# EXTRACTION AND CHARACTERIZATION OF NATURAL DYES FROM *ANTHOCLEISTA VOGELLI, JUSTICIA CARNEA* AND *MANGIFERA INDICA*

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## ABSTRACT

The extraction of natural dyes from *Anthocleista vogelii, Justicia carnea* and *Mangifera indica* are reported. The optimization of the extraction process was evaluated using acetone, ethanol, and water, pressurized liquid extraction and Soxhlet extraction. The optimal natural dye extraction was observed with acetone using pressurized liquid extraction for 1 h. The extracted natural dyes were isolated using column chromatography and were characterized by HPLC. The *Anthocleista vogelii* HPLC test for alkaloids revealed the presence of Hordenine, Cytisine and Galanthamine; *Anthocleista vogelii* HPLC test for flavoids revealed the presence of Benzoic acid, Isoquercetin, Rutin and Chlorogenic acid; *Anthocleista vogelii* HPLC test for phenol revealed no compound. *Justicia carnea* HPLC test for alkaloids revealed the presence of Hordenine, Cytisine, Methyl Jasmonate and Galanthamine; *Justicia carnea* HPLC test for flavonoids revealed the presence of Benzoic acid, Isoquercetin, Rutin, Hesperidin, Chlorogenic acid and Ellagic acid; *Justicia carnea* HPLC test for phenols revealed the presence of Ferulic acid. *Mangifera indica* HPLC test for flavonoids revealed the presence of Benzoic acid, Isoquercetin, Rutin and Chlorogenic acid; *Mangifera indica* HPLC test for phenol revealed the presence of Eugenol and Ferulic acid. The toxicity test $LD_{50}$ for the natural dyes were evaluated. It was observed that the $LD_{50}$ value of the *Anthocleista vogelii* was above 5000 mg/kg and that no deaths was recorded; the $LD_{50}$ value of the *Justicia carnea* was above 5000 mg/kg and that no deaths was recorded; and the $LD_{50}$ value of the *Mangifera indica* was 5054.77 mg/kg and two deaths was recorded.

**Keywords:** *Anthocleista vogelii, Justicia carnea, Mangifera indica, extraction, characterization.*

## INTRODUCTION

Colour plays a dominant role in the cultures of the world as it influences our moods and emotions, and generally enhances the way we enjoy our surroundings (Abba *et al*., 2016; Yusuf *et al*., 2017). Throughout history, humans have used natural dyes for purposes varying from cosmetics, to coloration of food, and textiles (Yi and Cho, 2008). The ancient civilisations of the valleys of the Nile, Tigris, Euphrates, Indus and Yellow Rivers made use of natural dyes; during the dynastic period of 3200 B.C. There was evidence of alum being used as a mordant in the dyeing of textiles (Bechtold *et al.*, 2003). By 1960, synthetic dyes were the dominant dyes used. More than 90% of the thousands of dyes used were synthetic. Synthetic dyes led to an almost complete replacement of natural dyes, due to the favourable application properties of synthetic dyes. The causes of their boom were brilliance, rich palette of colours, superior colour fastness, greater reproducibility, generally lower cost, and greater stability than natural dyes (Bukhari *et al.*, 2017; Gajendra and Kumaran, 2019a; Rather *et al*., 2018). The methods of application were frequently simpler, and they gave more consistent results than natural dyes (Ain *et al*., 2016; Rather *et al*., 2017). Rising environmental consciousness among consumers led to eco lobby groups (Haji, 2012; Parton, 1998). ). An increase of public interest in natural products has also revived an interest in the use of natural dyes (Ali *et al*., 2016; Deo *et al*., 2018; Khan *et al*., 2014). Today, many businesses, both large and small, have started exploring the use of natural dyes in producing an ecologically sound product that would appeal to the 'green' minded consumer (Babatunde, 2017; Dalby, 1993; Gafai *et al.*, 2019; Soaga *et al.*, 2014; Swamy *et al*., 2016). Some experts have argued against the re-emergence of natural dyes in modern dye-

houses (Glover, 1998; Glover and Pierce, 1993), while other experts have argued in its favour (Ali, 1993; Hill, 1997). ). Natural dyes currently account for about 1% of the total amount of dyes used worldwide (Geelani *et al.*, 2015). Recent global efforts to promote the cultivation and application of natural dye plants have been made. DOBAG (the Turkish acronym for Natural Dye Research and Development Project) was launched in Turkey with German assistance in cooperation with the Marmara University, Istanbul in 1981; Project DOBAG was successful in reviving the lost art of producing naturally dyed carpets in the Turkish carpet sector (Křížová, 2015). Other projects include the PrisCA in Italy, the INDINK in the United Kingdom, and the recently concluded European SPINDIGO project on indigo plants (Guinot *et al*., 2006).

## MATERIALS AND METHODS

The leaves of *Anthocleista vogelii, Justicia carnea* and *Mangifera indica* were collected at Abraka and Obiaruku, Delta State. The plant materials were identified by a taxonomist, Dr. W. Egboduku of the Botany department in the Delta State University (*Mangifera indica*, voucher number: UBH-M257; *Justicia carnea*, voucher number: UBH-J386; and *Anthocleista vogelii*, voucher number: UBH-A428).

Silica Gel (60-120 Mesh), cotton, treated white sand, acetone, chlorofoam, ethanol, methanol, n-hexane and petroleum ether were procured from procured from Sigma Aldrich Chemicals and were used for the extraction, and purification processes.

The purified dye extracts were characterised by using HPLC. Agilent Technologies Rev. A.10.02 [1757] was used for the HPLC. Soxhlet apparatus, column, round bottom flask, heating mantle, condenser and an

electrical grinder were used for the extraction and purification of the colourant.

**Extraction of plant dyes**

The leaves of the plant samples were collected, air-dried and ground to a fine particle size. 5 g of the plant powder samples were subjected to solvent extraction using 250 mL acetone in a Soxhlet extractor and heated at 20, 40, 60, 80, and 100 $^{o}$C for 1 h respectively. Extraction was repeated with other solvents such as ethanol and water. Pressurized liquid extraction was also carried out using a pressure pot and 5 g of the plant powder sample was subjected to solvent extraction using 250 mL acetone and heated at 20, 40, 60, 80, and 100 $^{o}$C for 1 h respectively. Extraction was also repeated with other solvents such as ethanol and water. Plots of the extraction time and pressure against absorbance were carried out for each plant sample.

**Purification of plant dyes**

The main colourant component of the extracts was purified and isolated using column chromatography. N-hexane, petroleum ether, chloroform and methanol were the solvents used. The extracts were combined, solvent-stripped and the colour components of the residue separated by chromatography on a silica column, eluting with chloroform/methanol (70:30), monitoring by thin layer chromatography (TLC).

**Toxicity testing $LD_{50}$**

45 wistar rats are divided into three groups of 15 rats each, one group for each plant. In each group of 15 wistar rats, the animals are divided into 5 groups of 3 rats each, and the test substance is given at one of the five fixed-doses (100, 1000, 2000, 3000, and 5000 mg/kg) in order to establish the dose range of producing any toxic effect. The number of deaths in each group is recorded after 24-hours and the $LD_{50}$ is calculated as the shown below

$LD_{50}=\sqrt{a\ x\ b}$

(1)

Where a is the highest non-lethal dose and b is the least toxic dose

## RESULTS AND DISCUSSION

### Optimization of solvent extraction

Several experiments were performed for the extraction of natural dyes from *Anthocleista vogelii, Justicia carnea* and *Mangifera indica* leaves using acetone, ethanol and water as solvents, and using Soxhlet extraction and pressurized liquid extraction. The results of the experiments are shown in Figures 1 to 4 and on table 1-4. It was observed that the yield of *Anthocleista vogelii, Justicia carnea* and *Mangifera indica* using pressurized liquid extraction and acetone as solvent was higher compared to those obtained using ethanol and water or other forms of extraction. The absorbance readings were taken at a wavelength of 505 nm.

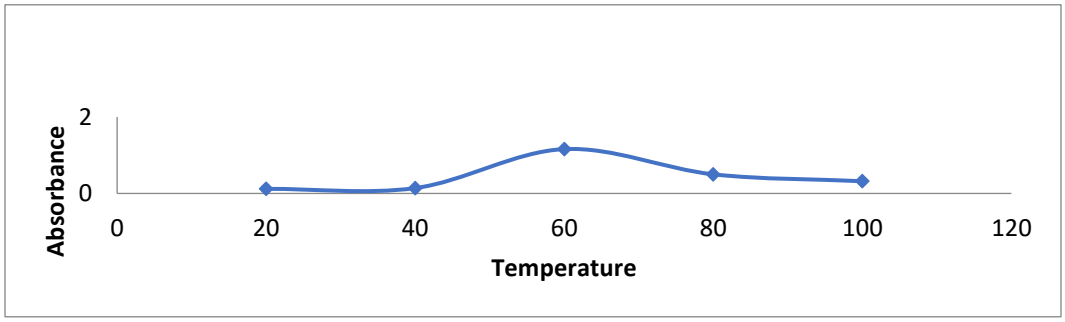



**Figure 1. Effects of dyeing temperature on absorbance of *Mangifera indica* under conditions of 1 h and acetone (300 mL)**

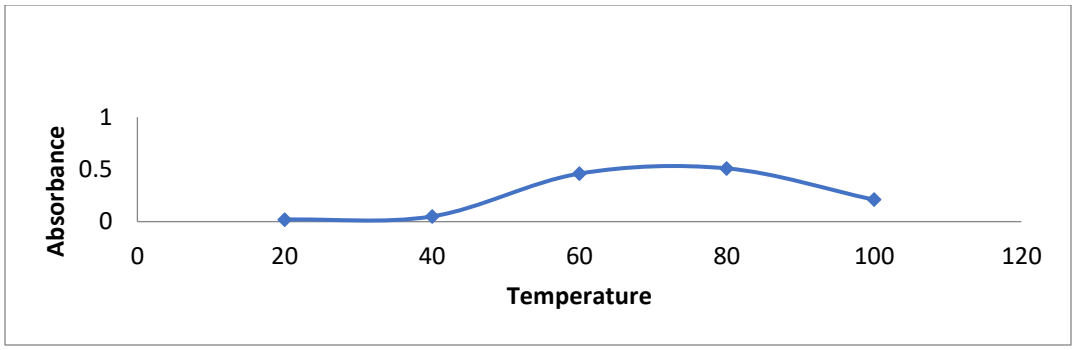
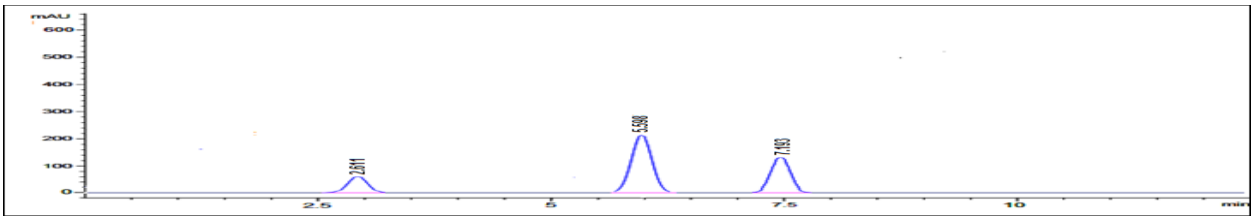



**Figure 2. Effects of dyeing temperature on absorbance of *Anthocleista vogelii* under conditions of 1 h and acetone (300 mL)**



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========Area Percent Report

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========Sorted By: Signal

Multiplier : 1.0000

Dilution : 1.0000

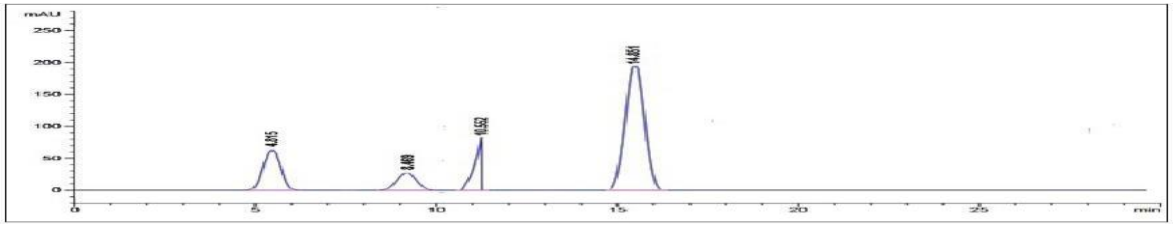
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength= 270 nm

**Fig 3. *Anthocleista vogelii* HPLC test for alkaloids**

**Table 1. *Anthocleista vogelii* HPLC test for alkaloids**

| Peak # | Ret Time (min) | Type | Width (min) | Height (mAU) | Area % | Compound |
|---|---|---|---|---|---|---|
| 1 | 2.611 | VV | 0.642 | 60.004 | 13.46943 | Hordenine |
| 2 | 5.598 | BB | 0.734 | 218.182 | 55.99496 | Cytisine |
| 3 | 7.719 | BB | 0.683 | 127.821 | 30.52508 | Galanthamine |

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Area Percent Report

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Sorted By : Signal

Multiplier : 1.0000

Dilution : 1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength = 320 nm

**Fig 4. *Anthocleista vogelii* HPLC test for flavonoids**

**Table 2. *Anthocleista vogelii* HPLC test for flavonoids**

| Peak # | Ret Time | Type | Width | Height | Area % | Compound |
|---|---|---|---|---|---|---|
| 1 | 4.815 | VV | 1.479 | 60.004 | 11.465 | Benzoic acid |
| 2 | 8.469 | VP | 1.474 | 28.736 | 5.472 | Isoquercetin |
| 3 | 10.552 | BB | 0.871 | 84.991 | 9.563 | Rutin |
| 4 | 14.851 | BB | 1.481 | 196.004 | 37.500 | Chlorogenic |

**Table 3. Extract one Mangefera indica (mango leaves)**

| Groups | Number of | Death | Behavioral | Fatigue | Writing | |
|---|---|---|---|---|---|---|
| 100 mg/kg | 3 | 0 | Nil | Nil | Nil | |
| 1000 mg/kg | 3 | 0 | Nil | Nil | Nil | |
| 2000 mg/kg | 3 | 0 | Mild | Nil | Nil | |
| 3000 mg/kg | 3 | 0 | Moderate | Yes | nil | |
| 5000 mg/kg | 3 | 2 | Sever | Yes | Yes | |

LD50 value of the *Mangefera indica* (mango leaves) = 5054.77 mg/kg

**Table 4. Second Extract *Justicia carnea* (Brazilian plume leaves)**

| Groups | Number of rats | Death | Behavioral change | Fatigue | Writing effect | |
|---|---|---|---|---|---|---|
| 100 mg/kg | 3 | 0 | Nil | Nil | Nil | |
| 1000 mg/kg | 3 | 0 | Nil | Nil | Nil | |
| 2000 mg/kg | 3 | 0 | Nil | Nil | Nil | |
| 3000 mg/kg | 3 | 0 | Nil | Nil | Nil | |
| 5000 mg/kg | 3 | 2 | Nil | Nil | Nil | |

LD50 value of the *Justicia carnea* = above 5000 mg/kg

The optimization of extraction of natural dyes from *Anthocleista vogelii, Justicia carnea* and *Mangifera indica* using various solvents was investigated. The optimization of extraction procedure forms the basis of the increase in the concentration of the natural dye molecules. This enables the improvement of the fastness properties of the natural dyes on the fabrics. It was observed that the extraction yield of *Anthocleista vogelii, Justicia carnea* and *Mangifera indica* using acetone as solvent and pressurized liquid extraction was higher compared to those obtained using ethanol and water as solvents or other forms of extraction.

*Anthocleista vogelii* HPLC test for alkaloids revealed the presence of Hordenine, Cytisine and Galanthamine; HPLC test for flavoids revealed the presence of Benzoic acid, Isoquercetin, Rutin and Chlorogenic acid; HPLC test for phenol revealed the presence of no compound. *Justicia carnea* HPLC test for alkaloids revealed the presence of Hordenine, Cytisine, Methyl Jasmonate and Galanthamine; HPLC test for flavonoids revealed the presence of Benzoic acid, Isoquercetin, Rutin, Hesperidin, Chlorogenic acid and Ellagic acid; HPLC test for phenols revealed the presence of Ferulic acid only. *Mangifera indica* HPLC test for flavonoids revealed the presence of Benzoic acid, Isoquercetin, Rutin and Chlorogenic acid; HPLC test for phenol revealed the presence of Eugenol and Ferulic acid.

The test of toxicity of the natural dyes revealed that the $LD_{50}$ value of the *Anthocleista vogelii* was above 5000 mg/kg and that no deaths was recorded, the $LD_{50}$ value of the *Justicia carnea* was above 5000 mg/kg and that no deaths was recorded, and the $LD_{50}$ value of the *Mangifera indica* was 5054.77 mg/kg and two deaths was recorded. In conclusion, all ethanol extracts of the natural dyes above 3250mg/kg were

safe according to world health organization (WHO) plant substances guidelines.

## CONCLUSION

Natural dyes were extracted from *Anthocleista vogelii*, *Justicia carnea* and *Mangifera indica*. Optimal extraction of natural dyes from *Anthocleista vogelii*, *Justicia carnea* and *Mangifera indica* was achieved by using acetone as solvent and pressurized liquid extraction at 1 h. The natural dyes obtained by column chromatography was characterized using HPLC to identify to identify the compounds present in the natural dyes. *Anthocleista vogelii* HPLC test for alkaloids revealed the presence of Hordenine, Cytisine and Galanthamine; HPLC test for flavoids revealed the presence of Benzoic acid, Isoquercetin, Rutin and Chlorogenic acid; HPLC test for phenol revealed the presence of no compound. *Justicia carnea* HPLC test for alkaloids revealed the presence of Hordenine, Cytisine, Methyl Jasmonate and Galanthamine; HPLC test for flavonoids revealed the presence of Benzoic acid, Isoquercetin, Rutin, Hesperidin, Chlorogenic acid and Ellagic acid; HPLC test for phenols revealed the presence of Ferulic acid only. *Mangifera indica* HPLC test for flavonoids revealed the presence of Benzoic acid, Isoquercetin, Rutin and Chlorogenic acid; HPLC test for phenol revealed the presence of Eugenol and Ferulic acid. The test of toxicity of the natural dyes revealed that the $LD_{50}$ value of the *Anthocleista vogelii* was above 5000 mg/kg and that no deaths was recorded, the $LD_{50}$ value of the *Justicia carnea* was above 5000 mg/kg and that no deaths was recorded, and the $LD_{50}$ value of the *Mangifera indica* was 5054.77 mg/kg and two deaths was recorded. In conclusion, all ethanol extracts of the natural dyes above 3250mg/kg were safe according to WHO plant substances guidelines.

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