



Phytochemical Profile of *Rothmannia Whitfieldii* and *Pentaclethra Macrophylla*, *Lonchocarpus Cyanescens*, *Curcuma Longa* and *Duranta Repens* from Different Endemic Plant Species in South East Nigeria.

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ABSTRACT

The study was carried out to explore the potentials of selected endemic plant extracts as alternative counter stain in biological studies. Selected endemic plant samples were *Rothmannia whitfieldii* and *Pentaclethra 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. Results obtained phytochemical analysis showed the presence or presence of bioactive compounds such as Alkaloids, Flavonoids, Saponins, Tannins, Phenols, Gluco, Terpenoid, steroid in the plant extracts. Terpenoids and steroids were present in *Pentaclethra macrophylla* while Alkaloid was present in all the plant extracts assayed; flavonoids, saponins, and phenols were present in *Rowthmania whitfieldii* Seed, *Lonchocarpus cyanescens* and *Rowthmania whitfieldii* peel respectively. *Pentaclethra macrophylla* extracted with absolute ethanol yielded the highest Alkaloids with a mean value of 15.83 ± 1.77 while the least value of Alkaloids were obtained from *Rowthmania whitfieldii* Seed, Rw (Sh) with a mean value of 0.63 ± 0.11 . Results from the quantitative analysis of the plant extracts further showed that Gluco (mg/g) yielded the highest value of phytochemicals with a mean value of 412 ± 41.54 while the least phytochemical was obtained from *Rowthmania whitfieldii* with a mean value of 22 ± 2.82 .

Introduction

The discovery of synthetic dyes in the late nineteenth century brought the collapse of the use of natural dyes from plants, animals, and minerals in the staining of textile, food, cosmetics, and paint industries (Carvatho & Santos, 2016). Synthetic dyes were able to fix up the age-long problems of the complexity of dye processing, reproducibility results, limited shades, blending, and inadequate fastness posed by natural dyes (Church, 2014). Many brilliant colors formed through different chemical combinations emerged. People's appetite for them increased and more were produced. Malik et al, (2014) reported that many chemicals used in these industries causes environmental and health problems. About 40% of globally used dyes contain organically bound chlorine, a known carcinogen (Malik et al., 2014). These chemicals are discharged as waste into the environment, evaporated into the air we breathe or absorbed through our skin. Because of this chemical pollution, normal functioning of cells is disturbed, thus affecting the physiological and biochemical mechanisms in animals, causing impairment of important functions like respiration, osmoregulation, reproduction, anaphylactic reaction, and even mortality. They also may contain heavy metals that are non-biodegradable, which are capable of accumulating in primary organs of organisms and canverse through the ecosystem, affecting both aquatic and terrestrial lives adversely (Dapson & Dapson, 2005).

Synthetic dyes are problematic despite their undeniable importance, this is because the families of chemical compounds that make good dyes are also toxic to organisms and affect the environment generally (Bhatia, 2017; Muthu, 2017). Oxides of nitrogen, sulphur and volatile organic compounds released from dyes among the list of air pollutants (Bhatia, 2017). Studies have shown that discharge of untreated dye effluents from industries associated with dyes into the aquatic environment causes aesthetic damage to water bodies due to colouration (Setiadi, Partwi, & Widjarsa, 2006), prevents light penetration through water which affects aquatic photosynthetic activities and dissolve oxygen levels (Imran, Crowley, Khalid, Hussain, Mumtaz, & Arshad, 2015; Hassan & Carr, 2006).

Substances released into the environment, bioaccumulates and biomagnifies thereby causing ecological problems such as pollution of the environment, alteration of ecological niche, loss of biodiversity, extinction of important flora and fauna etc (Sandhya, 2010; Newman, 2015).

Dyes used in biological studies are known as stains. Staining makes biological tissues optically distinct (Tifford, 2009; Prescott, Harley, & Klein 2009). It increases visibility, accentuates morphological features, fixes and same times preserve these specimens for further studies (Sandhya, 2010), thereby aiding histological studies in identification of tumour and cancerous cells. Other studies include, bioassay study of nematodes and nucleic acids (Ghosh,

Panda, Rath, Pal, Sharma, & Das, 2015) and microbial staining (Adevemo, Olukemi, Amodu, & Olayemi 2018; Braide, Akobundu, Nwaoguikpe, & Njiribarko, 2011).

Currently in use are synthetic dyes of petroleum origin (Hunger, 2003). They are majorly compounds containing Azo groups, the largest group of colorants constituting 60-70% organic dyes produced in the world (Adevemo et al., 2011). They are made of two cleared products, benzidine which induces various human and animal tumors and P-phenylenediamine a contact allergen (Chun, 2016). Most synthetic dyes are known carcinogenic, mutagenic and disease-causing agents in animals and humans (Bhuyan, Singh, & Bhuyan, 2013). Triple primary cancers involving skin, kidney, urinary bladder and liver of workers have been associated with dye stains (Aquino, Rocha-Filho, Ruotolo, Bocchi, & Biaggio, 2014), allergies such as contact dermatitis, rhinitis and conjunctivitis have also been reported, (Hunger, 2003). More so, the effects of dye on terrestrial ecosystem have been well documented. Dyes reduce soil microbial diversity, seed germination and plant growth (Imran et al., 2015; Rahman et al., 2018). Crystal violet dye is a known mitotic poison promoting fish tumour (Ali, Shehata, & Ramadan, 2016). High risk of liver disorder, nerve damage and life-threatening cancers were reported in exposure to Sudan dye (Fonovich, 2012; Alim-un-Nisa, Naseem, & Yasha, 2016). Congo red, aniline and fuchisin are known carcinogens and mutagens (Dapson & Dapson, 2005). Azure-B intercalates with the helical structure of DNA (Hunger, 2003). The deleterious effect of dyes on man and his environment has pushed countries that were the first producers of synthetic dye to ban their manufacturing and use of synthetic dyes (Priyadarsini, 2014).

The current era of sustainable development, environmental protection and conservation have created the awareness that the necessities of human beings are not met with food, clothing and shelter alone, but the presence of pure and safe air, and water in a clean and loveable environment. The need for re-introduction of natural dyes is of utmost importance. This has aroused more interest in the search for alternative natural dyes for staining microbial cells, tissues, food samples and other materials which will be relatively cheaper, eco-friendly and biodegradable. The extraction of natural dyes from plants and animals stands as an alternative to replacing synthetic dyes whose deleterious and hazardous effects to living things and the environment have been receiving increased global awareness targeted to reducing their effects (Ali, et al., 2016).

The extraction, characterization and evaluation of the staining potentials of dye extracts from parts of these plants, *Rothmania whitfieldii*, *Duranta repens*, *Penthacletra macrophylla*, *Lonchocarpus cyanescens*, and *Curcuma longa* will add to the scarce knowledge on indigenous plant species and staining potentials needed to curb the menace of synthetic dyes.

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 km² 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* was procured 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 3.1.

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°N	4.5370°E
3	Obinze	4.8396° N	6.9112° E

Preliminary Qualitative Phytochemical Screening/Analysis

The preliminary qualitative phytochemical screening was carried out according to the method described by Harbone (1998), Parekh and Chanda (2007). The extracts from the different samples was assessed for the presence/absence of the following phytochemicals parameter: saponins, flavonoids, alkaloids, tannins, and cardiac glycosides.

Test for Saponins (Foam test): Fifty (50) mg of the extract was diluted with distilled water and made up to 20 mL. The suspension is shaken in a graduated cylinder for 15 minutes. Saponins are detected by the formation of a two-cm layer of foam.

Test for Tannins (Ferric chloride test): The extract (50 mg) was dissolved in 5 mL of distilled water. A few drops of neutral 5% ferric chloride solution are then added. Dark green colour indicates the detection of tannins.

Test for Phenols: The extract (50 mg) was dissolved in distilled water. Then 3 mL of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Test for Flavonoids

Alkaline reagent test: Extract was combined with a few drops of sodium hydroxide solution. The appearance of intense yellow colour, which turns colourless on the addition of dilute acid, signifies the presence of flavonoids.

Shinoda's test: One mL of the extract was added with 0.5 mL of hydrochloric acid and magnesium metal. The presence of flavonoids is confirmed by reddish coloration.

Test for Triterpenoids: The different extracts will be treated with chloroform and then filtered and added with a few drops of conc. sulphuric acid (H₂SO₄). It is then shaken and allowed to stand. The appearance of golden yellow colour or red-brown colour indicates the presence of triterpenes.

Test for Fixed oils and fats (Spot test): A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils

Test for Proteins: The extracts (100 mg) was dissolved in 10 mL of distilled water and filtered through Whatman No. 1 filter paper. The filtrate is subjected to test for proteins.

Millon's test: To 2 mL of filtrate, a few drops of Millon's reagent (metallic mercury in nitric acid) is added. A flesh to red precipitate indicates the presence of proteins.

Xanthoproteic test: The extract is treated with concentrated nitric acid and observed. A positive result is indicated by yellow precipitate formation.

Test for Glycosides: Fifty (50) mg of the extract will be hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered, and the hydrolysate is subjected to the following:

Legal's test: 50 mg of the extract was dissolved in pyridine and then sodium nitroprusside solution is added. The solution is made alkaline using 10% NaOH. Pink colour means glycosides are detected.

Borntrager's test: To 2 ml of filtered hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. A pink colour indicates the presence of glycosides.

Test for Carbohydrates

Molish's test: To 2 mL of extract, two drops of alcoholic solution of α -naphthol was added. The mixture is shaken well and a few drops of concentrated sulphuric acid are added slowly along the sides of the test tube. The appearance of the violet ring at the junction means carbohydrates are detected.

Test for Terpenoids: 2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water bath and then boiled with 3 ml of concentrated H₂SO₄. A grey color is formed which showed the entity of terpenoids.

Test for Steroids: 2 ml of chloroform and concentrated H₂SO₄ was added with the 5 ml aqueous plant crude extract. In the lower chloroform layer, red color appeared that indicate the presence of steroids

Test for gum and Mucilages: The extract (100 mg) was dissolved in 10 ml of distilled water and to this 2 ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of Gums and Mucilages.

Statistical Analysis

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

Results and discussion

Qualitative Phytochemical Properties of the Sample Extracts

Results of qualitative analysis of plant extracts (v.) used for the study is shown in Table 1. Results obtained showed the presence or presence of bioactive compounds such as Alkaloids, Flavonoids, Saponins, Tannins, Phenols, Gluco, Terpenoid, steroid in the plant extracts. Terpenoids and steroids were highly present in *Pentaclethra macrophylla* while Alkaloid was present in all the plant extracts assayed; flavonoids, saponins, and phenols were moderately present in *Rowthmania whitfieldii* Seed, *Lonchocarpus cyanescens* and *Rowthmania whitfieldii* Sheel respectively.

Table 1: Qualitative Phytochemical Properties of the Sample Extracts

Sample Extracts	Phytochemical contents									
	Alkaloids			Flavonoids	Saponins	Tannins	Phenols	Gluco	Terpenoid	steroid
	M	W	P							
Cp _{Ab} ethanol	+	+	+	++	++	+	+	-	+	++
Cp _{Aq}	+	+	+	+	++	-	+	-	+	++
Cp _{HotH2O}	+	+	+	+	+	-	+	-	+	++
Cl _{Ab} ethanol	+	+	+	+	+	+	+	-	+	++
Cl _{Aq}	+	+	+	++	++	-	+	-	+	++
Cl _{hot H2O}	+	+	+	+	++	-	+	-	+	++
Pm _{Ab} ethanol	+	+	+	+	-	+	++	-	+++	+++
Pm _{Aq}	+	+	+	++	+	-	+	-	-	+
Pm _{hotH2O}	+	+	+	++	+	-	+	++	-	+
Dr _{Sox}	+	+	+	++	-	+	+	-	+	++
Dr _{Ab}	+	+	+	+	-	-	+	-	+	+
Dr _{Aq}	+	+	+	+	+	-	+	-	+	++
Lc _{Sox}	+	+	+	+++	+	+	++	-	+	++
Lc _{Ab}	+	+	+	+	-	-	+	-	+	++
Lc _{Aq}	+	+	+	+	+++	-	++	-	+	++
Rw(S) _{NaOH}	+	+	+	+	-	+	+	-	+++	+
Rw(S) _{Aq}	+	+	+	+	+	-	+	++	-	+
Rw(Sh) _{NaOH}	+	+	+	++	+	-	+	++	-	+
Rw(Sh) _{Aq}	+	+	+	+	+	-	+	-	+	+
Rw(Mix) _{NaOH}	+	+	+	+	-	-	+	-	+	++
Rw (Mix) _{Aq}	+	+	+	+	+	-	+	+	+	++

Legend: +++ = highly present, ++ = Moderately present, + = Present, - = Absent

Cp = *Curcuma longa* Peeled, Cl = *Curcuma longa* unpeeled, Pm = *Pentclethra macrophylla*, Dr = *Duranta repens*, Lc = *Lonchocarpus cyanescens*, Rw (S) = *Rowthmania whitfieldii* Seed, Rw (Sh) = *Rowthmania whitfieldii* Sheel, Sox = Soxhlet extract, Ab = Absolute ethanolic extract, Aq = Aqueous extract

Quantitative Phytochemical contents of the Sample Extracts Used

Mean concentrations of the quantitative phytochemical constituents of the plant extracts (*Curcuma longa*, *Pentclethra macrophylla*, *Duranta repens*, *Lonchocarpus cyanescens*, and *Rowthmania whitfieldii*) used for study is presented Table 2. Results obtained showed the presence of phytoconstituents such as Alkaloid (%), Flavonoids (%), Phenols (mg/g), Gluco (mg/g), Terpenoid (mg/g) and Steroid (%). *Pentclethra macrophylla* extracted with absolute ethanol yielded the highest Alkaloids with a mean value of 15.83 ± 1.77 while the least value of Alkaloids were obtained from *Rowthmania whitfieldii*

Seed, Rw (Sh) with a mean value of 0.63 ± 0.11 . Similarly, Flavonoids (%) content of *Pentclethra macrophylla* extracted with absolute ethanol yielded the highest phytoconstituents with a mean value of 10.21 ± 1.23 (%) while the least value was gotten from *Duranta repens* with a mean value of (1.13 ± 0.01) . *Pentclethra macrophylla* quantitatively yielded the highest value of phenols with 356 ± 39.41 while *Duranta repens* recorded the least concentration with a mean value of 11.42 ± 1.18 . Results from the quantitative analysis of the plant extracts further showed that Gluco (mg/g) yielded the highest value of phytochemicals with a mean value of 412 ± 41.54 while the least phytochemical was obtained from *Rowthmania whitfieldii* with a mean value of 22 ± 2.82 .

Table 2 Quantitative Phytochemical contents of the Sample Extracts Used

Sample Extracts	Phytochemical contents					
	Alkaloid (%)	Flavonoids (%)	Phenols(mg/g)	Gluco (mg/g)	Terpenoid (mg/g)	Steroid (%)
Cp_{Ab} ethanol by sox	15.83 ± 1.77	10.21 ± 1.23	39.34 ± 4.52	153 ± 23.12	54.95 ± 9.06	53 ± 8.43
Cp_{Aq}	8.55 ± 0.22	6.18 ± 0.03	26.11 ± 2.43	234 ± 30.47	30.24 ± 5.23	2.7 ± 0.13
Cp_{hotH2O}	4.43 ± 0.14	2.55 ± 0.05	13.42 ± 0.23	87 ± 16.28	17.19 ± 1.97	0.7 ± 0.05
Cl_{Ab} ethanol by sox	13.66 ± 1.43	2.61 ± 0.07	37.34 ± 3.52	173 ± 21.11	14.75 ± 1.64	34.56 ± 2.83
Cl_{Aq}	7.44 ± 0.38	4.45 ± 0.13	24.61 ± 2.21	284 ± 35.28	10.27 ± 1.03	2.14 ± 0.07
Cl_{hot H2O}	5.43 ± 0.24	3.88 ± 0.12	13.72 ± 1.47	89 ± 14.24	7.55 ± 0.82	0.5 ± 0.02
Pm_{Ab} ethanol by sox	5.73 ± 0.26	3.36 ± 0.10	356 ± 39.41	257 ± 29.32	173 ± 27.17	4.63 ± 0.14
Pm_{Aq}	3.53 ± 0.12	1.68 ± 0.01	98 ± 19.82	382 ± 40.01	243 ± 37.08	1.28 ± 0.13
Pm_{hotH2O}	4.43 ± 0.23	2.46 ± 0.04	168 ± 24.88	257 ± 32.09	173 ± 25.62	4.63 ± 0.24
Dr_{Sox}	21.23 ± 1.81	10.21 ± 0.43	42.32 ± 4.79	153 ± 27.10	54.95 ± 7.21	123 ± 21.42
Dr_{Ab}	9.75 ± 0.42	6.18 ± 0.03	26.11 ± 2.93	234 ± 24.73	33.21 ± 2.29	1.54 ± 0.12
Dr_{Aq}	4.53 ± 0.25	1.13 ± 0.01	11.42 ± 1.18	127 ± 23.25	18.19 ± 1.24	1.35 ± 0.11
Lc_{Sox}	4.53 ± 0.25	3.76 ± 0.11	276 ± 35.27	357 ± 36.12	166 ± 17.54	5.65 ± 0.33
Lc_{Ab}	3.73 ± 0.15	2.78 ± 0.08	158 ± 24.12	412 ± 41.54	233 ± 24.17	1.78 ± 0.13
Lc_{Aq}	4.63 ± 0.24	2.86 ± 0.09	248 ± 32.42	257 ± 32.32	153 ± 23.12	4.87 ± 0.26
Rw(S)_{NaOH}	0.83 ± 0.09	2.36 ± 0.03	67 ± 17.39	37 ± 3.23	13 ± 1.47	0.63 ± 0.11
Rw(S)_{Aq}	1.63 ± 0.14	1.58 ± 0.01	98 ± 19.82	32 ± 3.07	43 ± 3.93	0.28 ± 0.06
Rw(Sh)_{NaOH}	2.63 ± 0.31	2.33 ± 0.03	122 ± 28.05	137 ± 23.53	123 ± 22.27	3.27 ± 0.10
Rw(Sh)_{Aq}	0.63 ± 0.11	1.36 ± 0.03	37 ± 6.24	27 ± 3.03	13 ± 1.47	0.55 ± 0.07
Rw(Mix)_{NaOH}	1.52 ± 0.13	1.13 ± 0.01	48 ± 5.17	22 ± 2.82	23 ± 2.11	0.22 ± 0.03
Rw (Mix)_{Aq}	3.63 ± 0.19	1.26 ± 0.01	142 ± 23.23	231 ± 23.71	153 ± 23.12	2.87 ± 0.34

Discussion

Plant produce different chemical compounds or phytochemical which have been used in a wide range of commercial, Medicinal and industrial applications. The results of comparative preliminary phytochemical screening of the plant species used in this study (*Curcuma longa*, *Pentclethra macrophylla*, *Duranta repens*, *Lonchocarpus cyanescens*, and *Rowthmania whitfieldii*) revealed the presence of several groups of secondary metabolites such as Alkaloids, Flavonoids, Saponins, Tannins Phenols, Gluco-Terpenoid and steroids present in the plants. This aligns with the results of Onuegbu, et al. (2023) and Agidew et al. (2020).

The result obtained from the preliminary qualitative phytochemical screening of different solvent extracts of *Curcuma longa* Linn peel showed similar results in studies conducted Phytochemical Composition and Toxicological Profiling of *Curcuma longa* (Turmeric) Root Extract in Rats which reported cardiac glycosides, flavanoids, saponins, tannins and alkaloids (John, Kalu, Christopher, & Amarachi, 2024). It has been established that the presence of numerous phytochemical constituents in plants provide versatile biological properties for multipurpose utilization such as treating ailments, cosmetics, flavours and dyeing potentials. The absence of alkaloids in the leaves of *Duranta repens* is not consistent with earlier reports by Agidew et al. (2020) that detected an appreciable level of alkaloids in *Duranta repens* leaves. Saponins are known to reduce surface tension and this property enhances staining (Chukwu, Odu, Chukwu, Hafiz, Chidozie & Onyimba (2019). In fact the various phytochemical constituents portray *Curcuma longa*, *Pentclethra macrophylla*, *Duranta repens*, *Lonchocarpus cyanescens*, and *Rowthmania whitfieldii* plant extracts as a successful potential natural dye.

According to Boroushaki et al. (2016) Curcumin (Diferuloylmethane), the main yellow bioactive component of *Curcuma longa* has been shown to have a wide spectrum of biological actions - anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive and hypocholesteremic activities. (Hill et al. 2007; Navarrete, Pizzi, Pasch, & Rode, 2013). Since long turmeric or *C. longa* is valued for its principle coloring constituent curcumin, which imparts yellow color to textile fibers and food. The ability of a dye to stain specific tissue structures is determined by certain factors, one of which is the acidity of the stain. Acidic structures would be stained by basic dyes while basic structures would be stained by acidic dyes. Owing to the strong affinity of *C. longa* for the cytoplasm, it can be deduced that the *C. longa* extract dye is acidic in nature (Ghosh, Panda, Rath, Pal, Sharma, & Das, 2015). This deduction is corroborated by the phytochemical analysis of the active column fraction. It contained flavonoids, which are typically polyphenolic compounds (Mahomoodally et al. 2005; Mahomoodally, Gurib-Fakim, & Subraty 2005).

The quantitative analysis of the plant species used in this study, revealed methanol extract having a higher percentage of the secondary metabolites than the aqueous extract. It was observed that Phenols were the highest percentage found in the Methanol extract, however, this is different from similar studies which showed Flavonoids and saponins as the highest proportion of phytochemicals (Dicenso et al., 2020; Sapna, Dhruv, & Harmeet, 2019). The variation observed may be due to the difference in plants under study. With respect to the result of the study, the antimicrobial activities of the dye extract is recommended for further consideration.

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