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Original Article

EVALUATION OF PIGMENTS AS ANTIOXIDANT AND ANTIBACTERIAL AGENTS FROM *BETA VULGARIS LINN*.

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ABSTRACT

Objective: The work is aimed to evaluate the health beneficial properties of (*Beta vulgaris*) *Beetroot*. *Beetroot* ranks among the 10 most powerful vegetable as a natural antioxidant and has a potential source of natural food colourant**.** The present work is therefore organized to evaluate the Total Phenolic Content (TPC), Antioxidant activity and Antibacterial activity of the Ethanolic and Methanolic extracts of *Beetroot*.

Methods: In the present work the Total Phenolic Content was determined by using *Folin-Ciocalteu* reagent method of the Ethanolic and Methanolic extracts of *Beetroot* (*Beta vulgaris)*. The antioxidant scavenging activity of these extracts was determined by applying three different assay methods: (1) (1, 1-diphenyl-2-picryl hydrazyl) DPPH method, (2) Ferric thiocyanate (FTC) method and (3) Thiobarbituric acid (TBA). The antibacterial test was examined against gram positive (*B. subtilis, S. aureus*) and gram negative (*E. coli, S. dysenteriae*) bacterial strains.

Results: In the present work the Methanolic extract showed greater TPC value 394.8 mg/g GAE than the Ethanolic extract 316.8 mg/g GAE. A correlation between antiradical capacities of the extracts with TPC value was clearly observed. The Methanolic extract was found to be most effective in all the methods. 50% scavenging power of the Methanolic and Ethanolic extracts were (0.129 mg/ml and 0.254 mg/ml) in DPPH method respectively. Moreover, in TBA and FTC method, both the extracts of *Beetroot* exhibited strong percentage inhibition ranging from 49%-62%. The results of the antibacterial test indicated that the Ethanolic and Methanolic extracts of *Beetroot* are significantly effective, both in Gram-negative (*E. coli, S. dysenteriae*) and in Gram-positive (*B. subtilis, S. aureus*) bacterium.

Conclusion: Thus, from the above experimental observations, it can be clearly stated that the *Beetroot* is a promising source of natural antioxidant and antibacterial agent and definitely provides an alternative towards synthetic antioxidant because of its beneficial properties.

Keywords: *Beetroot*, Total phenolic content, Antioxidant, Antibacterial

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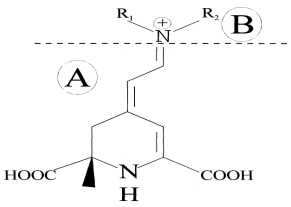
INTRODUCTION

In biological systems a variety of naturally occurring compounds and their association with the prevention in various ailments like cardiovascular diseases, liver, kidney disorders, chronic diseases and certain forms of cancer have been investigated and several studies have shown that diet rich in fresh food and vegetables like carrot, beetroot, tomato, grapes, Spanish, green tea, garlic and turmeric etc. provide a shield against degenerative diseases [1-4].

In the recent years, natural compounds isolated from several plants have attracted the focus of researchers for their medicinal and dietary values. There are a large group of naturally occurring compounds including vitamin A, vitamin E, vitamin C, plant pigments like betalain, carotenoids, flavonoids, phenolic acids and polyphenols which possess the capability to regulate our cellular metabolism, and maintain the oxidative balance in our body and retard many diseases linked to the oxidative stressed such as cancer, diabetes, nervous disorders, heart diseases etc. and play a significant role as an antioxidant, antiviral, antimicrobial, hepatoprotective and anti-cancerous agent [5-7].

Plant pigments and phenolic compounds have been studied and found to possess a free radical and ROS (reactive oxygen species like superoxide radical ion, singlet oxygen, hydroxyl radical, hydrogen peroxide, etc.) scavenging power and thus act as natural antioxidant either by termination of free radical chain reaction or by reduction of ROS and other free radical species of the biological system, which are generated during the oxidative stress conditions and are responsible for serious damages of macromolecules(like protein, lipid, DNA), peroxidation of food, many harmful diseases and aging [8, 9].

Beetroot (Beta vulgaris) is a member of *Chenopodiaceae* family. It is originally found in South Europe as an annual or biennial herb and is extensively cultivated in Europe, Russia, America, and Asia. It has been listed as one of the most beneficial vegetables due to its higher percentage of antioxidant activity in addition to its great application in the food industry with respect to the presence of a natural colourant Betalain [10, 11].



Betalain general formula (A) Betalamic acid moiety (B) The structure will be betacyanin or betaxanthin depending on the residue R1 and R2.

Red Beet Betalain pigment comprises an excellent natural food colourant and is effective against the oxidative stress and act as a scavenger of the free radical and ROS species which are associated with the many diseases [12]. It is a nitrogenous pigment, soluble in water and synthesised from amino acid Tyrosine. It is composed of two units Betacyanin (red-violet) and Betaxanthin (yellow). The basic structure of betacyanin consists of condensation of betalamic acid with cyclodopa which may be glycosylated with a sugar moiety, further, condensation of betalamic acid with amines or amino acid gives betaxanthin [13-15]. So far, it has been found that more than 50 betacyanins are reported and the most important is Betanin (5-O-β-glucoside) which is responsible for the color in *beetroot* and have

been used as a natural colourant in the modern food industry [16,17]. Along with the betanin, betalain also consists of isobetanin, neobetanin, betanidin, vulgaxanthin (I), and vulgaxanthin (II) and all acts as scavengers of free radical and ROS species [18, 19].

Besides this, *beetroot* contains about a one-tenth portion of pure sugar which are glucose, glucuronic acid or apiose. Moreover, it has been to be found that beetroot contains a significant amount of phenolic acids like chlorogenic acid, caffeic acid, ferulic acid, cinnamic acid and p-coumaric acid in addition to small amount of vitamin A, vitamin C, vitamin B12, iron, potassium, sodium, zinc and calcium [20-22]. The abundance of statements has been established suggesting that the *beetroot* is not only used as a harmless natural food colorant but also plays a major role in the reduction of oxidative stress because of its antioxidant ability. It is potentially believed to be associated with antioxidant, anti-inflammatory, antimicrobial, hepatoprotective activities [23-27].

The present study is thus designed to focus on the examination of antioxidant activity of *Beet Root* extracts (ethanol and methanol) using three different methods and antibacterial activity along with the evaluation of total phenolic content spectrophotometrically.

MATERIALS AND METHODS

Plant material

Beetroot (Beta vulgaris) were collected from local markets of Allahabad, Uttar Pradesh, India and identified in the post graduated department of Horticulture, SHIATS. Allahabad. The beetroot was cleaned and cut into small pieces and was subjected to dry at room temperature. The dried root was grinded and powdered.

Preparation of extracts

The 60 g of dried and powdered plant material were extracted with 150 ml of solvent ethanol and then with methanol by using soxhlet extractor for 48 h at a temperature not exceeding the boiling point of the solvents. The extracts were filtered by using Whatman No. 1 paper. The extracts were concentrated to a small volume by using rotary evaporator then concentrated to dryness and were used for further investigation.

Total phenolic content (Folin-ciocalteu method)

Total phenolic content in the extracts was determined using Folin-Ciocalteu method [28]. For this, 0.1 ml of stock solution (1 mg/ml) of the extract was mixed with 0.75 ml of Folin-Ciocalteu reagent (1 ml in 10 ml of DW.) and left to stand for 5 min. After which 0.75 ml of aqueous Sodium Carbonate (100 mg/ml) was added and the volume of the reaction mixture was made up to 10 ml by adding distilled water. The mixture was allowed to incubate for 90 min. The standard curve was prepared using different dilutions of Gallic acid (2, 1, 0.5, 0.25, 0.125, 0.0625 mg/ml). The absorbance was taken at 760 nm using UV-VIS Spectrophotometer. The total phenolic values were calculated and expressed in terms of milligrams of Gallic acid per 10 ml of extract.

Antioxidant activity

DPPH radical scavenging activity assay

The antioxidant activity of the extracts was measured by using stable radical $DPPH^{\cdot}$ as a reagent and the activity was determined in terms of hydrogen donating and electron releasing abilities of the extracts. The different working solutions of the extracts were prepared in methanol (1, 0.5, 0.25, 0.125, 0.0625 mg/ml). The DPPH solution (0.002%) was also prepared in methanol. In each of the test-tubes different concentration of the extract was taken and the made up the volume to 2 ml, to this was added 2 ml of DPPH solution and test tubes were then incubated for 30 min at room temperature. The same procedure was followed for BHT and Gallic acid as well. Methanol with DPPH was used as a control. The method given above is the same as used by Kahalaf [29]. With slight modification. The absorbance was recorded at 517 nm using a UV-Visible spectrophotometer. The radical scavenging activity of each extract was calculated by using the following equation-

$$(\%)\ \text{Scavenging effect} = [1-(A/B)]\ X\ 100$$

Where

A = absorbance of the sample

B = absorbance of the control

Ferric thiocyanate method

The FTC Method was used to determine the antioxidant activity of extracts [30]. 4 mg of each extract was dissolved in 4 ml of ethanol (99.5%), to it was mixed 4.1 ml of linoleic acid (2.5% in ethanol 99.5%), 8 ml of Phosphate Buffer (0.02M, pH 7) and 3.9 ml of distilled water. The mixture was then incubated at 40oC in an oven. After this, 9.7 ml of ethanol (75%) and NH4SCN (30%) was added to 0.1 ml of the reaction mixture to measure the extent of the antioxidant activity. After 3 minutes of the addition of 0.1 ml of ferrous chloride (0.02M) in 3.5% HCl to the reaction mixture, the absorbance was measured at 500 nm using UV-Visible Spectrophotometer. The absorbance was taken at every 24 h until the maximum value of control absorbance was obtained. BHT was used as standard here. The inhibition of lipid peroxidation was measured as follows-

$$(\%)\ \text{Inhibition} = 100-[(A_1/A_0)\ X\ 100]$$

Where

A_0 = absorbance of the control reaction mixture

A_1 = absorbance of the sample reaction mixture

Thiobarbituric acid method

The TBA test has been conducted according to Huda-faujan [30]. In this method, 2 ml of Trichloroacetic Acid (20%) and 2 ml of Thiobarbituric Acid (67%) were added to 1 ml of the sample that was prepared for FTC Method and the solution kept on water bath for 10 min at 100 ° C, it was then cooled and centrifuged at 3000 rpm for 20 min. The absorbance of supernatant was measured at 532 nm. The antioxidant activity has been described by percentage inhibition-

$$(\%)\ \text{Inhibition} = [1-(A_1/A_0)]\ X\ 100$$

Where

A_1 = absorbance of the sample

A_0 = absorbance of the control

Antibacterial activity

For the evaluation of antibacterial activity of the Beet root against some bacterial strains, agar well diffusion method was used [31]. The bacterial strains used in this study including gram-positive *Staphylococcus aureus, Bacillus subtilis* and gram-negative *Escherichia coli, Shigella dysenteriae* were obtained from the Laboratory of Microbiology and Fermentation Technology, SHIATS, Allahabad, India. 20 µl volume of the sample extracts of 2 mg/ml concentration poured into 5 mm well of inoculated agar plates. Ampicillin 10 mg/ml was used as a positive control. The resulting zone of inhibition (ZI) were measured after the incubation of 48 h at 370 C and expressed in mm. The antibacterial activity results were considered as inactive if<4.5 mm; 4.5-6 mm as partially active; while 6.5-9 mm as active and greater than 9 mm as very active [32]. The experiments were carried out in triplicate and averaged.

RESULTS AND DISCUSSION

Total phenolic content of the extract

The antioxidant activities of plants may be attributed to the Phenolic compounds due to their redox properties. These are secondary plant metabolites and contribute to the plant's antioxidant ability.

Folin-Ciocaltue method was used to determine the TPC of *Beetroot* extracts using Gallic Acid as the standard. The TPC was calculated with a regression equation based on a standard curve using Gallic acid at different concentration ($y=0.077x$, $R^2=0.998$) and expressed in milligrams of Gallic acid. From fig. 1, it could be interpreted that methanolic extract had the greater Phenolic concentration of 394.8

mg GAE/mg followed by an ethanolic extract which was 316.8 mg GAE/mg. Aris [33] reported that the TPC in fruits *of Ficus Deltoidea Var Angustifolia* ranges from 159.2-259.2 mg/g GAE. *Moringaolifera* has TPC value in three different climates (India, Nicaragua and Niger) ranged from 2940-4250 mgGAE/dry weight [34].

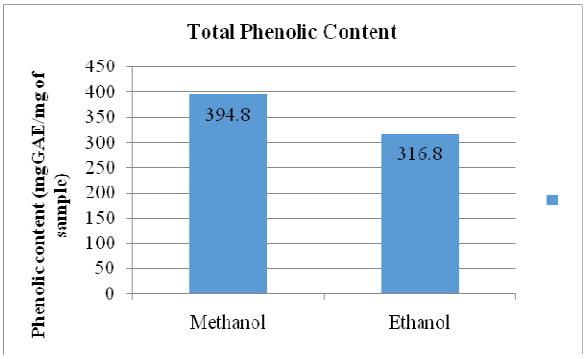


Fig. 1: Total phenolic content in ethanol and methanol extracts of *Beetroot*

Antioxidant activity

DPPH radical scavenging activity assay

In this study, the scavenging activity was determined by the DPPH˙ testing method, which was found to be rapid, easy and economical for measurement antioxidant activity [35-37]. The DPPH˙ is a free radical, stable in nature and accepts an electron or hydrogen radical to become a stable molecule. The reducing nature of DPPH˙ was determined by a decrease in its absorbance induced by antioxidants at 517 nm. In the DPPH˙ method, all the results were obtained from the extracts of *Beetroot* and were compared with Gallic acid and BHT taken as standard references. As illustrated in fig. 2 scavenging of DPPH˙ increases with increase in the extract concentration. The IC_{50} value is defined as the concentration of the extract at which 50% of radicals have been scavenged under experimental conditions. A smaller IC_{50} value corresponds to higher antioxidant activity [38]. In this study, highest DPPH scavenging activity was shown by methanolic extract with IC_{50}value 0.129 mg/ml and by ethanolic extract with IC_{50} value 0.254 mg/ml.

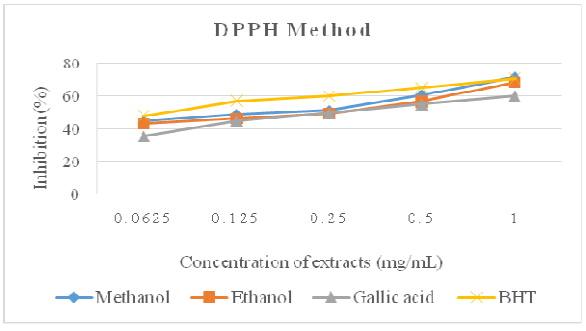


Fig. 2: DPPH scavenging activity of ethanol and methanol extracts of *Beet root* and standards BHT and Gallic acid with different concentration (mg/ml)

Ferric thiocyanate method

The amount of peroxide value in the beginning of liquid peroxidation was measured by the FTC method, where ferric ions are formed upon reaction of peroxide with ferrous chloride. The ferric ions then unite with ammonium thiocyanate producing ferric thiocyanate, it is red in color. The darker the color, the higher will be the absorbance [35]. Lower absorbance correlates to high antioxidant activity [33]. In FTC method, both the methanolic and ethanolic extract of beetroot had been oxidized when stored for seven days and exhibited strong antioxidant potential in inhibiting linoleic acid oxidation as compared to the control. From the fig. 3, the percentage of inhibition of linoleic acid of ethanolic extract, methanolic extract and BHT were found to be 51%, 62% and 69% respectively. The absorbance of control was 0.410 after seven days of storage. Initially, the highest percent inhibition is shown by methanolic extract (62%) and ethanolic exhibited the lower percent inhibition (51%) than methanol extract. Huda *et al.*, 2009 reported the percentage of linoleic acid inhibition of *P. koenigii, C. Caudatus, C. asiatica, O. javanica,* and *P. minus* to be 70.60, 68.67, 66.17, 65.41 and 63.66 % respectively. The antioxidant activity also increases with increase in the concentration of the plant extract.

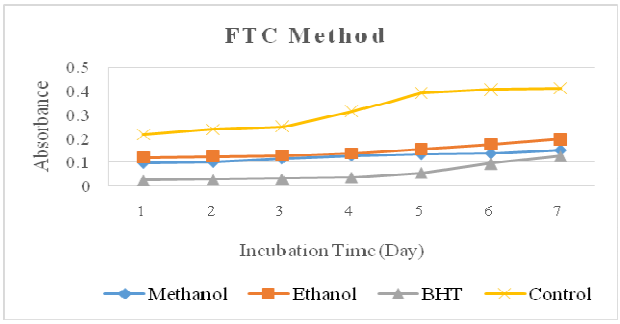


Fig. 3: Antioxidant activity of ethanol, methanol extracts of *Beet root* and standard BHT as measured by the FTC method

Thiobarbituric acid method

TBA method is used to measure the extent of lipid peroxidation at the secondary stage where peroxide decomposes to form carbonyl compounds. Both the extracts showed strong antioxidant activities. The percentage of antioxidant activities of methanolic extract and ethanolic extract and BHT were 58%, 49% and 65% respectively. The absorbance of control sample obviously showed the highest reading. This result is similar to that reported by Huda-faujan [30] and Aris [33] that the control sample had the highest absorbance reading in TBA after seven days of storage. Fig. 4 shows that the amount of peroxide in the initial stage of lipid peroxidation is greater than the amount of peroxide present in the secondary stage.

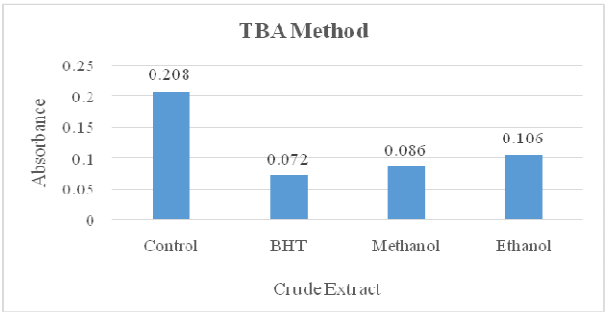


Fig. 4: Antioxidant activity of ethanol, methanol extracts of *Beet root* and Standard BHT as measured by the TBA method

Antibacterial activity

Along with this activity *in vitro* antibacterial activity of ethanolic and methanolic extracts of *Beetroot* was examined by using agar well diffusion method. According to the result given in fig. 5, methanolic extract exhibits strong activity against both gram positive and gram negative strains and showed the highest activity against *B subtilis* with 18.2 mm ZI. On the other hand, ethanolic extract showed weak antibacterial activity that was comparable with the standard.

Methanolic and ethanolic extracts exhibited antibacterial activity in descending order *B. subtilis>S. aureus>E. coli>S. dysenteriae*. This result have a similar observation with that of TPC as the methanolic extract possessed higher phenolic content than ethanolic extract. Thus *Beetroot* extracts could be used as an effective antibacterial agent.

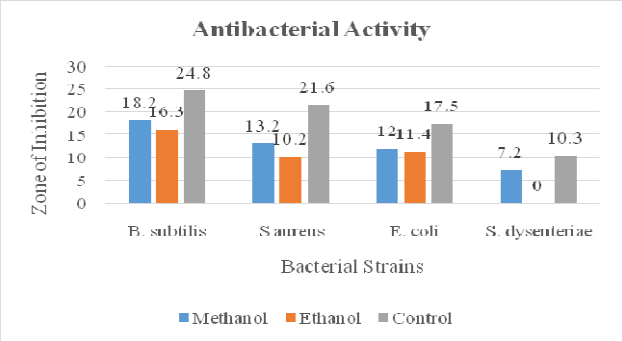


Fig. 5: Antibacterial activity of ethanol and methanol extracts of *Beetroot* (values are mean SD of three determinations)

Natural antioxidants are found to be powerful scavengers of ROS, and significantly provide healthy lifestyle along with the degree of protection towards various diseases in human body system [39]. Natural antioxidants advance over the synthetic antioxidant due to their non-toxic nature. Drugs based on the application of antioxidant for the protection of complex diseases like diabetes, stroke, cancer, heart and kidney damages have attracted a great deal of researchers towards the use of natural antioxidants. Much plant extracts exhibit efficient antioxidant properties due to their phytoconstituents including phenolic pigments like carotenoid, flavonoids, betalains, small amounts of vitamins and ions. [40, 41].

The present study supports the scavenging activity and antibacterial potential of the beet root extracts. In the food industry, potentially safe natural antioxidants have been isolated from beetroot. This study has shown that the methanolic and ethanolic extracts of beetroot possess a considerable amount of phenolic compounds and exhibit a positive correlation between the antioxidant, antibacterial activity and total phenolic content.

Formation of the non-radical form of DPPH (DPPH-H) obtained by the reduction of $DPPH^{\cdot}$ in the presence of radical scavengers or hydrogen donors present in plants extracts provides the basis of antiradical activity on DPPH radical scavenging assay. The measurement of antiradical activity was achieved by the application of the series of different concentrations of ethanolic and methanolic extracts of beetroot. The result shows that methanolic extract possesses strong antioxidant activity with IC_{50} 0.129 mg/ml which was greater than that shown by ethanolic extract with IC_{50} 0.254 mg/ml. Both the extracts prove themselves to be a potential antioxidant.

Methanolic and ethanolic extracts of *beetroot* were examined by FTC and TBA methods. The amount of peroxide formed at initial stages of linoleic acid peroxidation would be measured by FTC method. The antioxidant activity increases as the concentration of peroxide decreases when stored for seven days. The determination of evaluation of the extent of lipid peroxidation was done by the TBA method through measurement of the secondary products of oxidation like aldehyde and ketone. Initially, the control sample showed highest absorbance reading and lower level of antioxidant activity as compared to both the extracts and standards. Based on the absorbance rate, the methanolic extract possessed prominent antioxidant activity with 62% and 58% followed by ethanolic extract with 51% and 49% for FTC and TBA methods respectively. The beetroot extracts showed potential activities against the Gram positive and Gram negative bacteria such as, *B. subtilis, S. aureus, E. coli and S. dysenteriae.* The methanolic and ethanolic extracts prepared from the beetroot are effective against enterobacterial growth. Our results agree with the previous studies that show that the beetroot has significant activity against various pathogenic and opportunistic bacteria.

In this present work, after cumulating the results, it could be considered that the beet root extracts might be a potent source of antioxidant and possesses a high antibacterial potential of preventing and treating diseases like malaria, stroke, diabetes, heart diseases and cancer.

CONCLUSION

From the above experimental observations, it can be clearly stated that the beetroot is a promising source of natural antioxidant and antibacterial agent and definitely provides an alternative towards synthetic antioxidant because of its beneficial properties and opens a new aspect of research trend for beetroot as a natural antioxidant and viable food ingredient.

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CONFLICT OF INTERESTS

Declare none

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