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VARIATION IN REFLECTANCE, ABSORBANCE AND TRANSMITTANCE OF *Beta vulgaris* (BEETROOT) EXTRACT AT ULTRAVIOLET, VISIBLE AND NEAR INFRARED WAVELENGTHS OF SOLAR RADIATION

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Abstract

Variation in the behaviour of the optical properties of Beetroot extract was investigated. The solvent extraction method was adopted in the extraction process and the optical properties of the extract determined using UV- Vis – NIR absorption spectrum at the range 172nm ≤ wavelength ≤ 1100nm. The ranges of the ultraviolet, visible, and infrared regions considered include: 172nm ≤ wavelength ≤ 349nm, 350nm ≤ wavelength ≤ 700nm, and 701nm ≤ wavelength ≤ 1100nm respectively. Results show that the absorption coefficient and transmittance suggests a polynomial regression model. Also, there is a high negative correlation between the absorbance and the transmittance in the visible region, the findings show that beetroot extract is a suitable sensitizer in the bio-electricity application.

Keyword: Chlorophyll, polynomial regression, pigment, absorption coefficient, and carotenoids

Introduction

Natural dyes from leaves, flowers, or fruits are good absorber of light energy from the sun. In nature, some fruits, flowers, and leaves show different colours and have numerous pigments that can easily be extracted (Saelim *et al.,* 2010). Pigments convert and store sun powered energy as synthetic energy in organic compounds. Chlorophylls, carotenoids, flavonoids, and anthocyanins (Merzlyak *et al.*, 2003) are the essential retaining operators in pigments. These pigments comprise an electron donor, and an electron acceptor. The excitation from the contributor to the acceptor shows an intramolecular charge transfer. They stated that reflectance affectability clarifies how sensitive the reflectance is at a particular wavelength for estimating Chlorophyll. Pigments in the leaves and other parts of the plant absorb light at all wavelengths. Some transmit and reflect radiation at certain selected wavelengths and absorb radiation at other wavelengths. Ding *et al*. (2009) stated that different wavelengths have different levels of spectral sensitivity and accuracy for measuring Chlorophyll.

Robson and Aphalo (2019) estimated the vertical transect of spectral irradiance (290-900 nm) propagated through a settled occasional snowpack. The result shows peak transmission of radiation in the UV-A locale in the upper centimeters of the snowpack and decrease in transmittance at longer frequencies. Gorton and Vogelmann (1996) studied the impact of epidermal cell shape and pigmentation on tissue optical properties in the visible and ultraviolet (UV) regions utilizing the Mixfa + and mixfa-lines of Anfirrhinum majus as a model.

Scientists have considered the impact of sunlight based radiation particle on the optical properties of leaves pigments. It was discovered that maximum UV ingestion in the focal region of epidermal cells was marginally more noteworthy in Mixfa+ than mixfa-, and flawless Mixta+ blossoms mirrored less light in the phantom locales with moderate flavonoid absorbance. Merzlyak *et al*. (2002) considered another approach to evaluating optical reflection, retention, and transmission of leaves. The study indicated that, from an optical outlook, leaf tissue is a profoundly dissipating material, and the interminable reflectance of a leaf is exceedingly sensitive to follow measures of absorbing components. Singh *et al*. (2012) explored the impact of ultraviolet-B (UV-B) radiation on two cryptogamic plants (*Xanthoria elegans* and *Bryum argenteum*) developing at a high altitude of the central Himalayan locale of India. Result shows that the maximum average UV-B irradiance was 4.38 Minimal Erythemal Dose per hour (MED/ h) at the experimental site, while the minimum average UV-B irradiance was 1.72 MED/ h.

The UV-B absorbing compounds and phenolics secure these plants against UV-B radiation. Iolanda and Josep (1999) investigated the Claus and Sandor (2012) study on the reflectance spectra and images of green leaves with different tissue structures and chlorophyll content. The results demonstrate that absorption of chlorophylls and carotenoids in the pigment-protein complexes of chloroplasts, size of aerial interspaces between cells, and the structure of the leaf surface determine the leaf reflectance. This study investigates the variance in behaviour of the optical properties of *Beta vulgaris* (beetroot) extracts at the ultraviolet, visible, and near-infrared regions of solar spectra. The response patterns in the absorption coefficient and optical characteristics of each region were also explored.

Materials and Methods

Plant material

Beta vulgaris Linn. (*BV, Chenopodiaceae*), popularly known as *'ichukandarî'* or *'ibeet rootí'*, is an annual herb with erect trunk and tuberous root stocks. It is native to the Mediterranean region and widely cultivated in Europe, America, and all-over India. Several parts of this plant are used in traditional Indian medicine for numerous therapeutic properties. Roots are medicinal, diuretic, and are used in the cure of liver diseases and mental traumas. Leaves are also diuretic, tonic, and useful in alleviating inflammation, paralysis, and diseases of the liver and spleen. The presence of phytochemicals such as beta-lains i.e., *betacyanins* (red-violet pigments) and *betaxanthines* (yellow pigments), *flavonoids, polyphenols*, vitamins, and minerals are found in the leaves. Due to the high nutritional value, the leaves are widely consumed as vegetables worldwide. Beetroot (*Beta vulgaris*) is the major source of natural red dye, often referred to as "beetroot red." *Betanine* is the key constituent of the red colorant extracted from *Beta vulgaris*.

Extraction of beta vulgaris (beetroot) and determination of the optical properties

Beetroot was collected and washed in distilled water to remove impurities and contaminants. It is then blended separately with the addition of 50ml of absolute ethanol as solvent. This is filtered and stored in a closed dish and covered with aluminum foil to prevent contact with sunlight (Senthil *et al.,* 2014). The optical properties of the extracts were determined using the UV-Vis-NIR absorption spectrum within the range 172nm ≤ wavelength ≤ 1100nm with the ultraviolet, visible, and Infrared regions with the ranges 172nm ≤ wavelength ≤ 349nm, 350nm ≤ wavelength ≤ 700nm, and 701nm ≤ wavelength ≤ 1100nm respectively.

Data analysis

The statistical analysis was carried out using the SPSS program (IBM SPSS Statistics version 23). Analysis of variance (ANOVA) was used to ascertain the significance of differences between the optical property levels at different spectrum groups. A one-way between-group analysis of variance was conducted to compare the impact of the ultraviolet, visible, and infrared spectra on the transmittance, absorbance, and reflectance of the extract within these regions. Levene statistics was used to test for the homogeneity of variances, while Games-Howell procedure was used to determine whether there are statistical differences between the groups since the homogeneity of variances was violated. Pearson *r* correlation coefficients were used to determine the degree of relationship among the optical properties at the ultraviolet, visible, and infrared region of the spectra. A polynomial regression analysis was employed to fit the data with an appropriate model. The polynomial regression models consisting of successive power terms have the absorption coefficient as the dependent variable, and the transmittance at different solar radiation region as the independent variable specified implicitly as:

$$Y_i = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \beta_3 X_i^3 + e_i \quad (1)$$

$$Y_i = \beta_0 + \sum_{i=1}^{3} \beta_j X_i^j + e_i \quad (2)$$

Where,

$Y_i =$ *Absorption coefficient*

$\beta_0 =$ *Y – intercept of the regression surface*

$\beta_j =$ *Slope of the regression surface with respect to transmittance at degree j,*

j = 1, 2, ..., k

$X_i^j =$ *Transmittance of radiation region i at degree j*

$e_i =$ *random error term component for radiation region i*

The coefficient of determination (R^2) are given as (Ostertagora, 2012):

$$R^2 = 1 - \frac{SSE}{SST} = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y}_i)^2} \quad (3)$$

$n =$ *number of observations,*

$k =$ *degree of the polynomial,*

$y_i =$ *value of the absorption coefficient Y for radiation region i,*

$\hat{y}_i =$ *is the fitted values of the absorption coefficient Y for radiation region i,*

$\bar{y}_i =$ *is the arithmetic mean of the absorption coefficient Y for radiation region i,*

SST is the total sum of squares and

SSE is the sum of square of the residue

The optical characteristics of pigment at any given wavelength(λ) are related using the equation by Merzlyaketal. (2002)

$$A(\lambda) = \log\frac{1 - R(\lambda)}{T(\lambda)} \quad (4)$$

where $A(\lambda)$, $T(\lambda)$, and $R(\lambda)$ are the absorbance, transmittance and reflectance at a given wavelength λ

Results and Discussion

The descriptive statistics of the optical characteristics at different radiation region is displayed in Table 1. The Table shows that the transmittance gives a better representation of the behavior of the optical properties at the three different regions. The mean value of the absorbance and reflectance are relatively small. Hence, the response pattern of the absorption coefficient and transmittance at each region is considered (Table 7). The degree of relationship among the optical properties in the ultraviolet, visible, and infrared regions are presented in Table 2. The results of the Pearson correlation indicated that there was a strong negative association between the transmittance and the absorbance (r = -0.984, n = 295, p = 0.001), and a significant positive association between the transmittance and the reflectance (r = 0.946, n = 295, p = 0.001), the absorbance and the reflectance has a strong negative correlation (r = -0.989, n = 295, p = 0.001) in the ultraviolet region, while at the visible region, the transmittance and the absorbance has a strong negative correlation (r = -0.942, n = 604, p = 0.001), a significant positive association exists between the transmittance and the reflectance (r = 0.889, n = 604, p = 0.001). The association between the reflectance and absorbance is also significantly negative (r = -0.991, n = 604, p = 0.001). However, there is a strong negative association between the transmittance and the absorbance (r = - 0.852, n = 719, p = 0.001), and a very weak negative association between the absorbance and the reflectance (r = - 0.116, n = 719, p = 0.001), while the relationship between the transmittance and the reflectance is negative but insignificant (r = - 0.421, n = 719, p = 0.001), in the Infrared region. This implies that the infrared region of the spectrum has relatively irrelevant and insignificant contribution in the photovoltaic process. Table 3 shows the one-way analysis of variance for each of the ultraviolet, visible and infrared regions. Result shows a significant effect of the three region at $p < 0.05$ level for the optical properties; [F (2, 879) = 4547.815, p = 0.001], [F (2, 1809) = 1919.244, p = 0.001], and [F (2, 2154) = 7836.705, p = 0.001], respectively. The assumption of homogeneity of variance was violated (Ultraviolet $p < 0.05$, visible $p < 0.05$, and Infrared $p < 0.05$) (Table 4) hence, the welch robust tests of equally of means (Table 5) was considered. The Table shows that there was a significant impact of ultraviolet, visible, and infrared on the optical property; [F (2, 476) = 4038.12, $p < 0.001$], [F (2, 1041) = 2404.0, $p < 0.001$], and [F (2, 1177) = 4236.8, $p < 0.001$]. The location of these differences is evidenced in Table 6. The Table indicated a significant difference among transmittance (M = 24.02, SD = 6.08), absorbance (M = 0.64, SD = 0.13), and reflectance (M = 0.12, SD = 0.07), in the ultraviolet region; transmittance (M = 17.23, SD = 9.44), absorbance (M = 0.86, SD = 0.33), and Reflectance (M = -0.03, SD = 0.24), in the visible region; transmittance (M = 48.90, SD = 14.74), absorbance (M = 0.33, SD = 0.13), and reflectance (M = 0.18, SD = 0.08) in the infrared region. Table 7 shows the polynomial regression model fits and parameter estimate of the effect of transmittance on the absorption coefficient at the ultraviolet, visible and infrared region of the solar radiation. The table suggests that optical properties describes a curvilinear model fits much better than the linear and quadratic models. The cubic model was adopted for the three regions with the coefficient of determination of 1.000, 0.949 and 0.949 in the ultraviolet, visible and infrared regions respectively. Results from this study suggest that the optical characteristics of the extract behave differently in the solar radiation regions which could be attributed to the irregular physiological responses of the plant extract in different regions (Carter and Knapp, 2001). Pigment transmittance in the ultraviolet region is low (Grant *et al*., 2003) hence, a high level of reflectance. This could be responsible for their high negative correlation (Table 2). However, the presence of chlorophylls and carotenoids increases the absorption level in the visible region, which results in low reflectance and transmittance in this region. Previous investigators discovered that the leaf reflectance is low because of the absorbing agents (Gitelson and Merzlyak, 1994). The study also observed that the extract possesses no measurable absorption of sunlight in the near-infrared region; this corresponds to the report of Merzlyak *et al*. (2003).

Conclusion

The variation in behaviour of the optical properties of *Beta vulgaris* (beetroot) extracts at the ultraviolet, visible and near-infrared regions of solar spectra was studied. The response patterns of the absorption coefficient and transmittance at each region are explored. The optical properties of the extract were determined using UV-Vis-NIR absorption spectrum within the range 172nm ≤ wavelength ≤ 1100nm. The ultraviolet, visible, and infrared regions considered are in the ranges 172nm ≤ wavelength ≤ 349nm, 350nm ≤ wavelength ≤ 700nm, and 701nm ≤ wavelength ≤ 1100nm respectively. The outcome of the findings indicates that the absorption coefficient and transmittance describes a polynomial regression model in the three regions. Also, there is a high negative correlation between the absorbance and the transmittance in the visible region. The study also shows that beetroot extract is a suitable sensitizer in the bio-electricity generation.

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Table 1: Descriptive statistics of the optical characteristics distribution at different radiation region

Spectrum		N	Mean	Std Deviation	Minimum	Maximum
	Transmittance	295	24.029094	6.0847539	8.8437	42.7450
Ultraviolet	Absorbance	295	.636694422875591	.131513708362036	.3691146785827351	1.0533659981777195
	Reflectance	295	.123014641531189	.072455782964448	-.1418029981777196	.2034353214172650
	Valid n (list wise)	295				
	Transmittance	604	17.235623	9.4377437	2.4173	35.6540
Visible	Absorbance	604	.860057837367362	.328264451193934	.4478917398520170	1.6166694479541648
	Reflectance	604	-.032414069155441	.241486981453958	-.6408424479541648	.1955682601479829
	Valid n (list wise)	604				
	Transmittance	719	48.905404	14.7352411	.8702	146.5900
Infrared	Absorbance	719	.328151325623484	.134583453971532	-.1661043448416164	2.0603809210433020
	Reflectance	719	.182794629870257	.077622564888368	-1.0690829210433020	.2034898230096971
	Valid n (list wise)	719				

Table 2: Pearson r correlation coefficient of the optical properties at the different regions

SPECTRUM			TRANSMITTANCE	ABSORBANCE	REFLECTANCE
VISIBLE	TRANSMITTANCE	Pearson Correlation	1	-.942**	.889**
		Sig. (2-tailed)		.000	.000
		N	604	604	604
	ABSORBANCE	Pearson Correlation	-.942**	1	-.991**
		Sig. (2-tailed)	.000		.000
		N	604	604	604
	REFLECTANCE	Pearson Correlation	.889**	-.991**	1
		Sig. (2-tailed)	.000	.000	
		N	604	604	604
INFRARED	TRANSMITTANCE	Pearson Correlation	1	-.852**	-.421**
		Sig. (2-tailed)		.000	.000
		N	719	719	719
	ABSORBANCE	Pearson Correlation	-.852**	1	-.116**
		Sig. (2-tailed)	.000		.002
		N	719	719	719
	REFLECTANCE	Pearson Correlation	-.421**	-.116**	1
		Sig. (2-tailed)	.000	.002	
		N	719	719	719
ULTRAVIOLET	TRANSMITTANCE	Pearson Correlation	1	-.984**	.946**
		Sig. (2-tailed)		.000	.000
		N	295	295	295
	ABSORBANCE	Pearson Correlation	-.984**	1	-.989**
		Sig. (2-tailed)	.000		.000
		N	295	295	295
	REFLECTANCE	Pearson Correlation	.946**	-.989**	1
		Sig. (2-tailed)	.000	.000	
		N	295	295	295

**Correlation is significant at the 0.01 level (2-tailed).

Table 3: Analysis of variance

Spectrum		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	114183.414	2	57091.707	1919.294	.000
VISIBLE	Within Groups	53810.884	1809	29.746		
	Total	167994.298	1811			
	Between Groups	109066.818	2	54533.409	4547.815	.000
ULTRAVIOLET	Within Groups	10540.197	879	11.991		
	Total	119607.016	881			
	Between Groups	1134501.318	2	567250.659	7836.705	.000
INFRARED	Within Groups	155914.754	2154	72.384		
	Total	1290416.072	2156			

Table 4:Test of Homogeneity of Variances

Spectrum	Levene Statistic	df1	df2	Sig.
VISIBLE	1109.391	2	1809	.000
ULTRAVIOLET	519.223	2	879	.000
INFRARED	430.464	2	2154	.000

Table 5: Welch Robust Tests of Equality of Means

Spectrum	Statistic[a]	df1	df2	Sig.
INFRARED	4236.827	2	1177.998	.000
ULTRAVIOLET	4038.120	2	476.225	.000
VISIBLE	2404.013	2	1041.177	.000

Table 6: Games-Howell Multiple Comparisons

	(I) SPECTRUM	(J) SPECTRUM	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
VISIBLE	Transmittance	Absorbance	16.3771609163506*	.3842511091133	.000	15.474363777815	17.279958054886
		Reflectance	17.2679164338439*	.3841424318585	.000	16.365372108256	18.170460759432
	Absorbance	Transmittance	-16.3771609163506*	.3842511091133	.000	-17.279958054886	-15.474363777815
		Reflectance	.8907555174932*	.0166498753685	.000	.851680522930	.929830512056
	Reflectance	Transmittance	-17.2679164338439*	.3841424318585	.000	-18.170460759432	-16.365372108256
		Absorbance	-.8907555174932*	.0166498753685	.000	-.929830512056	-.851680522930
ULTRAVIOLET	Transmittance	Absorbance	23.3278294555452*	.3497721511678	.000	22.503879781050	24.151779130040
		Reflectance	23.8426929118018*	.3497144602657	.000	23.018876363360	24.666509460243
	Absorbance	Transmittance	-23.3278294555452*	.3497721511678	.000	-24.151779130040	-22.503879781050
		Reflectance	.5148634562566*	.0087200018809	.000	.494359505945	.535367406568
	Reflectance	Transmittance	-23.8426929118018*	.3497144602657	.000	-24.666509460243	-23.018876363360
		Absorbance	-.5148634562566*	.0087200018809	.000	-.535367406568	-.494359505945
INFRARED	Transmittance	Absorbance	48.5772531250023*	.5495546850262	.000	47.286580652277	49.867925597728
		Reflectance	48.7226098207556*	.5495393892788	.000	47.431972972319	50.013246669192
	Absorbance	Transmittance	-48.5772531250023*	.5495546850262	.000	-49.867925597728	-47.286580652277
		Reflectance	.1453566957532*	.0057940990392	.000	.131759394726	.158953996780
	Reflectance	Transmittance	-48.7226098207556*	.5495393892788	.000	-50.013246669192	-47.431972972319
		Absorbance	-.1453566957532*	.0057940990392	.000	-.158953996780	-.131759394726

*. The mean difference is significant at the 0.05 level

Table 7: Model summary and parameter estimates for transmittance

Spectrum	Equation	R Square	Sig.	Constant	b1	b2	b3
Ultraviolet	Linear	.968	.000	.026	.000		
	Quadratic	.997	.000	.031	-.001	1.053E-5	
	Cubic	1.000	.000	.034	-.001	3.218E-5	-2.972E-7
Visible	Linear	.726	.000	0.16	.000		
	Quadratic	.871	.000	.025	.000	2.161E-6	
	Cubic	.949	.000	.035	-.001	1.067E-5	-4.032E-8
Near-Infrared	Linear	.726	.000	.016	.000		
	Quadratic	.871	.000	.025	.000	2.161E-6	
	Cubic	.949	.000	.035	-.001	1.067E-5	-4.032E-8

Dependent Variable: ABSORPTION COEFFICIENT