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Original Research Article

Extraction of Natural Colour from Beet Root (*Beta vulgaris*) its Phytochemical Analysis and Antibacterial Activity

Parveen Zia[1], Mishra Sunita[2*], Singh Sneha[3]

[1]Students, Department of food and Nutrition, School for Home Science, Babasaheb Bhimrao Ambedkar University, A Central University, Lucknow, Uttar Pradesh, India
[2]Dean and Head, Department of food and Nutrition, School of Home science, Babasaheb Bhimrao Ambedkar University, A Central University, Lucknow, Uttar Pradesh, India
[3]Students, Department of food and Nutrition, School for Home Science, Babasaheb Bhimrao Ambedkar University, A Central University, Lucknow, Uttar Pradesh, India

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Abstract: Beet root (*Beta vulgaris*) is a rich source of Antioxidant and minerals including sodium, potassium, iron and magnesium. Beet root are low in calories value (about 45Kcal per 100g) and zero cholesterol. Food colour is the most important parameters improve food quality to a significant level. Beet root has several medicinal properties such as anti-hypertensive, anti-microbial, anti-inflammatory, hepato-protective, anti-cancer and diuretic. This study was conducted to improve the use of natural colorants in food and food products and also improve the nutritional quality of food. The chemical analysis of these study showed beet root have a phytochemical property. The present study was designed to evaluate phytochemical analysis and antibacterial activity of beet root and to provide a brief knowledge association with health benefits of beet. In conclusion, these results suggest that beet root have good phytochemical properties.

Keyword: Beet root, Extraction, natural colour, yield, phytochemical analysis, Antibacterial activity.

INTRODUCTION

Beet root (*Beta vulgaris*) is the taproot portion of a beet plant. It belongs to subfamily Betaoideae of the family Amaranthceae betalains Betacyanin, Betaxanthin and Anthocyanins pigment naturally present in beet root, thus Pigment have been widely used in food products as a natural colorants (Mamta Jaiswal and kiran Agrahari 2017). It is a perishable vegetable it may be dehydrated and its mineral content may increase due to reduction of water mass. And highly rich in fibre as well as sugars. Colour of food can play a vital role in flavour perception. (Deepika Dhawan *et al*., 2019). Food colour can be divided into four categories a. natural colour b. nature-identical colours c. inorganic colour. Synthetic colour (Mayank chauchan, Mohammad Alikhan, 2017). Betalains is a pigment which are widely used as natural food colorants in different food industries People associate certain colours with certain flavours, and the colour of food can influence the perceived flavour in anything from candy to wine sometime the aim is to simulate a colour that is perceived by the consumer as a natural, such as adding red colouring to glace cherries (which would otherwise be beige) but sometime it is for effect, like the green ketchup that Heinz launched in 1999 color additives are used in food for many reasons.

In recent years used in various chemical or synthetic colours are used in major field like Agriculture molecular biology and food technology etc. (Fariha Kabir *et al*., 2019). the replacement of these harmful chemical colours must be done using natural colorants the natural colours of beet root enhance the physical as well as nutritional properties of food and food products such as (vitamins, minerals, and antioxidant). (Tanmay Sarkar *et al*., 2015). Now a days, the new trend in food additives tends to use of natural food colorant in colouring of food. Red beetroot is a good tonic for human health. (Dorcus Masih *et al*., 2019).

Natural colorants can provide a comprehensive range of attractive food for use in the different food industries. According to (Diego dosS Bainao. 2017). Macronutrients in 100 g of beet root has an energy value 43 kcal ,9.56g of carbohydrates,1.61g of protein,0.17g of total lipids and 6.76g of total sugars. Mostly five natural colours Anthocyanin, beet root Annatto, caramine and turmeric are used in modern

***Corresponding Author:** Mishra Sunita

food industry. Beet root is more acceptable for nutrient content such as dietary fibre, minerals content (iron, zinc, calcium and sodium) vitamin contents (folic acid, vitamin A, niacin biotin and C) (Al- Mamun *et al*., 2020).

Natural colours added to food for different purposes such as uniformity of colour in food, to enhance the natural colour and appearance of food and food products whose colour has been reduce during the processing. It is mainly consumed in form of juice, powder, oven-dried or jam-processed across different food cultures. (Pravin Mirmiran *et al*., 2020) According to CAGR (Compound Annual Growth Rate) the global natural food colors market is an Anticipated to grow at a CAGR of around 5% during 2019-2024. The aim of our current research work to aware the community about to use of natural colorants in food products.

MATERIAL AND METHODS

The vegetable (beet root) for extraction of natural colour was purchased from local market (Prayagraj). Fresh beet root with uniform maturity and weight were used as a raw material and were stored at a room temperature prior to the experiments.

Chemicals and reagents

Ferric chloride ($F_eCl_{3)}$, Conc. Sulphuric acid (H_2So_4), sodium hydroxide (NaOH), Chloroform, ammonium solution, Nutrient Agar

Sample Preparation

All the 3 kg beet roots were washed in running tap water for removing any durt or foreign material from their surface. And selected on the basis of uniform size and physical injury. The average moisture % of beet root (*Beta vulgaris*) was found 87.20% (wb) (Venkatasubramanian Sivakumar *et al*.,).

Then peeled and chopped the beet roots into uniform size (1-3 mm) using knife, these uniform slices were dried in laboratory Hot air oven at 60-70^{0}C for 8-10 h. the dried beet root were subjected to grinding in mixer grinder (Sudipta Kumar Hazra *et al*., 2020). Then ground material was passed through mesh sieve and packed in air-tight container for further use. Flow chart of extraction of beet root is given in Figure-1.

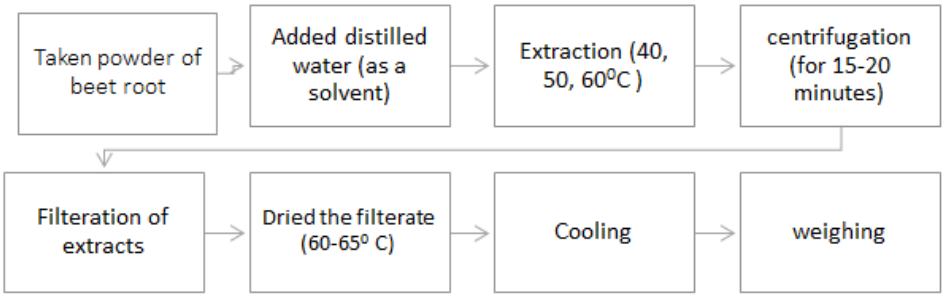

Figure-1: Flow chart of extraction of natural colour from beet root

Extraction of Colour

Extraction process was carried out by aqueous extraction method, taken finest powder of beet root, due to highest extraction yield. In Aqueous extraction process distilled water was used as a solvent under different aqueous extraction condition. Extraction temperature (40, 50, 60^{0}C) time (20, 60, and 100) min and solid- liquid ratio (1:5 g/ml) were taken in 150 ml Erlenmeyer flasks and it was incubated at different time temperature combination (J. Prakash Maran *et al*.,).

Extracts were taken at different time intetrvals (20, 60 and 100 min). After extraction for a selected time and temperature the mixture was centrifuged for 15-20 min. the obtained extracts were filtered using filter paper (What man No.1) then dried in hot air oven at 60-65^{0}C until all the water get evaporated, the petri plates were put in a desiccator for cooling and weighed. The weight of the natural colorants extract obtained per gram of beet root powder was then calculate. The beet root extract is used in food industry for improvement the redness in soups, sauces, jams, jellies, sweets, and breakfast cereals (Navnidhi *et al*., 2019).

$$\textbf{YIELD OF TOTAL EXTRACT} = \textbf{Total extract obtained in gram} \times 100 \div \textbf{Amount of beetroot used (gm)}$$

Qualitative Phytochemical Analysis

Phytochemical have a great antioxidant potential and their beneficial effects on human health, and they give greater health benefits to the consumers (Monika Thakur et.al, 2020).

(According to S. Ranganna) The extracts of beet root were analysed for tannin, saponin, quinone, flavonoid, glycoside, Terpenoids, coumarin, phenols, steroid, cardiac glycoside, anthocyanin/betalains and Betacyanin using standards procedures. The following biochemical analysis were performed for the detection of bioactive compounds (Sidra Rheman *et al*., 2021). Anthocyanin are mostly used in food in the food industry as a synthetic colorants, they can replace Allure red (FD&C Red No. 40) (M. T. M Assous *et al*.,

2014). betalains is also a natural pigment used in food industry as a natural colorants (Ashwini Gengatharan *et al*., 2015).

Test for tannin

Tannins, the second most abundant polyphenol, mainly function as defence compounds that protect plants against abiotic stresses, such as drought, heat, and high UV radiation (Vidya Suseela, 2019).

1 ml of the beet root extract was added 1 ml of 5% $fecl_3$ dark blue and greenish black color indicates the presence of tannin.

Test for Saponin

Saponin is a large family of structurally related compounds containing a steroid or triterpenoid aglycone (sapogenin) linked to one or more oligosaccharide moieties (V. R. Mohan *et al*., 2016).

1 ml of the beet root extract was added in 1 ml of distilled water shake it for 15 min formation of 1 cm layer of foam indicates the presence of the saponin.

Test for Quinone

1 ml of the beet root extract was added in 1 ml of Conc. H_2So_4. Red color indicates the presence of quinones.

Test for Flavonoid

Flavonoids are health promoting and disease preventing dietary supplements. Flavonoids also possess antiviral and antibacterial effects (Pon Velayuthan Anandh Babu *et al*., 2009).

1 ml of the beet root extract was added in1 ml of 2N sodium hydroxide (N_aOH) yellow color formation indicates the presence of flavonoids.

Test for Glycocides

Glycoside are secondary metabolites that comprise of a sugar portion that is linked to a nonsugar moiety (typically a monosaccharide) (Onaolapo A. Y *et al*., 2019).

1 ml of the extract was added in 3 ml of chloroform then added 10% ammonium solution. Pink color indicates the presence of glycoside.

Test for phenols

Phenols is a bio-active compounds derived from plants and foods (Kevin Robards *et al*., 2003).

1 ml of beet root extract was added in 2 ml of distilled water then added few drops of 10% $fecl_3$ formation of blue green color indicates the presence of phenols.

Test of coumarins

Coumarin is one of the natural compounds have a stability, solubility, and low toxicity (Shruti Mishra *et al*., 2020).

1 ml of extract was added in 1 ml of 10% N_aOH yellow color indicates the presence of coumarins.

Anthocyanin and betacyanins

Anthocyanins are natural bioactive compounds, the consumption of foods which is rich in anthocyanin is related to decrease the risk of cardiovascular diseases and cancer (Anna Rafaela Cavalcante Braga *et al*., 2018) betacyanins is responsible for the red-violet color of fruits and vegetables (Sri Priatni *et al*., 2015).

1 ml of extract was added in 1 ml of N_aOH then heat it for 5 min at 100^0C bluish green color indicates the presence of anthocyanin and formation of yellow color indicates the presence of betacyanin.

Antibacterial activity

Source of Microorganism for Antibacterial Activity

Escherichia coli, Salmonella Enteritidis and Staphylococcus Aureus were used in this study for antibacterial activity of beet root. These cultures were obtained from the laboratory of environmental microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow U.P, (INDIA).

Antibacterial activity of beet root

Sample for the determination of antibacterial activity of beet root (*Beta vulgaris*), which was dissolved in distilled water to a concentration of 100 mg/ml. (Markov *et al*., 2011).

Agar well diffusion method is mainly used for the evaluation of antibacterial activity of plants or microbial extract (Mouny Balouiri *et al*., 2016). The determination of antibacterial activity in beet root was done using the agar well diffusion method (Emoghene *et al*., 2014). The zone of growth was found only for Escherichia coli (ATCC 10536), Staphylococcus aureus (ATCC 11632) and Salmonella enteritidis for the well diffusion method, wells of 9mm diameter were made. Three wells are made on the surface of the agar plate. The beet root extract solution (50 and 100) microliter was then transferred into the wells of incubated agar plates (Kahkashan Perveen, Najat A. Bokahri, 2020). The plates were refrigerated at 8^0C for 1 h to allow the extract to diffuse into the medium, and then incubated at 37^oC for 24 h. after incubation period the diameters of the inhibition zone were measured and recorded in millimetres (mm). The measurement of diameter (mm) of the zone of inhibition was done by using a transparent scale, and then evaluated (Ramesa Shafi Bhat *et al*., 2014).

RESULTS AND DISCUSSION

Yield

The yield of extracted colour from beet root was 12.7% at 40˚C for 20 minutes, 5.98 % at 40˚C for 60 minutes and 9.95% at 40 ˚C for 100 minutes

respectively. It concluded that maximum yield was obtained at 40 ˚C For 20 minutes i.e, 12.7%.

Quantitative screening of phytochemical

The phytochemical analysis showed beet root (*Beta vulgaris*) contain some secondary metabolites. The below table.1 shows the presence (+) and absence of (-) of phytochemical constituents in the tested sample of beet root. In this analysis we obtain the presence of saponin, quinone, flavonoid, phenols, coumarin, steroid, anthocyanin and betacyanin, and the absence of tannin and glycoside. Secondary metabolites present in plants which is responsible for their therapeutic activity.

Table-1: Phytochemical analysis of beet root (*Beta vulgaris*)

Phytochemical constituents	Water extract
Tannin	+
Saponin	+
Quinone	+
Flavonoid	+
Glycoside	-
Phenols	+
Coumarin	+
Steroid	+
Anthocyanin & Betacyanin	+
+ **represents presence & - represents absence**	

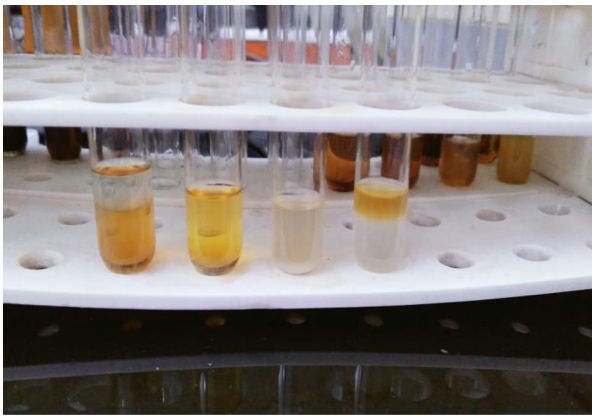

Figure-2: Presence of Phytochemicals

Antibacterial Activity

Microorganism are vary extensively in their degree of susceptibility to anti-bacterial agent. In this study we observed the various zone of inhibition in beet root extract after analysis. The zone of inhibition are represented in the below Table-2. The zone of inhibition in tested extract are, Escherichia coli (15mm), Staphylococcus aureus (10mm) and Salmonella enteritidis (10mm). On the basis of the result obtained in this probation it can be conclude that beet root extract had momentous in vitro broad spectrum antibacterial activity.

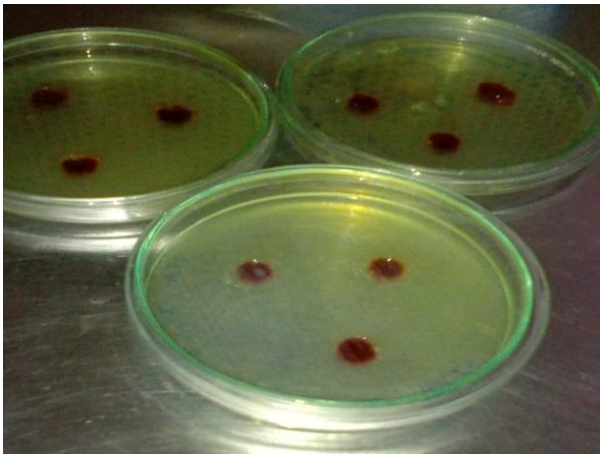

Fig-3(a): Well diffusion of beet root extract

Fig-3(b): Zone of inhibition

Zone of inhibition of beet root extract

Beetroot extract showed a varying zone of inhibition to the growth of tested microorganism Table-2.

Table-2: Antibacterial activity against zone of inhibition

Sample	Escherichia coli	Staphylococcus aureus	Salmonella enteritidis
Beet root extract	15mm	10mm	10mm

CONCLUSION

Beet root (*Beta vulgaris*) have a two major pigment (Anthocyanin & Betacyanin). Which are soluble in water both have aesthetic value and positive health effects in food, unlike artificial colorants which may cause adverse effects in humans. Beet root show the great potential as functional food. Beetroot is gaining popularity as a super food due to its health benefits. Betanine is the main component of the red colour, known as beetroot red, extracted from (*Beta vulgaris*). After extraction Betalains have been successfully used in commercial food colouring agent for a number of years. The phytochemical profile of beetroot presents positive health impact to human,

which would also allow the development of innovative foods.

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Conflicts of Interest: The author shows no conflict of interest.

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