Chemistry Africa (2020) 3:449–455
https://doi.org/10.1007/s42250-020-00135-6

ORIGINAL ARTICLE

UV–Vis Spectrometry for Quantitative Study of Tannin and Flavonoid Rich Dyes from Plant Sources

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Received: 18 February 2020 / Accepted: 6 April 2020 / Published online: 25 May 2020

Abstract
The UV–Vis absorbance method is modified as a faster and cheaper means for the study of extraction of natural dyes from plant sources. The method was validated using standard methods of analysis viz: egg albumin precipitation and aluminium colorimetric methods for quantification of tannins and flavonoids respectively. The solvents selected for extraction of dye from plant samples included; distilled water, methanol (5% v/v), ethanol (5% v/v), acidified water pH 3.5 (acidified with acetic acid), and sodium bicarbonate solution (0.1% w/w). Qualitative UV scans were conducted on dye extract of each plant obtained from previously dyed cotton fabrics and wavelengths at maxima for extract of each plant was recorded. In the validation step, data obtained from the modified UV absorbance method were compared to that obtained from standard methods. The modified and validated method was used for the study of extraction output of dye from selected dye-yielding plants using the selected solvents. Extracts from the selected plants registered absorbance maxima at wavelengths unique to each viz: *A. coriaria* (291 nm), *V. paradoxa* (294 nm), *M.lucida* (300 nm) and *H. madagascariensis* (428 nm) these were employed in setting the analytical instrument. Data obtained from the modified method closely correlated to that from egg albumin and aluminium colorimetric methods with $R^2 = 0.989$ and 0.9258 respectively. The modified method registered minimal errors in the range of (0.3–8.7%) for the *A. coriaria* extracts and errors of (2.3–6.7%) for *M. lucida* extracts. The errors recorded from the standard methods were in the range of (2.1–7.9%) and (2.29–6.8%) for egg albumin precipitation and aluminium colorimetric methods respectively. Both the standard and the modified methods exhibited similar accuracy of analysis. Through the application of the modified method, sodium bicarbonate solution (0.1% w/w) was identified to give the best dye extraction output. This modified method gives a faster and cheaper means for quantitative analysis where data is required rapidly for decision making.

Keywords UV–vis absorbance · Egg albumin · Validated · Aluminium colorimetric · Extraction

1 Introduction

Since prehistoric times, man has been involved in the art of dyeing with dyes from natural sources. The dyes from natural sources are mainly obtained from plants, animals, and minerals whose majority is from plants [1]. Various plant parts are used as sources of dyes viz; roots, berries, barks, leaves, seeds, and woods other sources are fungi and lichens [1]. Despite of the historical values attached to the use of natural dyes, the emergence of synthetic dyes in 1856 reduced its prominence mainly because the synthetic dyes had good color fastness, good reproducibility of shades, brilliance of color and they are also easy to use [2]. In spite of the advantages of synthetic dyes over the natural dyes, it has been reported that the use of synthetic dyes are; suspected to cause allergies, carcinogenic and detrimental to human health [3]. This awareness has drawn the attention of various researchers to the application of natural dyes in textile materials [4]. This is because they are biodegradable and eco-safe.

At present, plants are the main sources of natural dyes owing to their availability and diversity. Before their application, the natural dyes from plants have to be extracted.

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Process variables of importance for the efficient extraction and dyeing includes; concentrations of dye source material, extraction time, dyeing time, mordant concentration and pH among others [5]. To optimally derive advantage offered by natural dyes, they have to be extracted eco-efficiently. Several techniques have been improved to efficiently extract dyes from plants namely; ultrasonic, microwave and gamma rays assisted methods [6–8]. Gamma rays treatment of 15 kGy was an effective dose efficiently use for dye extraction and cotton pretreatment [8]. All these methods being developed are to be eco-efficient since the main purpose of extracting dyes from plants is to avoid environmental pollution [9]. Despite all the environmental concerns, the optimal extraction of dyes from plant samples is important in promoting their use since it is a function of dye yield. Commonly occurring dye yielding plants have tannins and flavonoids as the major color-forming compounds. Tannin and flavonoid rich dyes constitute of many phenolic compounds. The phenolic rings absorb UV light and many of them are colored compounds, i.e. show absorption features in the visible region.

These characteristics of dyes make UV–visible spectroscopy the best technique to investigate dye compounds. Ultraviolet and visible spectrometers are instruments whose applications are versatile in analytical chemistry. They are simply fast and generally easy to use and the technique has been in use for the last 35 years. It has currently become the most important analytical instrument in the present day laboratory [10]. Earlier studies have reported standard methods for quantification of total flavonoids and total tannins in plant samples viz: the aluminium colorimetric method [11] and egg albumin precipitation method [12] respectively. These methods employs UV spectrometry however, both methods have lengthy procedures and involve the use of many expensive reagents hence unsuitable for analysis involving many sets of experiments whose results are immediately required as in industrial applications.

Analytical methods used in routine operations like the dye industry is required to be fast, cheap and safe. This requirement is not achieved by the standard methods of analysis which were earlier reported. The present study is aimed at bridging this gap through modification of UV–Vis spectrometry for faster quantitative analysis. This is done by modifying and validating a direct UV absorbance method for application in a study involving the extraction of dyes from tannin and flavonoid rich dye-yielding plant samples. This effort is made because the direct UV absorbance method is simple, fast, accurate, safe and cheap.

2 Experimental Details

2.1 Materials and Equipment Used

Instruments and materials used include; centrifuge, UV spectrophotometer: Genesys 10S UV–Vis, analytical balance, constant temperature water bath and Lab sieve with vibrator. Other materials include glass microfiber filter papers, plain weave grey cotton fabrics acquired from Southern Range Nyanza Textile Mill in Jinja. Some reagents used are; egg albumin powder AR, potassium acetate AR., sodium bicarbonate AR, methanol, ethanol, distilled water and other common laboratory glass wares were used. Stem bark of plants were sampled within Uganda in districts of Mukono, Tororo and Gulu, they were dried in shades and pulverised to fine powder (≤ 715 µm).

2.2 Methods

2.2.1 The Qualitative UV Scan of Crude Dye Extracts from Selected Plants

Crude dye was extracted from each plant by aqueous method as described by Deo and Roshan [13]. Dried and pulverized plant parts (200 g) of *A. coriaria* (stem bark), *V. paradoxa* (stem bark), *M. lucida* (stem bark), and *H. madagascariensis*. (stem bark) were separately soaked in distilled water (500 cm^3) for 30 min. The mixtures were heated at 60 °C for 30 min and temperature was raised to boiling temperature (90 °C) and gently boiled for one hour to yield a dye extract. Distilled water was added to the mixture during boiling to maintain the material to liquor ration. The boiled mixture was made to stand for 30 min at ambient temperature and filtered. The dye solution was preserved for use in subsequent steps.

Before the application of dye on fabrics, they were pretreated as follows; pieces of plain woven cotton fabrics with dimensions of (8×10 cm) each with average weights of 1.4 g were scoured by soaking in a solution of sodium bicarbonate (0.5 gpl) and non-ionic detergent (Tweet®, 2 gpl) at 50 °C for 25 min. The material to liquor (M:L) ratio was maintained at 1:40. The scoured fabrics were thoroughly rinsed with tap water and dried at room temperature. The dye extracts were separately applied on cotton fabrics according to method described by Katy [14]. Samples of cotton fabrics (8×10 cm) were dyed using an open beaker (1000 cm^3). The material to liquor ratio (M: L) was maintained at 1:20 throughout dyeing. The dyed fabrics were removed from dye bath and washed with soap, rinsed with distilled water and dried in open air. The dyes were extracted from dyed fabrics by formic

acid and methanol (5:95 v/v) as earlier reported [15]. This method was modified by scaling up to suit the purpose of the experiment. The dye extracts obtained from fabrics for each plant were filtered through glass microfiber filter. Small fractions of filtrates were transferred in sample cells and qualitative UV scans were conducted on them. Wavelength at the point of maximum absorbance for each sample was recorded.

2.2.2 Determination of UV Absorbance of Dye Extracts Before and After Various Treatments

Two standard methods were used for validating the direct UV absorbance method viz: egg albumin precipitation method for quantification of tannins and aluminium colorimetric method for quantification of flavonoids. The methods were used for a tannin and flavonoid rich dye. Five solvent systems were used in the method validation step they include: distilled water, methanol (5% v/v), ethanol (5% v/v), acidified water pH 3.5 (acidified with acetic acid), and sodium bicarbonate solution (1% w/w).

In the validation step, dyes were extracted from plant samples using each of the selected solvents separately. The dried and pulverized plant samples (0.1 g) of *A. coriaria* and *M. lucida* were transferred to separate glass vessels and a solvent (10 cm^3) added to it and made to soak for 30 min. It was then boiled on a water bath for 20 min and filtered. The filtrate (crude dye liquor) from *A. coriaria* and *M. lucida* plants species were preserved for further treatment.

The Egg Albumin Precipitation Method The crude dye extracts from *A. coriaria* were acidified with acetic acid to pH of 3.5 (optimized pH for protein precipitation) and transferred to a volumetric flask (200 cm^3) and made to the mark. To obtain a suitable dilution, to a fraction of the dye extracts (5.0 cm^3) fresh solvent (15 cm^3) was added. This was done for each solvent system used separately. The UV Absorbance of the dye extract was determined at a predetermined wavelength of 291 nm for *A. coriaria.* The blank used in each case is the respective solvent system used in each extraction. This set was done in triplicate.

To another sample of dye extract (5.0 cm^3), egg albumin solution (100 mg/l, 15 cm^3) was added. A fraction of the mixture was transferred into a suitable glass tube and incubated at 50 °C for one hour and centrifuged at 10,000 rpm for 30 min. The supernatant was transferred to a cell and the UV absorbance determined at 291 nm. The blank used in each case is as in the previous step. All these sets of experiments were done in triplicate.

The Aluminium Colorimetric Method The aluminium colorimetric method as described by Woisky and Salatino [16] was used. In this case only one part of the procedure was adopted. Samples of crude dye extracts (0.5 cm^3) were placed in test tubes and the following solutions were added; aluminium chloride (10%, 0.1 cm^3), potassium acetate (1 M, 0.1 cm^3), methanol (80%, 1.5 cm^3) and distilled water (2.8 cm^3). The blank was prepared using the above reagents but without the extract and the aluminium chloride was replaced with distilled water. The test tubes were incubated at room temperature for 30 min and absorbance was taken at 415 *nm*. These sets were done in triplicates.

2.2.3 Evaluation of Extraction Output of Selected Solvents on the Dye Yielding Plant Species

The modified and validated UV absorbance method was used in the evaluation of the extraction output of dye from various plant samples. Extraction of dyes from the plant samples were conducted under similar experimental conditions. Dried and pulverized plant parts (0.1 g) of *A. coriaria* (stem bark), *V. paradoxa* (stem bark), *M. lucida* (stem bark), and *H. madagascariensis* (stem bark) were separately transferred to glass vessels and solvent (10 cm^3) added to it and made to soak for 30 min. It was then boiled on a water bath for 20 min and filtered. The filtrates (crude dye liquor) were scanned for absorbance. The wavelengths for the absorbance were set at predetermined values for each plant.

These experiments were conducted on all the selected plant species and for each solvent. The sets of experiments were done in triplicate.

3 Results and Discussions

In this study, the direct UV absorbance measurement was modified and validated for the evaluation of extraction output of dyes from selected plants using various solvents for extraction.

3.1 UV Absorbance of Dye Extracts

Most natural dye compounds in plants are phenolic compounds. The ability of the phenolic ring to absorb UV light and the fact that some of the phenolic substances are coloured compounds, i.e. show absorption features in the visible region, make UV–visible spectroscopy a suitable technique to quantify phenolic compounds. Owing to their strong UV–Vis absorbing characteristics, some natural green and red dyes were successfully used as sensitizer source for TiO_2 photoanode based dye sensitized solar cells [17]. As can be noted from Table 1, dye extract from each of the selected plant species absorbs at unique wavelengths. The

Table 1 The wavelengths at maximum UV absorbance for dye extracts from the selected plants

Plant	Wavelength at maxima (λ) nm
A. coriaria	291
V. paradoxa	294
M. lucida	300
H.madagascariensis	428

wavelengths at maximum absorbance for the compounds varied in the range of 291–428 nm. The wavelengths are characteristic of the color forming compounds. These differences show that the color forming compounds from these plants are unique to each. It also provides a means for the determination of the relative quantities of extracted dyes under various conditions by enabling appropriate setting of wavelength on the analytical instrument.

3.2 Correlation of UV Absorbance Values from Modified UV Absorbance Method and the Standard Methods

3.2.1 The Egg Albumin Precipitation Method

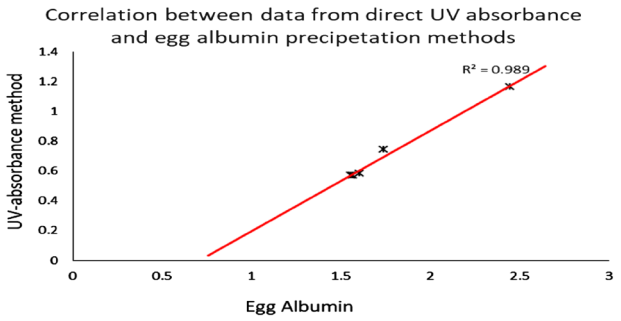
The egg albumin precipitation is a standard method used for the quantification of tannins in samples. It is based on the fact that egg albumin a protein is precipitated by tannins which if in solution, the tannin is removed in the precipitated complex. The precipitation is favored in an acidic pH of 3.5 and a warm temperature. The removal of tannins from a solution results to a decrease in the optical density hence the decrease in its UV absorbance in quantity that is consistent to amount of tannins removed. Used here is a part of the standard method that has been employed as earlier described. In this method, a tannin rich dye plant source *A. coriaria* was used as a dye source and selected solvents were used for extraction of dye from the plant samples. Table 2 contains data obtained from the modified UV absorbance method and the standard egg albumin precipitation method. The numerical values of UV absorbance of dye extracts obtained from the direct measurements were compared to the values obtained from the egg albumin precipitation method.

Data from both methods were analyzed and found to closely correlate and the value of coefficient of determination $R^2 = 0.989$ as can be noted in Graph 1. This demonstrates the strength of the linear relationship of data from both methods. The variance which is 0.011% is due to uncertainty that cannot be explained and no doubt it cannot alter the strong data correlation which is demonstrated. This information provides sufficient evidence that the two

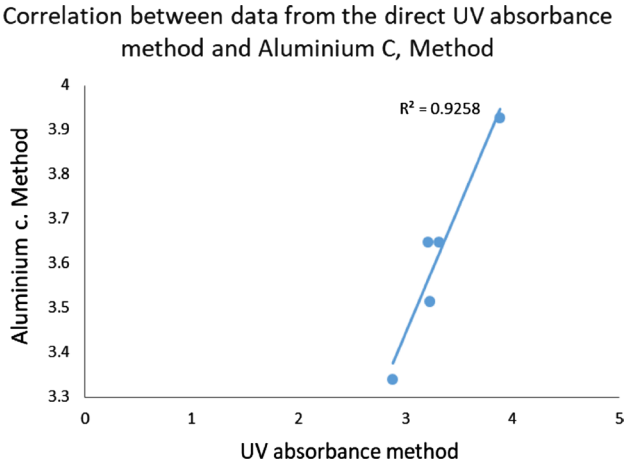
Table 2 UV absorbance and percentage errors registered on dye extracts from the modified method egg albumin precipitation method

Solvent	Analytical method with corresponding absorbance, SD and % error					
	Dye extract			Supernatant from egg albumin treatment		
	A	SD	% error	$(A_0\text{-}A_1)\Delta A$	SD	% error
Distilled water	1.739 ± 0.103	0.056	5.9	0.741 ± 0.059	0.044	7.9
Methanol (5% v/v)	1.566 ± 0.005	0.013	0.32	0.570 ± 0.056	0.003	3.6
Ethanol (5% v/v)	1.606 ± 0.141	0.072	8.7	0.582 ± 0.071	0.085	4.4
Acidified water (pH 3.5)	1.558 ± 0.111	0.027	7.1	0.570 ± 0.063	0.053	4.0
Basic soln. (1%$NaHCO_3$)	2.446 ± 0.111	0.060	4.5	1.164 ± 0.051	0.051	2.1

SD standard deviation, *A* UV absorbance, A_0 absorbance of crude dye extract, A_1 absorbance of supernatant after precipitation with egg albumin



Graph 1 Correlation of data from the Egg albumin and the Direct UV absorbance methods



Graph 2 Correlation of data from the direct UV absorbance and the aluminium colorimetric methods

methods provide consistent result in a study involving extraction outputs of dye from the selected plant.

The simple modified UV absorbance method registered errors in the range of 0.3–8.7% with average standard deviation of below 0.1 for the solvents used. The standard method had errors in the range of 2.1–7.9% with average standard deviation below 0.1. According to values obtained from descriptive statistics, both methods showed minimum variability in the analytical results and all showed similar percentage errors that falls within acceptable limits. The modified method provided results which are consistent and with minimum variability and percentage errors. Therefore, the modified UV–Vis absorbance analytical method has a good degree of accuracy of analysis. From this background information, the simple and fast modified UV–Vis absorbance method can be appropriately used for studying the extraction efficiency of natural dyes from these plants under various conditions.

3.2.2 The Aluminium Colorimetric Method

This is a standard method used for the quantification of flavonoids in samples. It uses standards like pure quercetin to develop calibration curves in the quantification procedure. This method principally uses the formation of stable complexes with the C-4 keto group and alternatively the C-3 or C-5 hydroxyl group of flavones and flavonols in the presence of aluminium chloride**.** The resulting complexes formed are stable in acids with the ortho-dihydroxy groups in either the A-ring or B-ring [18]. This is the principle on which the analytical method is based. The aluminium colorimetric method is adopted for studying relative quantities of flavonoids extracted in various samples in correlation to a modified direct UV absorbance method.

Part of the procedure involving the calibration curve was not used but only the preliminary stages that include the blending of required reagents in appropriate quantities and reading the UV absorbance was used. It is purposely done to study the potential use of the modified direct UV absorbance method in the quantitative study of extraction of flavonoids from plant sources**.**

From the value of R^2, there is a total variation of 0.0742% (the non-correlation) which also remains unexplained and it does not have a significant negative effect on the fact that data from the two methods are strongly correlated. Additionally, analysis of data from the modified method recorded errors of 2.3–6.7% with standard deviation of approximately 0.1. The standard method registered errors of 2.29–6.8% across various solvent systems used and the standard deviations were also approximately 0.1. Basing on these values of descriptive statistics, both methods demonstrated good level of accuracy in measurements. The narrow variability in data obtained proves the ability of the method in the study of extraction of flavonoid rich-dye from plant sources.

Table 3 contains UV absorbance obtained from the modified method and the aluminium colorimetric method. Correlation analysis of the data values gave the value of coefficient of determination $R^2 = 0.9258$ as illustrated in Graph 2.

In both cases, there is demonstrated evidence that, data from the two methods are strongly correlated and had minimal analytical errors. The techniques employed effectively validated the direct UV absorbance method for studies involving extraction output of dyes from tannin rich and flavonoid rich dye yielding plants. The use of the method in studying extraction of dyes from large samples, permit slight errors with no dire consequences. The modified direct UV absorbance method has the advantage that it is fast, simple, straight forward and cost effective. It can permit a study involving rapid and frequent analysis of samples whose results are immediately required for decision making. A typical example of this situation is the optimization study of extraction of dyes from samples where extraction parameters are varied to achieve optimum extraction.

Table 3 UV absorbance values and percentage errors of modified method and aluminium colorimetric method for dye extracts from selected solvents

Solvent	Analytical method with corresponding Absorbance, SD and % error					
	Modified method			Aluminium colorimetric method		
	A	SD	% error	A	SD	% error
Distilled water	2.888 ± 0.190	0.11	6.7	3.338 ± 0.187	0.13	5.60
Methanol (5% v/v)	3.316 ± 0.145	0.08	4.4	3.648 ± 0.248	0.14	6.80
Ethanol (5% v/v)	3.212 ± 0.073	0.04	2.3	3.647 ± 0.160	0.09	4.39
Acidified water (pH 3.5)	3.234 ± 0.116	0.06	3.6	3.513 ± 0.195	0.10	5.59
Basic soln.(1%$NaHCO_3$)	3.892 ± 0.121	0.06	3.1	3.926 ± 0.09	0.05	2.29

SD Standard deviation, *A* UV Absorbance of dye

3.3 Extraction Output of the Selected Solvents

The direct UV absorbance method was exclusively used for the study. Table 4 contains UV absorbance values for extracts from the selected solvents.

The values of absorbance are a function of the quantity of dye extracted from the plant samples. Earlier studies on the efficient extraction of natural dyes from plant samples using various solvents was successfully achieved by the use of UV absorbance technique alongside other analytical techniques [19]. The strength of UV absorbance as analytical technique is improved by its modification to suit interest of a given study. Extraction efficiency is also strongly dependent on the solvent system used and it varies with the nature of dye compounds in the sample. Dye extraction output for a given solvent depends on the solubility of dye compounds in it. In the present study, sodium bicarbonate solution registered a superior extraction output above the other solvents. For several years sodium bicarbonate has been used to fasten the cooking of vegetables. It does this by accelerating the rate of breakdown of pectin. Pectin strengthens plant cell walls and holds the cells together. The bicarbonate is used in very small amount because once started, the breakdown of pectin proceeds on its own. Pectin is largely found in dicotyledonous and non-grass monocotyledonous plants [20]. They are useful for the general growth and development of plants since they make plant tissues rigid [21]. Through the breakdown of pectin, sodium bicarbonate weakens cell walls and makes them more porous for solvents to penetrate into cells. It becomes easy to get out compounds which are inside cells hence the ease of their extraction.

From the values of absorbance recorded in Table 4, sodium bicarbonate solution has the best extraction output of all the solvents. It has further advantages over the other solvents in that it is readily available, cheap and eco-safe and dyes extracted with it can also be used in the food and cosmetic industries. This makes sodium bicarbonate solution a perfect choice for use in the extraction of natural dyes from plants.

4 Conclusions

With some modifications, the direct UV absorbance measurement is an effective method in the quantitative study of the extraction of natural dyes from tannin and flavonoid-rich plant samples. The method is perfectly validated by the egg albumin precipitation and aluminium colorimetric methods for tannin and flavonoid-rich plant sources respectively. Natural dyes from the selected plants absorb UV radiations at wavelengths unique to each which is characteristic of dye compounds in a given plant. This makes the UV–Vis spectrometry suitable for their study. The modified UV–Vis spectrometry can be successfully used to study extraction output of tannin and flavonoid rich dyes from plant sources especially in situations where many samples are handled and results are required rapidly. The extraction of natural dyes from plant samples can be optimally achieved through the

Table 4 UV Absorbance of dye extracts obtained from selected plants using various solvents

Plant species	Absorbance of dye extracts obtained from selected solvents				
	Distilled Water	Methanol (5% v/v)	Ethanol (5% v/v)	Acidified water (pH 3.5)	Basic soln. (1% $NaHCO_3$)
A. coriaria	1.739 ± 0.024	1.566 ± 0.231	1.606 ± 0.015	1.558 ± 0.122	2.446 ± 0.033
V. paradoxa	1.221 ± 0.129	0.665 ± 0.096	0.861 ± 0.455	0.666 ± 0.100	2.346 ± 0.279
M. lucida	2.888 ± 0.062	3.316 ± 0.058	3.212 ± 0.184	3.234 ± 0.063	3.892 ± 0.141
H. madagascariensis	0.039 ± 0.002	0.045 ± 0.006	0.057 ± 0.004	0.024 ± 0.003	2.124 ± 0.007

use of dilute sodium bicarbonate solution which is largely eco-safe and cheap.

Acknowledgements Recognition goes to Simon Peter a lab technician at Kyambogo University for his contribution in running the UV spectrophotometer. In a special way, thanks go to Dr. Justus Kwetegyeka the Dean of Faculty of Science, Kyambogo University for accepting a large part of the study at chemistry lab at Kyambogo. Appreciation goes to the African Development Bank (AfDB) for funding the research project

Funding This research project was supported by the African Development Bank (AfDB) under staff development for Academic staff.

Availability of data All information obtained during the study is available to all who have interest.

Compliance with Ethical Standards

Conflict of interest There is no conflict of interest with respect to the study and the need to publish the work.

References

1. Ferreira ESB, Hulme AN, Macnab H, Ouve A (2004) The natural constituents of historical textile dyes. Chem Soc Rev 33:329–336
2. Anderson B (1971) Creative spinning, weaving and plant dyeing. Angus and Robinson, Singapore, pp 24–28
3. Singh V, Singh RV (2002) Healthy hues, down to earth. Gen Rep 11:25–30
4. Bechtold T (2006) Extraction of natural dyes for textile dyeing from coloured plant wastes released from the food and beverage industry. J Sci Food Agric 86:233–242
5. Pubalina S (2020) A review on application of natural dyes on textile fabrics and its revival strategy. In: Chemistry and technology of natural and synthetic dyes and pigments. 10.5772/intechopen.90038
6. Sheikh J, Jagtap PS, Teli MD (2016) Ultrasound assisted extraction of natural dyes and natural mordants vis a vis dyeing. Fibers Polym 17:738–743
7. Teli MD, Sheikh J, Jagtap PS (2013) Microwave Assisted extraction of natural dyes and natural mordants vis a vis dyeing. Asian Dyer. https://doi.org/10.1007/s12221-016-5031
8. Khan AA, Iqbal N, Adeel S, Azeema M, Batoolal F, Bhatti LA (2014) Extraction of natural dye from red calico leaves: gamma ray assisted improvements in colour strength and fastness properties. Dyes Pigm 103:50–54
9. Geetha B, Judia Harriet Sumathy V (2013) Extraction of natural dyes from plants. IJCPS 1(8):502–509
10. Basic UV-Vis Theory (2000) Concepts and applications. ThermoSpectronic, A Guide, p 1
11. Hatamnia AA, Abbaspour N, Darvishzadeh R (2014) Antioxidant activity and phenolic profile of different parts of Bene (*Pistacia atlantica* subsp. kurdica) fruits. Food Chem 145:306–311
12. Atlabachew M, Chandravanshi BS, Redi M (2014) Selected secondary metabolites and antioxidant activity of Khat (*Catha edulis* Forsk) chewing leaves extract. Int J Food Prop 17(1):45–64
13. Deo HT, Roshan P (2004) Natural dyeing of derim with eco-friendly mordant. ITB Int Textile Bull 5(50):66–70
14. Katy H (1997) Cultivation and Extraction of Natural Dyes for Industrial Use in Textile Production. Bio Mat Net, Inter,AIR 2-CTT94- 80.
15. Zhang X, Laursen RA (2005) Development of Mild extraction methods for the analysis of natural dyes in textiles of historical interest using LC-diode array detector-MS. J Anal Chem 77:2022–2025
16. Woisky R, Salatino A (1998) Analysis of propolis: some parameters and procedures for chemical quality control. J Apic Res 37:99–105
17. Kabir F, Bhuiyan MMH, Manir MS, Rahaman MS, Khan MA, Ikegami T (2019) Development of dye-sensitized solar cell based on combination of natural dyes extracted from Malabar spinach and red spinach. Results Phys 14:102474
18. Chang C, Yang M, Wen H, Chern J (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 10:178–182
19. Zumahi, S. M. Amir-Al, Hossain, Md Kamal, Abser, M., Matin, Rummana, Bashar, M., Ahmed, Farid, Hossain, Md. Abul, (2018). Extraction of different natural Dyes from Flower Plants. International Conference on Recent Advances in Mathematical and Physical Sciences. At: Jahangirnagar University, Savar, Dhaka, Bangladesh. https://doi.org/10.13140/RG.2.2.22055.65443
20. Mohnen D (2008) Pectin structure and biosynthesis. Cur Opin Plant Biol 2008(11):266–277
21. Sila D,N, Van Buggenhout S, Duvetter T, Fraeye I, De Roeck A, Van Loey A, Hendrickx M, (2009). Pectins in processed fruits and vegetables: Part II—Structure–function relationships. *C R F S F S*.