Original Article

STUDY OF PHYTOCHEMICAL COMPOSITIONS AND SEPARATION OF COLOURS USING THIN LAYER COLUMN CHROMATOGRAPHY FOR *BIXA ORELLANA* (ANNATTO) SEED EXTRACTS.

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Abstract

Introduction: *Bixa orellana* is known to be the only specie of *Bixa*ceae family and is used in Africa for painting the lips by young ladies to attract males and beautification during the annual cultural festival of Iri ji (new yam festival) of Ugwu Odida people in Onicha Igbeze. Its use was also reported in South America for production of cosmetics and as a colourant in food industries. Recently, it has been observed that the *Bixa* plant commonly known as Annatto or *Apo* stain among Medical laboratory scientists is gradually becoming an ornamental shrub in institutions of learning and homes among others in Nigeria.

Objective: To carry out phytochemical analysis and thin layer column chromatography of *Bixa orellana* (Annatto) seed extracts.

Materials and Methods: The seeds of *Bixa orellana* were extracted in distilled water, absolute ethanol, acetone, Ammonia, and xylene using maceration methods. Phytochemical analysis was carried out and the fractions of the extracts were determined using Thin Layer Column Chromatography to obtain the different colour components of the eluents used for staining.

Results: The result of Phytochemical analysis was found to show the presence of Carbohydrate Saponins, Cardiac glycosides, Anthraquinones, Flavonoids, Tannins, Steroid and Alkaloids. The results of chromatograhy fraction procedure was shown and the colors obtained were as follow: Deep red, Light orange, Red and Golden.

Keywords: Bixa orellana, Phytochemical, chromatography, extracts.

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INTRODUCTION

Bixa orellana is a multi-use plant popularly known as Annatto seed and is popularly known among Medical Laboratory Scientists as Apo stain. Central and South American populations used these seeds to colour their bodies and lips. *Bixa. orellana* is the only species of *Bixa*ceae family[1]. In Nigeria it has been sighted in many institutions of high learning where it is used as ornamental shrub, perhaps planted by scholars after being used for research and to ease accessibility and availability for those who might wish to do further research.

The species name of this plant is named after the Spanish scientist conquistador, Francisco de Orellana. *Bixa orellana* (annatto) plant is popularly known as Achiote or lipstick tree due to the reddish orange dye of its seeds. The dye is highly

concentrated in the thin coloured resinous coating capsular fruits[2]. Annatto is used in the production of foods and drugs as a colourant[2]. The oil soluble annatto dye is used in dairy- and fats-based products like butter, margarine, cheese, and in baking, pharmaceuticals, tanning of leather and cosmetics. Available literature provides information on the chemical structure of *Bixa orellana*, its extraction, formulation, processing, pharmacology and toxicology[3].

According to many, technical factors have been identified to influence annatto extraction such as concentration of solvent, ratio of solute to solvent, extraction time, and extraction temperature[3]. However satisfactory results of annatto were obtained by the use of ultraviolet-xis spectroscope analysis and Fourier transform infrared spectroscopy analysis (FTIR). FTIR has also helped to identify the chemical structure of the extract corresponding to its carotenoid compounds[3]. Considering the restrictions placed on the use of synthetic dyes by World Health Organization, interest in natural dyes is increasing.

The annatto seed pigments – *bixin* and *norbixin* are among those mostly used in the food, pharmaceutical and cosmetic industries due to the intensity of their colours, their greater stability and wide variety of colours. This latter two features are an additional advantage of the annatto carotenoids over other carotenoids such as those of carrot and beetroot. In food production, extraction with CO_2 has shown satisfactory advantage since it is an inert gas under normal conditions that makes it easy to separate from the solute, leaving behind a pure final product with no organic solvent residues[4].

The aim of this study is to determine the phytochemical composition and thin layer column chromatography of *Bixa orellana* (annatto) seed extracts.

METHODOLOGY

The study was carried out in histopathology department Faculty of Medical Laboratory Sciences, and the Department of Pharmacognosy and Ethno-Pharmacy Faculty of Pharmaceutical Science, Usman Dan Fodio University, Sokoto, Sokoto State and in collaboration with Usman Dan Fodio University Teaching Hospital, Sokoto.

The plant taxonomic identification and assigning of specimen Voucher Number was carried out at the Botany unit, Department of Biological Sciences Usman Dan Fodio University Sokoto, and a voucher specimen was prepared and deposited in the herbarium of the same department.

The study design was experimental research. The seeds of *Bixa orellana* were extracted in distilled water, absolute ethanol, acetone, Ammonia and xylene using maceration methods. Phytochemical analysis was carried out to know the chemical components of the *Bixa orellana* and thin layer column chromatography of the *Bixa orellana* extracts was also carried out to establish the staining fractions, at Faculty of Pharmaceutical Sciences, in the department of Pharmacognosy laboratory, Usman Dan Fodio University, Sokoto. Column chromatography is a separating technique, which utilizes liquid as the mobile phase and solid as the stationary phase packed in a column. In this chromatography, separation of individual component is due to the affinity of the substance to the stationary phase, and its solubility in the mobile phase due to gravity.

Table 1: Experimental design was adopted for this research

Experiments	Methods	Location
Seed collection	Manual harvesting	Nigerian Air Force Base Makurdi
Seed extraction	Maceration	Faculty of pharmaceutical science Usman Danfodiyo University Sokoto
Solvent extraction	Water, acetone, ethanol, Ammonia and xylene	Faculty of pharma ceutical science Usman Danfodiyo University Sokoto
Preliminary phytochemical screening	Quantitative analysis	Faculty of pharmaceutical science Usm an Danfodiyo University Sokoto
Chromatography	TLC, Column	Faculty of pharmaceutical science Usman Danfodiyo University Sokoto

LABORATORY ANALYSIS

The extract of *Bixa orellana* seeds was subjected to phytochemical analysis and Thin Layer Column Chromatography (TLC) Procedure to obtain the fractions and eluents needed.**RESULTS**

Table 2: PHYSICAL CHARACTERISTICS AND PERCENTAGE (%) YIELD OF *BIXA ORELLANA* SEEDS

Texture	Weight of powder (g)	Extracting solvents	Weight of extracts	% yields	Colours
	80	Acetone	24.00	30.00	Deep red
Dried	80	Ethanol	18.38	22.97	Light orange red
	80	Distilled water	15.20	19.00	Golden yellow

TABLE 3: PRELIMINARY PHYTOCHEMICAL COMPONENTS OF ETHANOLIC EXTRACTS SOLUTION OF *BIXA ORELLENA* SEED

Phytochemicals	Test	Observation	Results
1. Carbohydrate	Molirch test	Dull violent colour	++
	Reducing sugar	Bricked precipitate.	++
2. Saponins	Frothing test	Frothing	++
3. Tannins	$Fecl_3$ test	green ppt	+

4. Flavonoid	Strong Lead Sub-acetate test	White ppt	+
	Na OH Test	Yellowish colour	+
	Shinoda's test	reddish colour	
5. Cardiac glycoside	Salkowski's	brownish colour interphase	+
	Kella-killiani test	purple –brown ring at the inter-phase	+
6. Anthraquinones	Borntrager test	No colour change	_
7. Steroid	Liberman burchad test		+
8. Alkaloid	Dragendoff's test	Brown ppt	++
	Meyer's test	white ppt	++
	Wagner's test	brown ppt	++
Key: Presence + , Absence -			

TABLE 4: PRELIMINARY PHYTOCHEMICAL COMPONENTS OF DISTILED WATER EXTRACTS SOLUTION OF *BIXAORELLENA* SEED

Phytochemicals	**Test**	**Observation**	**Results**
1. Carbohydrate	Molirch test	Dull violent colour	++
	Reducing sugar	Bricked precipitate.	++
2. Saponins	Frothing test	Frothing	++
3. Tannins	$Fecl_3$ test	green precipitate.	+
4. Flavonoid	Strong L ead Sub-acetate test	White precipitate.	+
	Na OH Test	Yellowish colour	+
	Shinoda's test	reddish colour	

5. Cardiac glycoside	Salkowski's Kella-killiani test	Brownish colour interphase purple –brown ring at the inter-phase	+ +
6. Anthraquinones	Borntrager test	No colour change	_
7. Steroid	Liberman burchad test		+
8. Alkaloid	Dragendoff's test Meyer's test Wagner's test	Brown precipitate. white precipitate. brown precipitate.	++ ++ ++
Key: Presence +, Absence -			

TABLE 5: PRELIMINARY PHYTOCHEMICAL COMPONENTS OF ACETONE EXTRACTS SOLUTION OF *BIXAORELLENA* SEED

Phytochemicals	Test	Observation	Results
1. Carbohydrate	Molirch test	Dull violent colour	++
	Reducing sugar	Bricked precipitate.	++
2. Saponins	Frothing test	Frothing	++
3. Tannins	$Fecl_3$ test	green precipitate.	+
4. Flavonoid	Strong Lead Sub-acetate test Na OH Test Shinoda's test	White precipitate. Yellowish colour reddish colour	+ +

5. Cardiac glycoside	Salkowski's Kella-killiani test	brownish colour interphase purple–brown ring at the inter-phase	+ +
6. Anthraquinones	Borntrager test	No colour change	_
7. Steroid	Liberman burchad test		+
8. Alkaloid	Dragendoff's test Meyer's test Wagner's test	Brown precipitate white precipitate brown precipitate.	++ ++ ++
Key: Presence +, Absence -			

Table 6: COLUMN CHROMATOGRAPHY FRACTIONS

The results of chromatograhy fraction procedure, the colors obtained were as follow: Deep red, Light orange red and Golden yellow:

S/No	Collection of eluents (ml)	Weight (g)	Distance Moved by spot	Distance moved by solvent front (ml)	Retardation factor
1	**1 – 8**	0	0	5.5	0
2	**9 – 25**	0.09	5.1	5.5	0.93
3	**26 – 40**	0.08	4.2	5.5	0.76
4	**41 – 48**	0.09	2.4	5.5	0.44
5	**49 – 66**	0.22	2.7	5.5	0.49
6	**67 – 77**	0.15	4.4	5.5	0.8
7	**78 – 86**	0.7	3.5	5.5	0.64
8	**87 – 100**	0.06	4	5.5	0.73

DISCUSSION

The maceration extraction method was used to extract the *Bixa orellana* powder using five reagents; Acetone, Ethanol, water, Ammonia and Xylene with a percentage yield of 30%, 22.97% and 19.00%, 39% and 16% respectively and these comparisons between them shows that *Bixa orellana* seed powder has significant extractive values in different solvents and it was in agreement with results reported by Sangeeta[5] and James[6] but was also observed that when immersed in Ammonia there was high extractive value but poorly extracted in Xylene and both reagents presented poor colour-carrying capacity and no further investigation was done since this property does not reflect hope of future staining or histochemical reactive capacity.

Phytochemical investigation of *Bixa orellana* seed powder extract with acetone, ethanol and distilled water showed the presence of carbohydrates, proteins, amino acids, steroids, glycosides, saponins, flavonoids, alkaloids, tannins, phenols at least in one of the extracts but they were all negative to anthraquinoid. These results were in line with other studies performed with *Bixa orellana* seed extracts, where the same secondary metabolites were found[5]. Similar results were obtained on comparative phytochemical screening of qualitative and quantitative parameters of *Bixa orellana* extract[6].

Thin Layer Chromatography (TLC) was used to identify the proper solvent to use and elute of fractions from the column and the TLC plates were developed and exposed to visible light, UV light and H_2SO_2 in 105 ^{0}C oven to visualize the plate and it was discovered that about seven spots were moved, the method used is in line with the method recommended[7]. Different elements collected have different distances, weights and as such have different retention factors as presented in the result; this result is similar to a previous study on isolation and antimicrobial evaluation of *Bixa orellana* and *Linum usitatissimum*[8].

CONCLUSION

Bixa orellana seed extract has a better yield with acetone but ethanol extract has better optical contrast when compared with acetone, or water. *Bixa orellana* contains many compounds as seen in phytochemical screening and Thin Layer Column Chromatography but with a single chromophoric group and stands to top as one of the economic trees if well utilized through research following its phytochemical compositions.

CONFLIT OF INTEREST

The authors declare that there are no conflicts of interest.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate research and ethics committee and have therefore been performed in accordance with the ethical standards. This work was carried out in collaboration with all Authors read and approved the final manuscript.

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