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BEETROOT (*BETA VULGARIS*) STEM - AN INEDIBLE WASTE PRODUCTS IS A POTENT SOURCE OF ANTIOXIDANT AND ANTIBACTERIAL AGENT

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ABSTRACT
Beetroot (*Beta vulgaris*) belongs to *Chenopodiaceae* family. Several parts of the beetroot plant are used for medicinal purposes such as lowering blood pressure, lowering the risk of diabetes, prevention of cancer, detoxification in liver, to treat anaemia. It is rich in vitamins and minerals. Beetroot stem has many nutritional and medicinal values. It acts as antioxidant, antimicrobial, anti-inflammatory agent. The present study analysed the phytochemical constituents foundin beetroot stem using various extracts (Aqueous, Acetone, Ethanol, Petroleum ether). The antioxidant activity of beetroot stem extracts showed better results by using DPPH, Flavonoids, Total phenol and FRAP assay. The antibacterial activity of beetroot stem was done and it showed measureable effect against *Bacillus subtilis, Klebsiella pneumoniae* and *Escherichia coli*. From this work, we provide the substantial evidence that beetroot stem is an inedible waste product is a potent source of antioxidant and antibacterial agent there by indicating its use as a value-added component for functional foods.

KEY WORDS: Beetroot stem, Phytochemicals, Antioxidants, Antibacterial activity.

1. INTRODUCTION
Beetroot crop belonging to the *Chenopodiaceae* family.[1] The bionomial name of beetroot is *Beta vulgaris*. It is known by several common names like beet, chard, European sugar beet, red garden beet, Harvard beet, blood turnip, mangelwurzel, mangel and spinach beet.[1] Beetroot is biennial plant that is cultivated for its thick fleshy roots in early spring. Members of this family are dicotyledonous. It is an erect annual herb with tuberous root stocks. It makes an excellent dietary supplement being not only rich in minerals, nutrients and vitamins but also has unique phytoconstituents, which have several medicinal properties.[3] In India, it is mainly grown for its juice and vegetable value.[2] It mainly contains vitamins A, B_1, B_2, B_6 and C. It is also a good source of calcium, magnesium, copper, phosphorus, sodium and iron.[2] Its powder is used as a natural red food colorant which used to applied in dry mixes (soups, Indian curry mixes), sweets, jams, jellies, etc., The bright red colour of beetroot is due to the red pigments known as betalains.[4] The main benefits are that it contains no fat, very few calories and is a great source of fiber. The best quality and root colour are obtained when the air temperature ranges between 10 and 18°C.[1] It is one of the natural foods which boost the energy in athletes as it has one of the highest nitrates and sugar contents plant.[3] The common vegetables, which form part of daily meals, have various medicinal properties, can prevent many of human aliments and retain health and vigour.[5]

Several parts of the beetroot plant are used in medicinal system.[3] Red beets are used as antioxidant, antimicrobial, anti-inflammatory, antiallergenic, antithrombotic, anti-atherogenic, cardio protective and vasodilatory properties.[6] The use of non-conventional foods such as beet stalks and leaves help reduce the liver damage caused by a high-fat diet.[7] The beetroot leaves were recommended by the father of medicine "Hippocrates" for faster healing of wounds.[8] Betalains have great importance for cardiovascular diseases by lowering the level of homocysteine.[9] Beetroots also can inhibit the cell proliferation of human tumour cells.[10] Beetroot ranks among the ten most potent vegetables with respect to antioxidant property.[3] Since beetroot is a perishable vegetable it may be dehydrated and its mineral content may increase (especially iron and calcium) quantitatively due to reduction of water mass. Though there is loss of antioxidants up to some extent, but still may possess health benefits along with its quality pinkish colour.[11] The aim of the work is to study the phytochemical analysis in different extracts of beetroot stemand to study its effect as anti-oxidant and antimicrobial activities.

2. MATERIALS AND METHODS

2.1. COLLECTION AND PROCESSING OF PLANT SOURCE

Figure 2.1. Collected and Processed Plant source.

The beetroot stem was collected from erode local market and then it wasshade dried, further crushed to powder and stored in air tight container.

2.2. PREPARATION OF EXTRACTS

The powdered plant material was mixed with aqueous, acetone,ethanol and petroleum ether solvents in the ratio of 1:10 (gram: millilitre). The mixture was placed in 100 ml glass bottle with Teflon lids. The bottles were capped and placed at 65°C in the Water bath for an hour and were swirled manually. The sample were removed from the water bath and cooled at room temperature. The supernatant was centrifuged at 10000 rpm and filtered through Whatmann No.1 filter paper. The filtrates were evaporated to dryness and crude obtained was stored at 5°C in refrigerator until further analysis.

2.3. PRELIMINARY PHYTOCHEMICAL SCREENING

2.3.1. Test for Carbohydrates: To a few drops of extract, 2 ml of alcoholic solution of alpha-napthol was added. The mixture was shaken well and 2.0 ml of Acid solution was added slowly along the sides of the test tube and allowed to stand. A reddish ring formed at the junction of two solutions indicates the presence of carbohydrates.

2.3.2. Test for Reducing Sugars: To a few drops of extract, 2ml of Fehling's reagent was added. The mixture was shaken well and kept in a boiling water bath for five minutes. Formation of brick red precipitate indicates the presence of sugar.

2.3.3. Test for Flavonoids: To a few ml of extract, few drops of Diluted sulphuric acid were added. Orange colour develops which indicates the presence of flavonoids.

2.3.4. Test for Amino Acids: To a few drops of extract, few drop of Ninhydrin solution was added in a test tube. A characteristic blue colour indicates the presence of amino acids.

2.3.5. Test for Proteins: To a few ml of extract, few drop of Millon's reagent was added. White precipitate indicates the presence of Proteins.

2.3.6. Test for Glycosides: To 2 ml of extract, 2ml of chloroform and 2 ml of acetic anhydride is added. Formation of violet to blue to green reddish-brown ring indicates the presence of glycosides.

2.3.7.Test for Cardiac Glycosides: In a test tube added 5 ml of extract and 2 ml of glacial acetic acid and 1 drop of ferric chloride and 1.0 ml of Conc. sulphuric acidis added slowly along the sides of the test tube and allowed to stand. Formation of brown, violet, greenish rings indicate the presence of cardiac glycosides.

2.3.8. Test for Total Phenols: To 2 ml of extract, Ferric chloride is added. Formation of deep blue colour indicates the presence of total phenol.

2.3.9. Test for Anthraquinones: To 2 ml of extract, 2 ml of 10% Ammonium hydroxide is added. Formation of bright pink colour indicates the presence of anthraquinones.

2.3.10. Test for Steroids: To 2 ml of extract, 2ml of chloroform and 2 ml of acetic anhydride is added reddish brown colour is formed. To this added 1 ml of Concentrated Sulphuric Acid. Formation of violet to blue green colour indicates the presence of Steroids.

2.3.11. Test for Anthocyanin: To 2 ml of extract, 2 ml of 2N Ammonium chloride and ammonium is added. Appearance of blue violet colour indicates the presence of anthocyanin.

2.3.12. Test for Phenols: To 2 ml of extract, 3 ml of ethanol and a pinch of ferric chloride are added. A greenish yellow colour appears which indicates the presence of Phenols.

2.4. DETERMINATION OF ANTIOXIDANT ACTIVITY

2.4.1. DPPH ASSAY: The radical scavenging activity (RSA) of different extracts was determined by using DPPH assay. According to this method, take 0.4 ml of plant extracts and 2.6 ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517 nm. 3 ml of DPPH was taken as control.Experiment was done in triplicate.

2.4.2. TOTALFLAVONOIDS ASSAY: Take0.5 ml of each extract, 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water. Sample and sample blank of all extracts were prepared and the tubes were incubated at room temperature for 30 minutes their absorbance was measured at 415 nm.Experiment was done in triplicate.

2.4.3. TOTAL PHENOLIC ASSAY: 1 ml of different extracts was taken in different test tubes added 0.5 ml of Folin-Ciocalteu reagent. After 3 minutes, added 2.0 ml of 20 % sodium carbonate solution. Mixed thoroughly and placed the tubes in boiling water for exactly one minute, cooled and measured the absorbance at 765 nm against the reagent blank.Experiment was done in triplicate.

2.4.4. FRAP ASSAY: 1 ml of each extract at various concentrations (0 – 500 mg/l) was added to a test tube. 1 ml potassium phosphate buffer (0.2 M, pH 6.6) and freshly prepared potassium ferricyanide (1 ml, 1%) were added to extracts. The mixture was incubated in a water bath (50°C for 20 minutes). 1 ml of trichloroacetic acid (10% TCA) was added to the mixture followed by centrifugation at 5000g for 5 minutes. From the upper layer of mixture, 1 ml was taken and mixed with 1 ml distilled water followed by 100 µl of freshly prepared ferric chloride (0.1%). The absorbance of samples was measured at 765 nm against blank.Experiment was done in triplicate.

2.5. ANTIBACTERIAL ASSAY BY DISC DIFFUSION METHOD

Antibacterial activity of *Beta vulgaris* stem was determined by disc diffusion method. The selective microorganisms (***E. coli, Klebsiella pneumonia*** and ***Bacillus subtilis)*** are collected in Nutrient agar plate from department of Biotechnology, KASC, Erode. For inoculum preparation, the colonies of bacteria such as were suspended in nutrient broth and turbidimetrically adjusted. The 250 µl of test organisms were spreaded in different petri plates containing nutrient agar medium and made4 wells in each plate. Test solution of 50 µl was poured into each respective well. These plates were incubated at 37°C. After 24 hours of incubation, the diameter of the clear zones that showed inhibition of bacterial growth was measured in millimetre (mm). Experiment was done in triplicate and mean value of zone inhibition was calculated with standard error. Experiment was done in triplicate.

3. RESULTS AND DISCUSSION

In the present study evaluated the phytochemical analysis, antioxidant activity and antibacterial activity of different extracts of beetroot stem.

3.1 YIELD OF EXTRACTS

The percentage of extracts obtained from different solvents are shown as follows

Table 3.1: Yield of Extracts.

Solvents used	%of extracts obtained
Aqueous	55%
Acetone	50%
Ethanol	42%
Petroleum ether	38%

3.2 PHYTOCHEMICAL SCREENING

The results of phytochemical screening were obtained as follows

Table 3.2: Results of phytochemical analysis.

S. No	TEST FOR PHYTOCHEMICALS	EXTRACTS			
		WATER	ACETONE	ETHANOL	PETROLEUM ETHER
1	Carbohydrates	+	+	+	+
2	Reducing Sugar	+	+	+	+
3	Flavonoids	+	+	+	+
4	Amino acids	+	+	+	+
5	proteins	+	+	+	+
6	Glycosides	+	-	-	-
7	Cardiac Glycosides	+	+	-	+
8	Totalphenols	+	+	+	-
9	Anthroquinines	+	+	-	-
10	Steroids	+	-	+	-
11	Anthocyanins	+	+	-	-
12	Phenols	+	+	+	+

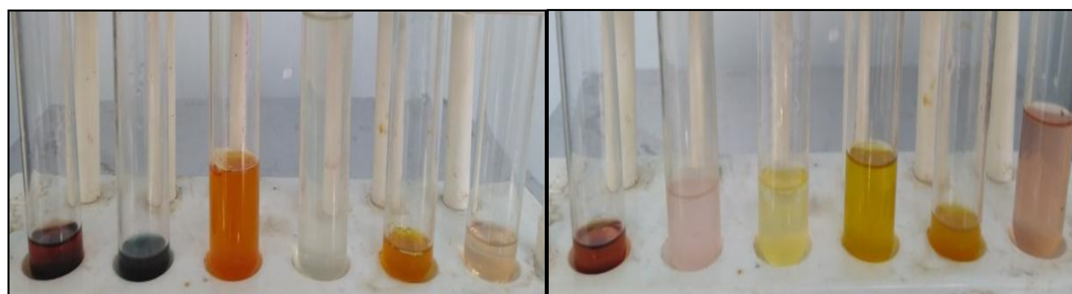

Figure 3.1: Showed the results ofphytochemical analysis.

The phytochemical screening of various extracts shows the presence of certain important components such as carbohydrates, flavonoids, amino acids, protein, cardiacglycosides, total phenols, anthocyanin and phenol. The above phytochemical constituents were highly present in the acetone, aqueous extract and ethanol.

3.3. ANTIOXIDANT ASSAY OF BEETROOT STEM

3.3.1 DPPH ASSAY

From the result it is clear that acetone extract of *Beta vulgaris* stem shows maximum DPPH activity when compared to the other extracts.

Table 3.3: Results of DPPH Assay.

EXTRACTS	DPPH (%)
Aqueous	27.65 ± 1.51
Acetone	65.95 ± 2.75
Ethanol	23.40 ± 0.16
Petroleum ether	40.42 ± 1.11

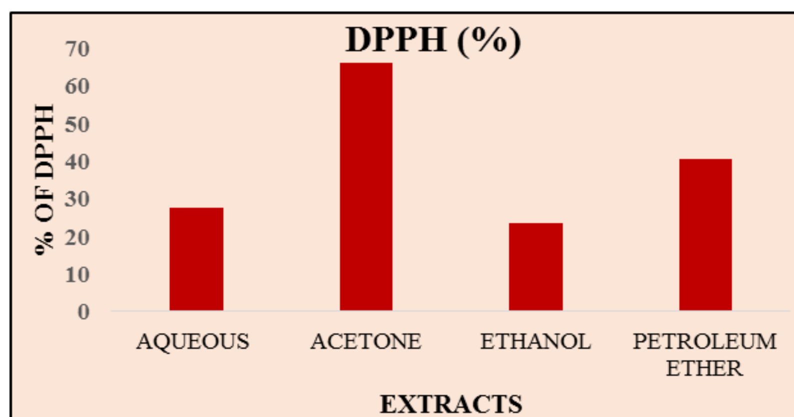

Figure 3.2: Levels of Antioxidants in extracts.

3.3.2 FLAVONOID ASSAY

From the above results it is clear that aqueous extract of *Beta vulgaris* stem shows maximum flavonoid activity when compared to the other extracts.

Table 3.4: Results of Flavonoid assay.

EXTRACTS	CONCENTRATION OF FLAVONOID (mg)
Aqueous	6.1 ± 1.1
Acetone	3.2 ± 0.8
Ethanol	2.3 ± 0.6
Petroleum ether	5.4 ± 1.1

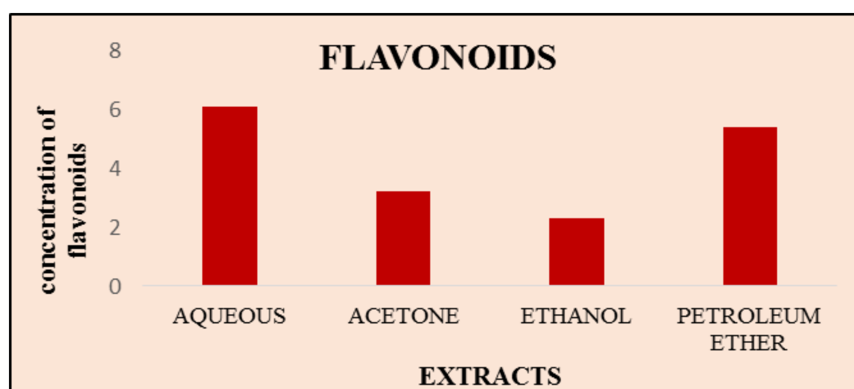

Figure 3.3: Concentration of Flavonoids in extracts.

3.3.3 TOTAL PHENOL ACTIVITY

From the result it is clear that aqueous extract of *Beta vulgaris* stem shows maximum total phenol activity when compared to the other extracts.

Table 3.4: Results of Total Phenol assay.

EXTRACTS	CONCENTRATION OF TOTAL PHENOL (mg)
Aqueous	1.60 ± 0.05
Acetone	1.15 ± 0.17
Ethanol	1.00 ± 0.01
Petroleum ether	1.35 ± 0.12

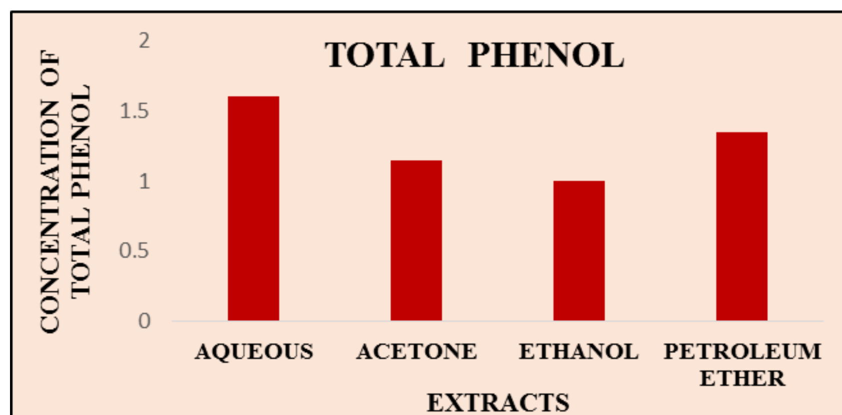

Figure 3.4: Concentration of Total Phenol in extracts.

3.3.4 FRAP ACTIVITY

From the results it is clear that that aqueous extract of *Beta vulgaris* stem shows maximum FRAP activity when compared to the other extracts.

Table 3.5: Results of FRAP assay.

EXTRACTS	FRAP (%)
Aqueous	0.37 ± 0.1
Acetone	0.31 ± 0.1
Ethanol	0.23 ± 0.3
Petroleum ether	0.33 ± 0.2

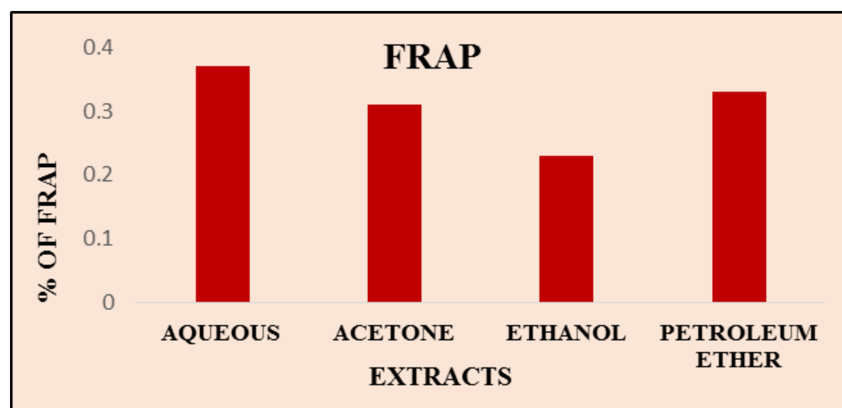

Figure 3.5: Percentage of FRAPin extracts.

3.4. ANTIBACTERIAL ASSAY OF BEETROOT STEM

The stem extract of *Beta vulgaris* had been tested for their antibacterial activities and an interesting antibacterial profile has been observed against gram positive (*Bacillus subtilis*) and gram negative bacteria (*Klebsiella pneumoniae and Escherichia coli*). The stem extracts showed enormous activity against all three bacteria tested. The activities of extracts are mentioned in the terms of zones of inhibitions (mm).

Table 3.6: Results of Antibacterial Activity.

S. No	EXTRACTS	ZONE OF INHIBITION (mm)		
		Bacillus subtilis	*Klebsiella pneumoniae*	*Escherichia coli*
1	Aqueous	5.0 ± 0.2	3.0± 0.2	3.0± 0.1
2	Acetone	18.0± 0.3	4.0± 0.1	7.0± 0.2
3	Ethanol	4.0± 0.1	7.0± 0.1	2.0± 0.1
4	Petroleum ether	2.0± 0.1	1.0± 0.1	6.0± 0.2

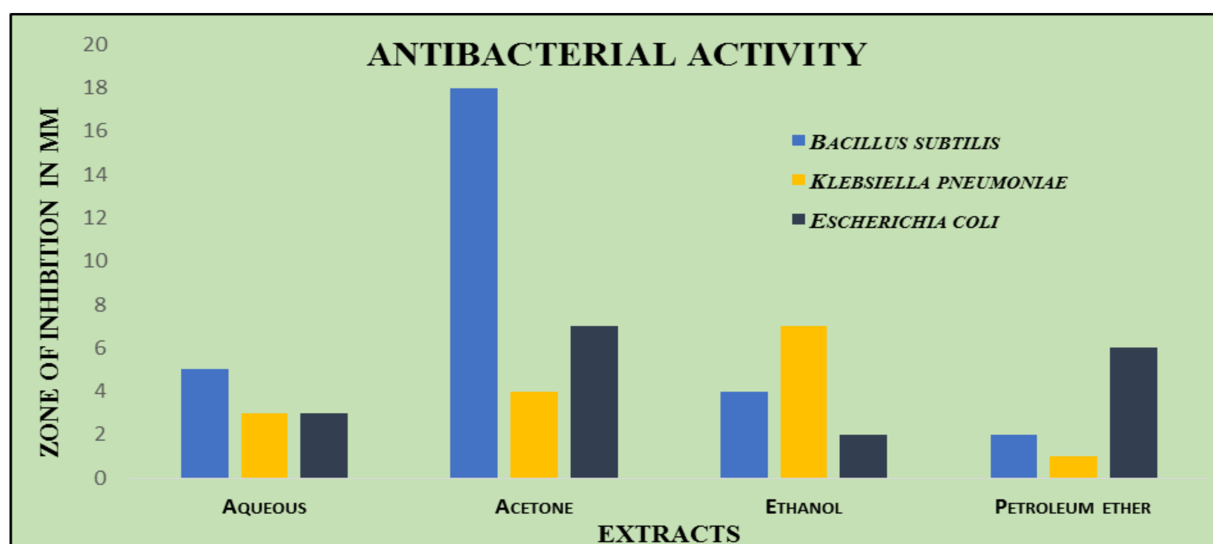

Figure 3.5: Antibacterial Activityof different extracts.

The diameter of inhibition zones (DIZ) against *Bacillus subtilis* was 5mm, 18mm, 4 mm and 2mm for aqueous, acetone, ethanol and petroleum ether extracts of*Beta vulgaris* stem respectively. The diameter of inhibition zones (DIZ) against *Klebsiellapneumoniae*was 3 mm, 4 mm, 7 mm and 1 mm for aqueous, acetone, ethanol and petroleum ether extracts of *Beta vulgaris* stem respectively. The diameter of inhibition zones (DIZ) against *Escherichia coli* was 3 mm, 7 mm, 2 mm and 6 mm for aqueous, acetone, ethanol and petroleum ether extracts of *Beta vulgaris* stem respectively. From the result, we observed that the zone of inhibition of *Bacillus subtilis* is higher in acetone and *Klebsiella pneumoniae* is higher in ethanol extract whereas the zone of inhibition of *Escherichia coli* is higher in acetone extract.

4. CONCLUSION

Beta vulgaris is a medicinal plant mainly its stem contains many therapeutic uses. From this study it can be concluded that various phytochemicals including phenols, flavonoids are present in different extracts (Aqueous, Acetone, Ethanol and Petroleum ether) of *Beta vulgaris.* But, the acetone solvent has a capability to extract the high amount of phytoconstituents from the *Beta vulgaris.*Antioxidant activity of all four extracts of *Beta vulgaris*was investigated and found. DPPH activity is high in ethanol extract whereas the flavonoid activity is high in aqueous and petroleum ether extracts. In the same way total phenol activity and FRAP activity were found to be maximum in petroleum ether.

Antibacterial activity test showed that *Beta vulgaris* has a measureable effect against certain gram positive and gram negative bacteria. The zone of inhibition of (*Bacillus subtilis*) and (*Klebsiella pneumoniae*) is higher in acetone extract whereas the zone of inhibition of (*Escherichia coli*) is higher in ethanol extract.In the present study, provides the substantial evidence that beetroot stem; an inedible waste product is a potent source of antioxidant and antibacterial agent there by indicating its use as a value-added component for functional foods

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