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Extraction And Characterization of Dye From Spinach (Spicia oleracia)

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A B S T R A C T :

This research work aimed at extraction and characterization of dye from spinach (Spinacia oleracea). Fresh leaf of the plant was dried, pulverized, extracted using methanol by maceration method, screened for antimicrobial activity. Infrared spectroscopy techniques and studied its application on cotton fabric. The methanolic extract of spinach leaf indicates that the extract had no activity on the micro-organisms studied at concentration level of 400, 200, and 100 mg/ml. The infrared spectroscopy analysis of methanol extract shows frequency of absorption at peak 3929, 3769, 3617, 3189, 2971, 2636, 2480, 2302, 1608, 1395 and 1069 Cm-1, which correspond to hydroxy, amides, aromatics, alkenes, and ketones respectively. Application of dye from spinach cotton fabric with pre mordanting indicate that K2Cr2O7 yield the best color fastness, while pre mordanting with Aloe vera has the worst color fastness. Pre mordanting with CuSO4 and K2Cr2O7 also perform better than Fe2SO4, while K2Cr2O7 was better than CuSO4. On the other hand, the natural mordant (Aloe vera, lemon juice and Alum) showed significant color fastness on the cotton fabric. Thus, spinach can be used as a good dye.

Keywords: Spinach, Characterization, Maceration method, Antimicrobial activity, Cotton fabric, Extraction.

Introduction

Dye is a natural or synthetic substance used to impart color to, or change the color, of textiles, paper, leather, and other materials, such that the coloring is not readily altered. (Abrahat et al., 2018, Bushra et al., 2018). Natural dyes have become a part of human life since time of immemorial. The Alchemy of color's started its use from an early time (Vankar, 2000). Use of natural dyes in coloration of textile materials and other purpose is just one of the consequences of increased environmental awareness (Gupta et al., 1998). Natural dyes exhibit better biodegradability and generally have a better compatibility with the environment. Also, they possess lower toxicity and allergic reactions than synthetic dyes (Kumar and Bharti, 1998). Today, in the world of growing environmental consciousness, natural colorants have attracted the attention of everyone. Natural dyes used in food are screened for safety but the information is not known for most of the natural dyes used in craft dyeing and with potentially wider use. There is a tendency to assume that consumable natural products are safer and better than synthetic product because they came naturally. The safety of natural dyes needs to be proved if they are used more widely and in commercial process (Kumar, 1998).

With the improvement of living standards, everybody is very much conscious about the environmental protection and health safety. Natural dyes have attracted more attention to the industry due to exhibiting better biodegradability and more compatibility with the environment. Characteristic colors that are gathered from common assets can be categorized as either plant, creature, mineral, or microbial colors and can be used for coloring a wide range of regular filaments (Bharti, 1998). Late examination shows that it can likewise be utilized to color a portion of the manufactured filaments too. Normal colors are not just utilized in the shading of material filaments, they are also utilized for food, prescriptions, handiwork articles, and leather preparing. Extraction and purification play a vital role in the processing of natural dyes. There are different types of extraction process currently available for these natural dyes, such as solvent extraction, aqueous extraction, enzymatic extraction and fermentation, extraction with microwave or ultrasonic energy, supercritical fluid extraction, and alkaline or acid extraction (Mia R et al., 2021).

Spinach (Spinacia oleracea) has been widely grown throughout Nigeria. spinach is one of the most important and nutritious vegetable eaten raw or cooked it provides a very good number of vitamins B6, riboflavin, folate, niacin, soluble dietary fiber, omega 3-fatty acid and minerals. Spinach is also rich with iron; its use prevents some of diseases like osteoporosis, anemia results of iron deficiency (Tahseen and Patricia, 2014). In addition to its food value, spinach has a numeral therapeutic use. Spinach is used for gastrointestinal disorder, blood-generating therapy, growth stimulation in children, appetite stimulation, convalescent support, and fatigue. It has been also suggested to be useful as an anticancer agent, antioxidant (Tahseen and Patricia, 2014), and for cancer prevention. Some fruits, plants, flowers and leaves displayed various colors and contained several pigments, which can be simply extracted and then used as sensitizer because most of green plants contained a lot of chlorophyll, which help in absorbing photon from sunlight, while anthocyanins, which give color to fruits and plant (Chang 2019 and Kumara et al., 2013), tannin and carotene. Chlorophyll can absorb light from red, blue and violet wavelengths and obtains its color by reflecting the green wavelength. chlorophyll extracted from spinach was used as a sensitizer. The influence of different extracts solvent from ethanol and distilled water to extract the chlorophyll pigments from spinach is has been investigated from its absorption spectrum, band gap and also absorbed coefficient. The chlorophylls are one of a number of pigments usually contained in green organelles of higher plants (Khan, 2018). Initially, it was assumed that chlorophyll was a single compound but in 1864 Stokes showed by spectroscopy that chlorophyll was a mixture. If dried leaves are powdered and digested with ethanol after concentration of the solvent, 'crystalline' chlorophyll is obtained, but if ether or aqueous acetone is used instead of ethanol, the product is 'amorphous' chlorophyll (Ramya, 2018).

Statement of the problem

Artificial dyes have been used widely since its inception. They are used in everyday items like clothes, food products, cosmetics, etc. the harmful effects of these dyes, however, go unnoticed by the people. In recent years, due to the scientific development, the adverse effects of these dyes are being discovered, and they have been found to cause or contribute to various allergic reactions, toxicity, mental disorder, tumor formations, etc. Recent studies have shown that many commonly used artificial dyes contribute to cancer, including brain and testicular tumors, colon cancer, and mutations. (Laurel, 2010). An alternative for the synthetic dyes is the natural dyes or pigment extracted from plants such as anthocyanin, carotenoid, chlorophyll and many others. These natural dyes are easily extracted from various parts of plants, such as from the fruits, flowers, leaves, and seeds. Regardless of the limited performance of natural dyes, the natural dyes exhibit advantages, including high absorption coefficients, highlight-harvesting efficiency, inexpensive, ecologically friendly, non-toxic, and are easily extractable. Hence the need to explore the extraction and application natural dye from Spinach.

Aim and Objectives

The aim of this research is extraction and characterization of dye from spinach (*Spinacia Oleracea)*

Specific Objectives

i.To extract natural dye from spinach (Spinacia Oleracea).
ii. To carry out IR analysis using a spectrophotometer.
iii. To carry out Antimicrobial Analysis of the dye extract.
iv. To apply natural dye extracted from spinach and cotton fabric using the following mordants Lemon, $KAl(SO_4)_2$, $CuSO_4$, Fe_2SO_4 and $K_2Cr_2O_7$ and Aloe vera.

Materials and Methods

Materials
The dried leaves of the spinach used for the work was collected from university of Maiduguri Agricultural farm, Borno state. Nigeria.
Apparatus
Apparatus used for the experiments include: Beaker, Conical flask, Funnels, Measuring cylinder, Cotton fabric (White), Thermometer, Stirrer, Nose mask, Lab coat, Water bath, Stove, Kerosene, Spatula, Filter paper, Sample bottles, Glass rod.
Equipment's
Weighing balance, UV-Spectrophotometer and IR spectrophotometer.
Solvents and Reagents
Distilled water, Methanol sodium carbonate.
Natural Mordants
Lemon juice and aloe vera were used as natural mordants. Fresh lemon was purchased from Maiduguri Monday market which were used as natural mordants. Fresh lemon was squeezed and the juice was collected and filtered. Fresh leaves of Aloe Vera were collected and washed thoroughly, the outer green surface was peeled off and the linear white mass was collected and crushed to semi solid consistency 200ml of the aloe vera semi liquid was mixed with 600ml of water and then filtered. (Abdu and Yusuf, 2015).
Synthetic Mordants
Ferrous sulphate, Copper sulphate, Potassium dichromate and Alum (Abdu and Yusuf, 2015).

Methods

Cold Maceration of dye
100g of the sample spinach leaves was added to a beaker (500ml) containing 300ml of distilled water and agitation was done at intervals for 24 hours for complete extraction. The resultant mixture was filtered (Nwonye *et al.,* 2017).
Scouring of the Cotton Fibre
As preparatory processes for dyeing of the cotton fabrics, the desized cotton fabrics samples were washed in a solution containing 0.5g/l of sodium carbonate and 2g/l of detergent at 50°C for 25minutes. The scoured material was thoroughly rinsed with water and dried at room temperature (Abdu & Yusuf, 2015).
Dyeing Procedure
The dyeing of the scoured cotton fabrics was conducted adopting the well-known vat dyeing procedure. The six (6) fabric samples were mordanted with Cupric Sulphate, Ferrous Sulphate, Potassium Dichromate, Alum, Lemon Juice and Aloe Vera respectively (Ashis *et al*., 2011). A separate fabric was also dyed with no mordant.
Pre mordanting for each of the selected synthetic mordants, 2g of the mordant was dissolved in 500ml of distilled water. The scoured cotton sample was heated for 30 minutes at a temperature of 100°C in the mordant solution. The fabrics were dried without washing.
On the other hand, the cotton fabric was pre-mordanted with the natural mordant via the following procedure; 60ml of lemon juice was mixed with 600ml of distilled water, the resulting mordant solution was used to heat the scoured cotton samples for 30 minutes at a temperature of 100°C.
The dried pre-mordanted fabrics were then placed in a 750ml of spinach dyebath, gradually raising the temperature to 100°C and allowed to simmer for 50 minutes. The dyed fabrics were removed and cool washed in a 2g/l detergent solution according to the ISO prescription (Abdu and Yusuf, 2015).

Color Fastness

Fastness to washing: All the dyed fabrics were soaked in laundry soap for 1 h, washed and rinsed with cold water. The fabrics were then allowed to dry at room temperature (Ezeokonkwo *et al.,* 2018).

Fastness to sunlight: The dyed fabrics were exposed to sunlight for 10 h every day for 2 days (Ezeokonkwo *et al.,* 2018).

UV-Visible Analysis

UV visible spectrometry provides a technique that may be used to detect one or more components is a solution and measure the concentration of these species. The primary advantage of this technique is that even traces of substance can be determined in a simple way which is not possible with classical analytical methods like gravimetric and volumetric procedure. In addition, it is used for obtaining structural information of substance particularly organic compound and identification of molecules (D Harvey, 2000).

Infrared Analysis

Infrared spectroscopy is primarily used in industrial and laboratory chemical research. It has wide applications in the control of quality of products during the process of production, dynamic measurement in geological analysis, and monitoring strategies such as the long-term unattended measurement of carbon dioxide concentrations in greenhouses and growth chambers using infrared gas analyzers (Bassan P *et al.,* 2014).

Screening for antimicrobial activity

The leaves extract of spinach was subjected to preliminary antimicrobial evaluation on the mentioned strains using the hole-in-plate disc diffusion technique as described by Forbes et al (21) and adopted by (Usman *et al*., 2009). Wells were bored on the media using 6 mm corn borer and were filled with 0.2 mL aliquots of various concentrations of the extracts (400 mg/ mL, 200 mg/mL and 100 mg/mL equivalent to 40, 20 and 10 mg/hole respectively). The agar plates were then kept in an incubator at 37ºC for 24 hours. After incubation, diameters of inhibition zone for each extract were measured in millimetres using a transparent meter rule. Each extract was tested in triplicates (Usman *et al.,* 2018).

Results and discussion

Results

Extraction Profile of Spinach

One hundred (100) grams of powdered leaf extract of spinach was extracted with 85% methanol by maceration process yielded five grams (5) which is green in color and gummy in texture. The result of the extraction profile is shown in the figure 1

Sample	Mass (mg)	Yield	% Yield	Color	Texture
Spinach (Spinacia Oleracea)	**100**	**5.00**	**10.00**	**Greenish**	**Gummy**

Fig. 1: The Extraction Profile of Methanolic Leaf Extract of Spinach

Infrared Spectroscopy Analysis of Methanol Extract of Spinach

The Infrared spectra of methanol extract of spinach showed frequency of absorption at peak 3929, 3769, 3617, 3189, 2971, 2636, 2480, 2302, 1608, 1395 and 1069 cm^{-1}, which corresponds the functional groups of hydroxy, amides, aromatics, alkanes, alkenes, ketones compounds. The summary of the results is shown in figure 1.

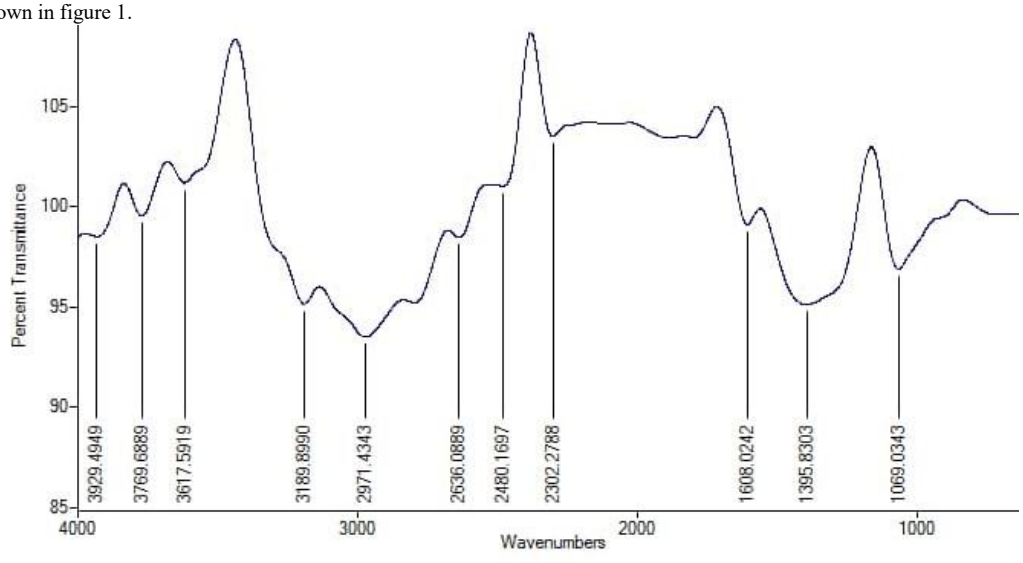

Fig. 2: Infrared Spectra of Methanolic Extract of Spinach

Absorption Frequency (Cm^{-1})	Functional Group	Compound
3929.5	OH	Hydroxy
3769.7	N-H	Amide
3617.6	N-H	Amide
3189.9	C-H	Aromatic
2971.4	C-H	Alkane
2636.1	C-H	Alkene
2480.2	C-H	Alkene
2302.3	C-H	Alkene
1608.0	C=C	Alkene
1395.8	C-H	Alkane
1069.0	C=O	Ketone

Fig.1.2: Infrared spectroscopy of methanol extract of spinach

Antimicrobial Susceptibility Study of Spinach (*Spinaceae Oleraceae*) Methanolic Extract

The antimicrobial susceptibility of spinach extract shows that the plant methanol extract has no activity on the micro-organism studied at concentration levels of 400, 200, and 100 mg/ml. The result of the study is shown in figure 3.

	Concentration (mg/ml) per mean zone of inhibition in diameter (mm)		
S/N organism	400	200	100
1 Staphylococcus aureus	0.00±0.00	0.00±0.00	0.00±0.00
2 Streptococcus pyogenes	0.00±0.00	0.00±0.00	0.00±0.00
3 Bacillus subtills	0.00±0.00	0.00±0.00	0.00±0.00
4 Corynbacteria specie	0.00±0.00	0.00±0.00	0.00±0.00
5 Escherichia coli	0.00±0.00	0.00±0.00	0.00±0.00
6 Klebsiella pneumonia	0.00±0.00	0.00±0.00	0.00±0.00
7 Salmonella typhi	0.00±0.00	0.00±0.00	0.00±0.00
8 Pseudomonas aeroginosa	0.00±0.00	0.00±0.00	0.00±0.00

Key: n = 3

Fig. 4: Antimicrobial Activity of Spinach Methanol Extract Using Disc Diffusion Method

Application of Dye from Cotton Fabric

Figure 5 and 5.1 respectively, shows that application of dye from spinach with pre-mordanting indicate that $K_2Cr_2O_7$ yield the best color fastness, while pre-mordanting with Aloe vera has the worst colure fastness also it may be seeing that pre-mordanting with $CuSO_4$ and $K_2Cr_2O_7$ perform better than Fe_2SO_4, it may be seeing that $K_2Cr_2O_7$ is better than $CuSO_4$. On the other hand, the natural mordant (*Aloe vera,* Lemon juice and Alum) show significant colure fastness on the cotton fabric but aloe vera had the worst performance compared to lemon juice and better than Fe_2SO_4. Finally, the application of spinach dye with no pre-mordanting shows better color fastness than Fe_2SO_4 and *Aloe vera*. Will be interesting to note that, the reason for color variation of dye fabric can be attributed to different chemical reaction between the mordant, the dye and the cotton fabric.

Fig. 5: pictorial representation of dye fabric using synthetic mordants

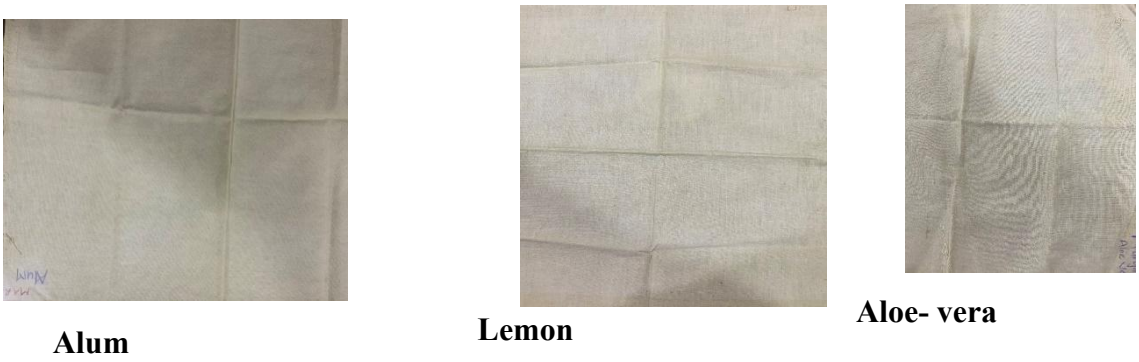

Fig. 5.1: pictorial representation of dye fabric using natural mordants

Discussion

The extract from leaves of spinach found soluble in distilled water and methanol, the dye extracted from leave of spinach showed prominent and lucid colors when applied on cotton fabric (Vankar, 2010). The antimicrobial ability of methanolic spinach extract was tested using disc diffusion method and the extract showed no inhibition to common pathogenic organism (Bushra khan *et al.,* 2018).

The Infrared spectra of methanol extract of spinach showed frequency of absorption at peak 3929, 3769, 3617, 3189, 2971, 2636, 2480, 2302, 1608, 1395 and 1069 cm^{-1}. Which corresponds to hydroxy, amides, aromatics, alkanes, alkenes, ketones functional group of compounds respectively, the functional group of compounds could likely be of phytochemicals which include flavonoids, tannins, alkaloids, terpenoids, phenolics etc., reported by (sowpartharu and Radhika, 2020). Natural dye on the rise ever since the harmful effect of artificial dye have begun to uncover. Presence of important metabolites and high antioxidant activity are an indication of beneficial purpose of dye which serve in food industries. Natural dye can thus, replace artificial dyes in the near future (Bushra Khan *et al*., 2018).

Conclusion

This work shows that the process of extraction of dye was eco-friendly. The use of mordant application is found to be fruitful to improving color adherence and shades of dyes. Spinach leaf is a very good plant that serve as part of raw material for obtaining shades of green dye that can be uses for fabric dyeing in future. The result of this study showed that, spinach does not have antimicrobial property against the tested microorganism and cannot inhibit the growth of enteric pathogens. The study of Infrared spectroscopy showed frequency of absorption which corresponds to functional groups of compounds.

Recommendation

1. The government should sponsor research work on the natural dye exploration as this will help to safeguard our environment from damage host by the use of synthetic dyes.
2. It is recommended that natural dye obtain from spinach leaf should be use for food coloring.

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