

Acknowledgement

First and foremost, I would like to thank God for lightening my way and enabling me to achieve all that I have achieved. May your name be exalted, honored, and glorified.

I would like to take the opportunity to thank many people who spent their time and shared their knowledge for helping me to complete my thesis with the best possible result:

I would like to express my deep and sincere gratitude to the members of the advisory committee; it is an honor for me to express my gratitude to **Prof. Dr. Abdel-Nasser B. Singab**, Professor of Pharmacognosy, Vice Dean for the Society Service Affairs and Environmental Development, Faculty of Pharmacy, Ain Shams University, for suggesting the research point and for his indispensable advice, continuous support, patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. This thesis would not have been possible without his kind supervision and valuable assistance from the initial to the final level. I am deeply indebted to him.

Ass. Prof. Dr. Nahla A. Ayoub, Associate Professor of Pharmacognosy and Head of the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, for her important support throughout this work. Her understanding, encouraging, valuable comments and personal guidance have provided a good basis for the present thesis. Truly, she is a role model for all women in science.

Prof. Dr. Eman Noaman Aly, Professor of Biochemistry, National Centre for Radiation, Research and Technology, Atomic Energy Authority, whose encouragement, guidance and support enabled me to develop an understanding of the subject. She taught me the skills for carrying out the biological studies I am heartily thankful to her.

I am deeply grateful to **Dr. Riham Tharwat**, National Research Centre, Cairo, Egypt for running NMR analysis of the isolated compounds.

Special thanks are due to **Prof. Dr Suzi Sobhy Atalla**, Professor of Histology, Faculty of medicine, Ain Shams University, for helping me with the histopathological studies.

I wish to extend my warmest thanks to all my colleagues in the Department of Pharmacognosy for their cooperation, support and the friendship we share. My sincere thanks are due to **Dr Rola Milad** and **Dr Sherweit El Ahmady** for their kind support and cooperation. Special thanks are due to **Dr Mohamed Ashour** and **Dr Mohamed El Shazly** for their valuable advice, encouragement and friendly help.

My deepest gratitude goes to my family for their unflagging love and support throughout my life; this thesis is simply impossible without them. I am indebted to my father, **Father Shenouda Ayoub**, who has always been my role model. His continuous encouragement and support helped me a lot throughout the course of this work. Thank you for believing in me always. I am indebted to my mother, **Eng. Mona Samy**; I have no suitable word that can fully describe her everlasting love to me. Her encouragement and support have meant so much to me all my life. My special gratitude is due to my sister, **Mariam Ayoub**, for her loving support and continuous encouragement. If we ever had a family motto that would have been – *If there's a will, there's a way* – a philosophy of life I have been carrying with me every day. Special thanks are due to my grandmother for her endless love and unlimited kindness.

I owe my deepest gratitude to my dear husband, **Dr. John Samir**, for his immeasurable assistance during the course of this work. Without his encouragement and understanding it would have been impossible for me to finish this thesis. Loving thanks to my everlasting joy, my lovely daughter **Angelina**, whom I hope will always be proud of me as I am of her.

Iriny M. Ayoub
Cairo, 2010

INTRODUCTION

Since early mankind, plants have been used for healing purposes. Even as modes of medicine changed throughout the centuries, methods and ideas on plant healing were passed down from family to family, and within communities. Although for a while, much of the medical community ignored plant medicine as old fashioned. Lately the trend in medical science has called out to the return of the natural methods of healing. Nevertheless, the use of plant products has once again become essential.

The family Asteraceae (Compositae) is one of the largest families of flowering plants with about 1100 currently accepted genera and 25000 species ⁽¹⁾. Chemical research in recent years has increased medical interest in the family and we now have a better knowledge of many almost discarded folk remedies as well as uninvestigated plants. It comprises two subfamilies, Tubuliflorae (Asteroideae) and Liguliflorae (Cichorioideae) ⁽²⁾.

Subfamily Liguliflorae: All flowers have ligulate corollas. Genera include *Cichorium*, *Crepis*, *Hieracium*, *Taraxacum*, *Lactuca*, *Scorzonera* and *Sonchus* ⁽²⁾. Vegetables obtained from this group include lettuce, scorzonera root, chicory and endive. Chicory root and dandelion root have been used, particularly in war time Europe, as adulterants of or substitutes for coffee ⁽²⁾. As might be expected from its size, the Compositae contains a wide variety of chemical constituents and the literature is enormous ⁽²⁾.

The genus *Cichorium* consists of two widely cultivated species *C. intybus* (chicory) and *C. endivia* (endive) and four wild species, *C. bottae*, *C. spinosum*, *C. calvum*, and *C. pumilum* ^(3, 4).

Plants of the genus *Cichorium* produce bitter sesquiterpene lactones as their characteristic secondary metabolites, some of which possess interesting

biological activities ⁽⁵⁻⁷⁾. Phytochemically, sesquiterpene lactones, coumarins, flavonoids, caffeic acid derivatives, triterpenoids, phytosterols and inulin were previously isolated from *Cichorium intybus* L., the most popular species ⁽⁷⁻¹²⁾.

Multiple uses of different plant parts of *Cichorium intybus* L. have been reported. Roots have been used as hepatoprotective agents ^(13, 14). It has been shown to be effective in protecting against free radical-mediated hepatic damage ⁽¹⁵⁾ and generally showed antioxidant activity ⁽¹⁶⁾. Root extracts have also been used as a remedy for malaria ⁽¹⁷⁾ and against Ehrlich ascites carcinoma ⁽¹⁸⁾. It also showed antibacterial activity ⁽¹⁹⁾. Leaves are very common in salad as they promote appetite and digestion ⁽²⁰⁾. Chicory seeds are used for treating liver diseases ⁽²¹⁾. *Cichorium intybus* L. was found to be a potent antihepatotoxic plant and is a major component of indigenous drugs in India viz. Liv-52, Geriforte, Acilvan, Livex ⁽²²⁾. The alcoholic extract of this plant is also used against pyorrhea or gingival inflammation ⁽²³⁾. In addition, aqueous and alcoholic extracts of *C. intybus* root showed anti-inflammatory activity against formalin-induced edema ⁽²⁴⁾.

The wild chicory, *Cichorium pumilum* Jacq. (syn. *Cichorium endivia* subsp. *divaricatum*), is widely used as a green salad in rural Egypt, especially in the Nile Delta ⁽²⁵⁾. A single flavonoid glycoside (isoquercitrin) has been reported from aerial parts of *Cichorium pumilum* Jacq. ⁽²⁶⁾. Also, norisoprenoids and cichoriin have been isolated ⁽²⁷⁾. In other studies, sesquiterpene lactones and simple phenolic compounds were isolated from roots of *Cichorium pumilum* Jacq. ^(28, 29).

Many wild edible plants are nutritionally rich and can supplement nutritional requirements, especially vitamins and micronutrients ⁽³⁰⁾. Wild edible plants have always been important in the folk traditions of the Mediterranean region. It is of outmost importance to obtain data about popular uses of wild edible plants before this knowledge disappears. In many Mediterranean countries these traditions are at risk of disappearing, and hence there is a crucial need to study such knowledge systems and find innovative ways of infusing them to the future generations ^(31, 32).

Though many phytochemical constituents and valuable medicinal uses were reported for *Cichorium intybus*, yet little phytochemical or biological studies were traced regarding *Cichorium pumilum* Jacq.; hence it was chosen for an in-depth study to explore the biological and phytochemical importance of this edible plant.

Based upon the cited literature, the aim of this thesis was to investigate both the biological activity and the chemical constituents of ***Cichorium pumilum* Jacq.**

Aim of Work

1. Determination of the biological activity of the plant extract and its various fractions.
2. The biologically active fraction(s) will be subjected to further phytochemical investigation including various chromatographic techniques.
3. Identification of the compounds responsible for the bioactivity, whenever possible.

Review of Literature

A. Family Asteraceae (Compositae)

The Compositae (Asteraceae) is one of the largest and most diverse families of flowering plants, comprising one-tenth of all known angiosperm species. It is characterized by the compound inflorescence that has the appearance of a single "composite" flower from which it derives its name. The Compositae is divided into two major subfamilies with 12 to 18 tribes, 1,100 to 2,000 genera and 20,000 or more species. Lettuce and sunflower are representatives of each of the two major subfamilies ⁽³³⁻³⁶⁾.

B. Botanical Description of Family Asteraceae

Members of this family are usually annuals, perennials or low shrubs, sometimes trees, rarely woody climbers, epiphytic or aquatic; tissues with latex or not ⁽³⁷⁾.

Leaves

Leaves are alternate or opposite, usually simple, often lobed or divided.

Inflorescence

A capitulum surrounded by an involucre of one or more series of protective bracts (phyllaries), capitula solitary and scapiform, in cymose or corymbiform inflorescences, or aggregated into glomerules or compound secondary inflorescences; receptacle naked or with scales (paleae) or bristles subtending the florets; florets small, 1-500 or more, sessile on the common receptacle, perfect, pistillate, staminate or sterile; corollas gamopetalous of (3-)5 united petals, ± regular and equally or unequally (3-)5-lobed, or variously zygomorphic, bilabiate with 2-lobed inner and 3-lobed outer lip, or pseudobilabiate with an outer 4-toothed lip and a single lobed linear lip, or ligulate with a strap-shaped 5-toothed limb, or radiate with strap-shaped 3- or fewer- toothed limb or ray; rarely all male or all

female and the plants are dioecious, or only the inner female-fertile and the outer functionally male, often with a distinctive anther or filament collar immediately below anther; anthers usually united into a tube around the style; style usually divided into 2 branches (style arms), these sometimes with apical appendages; ovary inferior, of 2 united carpels, 1-locular, with 1 erect basal ovule.

Fruit

Fruit is 1-seeded achene, usually crowned by a persistent or deciduous pappus; pappus of awns, scales, bristles, hairs or a $\pm$ crown-like or cup-shaped structure, or completely absent, never green or herbaceous.

C. Genus *Cichorium*

The genus *Cichorium* consists of two widely cultivated species *C. intybus* (chicory) and *C. endivia* (endive) and four wild species, *C. bottae*, *C. spinosum*, *C. calvum*, and *C. pumilum* ^(3, 4). *Cichorium spinosum* and *C. bottae* are morphologically well diagnosed species ⁽⁴⁾. The remaining four species in the genus, *C. intybus*, *C. endivia*, *C. calvum*, and *C. pumilum*, are usually difficult to distinguish ⁽⁴⁾. Based on genetic analysis, two main clusters were formed, one including *C. intybus* and *C. spinosum* and the other including *C. endivia*, *C. pumilum*, *C. calvum* and *C. bottae*. However, identification of *Cichorium* species and cultivars appeared to be difficult due to the absence of specific AFLP (amplified fragment length polymorphism) markers except for *C. bottae* ^(3, 4).

Cichorium endivia differs clearly from *C. intybus* only in long pappus scales, annual life span ⁽⁴⁾. *Cichorium spinosum* and *C. bottae* can be easily identified on the basis of unique spiny terminal branches and a cushion-like growth form, respectively ⁽⁴⁾. The remaining two wild species, *C. pumilum* and *C. calvum*, are morphologically very similar to *C. endivia* and *C. intybus*, but they resemble *C. endivia* in annual life span ⁽⁴⁾.

Chicory is a Mediterranean herbaceous plant belonging to the Asteraceae family ⁽³⁸⁾. It is mainly used as a food product, diffused as a pot herb or as a salad plant. In recent years cultivation of this plant has increased substantially, because

it can be produced in the autumnal months when some types of vegetable are not available. Many Italian regions are involved in this cultivation ⁽³⁹⁾.

According to Bischoff's classification there are three varieties of *Cichorium intybus* L. var. *silvestre*, or wild chicory; var. *sativus*, or chicory from roots; and var. *foliosum*, or chicory from leaves ⁽³⁹⁾.

Cichorium intybus is a perennial species containing several cultivar groups⁽⁴⁰⁻⁴²⁾ developed from breeding programs designed to meet the different commercial uses of the plant.

1. The cultivars of the **Root Chicory Group** are cultivated for their large roots (Fig. 1), which were formerly used as a coffee substitute or additive. Today, they are mainly cultivated for the production of inulin (a β -(2,1)-linked fructan, the major reserve carbohydrate in many Asteraceae) and for food and non-food applications ^(43, 44).
2. The **Witloof Group** (Fig. 2) contains witloof or Brussels chicories, a common vegetable in Belgium, France, and The Netherlands, which are used for producing witloof or French endive under artificial conditions ⁽⁴⁵⁻⁴⁷⁾.
3. The **Pain de Sucre Group** (Fig. 3) comprises the green-leaved cultivars which are mainly cultivated in northwestern Europe. Contrary to what their name suggests, the cultivars have a bitter taste.
4. The **Radicchio Group**, originating from Northern Italy, consists of leaf chicories that are essentially bred for their blond, red, or variegated leaves that are used as fresh or cooked food. The varieties of radicchio are named after the Italian regions where they originate; two popular red varieties of *Cichorium intybus* L. var. *silvestre*: Chioggia (Fig. 4) and Treviso (Fig. 5) ^(45, 48).

On the other hand, the wild chicory, *Cichorium pumilum* Jacq. (Fig. 6) is an annual species that grows as a weed of cultivation. It is widely used as a green salad in rural Egypt, especially in the Nile Delta ⁽²⁵⁾.

D. Botanical Description of Genus *Cichorium*

Members of genus *Cichorium* are usually annuals, biennials or herbaceous perennials ^(25, 48).

Leaves

Leaves are dentate to runcinate-pinnatifid or pinnatisect ^(25, 48).

Inflorescence

Capitula are in elongate spicate inflorescences, solitary or in clusters, in the axils of the branches; phyllaries 2-seriate, the outer short and forming a calyculus; florets blue, rarely pink or white; receptacle ± flattened, naked or nearly so ^(25, 48).

Fruit

Achenes are obovoid, 3-5-angled, glabrous, pappus of minute 1-3-seriate scales ⁽²⁵⁾.

E. Botanical Description of *Cichorium pumilum* Jacq.

Cichorium pumilum Jacq. is sparsely scabrid, otherwise glabrous. Annual, 10-80 cm; stems are erect or spreading, much branched ^(25, 48).

Leaves

Basal leaves are 6-15(25) x 2.5-6 cm, oblong or oblanceolate, dentate or runcinate- pinnatifid to pinnatisect, tapering to a short winged petiole, the margins are coarsely dentate or denticulate. Cauline leaves are much smaller, entire or denticulate, clasping, auriculate (Fig. 6 and Fig. 7) ^(25, 48).

Inflorescence

Capitula are sessile or pedunculate, 1- to several in the axils of cauline leaves, or terminal; peduncle indurate in fruit; involucre 0.8-1.2 x 0.6-0.8 cm, campanulate; outer phyllaries 0.8-1.2 x 0.4-0.5 cm, ovate-elliptic, the margins ciliate, with cilia to 1.5 mm, the apex is acute or spinulose; inner phyllaries 1-1.2 x 0.2-0.3 cm, oblong, with scarious margin and obtuse to subacute apex. Florets are 0.8-1.2 x 0.3-0.4 cm, blue, the apex 5-dentate (Fig. 8 and 9) ^(25, 48).

Fruit

Achene c. 2 x 1.2 mm, obconical, 5-angled, ribbed, the apex truncate; pappus c. 1 mm, of whitish scales ^(25, 48).



**Fig. (1): *Cichorium intybus* var. *sativus*
Root chicory**



Fig. (2): Witloof chicory



Fig. (3): Chicorée Pain de Sucre



Fig. (4): Radicchio (Chioggia)



Fig. (5): Radicchio (Treviso)

Taxonomical Classification of *Cichorium pumilum* Jacq. ⁽⁴⁹⁾

- **Kingdom:** Plantae
- **Subkingdom:** Tracheobionta
- **Super division:** Spermatophyta
- **Division:** Magnoliophyta
- **Subdivision:** Magnoliophytina
- **Class:** Magnoliopsida (Dicotyledoneae)
- **Subclass:** Asteridae
- **Order:** Asterales
- **Family:** Asteraceae
- **Genus:** *Cichorium* L.
- **Species:** *Cichorium pumilum* (Jacq.)



Fig. (6): Aerial parts and root of *Cichorium pumilum* Jacq.



Fig. (7): Aerial parts of *Cichorium pumilum* Jacq.



Fig. (8): Inflorescence of *Cichorium pumilum* Jacq.



Fig. (9): Flower of *Cichorium pumilum* Jacq.

Synonyms of *Cichorium pumilum* Jacq.

- *Cichorium pumilum* Jacq. (1771), [basionym] ⁽²⁵⁾.
- *Cichorium divaricatum* Schousb. (1800) ⁽²⁵⁾.
- *Cichorium endivia* L. (1753) subsp. *divaricatum* (Schousb.) P. D. Sell (1976) ⁽²⁵⁾.
- *Cichorium endivia* L. subsp. *pumilum* (Jacq.) Cout. (1913) ⁽²⁵⁾.
- *Cichorium intybus* L. subsp. *pumilum* (Jacq.) Ball (1878) ⁽²⁵⁾.

The standard author abbreviation *Schousb.* is used to indicate Peder Kofod Anker Schousboe (1766 - 1832), a Danish botanist ⁽⁵⁰⁾.

The standard author abbreviation *P. D. Sell* is used to indicate Peter Derek Sell (1929 - 1996), an English botanist ⁽⁵⁰⁾.

The standard author abbreviation *Jacq.* is used to indicate Nikolaus Joseph von Jacquin (1727 - 1817), a Dutch scientist who studied medicine, chemistry and botany ⁽⁵⁰⁾.

The standard author abbreviation *Cout.* is used to indicate António Xavier Pereira Coutinho (1851 – 1939), a Portuguese botanist ⁽⁵⁰⁾.

Derivation of the Botanical Name

- ***Cichorium***, the Latinized version of the Arabic name ⁽⁵⁰⁾.
- ***endivia***, from Late Latin *endivia*, from Late Greek *entybion*; probably of Eastern origin (perhaps from Egyptian *tybi* "January," which is when the plant grows in Egypt) ⁽⁵⁰⁾.
- ***pumilum***, dwarfish, little; dwarf ⁽⁵⁰⁾.

F. Distribution of *Cichorium pumilum* Jacq.

Cichorium pumilum Jacq. is native to ⁽⁵¹⁻⁵⁵⁾:

Northern Africa: Algeria; Egypt; Libya; Morocco; Tunisia

Macaronesia: Portugal: Madeira Islands; Spain: Canary Islands

Western Asia: Cyprus; Iran; Iraq; Israel; Jordan; Lebanon; Syria; Turkey

Caucasus: Armenia; Azerbaijan

Southeastern Europe: Albania; Bulgaria; Former Yugoslavia; Greece [incl. Crete]; Italy [Sicily]

Southwestern Europe: France [Corsica]; Spain

G. Economic Importance of Family Asteraceae

The Compositae contains over 40 economically important species including food (lettuce, Jerusalem artichoke), oil (sunflower, safflower), medicinal (chamomile) and many ornamental (*Chrysanthemum*, dahlia, zinnia, marigold) crops ⁽⁵⁶⁾.

The high quality edible oils of sunflower and safflower are low in saturated and high in mono- and di-unsaturated fatty acids. Additional novel industrial and edible safflower and sunflower oils have been developed (e.g., high oleic sunflower oil) ⁽⁵⁷⁾. The family is a rich source of powerful insecticides and industrial chemicals, e.g. pyrethrum (*Chrysanthemum*) ⁽¹⁾. Several species are grown as medicinal herbs. *Echinacea* and others may be sources of biologically active compounds with medical or nutritional benefits ⁽⁵⁶⁾.