TEXTILE DYEING AND PHYTOCHEMICAL CHARACTERIZATION OF CRUDE PLANT EXTRACTS DERIVED FROM SELECTED DYE-YIELDING PLANTS IN UGANDA

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Abstract

This paper reports an investigation of the phytochemical constituents present in the crude plant extracts of 8 selected dye-yielding plants i.e. Albizia coriaria (bark), Vitellaria paradoxa (bark), Syzygium cordatum (bark), Morinda lucida (bark and roots), Rubia cordifolia (roots), Curcuma longa L. (rhizomes), Indigofera arrecta (leaves), Justicia betonica (leaves) and their dye potential on 100% cotton fabrics. The phytochemical analysis was performed to identify the presence of colour compounds responsible for dyeing cotton fabrics in the presence of alum as mordant. The phytochemical analysis revealed the presence of flavonoid aglycones, anthraquinone aglycones, flavonoid glycosides, carotenoids, tannins, and anthracenoside glycosides as the potential colour components responsible for the dyeing of cotton fabrics to characteristic colour shades. The analysis revealed the presence of both the flavonoid aglycones and the glycosides as the main colour components in the crude bark and root extracts of Morinda lucida plant species. However, tannins constituted the most common colour component in all the plant species investigated. Further research on these plant extracts for possible isolation and characterization of the major colour components responsible for dyeing cellulosic cotton fabrics is recommended.

Key words: Textile dyeing, phytochemical characterization, crude plant extracts, dye-yielding plants, Uganda.

INTRODUCTION

The need to identify active chemical constituents in plant extracts requires phytochemical and analytical techniques. Different phytoconstituents have different degrees of solubility in different types of solvents depending upon their polarity and structure (El-Mahamood and Doughari 2008). Phytochemical surveys are being seen as the first step towards the discovery and structural elucidation of useful natural organic constituents for textile or medicinal applications (Hostettmann et al., 2000). Many plants are chemically very variable depending on the locality where they are found with some of the constituents occurring only at certain seasons of the year (Adelani, 2007).

The mode of action of plants producing dyeing effects on selected textile materials can be better investigated if the active ingredients are identified and characterized. Various parts of Vitellaria paradoxa plant species have been screened...
for plant metabolites with ethnomedicinal applications using the pulverized materials and the results revealed the presence of carbohydrates, saponins, steroids, tannins and alkaloids in its crude extracts (Ndukwe et al., 2007, EL-Mahamood et al., 2008). Further reports indicate that characteristic phytochemical constituents have been identified from the root bark of Morinda citrifolia and Morinda tinctoria plant species and used for textile colouration (Adelani, 2007).

Uganda is home to thousands of unknown and undocumented potential dye-yielding plants, many of them lying in the wild. However, only a small proportion of these plants has been investigated phytochemically mainly in search of medicinal metabolites but none has been evaluated for constituents with potential to dye textile materials. By identifying the class to which a coloured organic compound belongs, the problem of characterization is enormously simplified. Chemical screening is thus performed to target isolation of new or useful types of constituents with potential dyeing properties as it was the case in this study. Once the novelty of a given constituent is established, it is then necessary to purify the plant extract and isolate samples for full structure determination as reported by Hostettmann, et al. (2000).

Plant extracts from the bark of Albizia coriaria from the root of Morinda lucida, from the bark of Vitellaria paradoxa, from the bark of Syzygium cordatum, Albizia coriaria, Curcuma longa L., Indigofera arrecta, Justicia betonica and Rubia cordifolia potential dye-yielding plants were screened to determine the characteristic chemical constituents present in their crude plant extracts. Screening procedures and standard methods of qualitative chemical analysis of plant extracts reported by various authors (Faraz et al., 2003; Laity et al., 2002, Ndukwe et al., 2007, Venkatesan et al., 2009, El-Mahmood and Doughari, 2008) were used in the study, with the aim of further isolation and characterization of the major colour components and the possible use of these substances in the textile industry.

MATERIALS AND METHODS

Plant materials
Fresh plant parts( roots, bark, leaves, seeds) of Albizia coriaria, Vitellaria paradoxa, Curcuma longa L, Morinda lucida, Indigofera arrecta, Rubia cordifolia, Justicia betonica, Syzygium cordatum and Bixa orellana L. collected between 2004 and 2008 from Mukono, Kabwagasi and Wakiso selected districts of Uganda, were cut into small pieces, spread on newspapers and left to dry in open air. After drying, each plant sample was pounded separately using mortar and pestle into powder. The air dried powdered samples were then used for dyeing of cotton fabrics, qualitative chemical analysis and identification of the main classes of the dye constituents present in the plant extracts.

Textile dyeing
Pieces of cotton fabrics (100%) (plain weave, 23x24/cm², 0.001mm thickness) (8x10cm) weighing approximately 1.41g were dyed in a beaker (250ml) at the boil for 60 minutes in the presence of potassium aluminium sulfate (alum) as mordant (10%, o.w.f). A material to liquor ratio of 1:200 was used for dyeing each cotton fabric with the crude aqueous extracts derived from all the plants investigated in the study. The crude dye aqueous extracts contained characteristic colour components responsible for textile coloration which needed to be identified phytochemically.

Solvents used
Petroleum ether, ethanol (96.5%) and distilled water in increasing order of polarity were the three main solvents used for the separation of all the chemical constituents from the crude plant extracts according to their physicochemical properties.

Chemical Analysis of the crude plant extracts
10 -25g of each plant material, ground to powder were first extracted with petroleum ether solvent several times until no more residue was left after evaporation of the ether. The ether extracts were combined and concentrated to 50 ml. Analysis of each ether extract for various liposoluble chemical constituents was carried out as described below.

Tests on the ether extracts
Test for steroids/triterpenoids
Ten (10 ml) of the ether extract was evaporated to dryness. The residue was dissolved in acetic anhydride (0.5 ml) and then in 0.5 ml of chloroform. The solution was transferred to a dry test tube and then 0.5ml of chloroform. The solution was transferred to a dry test-tube and by means of a dropping pipette, conc. Sulphuric acid was added to the bottom of the test tube. At the point where the two layers met, a brownish-red or violet ring was formed and the supernatant layer turned green or violet denoting the presence of steroids and/or triterpenoids.

Test for carotenoids
Ten (10 ml) of the ether extract was evaporated to dryness after which 2 – 3 drops of concentrated sulphuric acid in chloroform were added. An intense blue colour developed to show the presence of carotenoids in the extract.

Test for fatty acids
Ten (10 ml) of ether extracts were exhaustively extracted with aqueous sodium hydroxide solution. The aqueous alkaline layer was then acidified with conc. Hydrochloric acid (pH = 3 – 4), thereby liberating the fatty acids from their alkaline salts. The acid solution was then shaken several times with small portions of petroleum ether in a separating funnel to extract the fatty acids. The ether was then
Figure 1: Shades of colour developed on cotton fabrics from selected dye-yielding plants in Uganda.

Albizia coriaria (bark)

Vitellaria Paradoxa (bark)

Curcuma longa L. (roots)

Morinda lucida (roots)

Morinda lucida (bark)

Indigofera arrecta (Leaves)

Rubia Cordifolia (roots)

Justicia betonica (leaves)
Syzygium Cordatum (bark) evaporated to dryness. If the residue was oily, then fatty acids were present.

**Test for flavonoid aglycones**
Three (3 mls) of the ether extract were evaporated to dryness. The residue was dissolved in 1 – 2 mls of methanol. A piece of magnesium ribbon was then added to the solution followed by 4 – 5 drops of concentrated hydrochloric acid. A pink or magenta-red colour developed within 3 minutes indicated the presence of flavonoid aglycones.

**Test for anthraquinone aglycones (emodols)**
To three (3 mls) of the ether extract in a test tube, 1 ml of 10% sodium hydroxide solution was added. A red colour showed the presence of anthraquinone aglycones.

**Test for coumarins**
Three (3 mls) of the ether extract were evaporated to dryness. The residue was then dissolved in 2 mls of hot water and the solution allowed to cool to room temperature. The cooled solution was divided into equal parts, one of which served as a reference. The second part of the solution was made alkaline by adding 0.5 mls of 10% ammonia solution. An intense fluorescent colour observed under ultraviolet light indicated the presence of coumarins and their derivatives.

**Tests on the alcohol extracts**
The material left after the ether extract was dried and extracted three times with 95% ethanol. The alcohol extracts were combined and concentrated to 50 mls. Analysis on the alcohol extracts was carried out for important phenolic compounds according to their physicochemical properties as described below.

**Test for tannins**
0.5 - 1 ml of the alcohol extract was diluted with 2 mls of distilled water to which 2 – 3 drops of iron (III) chloride were added. A blue-black colour indicated the presence of gallic tannins and a green-black colour showed the presence of catechol tannins.

**Test for reducing sugars**
One (1) mls of the alcohol extract was diluted with (1 - 2 mls) of distilled water. 0.5 - 1 ml each of Fehlings solutions (I and II) were added to the solution and the mixture was then heated. A brick-red precipitate indicated the presence of reducing sugars.

**Test for alkaloids**
Twenty (20) mls of each extract was transferred to a capsule and evaporated on a boiling water bath. 5 – 10 mls of dilute hydrochloric acid (10%) was added to the residue. The alkaloids present became salts of the mineral acid. The alkaloids were precipitated by adding ammonia solution (10%) up to a pH of 8 – 9 and then extracted with chloroform. The chloroform was evaporated to dryness and the residue dissolved in hydrochloric acid (about 2 mls, 2%) and the solution divided into two parts. One part was kept as a reference. To the second part of the solution, 2 -3 drops of Bertrand’s reagent were added. A yellow-white precipitate indicated the presence of alkaloid salts.

**Tests on the hydrolysed alcohol and aqueous extracts**
To the ethanol extract (25 mls) was added hydrochloric acid (15 mls, 10%) and the mixture heated under reflux for about 10 minutes. During the hydrolysis of the glycosides, the solution became opalescent due to the formation of aglycones as a precipitate. The mixture was cooled and extracted three times with ether (10 – 12 mls) using a separating funnel. The ether extracts (30 – 36 mls) were combined and dried over anhydrous sodium sulphate.

**Test for anthraacyanoside glycosides**
The ether extract (5 ml) was evaporated to dryness. The residue is then dissolved in methanol (1 – 2 ml, 50%) by heating and then magnesium ribbon added followed by 5 – 6 drops of concentrated hydrochloric acid. A red solution which turns to neither violet at a neutral pH nor to green or blue in alkaline medium indicated the presence of anthracyanosides in the crude plant extracts.

**Test for polyuronide glucosides**
The plant material that had been extracted successively with ether and alcohol was dried. It was then extracted with warm distilled water for about 20 minutes. The solution was filtered and concentrated to about 50 mls. Two (2) mls of this aqueous extract was added dropwise to a test tube containing 10 mls of methanol. A violet or blue precipitate showed the presence of polyuronide glucosides.

**Test for glucosides**
The aqueous extract (2 mls) is transferred to a porcelain dish and is evaporated to dryness. 2 – 3 drops of concentrated sulphuric acid were added and allowed to stand for 3 – 5
minutes. 3 – 4 drops of methanol saturated with thymol (molisch’s reagent) are then added. The appearance of a red colour indicated the presence of glucosides.

**Test for Saponins**
Two (2) mls of the diluted aqueous extract (1:1) with distilled water was shaken in a test tube of 1.6 cm diameter for about 15 minutes. The appearance of a foam column of at least 1.6 cm high and which persisted for a minimum of 15 minutes indicated the presence of saponins.

**Test for anthraquinone glycosides**
To the ethanol extract (25 mls) was added 15 mls of 10% dilute hydrochloric acid and the mixture heated under reflux for about 10 minutes. The solution became opalescent due to the formation of aglycones as precipitate during the hydrolysis of the glycosides. The mixture was cooled and then extracted three times with ether (10 – 12 mls) using a separating funnel. The ether extracts (30 mls) were then dried over anhydrous sodium sulphate. 4 mls for the ether extract were concentrated to 2 mls. Ammonia solution was then added to the concentrated solution with shaking. A red colour indicated the presence of aglycones of anthraquinones in an oxidized form.

**Test for flavonoid glycosides**
The ether extract (5 mls) from the hydrolysed alcohol extract was evaporated to dryness. The residue was dissolved in 2 mls of 50% methanol by heating and then adding a small piece of magnesium ribbon followed by adding 5 – 6 drops of concentrated hydrochloric acid. A red solution indicated the presence of flavonoid glycosides.

**Table 1:** Phytochemical analysis of selected dye-yielding plant species in Uganda

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Syzygium cordatum (bark)</th>
<th>Albizia coriaria (bark)</th>
<th>Morinda lucida (bark)</th>
<th>Morinda lucida (roots)</th>
<th>Vitellaria paradoxa (bark)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids and Triterpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid aglycones</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Anthracenoside aglycones (Emodols)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Fatty acids</td>
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<td>-</td>
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<tr>
<td>Tannins</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthracenoside glycosides</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Flavonoside glycosides</td>
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<tr>
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<td>+</td>
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<tr>
<td>Polyuronide glycosides</td>
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<td>-</td>
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<tr>
<td>Carotenoids</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Glucosides</td>
<td>+</td>
<td>-</td>
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</tr>
</tbody>
</table>

+= Present; - = Absent

**RESULTS AND DISCUSSION**
Table 2 shows the result of the phytochemical screening for the nine plant species considered in this study. The results indicate that qualitative chemical analysis was useful for a preliminary phytochemical characterization of the dye-yielding plants and possible prediction of the resulting colours on a substrate. The results provide an empirical basis for the potential use of these plants in dyeing of textile materials. The extracts from Albizia coriaria, Vitellaria paradoxa, Morinda lucida, Indigofera arrecta, Rubia cordifolia and Syzygium cordatum plant species revealed the presence of tannin moieties in their molecular structures. Tannins have been reported to be the most important ingredients which are necessary for dyeing with natural dyes, especially to brown shades of colour (Zin and Moe, 2008). However, the observation that some of these plants contain not only tannin compounds but possess flavonoid and anthraquinone aglycones in their molecular structures suggests that the dyeing potential may not be due to the presence of tannin dye constituents alone but from a combination of all the colour components present in the crude extracts. The different shades of colour developed from the individual interactions of the colour components from the crude extracts with the cotton fibres are shown in table 1. The extract from the Morinda lucida species contained mainly the flavonoid aglycones, flavonoid glycosides and anthraquinone aglycone phytochemicals in its molecular structure. Like many other plants, Morinda lucida is chemically very viable depending on the locality where it is
Table 2: Phytochemical analysis of selected dye-yielding plant species in Uganda

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Curcuma longa L. (rhizomes)</th>
<th>Indigofera arrecta (leaves)</th>
<th>Rubia cordifolia (roots)</th>
<th>Justicia betonica (leaves)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids and /or Triterpenoid glycosides</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid aglycones</td>
<td>+</td>
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</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Anthracenoside aglycones (Emodols)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Fatty acids</td>
<td>-</td>
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<tr>
<td>Tannins</td>
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<tr>
<td>Reducing sugars</td>
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<td>Alkaloids</td>
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<td>Anthracenoside glycosides</td>
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<td>Flavonoside glycosides</td>
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<tr>
<td>Saponins</td>
<td>-</td>
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<tr>
<td>Polyuronide glycosides</td>
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<tr>
<td>Carotenoids</td>
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<tr>
<td>Glucosides</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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found (Adelani, 2007), and variation of the principal colours on the same or different textile fibres is therefore expected. Whereas the colour from the Morinda species found in several West African countries is mainly red on cotton fabrics, indicating a greater involvement of anthraquinone dye components in the shade production, the results in the present study show that mainly yellow shades of colour were produced from the plant extracts of the Morinda lucida species found in Uganda indicating a greater involvement of flavonoids in colour production on the same substrate.

The extract from Curcuma longa L gave a characteristic bright yellow shade of colour on cotton fabrics attributed to the presence of both flavonoid and anthraquinone aglycones enhancing each other in their overall contribution to the depth of colour shown in table 1. Results from analysis of Rubia cordifolia plant species revealed the presence of anthraquinones in their root extracts responsible for the reddish-pink colour of the dyed cotton fabrics. The presence of carotenoids and anthraquinone glycosides characterized the blueish-purple colour from the leaf extracts of Justicia betonica on the cotton fabrics.

The presence of tannins, flavonoid aglycones and anthraquinone aglycones in the root and bark of all the plants investigated showed that the extracts were of textile importance. Various extracts from the bark and root of different plants belonging to the genus Morinda have been reported to contain mainly anthraquinone moieties in their molecular structures (Bhuyan and Saikia, 2005; Jansen and Cardon, 2005; Paitoon et al., 2002, Samantha and Agarwal, 2009). The same anthraquinone molecular compounds have been reported to have significant dyeing properties on cotton, silk and wool fibres (Paitoon et al., 2002; Jansen and Cardon, 2005).

The presence of saponins, tannins, alkaloids and steroids/triterpenoids in all the plants showed that the extracts are of various pharmacological importance, confirming their traditional widespread use in herbal medicine in Uganda (Katumba et al., 2004).

CONCLUSION
The general chemical composition of an unknown plant extract may be determined by qualitative chemical analysis by extraction with different solvents as indicated in this study. The separation of the main classes of chemical constituents present in an extract was obtained by successive and selective extractions with solvents of differing polarities according to the physicochemical properties of each group of the active principles.

By identifying the class to which the chemical compounds in each extract belonged, the problem of colour characterization was enormously simplified. The presence of tannins, carotenoids, flavonoid and anthraquinone aglycones and glycosides in the crude extracts of all the plant species investigated phytochemically makes them of significant textile importance as sources of natural dyes for textile coloration. Further study should be undertaken to isolate the major colour constituents and determine their molecular structures for proper identification and further research.
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REFERENCES


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