International Research Journal of Natural Sciences 12 (1),15-24, 2023
Print ISSN: ISSN 2053-4108(Print)
Online ISSN: ISSN 2053-4116(Online)
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development -UK

Extraction of Dye Pigments from Different Endemic Plant Samples Using Various Extraction Methods

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doi: https://doi.org/10.37745/irjns.13/vol12n11524 Published October 06, 2024

Citation: Adjero, L.A., Ezejiofor, T.I.N., Udebuani, A.C., Duru, C.M., and Nwachukwu, M.O. (2024) Extraction of Dye Pigments from Different Endemic Plant Samples Using Various Extraction Methods, *International Research Journal of Natural Sciences*, 12 (1),15-24

Abstract: *The study was carried out to explore the potentials of selected endemic plant extracts as alternative counter stain in bilological studies. Selected endemic plant samples were Rothmannia whitfieldiiand Penthacletra macrophylla, Lonchocarpus cyanescens Curcuma longa and Duranta repens. Preliminary qualitative and quantitative Phytochemical Screening Extraction were carried out using well-established laboratory protocols using different extraction techniques. The natural dye was extracted from the plant materials using Soxhlet, Cold water, Hot water and Ethanolic extraction methods and assayed on cytological, histological and mycological specimen. Results obtained showed that the extraction time varied with an increase in temperature and pH of plant yielding dyes. From all the solvents used for the extractions of dye extract from Lanchocarpus cyanesces, Duranta repens, Pentaclethra macrophylla and Curcuma longa), Soxhlet extraction method at 56.8 ^{O}C, 56.8 ^{O}C, 56.8 ^{0}C, 57.1, 56.8 and for two hours thirty minutes (2.30min) was observed to be the best solvent while 1% NaoH at 43.7 ^{O}C for less than an hour (0.45min) proves to be the best solvent for the extraction of Rothmania whitfieldii dye extract. The Cold-water extraction method is less effective to extract dye from the plant species as far as the time needed for extraction is concerned. Further study on the effects of the extracted dyes from Lanchocarpus cyanesces, Duranta repens, Pentaclethra macrophylla and Curcuma longa on cytological, histological and mycological specimen is recommended. The study has demonstrated the potential use of dyes from endemic plants as a suitable substitute for staining microorganisms in a very simple, cheap, easy and readily available procedure*

Keywords: Extraction, dye, endemic plant, *Lanchocarpus cyanesces, Duranta repens, Pentaclethra macrophylla* and *Curcuma longa* extraction methods

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INTRODUCTION

Dyes are referred to as substances natural or synthetic that have the ability to alter at least temporarily the crystal structure of the substrate to which it is applied, thereby conferring colour to it (Bafana, Devi, & Chakrabarti, 2011). Dyes are composed of chromophores and auxichromes. Chromophores impart colour and have major functional groups as Azo, anthroquinone, nitro and others, while auxichromes are colour intensifiers with substituents capable of donating or withdrawing hydroxyl electrons. Major compounds found in auxichromes are amines, carboxyls, sulfonates (Onuegbu, Nnorom, & Onyedika, 2023; Pangarthen, De-Carvalho, Araujo, Pinto, Borges, Souza, & Kuriyama, 2002).

There are two known sources of dyes; natural sources and synthetic sources. Natural sources are plant parts-roots, barks, leaves, flowers, berries, while animal sources are fungi, lichens, invertebrates providing dyes such as woad, indigo, saffron, madder (Pangarthen et al., 2002). Synthetic dyes are chemically produced dyes like fuchins, safaranin, and induline (Hunger, 2003).

It is estimated that over 10,000 different dyes and pigments are used industrially and over 7×10^5 tons of synthetic dyes are annually produced worldwide (Javid & Manoj, 2020). Dyes have been widely employed in textiles, pharmaceuticals, food, cosmetics, plastics, tannery, photographic paper, laboratory staining/diagnoses and other products making them colourful, pleasant, desirable and optically distinct (Pangarthen et al., 2002). Dye production and usage even at artisanal levels have brought about chemical technologies which have created employment, promoted trade and improved income (Nieto-Galan, 2001; Vital, Saibaba, & Shaik, 2016).

Recent study has shown, that dye production and usage, generates 1 trillion dollars, with over 35 million workers worldwide and 70% of the total world exports (Desore & Narulg, 2018). Apart from its economic advantages, dyes have contributed immensely in biological researches and diagnoses in medicine through enhanced contrast in images leading to proper differentiation of structures and forms (Bhatia, 2017). Ithas aided histological and histopathological diagnosis, leading to a better understanding of the disease patterns and treatment therein.

From time to time there are adjustments in people's expectations and lifestyles. Recently there is a growing concern on the significance of biodiversity's protection and sustainability (Carvalho & Santos, 2016). Development more than before is being planned to reverse natural resource destruction and conserve a healthy environment. Exploiting and confirming the potentials of plant dyes in staining could be a promising alternative to synthetic dyes. This could lead to the development of effective plant-based dyes, such dyes would be quite affordable, eco-friendly to combat the rising cases of health and environmental issues due to the use of synthetic dyes. Nigeria is blessed with a great and very rich diversity of plants capable of yielding dyes. This has been exploited by indigenous dyers majorly in the textile and recently cosmetic industries (Chukwu, Odu, Chukwu, Haftz, Chidozie, & Onyimba, 2011); Mohammad et al., 2016). There is paucity of information on the dye yielding potentials of plants in Nigeria, their best extraction methods, as well as their staining abilities.

MATERIALS AND METHODS

Study Area

The study was carried out at the Teaching and Research Laboratory of the Department of Biology, Federal University of Technology Owerri located in Owerri Zone lying on coordinates 5°28'3.59"N, 7°02'06.0E on a land spanning over 550 km^2 and comprising of three Local Government areas of the twenty-seven in Imo State, namely Owerri West, Owerri municipal and Owerri North (Okere, Abu, & Ndukwu, 2018).

Collection of Plant Samples

Plant samples were collected from different parts of Imo State; Akabor (Mbaise) (Ahiazu Mbaise Local Government area), Ihiagwa, and Obinze (Owerri West Local Government area) all in Imo state. *Rothmannia whitfieldii*and *Penthacletra macrophylla* were collected from Akabor in Mbaise, *Lonchocarpus cyanescens* and *Duranta repens* from FUTO campus, while the rhizome of *Curcuma longa* wasprocured from Ihiagwa market. Field location and character was captured using the Global Positioning System (GPS). The habit and morphological features of the specimens were captured using FUJI film digital camera, Fine pix S4250. The collected plant samples were taken to a taxonomist in the Department of Biology, Federal University of Technology owerri for proper identification and classification. Then, the samples were labeled appropriately and analyzed at the laboratories of the Departments of Biological science, Science Lab technology (SLT), and Crop science, Federal University of Technology, Owerri. The Location Name and Geographic Coordinates of plant sampling areas is shown in Table 1.

Table 1: Location Name and Geographic Coordinates of plant sampling areas

S/N	Name Of Location	Longitude	Latitude
1	Ahiazu Mbaise	5.7070° N	6.7909° E
2	Ihiagwa	6.4002^{0}N	4.5370^{0}E
3	Obinze	4.8396° N	6.9112° E

Sample Preparation

The methodology of Adeyemo et al., (2017) was used with slight modification. The leaves of *Lonchocarpus cyanescens* and *Durunta repens*, the rhizome of *Curcuma longa*, pods of *Rothmannia whitfieldii* and *Pentacrethra macrophylla* were washed with distilled water and air-dried at room temperature and triturated with a grinder. For *Rothmannia whitfieldii*, the pulp and seed were removed from the dried fruit and pulverized equally, the dry cover of *Pentaclethra macrophylla* was also be removed, washed, dried and pulverised. The powdered plant material was sieved to an average size of 100µm using a sieve with an aperture size of 100µm.

Extraction of Plant Material

The extraction of the five (5) different indigenous plant material was carried out using different extraction techniques as follows:

Extraction of rhizomes of *Curcuma longa* (Turmeric Rhizome)

1. Soxhlet Extraction

The methodology as described by Azmir et al., (2013) with slight modification was used. Twenty grams (20g) of the triturated rhizome of *Curcuma longa* was weighed in an electronic weighing balance and poured into a thimble, 70% ethanol was measured and transferred into a round bottom flask as the extraction solvent. Then, the Soxhlet apparatus was set-up and the reflex was run for 8-10 hours. Upon completion of extraction, the solvent was evaporated using a rotary evaporator to obtain a crude extract and further dried in an oven at 60^0c to obtain the stain powder. The crude powder was transferred into a screw-capped bijou bottle, corked tightly, labeled appropriately, and was kept in the refrigerator at 4^0C for further purification.

Maceration Extraction

The method of Majekodunmi, (2015) was used. Twenty grams (20g) of turmeric powder were measured using electronic weighing balance into three different flasks. 100ml of ethanol at 50%, 70%, and 99.9% were added into each of the three different flasks and label appropriately according to the percentage of ethanol used and the mixtures were shaken vigorously. Then, the mouth of each flask was stoppered with a ball of cotton wool wrapped with aluminum foil and was allowed to stand in the dark for two days (48 h) at room temperature. Then each of the mixture was filtered through a muslin cloth and then the filtrate obtained was filtered with Whatman No.1 filter paper and the resulting filtrate was subjected to evaporation using a rotary evaporator to obtain a crude extract, which was then be transferred into a reagent bottle, labeled and stored in the refrigerator at 4^0C for further purification.

Extraction of leaves of *Duranta repens*

Aqueous Extraction

This will be achieved using both hot and cold aqueous extraction

A. Hot Aqueous Extraction

Hot aqueous extraction was carried out according to the procedure described by Tandon & Rane (2008). 20g of the pulverized leaf of *Duranta repens* was weighed and steeped in 200ml of boiled distilled water contained in a flask whose mouth was stoppered with a cotton wool wrap with aluminum foil. The mixture was shaken and allowed to stand for 24 hrs. Then the resulting solution was filtered through a muslin cloth and the resulting filtrate obtained was filtered again using Whatman No. 1 filter paper and the filtrate obtained was subjected to evaporation using a rotary evaporator to obtain a crude extract, which was then be transferred into a reagent bottle, labeled and stored in the refrigerator at 4^0C for further purification.

B. Cold Aqueous Extraction

Cold aqueous extraction was carried out according to the procedure described by Handa, (2008). 20g of the pulverized leaf of *Duranta repens* was weighed and steeped in 200ml of cold distilled water contained in a flask whose mouth was stoppered with a cotton wool wrap with aluminum foil. The mixture was shaken and allowed to stand for 24 hrs. Then the resulting solution was filtered through a muslin cloth and the resulting filtrate obtained was filtered again using Whatman No. 1 filter paper and the filtrate obtained was subjected to evaporation using a rotary evaporator to obtain a crude extract, which was then be transferred into a reagent bottle, labeled and stored in the refrigerator at 4^0C for further purification.

Alcoholic Extraction

Alcoholic extraction was carried out according to the procedure described by Hafiz et al. (2012). 20g of the powdered plant material of *D. repens* leaf was soaked in 200ml of absolute ethanol and the mixture was shaken vigorously and allowed to stand undisturbed for 24 hours, The mixture was filtered through a muslin cloth and then the filtrate obtained was filtered again using Whatman No.1 filter paper and the resulting filtrate was subjected to evaporation using a rotary evaporator to obtain a crude extract, which was transferred into a reagent bottle and labeled and stored in the refrigerator at 4^0C for further purification.

Extraction of pods of *Rothmannia whitfieldii* (Uri)

Aqueous Extraction

This method was carried out according to the procedure described by Nnorom et al. (2018): using beakers containing mixtures of *Rothmannia whitfieldii* with distilled water as a solvent for 45 minutes at room temperature of 27°C and 65°C in each case for all the extracts.

For extraction at room temperature, 1g of the milled dried pods of *Rothmannia whitfieldii* was measured out into 100ml beaker after which, 50ml distilled water was added to it and stirred thoroughly. The solution was allowed to stand for 45minutes before carefully draining the supernatant liquid into a sample test bottle using a white synthetic fabric as the sieve. The solvent was evaporated using a rotary evaporator to obtain a crude extract that was placed inside a bijou bottle and label before storing it in a refrigerator.

To extract at 65°C, 50ml of distilled water was poured into a 100ml beaker, which was placed in a water bath with a temperature set at 65°C. When the distilled water temperature got to 65°C, 1g of the pulverized plant material was added to it and stir. The solution was allowed for 45minutes while maintaining the temperature at 65°C before draining off the liquid into a sample test bottle using a white synthetic fabric as the sieve. The solvent will be evaporated using a rotary evaporator to obtain a crude extract which was placed inside a bijou bottle and labeled before storing it in a refrigerator for further use.

Alkali Extraction

Alkali extraction was achieved using 1% NaOH as described by Nnorom et al. (2018). using beakers containing mixtures of *Rothmannia whitfieldii* with 1% NaOH as a solvent for 45 minutes at room temperature of 27°C and 65°C in each case for all the extracts.

For extraction at room temperature, 1g of the milled dried pods of *Rothmannia whitfieldii* was measured out into 100ml beaker after which, 50ml of 1% NaOH was added to it and stirred thoroughly. The solution was allowed to stand for 45minutes before carefully draining the supernatant liquid into a sample test bottle using a white synthetic fabric as the sieve. The solvent was evaporated using a rotary evaporator to obtain a crude extract that was placed inside a bijou bottle and label before storing it in a refrigerator at 4^0C.

To extract at 65°C, 50ml of 1% NaOH was poured into a 100ml beaker, which was placed in a water bath with a temperature set at 65°C. When the temperature of 1% NaOH got to 65°C, 1g of the pulverized plant material was added to it and stir. The solution was allowed for 45minutes while maintaining the temperature at 65°C before draining off the liquid into a sample test bottle using a white synthetic fabric as the sieve. The solvent was evaporated using a rotary evaporator to obtain a crude extract which was placed inside a bijou bottle and labeled before storing it in a refrigerator at 4^0C for further use.

Extraction of leaves of *Lonchocarpus cyanescens*

Soxhlet extraction

Twenty grams (20g) of the pulverized leaf of *L. cyanescens* was weighed in an electronic weighing balance and poured into a thimble and 200ml of absolute ethanol (99.9%) was measured into a round bottom flask as the solvent of extraction as described by Azmir *et al*., (2013). Then the Soxhlet apparatus was set-up and the extraction experiment was carried out within 8-10 hours. Upon completion of extraction, the solvent was evaporated with a rotary evaporator leaving behind a crude extract. The crude extract was placed in a screw-capped bijou bottle, cork tightly, label appropriately, and kept in the refrigerator for further purification.

Aqueous Extraction

In aqueous extraction of *L. cyanescens*leaf, 20g of the pulverized plant material was measured out into two flasks, 200ml of distilled water and absolute ethanol was measured out and was added into the flask separately and labeled accordingly, after which the mouth of the flask will be stoppered with a ball of cotton wool wrapped with aluminum foil. The mixture was shaken vigorously and allowed to stand for 24 hours as described by Mansour, (2018). The supernatant of each flask was drained into a new flask with muslin cloth as a filter. The filtrate was subjected to evaporation using a rotary evaporator and after evaporation, the crude extract was stored inside a bijou bottle in a refrigerator for further purification.

Extraction of seeds of *Pentaclathra macrophylla*

Soxhlet extraction

Twenty grams (20g) of the pulverized seeds of *P macrophylla* was weighed in an electronic weighing balance and poured into a thimble and 200ml of absolute ethanol (99.9%) was measured into a round bottom flask as the solvent of extraction as described by Azmir *et al*., (2013). Then the Soxhlet apparatus was set-up and the extraction experiment was be carried out within 8-10 hours. Upon completion of extraction, the solvent was be evaporated with a rotary evaporator leaving behind a crude extract. The crude extract was

placed in a screw-capped bijou bottle, cork tightly, label appropriately, and kept in the refrigerator for further purification.

Aqueous Extraction

In aqueous extraction of *P macrophylla* seeds, 20g of the pulverized plant material was be measured out into two flasks, 200ml of distilled water and absolute ethanol will be measured out and was be added into the flask separately and labeled accordingly, after which the mouth of the flask was be stoppered with a ball of cotton wool wrapped with aluminum foil. The mixture was be shaken vigorously and allowed to stand for 24 hours as described by Mansour, (2018). The supernatant of each flask was be drained into a new flask with muslin cloth as a filter. The filtrate was subjected to evaporation using a rotary evaporator and after evaporation, the crude extract was be stored inside a bijou bottle in a refrigerator for further purification.

Fresh Sequeezes Extracts

Fresh sequeezes of each of the plant materials was be extracted by pulverizing each sample when harvested fresh using clean motar and pestle, after which the pulverized samples was be squeezed in a musclin cloth to remove debris. The filtrate obtained was poured into a clean airtight container and the fresh extracts was stored in the refrigerator at 4^0C for further purification.

Statistical Analysis

The generated data was subjected to statistical analysis using appropriate statistical tools such as Statistical Package for Social Sciences software application.

RESULTS AND DISCUSSION

RESULTS

Methods and time of extraction of plant yielding dyes with their temperature and pH values

Methods and time of extraction of plant yielding dyes (*Lanchocarpus cyanesces, Duranta repens, Pentaclethra macrophylla* and *Curcuma longa*) along with their corresponding Temperature and pH values is presented in Table 1. Results obtained showed that the extraction time varied with an increase in temperature and pH of plant yielding dyes. The highest extraction time for *Lanchocarpus cyanesces* was recorded to be 2.30 hrs at 56.8^0C while the least yield was gotten within 24 hrs each using Cold water, Hot water and Ethanolic methods of extraction respectively. The results further showed that soxlet extraction method took a longer time (2.30hrs) with respect to *Pentaclethra macrophylla, Curcuma longa, Rothmania whitfieldii* compared to other methods of extraction used for the study.

From all the solvents used for the extractions of dye extract from *Lanchocarpus cyanesces, Duranta repens, Pentaclethra macrophylla* and *Curcuma longa*), Soxhlet method of extraction at 56.8 ^{O}C, 56.8 ^{0}C, 56.8 ^{0}C, 57.1, 56.8 and for two hours thirty minutes (2.30min) was observed to be the best solvent while 1% NaoH at 43.7 ^{O}C for less than an hour (0.45min) proves to be the best solvent for the extraction of *Rothmania whitfieldii* dye extract.

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Table 1: Methods and time of extraction of plant yielding dyes with their temperature and pH values

Plant materials	Methods of extraction	Quantity (g)	Temperature (^{o}C)	pH	Extraction time (Hrs)
Lanchocarpus cyanesces	Soxhlet	100	56.8	5.0	2.30
	Cold water	100	38.5	4.2	24
	Hot water	100	100	4.3	24
	Ethanolic	100	49.6	4.3	24
Duranta repens	Soxhlet	100	56.4	4.1	2.30
	Cold water	100	38.0	4.2	24
	Hot water	100	100	4.3	24
	Ethanolic	100	48.7	4.3	24
Pentaclethra macrophylla	Soxhlet	100	57.1	4.2	2.30
	Cold water	100	39.0	4.1	24
	Hot water	100	100	4.3	24
	Ethanolic	100	49.3	5.0	24
Curcuma longa	Soxhlet	100	56.8	4.1	2.30
	Cold water	100	38.1	4.2	24
	Hot water	100	100	5.0	24
	Ethanolic	100	50.0	4.2	24
Rothmania whitfieldii	Cold water	100	38.3	4.2	24
	Hot water	100	100	4.3	24
	1% NaoH	100	43.7	8.4	0.45

DISCUSSION

The study established variation in methods of extraction (Soxhlet, Cold water, Hot water, Ethanolic) with respect to Temperature (^{o} C), pH, and Extraction time (Hrs). The optimal extraction temperature in the present study for solvents used in the extraction was 100 °C for Hot water solvent extraction methods for *Lanchocarpus cyanesces Duranta repens, Pentaclethra macrophylla, Curcuma longa, Rothmania whitfieldii was* within twenty for hours (24hrs). An initial increase up to the optimum temperature could be due to the disruption of plant tissues' cell walls, which makes the dissolution of solutes easy, but a further increase in temperature beyond the optimum limit possibly degrades the phytochemicals, as natural compounds are temperature sensitive. At higher temperatures, the yields of the resulting extracts decreased and began to rise back up at 56.8 °C. This decrease could be because the phytochemicals substance in the plant leaves is vulnerable to heat. According to Li, Yin, Li, Huang, Ye, & Zhang (2014), the properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ration, the extraction temperature and the extraction duration will affect the extraction efficiency. Thus, the optimum temperature of the solvent in the plant species extraction was 100 ^{o}C. This is in the range of the findings of Vajed, et al. (2024) and Rahman, et al. (2018) who reported optimal extraction temperature of 85 °C and 90 ^{0}C respectively.

From the study, it can be deduced that maximum dye contents are extracted in acidic medium at pH 4.3. A study by Yusuf, Shabbir, & Mohammad (2017) reported optimum pH of 4.5, which is line with the findings in this study. In a similar study, Albuquerque, Prieto, Barreiro, Rodrigues, Curran, Barros, & Ferreira (2020) reported a pH of 4.8 in their study on Catechin-based extract optimization obtained from *Arbutus unedo* L. fruits using different extraction techniques, which aligns with the findings in this study. In a related study, Vajed, Mohammadi & Parham, (2024) also reported pH range of 4.2 to 6.4, which aligns with the findings in this study. All extracts had acidic pH. Extracts with acidic pH have affinity for cytoplasm, which is usually basic in nature. As opined by Ragaswami and Bagyaraj, (2021), extracts act as counter stains and not primary stains, which are basic, and so has affinity for the nucleus because of the nuleic acid. Phenols are weak acids as these are having –OH groups in their structure. At higher pH values these phenolic contents of extract transforms into their anionic forms and equilibrium yield lowers with an increase in pH value (Uslu & Bamu, 2016).

CONCLUSION

Natural dyes were successfully extracted from the plant species using several solvents. Dye extract from the plants was extracted most by water as a solvent. The Cold-water extraction method is less effective to extract dye from the plant species as far as the time needed for extraction is concerned.

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