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EVALUATION OF SOME CHEMICAL CONSTITUENTS, ANTIOXIDANT, ANTIBACTERIAL AND ANTICANCER ACTIVITIES OF *BETA VULGARIS* L. ROOT

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ABSTRACT

Beta vulgaris is belonging to the family Chenopodiaceae and has several varieties with bulb colors ranging from yellow to red. Deep red-colored beet roots are the most popular for human consumption, both cooked and raw as salad or juice. The ethanolic extract of beetroots contains valuable and active compounds such as carotenoids, phenols, flavonoids, tannin, alkaloids, vitamins C, B3, B6 and B9. Therefore, beetroot extract has antioxidant and antimicrobial activity against gram positive and negative bacteria. Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus* demonstrated higher susceptibility than Gram-negative *Escherichia coli* and *Pseudomonas typhimureum*. *Beta vulgaris* ethanolic extract exhibit significant anticancer activity against lung (A549) but slight effect against colorectal adenocarcinoma Caco-2 cell lines at the high concentrations of ethanolic extract (800 µg/ml).

KEYWORDS:

Beta vulgaris, phenols, flavonoids, tannin, carotenoids, vitamins, DPPH, antibacterial, anticancer activity.

INTRODUCTION

In the past few years, it's found that the use of synthetic drugs to protect the human from diseases is unsafe to human and environment. So that, it's very important to use the medicinal plants which have secondary metabolites and antioxidant compounds which decrease the effect of free radicals [1, 2].

Beta vulgaris L. subsp. vulgaris is belong to the family Chenopodiaceae (Angiosperm) [3] and it also called beetroot or garden beet [4]. Beetroot is annual crop, biennial herbaceous and cultivated for their edible roots and leaves [5]. The color of beetroot is differed from yellow to red according to its variety. In all over the world, red beets are used in human consumption [6]. The roots are used in mak-

ing salads, jam, soups and juice [7,8]. In addition, the leaves contain a large amount of antioxidant and vitamins, so it can be used as food and cooked as a spinach substitute [9]. Red beets have betalain pigments so that it has commercial and pharmaceutical uses such as natural food dye, cosmetics, drug formulations and paintings [10-12].

Red beets are the 10th vegetable in the world that have antioxidants [13, 14]. These antioxidants used as the scavengers of free radicals and prevent the oxidative damage on proteins, DNA and lipoproteins [15, 16]. The oxidative damage of macromolecules may lead to chronic diseases such as cancer, cataractogenesis, cardiovascular disease, neurodegenerative diseases, and stroke, which may prevent by the antioxidant compounds in red beets [17]. Red beets also have high concentrations of secondary metabolites (phenolic acids, flavonoids, ascorbic acid) [18-20].

The most important problems in the processing of the food industry are the contamination of microbes which affects the quality of foods and cause economic losses [21, 22]. So that, the important strategy to overcome this problem is to use natural antimicrobial compounds which presented in medicinal plants and protect from fungi and bacteria [23, 24]. Red beets used as antioxidant, antimicrobial, anti-inflammatory, antiallergenic, antithrombotic, antiatherogenic, cardioprotective, and vasodilatory properties [25].

The aim of this work is to study the chemical composition of red beet roots and to study its effect as antioxidant, antimicrobial and anticancer activity.

MATERIALS AND METHODS

Plant Materials. The roots of *Beta vulgaris* subsp. vulgaris var. Plano (sugar beet) was collected from local market in Egypt. *Beta vulgaris* was botanically characterized by Dr. Samah Azooz from Botany Department, Faculty of Agriculture, Cairo University, Egypt.

TABLE 1

Microbial strains used to test the antimicrobial activities of *Beta vulgaris* root extract

Microbial group	Indicator strain	Positive control	Cultivation conditions
Gram positive bacteria	<i>Staphylococcus aureus</i> (ATCC 25923)	Kanamycin	Muller-Hinton broth, 37°C / 24 h
	<i>Bacillus cereus</i> (ATCC 33018)		Muller-Hinton broth, 30°C / 24 h
Gram negative bacteria	<i>Escherichia coli</i> (ATCC 8739)	Polymyxin	Muller-Hinton broth, 37°C / 24 h
	<i>Salmonella typhimureum</i> (ATCC 14028)		Muller-Hinton broth, 37°C / 24 h
	<i>Aspergillus niger</i> (nrrl 326)		Sabouraud dextrose broth, 25 °C / 3days
Fungus	<i>Candida albicans</i> ATCC 10231	Nystatin	Sabouraud dextrose broth, 25°C / 24 h

Microbial strain. Table 1 illustrated the microorganisms which were used in this study and were obtained from the American Type Culture Collection (ATCC) as well as the culture collection of the Microbiology Lab, Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University.

Extraction method. The roots were cleaned and washed thoroughly under tap water, and then the roots were freeze-dried and grinded into fine powder using an electric blender. The powder was dried in an oven at 40°C for 24 h. The fine powder sample (500mg) was extracted in 10 ml ethanol or distilled H₂O for 24 h using a shaker, then the extract was filtered and the samples were stored at 4°C until use [26]. All analysis was done in the labs of Cairo University. Research Park (CURP), Faculty of Agriculture, Cairo University, Cairo, Egypt.

Total polyphenol content. The total phenolic content was estimated by Folin Ciocalteu method as described by Singleton et al. [27]. The absorbance was measured at 765 nm using a spectrophotometer Thermo Scientific HERIUS.

Total flavonoid content. The flavonoids content was determined by aluminium trichloride method as described by Zhishen et al. [28]. The absorbance was measured at 510 nm using a spectrophotometer.

Total tannin contents. Tannin content in red beet roots was determined by using Folin-Denis reagent as described by Saxena et al. [29]. The absorbance was read at 700 nm using spectrophotometer.

Total alkaloid contents. Alkaloids was measured according to the method described by Adham [30].

The percentage alkaloid was calculated as:
Percentage of total alkaloid = [Weight of residue / Weight of sample] × 100

Total Anthocyanine content. Fresh weight of *Beta vulgaris* root was homogenized in methanol containing 1% (v/v) HCl and then filtrate. The filtration was read at 530 and 657 nm using spectrophotometer as described by Mancinelli et al. [31].

Total carotenoid content. Total carotenoids of red beet root were extracted using a mixture of hexane: acetone (1:1 v/v) as described by Jeyanthi et al. [32]. The absorbance of carotenoid was read at 630 nm using spectrophotometer.

Water soluble vitamins. Sample Preparation. Water soluble vitamin were determined by HPLC analysis after extraction from the sample according to Albala-Hurtado et al. [33]. Dry weighed 0.2 g of red beet root powder was placed into centrifuge tube and add 15 mL of deionized water. After 15 min of ultrasonic extraction, centrifuge at 4000 rpm for 5 minutes, then quantitatively transfer to 25 mL volumetric flask, add water to the mark. Filter through 0.2µm nylon membrane before injection.

Instrument Conditions. Agilent 1260 infinity HPLC Series (Agilent, USA), equipped with Quaternary pump, a Kinetex XB-C18 column 100 mm x 4.6 mm (Phenomenex, USA), operated at 35°C. The separation is achieved using a binary linear elution gradient with (A) 25 mM NaH₂PO₄ pH = 2.5, (B) methanol. The injected volume was 20 µL. Detection: VWD detector set at 254 nm for ascorbic acids and 220nm for vitamins B3, B6, B9 and B12 [34].

Extraction of phenolic and flavonoid compounds. 0.2g dry sample extracted with 20 ml ethanol 80%, soak in brown bottle for 24 hr at room temperature, centrifuged for 5 min, volume adjusted to 25 ml by ethanol 80%, filtered through Whatman filter paper, 10 ml of the solution evaporated to dryness then dissolved in 5 ml HPLC grade methanol 50%, filtered through PTFE filter with pore size 0.2 µm.

Instrument Condition for phenolic compounds. Agilent 1260 infinity HPLC Series (Agilent, USA), equipped with Quaternary pump, a Zorbax Eclipse plusC18 column 100 mm x 4.6 mm i.d., (Agilent technologies, USA), operated at 30°C. The separation is achieved using a ternary linear elution gradient with (A) HPLC grade water 0.2 % H₃PO₄ (v/v), (B) methanol and (C) acetonitrile. The injected volume was 20 µL. Detection: VWD detector set at 284 nm.

Instrument Condition for Flavonoids. HPLC, Smart line, Knauer, Germany., equipped

with binary pump, a Zorbax Eclipse plusC18 column 150 mm x 4.6 mm i.d., (Agilent technologies, USA), operated at 35°C. Eluent: methanol: H₂O with 0.5% H₃PO₄, 50:50 with flow rate 0.7 ml/min, the injected volume was 20 µL. Detection: UV detector set at 273 nm and data integration by claritychrom® software. This method was the modified of methods Goupy et al. [35] and Mattila et al. [36] for fractionate the polyphenols and flavonoids, respectively.

DPPH free Radical Scavenging activity (RSA). The antioxidant activity of the *Beta vulgaris* root extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable DPPH method as modified by Hae-Ryong et al. [37]. The reaction mixture containing 1 ml of the extract at different concentrations (40, 80, 120, 150 µg/ml) and 1ml of DPPH (0.2mM) was vigorously shaken and incubated in darkness at room temperature for 30 minutes. The absorbance was read at 517nm using UV-visible spectrophotometer. Radical scavenging activity was expressed as percent of inhibition and was calculated using the following formula:-

$$\%DPPH = \left[\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \right] \times 100$$

Antibacterial activity. Agar disc diffusion method was used to evaluate antibacterial activity of red beet roots as describe by Bauer et al. [38]. The strains were grown on Mueller-Hinton agar slants at 37°C for 24 h and checked for purity. After the incubation, the cells were washed off the surface of agar and suspended in sterile physiological solution. The number of cells in 1 ml of suspension for inoculation measured by McFarland nefelometer was 5×10^7 CFU/ml. 1 ml of these suspensions was homogenized with 9 ml of melted (45°C) Mueller-Hinton agar and poured into Petri dishes. On the surface of the agar, 5 mm diameter paper discs (HiMedia®, Mumbai, India) were applied and impregnated with 15 µl of samples. The plates were incubated at the optimum temperature for each indicator strain (Table 1) and tested after 24, 48 and 72 h. Growth inhibition was scored positive in the presence of a detectable clear zone (ZI) around the disc and expressed in mm. Experiments were car-

ried out in triplicates and the inhibition zone was recorded as the average of the replicates ± SD.

In Vitro cytotoxicity assay. Human lung cancer (A549) and colorectal adenocarcinoma Caco-2 were purchased from CURP, faculty of agriculture at Cairo University (Egypt). Cells were maintained in (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 µg/ml streptomycin and 100 unit/ml penicillin g potassium, in a humidified 90% and 5% (V/V) CO₂ atmosphere at 37°C. The cytotoxicity of ethanolic extracts was tested by the neutral red (NR) assay as previously described [39]. Exponentially growing cells were collected using 0.25% Trypsin-EDTA and seeded in 96- well plates at 20000 cells/well. After incubation (overnight), extracts were added in various concentrations (10, 50, 100, 200, 400, and 800 µg/ml); 4 wells for each concentration. After treatment with extracts for 24h., media were removed and cells were exposed to neutral red solution for 4 hours at 37°C. Destin solution was used to dissolve the NR stained cells and color intensity was measured at 540nm microplate reader (Biotek, ELX808).

Statistical analysis. All results were expressed as mean values ± standard deviation. Comparisons were performed by analysis of variance (ANOVA). Statistical analyses were run using SAS software.

RESULTS AND DISCUSSION

Chemical constituents of red beet root. As illustrated in Table 2, the chemical constituents of ethanolic extract of red beet roots contain total phenolic (133.5 mg /g DW), total flavonoids (1.5 mg /g DW), total tannin (5.13 mg /g DW), total alkaloid (2.1 g /100g DW), total athocyanin (63.7 µg/100g FW) and carotenoids (1.7 mg/100g FW). These results are similar to previous studies [40-42], who found that the main components of red beet root extract are polyphenols, alkaloids, tannins, flavonoids, folic acid, reducing sugars and ascorbic acid. In addition, folic acid and vitamins A, B, and C can play important roles in brain development and motor function.

TABLE 2
Quantitative phytochemical analysis of *Beta vulgaris* root

Constituents	Values in ethanolic extract
Total phenolic (mg Gallic acid /g DW)	133.5±1.05
Total flavonoid (mg Quercetin /g DW)	1.54±0.047
Total tannin (mg Tannic acid /g DW)	5.13±0.085
Total alkaloid (g/100g DW)	2.10±0.040
Total athocyanin (µg/100g FW)	63.73±0.032
Carotenoids (mg/100g FW)	1.72±0.08

Values are mean ± SD of three replicate analyses

The presence of the secondary metabolites in red beet root has contributed to its medicinal value as well as physiological activity [43]. Phytochemical components are responsible for both pharmacological and toxic activities in plants [44]. They are used for therapeutic purposes to cure various diseases and to heal injuries [45]. For instance, flavonoids have been shown to have antibacterial, anti-inflammatory, anti-allergic, antiviral, antineoplastic and antioxidant, which act as free radical scavenger and metal chelators [46, 47]. Alkaloids contribute to plant species fitness of survival and have pharmacological effects and are used as medication and recreational drugs [48]. They protect the plants against infection with insects by the production of the bitter taste that repels insects from feeding on plant leaves. Tannins may provide protection against microbial degradation of dietary proteins in the rumen [49]. In addition, carotenoids have protective effects against several diseases such as cancer, coronary heart disease, inflammatory reactions, and age-related macular degeneration [50] and act as antioxidant [51].

HPLC of soluble vitamins. The results in Table 3 reported that the ethanolic extract of red beetroot contains vitamin C (26.2 mg/100g DW), vitamin B3 (1.67 mg/100g DW), vitamin B6 (6.17 mg/100g DW) and vitamin B9 (2.60 mg/100g DW). These results are similar to Odoh and Okoro [41] who found that beetroot contains significant amounts of vitamins, especially vitamin C (4.36 mg/100 g).

The results showed that red beet root has a high concentration of ascorbic acid. This vitamin plays an important role in human nutrition, including growth and maintenance of tissues, the production of neurotransmitters, hormones and immune system responses. Vitamin C is an important antioxidant and reduces the adverse effects of reactive oxygen species which caused damage to macromolecules such as lipids, DNA and proteins, which are related to cardiovascular disease, cancer and neurodegenerative diseases [52].

TABLE 3
Water soluble vitamins contents of
***Beta vulgaris* root**

Vitamin contents	Values (mg/100g DW)
Vitamin C (Ascorbic acid)	26.23±0.32
Vitamin B3 (Niacin)	1.67±0.05
Vitamin B6 (Pyridoxine)	6.173±0.16
Vitamin B9 (Folic acid)	2.60±0.08

Values are mean ± SD of three replicate analyses

HPLC of phenolic compounds. Data in Table 4 showed that the ethanolic extract of red beet root contains a number of phenolic compounds such as gallic acid (11 mg/100g DW), catechol (7.4 mg/100g DW), *p*-Coumaric acid (0.74 mg/100g

DW), ferulic acid (0.68 mg/100g DW), *o*-Coumaric acid (1.31 mg/100g DW) and cinnamic acid (0.6 mg/100g DW). These results are similar to Vulić et al. [53] who reported that beetroot contain ferulic, vanillic, *p*-hydroxybenzoic, caffeic and protocatechuic acids.

In addition, the ethanolic extract of red beet root contains a number of flavonoids compounds such as myricetin (19.3 mg/100g DW), neringenin (19.9 mg/100g DW), kaempferol (3.0 mg/100g DW) and apigenin (2.56 mg/100g DW). Similar results reported by Pyo et al. [54] recognized the following: catechin (6.7 mg/100 g FW), myricetin (2.2 mg/100 g FW), quercetin (7.5 mg/100 g FW) and kaempferol (9.2 mg/100 g FW).

Also, Ben Haj Koubaier et al. [55] found that the presence of five phenolic acids (ferulic, vanillic, syringic, ellagic, and caffeic), three flavonoids (quercetin, kaempferol, and myricetin) for roots of red beet by using Liquid chromatography–mass spectrometry. These flavonoids act as antioxidation, antiinflammation and inhibition of tumor proliferation [56].

TABLE 4
HPLC analysis of phenolic and flavonoid
compounds of *Beta vulgaris* root

Phenolic compounds	Conc. mg/100g DW
Gallic acid	11.01
Catechol	7.38
<i>p</i> -Coumaric acid	0.74
Ferulic acid	0.68
<i>o</i> -Coumaric acid	1.31
Cinnamic acid	0.60
flavonoid compounds	
Myricetin	19.25
Neringenin	19.92
Kaempferol	3.02
Apigenin	2.65

Antioxidant activity of red beet root. The effect of antioxidants on DPPH radical scavenging was thought to result from their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. It is visually noticeable as a discoloration from purple to yellow. The scavenging of DPPH radicals increased with increasing extract concentration from 40, 80, 120 and 150 µg /mL (Table 5). The IC₅₀ value of ethanolic extract of beet root was 55.82 µg /mL concentration. IC₅₀ value indicate the concentration of the test sample required to inhibit 50% of the free radicals. The IC₅₀ value is a parameter widely used to measure the free radical scavenging activity [57]; a smaller IC₅₀ value corresponds to a higher antioxidant activity.

Enzymatic and nonenzymatic antioxidants are molecules that have the ability to scavenge free

radicals before they damage the cells. Antioxidants can be endogenous or obtained exogenously, either through diet or by dietary supplements [58]. Scientific evidence suggests that antioxidant compounds in food play an important role as a health protecting factor. Beet root ranks among the top ten most powerful vegetables with respect to its antioxidant capacity ascribed with a total phenol content of 50–60 $\mu\text{mol/g}$ dry weight [13]. Kähkönen et al. [14] reported that beet root contains considerable amount of phenolic acids such as ferulic, protocatechuic, vanillic, *p*-coumaric, phydroxybenzoic, and syringic acids [59].

TABLE 5
Antioxidant activity of *Beta vulgaris* root against DPPH method.

Conc. ($\mu\text{g/ml}$)	DPPH % in ethanolic extract
40	49.20
80	50.638
120	52.763
150	70.351
IC ₅₀ ($\mu\text{g/ml}$)	55.823

Phenolic compounds present in red beet decrease oxidative damage of lipids improves antioxidant status in humans, scavenges free radicals, exhibits inflammatory effect, anticancer property and reduces the risk of chronic illnesses such as cancer and cardiovascular diseases [18]. In addition, the antioxidant properties of phenolic compounds are mainly because of their redox potential, which allows them to act as reducing agents, hydrogen donors, metal chelators and singlet oxygen quenchers.

Antimicrobial activity of red beet root. The agar diffusion method used to evaluate the antibacterial and antifungal activity of ethanolic extract of red beetroot by using selected gram-positive, gram-negative bacteria and fungus. The diameter of the inhibition zone (ZI) is shown in Table 6.

The data indicate that the extract exhibited the activity against the investigated food pathogens. Gram positive bacteria *Staphylococcus aureus* and *Bacillus cereus* demonstrated higher susceptibility than Gram-negative *Escherichia coli* and *Salmonella typhimureum*. The extract showed antibacterial activity against *Staphylococcus aureus* (ZI = 12.5 mm), one of the most common gram-positive bacte-

rium causing food poisoning. On the other hand, a weak antimicrobial activity was found against *Salmonella typhimureum* (ZI = 7.11 mm). The extract showed no effect on the fungus used (*Aspergillus niger* and *Candida albicans*). In general, red beet extracts have exhibited antibacterial activity against a wide range of gram-positive bacteria and gram-negative bacteria; however, no inhibitory activity was found against the fungi and yeasts studied [60-63].

In terms of antimicrobial potential, gram-positive bacteria (*Bacillus*, *Micrococcus*, *Staphylococcus*, and *Streptococcus*) have been found more susceptible to red beets than gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) [62-63, 53]. The red beetroot extract contains a high amount of phenolic compounds which may cause the disrupting of the cell wall structure of gram-positive bacteria [64-66]. The inhibitory effect of ethanolic extract on gram-negative bacteria is attribute to their outer membrane, consisting of double-layered, highly hydrophilic lipopolysaccharide molecules, and unique periplasmic space [67-68].

These bacteria caused the infectious diseases on human health and also affect on food safety. The phytochemicals derived from red beetroot have been widely exploited for their colorant properties; however, there is much potential for utilizing their antimicrobial properties, particularly in food-related applications and consumer products targeting human health and beauty. For example, red beet compounds could be incorporated into products such as active antimicrobial food packaging [69], cosmetic products [70-72] and also in active drug formulations for the treatment against disease in the form of supplements, topical sprays, and ointments.

Anticancer activity of red beetroot. Data in Table 7, showed that the cytotoxic activity of red beetroot as an anticancer agent (towards to lung) and IC₅₀ dose. The percentage of lung cancer cell line (A549) viability was decreased with increasing the concentrations of the methanolic extract of red beetroot (Figure 1). On the other hand, the viability of colorectal adenocarcinoma Caco-2 is not affected by all concentrations of red beet root except the high concentrations (800 $\mu\text{g/ml}$) which showed slight decrease in the viability of Caco-2 cell line. Cancer is often associated with increased risk of death and the toxic side effects caused by the modern medicine.

TABLE 6
Antibacterial activities of *Beta vulgaris* root against selected bacterial strains and fungus.

Samples	Inhibition zone (mm)*					
	Gram positive bacteria		Gram negative bacteria		Fungus	
	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>S. typhimureum</i>	<i>A. niger</i>	<i>C. albicans</i>
<i>Beta vulgaris</i> root in ethanolic extract	12.54±0.35	9.25±0.16	8.37±0.21	7.11±0.0	-	-

Values are mean \pm SD of three replicate analyses, *Well size = 5 mm

TABLE 7
Anticancer activities of *Beta vulgaris* root.

Concentrations ($\mu\text{g/ml}$)	Lung cell line (A549)	Colorectal adenocarcinoma Caco-2
	Viability %	
10	100	100
50	99.8	100
100	88.6	100
200	86.4	100
400	82.8	100
800	78.0	95.5
IC ₅₀ ($\mu\text{g/ml}$)	17800	4831

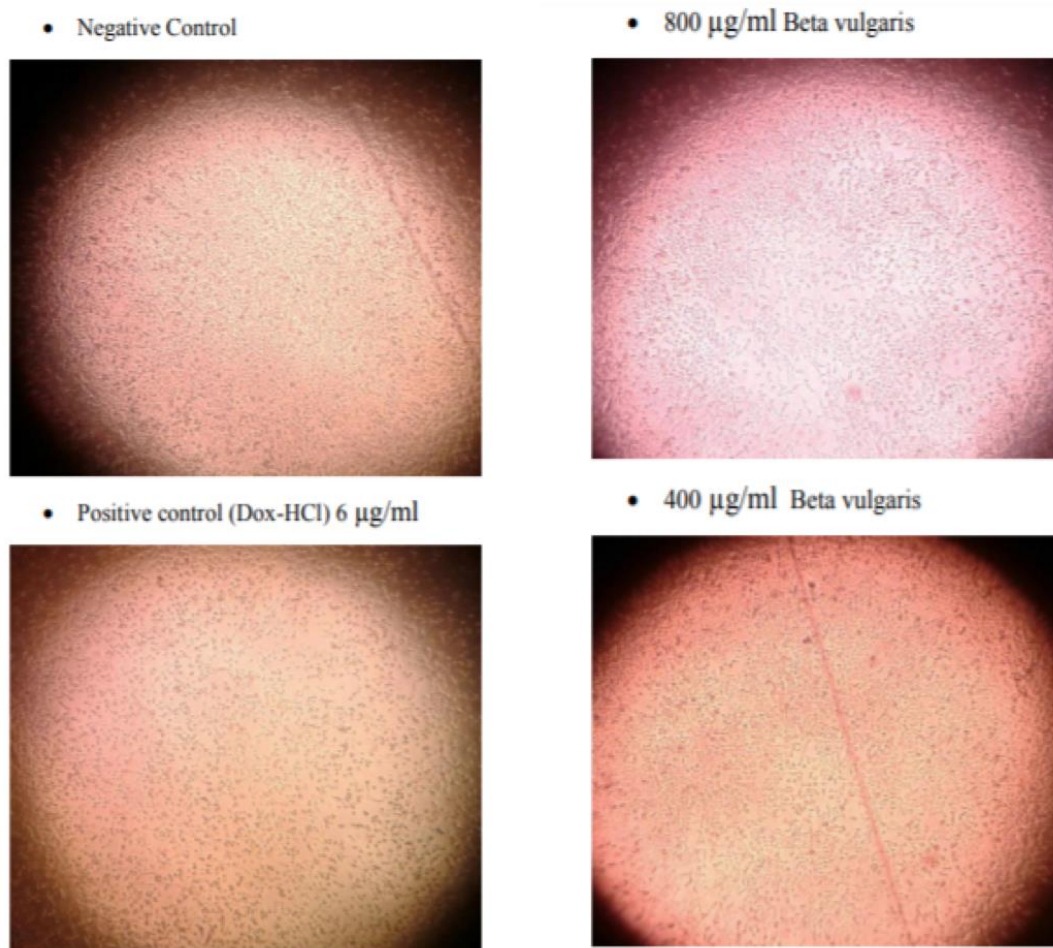


FIGURE 1
Morphological observation of cancer cell lines (A549) by 40X magnification power.

Many cancer patients seek alternative and complementary methods of treatment such as usage of phytomedicine. Natural dietary agents have drawn a great deal of attention because of their potential to suppress cancers and to reduce the risk of cancer development by decreasing oxidative stress, which plays a significant role in the pathogenesis and pathophysiological process of cancer [73]. Previous studies have shown that beetroot has an excellent antioxidant property which can contribute to the anticancer activity [74-75]. Previous phytochemical studies of *Beta vulgaris* indicate the

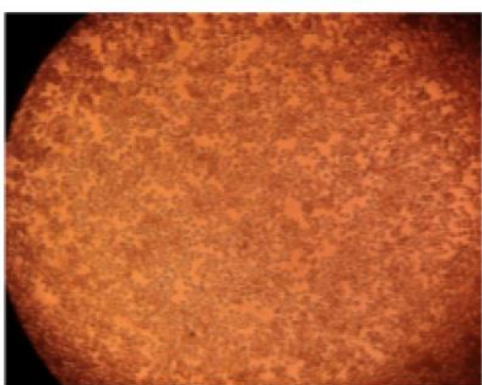
presence of phenolic groups, flavonoids, betaxanthins and betacyanins [74]. Polyphenolic compounds might inhibit cancer cells by xenobiotic metabolizing enzymes that alter metabolic activation of potential carcinogens, while some flavonoids could also alter hormone production and inhibit aromatase to prevent the cancer cells [76, 77]. The mechanism of action of anticancer activity of phenols could be by disturbing the cellular division during mitosis at the telophase stage. It was also reported that phenols reduce the amount of

cellular protein and mitotic index and colony formation during cell proliferation of cancer cells [78].

• Negative Control



• Positive control (Dox-HCl) 6 µg/ml



• 800 µg/ml Beta vulgaris



FIGURE 2
Morphological observation of Colorectal adenocarcinoma Caco-2 by 40X magnification power.

CONCLUSION

This research is following a trend to effectively identify various compounds found in the root of red beet and find its prophylactic role in designing and developing pharmacological drugs with less

side effects. In vitro investigations in the present study provide substantial evidence that beetroot peel; an inedible waste product is a potent source of antioxidant, antimicrobial agent and anticancer activity thereby indicating its use as a value-added component for functional.

REFERENCES

- [1] Zaidi, S.K., Hoda, M.N., Tabrez, S., Ansari, S.A., Jafri, M.A., Shahnawaz, K.M. (2014) Protective effect of *Solanum nigrum* leaves extract on immobilization stress induced changes in rat's brain. *Evid. Based Complementary Altern Med.* 3, 22-34.
- [2] Sulakhiya, K., Patel, V.K., Saxena, R., Dashore, J., Srivastava, A.K., Rathore, M. (2016) Effect of *Beta vulgaris* Linn. leaves extract on anxiety and depressive-like behavior and oxidative stress in mice after acute restraint stress. *Pharm. Res.* 8, 11-12.
- [3] Oyen, L.P.A. (2004) *Beta vulgaris* L. In: *Plant Resources of Tropical Africa 2. Vegetables*. Grubben, G.B.H., Denton, O.A. (Eds.), Wageningen: PROTA Foundations, 110-113.
- [4] Goldman, I.L., Navazio, J.P. (2003) History and breeding of table beet in the United States. *Plant Breed. Rev.* 22, 357-388.
- [5] Neelwarne, B., Halagur, S.B. (2012) Red beet: an overview. In: *Red Beet Biotechnology: Food and Pharmaceutical Applications*. Neelwarne, B. (Ed.), New York: Springer, 1-43.
- [6] Singh, B., Hathan, B.S. (2014) Chemical composition, functional properties and processing of beetroot—a review. *IJSER.* 5, 679-684.
- [7] Wruss, J., Waldenberger, G., Huemer, S., Uygun, P., Lanzerstorfer, P., Müller, U., Höglinger, O., Weghuber, J. (2015) Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. *J. Food Compos. Anal.* 42, 46-55.
- [8] Guldiken, B., Toydemir, G., Nur Memis, K., Okur, S., Boyacioglu, D., Capanoglu, E. (2016) Home-processed red beetroot (*Beta vulgaris* L.) products: changes in antioxidant properties and bioaccessibility. *Int. J. Mol. Sci.* 17, 858.
- [9] Biondo, P.B.F., Boeing, J.S., Barizo, R.O., Souza, N.E.D., Matsushita, M., Oliveira, C.C.D., Boroski, M., Visentainer, J.V. (2014) Evaluation of beetroot (*Beta vulgaris* L.) leaves during its developmental stages: a chemical composition study. *Food Sci. Technol. (Campinas).* 34, 94-101.
- [10] Pai, S.R., D'Mello, P. (2004) Stability evaluation of beetroot colour in various pharmaceutical matrices. *Indian J. Pharm. Sci.* 66, 696-699.

- [11] Esatbeyoglu, T., Wagner, A.E., Schini-Kerth, V.B., Rimbach, G. (2015) Betanin—a food colorant with biological activity. *Mol. Nutr. Food Res.* 59, 36–47.
- [12] Sainath, M., Kumar, K.S., Babu, K.A. (2016) Formulation and evaluation of herbal lipstick. *IJARMP*, 2058–60.
- [13] Vinson, J.A., Hao, Y., Su, X., Zubik, L. (1998) Phenol antioxidant quantity and quality in foods: vegetables. *Journal of Agricultural and Food Chemistry.* 46, 3630–3634.
- [14] Kähkönen, M.P., Hopia, A. I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S. (1999) Antioxidant activity of plant extracts containing phenolic compounds. *J. Agr. Food Chem.* 47, 3954–3962.
- [15] Chang, S., Hsieh, C., Yen, G. (2008) The protective effect of *Opuntia dillenii* Haw fruit against low-density lipoprotein peroxidation and its active compounds. *Food Chem.* 106, 569–575.
- [16] Uttara, B., Singh, A.V., Zamboni, P., Mahajan, R.T. (2009) Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr. Neuropharmacol.* 7, 65–74.
- [17] Halliwell, B., Gutteridge, J. (1999) The chemistry of free radicals and related 'reactive species'. *Free Radic Biol Med.* 3, 1–7.
- [18] Georgiev, V.G., Weber, J., Kneschke, E., Denev, P.N., Bley, T., Pavlov, A.I. (2010) Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot *Beta vulgaris* cv. Detroit dark red. *Plant Foods Hum. Nutr.* 65, 105–111.
- [19] Vulić, J., Čanadanović-Brunet, J., Četković, G., Tumbas, V., Djilas, S., Četojević-Simin, D., Čanadanović, V. (2012) Antioxidant and cell growth activities of beet root pomace extracts. *J. Funct. Foods.* 4, 670–678.
- [20] Vulić, J.J., Čebović, T.N., Čanadanović-Brunet, J.M., Četković, G.S., Čanadanović, V.M., Djilas, S.M., Tumbas Šaponjac, V.T. (2014) *In vivo* and *in vitro* antioxidant effects of beetroot pomace extracts. *J. Funct. Foods.* 6, 168–175.
- [21] Brooks, J.D., Flint, S.H. (2008) Biofilms in the food industry: problems and potential solutions. *Int. J. Food Sci. Technol.* 43, 2163–2176.
- [22] Yeung, M. (2016) Microbial forensics in food safety. *Microbiol spectr.* 4(4), EMF-0002-2013.
- [23] Davidson, P.M., Taylor, T.M., Schmidt, S.E. (2013) Chemical preservatives and natural antimicrobial compounds. In: *Food microbiology: Fundamentals and Frontiers*. 3rd ed. Doyle, M., Beuchat, L. (Eds.), Washington, D.C.: American Society of Microbiology, 765–801.
- [24] Gyawali, R., Ibrahim, S.A. (2014) Natural products as antimicrobial agents. *Food Control.* 46, 412–429.
- [25] Balasundram, N., Sundram, K., Samman, S. (2006) Phenolic compounds in plants and agricultural by-products: antioxidant activity, occurrence, and potential uses. *Food Chem.* 99, 191–203.
- [26] Sumathy, N., Sumathy, J. (2011) Antibacterial and antifungal activity of musa fruit peels against skin and gastrointestinal tract diseases. *Herbal Tech Industry.* 2, 9–11.
- [27] Singleton, V., Rossi, J. (1965) Colorimetry of total phenolics with phosphomolibdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16, 144–158.
- [28] Zhishen J., Mengcheng T., Jianming W. (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64, 555–559.
- [29] Saxena, V., Mishra, G., Saxena, A., Vishwakarma, K.R. (2013) A comparative study on quantitative estimation of tannins in *Terminalia chebula*, *Terminalia bellerica*, *Terminalia arjuna* and *Saraca indica* using spectrophotometer. *Asian J. Pharm. Clin. Res.* 6(3), 148–149.
- [30] Adham, A.N. (2015) Comparative extraction methods, phytochemical constituents, fluorescence analysis and HPLC validation of rosmarinic acid content in *Mentha piperita*, *Mentha longifolia* and *Osimum basilicum*. *J. Pharmacogn. Phytochem.* 3(6), 130–139.
- [31] Mancinelli, A.L., Yang, C.H., Rabino, I., Kuzmanoff, K. (1976) Photo control of anthocyanin synthesis. *Plant Physiol.* 58, 214–219.
- [32] Jeyanthi, R.L., Sharmila, S., Das, M.P., Seshiah, C. (2014) Extraction and purification of carotenoids from vegetables. *J. Chem. Pharm. Res.* 6(4), 594–598.
- [33] Albala-Hurtado, S., Veciana-Nogues, M.T., Izquierdo-Pulido, M., Marine-Font, A. (1997) Determination of water-soluble vitamins in infant milk by high performance liquid chromatography. *J. Chromatogr. A.* 778, 247–253.
- [34] Jedlicka, A., Klimes, J. (2005) Determination of water and fat-soluble vitamins in different matrices using High-Performance Liquid Chromatography. *Chem. Pap.* 59, 202–222.
- [35] Goupy, P., Hugues, M., Biovin, P., Amiot, M.J. (1999) Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. *J. Sci. Food Agr.* 79, 1625–1634.
- [36] Mattila, P., Astola, J., Kumpulainen, J. (2000) Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. *J. Agr. Food Chem.* 48, 5834–5841.

- [37] Park, H.R., Park, E., Rim, A.R., Jeon, K.I., Huang, J.H., Lee, S.C. (2006) Antioxidant activity of extracts from *Acanthopanax senticosus*. *African Journal of Biotechnology*. 5(23), 2388-2396.
- [38] Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M. (1966) Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 45(4), 493-496.
- [39] Repetto, G., del Peso, A., Zurita, J.L. (2008) Neutral red uptake assay for the estimation of cell viability/cytotoxicity. *Nat Protoc.* 3, 1125-1131.
- [40] Mroczek, A., Kapusta, I., Janda, B., Janiszowska, W. (2012) Triterpene saponin content in the roots of red beet (*Beta vulgaris* L.) cultivars. *J. Agr. Food Chem.* 60, 12397-12402.
- [41] Odoh, U.E., Okoro, E.C. (2013) Quantitative phytochemical, proximate/nutritive composition analysis of *Beta vulgaris* linnaeus (chenopodiaceae). *Int. J. Curr. Res.* 5(12), 3723-3728.
- [42] Mokhtari-Dehkordi, S., Hojjati, M., Rouhi, L., Rabiei, Z., Alibabaei, Z. (2014) Effect of ethanolic extract of beet roots and leaves on motor coordination in male Wistar rats. *J. Shahrekord Univ. Med. Sci.* 16, 14-23.
- [43] Sofowara, E.A. (1993) Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, 55 – 71.
- [44] El-Beltagi, H.E.S. (2011) Effect of roasting treatments on protein fraction profiles, some enzyme activities of Egyptian peanuts. *Int. J. Food. Sci. Nutr.* 62, 453-456.
- [45] Okwu, D.E., Josiah, C. (2006) Evaluation of the chemical composition of two Nigerian medicinal plants. *Afr. J. Biotechnol.* 5, 357-361.
- [46] Nakayama, N.G., Lindsey, M.L., Medical, L.H. (1993) Inhibition of the infectivity of influenza virus by tea polyphenol. *Antiviral Res.* 21, 289 – 299.
- [47] Mishra, A.K., Okoli, J.O., Ohaju-obodo and Eifidiyi, K. (2009) Inhibitory activity of India specie plant *Cinnamomum zeylanicum* extracts against *Alternaria solani* and *Curvularia lunata*, the pathogenic dematiaceous moulds. *Ann. Clin. Microbiol. Antimicrob.* 8(9), 1-7.
- [48] Roger, M.F., Wink, M. (1998) Alkaloids: Biochemistry, ecology and medicinal applications. Plenum press, 2-3.
- [49] Aletor, V.A. (1993) Allelochemicals in plant food and feedingstuffs: 1. Nutritional, biochemical and physiological aspects in animal production. *Vet. Hum. Toxicol.* 35, 57-67.
- [50] Eisenhauer, B., Natoli, S., Liew, G., Flood, V.M. (2017) Lutein and zeaxanthin-Food sources, bioavailability and dietary variety in age-related macular degeneration protection. *Nutrients.* 9(2), 1-14.
- [51] Sözgen Başkan, K., Tütem, E., Özer, N., Apak, R. (2013) Spectrophotometric and chromatographic assessment of contributions of carotenoids and chlorophylls to the total antioxidant capacities of plant foods. *J. Agr. Food Chem.* 61(47), 11371–11381.
- [52] Naidu, K. (2003) Vitamin C in human health and disease is still a mystery? An overview. *Nutr. J.* 2(1), 1–10.
- [53] Vulić, J.J., Čebović, T.N., Čanadanović, V.M., Četković, G.S., Djilas, S.M., Čanadanović-Brunet, J.M., Velićanski, A.S., Cvetković, D.D., Tumbas, V.T. (2013) Antiradical, antimicrobial and cytotoxic activities of commercial beetroot pomace. *J. Food Funct.* 4, 713–721.
- [54] Pyo, Y.H., Lee, T.C., Logendra, L., Rosen, R.T. (2004) Antioxidant activity and phenolic compounds of Swiss chard (*Beta vulgaris* subspecies *cycla*) extracts. *Food Chem.* 85, 19–26.
- [55] Ben Haj Koubaier, H., Snoussi, A., Essaidi, I., Chaabouni, M.M., Thonart, P., Bouzouita, N. (2014) Betalain and phenolic compositions, antioxidant activity of tunisian red beet (*Beta vulgaris* L. *conditiva*) roots and stems extracts. *Int. J. Food. Prop.* 17(9), 1934-1945.
- [56] Birt, D.F., Hendrich, S., Wang, W. (2001) Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacol. Therapeut.* 90, 157–177.
- [57] Cuvelier, M.E., Richard, H., Berset, C. (1992) Comparison of the antioxidative activity of some acid-phenols: Structure–activity relationships. *Biosci. Biotechnol. Biochem.* 56, 324–325.
- [58] Li, Y., Brown, R.W., Bonner, M.R., Deng, F., Tian, L., Mu, L. (2014) Positive relationship between total antioxidant status and chemokines observed in adults. *Oxidative Medicine and Cellular Longevity*. Article ID 693680, 6p.
- [59] Kujala, T., Loponen, J., Pihlaja, K. (2001) Betalains and phenolics in red beetroot (*Beta vulgaris* L.) peel extracts: extraction and characterization. *Zeitschrift fur Naturforschung.* 56, 343–348.
- [60] Koochak, H., Seyyednejad, S.M., Motamedi, H. (2010) Preliminary study on the antibacterial activity of some medicinal plants of Khuzestan (Iran). *Asian Pac. J. Trop. Med.* 3, 180–184.
- [61] Velićanski, A.S., Cvetković, D.D., Markov, S.L., Vulić, J.J., Djilas, S.M. (2011) Antibacterial activity of *Beta vulgaris* L. pomace extract. *APTEFF.* 42, 263–269.
- [62] Canadanovic-Brunet, J., Savatovic, S.S., Cetkovic, G.S., Vulić, J.J., Djilas, S.M., Markov, S.L., Cvetkovic, D.D. (2011) Antioxidant and antimicrobial activities of beet root pomace extracts. *Czech J. Food Sci.* 29, 575–585.

- [63] Boo, H., Hwang, S., Bae, C., Park, S., Heo, B., Gorinstein, S. (2012) Extraction and characterization of some natural plant pigments. *Ind. Crop Prod.* 40, 129–135.
- [64] Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C.I., Angioni, A., Dessi, S., Marzouki, N., Cabras, P. (2007) Antimicrobial activity of Tunisian quince (*Cydonia oblonga* Miller) pulp and peel polyphenolic extracts. *J. Agr. Food Chem.* 55, 963–969.
- [65] Daglia, M. (2012) Polyphenols as antimicrobial agents. *Curr. Opin. Biotechnol.* 23, 174–181.
- [66] Kchaou, W., Abbès, F., Mansour, R.B., Blecker, C., Attia, H., Besbes, S. (2016) Phenolic profile, antibacterial and cytotoxic properties of second grade date extract from Tunisian cultivars (*Phoenix dactylifera* L.). *Food Chem.* 194, 1048–1055.
- [67] Nikaido, H. (2003) Molecular basis of bacterial outer membrane permeability revisited. *Microbiology and Molecular Biol. Rev.* 67, 593–656.
- [68] Delcour, A.H. (2009) Outer membrane permeability and antibiotic resistance. *Biochim. Biophys. Acta (BBA)-Proteins and Proteomics.* 1794, 808–816.
- [69] Manohar, C.M., Kundgar, S.D., Doble, M. (2016) Betanin immobilized LDPE as antimicrobial food wrapper. *LWT-Food Sci. Technol.* 80, 131-135.
- [70] Kapoor, V.P., Katiyar, K., Pushpangadan, P., Singh, N. (2008) Development of natural dye based sindoor. *Nat. Prod. Rad.* 7, 22–29.
- [71] Yilar, M., Kadioglu, I., Telci, I. (2018) Chemical composition and antifungal activity of *Salvia officinalis* (L.), *S. cryptantha* (montbret et aucher ex benth.), *S. tomentosa* (Mill.) plant essential oils and extracts. *Fresen. Environ. Bull.* 27, 1695-1702.
- [72] Sevindic, M., Akgul, H., Dogan, M., Akata, I., Selamoglu, Z. (2018) Determination of antioxidant, antimicrobial and protective activity and heavy metals content of *Leatiporus sulphureus*. *Fresen. Environ. Bull.* 27, 1946-1952.
- [73] Bennett, L., Rojas, S., Seefeldt, T. (2012) Role of antioxidant in the prevention of Cancer. *J. Exp. Clin. Med.* 4(4), 2015-22.
- [74] Jasna, M., Canadanovic, B., Sladjana, S., Gordana, C., Jelena, J., Sonia, M., Dragoljub, D. (2011) Antioxidant and antimicrobial activities of beetroot pomace extract. *Czech J. Food Sci.* 29(6), 575-85.
- [75] Slavov, A., Karagyozev, V., Denev, P., Kratchanova, M., Kratchanov, C. (2013) Antioxidant activity of red beet juice obtained after microwave and thermal pretreatment. *Czech J. Food Sci.* 31(2), 139-47.
- [76] Abdel-Rahim, E.A., El-Beltagi, H.S. (2010) Constituents of apple, parsley and lentil edible plants and their therapy treatments for blood picture as well as liver and kidneys functions against lipidemic disease. *EJEAFChe* 9, 1117-1127.
- [77] El-Desoky, A.H., Abdel-Rahman, R.F., Ahmed, O.K., El-Beltagi, H.S., Hattori, M. (2018) Anti-inflammatory and antioxidant activities of naringin isolated from *Carissa carandas* L.: *In vitro* and *in vivo* evidence. *Phytomedicine.* 42, 126-134.
- [78] Afify, A.M.R., El-Beltagi H.S., Fayed, S.A., El-Ansary, A.E. (2018) Enhancing effect of olive leaves extract on lipid profile and enzymes activity in streptozotocin induced diabetic rats. *Fresen. Environ. Bull.* 27, 1875-1883.
- [79] Anand, G., Sumithira, G., Chinna, R., Muthukumar, A. (2013) *In vitro* and *In vivo* anticancer activity of hydro alcoholic extract of *Ipomoea cornea* leaf against Ehrlich Ascites Carcinoma cell lines. *Int. J. Adv. Pharmaceutical. Genuine Res.* 1(1), 39-54.

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