Research Article

Phytochemical and Proximate Analysis of Foliage and Seed of *Bixa orellana* Linn

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ABSTRACT

The phytochemical and proximate evaluation of the foliage and seed of *Bixa orellana* for useful bioactive contents was determined in this study. Phytochemicals and crude contents profiles from aqueous and ethanolic extracts of the plant showed tannins, flavonoids, terpenoids, glycosides, alkaloids, steroids, phenols and saponins. Quantitatively, 21.89 g, flavonoids; 31.86 g, glycosides; 11.11 tannins; 121.89 phenols was recorded for the leaf and 8.86 g, flavonoids; 93.22 g, glycosides; 44.79 tannins; 82.99 phenols and 10.11 g, saponins for seed. Proximate analysis showed 6.13±0.05 %, moisture; 4.5±0.36 %, ash; 10.40±0.25 %, crude fats; 52.80±0.5 %, crude fibre; 4.24±0.02 %, protein and 21.85±0.00 % carbohydrate for leaf and 4.89±0.19 %, moisture; 5.62±0.12 %, ash; 7.20±0.07 % , crude fats; 53.31±0.07 %, crude fibre; 12.55±0.28 %, protein and 16.26±0.39 %, carbohydrate content for seed. The high amount of phenolic compounds recorded indicates that *Bixa orellana* possess high antioxidant, anticarcinogenic and antimalarial potentials. The leaves showed significantly higher moisture, flavonoids, phenols and saponins content; hence to ensure sustainable management of the plant resources, the leaves should be the primary target of any phytochemicals extraction activities for B. orellana.

Keywords: *Bixa orellana*, Phytochemicals, Proximate analysis, Antioxidant, Sustainable management.

INTRODUCTION

Plants possess therapeutic properties or exert pharmacological effects on the animal body. Such plants are designated as "Medicinal Plants" and serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine[1]. The medicinal value of plants lies in the chemically bioactive substances such as tannins, carbohydrates, terpenoids, steroids and flavonoids that generate definite physiological action on the human body.[2]

The increasing demand for plant constituent in the cosmetic, food and pharmaceutical industries suggests that systematic studies of medicinal plants are increasingly important in the drive to find active compounds for prospective applications.[3] Traditional medicine is an integral part of the health system in developing countries.[4] Medicinal plants play a key role in the world health care and about 80% of Africans depend on phytomedicine. There is the increase need to search for potential drug-agent plants for the treatment of diseases, especially priority diseases in Africa such as HIV/AIDS, hypertension, sickle cell anaemia, diabetes and malaria.[5]

Unsustainable use of bioresources often include among others, uncontrolled and indiscriminate harvest of plants materials, plant parts and regrettably whole plant removal. These activities perpetuated by indigenous people as well as the industry and scientific community is a major drawback on the global conservation of biodiversity and sustainable land management (SLM) efforts.[6] One of the factor behind these destructive activities is the lack of knowledge on the part of the plant with the most concentration of the bioactive constituents.

Bixa orellana – Annatto, main use of the bright red fruit (seedpods) as a natural colouring for food, textiles, objects, body, hairs and face hence it's common name "Lipstick tree". The ancient Maya and Aztec regarded Annatto as a symbolic plant. Ancient Maya scriptures were penned with ink made from annatto juice and both civilizations considered juice a substitute for blood and thus ascribed to it sacred connotations. The Armerindians consumed the seed for bravery and as an aphrodisiac. The whole tree has a long history as a valued medicinal plant that has been used to treat a wide variety of conditions from fevers to cancer.[7,8] The decoction of the leaves, mixed with the seed colorant, yields a drink that helps alleviate disorders associated with the female reproductive system. A drink prepared from the dried fruit pulp is administered as antidote against prussic acid present in *Manihot esculenta* as well as some tropical venomous plants, such as *Jatropha curcas* and *Hura crepitans*.[9]

In Nigeria, the various trado-medical systems have highlighted the medicinal value of *Bixa orellana* as it has been traditionally employed for the treatment of malaria and fevers, hypoglycemia, scrapes and burns.[10,11] The present study was carried out to determine; the bioactive constituents of *Bixa orellana* through the pharmacognostic and phytochemical evaluations (qualitative and quantitative) of the leaves and seeds as well as to ascertain the plant part/organ with the most concentration of the phytoconstituents.

MATERIALS AND METHODS

Plant collection

Fresh leaves and seeds of *Bixa orellana* were collected from rainforests in South West Nigerian. Plant samples were identified by Dr. Conrad Omonhinmin of the Department of Biological Sciences. Plant sample and voucher details provided are: ... with the sample voucher deposited in the department's herbarium. The samples were air-dried for 2 weeks, ground into fine powder, and stored in airtight containers.

Analysis Reagents

All reagents for the analysis were all analytical grade.

Methods

Preparation of aqueous and ethanol extracts

10 g of powdered leaf and seed samples were steeped in 180ml of ethanol and 200 ml of distilled water for 12 hours. The extracts were filtered and concentrated to ¼ of the initial mixture volume.

Proximate analysis.[12]

Proximate analysis of the samples was performed for percentage moisture, ash, crude fibre, crude fat, and crude protein content.

Moisture content:

$$\% \text{ Moisture} = \frac{W_1 - W_2}{\text{Weight of sample}} \times 100$$

Ash content:

$$\% \text{ Ash} = \frac{\text{Difference in weight of ash}}{\text{Weight of sample}} \times 100$$

Crude Fat content:

$$\% \text{ Crude fat} = \frac{\text{Weight of Ether extract}}{\text{Weight of sample}} \times 100$$

Crude Fibre content:

$$\% \text{ Crude fibre} = \frac{W_1 - W_2}{Wo\ (\text{Weight of sample})} \times 100$$

Crude Protein content:

$$\% \text{ Crude protein} = 6.25 \times \text{N (Correction factor)}$$

$$\%N = \frac{S - B \times N \times 0.014 \times D}{\text{Weight of sample} \times V} \times 100$$

Phytochemical analysis

Qualitative analysis.[13-16]

Qualitative chemical confirmatory tests for alkaloids, flavonoids, glycosides, phlobatannins, saponins, tannins and terpenoids were carried out on the aqueous and ethanol extracts of the leaves and seeds of *Bixa orellana* using standard procedures.

Test for Tannins: Boil 0.5g of powdered samples of leaves and seeds in 20 ml of distilled water in a test tube, filter and add 0.1% FeCl3. A brownish green or a blue black colouration is confirmatory for tannins.

Test for Phlobatannins: Boil 10 ml of aqueous extract of plant sample with 1% HCl acid. A deposition of red precipitate indicates the presence of phlobatannins.

Test for Flavonoids: Heat powdered sample with 10 ml of Ethyl acetate over a steam bath for 3 min, filter, shake 4 ml of the filtrate with 1 ml of dilute Ammonia solution. A yellow colouration indicates the presence of flavonoids.

Test for Terpenoids (Salkowski test): Mix 5 ml of aqueous extract of plant sample with 2 ml CHCl3 in 3 ml concentrated H2SO4. A reddish brown interface is confirmatory for terpenoids.

Test for Glycosides (Keller-Killani test): Mix 1 ml of concentrated H2SO4 in 5 ml of aqueous extract of the plant sample with 2 ml glacial CH3CO2H containing 1 drop of FeCl3. A brown ring is confirmatory for cardiac glycosides.

Test for Alkaloids: Mix 5 g of powered sample in 200 ml of 10% CH3CO2H in C2H5OH. Allow mixture to stand for 4 hours covered. Filter mixture and concentrate in water bath to ¼ initial volume. Add concentrated NH4OH until precipitation is complete. Wash precipitate with dilute NH4OH, filter, dry and weigh as alkaloids.

Test for Steroids: Mix 2 ml of Acetic anhydride in 0.5 g ethanolic extract of sample with 2 ml H2SO4. A violet to blue or green colour is confirmatory for steroids.

Test for Saponins: Boil 2 g of powdered sample with 20 ml of distilled water in a water bath and filter. Mix 10 ml of the filtered sample with 5 ml of distilled water, agitated to obtain a stable persistent froth. Mix emulsion from frothing with 3 drops of olive oil to confirm saponins.

Quantitative analysis

Quantitative determination of tannins and phenols were done using a spectrophotometer. Absorbance was measured at 395 nm (tannins) and 505 nm (phenols). Flavonoids and saponins quantities were determined by drying to constant weight.13 Data were analysed using Microsoft excel and SPSS (15.0) for Windows.

RESULTS AND DISCUSSION

Medicinal attributes of a plant is conferred by its secondary metabolites content and the beneficial medicinal effects of plant materials typically result from the combinations of phytochemicals present in the plant.[17] Plants are believed to be rich in a variety of secondary metabolites such as alkaloids, flavonoids, terpenoids and saponins that have therapeutic values. These metabolites have received attention as active agents for the management of several disease conditions and as such, researches have focused on evaluating the phytochemical profile of plants to ascertain the various

properties such as antioxidant attributes of plants used in ethnomedicine.[11]

Qualitative analysis of leaf and seed extracts

In the present phytochemical profiling of *Bixa orellana* from aqueous and ethanolic leaf and seed extracts, tannins and glycosides were recorded in the plant parts regardless of the solvent; flavonoids and phenols were present in the leaves and seeds but in water only (aqueous); terpenoids were present in ethanol and aqueous extracts of the seeds; saponins were recorded in both solvents extracts of the leaves; and alkaloids were present in ethanol only extracts of the leaves and seeds. Phlobatannins and steroids were not recorded in any of the plant parts (Table 1). Similarly, aqueous extract analysis by earlier works; confirmed the presence of flavonoids, saponins and alkaloids in *Bixa orellana* and phytochemical analysis of *B. orellana* ethanolic leaf extracts recorded tannins, glycosides, alkaloids and saponins.[18,19]

The difference in phytochemical content of the aqueous and ethanol extracts is attributable to the difference in the concentration of phytochemicals in various part/organs of the plants and the differential in the dissolution of phytochemicals in either solvents. These differences have been employed by indigenous people for medicinal preparations in the management of diseases. It is not uncommon among indigenous practitioners to have different parts of the same plant steeped in water and alcohol for the treatment of different diseases. These facts should particularly drive the quantitative analysis of various plant parts/organs to generate phytoconstituent and pharmacological profiles on parts/organs of important plants. This will greatly improve the conservational use of plants as well as help achieve to a considerable extent; the much advocated sustainable utilization of bioresources by all.

Quantitative analysis of leaf and seed extracts

Bixa orellana seeds recorded significantly ($\rho < 0.05$) higher tannin and glycosides content, and the plant leaves recorded significantly ($\rho < 0.05$) higher flavonoids, saponins, and phenol content (Table 2). This implies that B. orellana leaves have higher concentration of anticarcinogenic and antioxidant phytochemicals than the seeds. Hence, the leaves may constituents a better source for extraction of these phytochemicals. Conversely, higher phenol content were recorded for *B. orellana* seeds.[20]

The concentration of a given phytoconstituent in plant parts can be influenced by seasonal and environmental factors like soil type and other climatic factors[21,22]. This may account for the difference in concentration of phyto-constitutents recorded for *Bixa orellana* in the present and previous studies.

Table 1: Phytochemical analysis of *Bixa orellana* leaf and seed

S/N	Sample	Aqueous leaf	Extract seed	Ethanolic leaf	Extract seed
1	Tannins	+	+	+	+
2	Phlobotannins	-	-	-	-
3	Flavonoids	+	+	-	-
4	Terpernoids	-	+	-	+
5	Glycosides	+	+	-	+
6	Alkaloids	-	-	+	+
7	Steroids	-	-	-	-
8	Phenols	+	+	-	-
9	Saponins	+	-	+	-

+ = Present ; - = Absent.

Table 2: Proximate and Quantitative Analyses of *Bixa orellana* leaves and seeds

Sample	Leaf	Seed	Significance (α=0.05)
Proximate Analysis			
Moisture (%)	6.13±0.05	4.89 ± 0.19	0.07*
Ash (%)	4.58 ± 0.36	5.62 ± 0.12	0.07*
Crude Fats (%)	10.40 ±0.25	7.20 ± 0.07	0.11*
Crude Fibre (%)	52.80 ± 0.5	53.31 ± 0.07	0.03
Protein (%)	4.24 ± 0.02	12.55 ± 0.28	0.29**
Carbohydrate (%)	21.85±0.00	16.26±0.39	0.09*
Quantitative Analysis			
Tannins	11.11±0.04	44.79±0.03	0.35**
Flavonoids	21.89±0.09	8.86±0.06	0.26**
Glycosoides	31.86±0.05	93.22±0.05	0.29**
Phenols	121.89±0.03	82.99±0.03	0.12*
Saponins	10.11±0.02	--	0.00

Proximate analysis of leaves and seeds

Significantly ($\rho < 0.05$) higher ash and crude protein content was recorded for *B. orellana* seeds and significantly higher amount of moisture, crude fat and carbohydrate was recorded for the plant's leaves. However, the crude fibre content of the leaves and seeds were not significantly ($\rho < 0.05$) different (Table 2).

The high ash content recorded for *B. orellana* seeds implies that the plant seed contain a higher mineral content and this may be the reason for its used in Carribean, Latin American, Filipino and Mexican cuisines[23]. Annato has been used in soups, stews, chicken and pork in annatto sauce preparations as well as employed as spice for beef, eggs, fish, shrimps, sweet potatoes, and tomatoes. It has also been employed in yolk colour enhancement for improved poultry products by its addition to poultry feeds.[24]

The low fatty properties of the seeds are validated by the present result and it indicates that *Bixa orellana* seeds have significantly ($\rho < 0.05$) lesser crude fats than the leaves. This non-fat property have endeared it to vegetarians, non-vegetarians and manufacturers as seen in its use as a major additive in high-fat dairy products, margarine and hard candy.[25] Annatto seeds appeal in culinary uses may also be linked to the absence or low content of antinutritional factors.[26,27] Saponins (characterized by its bitter taste and foaming properties) were not recorded for the seeds; glycosides were recorded in lower amounts than in the leaves and though the seeds recorded higher tannin content, the generally low tannin amounts observed for the leaves and seeds imply low tannin-related antinutritional effects.

Despite the significance in moisture content between the leaves and seeds, the total moisture content of the plant materials are considered low and this low moisture content of both seeds and leaves indicates that the plant materials can be stored over a long period with lesser susceptibility to microbial attack.[28]

Medicinal properties and Cytotoxicity

Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's) and the aggravated stages of malaria.[29] Tannins protect cells against oxidative damage caused by free radicals, hence the anticarcinogenic and antimutagenic potentials of tannins can be linked to their antioxidative properties against cellular oxidative processes like lipid peroxidation. Saponins are anti-inflammatory with expectorant effect. Saponins also act as adjuvant in enhancing immune response in the body. Alkaloids confer antibacterial, antipyretic, antimalarial, antifungal, and antitumoric effects; terpenoids exhibits antitumoric property, by acting as a potent inhibitor of cell division, inhibiting the formation of solid tumours in the body. The anticarcinogenic activity of phenols has been attributed to stimulation of detoxification enzymes, ability to block carcinogens from damaging cellular DNA, antioxidant activities, protection against mutagenicity, inhibition of tumor initiation, and delay in tumor promotion.[30,31,32]

Comparatively, and as substantiated by the evidence-based reports; the higher flavonoids, glycosoides, phenols and saponins contents observed for the leaves predisposes that the leaves rather than the seeds are preferable material for use in most medicinal preparations and thus the target of analysis of the validity of most medicinal claims linked with Annatto.[23]

Although Annatto is reported to be well tolerated with particular reference to the use of the seeds in several food colouring and additive approaches; the leaf extracts have also been reported to be safe, albeit under certain conditions.[23] Additional structure-function activities as well as safety and efficacy studies, are required to situate the cytoxicity of Annatto. This position is more relevant considering the concentration and number of phytochemicals reported for *B. orellana* leaves in this study.[33]

CONCLUSION

Bixa orellana offers a range of potentials for the food and pharmaceutical industries, beyond its presently established role as colourant and additive for food and feeds. Its phytochemical constituents and contents depict a plant that constitutes a good source of phytochemicals like tannins, glycosides, phenols, saponins and flavonoids. The leaves and possibly other vegetative parts (stem and root) rather than seed constitute the primary target for sourcing such phytochemicals in *B. orellana*. The seed may find further applications in food preparations due to its low antinutritional factors contents. The versatility and hardiness of the plant, such that it can grow in almost all types of soils imply that Annatto can be cultivated in high volume with minimal inputs. This will reduce the destructive impacts of indiscriminate harvest of whole plants by indigenous people, researchers and bioprospectors for such phyotchemicals.

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