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Chemical Composition and Biological Activities of Red beetroot (*Beta Vulgaris* Linnaeus) Roots

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PLANTS have been used for many years as a source of traditional medicine to treat various diseases and conditions. *Beta Vulgaris* Linnaeus ranks among the 10 most powerful vegetables as excellent sources for phytochemicals, which showed potent antioxidant and anticancer activities.

The aim of this study is to characterize the chemical composition and determine the cytotoxicity and antibacterial activity of *B.Vulgaris* roots extract.

The results of GC-Ms analysis of the unsaponifiable matter of *B.Vulgaris* roots showed the presence of 18 components. The undecane is the major hydrocarbon compound and α-citronellol is the major oxygenated compound.

The methanolic extract of *B.Vulgaris* roots were enriched with a complicated mixture of phenolic acids and flavoniod which elucidated for the first time from this plant, including polyphenolic acid; ellagic acid (1); pyrogallol(2); salicylic(3); catechol(4); benzoic(5); protocatchuic (6); chlorogenic(7); *p*-hydroxy benzoic acids (8). The molecular components of a flavoniod fraction, obtained from liquid chromatography extract, were identified using HPLC -ESI -MS. The major components were vitexin -2" -*O*-rhamnoside (1); demethylated -2"-xylosylvitexin (2); isorhamnetin -3-gentiobioside (3); rutin(4); beside minor components, luteolin-6-arabinose-8-glucose(1); hesperidin (2); acacetin (3); kaempferol-3-(2 -*p* -comaroyl) glucose (4); apigenin 6 -arabinose -8-glactose (5); quercetin (6); naringin (7). The structures of all isolated compounds were elucidated by conventional methods, spectroscopic analysis alongside their mass spectrometric investigations.

The search for new, potentially biologically active extract becomes much more efficient after identification of all compounds in that mixture.

The cytotoxic activity of methanolic extract was evaluated against pancreatic carcinoma (PANC-1), hepatocellular carcinoma cell line HEPG-2 and, Lung carcinoma (A549) using MTT assay and vinblastine as a reference drug. methanolic extract showed higher activity with (IC_{50}=3.69 µg/ml), mild cytotoxic activity against HepG2 with (IC_{50}=4.43 µg/ml),Lung carcinoma (A549) with (IC_{50}=4.9 µg/ml) and a weak activity against prostate carcinoma PC-3 with (IC_{50}=6.1 µg/ml).

The antimicrobial activity of *B.Vulgaris* roots methanolic extract was evaluated using agar well diffusion method towards two representatives for each of Gram positive and Gram negative bacteria. The extract demonstrated inhibitory effect against pathogenic microorganisms: *Staphylococcus aureus*, ATCC 29213; (ZI=24mm) strains and *Pseudomonas aerogenasa* ATCC 27853); (ZI=19.3 mm) at 100 µL concentration.

Keywords: Beetroot (*Beta Vulgaris* Linnaeus) roots, Phenolics, Flavonoids, Antimicrobial, Anticancer activities

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Introduction

The *Beta vulgaris* L., belongs to Amaranthaceae family [1]. According to Judd (1999) as well as Stevens (2001). Amaranthaceae and Chenopodiaceae are merged into one family Amaranthaceae. which contains 1400 species classified into 105 genera [2]. The *Beta vulgaris* is native to the Mediterranean regional and widely cultivated in America, Europe and through India. The purple root vegetable known as beetroot or table garden beet. which have been used in traditional medicine for hundreds of years. The modern pharmacological studies showed that extracts from Amaranthaceae plants exhibited antioxidant [3,4], antidiabetic [5], immunostimulatory, antitumor,antibacterial, antiinflammatory, anti-osteoporotis [6], antiulcer [7], hypoglycemic activities [8].

High Pressure Liquid Chromatography analysis of *B.Vulgaris* roots extract confirmed the presence of bioactive polyphenols such as quercetin, sinapic acid, *p*-coumaric acid, syringic acid, gallic acid, coumarin, caffeic acid, chlorogenic acid and catechin [9]. *B. vulgaris* contains some secondary metabolite compounds such as tannin saponins, alkaloids, flavonoids, terpenoids and steroids. [10].

The promising results of phytochemicals in health protection suggest the opportunity for their use in functional foods.[11].

In the present study the methanol extract of *B.vulgaris* was investigated by determining the cytotoxicity and antibacterial activity of beetroot extract. The *B. vulgaris* extracts (root) antioxidant, antihypertensive, hypoglycemia, anti-inflammatory, and hepatoprotective activities

Materials and Methods

Plant Material

Fresh roots of *B.vulgaris* were purchased from local market at Egypt on November 2017, The plant samples authenticated by Botany Department, Faculty of Science, Zagazig University. A voucher specimen is deposited in the herbarium.

Extraction of Roots

The dried powdered roots *B.Vulgaris* roots (2 Kg) was followed by using Soxhlet [12], beginning with petroleum ether (60-80^{o}C) and extracted with absolute methanol with 2 L solvent every time. The extracts were then concentrated in rotary evaporator at 40-50^{o}C. The dried extracts weights were calculated to give (20.7 and 42.8 gm). The petroleum ether and methanol extracts were preliminary investigated for their biological activity.

*Chemicals***:**

The standards chemicals, phenolic acids (gallic, caffeic, syringic, *p*-coumaric, ferulic, and sinapic), flavonoids (catechin, rutin, myricetin, quercetin, apigenin and kaempferol) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and the HPLC-grade solvents such as acetonitrile, methanol, water and acetic acid were purchased from Merck KGaA, Darmstadt, Germany.

The stocks standard solutions were prepared by dissolving the standard phenolic compounds and flavonoids in the appropriate volume of 70% aqueous methanol to produce a solution of the standards for HPLC

High Performance Liquid Chromatography:

Experimental data were obtained using an HPLC system consisting of a Agilent 1200 HPLC pump, equipped with auto sampling injector, solvent degasser, with diode array detector (DAD), a quaternary Hewlett Packard pump series 1100, a column Agilent C_{18} ZORBAX ODS 5µm (4.6 × 250 mm).

GC-Mass Spectrometer:

GC-Ms Hewlett Packard (model 6890 series) connected to Mass Spectrometer Hewlett Packard (model 5973 series) mass selective detector equipped with a fused silica capillary column HP-5MS (30m length × outside diameter 0.25 mm × 0.25 µm film (manufactured: Helwlett – Packard, Palo Alto, USA).

General

UV recording is Shimadzu UV–Visible-1601 spectrophotometer. Paper chromatographic analysis was carried out on Whatman 1 MM and 3 MM, using solvent systems:(1) H_2O; (2) 6% HOAc; (3) BAW (n-BuOH–HOAc–H_2O, 4:1:5, upper layer).

Biological Assays

Antimicrobial Activity of Extracts.

Test organisms

Microbial pathogens and culture media:

Two strains of bacteria and two fungi strains were obtained from the stock microbial cultures collection, at Microbiology Laboratory, Faculty of science, Al-Azhar University, Gram-positive bacteria: *Staphylococcus aureus*, ATCC

29213, Gram-negative bacteria: *Pseudomonas aerogenasa,* ATCC 27853, unicellular fungi: *Aspergillus niger* ATTC 16404, *Fusarium sp*. and yeast *Candida albicans*, ATTC 10231.

The strains were preserved at freeze temperature till usage. Nutrient broth used as cultivation medium for bacteria and Malt extract agar used as Fungi and yeast. [13,14].

Determination of anticancer activity:

Effect of roots as anticancer assays of potential cytotoxicity activity against the Prostate carcinoma cell line (PC-3), liver carcinoma cell line (HEPG2),Lung carcinoma cell line (A549) and Pancreas carcinoma cell line (PANC-1) carcinoma cell line were maintained at 37°C, 5% CO2, 95% air and 100% relative humidity were tested by the MTT assay and vinblastine as a reference drug. [15]. This experiment was carried out at Egyptian Cancer Institute, Cairo, Egypt.

Methods

Instrument Circumstance for phenolic compounds: HPLC

Separation and identification of phenolic acids by reversed phase HPLC (RP-HPLC) equipped with diode array detector (DAD). A gradient of water with 0.1% formic acid and acetonitrile (ACN) were applied from 5% to 30% ACN over 60 min with flow rate of 1 mL/min with, the injection volume was 10 µl The detection wavelength at λ 280 nm.

Determination of fatty acid composition

The fatty acid composition of the samples was determined by GC. For this purpose, the sample was converted into fatty acid methyl esters [16]. Use (30 mg) was taken in a test tube and methanolic NaOH (2 mL) (0.5N) was added and saponified by heating at 50°C for 20min. After saponification, the unsaponifiable matter was extracted with n-hexane (2 mL) and the hexane extract rejected. The aqueous layer was acidified by adding conc. HCl (0.2 mL) to break up the soap and to facilitate the release of fatty acids. Then n-hexane (2 mL) was added to recover the fatty acids. The obtained fatty acids were converted into fatty acid methyl esters by treating with boron trifluoride (1mL) in methanol. The identification of fatty acids was carried out using standard fatty acid methyl esters. The prepared fatty acid methyl esters were analysed using a Gas chromatograph [17]

Antimicrobial Activity of Extracts.

Antimicrobial assay:

The antimicrobial activity of B.vulgaris roots methanolic extract was examined using nutrient broth for bacteria and malt extract agar for fungi. The DMSO was used as solvent for the tested samples, the methanolic extract of B.vulgaris was dissolved in 10 % aqueous DMSO to obtain the final concentration (5mg/ml).

The diameters of zones of inhibition were measured for antibacterial and antifungal activities. The data have been recorded in the form of inhibition zones (diameter, millimetre). The experiment was conducted out in triplicate and the mean value calculated [18]

Isolation and Identification of Phenolics

The phenolic fractions, which was submitted to HPLC analysis. The HPLC showed four major peaks. The peaks were analyzed by ESI-MS and was identified as vitexin 2”-O-rhamnoside, 2”-xylosylvitexin [19] and as isorhamnetin-3-gentiobioside. The minor peak contained five components: rutin, quercetin 7-glucuronide, isorhamnetin, apigenin 7-rutinoside and an unknown flavonol-glycoside.

Isolation and identification of the phenolic metabolites

The defatted aqueous *B.vulgaris* root extract (20 gm) was dissolved in water and applied to a polyamide column and eluted with H_2O followed by H_2O-MeOH mixtures of decreasing polarities. The collected fractions were investigated by TDPC using BAW as the first solvent and 6% acetic acid as the second solvent.

GC-MS analysis of the unsaponifiable components of B.vulgaris roots petroleum ether extract.

The identified compounds were found in Figure (1) and Table (1),which revealed the presence of 18 compounds were classified as hydrocarbon (acyclic and cyclic) (72.71 %) and oxygenated compounds (6.31%). Undecane is the major hydrocarbon (11.03%) and α-citronellol is the major oxygenated compounds.

TABLE (1): Identification of the components of the Unsaponifiable matter of *B.vulgaris* roots petroleum ether extract.

S. No	R_t	Compound Name	Area%	M.W	Molecular Formula
1	5.58	2-Hexadecenol	0.68	296	$C_{20}H_{40}O$
2	5.70	1,2-epoxy octadecane	0.41	268	$C_{18}H_{36}O$
3	5.99	Cis-7-hexadecan	0.10	238	$C_{16}H_{30}O$
4	6.45	n-decan	5.85	142	$C_{10}H_{22}$
5	7.43	2-hexyl-1- decanoyl	1.14	242	$C_{16}H_{34}O$
6	7.59	n-butyl cyclohexane	2.12	140	$C_{10}H_{20}$
7	7.78	Nonadecane	3.35	214	$C_{19}H_{40}$
8	8.58	α-citronellol	3.98	156	$C_{10}H_{20}O$
9	9.91	Undecane	11.03	156	$C_{11}H_{24}$
10	10.63	2-Methyl decalin	2.14	152	$C_{11}H_{20}$
11	10.89	5-Phenyl decan	6.29	218	$C_{16}H_{26}$
12	11.84	(1-methyl-1-propyl pentyl) benzene	2.92	204	$C_{15}H_{24}$
13	13.96	n-dodecane	9.35	170	$C_{12}H_{26}$
14	14.43	2,6 – dimethyl undecane	2.02	184	$C_{13}H_{28}$
15	15.10	Octadecyl benzene	4.41	330	$C_{24}H_{42}$
16	31.64	5-Phenyl- undecane	8.85	232	$C_{17}H_{28}$
17	35.13	6-Phenyl-dodecane	7.82	246	$C_{18}H_{30}$
18	38.60	6-Phenyl –tridecane	5.93	260	$C_{19}H_{32}$

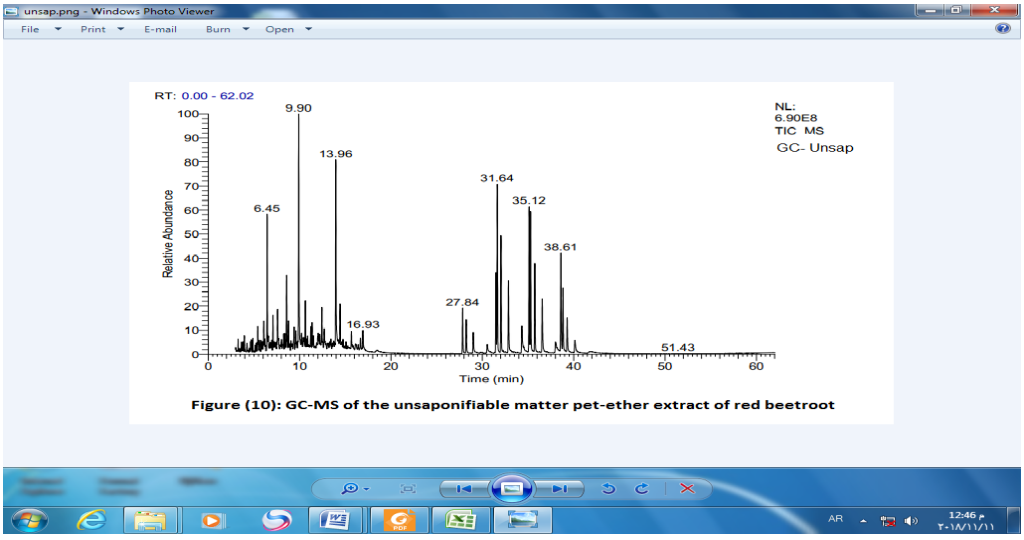

Figure (10): GC-MS of the unsaponifiable matter pet-ether extract of red beetroot

Figure (1): GC/ MS chromatogram of Unsaponifiable matter of *B. vulgaris* root

The High-Performance liquid chromatography (HPLC).

The phenolic profiles of the methanolic extract of *B. vulgaris* applied by High Performance Liquid Chromatography. By using external standards Fig (2), enabled to identification of the major phenolic acids: Gallic(1),ellagic acid (2); catechin (3); salicylic (4); catechol(5); caffeic acid (6); protocatchuic (7);chlorogenic (8); *p*-hydroxy benzoic (9); ferrulic acid (10); *p*-Coumaric (11).

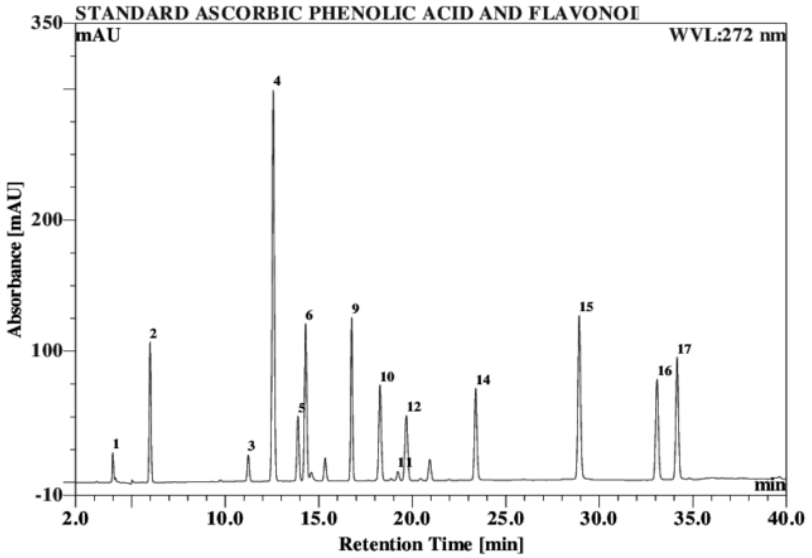

Figure (2): HPLC Chromatogram of standard phenolic acids and flavonoids

HPLC Chromatogram of standard phenolic acids and flavonoids.1.Ascorbic acid, 2. Gallic acid, 3. Catechin, 4. Methyl gallate, 5.Caffeic acid, 6. Syringic acid, 9. Rutin, 10. *p*-Coumaric acid, 11. Sinapic acid, 12. Ferrulic acid, 14. Myrecetin, 15. Quercetin, 16. Apigenin, 17.Kaempferol

Identification of flavoniod using HPLC.

The major identified 19 flavonoid compounds were: Luteolin 6-arabinose-8-glucose, Hisperidin, Acacetin, Kaempferol-3-(2-*p*-comaroyl)glucose, Quercetrin, Naringin, Apiginin.6-arabinose-8-glactose, Rutin.Table (2)

TABLE (2): HPLC of flavonoids of methanolic extract of Beta vulgaris L

S. No	Flavonoids	RT	Area %	mg/g
1	Luteolin 6-arabinose-8-glucose	9.47	0.95	0.960
2	Luteolin 6-glucose-8-arabinose	10.70	0.99	0.052
3	Apiginin.6-arabinose-8-glactose	11.64	2.32	0.198
4	Apiginin.6-rhamnose-8-glucose	12.10	0.53	0.036
5	Narengin	12.24	1.64	0.215
6	Hisperidin	12.45	1.29	0.728
7	Rutin	12.61	0.85	0.095
8	Apiginin.7-*O*-neohespiroside	13.14	0.66	0.023
9	Kaempferol 3,7-dirhamoside	13.31	0.63	0.024
10	Quercetrin	13.41	3.37	0.228
11	Rosmarinic	14.21	1.13	0.011
12	Quercetin	14.85	1.31	0.048
13	Naringenin	15.01	1.30	0.031
14	Acacetin	15.12	0.66	0.084
15	Kaempferol3-(2-*p*-comaroyl) glucose	15.22	0.63	0.229
16	Hespertin	15.37	0.84	0.081
17	Kaempferol	16.23	0.46	0.104
18	Rhamnetin	16.48	1.04	0.0204
19	Apigenin	16.66	0.31	0.005

In vitro cytotoxic activity of methanol extracts of B. vulgaris:

The cytotoxicity was determined using MTT assay of methanolic extract Figure (3) and Table (3), which showed significant anticancer activity against pancreatic carcinoma (PANC-1) with (IC_{50}=3.69 µg/ml), mild cytotoxic activity against HepG2 with (IC_{50}=4.43 µg/ml), Lung carcinoma (A549) with (IC_{50}=4.9 µg/ml) and a weak activity against prostate carcinoma PC-3 with (IC_{50}=6.1 µg/ml).

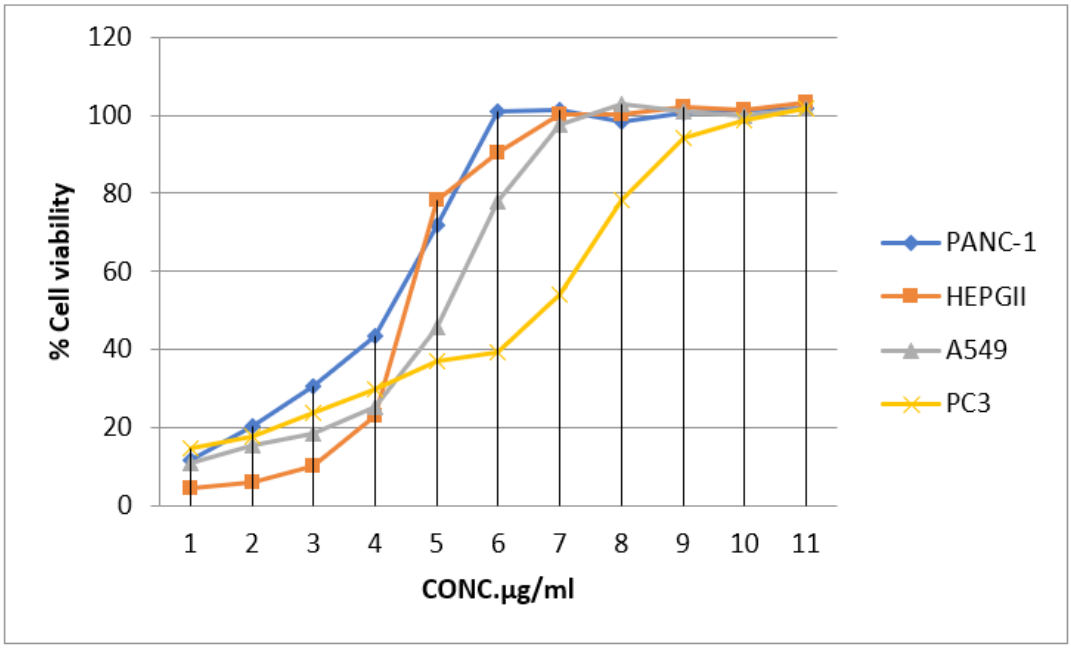

Figure (3): Percentage of Cell viability of methanolic extract of *B. vulgaris*

TABLE (3): Percentage of Cell viability of methanolic extract of *B. vulgaris*

Conc. of the extract, µg/ml	% of cell viability			
	PANC-1	HepG2	A549	PC-3
0	100	100	100	100
1	11.79941	4.377104	10.92896	14.56583
2	20.35398	6.060606	15.57377	17.92717
3	30.67847	10.10101	18.57923	23.80952
4	43.65782	23.23232	25.40984	29.69188
5	71.9764	78.11448	45.90164	36.97479
6	100.885	90.23569	77.86885	39.21569
7	101.4749	100.3367	97.54098	54.06162
8	98.23009	100.3367	102.7322	78.43137
9	100.59	102.0202	100.8197	94.11765
10	101.4749	101.3468	100	98.59944
11	101.7699	103.367	101.6393	101.6807

Antibacterial activity of the B.VulgarisL. methanolic extract:

The *B.Vulgaris* roots methanolic extract demonstrated inhibitory effect against pathogenic microorganisms: *Staphylococcus aureus*, (ATCC 29213; with zone (ZI=24mm) and gram positive strains *Pseudomonas aerogenasa* (ATCC 27853); with zone (ZI=19.3 mm) at 100 µL concentration.

Conclusions

The *B.Vulgaris L* roots extract capable of synthesizing and accumulating different types of phenolics and flavonoids which is a promising source for bioactive compounds.

The *B.Vulgaris L* roots extract showed significant anticancer activity against pancreatic

carcinoma, mild cytotoxic activity against HepG2 and a weak activity against prostate carcinoma.

In *vitro* antibacterial activity of the extract against two bacteria strains, showed a sensitizing effect against *Staphylococcus aureus*, (ZI=24mm) and gram positive strains *Pseudomonas aerogenasa* (ZI=19.3 mm) at 100 µL concentration.

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