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Original Article

Antioxidant and Antibacterial Activities of *Beta vulgaris* L. Peel Extracts.

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ABSTRACT

Objective: Research in the past few decades indicates that vegetable peels are accumulated with numerous biologically active compounds which impart various health benefits. Considering the biological activities of beetroot as well as the promising potential utilization of plant by products, the objective of the present study was to determine the antioxidant and antimicrobial activity of beetroot peel. **Methodology:** The antioxidant activity was determined using DPPH radical scavenging assay, FRAP and phosphomolybdenum reduction assay while the antibacterial activity was tested against four bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri* and *Klebsiella pneumoniae*. **Results:** The result illustrates that beetroot peel exhibits nutraceutical potential which was evident through its ability to scavenge free radicals and in inhibiting the growth of micro-organisms. **Conclusion:** Hence the present study thereby indicates the use of beetroot peel as a functional food constituent due to increased attention to fresher and greener food supplements.

Key words: beetroot peel, DPPH radical, phosphomolybdenum reduction, antimicrobial agent.

1. INTRODUCTION

Beetroot (*Beta vulgaris* L.) which belongs to Chenopodiaceae family are of different variety of colours ranging from yellow to red of which, deep red-coloured beetroots are usually consumed by humans either in the raw or in the cooked form. Red beetroot is one of the most potent vegetables with respect to antioxidant activity due to the presence of several active compounds such as carotenoids, glycine betaines, saponins, betacyanines folates, betanins, polyphenols and flavonoids. [31, 27, 6]

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Nowadays waste materials such as peels and seeds obtained from fruit and vegetable processing units are considered to be a rich source of phytochemicals and antioxidants due to the presence of high amount of phenolic compounds.[9] Moreover the peels obtained constitute about 40%-45% of the total weight and if they are not processed in a proper manner, it results in serious environmental pollution. [10] Hence reutilization of plant byproducts involves the process of converting them into commercially novel sources that are useful to the community with the additional benefit of decreasing environmental pollution.

Beetroot peel possesses antioxidant compounds thereby indicating its use in food and nutraceuticals industries. Beetroot extracts, especially peel extracts, have shown strong antioxidant activity in comparison to other vegetable peel extracts. [18] Betanin the main beta-cyanine present in red beetroot is distributed mostly towards the outer parts of root, decreasing in the order of peel, crown and flesh. Both betanin and isobetanin are present in greater amounts in the peels than in the flesh of beet. [24]The objectives of the study were to screen the presence of various secondary plant metabolites (phytochemicals) and to determine the antioxidant and antimicrobial activity of beetroot peel.

2. MATERIALS AND METHODS

Plant material and preparation of extract

Fresh red beetroots purchased from a local market in Chennai, were washed thoroughly under tap water. The peels were removed using a sterile sharp knife and shade dried for 4-5 days at room temperature. The dried peels were powdered using an electric blender and stored in airtight containers for further use.

The peel powder (5grams) was soaked in 100 mL of methanol and acetone separately for 72 hours by maceration technique. The supernatant was filtered using Whatmann filter paper, concentrated using rotary evaporator and dry residue was preserved at 5°C in airtight bottles until further use. The percentage yield obtained was calculated using the following formula:-

$$\% \text{ yield of extract: } \frac{\text{Extract weight}}{\text{Dry weight powder}} \times 100$$

Qualitative phytochemical screening

Phytochemical screening was carried out to analyze the presence of various phytoconstituents such as alkaloids, glycosides, saponins, phenols, flavonoids, terpenoids, steroids, carbohydrates and proteins according to standard method. [17, 27, 21, 28, 7, 13] General reactions in this analysis revealed the presence or absence of these compounds.

Estimation of total phenol content

Total phenolic content was estimated by the Folin-Ciocalteau reagent method. [23] Hundred µL of extracts (1 mg/mL) were made up to 1 mL by methanol and mixed with 1 mL of Folin Ciocalteu reagent (1:10 diluted with distilled water) and after 5minutes later, 1 mL of 20% sodium carbonate (Na_2CO_3) solution was added. The mixture was incubated at room temperature for 30 minutes and the absorbance was measured at 760 nm by spectrophotometer. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common reference standard.

Estimation of total flavonoid content

The total flavonoid content was determined by the aluminum chloride reagent method. [5] The extracts (500µg/mL) were made up to 1 mL by methanol and were mixed with 0.5 mL of 5% sodium nitrate ($NaNO_2$) solution and allowed to stand for 5 minutes. Then 0.3 mL of 10% aluminium chloride ($AlCl_3$) solution was added and the mixture was allowed to stand for further 5 minutes. Finally, 1 mL of 1M sodium hydroxide (NaOH) solution was added, and the final volume of the mixture was brought to 5 mL with distilled water. The mixture was incubated for 15 minutes at room temperature and absorbance was measured at 510 nm. The total flavonoid content was expressed as quercetin equivalent (µg/mg of extract), which is a common reference standard.

Antioxidant activity

DPPH assay

The free radical scavenging activity was determined using DPPH assay. [4] One mL of the extracts were taken in various concentrations (20-120 µg/mL) and mixed with 1 mL of 0.1 mM of DPPH solution in methanol. The setup was left at dark in room temperature and the absorption was monitored after 30 minutes. Absorbance was read at 517nm in spectrophotometer. The ability of the test sample to scavenge DPPH radical was calculated by the following formula:

$$\frac{Absorbance\ in\ control - Absorbance\ in\ sample}{Absorbance\ in control} x\ 100$$

Absorbance control was the absorbance of DPPH and methanol. Absorbance sample was the absorbance of DPPH radical and the test sample.

FRAP assay

Different concentrations of the extracts (20-120µg/mL) were mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20 minutes. 1 mL of 10% trichloroacetic acid was added to the mixture. Then 1 mL of 0.1% of freshly prepared ferric chloride was added and the absorbance of the resultant solution was measured at 700 nm. [30]

Phosphomolybdenum assay

The antioxidant activity was evaluated by reduction assay method which is based on the formation of green phosphomolybdenum complex. [20] Various concentrations of peel extracts were combined with 1 mL of reagent solution (4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulphuric acid). The tubes were capped and

incubated in a water bath at 95°C for 90 minutes. The samples were cooled to room temperature and the absorbance of the mixture was measured at 695 nm against blank.

Antibacterial activity

The microorganisms used for antibacterial activity were *Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri*, and *Klebsiella pneumoniae* respectively. The antibacterial activity of beetroot peel extracts was evaluated by agar well diffusion method. [16] 20 mL of nutrient broth agar prepared according to the standard procedure were poured into the plates and was allowed to solidify. The standard inoculum suspension was streaked over the surface of the media using a sterile cotton swab to ensure the confluent growth of the organism and the plates were allowed to dry for 5 minutes. Wells were made using a sterile stainless steel borer. Different concentrations of the extracts (50, 75 and 100 µL) were poured into the wells. Tetracycline was used as standard (1mg/mL). Diffusion of the extracts was allowed for 30 minutes at room temperature. Finally the inoculated plates were incubated for 24 hours at 37ºC. The zone of inhibition was measured and noted.

Thin Layer Chromatography

The thin layer chromatography method of the sample spotting was used to determine the R_f value of the separated compounds. TLC plate was prepared using silica gel G and was left overnight for drying. Aliquots of the extracts were applied on the TLC plates which was 0.2 mm above from the bottom with the help of a capillary tube. Then the plate was placed in a glass beaker containing solvents such as toluene: ethyl acetate: acetic acid as mobile phase. The zone of spot in the chromatogram corresponding to the band was noted. The R_f value was calculated using the following formula:-

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

3. RESULTS AND DISCUSSION

The percentage yield of extract was found to be 26.55% for methanol extract and 4.53% for acetone extract of beetroot peel respectively. It is evident from the results that secondary plant metabolites such as saponins, phenols, flavonoids, diterpenes, phytosterols and cardiac glycosides were present in both methanol and acetone extract while alkaloids and glycosides were absent. The phenol content and flavonoid content of methanolic extract was 99.1µg/mg GAE and 4.76µg/mg QE while for acetone extract the phenol content and flavonoid content was found to be 46.53µg/mg GAE and 4.51 µg/mg QE respectively.

DPPH (1,1-diphenyl-2-picrylhydrazl) is a stable nitrogen centered free radical that can be effectively scavenged by antioxidants. On accepting a hydrogen from a donor, DPPH which is dark purple in colour changes to yellow color and gets converted into a non-radical form known as 1,1-diphenyl-2- picrylhydrazine. Methanol extract of beetroot peel exhibited potent free radical scavenging activity that increased with increase in concentration when compared to acetone extract (Table 1). The IC_{50} values of methanolic and acetone extracts of beetroot peel was 118.90 µg /mL and 570.34 µg /mL concentration. IC_{50} value indicates the concentration of the test sample required to inhibit 50% of the free radicals.

Antioxidants are molecules that have the ability to scavenge free radicals before they damage the cells. There are highly complex antioxidant systems (enzymatic and non-enzymatic) in human cells, which work synergistically to protect the body against free radicals. Antioxidants can be endogenous or obtained exogenously, either through diet or by dietary supplements. [29] Scientific evidence suggests that antioxidant compounds in food play an important role as a health protecting factor. Beetroot (*Beta vulgaris* L.) ranks among the top ten most powerful vegetables with respect to its antioxidant capacity ascribed with a total phenol content of 50–60 µmol/g dry weight. [26, 12] Peels are reported to contain considerable amount of phenolic acids such as ferulic, protocatechuic, vanillic, *p*-coumaric, *p*-hydroxybenxoic, and syringic acids. [15]

Reducing power assay was also used in evaluating the antioxidant activity as the reducing capacity of a compound serves as a notable indicator of its potential antioxidant activity. The reducing power of beetroot peel was determined using FRAP assay and Phosphomolybdenum assay. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of compounds present in the extract. Presence of reducers causes the conversion of the $Fe^{3+/}$ferricyanide complex to the Fe^{2+}/ferrous form. The Phosphomolybdenum reduction assay was based on the reduction of Mo(VI) to Mo(V) in presence of electron donating compounds and subsequent formation of a green phosphate/Mo(V) complex at acidic pH and at higher temperature. From the results obtained it is evident that acetone extract exhibited good reducing capacity which increased with increase concentration of the extract (Table 2 and Table 3).

Table 1: Antioxidant activity using DPPH Assay

S.No	Methanol extract		Acetone extract	
	Concentration µg/mL	% inhibition of free radicals	Concentration µg/mL	% inhibition of free radicals
1	20	23.24	100	20.50
2	40	28.75	200	29.80
3	60	31.80	300	43.78
4	80	33.64	400	46.09
5	100	37.30	500	47.92
6	120	50.46	600	52.60

Table 2: Antioxidant activity using FRAP Assay

S.No	Concentration µg/mL	Absorbance at 700 nm	
		Methanol Extract	Acetone Extract
1	20	0.18	0.89
2	40	0.19	0.98
3	60	0.23	1.04
4	80	0.24	1.05
5	100	0.27	1.32
6	120	0.31	1.81

Table 3: Antioxidant activity using phosphomolybdenum Assay

S.No	Concentration µg/mL	Absorbance at 695 nm	
		Methanol Extract	Acetone Extract
1	20	0.05	0.40
2	40	0.07	0.47
3	60	0.13	0.48
4	80	0.14	0.52
5	100	0.15	0.53
6	120	0.24	0.61

Table 4: **Antimicrobial efficacy of *Beta vulgaris* peel**

Extracts	Pathogens	Standard Tetracycline	Zone of inhibition (mm)		
			50µL	75µL	100µL
Methanol	*Escherichia coli*	29	15	16	18
	Staphylococcus aureus	23	-	10	12
	Shigella flexneri	25	-	-	12
	Klebsiella pneumoniae	19	14	17	22
Acetone	*Escherichia coli*	21	11	14	17
	Staphylococcus aureus	19	-	-	-
	Shigella flexneri	26	-	-	11
	Klebsiella pneumoniae	26	11	12	14
50 µL - 250 µg 75 µL - 375 µg 100 µL - 500 µg					

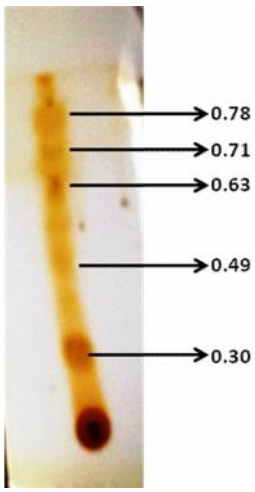

Fig 1: TLC of methanolic extract of beetroot peel

Beetroot peel contains maximum amount of water soluble nitrogenous pigments called betalains (54%) that scavenge free radicals and is also utilized as a natural food colorants in food industry. [2] Betalains and other phenolic compounds present in red beet decreases 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. [8, 30] In addition phenols are secondary plant metabolites that also account for antioxidant activity. The antioxidant properties of phenolic compounds are mainly because of their redox potential, which allows them to act as reducing agents, hydrogen donators, metal chelators and singlet oxygen quenchers.

Table 4 illustrates the antimicrobial efficacy of beetroot peel. Maximum inhibitory effect was seen in methanolic extract while acetone extract exhibited the least antimicrobial activity A diameter zone of inhibition of 11 to 15 indicates the test product having an intermediate antimicrobial activity against the test organisms. A diameter with a zone of inhibition of 16 or more indicates a high antimicrobial activity against the test organisms. [11]

Bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. [1] Use of natural active components with antimicrobial property obtained from plants or plant based agricultural products have enormous therapeutic properties in the current situation as modern drugs are associated with side effects such as allergies, hypersensitivity and immune suppression. Presence of various secondary plant metabolites exerts physiological actions such as antioxidant and antimicrobial properties. The characteristic intense color of beetroot is due to the presence of phenolic compounds which exhibits antimicrobial activity. [3]

Koochak *et al.* (2010) [14] tested the antibacterial activity of ethanolic extract of *Beta vulgaris* L. and reported that the extract did not possess inhibitory activity. Rauha *et al*. (2000) [22] tested the antimicrobial activity of methanolic extract of *Beta vulgaris* L. (500 µL of extract, 1 mg/mL) and observed only slight antibacterial activity against *Escherichia coli* and *Staphylococcus aureus.* Parekh and Chanda (2008) [19] also reported slight inhibitory activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* on aqueous leaf extract of *Beta vulgaris* L. while ethanolic leaf extract inhibited growth of *Staphylococcus subfava*. Though various parts of beetroot such as leaf and pomace extracts exhibits antimicrobial activity the present study indicates that the peels exhibits greater antimicrobial property because bioactive components are mostly present towards the outer parts of root, decreasing in the order peel, crown and flesh. Both betanin and isobetanin were found in greater amounts in the peel than in the flesh of beet. Hence the results of the study indicate the effectiveness of beetroot peel as an antimicrobial agent.

Thin layer chromatography

The presence of various phytoconstituents was further confirmed using thin layer chromatography technique. Thin layer chromatography is commonly used in the detection of compounds through a separation process. The number of

spots observed and their corresponding R_f values is shown in Figure1.

4. CONCLUSION

Current research on fruits and vegetable peels are following a trend to effectively identify various compounds and find its prophylactic role in designing and developing pharmacological drugs with less of 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 and antimicrobial agent thereby indicating its use as a value-added component for functional foods.

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