Curcuma longa extract as a histological dye for collagen fibres and red blood cells

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Abstract

Crude ethanolic extract and column chromatographic fractions of the Allepey cultivar of *Curcuma longa* Roxb, commonly called turmeric (tumeric) in commerce, were used as a stain for tissue sections. Staining was carried out under basic, acidic and neutral media conditions. Inorganic and organic dissolution solvents were used. The stain was used as a counterstain after alum and iron haematoxylins.*C. longa* stained collagen fibres, cytoplasm, red blood cells and muscle cells yellow. It also stained in a fashion similar to eosin, except for its intense yellow colour. Preliminary phytochemical evaluation of the active column fraction revealed that it contained flavonoids, free anthraquinone and deoxy sugar. A cheap, natural dye can thus be obtained from *C. longa*.

Keywords: *Curcuma longa*, flavonoid and stain, rhizome, turmeric (tumeric), Zingiberaceae

Introduction

The rhizome of *Curcuma longa* Roxb, belonging to the family Zingiberaceae, appears morphologically similar to *Zingiber officinale* except for the intense yellow colouring matter. *Curcuma longa* has an aromatic odour and a warm somewhat bitter taste. The dried and ground rhizome of *C. longa* is called turmeric (tumeric). It has been in use for centuries as a dye and as a component of curry powder, and more recently as a pH indicator; therefore, it is a common commercially used substance. It contains about 5% diaryl heptanoid colouring materials known as curcuminoids, the chief of which is curcumin (diferuloylmethane) together with smaller quantities of dicaffeoylmethane, caffeoylferuloylmethane and dihydrocurcumin. The volatile oil (about 5%) contains sesquiterpenes (e.g. zingiberene, 25%), sesquiterpene alcohols and ketones, and also monoterpenes (Evans, 1998). Furthermore, Evans (1998)stated that the characterization of the constituents of a polysaccharide fraction of *Curcuma longa*demonstrated new acid glycans designated as ukanons A, B, C and D in addition to small amounts of peptide moieties. Based on the method of production, there are two types of stains, natural and synthetic (Carleton, 1976). Haematoxylin obtained from the Mexican tree *Haematoxylon campechianum* is an example of a natural dye (Baker & Silverton, 1976) that is widely used in histochemistry, whereas eosin is a synthetic dye. Synthetic dyes are often efficient but may display hazards to human and animal health. This has resulted in the withdrawal of several dyes as their hazards became recognized (Bhuyan & Saikia, 2005). With the worldwide concern over the use of eco-friendly and biodegradable materials, the use of natural dyes obtained from plants has again gained interest (Eom et al. 2001). Moreover as many developing countries can no longer afford the ever increasing cost of synthetic dyes, the use of cheaper, naturally occurring dyes from plants is being viewed as an alternative to synthetic dyes. Based on the

aforementioned, the yellow dye of the Allepey cultivar of *Curcuma longa* was investigated as a natural dye with potential histopathological application.

Materials and methods

Preparation of plant extract

Rhizomes of the Allepey cultivar of *C. longa* were collected fresh from the Medicinal Garden of the Drug Research and Production Unit (DRPU), Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife, Nigeria, by J.M.A. A voucher specimen numbered IFE 153554 was deposited at the Botany Department Herbarium, Obafemi Awolowo University. The rhizomes were cut into small pieces and dried at 40 °C for 48 h in an open air oven (Gallenkamp, UK). They were milled to a fine powder. Then, 1.5 kg of the powdered plant material was treated with 2 L 70% ethanol in a Soxhlet extractor for 72 h. The extract was filtered and concentrated in vacuo at 50 °C and finally dried in a descicator to remove residual water. In total, 230 g (18.67% yield) of the crude ethanolic extract was obtained.

Purification of extract of *C. longa*

The crude ethanolic extract was purified using the column chromatography (CC) technique. Total extract was loaded on a wet packed silica gel (60–120 mesh) column and eluted sequentially with *n*-hexane (100%); a gradient of *n*-hexane/chloroform ranging from 1 : 9; 1 : 1, 3 : 2 and 1 : 1 chloroform/ethanol mixtures; and ethanol (100%). One hundred and twenty-three 50-mL eluent fractions were collected and monitored via thin-layer cromatography (TLC). The eluents were subsequently bulked into seven groups, CL/A–CL/G (these being fractions 1–38, 39–47, 48–61, 62–70, 71–88, 89–104 and 105–123) successively. The bulked fractions were in various colours ranging from amber, light yellow to yellowish wine and finally colourless.

Preparation of sections

Human tissue samples, 3 mm, thick were obtained from the skin, liver, intestine, kidney, lung and spleen at post-mortem examination. They were fixed in 10% formol saline for 24 h and processed for paraffin wax embedding with an automatic tissue processor (Sakura Fine Tech., the Netherlands) by dehydrating through 70, 90, 95 and two changes of absolute ethanol for 1.5 h. Clearing was achieved through changes of xylene twice for 2 h each, infiltrating through two changes of paraffin wax at 70 °C following which tissues were embedded in paraffin wax. Sections were cut at 4 µm with a rotary microtome (Sakura Fine Tech.), attached to slides and dried at 65 °C for 45 min.

Preparation of *C. longa* staining solution

Crude ethanolic extract (0.5 g), bulked column fractions (CL/A to CL/G) of *Curcuma longa*, were dissolved in 100 mL each of the following solutions: distilled water, 70% ethanol, 1%

acetic acid in 70% ethanol, 70% ethanol saturated with potassium aluminium alum (mordant) and 1% ammonium hydroxide in 70% ethanol.

Staining method

Sections were dewaxed in xylene for 4 min and hydrated through graded solutions of alcohol and stained in either Weigert's or alum haematoxylin for 10 min. Sections were then differentiated in 1% acid alcohol for 2–5 s and blued in tap water for 10 min. Sections were then counterstained with each of the *C. longa* extracts CL/A to CL/G solutions for 1–30 min and up to 48 h at room temperature and at 56 °C. Sections were finally rinsed in water, dehydrated in ascending grades of alcohol, cleared in xylene and mounted in a synthetic mountant.

Preliminary phytochemical screening

Fraction CL/D eluted with chloroform/ethanol (9 : 1) was screened to determine the presence of the following metabolites through preliminary phytochemical screening according to the method ofSofowora (1993). Alkaloids were screened using Dragendoff's reagent, Mayer's reagent, Wagner's reagent and tannic acid. Anthraquinones were also screened using Borntrager's test for free and combined anthraquinones after hydrolysis; flavonoids by the ferric chloride test, lead acetate test, sodium hydroxide test and ethyl acetate test; tannin by ferric chloride test and bromine water test; phlobotannins with hydrochloric acid; saponin with the froth tests and haemolytic test; and cardiac glycosides using the Salwoski's test, Libermann's test and Keller Killiani's test.

Results

Staining reaction

A 1% aqueous solution of *C. longa* stained collagen fibres, red blood cells, muscle fibres and cytoplasm deep yellow within 5 min. When used as a counterstain for haematoxylin, the staining reaction was similar to eosin except for its yellow colour (Fig. a1–5, b1–5). The blue–black nuclear staining of haematoxylin is more intense in Weigert's iron haematoxylin than in Erhlich's haematoxylin (Fig. b1).

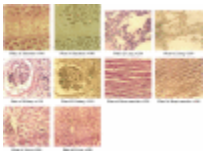

Fig. 1

a1, intestine ×100; a2, Lung ×250; a3, kidney ×150; a4, heart muscles ×250; a5, liver ×250 were stained with haematoxylin and eosin technique as controls.

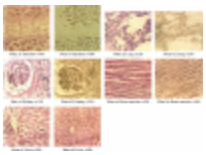

Fig. 2

Plate b1 was stained with Weigert's Haematoxylin and counterstained with *C. longa* extract while Plates b2, b3, b4 and b5 were stained with Mayer's Haematoxylin and counterstained with *C. longa*. Notice the general staining of tissue structures in a manner**...**

The effects of acidity, alkalinity and mordant on the stain

C. longa stained tissue sections yellow in aqueous solutions of acetic acid and ammonium hydroxide (acidic and alkaline solutions, respectively). The addition of potassium alum as a mordant did not improve the staining qualities of *C. longa*.

The effect of fractions of *C. longa* CL/A to CL/G on tissue sections at 1% concentration

Fraction CL/D stained the tissue sections shades of yellow with an optimum staining time of 5 min at room temperature. None of the other coloured fractions showed staining ability even after applying them for 48 h at room temperature or at 56 °C. When fraction CL/D was used as a counterstain for haematoxylin, nuclei were stained blue, and collagen fibres, red blood cells and muscle cells were stained yellow.

Phytochemical evaluation of the staining fraction (CL/D)

The fraction which stained the tissue sections contained flavonoids, deoxy sugars and free anthraquinone.

Discussion

C. longa contains several colouring compounds, chief of which is curcumin (Evans, 1998) but it is not clear if the active staining principle is curcumin because the column chromatographic evaluation of *C. longa* revealed the presence of several coloured fractions and characterization of the active fraction was not determined. 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 (Baker & Silverton, 1976; Carleton, 1976). 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 because it stained collagen fibres, red blood cells and cytoplasm yellow. This deduction is corroborated by the phytochemical analysis of the active column fraction. It contained flavonoids, which are typically polyphenolic compounds. Phenols are acidic, due to their ability to release the hydrogen from their hydroxyl group, hence the ability of *C. longa* to stain the basic parts of the cell. However, the lack of pH sensitivity is puzzling. Regardless, when *C. longa* was used as a counterstain for haematoxylin, the nuclei took the blue colouration which enabled a clear contrast to be made between the different structures of the cells. This shows that the reaction of the *C. longa* stain is similar to the reaction of eosin

in the haematoxylin and eosin technique except for its yellow colouration. For this reason, the Allepey cultivar of *C. longa* was tried as a counterstain because of its high pigment content and used as a substitute for eosin in the haematoxylin and eosin technique (Carleton, 1976). Consequently, *Curcuma longa* extract is a promising histological dye that is not only cheap but readily available. It could serve as a useful alternative to eosin in developing countries.

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