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STANDARDIZATION OF *ANNONA SENEGALENSIS* PERS. (ANNONACEAE) FRUITS AND STEM BARK FOR QUALITY CONTROL AND BIOLOGICAL REFERENCES

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ABSTRACT

Because of the overwhelming use of the fruits and bark of Annona Senegalensis for medicinal purposes in various parts of Africa and other developing world, this research was aimed at standardizing these two major parts of the plant for biological references and quality control in Pharmaceutical industries, research organizations, commercial use and monographs of tropical shrubs. Sample of the fruits (ripped) and the barks were collected and analyzed for the following parameters: physical constants (macro/ microscopical characters, extractive values, mineral compositions of seed), phytochemical constituents, toxicological standards, amino acid contents (of seed), aflotoxins, saponification value and chromatographic fingerprint of the parts. Results showed that values most of the parameters fall within WHO/FAO standards. Preliminary Phytochemical screening of revealed that the fruit contains tannins, Saponins, alkaloids, Steroids, flavonoids and essential oils (seeds) while the bark contains no steroids and essential oils. Amino acids, moisture contents, and saponification values were high in the parts slightly above the results obtained by other researchers. The study showed that these parameters are standards for quality control of that plant and reference for the A. senegalensis Pers.

KEYWORDS : *Annona senegalensis, Quality control, Parameters, Standardization, Biological references.*

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INTRODUCTION

Annona senegalensis Pers. (**Annonaceae**) belongs to one of the family of plants mostly used as ethno-medicinal prescriptions for many ailments like anthelmintic, diarrhea, stomach complaints, snake bite, oral trosh, veneral diseases, hypertension, dysentery and other commercial and veterinary uses [1]. It is called wild custard apple in English or wild sour sop.

The plant is found throughout Africa and all over the Sahel where rainfall is higher than 500 mm; typically in tall grass savannah areas from

semi-arid to sub humid areas most often growing as an under-story shrub. There areas are 4 synonyms that exist viz: *A.arenaria* Thon. ex Schum, *A.chrysophylla* Sil., *A. senegalensis* Var. Lat and *A. porpetac* (Boj.) R. Baill [2] .Because of these synonyms, identifying *A.senegalensis* sometimes could be difficult as all the species present almost identical features[3].Standardization of *A. senegalensis* means confirmation of its identity and determination of its quality and purity as well as detection like morphological, microscopical, physical and biological observations. This will create a clear distinction between allied species and adulterated products of fruit (seed) and stem bark [4].

Therefore, the aim of this research is to standardize *A. senegalensis* Pers. fruits (seeds) and stem bark for quality control, biological applications and references.

Materials and Methods

Collection, Identification and Preparation of Plant Materials

Fruits and stem bark of *A.senegalensis* Pers. were collected fresh from a forest in Ogurugu town, Enugu state, Nigeria in dry season of February 2011. They were identified by a taxonomist at the Department of Botany University of Nigeria Nsukka where a voucher number was deposited for the plant. The parts were air -dried for seven days and separately grinded into fine powder using electric grinder (Fritsch Idar-Oberstein Germany) and stored in an air-tight polythene bags for further analysis.

Extraction of Powder Plant Materials

Powdered plant materials weighing 200 g each were extracted in 95 % w/v absolute ethanol in 500 ml of water using Soxhlet apparatus and later de-fatted with pet-ether for 6 h. Filtrates were evaporated to dryness in water bath, weighed and stored in desiccators for use [4].

Macroscopical Examination of Drug

Sensory characters of the plant material were observed and recorded according to the method described by Trease and Evans [5].

Microscopical Features of Powder Drug

Microscopical character such as starch grains, calcium oxalate, sclereids, fibers, aleuron grains, calcium carbonates, lignin, tracheids and tannins were observed using x40 objectives of the compound microscope [6].

Physico- Chemical Evaluation of Drugs

The method of Harbone [7] was used to determine the values of the following parameters: moisture content, ash value, water soluble and insoluble ash (solubility), specific gravity, volatile and melting point, viscosity, bitterness value, crude fiber, protein, carbohydrate, minerals composition, extractive values, saponification value, and total alkaloids.

Preliminary Phytochemical Screening of Extracts

Active-constituents in the parts under investigation were determined using the method described by Trease and Evans 2007 [5] as well as Sofowora 2006 [8].

Chromatographic Analysis of Extracts

TLC (Thin Layer Chromatography) was carried out on the extract in ethanol -pet-ether-water (1:4:2) solvent system. Extracts were partitioned in a column that was left to stand for 72 hrs in order to obtained pure fractions of the extracts. On the TLC plates, the plates were sprayed with various detecting agents to know the class of compound of the spots. The retardation factors (R_F) were calculated using:

$$R_F = \frac{\text{Distance moved by spot}}{\text{Distance of solvent}} \quad \text{(no unit)}$$

Spectrophotometric shot was taken to produce the finger prints of the extracts [9].

Toxicological Standardization of Extract

Arsenic, heavy metals, radioactive contamination and aflotoxins were evaluated using standard procedures as described by Lorke [10] and UNIDO [11]. Arsenic is determined by matching the depth of colour with that of a standard stain. Coarsely ground plant materials were each put in a Kjeldah flash and 5 ml water, 10 ml nitric acid and 2 ml H_2S0_4 were added. This destroyed the harmful material in the drug. The solution was then cleared with sulphur trioxide vapours, cooled, and 30 ml ammonium oxalate was added and heat with sulphur trioxide vapours and left to cool. After this, 5 ml potassium iodide and granulated zinc were added and kept for 40 min. The colour of was compared with standard solution on mercuric bromide paper.

Bitterness Value Determination

Bitterness value was determined by comparing the threshold bitter concentration of the extracts with that of quinine hydrochloride (300 mg B.P, Embassy Pharmaceuticals) in 2000 ml. 0.1g of quinine was dissolved in 100 ml of distilled and extracts stock solution were prepared and diluted with it. The solution was tested and compared with the standard quinine solution. Bitterness value = *2000CAB (ml/g)* [11].

Where, A = concentration of the stock solution

B = volume of test solution in test tube

C = amount of quinine hydrochloride in the tube

Determination of Hemolytic Activity of the Extracts

Hemolytic activity was determined by first preparing the standard; A glass stopper flask was filled 1/10 of its volume with sodium citrate. Sufficient volume of freshly collected blood from healthy cat was added and shaken vigorously. The solution was stored in a refrigerator for 8 days at 4 ^{o}C. 1 ml of citrated blood was placed in a volumetric flask with phosphate buffer pH 7.4. Hemolytic activity = *1000 x a/b*

Where, 1000 = defined hemolytic activity of saponins standard

a = quantity of saponins standard that produced total haemolysis (g)

b = quantity of plant materials that produced total haemolysis (g)

RESULTS AND DISCUSSION

Macroscopic features present the sure and physical way of identifying the drug in the market and differentiate it from other adulterants [Table 1], while microscopical cell inclusions are very important for quality control of the drug [Table 2]. The starch grain varies in the two parts due to absence and present of striations in the stem bark and fruit respectively. Microscopical features vary slightly in the parts [Table 2]. Physico-chemical values do not exceed or fall below the acceptable standards in all the parts [Table 3].

Table 1 Macroscopical Parameters of *A.senegalensis* Pers. Fruits and Stem bark

Parameters	**Morphology group**	
	Barks	**Fruits**
Texture	Glabrous	Glabrous
Colour	Silvery grey	Yellow pink
Shape	Cylindrical	Heart-shaped (5-10 cm long)
Taste	Sour	Custard-like
Fracture	Brittle	Fibrous
Seeds	-	Numerous (Brown)
Powder	Coarse	Fine with nice smell

- (not applicable).

Table 2 Microscopical Examination of Powdered *A. senegalensis*

Inclusions / Cell Compositions	Morphology group	
	Bark	**fruit**
Starch	non-striated	striated
Calcium oxalate	Prism-like	-
Sclereids	Brachy	-
Fibers	Single/Crossed	Single
Aleuron grain	Minute	Large
$CaCO_3$	+	+
Lignin	Thickened	-
Tracheids	xylem	-
Tannins	++	+

+ (slightly present), ++ (highly present), - (not present or applicable).

Table 3 Physico-chemical Parameters of *A.senegalensis* Stem bark and fruits

Parameters	Bark	Fruit
Ash value	10 ± 0.04 w/w	10 ± 0.01 w/w
Moisture content	9 ± 0.02 w/w	11 ± 0.30 w/w
H_2O soluble ash (solubility)	15 ± 0.01 w/v	11 ± 0.01 w/v
Acid insoluble ash	12 ± 0.01 w/v	6.0 ± 0.001 w/v
Specific gravity	15 ± 0.01	18 ± 0.20
Volatile matter	8.2	12.3
Melting point	31 ^{o}C	30 ^{o}C
Viscosity	6.1 %	8.0 %
Bitterness value	420 m/g	300 ml/g
Refractive index	1.53	1.52
Crude fiber	11.21 %	18.60 %
Protein	5.3 %	10.8 %
Carbohydrate	6.2 %	22.5 %
Minerals	21.2 %	32.5 %
Water Extractive	14.3 %	15.6 %
Alcohol Extractive	10.4 %	12.8 %
Saponification values	7.5 %	5.2 %
Total alkaloids	12.1 %	6.3 %
Hemolytic activity	120 m/g	30 ml/g
Foaming Index	4.2 %	1.9 %
Optical rotation	+20.6^{o}	+23^{o}

*Results are means ± SE , n = 5.

Moisture content in drug should be minimized in drug in order to prevent microbial attack and deterioration of the herbal drugs [Table 3]. Higher hemolytic activity of the stem bark could be

attributed to the level of saponins in the extract as compared to fruit extract [Table 3]. The relevant of these parameters in herbal drug had been described by Ahamad *et al*. [12]. The values obtained for this species (*A.senegalensis* Pers.) could be use as fingerprint for the family *Annonaceae* .The fact that toxicological parameters presented in significant values and trace values as well not seen (NS) in this research, showed that the plant parts are were tolerated in the body for medical prescription in traditional medicine [Table 5] [13].

Phytochemical constituents in the plant are mainly glycosides and alkaloids, [Table 4], and these constituents are responsible for the biological activities of the plant that had been reported various researchers [5,6,12] chromatographic finger print using spectrophotometer showed normal peaks of unequal diameters and crest [Fig. 1 a and b], and this is peculiar to most Annonaceae family [8].

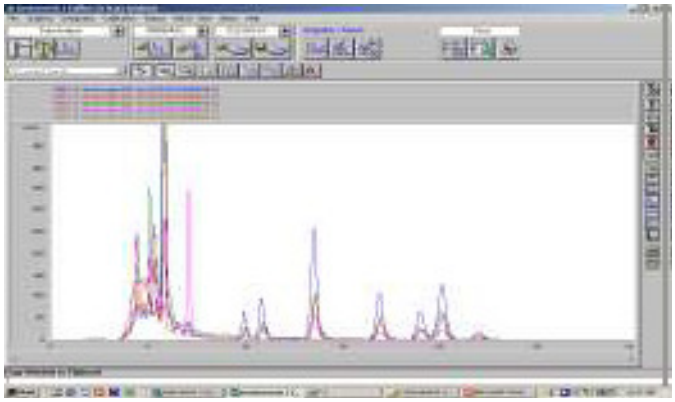

Figure 1a: TLC finger prints of ethanol extract of stem bark of *A. senegalensis* Pers. UV Spectrophotometer Model K231V11-A, made in Japan.

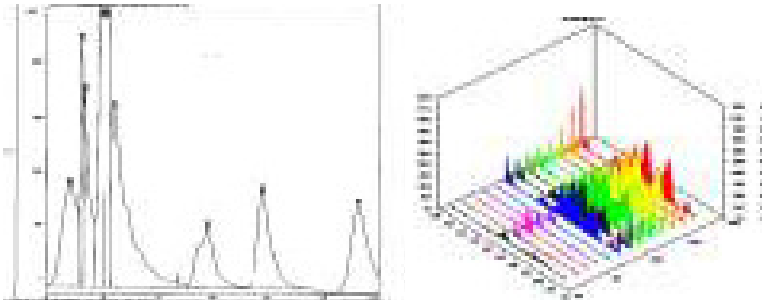

Figure 1 b: TLC finger prints of ethanol extract of fruits of *A. senegalensis* Pers**.**

Table 4 Phytochemical Screening of Extracts

Constituents	**Stem Bark**	**Fruits**
Tannins	+	+
Saponins	+	+
Alkaloids	+	+
Steroids	-	+
Flavonoids	-	+
Essential oils	-	+
Terpenes	+	-
Cyanogenetic glycosides	-	-
Cardiac glycosides	-	+

Anthraquinones	+	+

Constituents were determined after many tests [5, 6], TLC was used for confirmation, + (Present), - (not seen).

Table 5 :Toxicological Standardization of Extracts of *A.senegalensis* Pers.

Substances	Stem Bark (mg/kg)	Fruits (mg/kg)
Pesticides	0.04-0.5	0.0062-0.0065
Arsenic	NS	NS
Heavy metals	TS	TS
Radio actives	0.002-0.003	0.001-0.009
Aflotoxins	NS	NS
Cadmium	0.02-0.4	0.1-0.12

NS (not seen), toxicological experiment was conducted *in vitro* with no visible reaction with reagents in some of the substances, TS (values are in trace).
Phytochemical constituents in the plant are mainly glycosides and alkaloids, [Table 4], and these constituents are responsible for the biological activities of the plant that had been reported various researchers [5,6,12] chromatographic finger print using spectrophotometer showed normal peaks of unequal diameters and crest [Fig. 1 a and b], and this is peculiar to most Annonaceae family [8].

CONCLUSION

The study showed that the standardization of *A.senegalensis* Pers. stem bark and fruits ensures quality control of this herbal drug as it used for various purposes. However, standardization of other parts of the plant used for other purposes such as the leaves, roots and flowers, should be carried out towards holistic quality control of herbal drugs from the plant products, which will be used for ethnomedical prescription.

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