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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (2): 220-226, 2005
ISSN 1028-8880

The Morphological, Anatomical Properties and Antimicrobial Activity of Endemic *Linaria corifolia* Desf. (*Scrophulariaceae*) in Turkey

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Abstract: In this study, morphological, anatomical, properties and antimicrobial activities of *Linaria corifolia* Desf. which is an endemic plant of the Irano-Turanien phytogeographic region were investigated. Morphologically, it was observed that the species have a perennial root system, the herbaceous stem is cylindrical, erect and glaucous, leaves are filiform type. Inflorescence is many-flowered, bracts are linear-lanceolate, acute. Calyx lobes are lanceolate to oblong-ovate, Corolla pale lilac and spur narrowly conical. Stamens are didinam. Seeds are crescenti-trigonous, to tetrahedral, black. Anatomically, the internal morphological properties of root, stem and leaf were determined. It is observed that the extracts obtained from these plants have strong antimicrobial effect against the tested microorganisms used in this study.

Key words: *Linaria,* morphology, anatomy, ethnobotany, antimicrobial activity

INTRODUCTION

The genus *Linaria* Miller (Nevruz otu) has c. 200 species throughout the world[1]. It is distributed throughout the northern hemisphere and its distribution centre is located in the Mediterranean Basin as the majority of its species are found in Europe, south-western Asia and North Africa[2,3]. There are 20 *Linaria* species (29 taxa) in Anatolia where 34.4% of the 10 taxa are endemic in Turkey[1,4]. This genus comprises annual or perennial herbs with heteromorphic shoots in a wide variety of habitats, including dry and sandy places, cultivated ground and rocky slopes[3-5].

Sutton[6] recognized seven section in this genus (*Diffusae, Linaria, Pelisserianae, Speciosae, Supinae, Macrosentrum and Versicolores*). *L. corifolia* Desf. is represented in *Linaria* section Speciosae. According to Sutton[6] there are very few characters which distinguish sections *Linaria* and *Speciosae*, except for one or two characters of the seeds. Species from different sections are frequently confused in the absence of fruiting material and the commonest reported hybrid in the genus is between species of these two sections. The sections are undoubtedly far more closely related to each other than they are to sections *Macrocentrum, Pelisserianae* or *Versicolores* and it would be possible to unit the two sections at some higher rank in order to reflect the presumed phylogeny more closely. Plants of section *Speciosae* s. str. usually have yellow flowers while those of section *Repentes* have mostly purple, lilac, pink or white flowers. However, the lilac flowered *L. corifolia* has a rare yellow-flowered variant while the yellow-flowered *L. peloponnesiaca* has a rare purple-flowered variant. *L. antilibanotica* closely resembles *L. corifolia,* particularly in the scarious margin to the calyx-lobes, though it has greenish-yellow flowers rather than lilac flowers[6].

Wolfe[7] reported on correlation between the flower color polymorphism and flower size. According to Wolfe[7] light-colored flowers produce heavier fruits than dark-colored flowers may be a direct consequence of variation in flower size and the size of plant organs is dependent upon the amount of vascular tissue supplying them.

However, recently Pauchard[8] reported that *Linaria vulgaris* is a invasive plant and significant treat to native biodiversity in open, human-or naturally disturbed environments in protected areas of the rocky mountain in Yellowstone National Park.

Several authors have recorded the presence of *L. corifolia, L. genistifolia* subsp. *confertiflora, L. genistifolia* subsp. *Genistifolia, L. grandiflora* and *L. simplex* in Afyon-Baskomutan National Park in Turkey[9].

In Turkey *L. corifolia* grows between an altitude of s.I. 1000-2200 m and distributed Steppe, rocky, often

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calcareous slopes (rarely with *Pinus brutia* or *Pinus sylvestris*), screes, fallow fields and other open habitats (rarely dunes)[4]. The fresh and herbarium samples of *L. corifolia* were found near Afyon-Suhut roadside and near of Emirdag at 1050 m during our researches.

Linaria species are important plants for human health; leaves and flowers of some species which have flavonoids, iridoids, alkaloids, monoterpens and diterpens are used for treatment of some illnesses concerning folk medicine[10]. Bianco[11] has reported on the glycosidic components of *L. arcusangeli* and *L. flava* subsp. *sardoa*, in Sardinia.

Many authors have mentioned the chromosome number of different species of *Linaria*[12-15]. The reported chromosome numbers are 2n=12[12-15].

Linaria species have prosuse glandular hairs with unicellular or uniseriate stalks of varying length and heads usually composed of 1-4 cells. In transverse sections of petiol through the distal and, usually exhibiting a variously shaped median arc of xylem and phloem, or a crescentic group of separate bundle in specie *Linaria*. Also, Endodermis often conspicuous and commonly provided with casparian thickenings in *Linaria*. Pericyclic fibres arranged in isolated strands in species *Linaria*[16].

Erdemoglu[17] reported anatomical properties of *Linaria genistifolia* ssp. *confertiflora*. According to Erdemoglu[17], this species is widely spread in Turkey and used as a folk medicine. Anatomically, there are prysmatic crystals and starch grain in leaf mesophyll. In addition this species has no glandular hairs.

The studies on root, stem and leaf anatomy of this genus are limited. Although recent studies have been done on the morphological properties, chemical components, chromosome numbers, pollen morphologies and taxonomic characters of the genus, there is no report on their antimicrobial activity. Morphological, anatomical and antimicrobial properties of *L. corifolia* has not been studied before. The present report gives an account of the morphological, anatomical and antimicrobial properties of *L. corifolia*.

MATERIALS AND METHODS

Material: The specimens were collected during the flowering period. *L. corifolia* were collected from around Afyon-Suhut and Emirdag. Herbarium samples were prepared and deposited at the Afyon Kocatepe University and Canakkale Onsekiz Mart University, Science and Arts Faculty. Herbarium and fresh samples were used for morphological features. A part of the material was fixed in 70% alcohol for anatomical studies of root, stem and leaf. Anatomical studies were carried out on fresh samples kept in alcohol. Sartur and Sudan III[18] reactives were applied to the sections for a better understanding of some anatomical structures.

Extraction of plants: The aerial parts of the plants were air-dried and powdered under steril conditions. Twenty gram of the plant was extracted with 150 mL ethanol for 24 h by using a soxhlet apparatus[19]. All the extracts thus obtained were injected into empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher and Schul No: 2668, Germany) in the amount of 20 mL. Discs injected with pure ethanol served as negative control.

Microorganisms: The bacteria *Escherichia coli* ATCC 11230, *Enterobacter aerogenes* ATCC 13048, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC 12228, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Proteus vulgaris* ATCC 6895, *Pseudomonas aeruginosa* ATCC 27853, *Yersinia enterocolitica* ATCC 9610, *Mycobacterium smegmatis* CCM 2067, *Micrococcus luteus* CCM 169 and *Salmonella typhimurium* CCM 5448, the yeast cultures *Kluyveromyces fragilis* ATCC 8608, *Rhodotorula rubra* DSM 70403, *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763 were used in this study as the test microorganisms.

Preparation of microorganism cultures: All the bacteria mentioned above incubated at 30±0.1°C for 24 h by inoculation into Nutrient Broth (Difco) and the yeast cultures studied were incubated in Malt extract Broth (Difco) for 48 h. Mueller Hinton Agar (Oxoid) sterilized in a flask and cooled to 45-50°C was distributed to sterilized petri dishes having a diameter of 9 cm, by using pipettes in the amount above and yeast for 24 h in the amount of 0.01 mL (10^5 bacteria and yeast cultures per mL) and providing the distribution of food medium in petri dishes homogeneously. Dishes injected with extracts were located on the solid agar medium by pressing slightly and incubated at 37±0.1°C for 24 h for bacteria and 30±0.1°C for 48 h for yeast cultures[20,21]. On each plate an appropriate reference antibiotic disc was applied depending on the test microorganisms.

Morphological properties: *L. corifolia* is a perennial herbaceous plant. Morphological properties of its root, stem and leaf are given below:

Fig. 1: General appearance of *Linaria corifolia*

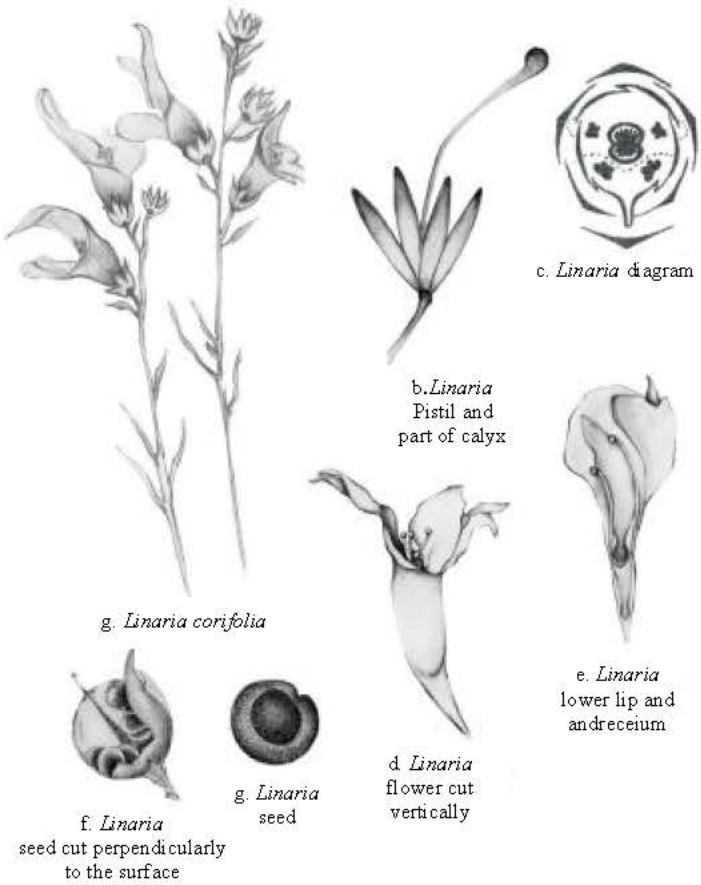

Fig. 2: The flower segments of *L. corifolia,* **a) The longitudinal appearance of flower, b) Pistil and calyx, d-e) Corolla, f-g) Seed**

Root: The top root of the taxon is 5-13x6-0.2-0.4 cm in length. Non-dense, pale brown, non hard bark surround the root (Fig. 1).

Stem: The stems are 15-57 cm long and clearly cylindrical in shape. The perennial herbaceous stem is glaucous, slender, virgate and glabrous or shortly glandular-pubescent. Fertile stems 1-several, ascending to erect and usually laxly branched above. Sterile stems often present (Fig. 1).

Leaf: Leaves of fertile stems are filiform to linear, acute 10-45x0.4-1.4 mm, the length 25-50 mm. The margins of leaves revolute, mostly alternate. Leaves of sterile stems are similar but mostly alternate or irregularly verticillate. Leaves generally little and venation is not clear at the leaf (Fig. 1).

Flower: Inflorescence many-flowered and lax in flower and fruit. Bracts, linear-lanceolate, acute and 1-3 mm length. Pedicels erect, 1.5-4 mm in flower, 2-8 mm in fruit. Calyx lobes lanceolate to oblong-ovate, acute, the margins scarious, white, 1.3-2.5x0.5-0.8 mm. Corolla 9-11 mm, pale lilac or rarely greenish-yellow; tube 1.5-2.5 mm broad; adaxial lip sinus 2-4.5 mm; abaxial lip sinus 1.2-1.5 mm; spur 2-3.5 mm, 0.6-1 mm broad at base, narrowly conical, straight, acute to subobtuse, much shorter than rest of corolla. Capsule 2-4x2.2-3.2 mm, more or less globose, emarginate, glabrous. Seeds 1.2-1.5 mm, crescenti-trigonous to tetrahedral, black; longitudinal marginal ridges 0.05-0.1 mm high, the intervening faces with rounded, anastomosed ridges; periclinal wall of testa-cells tabular or tabular-concav, rugulate except towards margins, that of cells from interstices usually with median papilla, the papillae slender, conical, acute (Fig. 2).

Anatomical properties

Root: A transverse section taken from the young middle part of the root was observed as follows (Fig. 3). There is a thick cuticular layer (7.5-10 μ) outer side. The epidermis is composed of almost square cells. Cortex is multilayered (4-6 cells) and parenchymatic. Parenchymatic cells are 15-27.5 x 25-42.5 μ. The sclerenchyma is 1-2 layered and is on the inner side of the cortex. Under the sclerenchyma layer phloem tissue forms a thin layer followed by xylem which covers a large area in root. The center of vascular cylender is composed of parenchymatous pith cells.

Herbaceous stem: The dicotyl stem is cylindrical. In the upper part there is a thick cuticular layer followed by a thick wall and single layer of epidermis. There are mesophytic type stomata on epidermis. Below the epidermis has no collenchymatic tissue. Cortex parenchymatous, multilayered and has far too much chloroplasts. There is starch sheath which contains uniseriate cells between cortex and vascular tissue. The vascular tissues are collateral type and pith region is parenchymatous (Fig. 4 and 5).

Leaf: Thickness of cuticle is 2.5-5.0 μ. There is a single layered epidermis on upper and lower surface of leaf. The shape of epidermal cell is regular. Epidermis cells are 15x20 μ. Stoma cells are present on both upper and lower epidermis. Stoma type is anomositic. Leaf is ecvifasial. Mesophyll cells are 3-5 layered and 15x27.5 μ. Upper and lower epidermis has no glandular or aglandular hairs (Fig. 6 and 7).

DISCUSSION

In this study, the morphological characters such as the shape of the leaf, the corolla, the structure of the bract and calyx have been used as taxonomical characters to identify endemic *Linaria corifolia*. Although present results are generally similar to those in the flora of Turkey[4,6], a few differences were determined. It was reported that the fertile stems were 12-40 cm, leaves of fertile stems 1-4.5 cm length and 0.4-1.4 mm broad, bracts were 1-3 mm, calyx lobes were 1.3-2.5 X 0.5-0.8 mm, corolla were 9-11 mm, corolla tube was 1.5-2.5 mm broad, spur was 2-3.5 mm length and 0.6-1 mm broad at base, capsules were 2-4x2.2-3.2 mm, Seeds were 1.2-1.5 mm[6]. In this study, it was determined that the root was 5-13x6-0.2-0.4 cm length, the fertile stems were 15-65 cm length, the leaves of fertile stems were 1.1-4.3 cm length and 0.4-1 mm broad, bracts were 1.9-4 mm, calyx lobes were 1.6-2.2x0.4-0.7 mm, corolla were 7-9 mm, spur was 2-2.5 mm, Capsules were 2-3 x 2.2-3.3 mm, Seed length were 0.8-1.2 mm.

Some researchers[3-5] reported that the members of the genus of *Linaria* annual or perennial herbs with heteromorphic shoots in a wide variety of habitats, including dry and sandy places, cultivated ground and rocky slopes. In this research it was also found that *Linaria corifolia* is perennial with heteromorphic shoots, grows high altitude, roadside and dry habitats. Although, it was reported that the colour of corolla was pale lilac and rarely greenish-yellow[6] we found only lilac in our samples.

The general anatomical characteristics of the family Scrophulariaceae were reported by Metcalfe and Chalk[16] but there is no any specific information about the anatomical structure of endemic *Linaria corifolia*. For this reason, in this study, the root, stem and leaf transverse

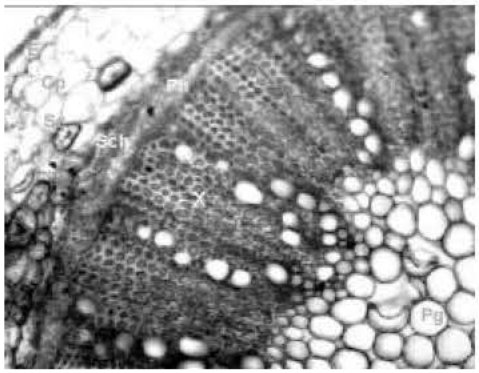

Fig. 3: Cross-section of root of *L. corifolia*
Cu: Cuticle E: Epidermis,
Cp: Cortex parenchyma, Sc: Stone cell,
Sch: Sclerenchyma, Ph: phloem,
X: Xylem, Pg: Pith region (10x10)

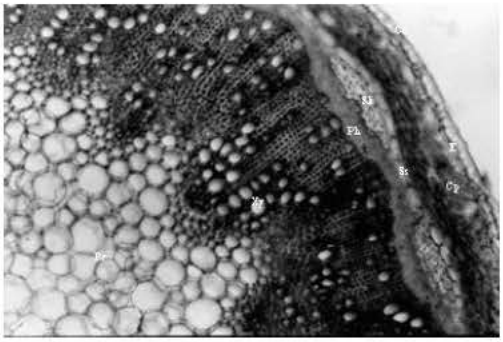

Fig. 4: Cross-section of herbaceous stem of *L. corifolia*
Cu: Cuticle, E: Epidermis,
Cp: Cortex parenchyma, Ss: Starch sheat,
Ph: Phloem, X: Xylem.
Pg: Pith region (6x10)

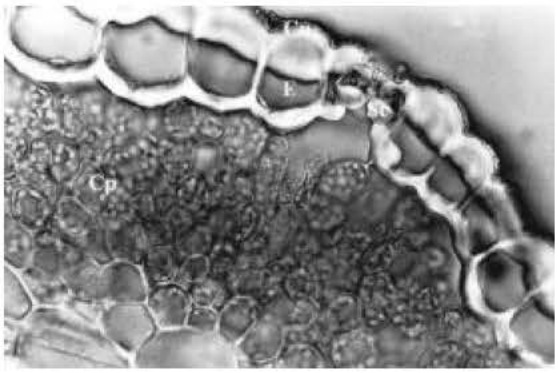

Fig. 5: The stoma of herbaceous stem of *L. corifolia*
Cu: Cuticle, E: Epidermis,
Sc: Stoma cell, Cp: Cortex parenchyma (10x40)

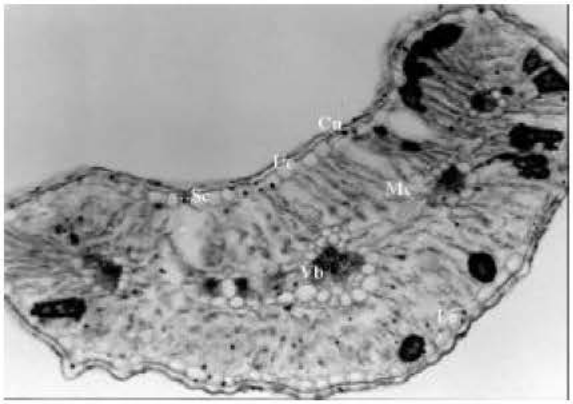

Fig. 6: Cross-section of leaf of *L. corifolia*.
Cu: Cuticle, Ue: Upper epidermis,
Mc: Mesophyll cell, Le: Lower epidermis,
Sc: Stoma cell, Vb: Vascular bundle (10x10)

Fig. 7: The stoma of leaf of *L. corifolia*
Cu: Cuticle, E: Epidermis, Sc: Stoma cell, Mc: Mesophyll cell (10x40)

Table 1: Antimicrobial activity of *Linaria corifolia* Desf

Microorganisms	Zone of inhibition (mm)				
	The ethanol extract	SAM20	CTX30	VA30	NY100
Escherichia coli	-	12	10	22	-
Enterobacter aerogenes	-	12	12	26	-
Staphylococcus aureus	19	17	12	13	-
Staphylococcus epidermidis	18	16	14	15	-
Bacillus subtilis	14	15	15	18	-
Bacillus cereus	15	16	16	20	-
Proteus vulgaris	-	16	18	22	-
Pseudomonas aeruginosa	-	10	54	20	-
Salmonella typhimurium	-	16	18	15	-
Yersinia enterocoliica	-	18	22	20	-
Mycobacterium smegmatis	16	21	11	18	-
Micrococcus luteus	12	36	30	32	-
Kluyveromyces fragilis	16	-	-	-	14
Rhudotorula rubra	12	-	-	-	18
Candida albicans	12	-	-	-	15
Saccharomyces cerevisiae	10	-	-	-	12

*Values, including diameter of the filter paper (6.0 mm), are means of three replicates. SAM 20; Ampicillin (10 mg), CTX 30; Cefotoxime (30 mg), VA 30; Vancomycin (30 mg), NY 100; Nystatin (100 mg)

sections of endemic *Linaria corifolia* were investigated for the first time. The anatomical properties of endemic *Linaria corifolia* have the general characteristics of Dicotyledons[22]. In this study it was found that this species had perennial root, thick cuticula and uniseriate epidermis, multilayered and parenchymatic cortex. There were 1-2 layered sclerenchyma between cortex and phloem tissue. The xylem covers a large area in root and pith region was parenchymatic (Fig. 2 and 3).

Metcalfe and Chalk[16] reported that some of the *Linaria* species have stone cells in the cortex tissue of stem. However, in this study the stone cells were found in root cortex tissue.

A transverse section taken from the middle part of the stem was observed as follows (Fig. 4 and 5). The epidermis is composed of thick walled square cells and frequently bears mesophytic stomata. The cortex comprises parenchyma and starch sheath. Vascular tissue is colateral type. The transverse section of the lamina revealed the following elements (Fig. 6 and 7). In transverse section, the upper and lower epidermis tissues comprise uniseriate, almost square and orbicular cells. Both epidermis tissues are covered with a thick cuticle. The stomata occur on the surface of both sides of the leaves. The leaf is ecvifasial. The mesophyll consist of 3-5 layered palisade parenchyma cells and 1-2 layered spongy parenchyma cells. There are bundle sheats around of vascular bundles (Fig. 6 and 7).

Metcalfe and Chalk[16] were emphasized that, *Linaria* species have prosuse glandular hairs with unicellular or uniseriate stalks of varying length and heads usually composed of 1-4 cells. In present research concerning this feature, *Linaria corifolia* has no glandular hairs.

Table 1 shows antimicrobial activities of the plant extracts. Besides, the inhibition zones formed by standard antibiotic discs.

Linaria L. species contain a wide range of flavonoids and glycosides, as well as ianol glucosides, iridoids, alkoloids, diterpanoids and phenylethanoids[23]. Members of the family *Scrophulariaceae* have been reported contain a group of unusual macrocyclic spermine alcoloids[23,24]. As can clearly seen from Table 1, the extracts obtained from *Linaria corifolia* showed antibacterial activity against especially Gram-positive bacteria, but no significant activity was found against the gram-negative bacteria. Notably, *Staphylococcus epidermidis* and *S. aureus* are more susceptible to the extract, as compared to all bacterial standard antibiotics. Similarly, in comparison to CTX30, the extracts of the plant have strong effect against acid fast bacterium *Mycobacterium smegmatis*. In general, Gram-negative bacteria have been found to be more resistant to extracts than Gram positive bacteria, possibly because of their cell wall lipopolysaccharite[25-27]. In addition, the extracts have antiyeast effects against especially *Kluyveromyces fragilis*.

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