Summary

Phytochemical and biological study of Sugar beet "*Beta vulgaris* L. subsp.*vulgaris* var. *altissima* Döll" cultivated in Egypt.

Introduction

Sugar beet is a plant whose root contains a high concentration of sucrose. It is cultivated commercially for sugar production, this root contains 75% water, about 20% sugar, and 5% pulp (the exact sugar contents can vary between 12 and 21% sugar, depending on the cultivar and growing conditions). Sugar is the primary value of sugar beet as a cash crop. The pulp, insoluble in water and mainly composed of cellulose, hemicellulose, lignin, and pectin, is used in animal feed (FAO, 2009).

Part I

Phytochemical investigation of sugar beet (*Beta vulgaris* L. subsp. *vulgaris* var. *altissima* Döll) leaves.

Chapter 1

Preliminary Phytochemical screening of sugar beet (*Beta vulgaris* L. subsp. *vulgaris* var. *altissima* Döll) leaves.

The preliminary phytochemical screening of the different extractives of the sugar beet leaves revealed the presence of carbohydrates and/or glycosides, sterols and/or triterpenes, saponins, alkaloids and flavonoids.

Chapter 2

Phytochemical screening of sugar beet (*Beta vulgaris* L. subsp. *vulgaris* var. *altissima* Döll) leaves.

The total alcoholic extract of the leaves was subjected to fractionation by different organic solvents: petroleum ether, methylene chloride, ethyl acetate and n-butanol.
Chromatographic investigation of the petroleum ether extract afforded three pure compounds which are:

- 24-methylene-cycloarta-24(30)-en-3β-ol, it is reported here for the first time from genus Beta.
- 24-methylene-29-norcycloarta-24(30)-en-3β-ol (cycloeucaalenol) (4-monomethyl cycloartane triterpene), this is the first report of separation of this compound from genus Beta.
- β-sitosterol, it was isolated before from Beta vulgaris.

Chromatographic investigation of the ethyl acetate extract afforded one compound which is:

- 1(1, 2, 5-trihydroxy hexan-2-yl)-2, 6, 6-trimethyl cyclohexane-1, 2, 4-triol a new rearranged cyclofarnesane sesquiterpene named as betacyclofarnesane.

Chromatographic investigation of the n-butanol extract afforded one compound which is:

- 4-methyl heptyl benzoate, this is the first report of isolation of this compound from any natural source

Chapter 3

GC/MS analysis of oily fractions of sugar beet (Beta vulgaris L. subsp. vulgaris var. altissima Döll) leaves.

The subfraction (1-22) of petroleum ether extract of leaves (fixed oil) found to be oily in nature so it was subjected to GC/MS after esterification, found to contain 23.22% hydrocarbons and 74.32% oxygenated compounds, the most abundance compounds are 1-Decanol, 2-hexyl (41.89%) and 2-Hexadecen-1-ol (12.2%).

Part II

Phytochemical investigation of sugar beet (Beta vulgaris L. subsp. vulgaris var. altissima Döll) fruits.

Chapter 1

Preliminary phytochemical screening of sugar beet (Beta vulgaris L. subsp. vulgaris var. altissima Döll) fruits.

The preliminary phytochemical screening of the different extractives of the sugar beet fruits revealed the presence of carbohydrates and/or glycosides, sterols and/or triterpenes, alkaloids and flavonoids.
Chapter 2
Phytochemical screening of sugar beet (Beta vulgaris L. subsp. vulgaris var. altissima Döll) fruits.

The total alcoholic extract of the fruits was subjected to fractionation by different organic solvents: petroleum ether, methylene chloride and ethyl acetate. Chromatographic investigation of the petroleum ether extract afforded two compounds which are:

- 1-nonadecanol, this compound wasn’t isolated before from genus Beta.
- palmitoleic acid, this compound wasn’t isolated before from genus Beta.

Chromatographic investigation of the methylene chloride extract afforded two compounds which are:

- 1, 2, 2-trimethyl hydrazine carboxylic acid. It is a new compound reported in the present study for the first time from a natural source.
- Atisine-11, 20-dione, 16. 17-dihydro, 17-benzoyloxy, 21-isopropyl-4-methylene hydroxyl and was named Beta-atisine. To the best of author knowledge, this is the first time to separate this compound from any natural source.

Chapter 3
GC/MS analysis of oily fractions of sugar beet (Beta vulgaris L. subsp. vulgaris var. altissima Döll) fruits.

The sub fraction (47-76) of mother liquor of the petroleum ether extract of fruits was oily in nature so it was subjected to GC/MS after esterification found to contain methyl esters of several types of fatty acids the most abundance are Hexadecanoic acid, methyl ester (saturated fatty acid methyl ester) 27.69% and 9-Octadecenoic acid(Z)-, methyl ester (monounsaturated fatty acid methyl ester ) 32.31%, the medium abundance are Cetene (monounsaturated hydrocarbon) 4.69% and 1-Nonadecene (monounsaturated hydrocarbon) 6.42% and the lower abundance are Cyclopropaneoctanoic acid, 2-hexyl-,methyl ester (cyclic saturated fatty acid methyl ester) 0.12%.
The saturated and unsaturated fatty acid methyl esters were equivalently present in the oil (39.66% and 37.67 % respectively). On the other hand, the unsaturated hydrocarbons were more prominent in the oil (14.01%) than the saturated hydrocarbons (1.04%).

Chapter 4

Study of sugar beet (*Beta vulgaris* L. subsp. *vulgaris* var. *altissima* Döll) fruit pigments.

Methylene chloride extract of sugar beet fruits was containing pigments of betalain type and there were identified by UV and Mass spectroscopy as amaranthin or iso amaranthin and indicaxanthin.

These pigments were subjected to cytotoxicity assay adopting MTT assay, the results show potent cytotoxic activities against HFB4 (Human skin cancer) cell line comparable to 5-fluorouracil and moderate cytotoxic activities against HCT-116 (colorectal carcinoma) cell line comparable to 5-fluorouracil.

Part III

Biological study of fruits and leaves of sugar beet (*Beta vulgaris* L. subsp. *vulgaris* var. *altissima* Döll).

I- Measurement of Potential Cytotoxicity of the different extracts and compounds of Sugar beet.

a- Sulphorhodamine (SRB) assay:

Decoction of sugar beet fruits showed very weak cytotoxic activities against all tested cell lines HEP2, HELA, HCT116, HFB4, MCF7 and HFPG2 with IC$_{50}$ (24, 24.3, 15.6, 21.6, 31.5 and 15.6) respectively. There results proved that; there is no scientific evidence on the use of the decoction of sugar beet fruits in folk medicine as anticancer.

b- MTT assay:

BV1F, BV2F, BV3F, BV4F, BV2L, BV4L and BV5L showed potent cytotoxic activity against HFB4 (Human Skin Cancer) cell line IC$_{50}$ = (2.68, 2.80, 2.30, 2.44, 2.67, 2.46 and 2.50 μg/ml) respectively comparable to standard 5-Flourouracil IC$_{50}$ = 2.10. Compound BV3F was the most potent one. Meanwhile, these compounds, petroleum ether, methylene
chloride and ethyl acetate extracts of the fruits and petroleum ether, methylene chloride and ethyl acetate extracts of the leaves showed moderate cytotoxicity against HCT-116 (Colorectal Carcinoma) cell line with IC50 = (4.45, 3.92, 3.00, 3.02, 4.75, 3.80, 3.99, 4.19, 5.07, 6.10, 5.10, 5.99 and 4.30 µg/ml) respectively comparable to standard 5-Flourouracil IC50 =2.55 µg/ml

II- Screening of the antioxidant activity of the methanol extracts of sugar beet leaves and fruits

ABTS antioxidant assay

The methanolic extract of the fruits showed strong antioxidant activity (86.15%) comparable to standard ascorbic acid, while the methanol extract of the leaves showed weak antioxidant activity (50.48%). The strong antioxidant activity of the methanolic extract of the fruits may be attributed to its betalain content.

III- Acetylcholine esterase inhibition assay

The methanolic extract of the fruits exhibited potent inhibition against AChE which was significantly higher than that of standard donepezil. So the methanolic extract of the sugar beet fruit is a promising potent and safe cholinesterase inhibitor which can be used in treatment of the Alzheimer's disease and for other possible therapeutic application as in the treatment of Parkinson’s disease, aging and myasthenia gravis.

Part IV

Botanical study of the leaves and petiole of sugar beet (Beta vulgaris L. subsp. vulgaris var. altissima Döll).

The Sugar-beet is native to Europe and the Middle East but is now cultivated worldwide as a commercial sugar crop in temperate climates. About a third of all sugar production in the world is derived from this plant, but this sugar is considered inferior to cane sugar because it does not crystallise as well and is absorbed more quickly by the body (Nielsen et al., 1997) and (Berglund et al., 1995).

Macromorphology of the leaves:

The leaves are dark green in color, commonly ovate to cordate, whorled, the apex is obtuse and the margin is entire. The venation is pinnate reticulate and and the midrib is prominent.
They have a papery texture, have long petiole exceeding 10 cm and asymmetric leaf base. The mature leaf measures 11-13(l.) x 5-6 (w.) cm.

**Micro-morphology of sugar beet leaves.**

A transverse section in the leaf shows projection on the upper side at the midrib region and convex on the lower side. The lamina is narrow, ribbon shaped. It shows a homogenous mesophyll formed only of spongy tissue interrupted in the midrib region by a cortical tissue. The **upper epidermis** is formed of polygonal cells with thick wavy cellulosic walls covered with thick smooth cuticle showing anisocytic, frequent anomocytic stomata both of them showing 3-4 subsidiary cells and showed scattered pigments and no hair. The **lower epidermis** is formed of polygonal cells with sinuous irregular thick cellulosic anticlinal walls covered with thick smooth cuticle. It shows abundant anisocytic stomata, frequent anomocytic stomata both of them showing 3-4 subsidiary cells and showed scattered pigments and no hairs. The **hypodermis** is composed of 5-6 rows of lacunar collenchyma under upper epidermis and 3-4 rows of lacunar collenchyma above lower one at the midrib region. The **mesophyll** is homogenous, formed of spongy tissue only. The spongy tissue is formed of 12-15 layers of homogenous tissues of rounded cells with thin cellulosic walls. The **cortical tissue** showed 16-18 layers of parenchyma under the upper hypodermis and 6-8 layers of lower parenchyma above the lower one. It consists of polyhedral parenchymatous cells with cellulosic walls, sometimes some of parenchymatous cells filled with pigments. The **vascular tissue** is formed of 3-7 separated open bicollateral vascular bundles in the midrib region arranged in arch shape. There are few other lateral small vascular bundles scattered in the mesophyll. The xylem is formed of lignified spiral, scalariform and banded pitted vessels. The phloem is formed of small cells with thin cellulosic walls and hardly differentiated into sieve tubes, companion cells and phloem parenchyma. The **pericycle** is consists of a group of non lignified fibers, with thin wall, wide lumen and tapering ends, measuring 450- 600- 650 μm in length and 10- 11.67- 15 μm in width. They form an arc around vascular bundle towards the periphery.

**The powdered leaves**

The powder is light green in color and no characteristic taste and odor. It's characterized microscopically by the presence of fragments of upper and lower epidermises are showing polygonal cells, wavy anticlinal walls, lower more sinuous, covered with smooth cuticle. Stomata are of anisocytic and anomocytic types (anisocytic type more abundant) and showing
3-4 subsidiary cells, fragments of Lignified spiral, scalariform and boded pitted xylem vessels, fragments of parenchymatous cells filled with orange pigments, Fragments of pericyclic fibers which are non lignified with thin walls, wide lumens and tapering ends.

**Micro-morphology of Beta vulgaris L. petiole.**

A transverse section of petiole has a cordate shape with minute projections uniformly distributed all over the outline. The epidermis composed of a single layer of cells with polygonal shape and shows no hairs followed by thin layer of hypodermal lacunar collenchyma located at each projection. The cortical tissue is composed of rounded parenchyma cells and about (15-20) open bicollateral vascular bundles which arranged in more or less V shape with an arc of pericyclic fibers.