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Eggplant Peel Ethanolic Extract: A Novel and Alternative Stain for Chromosome Banding

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Abstract

Rat and mouse have become important animal models to study various human diseases such as cancer. Here, we use eggplant peel ethanolic extract to visualise chromosome banding. This extract is easy to prepare, a novel, available alternative. The colour of this extract is bright dark green colour. This stain gave us good result for show the chromosomes banding, we used Thin Layer Chromatography (TLC) examination, Infrared (IR) spectroscopy and the spectrum ultraviolet UV to investigate about the compound and functional groups in this extract. Use of this extract represents a new method for chromosome banding and a viable alternative to existing chromosome staining methods.

Keywords: Ethanolic extract; Eggplant; Chromosomes bandings

Introduction

A wide variety of stains are used to visualise chromosomes under the microscope. The aceto-orcein, acetocarmine, gentian violet, and haematoxylin readily stain chromatin are classical cytological stains allowing for easy visualisation of chromosomes under a standard light microscope [1].

Numerous methods are available for identifying chromosomes and preparing karyotypes for clinical and research purposes. The most common methods of dye-based chromosome banding are Giemsa- (G), reverse- (R), centromere- (C), and quinacrine- (Q) banding. Q- and G-banding introduced a new era in which individual chromosomes could be definitively identified. With this capability, it also became possible to localize regions of variable size and staining to specific chromosomes. In particular, Q- and C-banding can reveal distinct classes of heteromorphisms that are not necessarily detectable in non-banded chromosomes. The most distinctive heteromorphism revealed by Q-banding was the brightly fluorescent distal long arm of the Y chromosome. The size of this brightly fluorescent segment varies from being almost negligible in size to being the longest segment on the Y long arm. Q-banding also revealed variations in staining of chromosomes 3, 4, 13-15, and 21-22 of the human karyotype [2-6]. Although G-banding techniques have become widely used for chromosome identification, C-banding also revealed size variations of heterochromatin around the centromeres of every chromosome; these could be more easily quantified in banded than in non-banded chromosomes. The heterochromatin regions of chromosomes 1, 9, and 16, and in the distal long arm of the Y, evident in non-banded chromosomes, were especially visible by C-banding [6-10].Since banding may be a reflection of the difference in the structure along the length of a chromosome, studying the mechanism of banding can improve our understanding of chromosome structure [11-13]. Banding can be used for chromosome identification (karyotyping), and for identifying abnormalities of chromosome number, translocations of material from one chromosome to another, and deletions, inversions or amplifications of chromosomes. Q-banding was discovered by Caspersson et al. [2] who applied quinacrine mustard dihydrochloride (QM) staining to human chromosome X. This resulted in the discovery that the end of the long arm of the Y chromosome was brightly fluorescent: bright enough that the human Y chromosome could be easily detected in interphase as well as in metaphase cells [14].

G-banding is the most widely used banding technique in clinical laboratories. The G-banding method uses acid fixation with saline treatment, followed by Giemsa staining [15]. Application of proteolytic enzymes such as trypsin [16,17] or pancreatin [18,19] improved the banding pattern. Dark-staining G-bands indicate AT-rich regions of chromosomal DNA that are more condensed replicate their DNA later than less condensed GC-rich regions [20].

R-banding was discovered by Utrillaux and Lejeune [21]. Due to technical difficulties and fluorescent requirements, it is not a widely used method of banding.

The observation by Pardue and Gall that the centromeres of mouse chromosomes stained darker than other chromosomal regions led to the discovery of C-banding [22]. Arrighi and Hsu [23,24] developed a modified technique C-banding by applying Giemsa staining; other modified versions of C-banding have since been developed.

The technique Cd-banding was described by Eiberg [25]. The G-11 technique and finally Silver Staining was developed by Howell and Black [27].

Eggplant, also called aubergine or brinjal (*Solanum melongena* L.) is an edible fruit, which is cultivated globally, but particularly in Asia and Europe [28,29]. Two kinds of anthocyanin have been isolated and identified from the purple cultivar [30,31]. The role of anthocyanin pigments as medicinal agents is well-accepted dogma in folk medicine throughout the world; indeed, eggplant is used in traditional medicines [30]. The anthocyanin delphinidin-3-rutinoside was identified from eggplant by HPLC-DAD-MS3 analyses [32]. A second anthocyanin, delphinidin-3-(p-coumaroylrutinoside)-5-glucoside (nasunin), was isolated as purple coloured crystals from eggplant peels [33].

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Material and Methods

Ethanolic extract

Eggplant peel was removed and dried in the shied. 25 grams of the resulting powder was extracted in 250 ml 96% ethanol alcohol by using the socholite for 6 hours. The colour of this extract is bright dark green colour.

Thin layer chromatography (TLC) examination

TLC was carried out using ethyl acetate: hexane (1: 9) solvent. Chemical analysis with ethyl acetate: hexane (6: 4) (1: 9) determined the presence of one separated compound (Figure 1).

Infrared (IR) spectroscopy

IR spectroscopy (250-4500 cm) indicated the presence of C-O, C=O, C=C, C-H and CHO=O bonds, and the presence of benzene ring (Figure 2).

The spectrum ultraviolet UV of Eggplant ethanolic extract

Scan spectrum curve show the presence of absorption at the wavelength packages (max = 200.00-780.00 nm) has shown in Figure 3

Cytogenetic examinations

Rat chromosomes were prepared for cytogenetic examination. The animals were sacrificed and chromosome aberrations relieved using the method of Giri et al. [34]. Prepared slides were rinsed in ammonium alum solution for 2-3 minutes and stained for 15 min in an eggplant alcohol extraction solution. The staining solution was filtered immediately before use. Stained slides were air-dried before being examined and photographed under a light microscope.

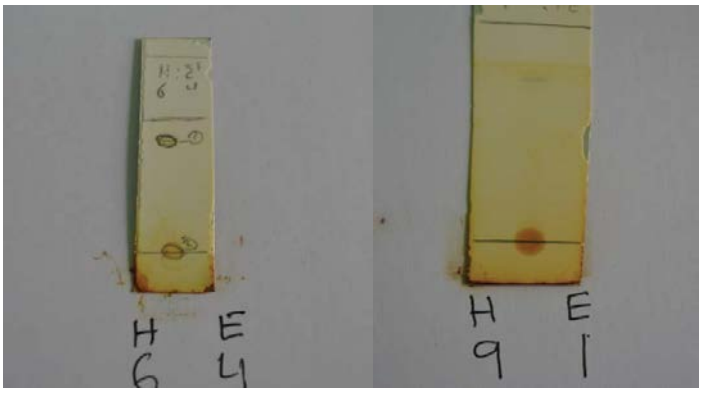

Figure 1: Location of the standards on the thin layer chromatography plate using hexane:ethanol (6:4) (1:9) as the mobile phase

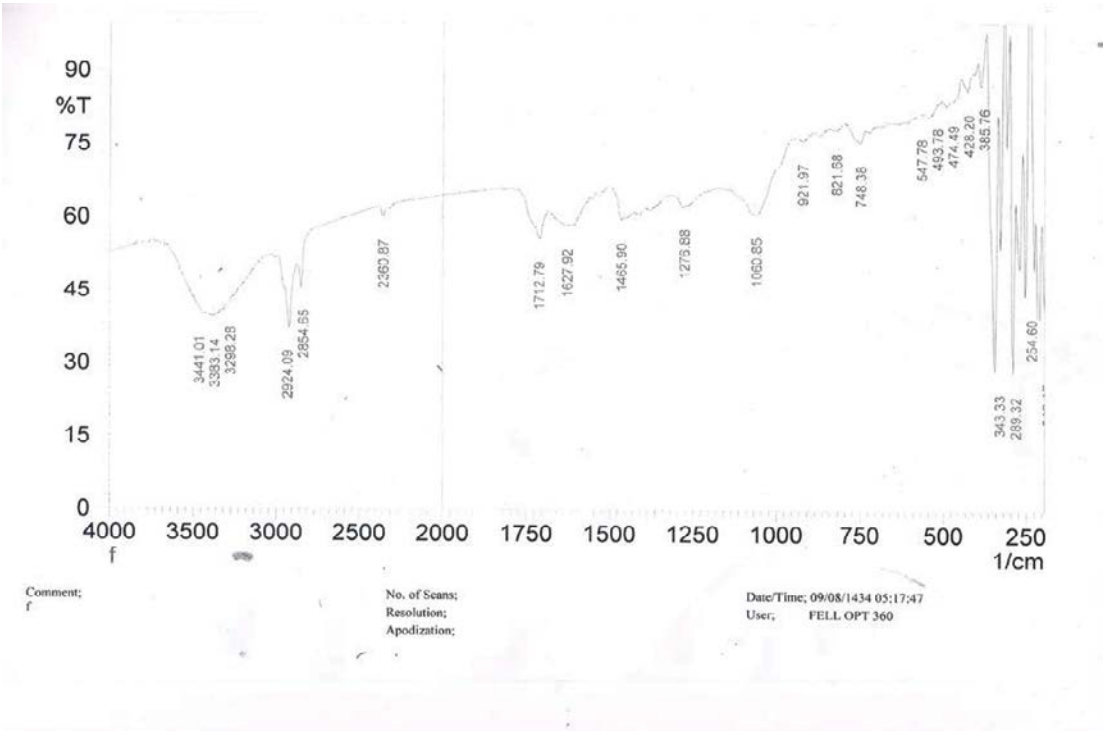

Figure 2: Infrared (IR) spectroscopy of Eggplant ethanolic extract

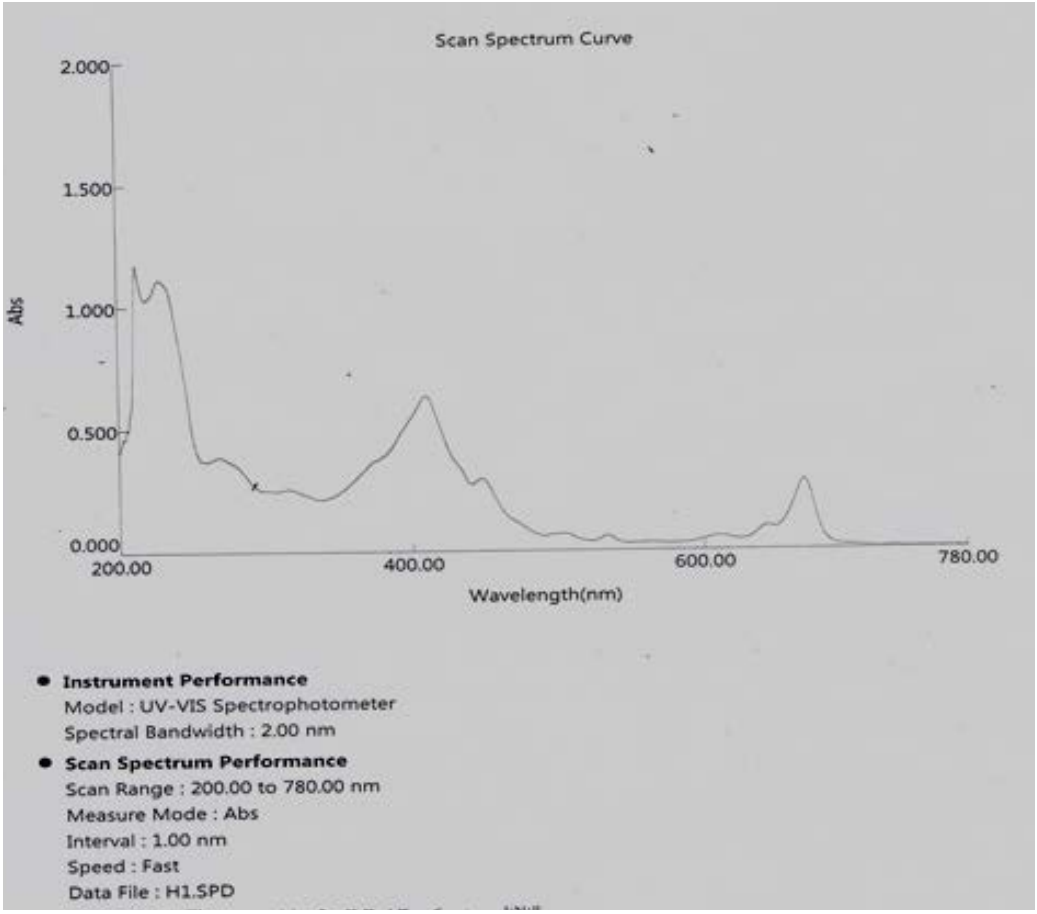

Figure 3: The spectrum ultraviolet UV of Eggplant ethanolic extract

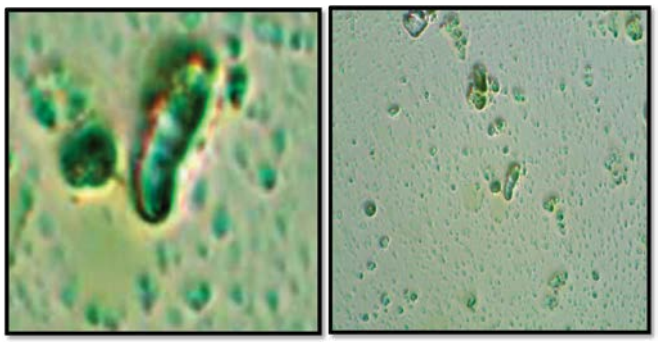

Figure 4A 4B: Normal rat male metaphase spread chromosome stained with eggplant peel ethanolic extract (800X) and (300X)

Results

As shown in Figure 4, stained chromosomes, with banding, could be clearly visualised after treatment with eggplant peel ethanolic extract, and Figure 5 which stained with Gemza shows no banding.

Discussion

Chromosome staining is used to enhance the contrast between different cellular components. Each chromosome arm is divided into regions, or cytogenetic bands, that can be seen using a microscope and special stains. The cytogenetic bands are labelled p1, p2, p3, q1, q2, q3, etc., counting from the centromere out toward the telomeres. These bands provide further information about the chromosomes. Since each chromosome number produces unique bands, this method can be used to identify individual chromosomes [35].

The result of this study showed that staining with eggplant alcohol extract is a viable method of staining rat chromosomes, and produced visible banding regions. In normal chromosomal stain procedure, metaphase chromosomes are treated with trypsin and stained with Giemsa stain. The major bands regions in chromosome are constitutive heterochromatin, facultative heterochromatin, and euchromatin. Heterochromatic regions, which tend to be AT-rich and relatively gene-poor, stain more darkly in G-banding. In contrast, less condensed chromatin, which tends to be GC-rich and more transcriptionally active, incorporates less Giemsa stain, and hence these regions appear as light bands in G-banding. In this study ammonium alum solution has been used. This compound may bind to the DNA through intercalation, minor or major groove binding, or external binding, and thus have an effect on the nucleotides in the DNA. The mode of binding depends on the nature of the interaction between the stain and the DNA, which may be either covalent or noncovalent [35].

Using IR spectroscopy (IR), we detected the presence of functional groups including C-O, C=O, C=C ,C-H, COH=O and benzene ring . This range of functional groups could produce more than one type of DNA-stain interaction, suggesting that our eggplant alcohol extract

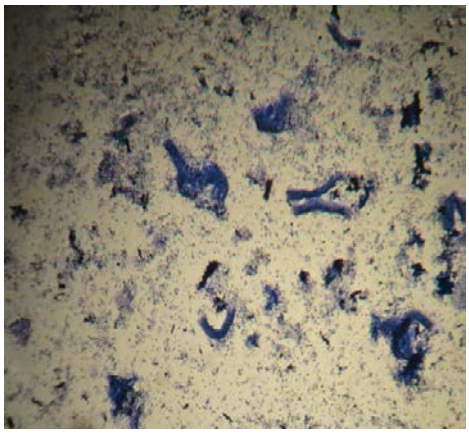

Figure 5: Normal rat male metaphase spread chromosome stained with Gemsa stain (300X)

stain can bind to DNA through several different binding modes [36]. The type of bands produced may also depend on the extent of denaturation undergone by the chromosome structure.

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