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PHYTOCHEMICAL EVALUATION AND BIOACTIVITY SCREENING OF UNDERUTILIZED HYDROALCOHOLIC *Beta vulgaris* L.PEEL EXTRACT

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ABSTRACT:

Vegetables are crucial because of their vitamins, minerals, fiber content and phytochemical compounds.The peels of vegetables are a source of vitamins, minerals and organic acids. The distinctive biological activity of the plants can be identified by their phytochemical properties. The antioxidant, anti-inflammatory activity and phytochemical evaluation was assessed in Hydro-alcoholic *Beta vulgaris* peel extracts (Laila Naif AI-Harbi *et al.*,2021). *Beta vulgaris* peel extracts have shown strong antioxidant activities compared to other vegetable peel extracts. The focus of this study was to examine systematically its biological activities and seek out its chemical constituents.

Keywords:

Beta vulgaris Peel, Phytochemical analysis, Antioxidant, Anti-inflammatory activity

INTRODUCTION

Beetroot (*Beta vulgaris* L.) which belongs to Chenopodiaceae family are of different varieties of colour ranging from yellow to red of which, deep red coloured are usually consumed. Red Beetroot is one of the most potent vegetable for their antioxidant activity due to several bioactive compounds like polyphenols, flavonoids, betalains, ascorbic acid, dehydroascorbic acid and inorganic nitrate (Laila Naif AI- Harbi *et al.*,2021).

The phytochemistry of plants has the predominant role of applying plant products as a potential remedy to different ailments. Antioxidants have a crucial role in inhibiting the production of reactive oxygen and nitrogen species (M. E., S., El-Sherbiny *et al.*,2024).The main bioactive compounds isolated from beetroot waste includes polyphenols, such as tannins, flavonoids and anthocyanins, vitamins(A and E), essential minerals, fatty acids, volatile compounds and pigments (Vilas-Boas *et al*.,2021).

Detrimental conditions for plants like extreme temperature, drought, heavy metals, nutrient deficiencies and high salinity, generate high concentrations of reactive oxygen species (ROS), which can cause oxidative stress. To avoid this, cells have a complex antioxidant system with enzymatic and non-enzymatic systems. The compounds derived from secondary metabolism, specifically phenolic compounds, play an elementary role against oxidative stress (Natividad Chaves *et al*.,2020).

Inflammation is a biological defence mechanism that enables living cells to protect themselves against diseases such as bacteria, fungi, viruses, physical agents and defective immune systems.The aim of this study was to

examine the effect of hydro-alcoholic extract on the total polyphenol content, total flavonoid content and antioxidant activity in terms of reducing power and anti-inflammatory activity of *Beta vulgaris* peel extract.

METHODOLOGY

The materials and methods used for the present study are discussed below;

EXTRACTION

Beta vulgaris was gently washed and peeled. It was then shade dried and ground into a fine powder .Taken 2 g of peel powder and is mixed with 20ml of solvent in a ratio of 1:10 , covered and left for 1 day and stirred occasionally and filtered using Whatman No.1 filter paper (Suryani *et al*.,2022).The Hydro-alcoholic extract was stored in the refrigerator for further use (S Keser *et al*., 2012).

PHYTOCHEMICAL ANALYSIS

Qualitative Tests

Test for Alkaloids

To 1ml of extract added few drops of diluted Hydrochloric acid and filtered. To this Wagner's reagent is added. A Reddish brown precipitate indicates the positive result

Test for Flavonoids

To 1 ml of extract add few drops of concentrated sulphuric acid. Orange colour indicates the presence of flavonoids

Test for Phenols

To 2ml of extract added 1ml of 5% ferric chloride. A red or purple colour indicates the presence of phenols

Test for Terpenoids

To 1ml of extract add 1ml of chloroform and 1ml of concentrated sulphuric acid along the sides of test tubes. A reddish brown colour indicates the positive result

Test for Tannins

Add 1ml of extract and few drops of 5% ferric chloride solution. A Brownish green colour indicates the presence of tannins

Test for Coumarins

Added 2ml of extract and 3ml of a 10% aqueous solution of sodium hydroxide. A yellow colour indicates the presence of coumarins

Quantitative Tests

Flavonoids

To 1ml of extract 1.25 ml of distilled water and 0.07ml of 5% $NaNo_2$ solution were added to the extract. It was incubated for 6 minutes and after that 5ml of 0.5% $AlCl_3$ was added. After another 5 minutes 0.5ml of 0.1M NaOH solution was added and the volume was made upto 2.5ml with distilled water. The absorbance was read at 510 nm using VIS Spectrophotometer (Sankhalkar & Vernekar, 2016).

Phenols

To 1ml of extract 0.5ml of Folin-Ciocalteu reagent and 2.5ml of 7.5% sodium carbonate solution were added and then the reaction was incubated for 45 minutes at room temperature. The absorbance was recorded at 725nm using UV/VIS spectrophotometer (Nahla *et al*., 2018).

ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Assay

The free radical scavenging activity of *Beta vulgaris* peel was determined using DPPH assay. To 1ml of solution containing from (0.2-1.0ml) of extract concentrations (200-1000µg/ml) and mixed with 1ml of DPPH solution in ethanol. Ethanol serves as control and ascorbic acid as standard. The test tubes were shaken vigorously and incubated in the dark at room temperature for 30 minutes. It was read in a UV spectrophotometer at 517nm (S John *et al*.,2017).

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

H_2O_2 Scavenging Activity

A solution of hydrogen peroxide (40mM) was prepared in Phosphate buffer (0.1 M, pH 7.4). Extracts (0.2-1.0ml) in distilled water were added at various concentrations and mixed with 1ml of H_2O_2 solution (0.6 mL,40mM).Ascorbic acid was used as a standard. This was incubated for 10 minutes and after incubation absorbance was read at 220nm (Serhat Keser *et al*.,2012).

$$\% \text{ Scavenged } [H_2O_2] = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

ANTI-INFLAMMATORY ACTIVITY

Inhibition of protein denaturation

To 5ml of the reaction mixture add 0.2ml of 1% bovine serum albumin, 2.8ml of phosphate buffered saline (PBS, pH 6.4), and extracts (0.5-2.5ml) and the mixture was incubated for 15 minutes and heated for 5 minutes. After cooling the turbidity was measured at 660 nm using a UV/VIS spectrometer.A Phosphate buffered solution was used as a control (Gunathilake *et al*.,2018).

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical analysis for *Beta vulgaris* peel showed the results for active secondary metabolites present in the hydro-alcoholic extract. The table 1 and 2 below shows the presence (+) or absence (-) of phytochemical constituents in the test sample. The Hydro-alcoholic extract shows the presence of flavonoids, phenols, tannins, coumarins and terpenoids and the absence of alkaloids and the percentage present.

Table 1: Qualitative phytochemical analysis of Hydro-alcoholic *Beta vulgaris* peel

Parameters	*Beta vulgaris* peel
Flavonoids	+
Phenols	+
Alkaloids	-
Coumarins	+
Terpenoids	+

Tannins	+

Presence of phytochemical constituents is represented as "+" and absence are represented as"-".

Table 2: Quantitative phytochemical analysis of Hydro-alcoholic *Beta vulgaris* peel

Sample	Extraction	Contents Percentage	
Beta vulgaris peel	Hydro-alcoholic	Phenolic content	14.98
		Flavonoids content	6.90

Antioxidant Activity

DPPH Radical Scavenging Activity

Antioxidant activity was inferred that it has shown higher activity with the *Beta vulgaris* peel extract. The figure 1 shows the Inhibition from 33.05 to 89.48%. Ascorbic acid was used as a standard for DPPH activity concentrations from 200-1000µg/ml for Hydro-alcoholic extracts.

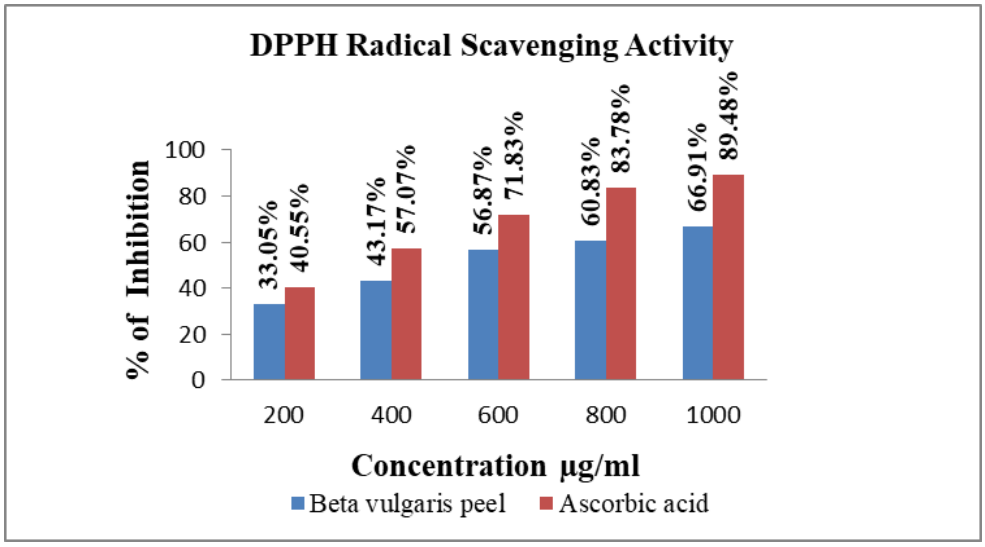

Figure 1: DPPH Radical Scavenging Activity

H_2O_2 Scavenging Activity

The Hydrogen peroxide radical scavenging assay of *Beta vulgaris* peel was evaluated with higher inhibition (20.24-66.55%). Ascorbic acid was used at standard concentrations of 10 to 50µg/ml.

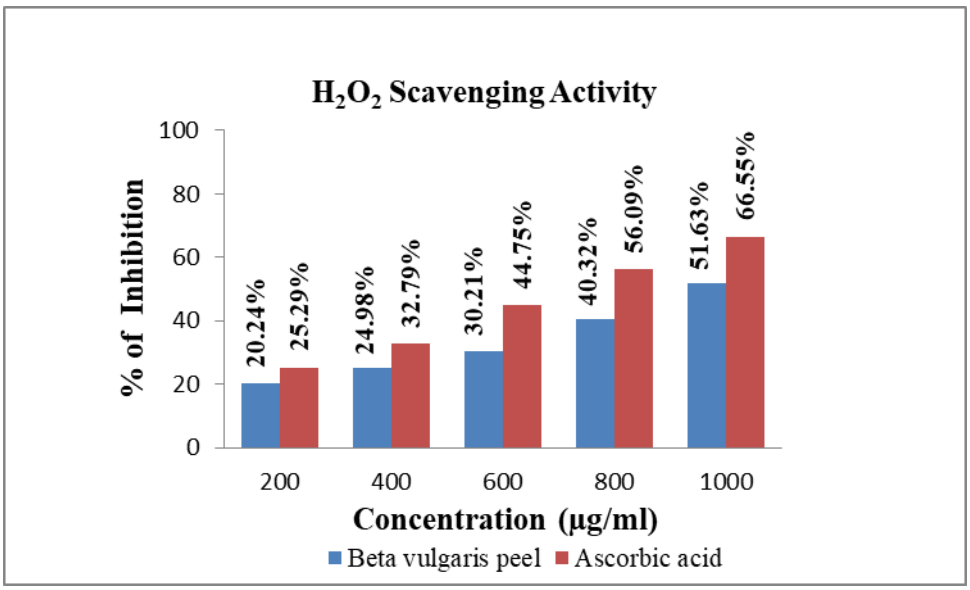

Figure 2: H_2O_2 Scavenging Activity

Anti-Inflammatory Activity

The anti-inflammatory activity was performed and the results show high inhibitory activity from (20.06-72.5%) for Hydro-alcoholic extracts of *Beta vulgaris* peel and concentrations ranging from 10-50µg/ml. Aspirin was used as standard.

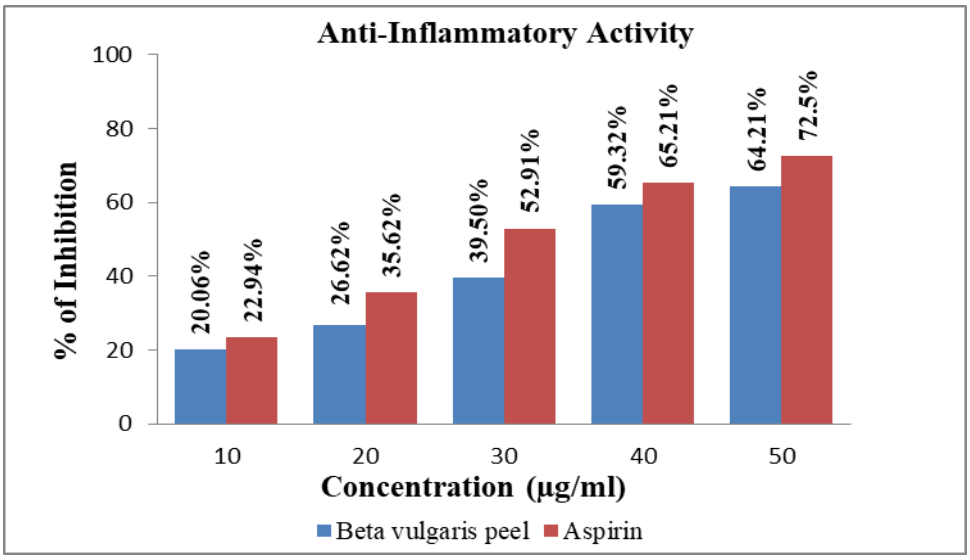

Figure 3: Anti-Inflammatory activity

These results reveal that the extract possess significant free radical scavenging capabilities.

SUMMARY AND CONCLUSION

In the present study the scrutinization of Hydro-alcoholic extracts of *Beta vulgaris* peel were examined for the presence of flavonoids, phenols, tannins, terpenoids, and coumarins. The presence of these phytochemicals indicates their bioactive compounds which have potent health benefits. In vitro investigations in the present study provide substantial evidence that *Beta vulgaris* peel, an underutilized product showed Antioxidant activity by DPPH and H_2O_2 Activity. Anti-inflammatory activity by protein denaturation method showed higher potency. Accordingly, future studies can be extended by performing other activities with the Hydro-alcoholic extracts of *Beta vulgaris* peel extract.

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