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Antioxidant and Antibacterial Properties of bio-pigment from *Beta vulgaris*

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Plants are essential for sustaining life on our planet, providing a diverse range of species that possess therapeutic properties. With a growing awareness of health and well-being among consumers, there has been a rise in the popularity of natural colorants sourced from plant-based materials. In this context, the main objective of this research was to extract valuable natural pigments from Beta vulgaris (commonly known as beetroot), with the aim of increasing the availability of pigments from natural sources while simultaneously minimizing environmental and health risks. The crude extract of pigments was obtained using the maceration method during the extraction process. The crude extract was then purified using flash column chromatography with various solvents as the mobile phase. Preliminary phytochemical screening revealed the presence of active compounds like phenols, carbohydrates, glycosides, phytosterols, tannins, flavonoids, alkaloids, terpenoids, and saponins. High-Performance Thin-Layer Chromatography (HPTLC) was performed to analyze the betalain profile, showing distinct bands at specific Rf values. The specific functional groups were identified in the Fourier-Transform Infrared (FTIR) spectrum based on the presence of characteristic bands. Antibacterial activity testing demonstrated varying degrees of inhibition against test organisms like Bacillus cereus, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The evaluated plant pigments exhibited positive results in terms of antioxidant activity, exhibiting free radical scavenging capabilities. The aqueous methanol extract showed the highest extraction yield at 36.71%. The aqueous methanol extract of Beta vulgaris showed the maximum antibacterial activity at 20 mm against Bacillus cereus. The methanol extract and aqueous ethanol extract displayed the lowest IC50 values (45.56 μg/ml and 43.23 μg/ml, respectively), indicating their high antioxidant potential. These findings highlight the promising potential of Beta vulgaris as a valuable source of natural pigments with both antibacterial and antioxidant properties.

Keywords: Antioxidant activity; Antibacterial activity; Beta vulgaris; Extraction; FTIR; HPTLC fingerprinting; Natural colour.

Plants play a vital role in sustaining life on Earth, and there is a growing global trend towards the production of natural colorants. The characteristic colors of plants are attributed to a diverse group of chemicals known as plant pigments, with each pigment having its own distinct name, chemical structure, and color properties.[1]Synthetic pigments tend to remain quite stable to common oxidation and reduction processes as per their design, and so are very

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difficult to remove from textile industry effluents. Many carcinogenic and allergic synthetic pigments are banned now. Many dyes, though still being banned, may not be completely safe. Synthetic pigment-based effluents can cause a serious hazard to the water stream and environment due to their synthetic origin and complex molecular structures, which decrease their ability to biodegrade. Most synthetic dyes are not biodegradable; they accumulate on lands and rivers, causing ecological problems. Regulatory bodies such as the FDA (Food and Drug Administration) and EFSA (European Food Safety Authority) set limits and guidelines for using these pigments in different products to ensure consumer safety.

In response to these environmental and health issues, there is an increasing focus on developing and utilizing natural and eco-friendly pigments. Natural pigments derived from plants, minerals, or other organic sources offer a more sustainable and biodegradable alternative to synthetic pigments. These natural pigments not only reduce the environmental impact but also provide an opportunity for the revival of traditional and cultural practices related to dyeing and coloring.

The Indian natural colorant market from plant pigments is witnessing significant growth and gaining prominence across various industries. The food and beverage sector is a major consumer, driven by rising demand for clean-label products. India's rich biodiversity offers a diverse range of plant-derived colorants for traditional dishes and beverages. The cosmetics and personal care industry is adopting natural colorants to cater to the demand for organic products. In pharmaceuticals, plant-derived colors are used for aesthetic appeal. The Indian government's regulations by FSSAI promote safer and natural alternatives. The market is expanding internationally as India emerges as a notable exporter.

Beta vulgaris contains various bioactive substances like ascorbic acid, flavonoids, polyphenols, saponins, and nitrate, which may have positive effects on health.[2] The Betalain pigments have shown the ability to protect biological components from oxidative damage, as demonstrated by several in vitro studies. With the growing demand for natural and nutritious food products, the potential applications of plant-derived pigments are becoming increasingly promising.[3]

Extraction is a crucial process used to obtain natural extracts and specific pigments from plants. Phytochemical studies have shown that plants contain various beneficial compounds such as tannins, saponins, flavonoids, alkaloids, and phenolic components. High performance thin layer chromatography fingerprinting has revealed the presence of betacyanins and betaxanthins, which are important phytoconstituents present in betalain. Oxidative stress in the body generates reactive oxygen species and free radicals, which can lead to damage to proteins, lipids and DNA. Using natural pigments with high antioxidant content is considered an effective approach to address the potential harm caused by synthetic colors to both individuals and the environment. These natural pigments not only offer a safer alternative but also possess therapeutic properties that can help in treating diseases and reducing the detrimental effects of free radicals.

Beta vulgaris contains bioactive substances like betalains and nitrates. Betalains act as antioxidants and anti-inflammatories, reducing oxidative stress and inflammation. Nitrates convert to nitric oxide, aiding cardiovascular health by relaxing blood vessels and improving blood flow. Previous research and studies on *Beta vulgaris* (beetroot) have provided confirmation of various potential positive effects on health. The dietary nitrates in beetroot can lead to vasodilation and improved blood flow, which may help reduce blood pressure and support overall cardiovascular health. Beetroot juice, rich in nitrates, has been found to enhance exercise performance by reducing the oxygen cost of exercise and increasing endurance, making it beneficial for athletes and active individuals. Betalains in *Beta vulgaris* act as potent antioxidants, protecting cells from oxidative damage and potentially reducing inflammation in the body.Some research suggests that the nitrates in beetroot may have positive effects on cognitive function and brain health, particularly in older adults. The extract of *Beta vulgaris* may have hepatoprotective effects, supporting liver health and function. It is a good source of dietary fiber, which can promote digestive health and regular bowel movements. Immune *Beta vulgaris* contains

essential vitamins and minerals that can support the immune system and overall health.

Review literature

Non-conventional extraction methods were reviewed for improved yield and eco-friendliness, considering thermal sensitivity and polarity. Microwave-Assisted Extraction (MAE) was effective for carotenoids, chlorophylls, and anthocyanins, while Ultrasonic-Assisted Extraction (UAE), Enzyme-Assisted Extraction (EAE), and Pulsed Electric Field Extraction (PEF) were suitable for betalains.

Parveen Zia *et al.* (2021) reported the zone of inhibition in the tested extract of *Beta vulgaris* against *Escherichia coli* (15mm), *Staphylococcus aureus* (10mm), and *Salmonella enteritidis* (10mm).

Yizhong Cai *et al.* (2003) demonstrated that betalains found in plants of the Amaranthaceae family, particularly red-violet gomphrenin-type betacyanins and yellow betaxanthins, exhibited remarkably potent antioxidant activity compared to traditional antioxidants like ascorbic acid, rutin, and catechin. The study indicated betalains as potential sources of natural antioxidants and colourants. Antioxidant activity varied with betalain chemical structures, with those containing more hydroxyl and imino groups exhibiting higher scavenging activity. The presence of hydroxyl groups at the C-5 position enhanced activity, while increased glycosylation reduced it.

Hee-Ock Boo *et al.* (2012) concluded that pigments derived from natural plants exhibited significant biological activities, with properties varying based on the specific type of pigment. As a result, these plant resources, containing active functional components, possess great potential as excellent materials for natural cosmetics and food supplements.

MATERIAL AND METHODS

Collection and Processing of plant sample

The plants used in this study, including the cone-shaped taproot of *Beta vulgaris*, were sourced from the Serenity Botanical Garden and authenticated with the code GU/BOT/A/V12. After the process of washing, drying, and grinding, the obtained powder was stored at a reduced temperature of 4°C for subsequent analysis.

The physicochemical properties of the plant material were evaluated following the guidelines of the Indian Unani Pharmacopoeia5. The analysis encompassed the assessment of various parameters, such as extraction yield, moisture content, flavor, color, and total ash value. Additionally, the determination of moisture content and total ash value was specifically conducted for Beta vulgaris.[4,5]

Extraction Process

The dried plant material was finely ground using a mortar and pestle and then sieved through a 1 mm sieve. The 10 grams of finely ground plant material were immersed in 100 ml of various solvents, including methanol, acetone, ethanol, and water for a period of 5 to 7 days.[6,7] A 50% aqueous methanol extract was prepared using a 1:1 mixture of methanol and water. The plant material was then filtered, and the remaining solid was further extracted to remove any remaining liquid. The obtained liquid was purified through filtration. Low-pressure rotary vacuum evaporators were employed to extract the solvent. Finally, the dried extract was stored in an airtight container at 4°C until further analysis.[8,9,10] The following formula was used to determine yield percentages:

$$\text{Extract yield \%} = \text{weight of dried extract/weight of dried leave} \times 100$$

Purification of pigment by Flash column chromatography

The crude plant extracts were subjected to purification through flash column chromatography, employing different solvents as the mobile phase and alumina as the adsorbent. The sample was carefully applied to the column using a pipette, and air pressure was used to force it into the column. The column walls were washed with a small volume of solvent, also forced into the column under pressure. Once the column was filled, it was connected to a fraction collector. The air pressure was adjusted to achieve an appropriate flow rate through the column and fractions were collected accordingly. The colored fractions containing the desired components were collected and further utilized for eluting various components from the column.[11]

Preliminary phytochemical screening

Qualitative preliminary phytochemical

tests were performed to determine the presence of various chemical groups in the extract. Standard methodologies were used to identify primary metabolites like proteins, carbohydrates, fixed oils, and fats. For the qualitative screening of phytochemicals, a small amount of the dry plant extract was used. The analysis revealed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, polyphenols, tannins, terpenoids, and glycosides in the plant extracts.[12]

HPTLC fingerprinting analysis of pigment

HPTLC fingerprinting, following the procedure outlined by Wagner *et al*. (2015), was performed. A TLC plate with Silica gel was used, and 10µl of the sample was applied. The plate was placed in a TLC twin trough developing chamber, pre-saturated with solvent vapour using methanol: water (9:1) as the mobile phase for betalain separation. Development of the plate was done up to 90mm. Subsequently, hot air was used to dry the plate and remove any remaining solvents. The plate was then stored in a photodocumentation chamber, and images were taken under visible light, UV 254 nm, and UV 366 nm. Peak table, peak display, and peak densitogram were observed during the analysis.[13]

Characterization of pigment by FTIR spectroscopy

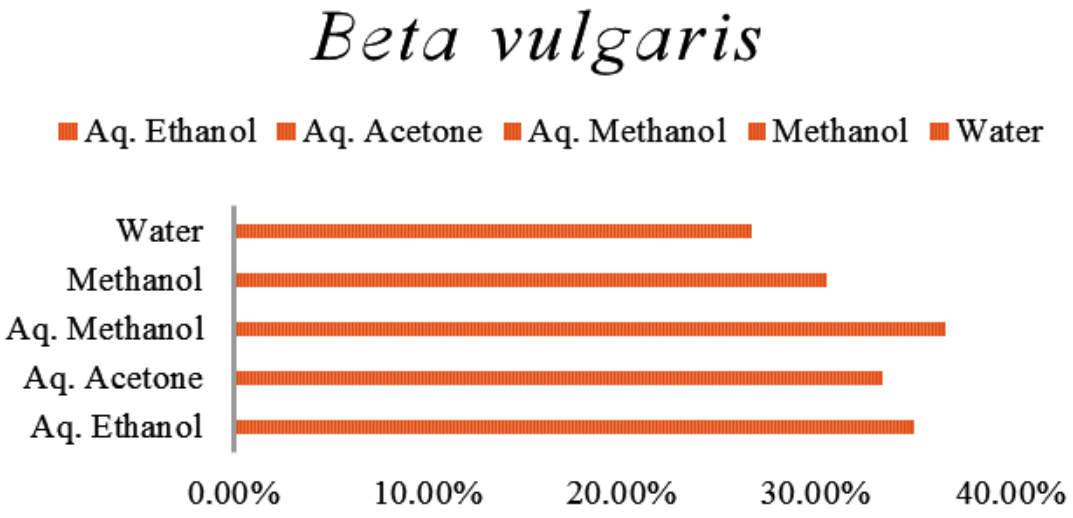
Plant pigment was analyzed by FT-IR for the detection of functional groups. 2 µl of sample (*Beta vulgaris*) was analyzed by using diamond ATR top plate.[14] A detector reads the analogue signal and converts the signal to a spectrum. The analysis conditions for FT-IR were 32 scans at a resolution of 8 cm^{-1}, measured between 400- 4,000 cm^{-1}.

Antioxidant activity of pigment

The antioxidant activity of the plant pigments was evaluated using the DPPH free radical scavenging assay. Different concentrations of the samples, such as 25 µg/ml, 50 µg/ml, 75 µg/ml, and 100 µg/ml were prepared. A 0.001% concentration of DPPH solution in ethanol was also prepared. Ascorbic acid was used as a positive control. The plant extract was mixed with the DPPH solution, and the mixture was vortexed and left to stand for 30 minutes at room temperature in a dark environment. The absorbance of the mixture was then measured using a spectrophotometer at 517 nm. Three measurements were taken for each sample. A negative control was included by adding DPPH solution to 1 ml of methanol.[15,16,17] The DPPH scavenging effect (%) was measured by using the following formula:

$$\text{DPPH Scavenging effect}(\%)=\{(Ao-A)/Ao\}x100$$

Where Ao is the absorbance of negative control (0.001% DPPH solution) and A is the absorbance in presence of extract.



Graph 1. Graphical representation of extraction yield

Antibacterial activity

The antibacterial activity of the selected plant extracts was assessed using the agar well diffusion method. The experiment was conducted three times for each treatment. The plant extracts containing antibacterial compounds were allowed to diffuse into the medium of freshly seeded plates with test organisms, including *Bacillus cereus* (MTCC 736), *Staphylococcus aureus* (MTCC 2408), *Escherichia coli* (MTCC 1650) and *Pseudomonas aeruginosa* (MTCC 424). After a 24 hour incubation period, the zone of inhibition around each well was measured in millimeters. The final concentrations of the extracts tested ranged from 2 to 10 mg/ml. Negative controls contained plates with DMSO reagent, while positive controls contained plates with chloramphenicol antibiotic solution.[18]

RESULTS

Plant powder was shown to have a wide range of physicochemical properties. The following table 1 shows the observed result:

Table 2 lists the physical characteristics of plant extracts as well as the extraction yields. Methanol, aqueous ethanol, aqueous methanol, aqueous acetone, and water systems all achieved extraction yields of 30.58%, 35.02%, 36.71%, 33.45%, and 26.73, respectively. The lowest extraction yield was found in methanol extract at 30.58%. Aqueous methanol extract produced

Table 1. Moisture content and ash value of *Beta vulgaris*

Plant	Moisture content (%)	Ash value (%)	Acid insoluble ash	Water soluble ash
Beta vulgaris	4.45 %	9.6 %	3.3 %	6.25 %

Table 2. Physical characteristics and % yield of Extract: *Beta vulgaris*

Plant	Solvent	Colour of Extract	Sense of Touch	Amount of Extract (gm)	% Yield
Beta vulgaris	Aq. Ethanol	Intense red	Excessive viscous	10.50	35.02
	Aq. Methanol	Intense red	Excessive viscous	11.01	36.71
	Aq. Acetone	Intense red	Minor viscous	10.03	33.45
	Methanol	Intense red	Viscous	9.17	30.58
	Water	Intense red	Viscous	8.02	26.73

Table 3. Preliminary tests for *Beta vulgaris* extract

Phytoconstituents	Test	BVAE	BVAA	BVM	BVAM
Alkaloid	Wagner's test	+	+	-	+
	Hager's test	-	++	-	-
Total phenol	Ferric chloride test	+++	-	+	++
Tannin	Lead acetate test	+++	++	++	++
Flavonoids	Alkaline reagent test	++	-	+	-
Phytosteroids and terpenoid	Libermann-Burchard's test	++	++	++	+
Carbohydrates	Molisch's test	++	++	+	++
	Fehling's test	++	++	+	+
	Benedict's test	++	++	++	+
Proteins and amino acids	Ninhydrin test	++	+	-	+
Saponins	Foam test	++	++	+	+
Fixed oil and fats	Spot test	++	+	++	+

a maximum extraction yield of 36.71%. The physicochemical properties of plant pigment include, colour and tactile sensation were also observed.

The highest extraction yield was obtained with the aqueous methanol extract, which had a yield of 36.71%. This indicates that aqueous methanol is the most efficient solvent for extracting the desired yield from *Beta vulgaris*. The water extract had the lowest extraction yield of 26.73%, suggesting that it might not be as effective in extracting the target compounds compared to the other solvents used in the study.

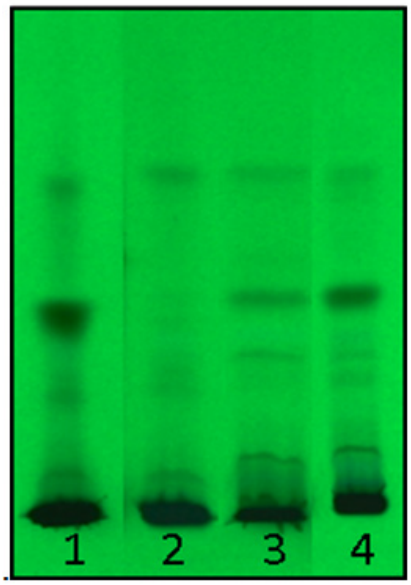


Fig. 1. Chromatogram for HPTLC Fingerprinting for Betalain pigmented compound;1: BVM, 2: BVAA, 3: BVAE ,4: BVAM; Range of R*f* value from 0.05 to 1.0

A phytochemical assay was used to determine the presence of major phenolic compounds, saponins, tannin, flavonoids, and other components in the plant sample.

Alkaloids were detected in all extracts obtained from *Beta vulgaris* L. in relatively low or moderate quantities. Total phenol concentrations were found to be higher in the aqueous ethanol (BVAE), extremely low in the methanol extract (BVM) and moderate in aqueous methanol extract (BVAM). Tannin was observed in moderate or high concentrations in all extract of *Beta vulgaris*. The alkaline reagent test shows a positive result for aqueous ethanol (BVAE), and methanol extract (BVM), and a negative result for aqueous acetone (BVAA) and aqueous methanol (BVAM). The Libermann-Burchard's test indicates the presence of phytosteroids and terpenoids. It shows a positive result for all extract of *Beta vulgaris*. All extracts contained a higher concentration of carbohydrate. The Ninhydrin test shows a moderate positive result (++) for BVAE, a lower positive result (+) for BVAA and BVAM, while a negative result observed for BVM. Fixed Oil was present in less amount among all extracts of *Beta vulgaris*.

• HPTLC fingerprinting analysis of pigments

• Betalain profile

Figure shows the chromatogram, densitogram, and HPTLC fingerprint profile for betalain. HPTLC was performed to analyse the betalain profile, which showed the presence of bands with Rf values between 0.05 and 0.70, 0.05 and 1.00, 0.03 and 1.00, and 0.06 and 1.00, respectively

The betalain compound found such as Betanin, Isobetanin and Vulgaxanthin I were found in different extract of *Beta vulgaris*. The

Table 4. Obtained R*f* value were compared with standard values to check the presence of betalain pigment compound on the basis of R*f* value

No.	Sample code	R*f* values	Presence of betalain compound No. of bands	R*f* value
1	BVM	0.05, 0.07, 0.11, 0.18, 0.23, 0.35, 0.43, 0.58, 0.64,0.700.07	2	0.35, 0.58
2	BVAA	0.05, 0.23, 0.35, 0.45, 0.58, 0.70,0.78,0.89, 0.98	2	0.35, 0.58
3	BVAE	0.03, 0.09, 0.23, 0.35, 0.38, 0.47, 0.58, 0.75, 0.93	3	0.35, 0.38, 0.58
4	BVAM	0.06, 0.09, 0.12, 0.20, 0.26, 0.38, 0.47, 0.58, 0.65, 0.74, 0.84, 0.92, 0.98	2	0.38, 0.58

R*f* value of 0.38 revealed the presence of basic betanin compound in BVAE and BVAM sample. The isobetanin was observed with R*f* value of 0.35. The Vulgaxanthin I showed R*f* value of 0.58 which was found to be common in *Beta vulgaris*.

FTIR spectoscopy

The FTIR spectra obtained for the extract showed all characteristic bands of betalain pigment. The spectrum shows a band at 2992 cm^{-1}, 2912 cm^{-1}, 2915 cm^{-1}, 2993 $cm^{-1}$and 2921 cm^{-1} associated with the stretching of C-H bonds. At 1711 $cm^{-1}$and

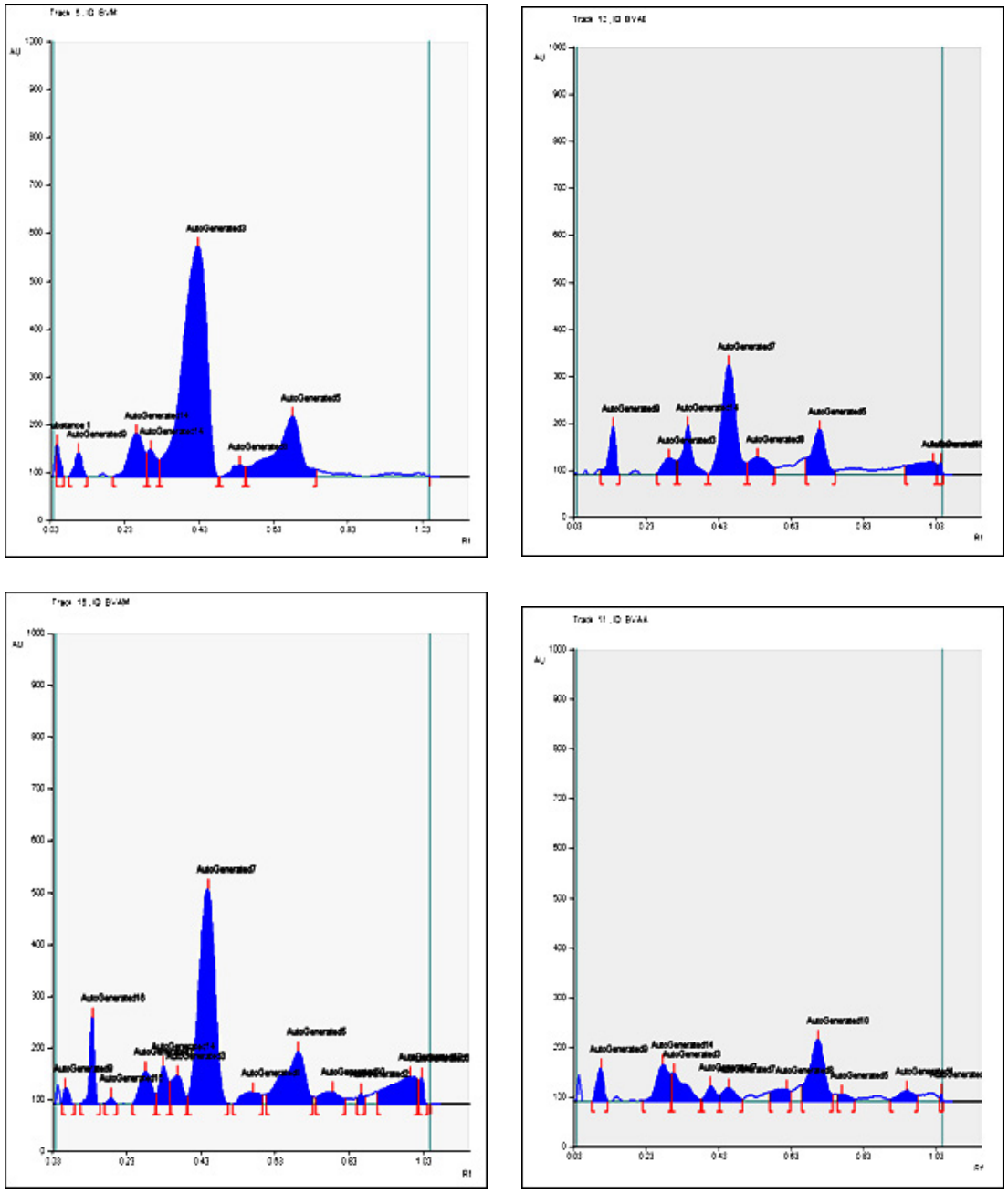


Fig. 2. HPTLC peak densitogram of betalain profile (at 254 nm) of *Beta vulgaris* plant pigment; BVM- Methanol extract of *Beta vulgaris*, , BVAA- Aqueous acetone extract of *Beta vulgaris*, BVAE- Aqueous ethanol extract of *Beta vulgaris*, BVAM- Aqueous methanol extract of *Beta vulgaris*

1706 cm^{-1} was observed a characterstic band of the C=O stretching vibration and at 1416 Cm^{-1}, 1405 $cm^{-1}$and 1403 cm^{-1} were observed a characteristic band of the O-H bending (carboxylic acid group). A band at 1603 cm^{-1}, 1623 cm^{-1}, 1627 cm^{-1} and 1614 cm^{-1}corresponding to C=C stretching. A band at 3294 cm^{-1} and 3267 cm^{-1} corresponding to O-H stretching vibration.

Antioxidant activity

The plant pigments exhibit antioxidant activity, as evidenced by their ability to scavenge DPPH free radicals. Higher concentrations of plant pigments result in greater antioxidant activity. The antioxidant activity of plant pigments vary depending on the specific pigment and concentration tested. The results showed that among the four extract, methanol extracts has the highest % scavenging activity for all the concentration (25, 50, 75 and 100 ìg/ml).

The IC_{50} values have been represented in the table. Graphpad prism software was used to calculate the IC_{50} value. The lowest IC_{50} was shown by the methanol extract and aqueous ethanol extract (45.56 µg/ml and 43.23 µg/ml) thus having the highest antioxidant activity.

The IC_{50} value represents the concentration of the extract required to inhibit 50% of a particular activity in the antioxidant activity. A lower IC50 value indicates a stronger antioxidant activity. When the IC50 value is low, it implies that the compound has a high antioxidant capacity because it can effectively inhibit or reduce the oxidative

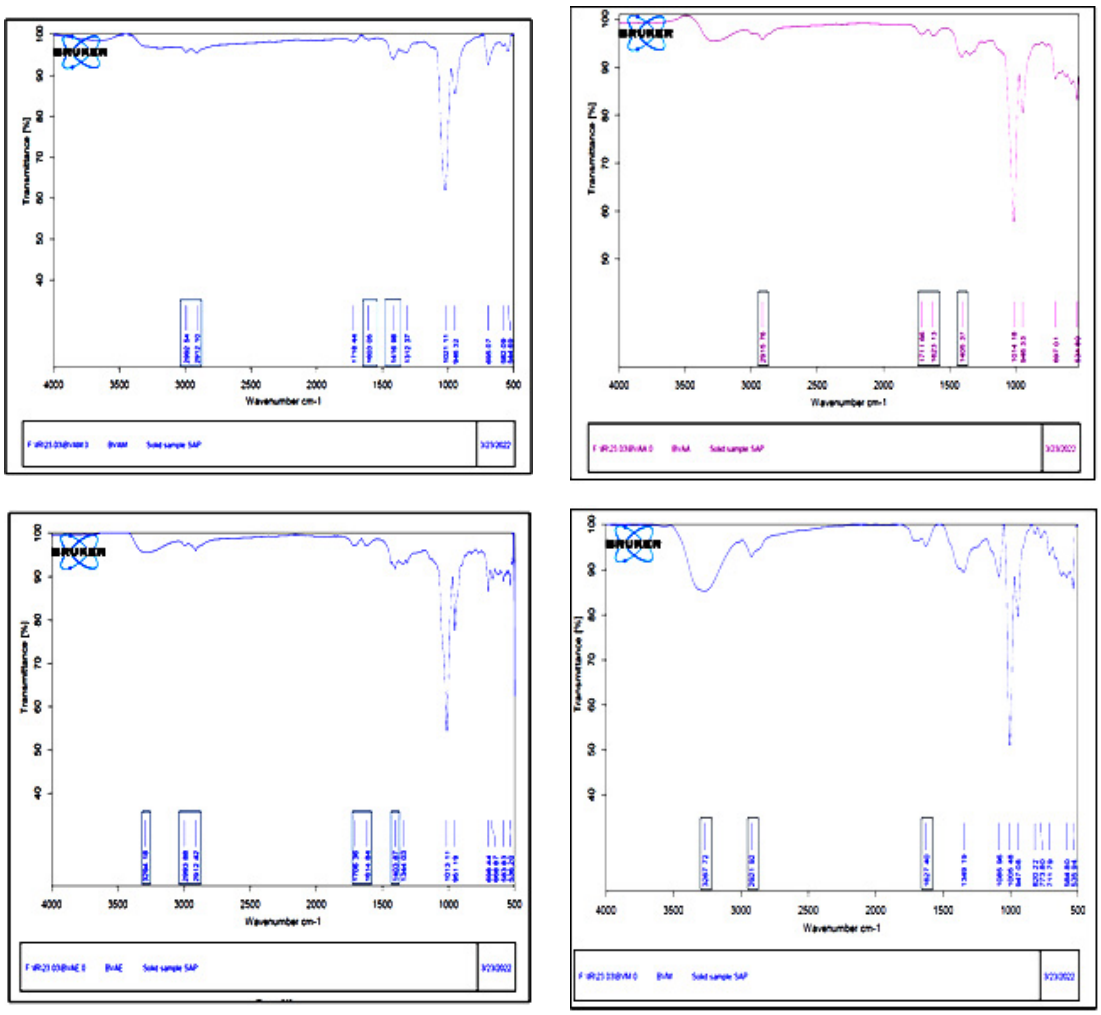


Fig. 3. FTIR spectroscopy of Beta vulgaris (BVAM, BVAA, BVAE and BVM)

damage caused by free radicals. These compounds have a greater ability to donate electrons or hydrogen atoms, thereby neutralizing free radicals and preventing their harmful effects on cells and tissues.

Antibacterial activity

The antibacterial activity of different extract of *Beta vulgaris* was tested against bacterial pathogens such as *Bacillus cereus* (MTCC 736), *Staphylococcus aureus* (MTCC 2408), *Escherichia coli* (MTCC1650) and *Pseudomonas aeruginosa* (MTCC 424). The specific zone of inhibition against various types of pathogenic bacteria was shown in Table.

Among these, the aqueous extract of methanol was found more effective against all selected bacteria. The maximum antibacterial activity of aqueous methanol extract of *Beta vulgaris* was found at 20 mm against *Bacillus cereus* (MTCC 736) and minimum 9 mm against *Staphylococcus aureus* (MTCC 2408). Minimum antibacterial activity was observed in acetone extract of *Beta vulgaris*. This may be the indication of the broad spectrum of antibiotic compounds present in the pigment due to the use of different solvents. The antibacterial activity of pigment varied according to the solvents.

DISCUSSION

Synthetic pigment-based effluents can cause a serious hazard to the water stream and environment due to their synthetic origin and complex molecular structures, which decrease their ability to biodegrade, making them go for alternative sources of safe pigments. The

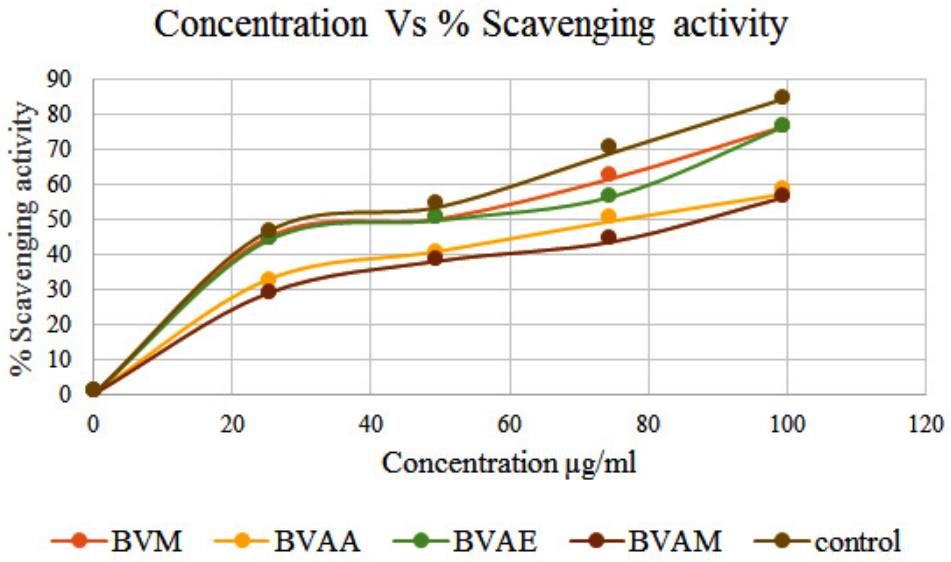
Table 5. FTIR spectrum range of *Beta vulgaris*

Sr. No.	Plant extract	Functional Group	Frequency (cm^{-1})
1	BVAM	C-H alkane (stretching)	2992, 2912 (3000-2840)
		C=C (stretching)	1603 (1650-1600)
		O-H bending (carboxylic acid)	1416 (1440-1395)
2	BVAA	C-H alkane (stretching)	2915 (3000-2840)
		C=O stretching (carboxylic acid)	1711 (1720-1706)
		C=C stretching	1623 (1650-1600)
		O-H bending (carboxylic acid)	1405 (1440-1395)
3	BVAE	O-H stretching (carboxylic acid)	3294 (3300-2500)
		N-H stretching	3294 (3250-3310)
		C-H alkane (stretching)	2993, 2912 (3000-2840)
		C=O stretching (carboxylic acid)	1706 (1720-1706)
		C=C stretching	1614 (1650-1600)
		O-H bending (carboxylic acid)	1403 (1440-1395)
4	BVM	O-H stretching (carboxylic acid)	3267 (3300-2500)
		N-H stretching	3267 (3250-3310)
		C-H alkane (stretching)	2921 (3000-2840)
		C=C stretching	1627 (1650-1600)

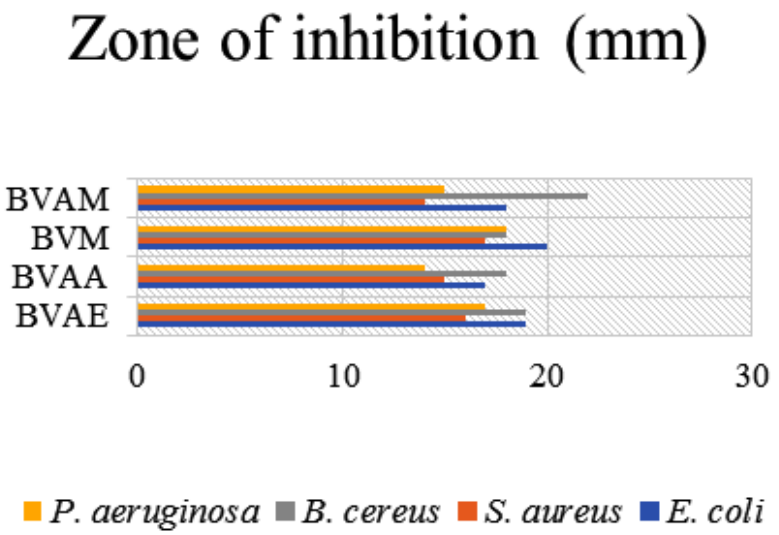
Table 6. Antioxidant activity of plant pigments

	DPPH Scavenging Effect (%), Mean± SD for three replicates					
		Beta vulgaris				
	Extract	BVM	BVAA	BVAE	BVAM	Control
Conc.	25µg/ml	44.75±0.91	32.71±0.85	43.82±0.39	28.80±0.26	46.48
	50µg/ml	50.21±0.17	40.97±0.20	49.78±0.46	38.15±0.30	53.64
	75µg/ml	61.88±0.78	49.64±0.85	56.65±0.32	43.71±0.56	69.22
	100 µg/ml	76.97±0.65	57.43±0.86	76.97±0.46	56.59±0.10	84.85
IC50		45.56 µg/ml	77.40µg/ml	43.23µg/ml	53.92µg/ml	38.54 µg/ml

present study was conducted to develop an eco-friendly biocolour from *Beta vulgaris*. Boles³aw Szadkowski *et al*. (2022) explored the potential of diverse natural lake pigments derived from plant-based natural dyes as environmentally friendly colorants for plastic packaging.[19] The extraction yield of methanol extract of *Beta vulgaris* was the lowest at 30.58%. Aqueous methanol extract had the highest extraction yield of 36.71%. Nawaz *et al*. (2020) described the effect of solvent polarity on extraction yield and antioxidant properties of phytochemical compounds in bean seeds. The results suggested that the extract yield was calculated as total extractable components (TEC) which ranged from 0.24±0.11-7.23±0.47 g/100 g dry wt.[8] Similar research managed by Oderay, C. *et al*. (2018) examined extraction methods for natural dyes (anthocyanin) from blackberries of Castilla



Graph 2. Graphical representation of % scavenging activity and concentration



Graph 3. Graphical representation of zone of inhibition (mm) for *Beta vulgaris*

Table 7. Zone of inhibition of *Beta vulgaris* extract against *Bacillus cereus* (MTCC 736), *Staphylococcus aureus* (MTCC 2408), *Escherichia coli* (MTCC1650), *Pseudomonas aeruginosa* (MTCC 424)

Sample	Zone of inhibition (mm)																			
	Escherichia coli (MTCC1650)				*Pseudomonas aeruginosa* (MTCC 2408)				*Staphylococcus aureus* (MTCC 2408)				*Bacillus cereus* (MTCC 736)			
	2mg	4mg	6mg	8mg	10mg	2mg	4mg	6mg	8mg	10mg	2mg	4mg	6mg	8mg	10mg	2mg	4mg	6mg	8mg	10mg
BVAE	12	13	17	18	19	12	13	15	16	17	12	13	14	15	16	11	12	17	18	19
BVAA	11	12	15	16	17	11	12	12	13	14	11	12	12	14	15	11	11	17	17	18
BVM	13	14	17	19	20	13	15	16	17	18	13	14	15	16	17	13	14	20	21	22
BVAM	10	11	16	17	18	10	11	13	14	15	09	11	12	13	14	12	13	18	19	20
Positive control (chloramphenicol)	18	19	22	24	25	18	20	21	22	23	17	19	20	21	22	18	19	25	26	27

and their application in yoghurt.[7] Support for the current work was obtained from Sturzoiu, A., *et al*., (2011) who had conveyed betanine extraction from *beta vulgaris*-experimental research and statistical modeling. The experimental study showed that aqueous solution of 0.2% citric acid, 0.1% ascorbic acid and 20% aqueous solution of ethanol were best suited for high yield of extraction.[20]

Physicochemical properties of plant extract such as colour and feeling of touch were observed. The phytochemical screening of different extracts of plant samples of *beta vulgaris* revealed the presence of some secondary metabolites such as alkaloids, phenolics, flavonoids, steroids, and terpenoids. HPTLC can be used as a phytochemical marker and is more effective in the field of plant taxonomy for secondary metabolite identification. The HPTLC results determined the presence different types of betaxanthin bands and validated different Rf values ranged from 0.05 to 1.00 (Table 4). The FTIR spectra obtained from the extracted pigment showed all characteristic bands of the selected pigment. The ability of pigments to minimize and scavenge free radicals may be one of the most significant indicators of their antioxidant activity. *Beta vulgaris* may be able to scavenge both hydroxyl and DPPH radicals. Support for the current work was obtained from Guine, R. P. *et al*, (2018) who had reported Extraction of phenolic compounds with antioxidant activity from beetroot (*Beta vulgaris*).[21] In the study conducted by Zakaria *et al*. (2018), the anthocyanin extract from blue pea flowers exhibited significant scavenging activity against DPPH and ABTS radicals. The IC50 values of the trolox standard for both DPPH (IC50 = 3.32 µg/mL) and ABTS assays (IC50 = 6.51 µg/mL) were significantly compared with blue pea flower extract.[22]

CONCLUSION

The plant pigment derived from *Beta vulgaris* exhibits promising antioxidant and antibacterial activities, making it effective potential for developing safer and eco-friendly biocolours. Among the different solvents tested, the methanol extract of *Beta vulgaris* demonstrated the highest efficiency in extracting phytoconstituents due to its polar nature. The results indicate that the methanolic extract possesses significant

antioxidant activity in aqueous systems, making it a valuable natural source of antioxidants for potential use in the food and pharmaceutical industries as a colorant or additive. The study revealed that the plant pigment and isolated compounds display potent antibacterial activity against both Gram-positive and Gram-negative bacteria. These findings highlight the potential of *Beta vulgaris* as a valuable resource for developing antimicrobial agents that could contribute to combating bacterial infections. The use of methanol as the solvent for extraction was found to be appropriate, as it efficiently extracted the beneficial compounds from the plant material.

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Conflict of Interest

There is no conflict of interest.

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