Towards technical development of natural dyes for the textile industry in Kenya: A case study of B*ixa orellana* solvent extracts

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Abstract

The aim of the study is to develop dyes of textile fibres using the natural dyes extracted from the seeds of Bixa orellana Linn. cultivated in tropical part of the coastal region of Kenya. Natural dyes originating from plants have fiscal advantage over synthetic dyes owing to their non-allergic, non-toxic, non-carcinogenic and eco-friendly natural dyes properties. The seeds are covered by a red resin which contains a number of carotenoid compounds that constitute the main colouring agents; Bixin and Norbixin are the predominant colouring compounds. The dye was extracted using solvent extraction method. Annatto colouring compounds can be identified by (GC-MS) technique. Analyses of the essential oils were carried out using GC, GC-MS and GC co-injection (of some of the available essential oils with authentic samples) using capillary gas chromatograph Hewlett Packard (HP) 5890 A Series II equipped with a split-less capillary injector system, cross-linked Hewlett. The extract was then used to dye cellulosic fibre by Exhaustion method. Dyeing was carried out using mordants through pre and post mordant in order to compare the fastness properties of the dye. Reflectance curves of the dyed materials were made using GretagMcBeth colour Eye 700 A^0spectrophotometer. From the results, the plant studied is a promising dye-yielding plant and produces a yellow orange colour.It could be exploited as a source of textile dyes and an economic plant. Additionally provide more in depth knowledge on behavior of annatto dyes and dyed cotton textile fibres.

Key words: Natural Dye, Bixa orellana Linn Seeds, Textile Fibres

INTRODUCTION

Globally, natural dyes for decades have been used as source of colorants in the production of home-based craft materials and food substrates among others (Delgado-Vargas, & Paredes-López, 2002). Natural dyes originating from plants have lately gained economic lead over synthetic dyes due to their non-allergic, non-toxic, non-carcinogenic and eco-friendly natural dyes properties (Bhuyan and Saikia, 2005; Arunkumar & Yogamoorthi, 2014; Alemayehu and Teklemariam, 2014).

Part of the plants such as roots, leaves nuts, flowers seeds fruits and young shoots are source of natural dyes. The outer, inner bark and heart wood of trees also produce natural dyes (Adrosko, 2012). Manufacturing of synthetic dyes is reliant on petrochemical source which some of the sources may contain carcinogenic amines (Hunger, 2003). The application of such dyes causes serious health hazards and influences negatively the eco-balance of nature (Goodarzian and Ekrami 2010; Jothi 2008: (Lorek and Lucas, 2003). Moreover, many countries already imposed stringent environment standards over these dyes. Due to this environmental and health implications of synthetic dyes, most of the dyes have started looking at the potential of using natural dyes day in day out dyeing (Samanta and Agarwal, 2009: Ado *et al.*, 2014).

In Kenya, textile industries use synthetic dyes and yet there are approximately 600 plant species especially the dye-yielding plants which some of the have not yet been characterized (Mayunga, 2007). They have neither been identified nor documented except for use in local herbal medicine and represent an enormous reservoir of new molecules with potential dye activities which are waiting to be discovered. One such product from nature is annatto dye. Little has been done on natural dyes in Kenya and hence there was need for this research to avail data for future reference and application.

In endeavor to classify plants with the probable dye-yielding, we chose to work on *Bixa orellana.L;* a plant that has been reported to have numerous essential oils of which geranylgenaniol and Ishwarane being the major components (Lawrence and Hogg, 1973; Jondiko and Pattenden, 1989).

Bixa orellana L. is a versatile plant commonly known as Achiote or lipstick tree and its well known as a source of red pigment known as annatto (bixin) produced from the seeds. Bixa has long been used to colour food products like cheese, fish and salad oil (Lawrence and Hogg, 1973). Despite the abundance of essential oils in seeds, fruits and leaves, there is no scientific documentary evidence of the plant on its use as a textile dye in Kenya (Sinha *et al.,* 2013) and (Paiva Neto *et al.,* 2003). Moreover, no laboratory experiments had been conducted using the plant`s essential oil or other products to investigate the dyeing of fibres. The seeds were used as a source of natural dyes. The current work reports developing textile fibres dye using *Bixa orellana.L* seeds.

METHODOLOGY

Plant materials, seeds

Commercial seeds of *Bixa orellana* were obtained from Kenya Bixa Limited in Tiwi, Kwale County along Likoni-Ukunda road at Latitude 4.2384^0S, Longitude 39.59502 ^{o} E in April 2014. Air dried seeds of *Bixa orellana* were separately subjected to steam distillation and solvent extraction.

Steam Distillation of Bixa

Four kilograms of the seeds were put into a tank that has a wire mesh to suspend the seeds. Three liters of water was added to the tank. The tank was then covered. The lid of the tank had a hole to which Dean and Stock apparatus was used (Chowdhury *et al.*, 2006). The tank was then heated from below. The heat generates the steam below the wire mesh and breaks the oil globules thereby releasing the essential oils. The oil is then forced by the steam up through the dean stock apparatus to the condensation tube where the steam is condensed to form an aqueous phase and essential oil floating on top (Chowdhury *et al.*, 2006). The water and the essential oils were separated at the top of the dean-stock apparatus by decantation. During the process of distillation, the oil came out in three different colors, first light yellow oil, followed by light green oil and finally dark green oil.

Analysis of these oils by thin layer chromatography and GC-MS indicates that the three oils had the same components varying only in abundance of the extraction. Alternative method of extraction discussed below was then used (Rath *et al.*, 1990).

Solvent Extraction of Annatto Dye

Dry commercial seeds of four kilograms were shaken in five liter conical flask using a rotary orbital shaker at a speed of 200 revolutions per minute under distilled hexane of three liters for four hours at room temperature. Aluminum foil was used to cover the flask to minimize light (Scotter *et al.*, 1998). The solution was then decanted and the seeds shaken further under two liters of hexane for two hours then decanted. The combined extract was then filtered and the solvent removed using a rotary evaporator at 60^oC leaving behind 114.5 grams of a deep red colored solution (Scotter *et al.*, 1998).

Column Chromatography of Bixa

Seven grams of yellowish green oil was chromatographed on a column packed with 150 grams of Silica gel. The column was the first eluted with 500 ml of 100% analar grade 40-60^oC petroleum ether. Five fractions of 100 ml each were collected (Selvi *et al.*, 2013). The first three portions did not contain any compound as was revealed by analytical thin layers chromatography (TLC). The fourth and the fifth fractions resulted in a bluish liquid when it was later on concentrated. The column was then eluted with 500ml 90% petroleum ether and 10% ethyl acetate. Five other 100ml fractions were collected again. Each of them gave colorless solutions that became greenish when concentrated (Selvi *et al.*, 2013).

This was followed by a change of solvent system once more this time using 2.5 liters of 70% petroleum ether and 30% ethyl acetate. Twenty fractions each of 50ml were then collected and analyzed by TLC. The next fifteen fractions resulted in light yellow oil component which gave almost similar spots on TLC plates (two large peaks and some other smaller ones) while the remainder gave colorless solutions even after concentration. Concentration of the yellow solutions yielded 4.2 grams of oil. The column was then rinsed with another 500ml of 60% and 40% petroleum ether and ethyl acetate respectively (Selvi *et al.,* 2013).

Five fractions each of 100ml were then again collected. These fractions were colorless and the TLC did not reveal any major spots. The yellow fractions from the above column were re-chromatographed on a column packed with 130 grams silica gel because it was suspected that one of the major spots revealed by the TLC could have been bixin (Mercadante *et al.,* 1997).

GC and GC-MS analysis of Bixa components

Analyses of the essential oils were carried out using GC, GC-MS and GC co-injection (of some of the available essential oils with authentic samples). GC and GC co-injection were performed on capillary gas chromatograph Hewlett Packard (HP) 5890 A Series II equipped with a split-less capillary injector system,cross-linked Hewlett Packard Ultra Methyl Silicone (50m length, 0.22m internal diameter, 0.33µm Carbowax film thickness) capillary column and flame ionization detector coupled to Hewlett Packard 3396 series II integrator (Fig 3 and 4). Hydrogen gas was used as a source of fuel, while nitrogen gas flowing at a speed of 0.8ml/min was used as carrier gas. Before the sample was injected a compensation run was made for a period of one hour. Temperature programs consisted of an (i) initial temperature of 50^{o}C for 5 minutes then raised to 280^{0} c at a rate of 5^{0}C per minute finally maintained at 280^{o}C for 20 minutes for the steam distillate (Fig 1). Bixa fraction and Bixa solvent extract (Fig 2) and (ii) initial temperature of 60^{o}C for 5 minutes rose at 130^{o}C at a rate of 4^{o}C per minute, raised again to 175^{o}C at a rate of 1^{o}C per minute, then raised once more to 295^{o}C per minute at 30^{o}C per minute and finally held at a constant temperature of 295^{o}C for a period of 20 minutes (Scotter *et al.,* 2000).

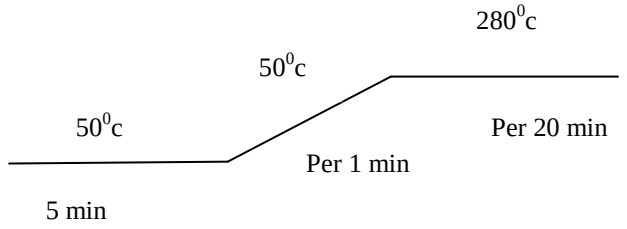


Figure 1: GC and GC-Ms temperature programme for steam distillate and solvent extracts

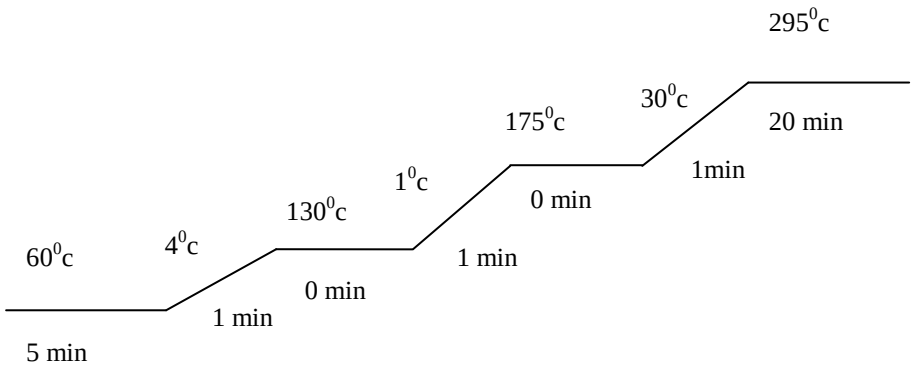


Figure 2: GC and GC-MS temperature programme for vacuum distillate

GC-MS analysis was carried out on a Hewlett Packard 5790A series II Gas chromatograph coupled to a VG analytical organic mass spectrophotometer manufactured by Micro mass, United Kingdom formally known as a VG Biotech. The mass spectrophotometer (Ms) was operated in the electron ionization (EI) mode at 70 electron volts (eV) and an emission current of 200 micro amperes (µA). The temperature of the source was held at 180^{o}C and a multiplier voltage of 300 volts. The pressure of the ion source and MS detector were held at 9.4x10^{-5} milliards respectively and1.4 x10^{-5} millibar respectively (Scotter *et al.*, 2000).

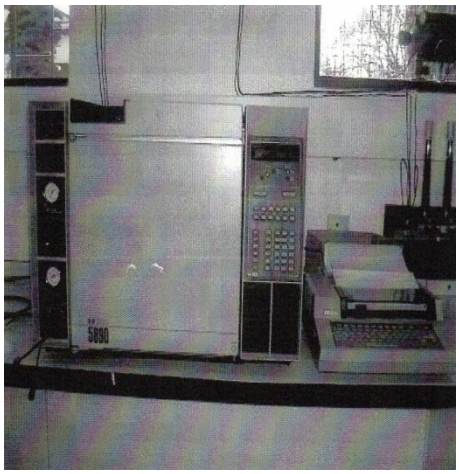


Figure 3: A HP 5890 series gas chromatograph with an integrator (Source: Author, 2014)

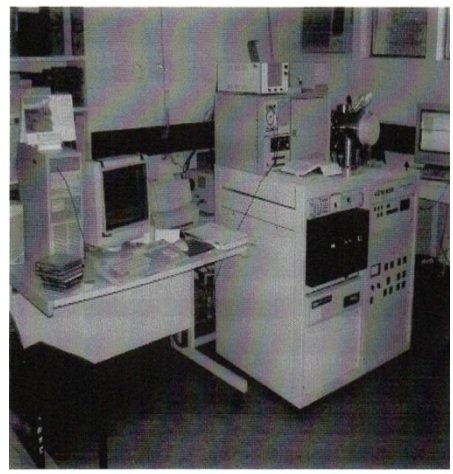


Figure 4: GC-MS integrated with computers (Source: Author, 2014)

The MS had a scan cycle of 1.5 seconds (scan duration of IS and inter-scan delay of 0.5s). The mass and scan ranges were set at mass charge ratio (m/z) of 1-1400 and 38-650, respectively. The instrument was calibrated using heptacosafluortributyl amine.

The column used for the GC-MS was the same as the one described for GC above except that the film thickness was 0.5µm. The temperature programme was similar to the ones described for GC above. Helium was used as a carrier gas. In both GC and GC-MS, high performance liquid chromatography (HPLC) grade dichloromethane (DCM) was used as a dilution solvent (Chao *et al.*, 1991).

Sample volume for Bixa crude extract are steam distillate (referred to here as Bixa D.G) was 2µL while a dilution ratio of 1:200 µL was used. Sample volume for vacuum distillate referred to here as Bixa D.B) and all column chromatographic fractions was 6µL whereas the dilution ratio was 1:40. Sample volume for the non-polar hexane column chromatography eluent of oleoresin was 6µL while sample volume and sample dilution ratio was in a ratio of 1:40. The differences in sample size and sample dilution ratio were as a result of differences in complexity of essential oil components and therefore the sample volume and dilution ratios above were the ones that gave the best separation after several attempts (Chao *et al.*, 1991).

Dyeing Procedures

Dyeing fabric

Cotton (cellulose based) fabric articles 002A and 053A in both bleached and non bleached (100m state or grey cloth) conditions were obtained from the processing department within Rivatex for experimental dyeing. Article 002A at Rivatex is constructed as follows; warp/weft count 43/43 nm; 13 picks/cm and 25 ends/cm while article 053A has its construction as warp/weft count 18/18 nm; 16picks/cm and 23 ends/cm (Bechtold *et al.*, 2003).

Fabric scouring

The cotton fabric articles cut into small size pieces whose total weight was 100 grams (g) were first washed in hot water for about 10 minutes using a non-bleach washing powder. A stainless steel pot was filled with 5 liters of water in which 35g soda ash was carefully added. The wet fabric articles were then put into the water and swished around using a stirring rod. The glass rod was left inside the pot to prop the lid slightly open to prevent the liquid from boiling over. The water was eventually brought to the boil (Sun and Stylios, 2004).

The heat was adjusted to low boil or hard simmers and allowed the fiber to boil half covered for 2 hours. The fabric articles were stirred every 15 minutes to ensure adequate scouring. The sauce pan was removed from the heat source after 2 hours and the fabric articles allowed to cool down until they could safely be removed from the water. The fabric articles were finally rinsed in clean cold water (Sun and Stylios, 2004).

Fabric mordanting

Mordanting was done in two sets as follows;

Single mordanting in an Alum/Vinegar bath
An alum [hydrated aluminium potassium sulphate],[$KAl(SO_4)_2.12H_2O$] mordant bath was prepared for this procedure. A solution containing 25g of alum, 30ml of vinegar (3% acetic acid) in three liters of water was prepared. The solution was brought to boil and then let to cool. The fabric articles were inversed into the cool mordant bath whose temperature was eventually brought to the range 80-90^oC by heating. This was simmered for 1 hour, removed and then let to cool (Siva, 2007).
Tri-mordanting in three successive mordant baths of Alum, Tannin and alum

Bath 1 (Alum mordant)

The dye pot was filled with 3 liters of water and nearly brought to boil. Alum 25g were first dissolved in a small container with boiling water and slowly added to the pot and stirred well.

Soda ash (Na_2CO_3) of 6g were then weighed out and added slowly and carefully to the water in the pot. The cleaned and scoured fabric articles were then added to the pot heated to simmering point and simmered for 1 hour. The fabric articles were stirred every fifteen minutes during simmering and eventually left in the pot for 24 hours. The fabric article pieces were wringed well, dried in the open air and left to age for a week. The pieces were washed well before treating them in the second bath (Balakina *et al.*, 2006).

Bath 2 (Tannin mordant)

The dye pot was filled with 3 liters of hot water. Tannic acid (6g) were dissolved in a small container with boiling point and added to the pot to which the cotton fabric articles were added. Simmering was done for 1 hour and left for 24 hours. The cotton pieces were then wringed well, dried and left to age for a week. The pieces were washed well before treating them in the third bath (Bai, 2014).
Bath 3 (Second Alum Bath)

Alum mordanting was repeated as described in Bath 1.

Dye Extraction from *Bixa orellana*

50g of Annatto seeds in a flat bottomed flask together with 100ml of ethyl alcohol was agitated in a magnetic agitator for 15,30, 60 and 120 minutes. After agitation, the mixture was sieved using a 200 mesh screen. The filtrate was saved for future distillation purposes and the reuse of alcohol used. The filtered dye was dried at a temperature of 60^oC in an air circulating oven for 30 minutes. After drying, the dye was pulverized and conditioned in a suitable container (Siva, 2007).

Dyeing

The dye bath was prepared with 5% (W/W) annatto dye which was solubilised with a solution of 0.5% Na_2CO_3 filtered through a 200 mesh screen in order to eliminate any impurities. Cotton samples weighing 100g were dyed in a COLOUR PET 12 with a liquor ratio 100:1 and at temperature of 60^oC for 30, 60 and 90 minutes. Tartaric acid, tannin and Alum were used as mordants. The mordants were added at the beginning of dyeing at concentrations of 5 and 10% (W/W). After dyeing the samples were washed with distilled water at room temperature in the same equipment for 20 minutes. Later they were dried (Sudhir, 2003).

Testing dyed fabric

Fastness to washing

A soap solution made up of 5g soap and 2g of anhydrous sodium carbonate (Na_2CO_3) in one litre of distilled water in a beaker was prepared and heated to 60^0C.

Specimens of dyed cotton fabrics measuring 10 cm by 4 cm were cut out. They were separately placed between two pieces of undyed cloths free from finishing chemicals of cotton measuring 5cm by 4cm and each of the now three pieces held together by stitching round the edges leaving 5cm by 4cm exposed.

The specimens were then placed in a beaker for 30 minutes with constant stirring while maintain the temperature at 60^oC by steady heating. They were removed and rinsed in cold water for 10 minutes. The specimens were then squeezed and dried in air not exceeding 60^oC (Siva, 2007)).

Bleaching test on dyed fabric

Pieces of dyed fabrics were immersed into a 3.85% V/V solution of Sodium hydrochlorite (NaOCl) and left to stand for a period of one hour (Eren *et al.,* 2009).

Testing for type of dye produced by *Bixa orellana*

One half of a liter of 5% W/V caustic soda (NaOH) solution was prepared and put in a saucepan. Pieces of dyed fabrics were added, then heated and brought to boil for a period of 2 minutes (Siva, 2007)

RESULTS AND DISCUSSION

Composition of *Bixa orellana* extracts

In steam distillation of the seeds, analysis of the essential oils by thin layer chromatography indicated that three layers of oils had the same components varying only in abundance of the extraction. Also during solvent extraction of the dye, the solvent removed using rotary evaporator at 60^0C leaving left behind 114.5 grams of a deep red coloured solution.

Bixa orellana is a mixture of compounds and on comparing the peaks on solvent extract and on authentic samples C_8 to C_{20} alkane standards (Fig.5), it shows that the sample was present. The tallest peaks are due to phthalate internal standard .Identification of each compounds was done on matching with libraries. The fibre constituents such as alkanes are similar to those reported by Jayawikrama (2006).

A study done by Pino and Correa, (2003) on the chemical composition of the essential oil from annatto (Bixa orellana L.) seeds identified 35 components by the use of GC/MS where some are alkanes.

Galindo-Cuspineira *et al*., (2003) by GC-MS recorded the existence of 107 compounds where some oil and other soluble in water. The following compounds were identified in the aqueous and oily: alcohols, aldehydes, alkanes, alkenes, ketones, esters and acids among others.

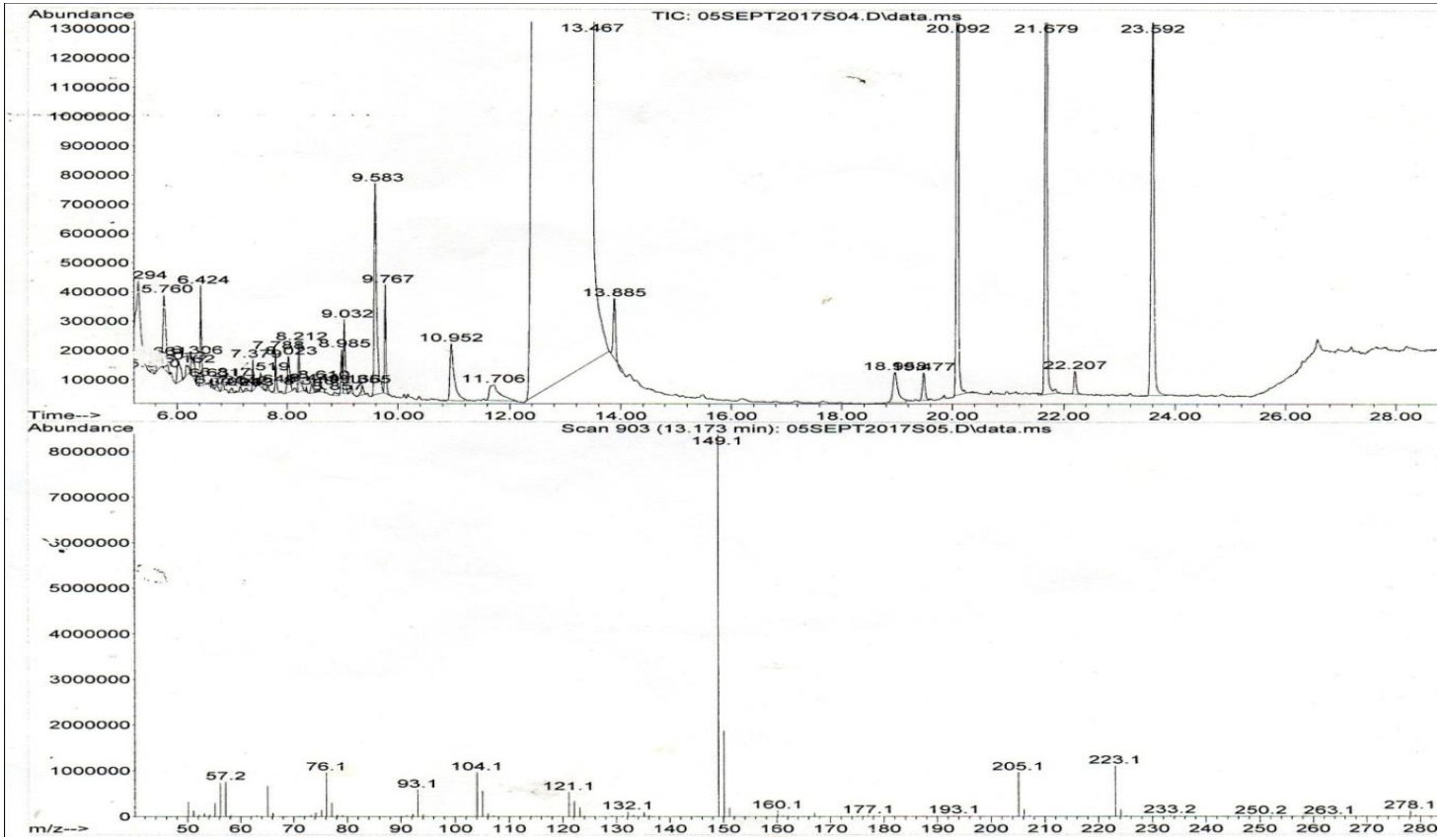


Figure 5: The GC-MS Spectra of C_8 to C_{20} n-alkane standard and Ion Chromatogram of identified compunds at each retention time

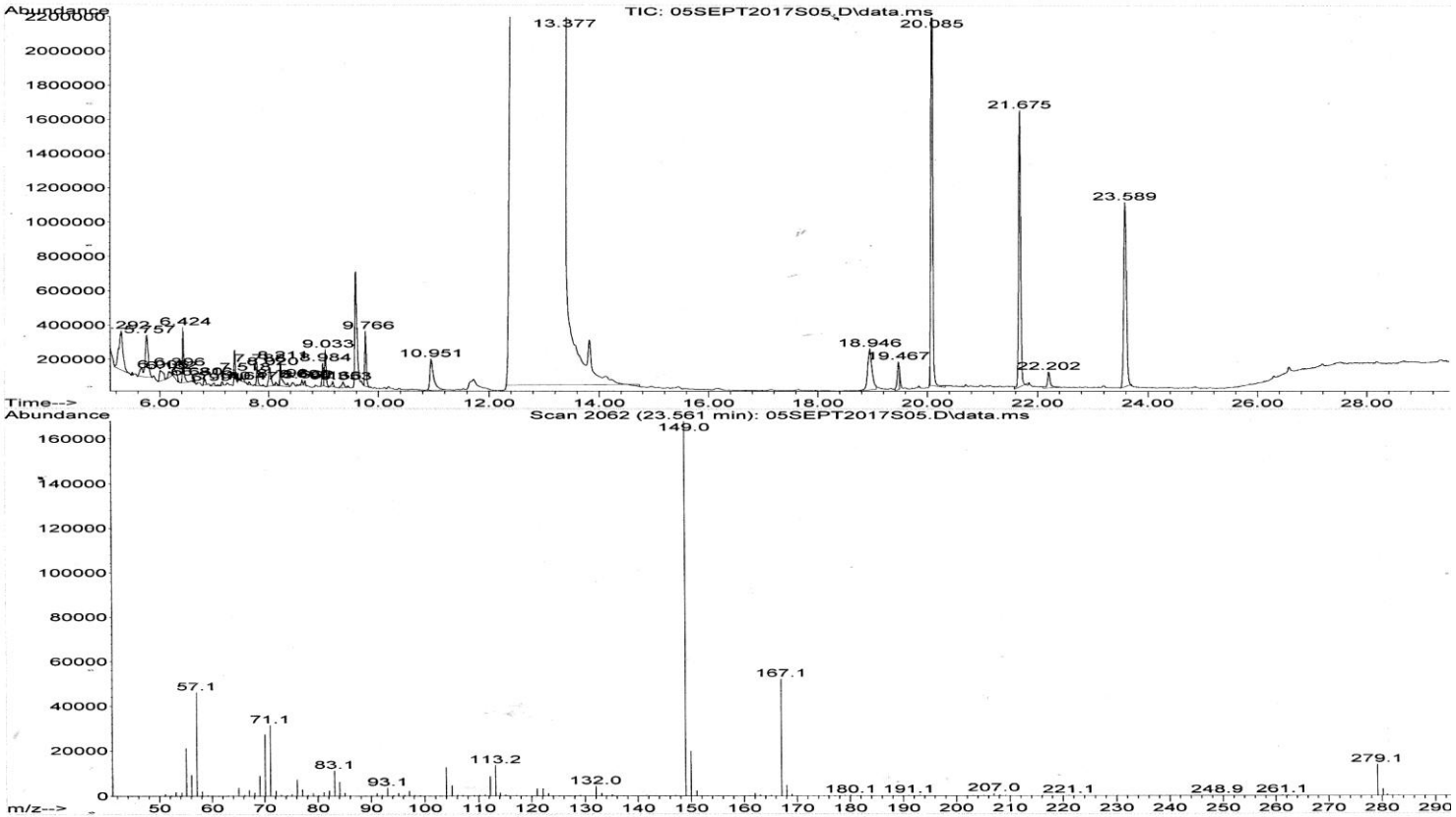


Figure 6: The GC-MS Spectra for *Bixa* Solvent extract

Dye extraction from *Bixa orellana.L*

The dye-bath obtained from the seeds had a yellow orange appearance as illustrated in Figure 8.

Figure 7: Bixa dye bath
(Source; Author, 2014)

Fabric mordanting and dyeing

Conspicuous change of colour on fabrics was observed. The fabric articles took up a yellow orange colour (Fig.7) early in the dying process. Depending on the concentration in solution *Bixa orellana.L* form the colors ranging from yellow orange to reddish orange (Yusa Marco *et al.*, 2008). Fabric articles tri-mordanted and dyed at room temperature and pressure and those at the range of 50 to 60^{o}C showed inconsistency in colour absorption especially at room temperature and pressure.

However all articles dyed in the temperature range of 80-90^{o}C exhibited uniform absorption of colour, Figures 8.Single mordanted articles were dyed in the temperature range of 80-90^{o}C (Fig 8b). There seemed to be no clear difference except for brighter colours for the tri-mordanted 002 articles compared with the single mordanted,

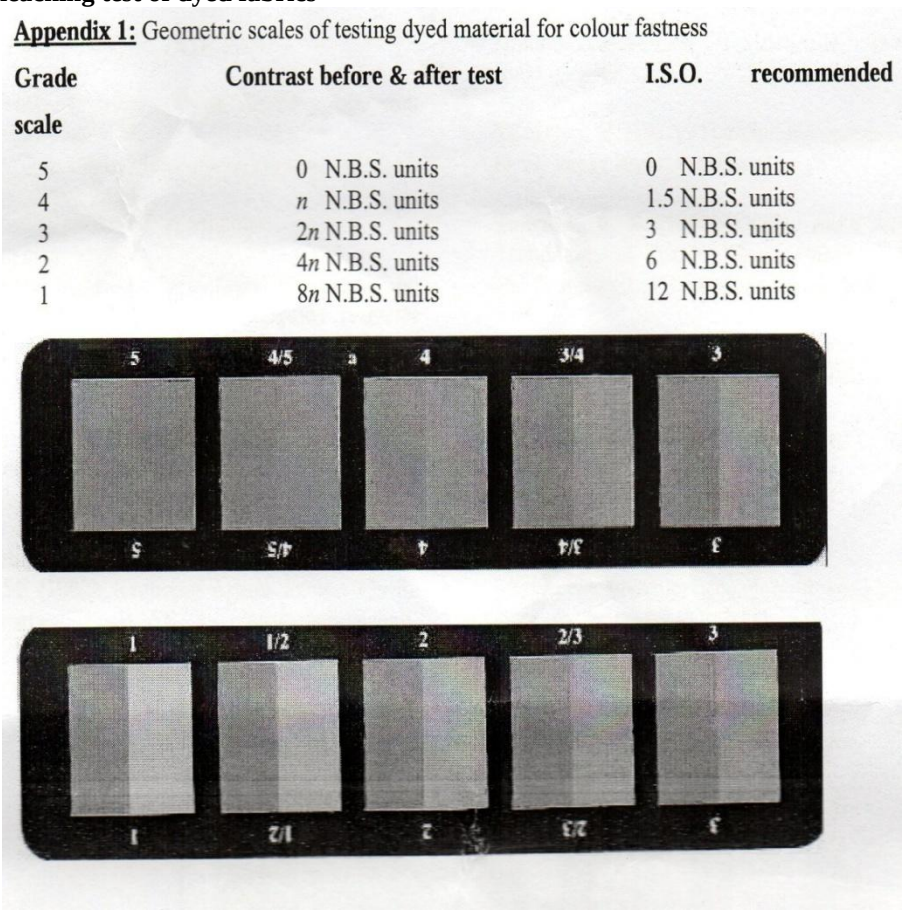
This could be attributed to other prevailing factors and conditions during the dyeing process that brought about the differences. Visual observations showed that unbleached fabric articles had a brighter intensity of colour as compared to the bleached ones especially for the articles 002 whereas brightness was greater in the bleached fabrics for both articles 002 and 053, (Figures 8c).

The characteristic odour of *Bixa orellana.L* which could have repulsive effects to different people was totally absent after dyeing and drying the fabric articles. The plant has a peculiar sweetish odour and an unpleasant saline bitterish taste (Giridhar *et al.*,

2014). This is a very positive attribute indicating that factors contributing to the odour emanating from *Bixa orellana* plant prior to the dye extraction are absent in the dye produced. Addition of caustic soda 8ml per liter (as a bleaching test) to the dye bath and dyeing produced dark brown shade, Figure 7.

Physico-chemical testing of cotton fabrics

Bleaching test of dyed fabrics



Appendix 1: Geometric scales of testing dyed material for colour fastness

Grade scale	Contrast before & after test	I.S.O. recommended
5	0 N.B.S. units	0 N.B.S. units
4	*n* N.B.S. units	1.5 N.B.S. units
3	2*n* N.B.S. units	3 N.B.S. units
2	4*n* N.B.S. units	6 N.B.S. units
1	8*n* N.B.S. units	12 N.B.S. units

Figure 9: Geometric scales of dyed cotton material for colour fastness
(Source; Lab manual)

There was no reaction of NaOCl on dyed fabric when pieces were immersed in concentrated bleaching solution for one hour except for the brightening effect. Thus, the dye is not easily bleached by ordinary bleaches because it sticks firmly to the fabrics

FIGURES:

Figure 8 a: Tri-mordanted cotton fabric bleached) article 002 dyed at rtp.Control on the left.

Figure 8 b: Tri-mordanted cotton fabric (un-bleached) article 002 dyed at rtp. Control on the left

Figure 8 c: Tri-mordanted cotton fabric (bleached) article 053 dyed at rtp. Control on the right.

Figure 8 d: Tri-mordanted cotton fabric (un-bleached) article 053 dyed at rtp. Control on the left.

Figure 8 e: Tri-mordanted cotton fabric (bleached) article 002 dyed at 50- 60^0 C. Control on the left.

Figure 8 f: Tri-mordanted cotton fabric (un-bleached) article 002 dyed at 50-60^0c. Control on the left.

Figure 8 g: Tri-mordanted cotton fabric (bleached) article 053 dyed at 50-60^0 C. Control on the left.

Figure 8 h: Tri-mordanted cotton fabric (bleached) article 053 dyed at 50-60^0 C. Control on the left.

Testing for the dye obtained from *Bixa orellana.*

There was considerable stripping on the dye from the dyed fabrics. Preliminary dye testing using 5% NaOH whereby not so much of the *Bixa orellana* dye was observed in the alkali solution though the fabric showed a considerable removal of the dye was a clear inference that the dye was a reactive dye.

Fastness to washing test

The fabric articles showed no fading in the shade acquired during the dying process after carrying out the fastness to washing test. Fastness test carried out on the fabric according to the McLaren (1952) grade scale gave grade 5 (Figure 9) (Textile laboratory manual). A study done by Selvi *et al.,* (2013) on studies on the application of natural dye extract from Bixa orellana seeds for dyeing and finishing of leather showed that he leathers dyed and finished using the *Bixa orellana* seeds extract showed better coloring properties

CONCLUSION AND RECOMMENDATIONS

Conclusion

The results showed that *Bixa orellana.L* plant is capable of producing viable dyes that can be used for dyeing cellulose-based fabrics. *Bixa orellana.L* produces reactive yellow orange dyes as evidenced in this study. However, different colours could be produced on the fabrics depending on the type of mordant used in fixing the colours on to the fabrics. In this study it was found out that high temperatures of between 80-90^{o}C are optimal for dyeing. This is because higher temperatures with a component of pressure could give better results in terms of brightness and fastness. This calls for further research. GC, GC-MS and co-injection remain the most appropriate methods of analysis of complex mixtures as evidenced from this study. By modified GC parameter it was possible to identify several components in the seeds of *Bixa orellana.L.* The more important red pigment present in this seeds is oxygenated carotenoid alpha bixin or Cis-bixin which forms 80% of the total carotenoids. The annatto dye extracted has the potential of being used as natural dye because they show dyeing properties at relatively low concentrations and for relatively longer period of time.

Recommendations

It is therefore recommended to evaluate the potential of using the annatto dye as one of the means of dyeing in the textile industries in Kenya and especially in the coastal regions where *Bixa orellana.L* plant is grown as a commercial crop. Isolation of individual components of the Bixa dye was not successfully achieved. It is therefore recommended that other spectroscopic isolation procedures such as preparative HPLC be made use of to isolate the compounds.

Moreover, isolation of the individual compounds, bixin and norbixin, would make it possible to conduct further analysis to determine whether the activities demonstrated by the annatto dye is as a result of these major components or not. Alternatively, the

synthetic dyes similar to the components of the annatto dye from the seeds could also be used for comparison.

Since in this study, only short term toxicological effects of the components was conducted, this does not give clear picture of any serious dermatological effects the dye might have on the users of the dyed fabric. It is therefore recommended that a specialist in the area of toxicology to carry out more detailed study on this dye. This would ensure that the potential users of these dyes do not expose their skins to a health hazard resulting to diseases.

The findings of this study points to the fact that dye extracted from *Bixa orellana* plant is possible. The properties of the dye obtained compare favourably with commercial reactive dyes that are petro-chemicals in nature. *Bixa orellana* dye is a primary colour, yellow which together with the other two red and blue could yield all other desired colours, for dyeing purposes. This study is a milestone in the dyeing industry as the world seeks to protect environment from synthetic dyes. The study however reflects the tip of the iceberg of a research that should be carried out in more detail and over a longer period of time.

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