Analysis of Bioactive Substances and Colouration Potential of the Stem of *Annona senegalensis* grown in Ankpa, Nigeria.

By

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ABSTRACT

This work analyzed the bio-active substances in the stem of Annona seneganlensis and its colouration potential on substrates. Analysis conducted on the plant extract showed the presences of some medicinal bioactive agents used in curing some ailment. Colouration on the fabric showed varied colour shades with potassium dichromate and copper sulphate mordants displaying fastness superiority.

Keywords: Bio-active, Fastness, Mordant, Substrates.

INTRODUCTION

Medicinal plants are the richest bio-resources of drugs of traditional system of medicine, modern medicine, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al,* 2008). Plants are source of large amount of drugs that posses anti-microbial, anti-cancer, anti-inflammatory, antiviral activities. Photochemical screening conducted on the bark of *Anacardium occidentale* showed the presence of medicinally bio-active substances as alkaloid, flavonoids, phytosterols, phenols protein and amino acid and carbohydrates (Egu, *et al,* 2016).

Apart from bio-active substances, plants (root, stem, fruit, and seed) also contain colouration materials (Gill, 1992; Keay, 1989). Colour is a psychological sensation produced when rays of visible range approach the eye (Sharma, 2011). A dye is a colour substance capable of colouring fabrics in such a manner that the colour cannot be removed by rubbing or washing. All dyes basically were of natural origin. The recent interest in the use of natural dyes is based on the fact that they display better biodegradability and general compatibility with the environment (Eleftheraidis and Tsatwaroni, 1989). Egu *et al* (2016) recommend the return to natural plants as some are friendly, less toxic, biodegradable and compatible with the environment.

The plant under study, A*nnona senegalensis* belong to the order-magnoliales and family-*Annonaceae*. It is commonly known as African-custard apple and wild soursop. The Yorubas call it *Abo-ewe-aso*, Hausa *Gwandar daji* (Pinto *et al,* 2005; Suleiman *et al,* 2008). The Igala's called it *Ukpokpo*; it grows mainly as shrub about 2 – 6 meter tall and occasionally, it grows as tall as 11

meter. The leaves bark and roots have been in use for years in curing various illnesses (Orwa et al, 2009).

METHODOLOGY

The stem of *Annona senegalensis* was collected, chopped to pieces and dried, and then pulverized to fine particle sizes (Stahl, 1965). 50 g of the pulverized sample was weighed into the thimble of the soxhlet extractor and 150 ml absolute ethanol was added as the solvent for extraction. The mixture was reflux for 3 h. The extract was collected, distilled and evaporated to dryness to obtain the dried solute.

Phytochemical Analysis

Standard phytochemical screening method was employed for each of the parameters as described by Prashant *et al*, (2011).

Alkanoids:

Wagner test. 0.5 g of the extract was dissolved in dilute hydrochloric acid (HCl) and filtrated. The filtrate was treated with 2 ml Wagner reagent, the mixture was then observed for formation of yellow colour precipitate.

Carbohydrates:

1 g of the extract was dissolved in 5 ml distilled water and filtered. The filtrate was treated with Benedict reagent and heated gently, and formation of Orange-Red precipitate was observed.

Glycosides:

Modified Borntrager's reagent. 0.5 g of the extract was treated with 2 ml dilute hydrochloric acid and 2 ml ferric chloride solution and immersed in boiling water for about 5 min. The mixture was then cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with 2 ml ammonia solution, and then it was observed for the formation of rose-pink colour in the ammonical layer.

Saponins:

Foam test. 0.5 g extract was diluted with 2 ml distilled water and was shaken. The mixture was allowed to stand for 10 min and the foam produced was observed for persistence for 10 min.

Phytosterols:

Salkowski's test. 0.5 g of the extract was treated with chloroform and filtered. The filtrate was treated with 2 drops of concentrated tetraoxosulphate (VI) acid, shaken and allowed to stand for the appearance of golden yellow colour.

Phenols:

Ferric chloride. 0.5 g of the extracts was treated with 4 drops of ferric chloride solution. The mixture was then observed for bluish black colouration.

Flavonoid:

Alkaline reagent test. 0.5 g of the extract was treated with 3 drops of sodium hydroxide solution and was observed for a yellow colouration to which was then added dilute hydrochloric acid and was observed for a colourless change.

Proteins/Amino Acid:

Xanthoprotic test. 0.5 g of the extracts was treated with 4 drops of concentrated nitric acid, the mixture was observed for a yellow colouration.

DYEING METHOD

Preparation of the Dye solutions:

The dried sample of the extract (1.0 g) was weighed into a beaker. 5 ml of water was added to the dye sample to make it into paste, and washed into 250 ml beaker with water at 50 ^{o}C to bring the total volume of the solution to 100 ml. The solution was allowed to boil for 5 min to aid proper dissolution of the dye and the solution was then cooled.

Preparation of dye bath:

From the dye solution already prepared, 25 ml was measured into a bath (beaker), and this was further diluted to 100 ml.

Preparation of the Fabric:

The fabric material to be dyed was scoured with soap and rinsed before placing it in the dye bath; this helps the dye liquor to penetrate properly through the fibre giving uniform and level dyeing (Sharma, 2011).

DYEING OF THE FABRIC:

Unmordanted Dyeing

To the dye bath solution, 2 g of the scoured fabric was introduced. The dyeing process commenced and lasted for 30 min at 60 ^{o}C. When the dyeing process was completed, the material was removed from the dye bath and allowed to air oxidize for 10 min after which it was rinsed with cold water to remove loose dye particles that adhered to the surface of the dyed material. The dyed fabric was then air dried after which the fastness properties were tested (Otutu *et al.,* 2010).

Mordanted Dyeing

Mordanting of the fabric was conducted by introducing 2 g of the scoured fabric into 2 % solution of potassium dichromate, alum, copper (II) sulphate, iron (II) sulphate and warmed for 30 min at 60 ^{o}C. The fabrics were then dyed in the dye bath for another 30 min at 60 ^{o}C, it was then air oxidized and air dried after which the colour shade/hues and fastness properties were tested and compared with the unmordanted fabric. (Otutu *et al.,* 2010).

FASTNESS PROPERTIES OF DYED TEXTILE

Light fastness:

One set of the dyed textile fabrics was exposed to sunlight for one week, while the other set was kept in wrapped black polyethene bag for the same period of time. The two sets were rated on a grey scale. (Otutu *et al.,* 2010).

Wash fastness:

One set of the dyed fabrics was washed using mild soap and the other set was washed with detergent at 60 ^{o}C for 30 min and were air dried. The washed fabrics were compared with the unwashed on the grey scale. (Otutu, *et al*., 2007)

Cosmetic Colouration:

100 g petroleum jelly was weighed and melted. Then 2.0 g of the dye sample was weighed into a test tube and was dissolved using ethyl alcohol. The dissolved dye was introduced into the melted petroleum jelly and the mixture was heated to 80 ^{o}C for an even mixture of the dye molecule. The mixture was cooled and the colour imparted was visually identified. (Otutu & Emmanuel, 2009).

Food Colouration (Pap):

100 g of pap (akamu) was first weighed and dissolved in water. 2.0 g of the dye sample was weighed into a glass beaker; water was then added to make it up to 200 ml at 60 ^{o}C with continuous stirring. The dye solution was heated to boiling point. The boiling dye solution was poured into the pap solution in a 500 ml beaker and the mixture was stirred vigorously until a uniform semi (molten) solid pap meal was obtained. The imparted colour was determined visually (Osabohien & Ukponmwan, 2002).

Alcoholic drink colouration:

2 g of the dye sample was weighed into a glass bottle, 100 ml of illicit gin was measured into the glass bottle and corked. The mixture was vigorously shaken for 10 min until a homogeneous mixture was obtained and the colour produced was fiscally determined (Osabohien, 2009).

RESULTS AND DISCUSSION

Table 1: Preliminary Results

Plant	pH Value	Melting Point	Colour (visual)	Solubility in cold H_2O	Solubility in warm H_2O
Stem of *Annona senegalesis*	4.7	108 – 110	Dirty brown	Soluble	Soluble

Table 2: Percentage Yield

Plant	X_e (g)	Y_p (g)	Percentage yield (%)
Stem of *Annona Senegalensis*	5.60	50	22.56

Table 3: Phytochemical Screening Result

Parameters	Test Observation	Observation	Inference
Alkaloids	Wagner's test	Brown precipitate(ppt)was not formed	-
Carbonhydrates	Benedict test	Orange red ppt was formed	+
Glycosides	Modified Borntrager's test	Pink colour was not formed	-
Saponins	Foam test	Foam persist after 10mins	+
Phytosterols	Salkwoski's test	Golden yellow was formed	+
Phenols	Ferric chloride test	Bluish black colour was formed	+
Flavonoids	Alkaline reagent test	Intense yellow colour formed in NaOH which turned colourless in dilute HCl	+
Proteins and amino acid	Xanthoproteic test	Yellow colour was formed	+

Keys:
(+) = Present
(-) = Absent

Table 4: Colour Shades/Hue and Fastness Properties of Dyed Fabrics

Dyed Sample	Colour of Fabric	Light Fastness	Washed Fastness strong soap	Washed Fastness mild soap
Unmordented	Light pink	1 – 2	1 – 2	3 – 4
$FeSO_4$	Ash	2 – 3	1 – 2	2 – 3
Alum	Milk	1 – 2	2 – 3	2 – 3
$K_2Cr_2O_7$	Light brown	3 – 4	3 – 4	3 – 4
$SnCl_2$	Pinkish milk	1 – 2	2 – 3	2 – 3
$CUSO_4$	Pinkish brown	3 – 4	3 – 4	3 – 4

Scale:
Key: 1 – 2 = Colour change; 2 – 3 = Slight colour change; 3 – 4 = Very slight colour change
4 – 5 = Colour retained

Table 4.4: Colour Imparted on Substrates

Plant	Colour Imparted
Pap	Light milkfish brown
Petroleum jelly	Brown
Illicit gin	Deep red

The results obtained showed the sample to be soluble in both cool and warm water with a slightly acidic pH (Table 1) and a poor yield (Table 2). The bio-active substances analyzed indicate the presence of all the bioactive ingredients except alkaloids and glycosides (Table 3). The dyed cotton fabrics presented wonderful colour hue with Potassium dichromate and Copper sulphate mordants displaying the best fastness properties (Table 4). Varied colour shades were imparted on the substrates (Table 5).

CONCLUSION

Natural plants are friendly and comfortable to use and handle, with diverse application for human utilization. Full exploration of these plants within our locality to serve man-kind in various positive ways will continue to be a welcome idea.

RECOMMENDATION

Our finding shows substantive bio-active ingredients in the plant, hence we recommend its usage for medicine and as dyestuff in the presence of mordants.

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