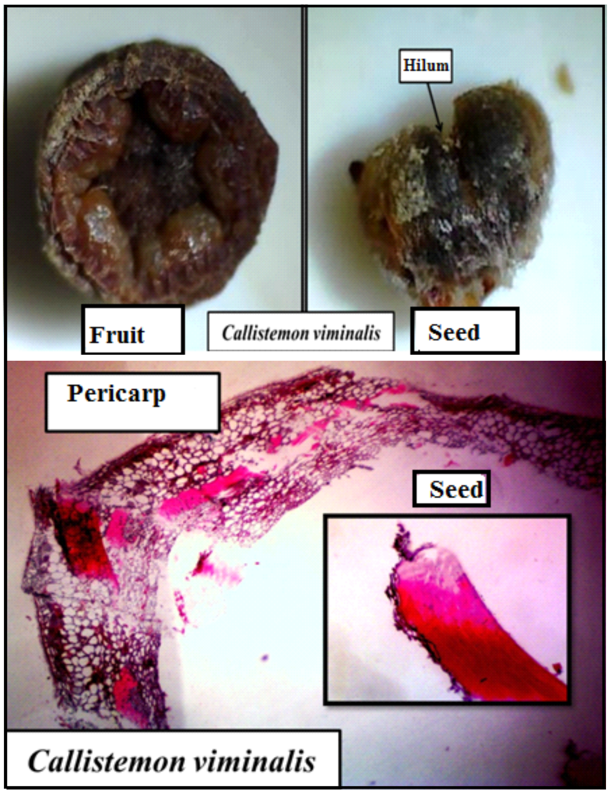

Plate 6. Cross section of *Callistemon viminalis* seed coat and fruit coat.

Most of the studied species contained the chemical compounds, except for some species that did not have tannins (Table 3). Plant extracts, especially wild ones, contained many active ingredients, with concentrations that differed from one plant to another. That's made the tests related to using chemical reagents to investigate them in the plant a necessity when studying them taxonomically. The study, including Hameed *et al.* (2021), relied on qualitative and quantitative chemical detections to separate species and divide them into approximate hypothetical groups based on this characteristic. Dwivedi *et al.* (2018) agreed that *E. globulus* contained all active compounds. In contrast, *colocynthis* contained all the mentioned active substances except for the two compounds, tannins and saponins, and *R. communis* did not contain saponins and glycosides. However, this did not agree with Bano *et al.* (2020).

Changes in the content of the same plant type for secondary metabolites were affected by several factors, including the degree of maturity, the method of extraction and preservation, and the harsh climatic and environmental factors, especially high

Table 3. The chemical detection of the active compounds in the studied species

Plant species	Phenol	Glycosides	Flavonoids	Spanin	Ratings	Tannins
Alcea rosea L.	_	_	+	_	+	_
Alhagi maurorum	+	+	+	+	+	+
Amaranthus spinosus	_	+	_	_	+	+
Amaranthus blitum	+	_	+	_	+	_
Amaranthus retroflex	+	+	+	_	_	+
Callistemon viminalis	+	+	_	_	+	+

temperature and drought, in addition to the soil chemical composition of pH and salts. All of these had a significant impact on the manufacture of these compounds and their concentration inside the plant (Ashraf *et al.* 2018). Hameed *et al.* (2021) indicated when studying seventeen genera of the Asteraceae family in the western regions of Iraq, Anbar Governorate, that their seeds contained many active substances and enzymes, one of which was peroxidase, which was an important enzyme in biological systems. Thus, the results remained to be compared. As for the mixed results of researchers, it is not possible, as it requires identifying many other factors related to the environment of the plant, functional efficacy, genetic and genetic factors, origin and formation (Verma and Shukla, 2015). These effective therapeutic compounds were alkaloids, glycosides, saponins and flavonoids (Hamood *et al.*, 2022). The species belonging to the compound family, including Achellia, Artemisia, Wild lettuce (Lactuca), and Silypum growing in western Iraq, contained some essential phenolic compounds such as pyrogallol, cinnamic acid, and gallic acid (Issa *et al.*, 2020).

CONCLUSION

The presence of these active groups in these plants indicated their great importance. Botanical diagnosis and classification along with chemical compounds are important as these plants have medicinal values. These plants control many diseases and enhance immunity in humans.

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