JOPAT Vol. 16(1) 86- 94, January – December, 2017.

ISSN 1118 – 1028

MICROSCOPIC, PHYSICOCHEMICAL AND CHROMATOGRAPHIC FINGERPRINTS OF LEAVES OF NIGERIAN *CASSIA TORA* LINN

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ABSTRACT

Introduction: *Cassia tora* Linn (Leguminosae–Caesalpinioideae), commonly known as Fotid in English and Tafasa in Hausa, is a perennial tree native to Africa. It has been reported to be used to manageskin infections, haemorrhoids, stomach ache and coughtraditionally. *C. Tora* leaves have been shown to possess anti-hepatotoxic, anti-allergic, anti-mutagenic, anti-fungal, radical scavenging, and anti-microbialactivities. Compounds such as anthraquinones, including chrysophanol, emodin, rhein have been isolated from *C. tora*.

Objective: To investigate the pharmacognostic and physicochemical characteristics of the leaves of *Cassia tora* Linn.

Methods:

Pharmacognostic investigations including microscopy, chemomicroscopy, proximate analysis and phytochemical investigations including Thin Layer Chromatographic finger-printing were conducted.

Results: The macro and microscopic studies revealed the leaves to be simple, papery, cordate and pinnately-veinnated. Both the adaxial and abaxial epidermal surfaces were characterized by abundant diacytic and anomocytic stomata respectively.Polygonal epidermal cells and numerous uniseriate, unicellular trichomes were also present. Quantitative leaf analysis revealed the following: stomata number on abaxial surface 445, stomata index 30.7, palisade ratio 17.1, vein-islet number 17.3 and vein- termination number 14.2. Chemomicroscopic characters present included lignin, tannin, mucilage, starch, oil and calcium oxalate crystals. The physicochemical parameters evaluated were: moisture content 4.6%, total ash 7.9%, acid-insoluble ash 1.5%, sulphated ash 19.6%, water-soluble ash 6.8%, alcohol-soluble extractive 9.5% and water-soluble extractive 24.1%. Chromatographic fingerprints of ethanol$_{(70\ \%)}$ extract showed major spots at R_f = 0.76 daylight (light green), UV_{366} (brown), spray reagent at 100°C (brown); R_f = 0.85 daylight (green), UV_{366} (red), spray reagent at 100°C (brown); R_f = 0.90 daylight (yellow), UV_{366} (brown), spray reagent at 100°C (brown).

Conclusion: The results of this research provide information which can be included in official monograph of the plant for its proper identification and quality control.

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Keywords: *Cassia tora*, Pharmacognostic studies, proximate analysis, Chromatographic fingerprints.

Introduction

*Cassia tora*Linn commonly known as Fotid (English), Tafasa (Hausa), Ndayaokon (Ibibio), Ako ire (Yoruba)is a stout, erect, smooth, half-woody annual herb with alternate leaves, 1-2cm long belonging to the Leguminosae family. It isnative to South-East Asia, Northern Australia, Africa and Latin America. *C. tora* is commonly found in waste grounds, secondary forests and grows wild along roadsides (Ajibesin*et et al*., 2008; Erinoso and Aworinde, 2012; Ingle *et al*., 2012).

The leaf of *C. Tora* has been used in Nigeria to manage skin infections, haemorrhoids, stomach ache, cough, pneumonia, ulcer and fever (Adamu*et al*., 2005; Dambata and Aliyu, 2011). Pharmacological activities including anti-hepatotoxic, anti-allergic, anti-mutagenic, radical scavenging, hypoglycaemic and anti-microbialactivities have been reported (Wu and Yen, 2004; Nam and Choi, 2008; Rejiya *et al*., 2009).The major secondary metabolites present in *C. tora* are alkaloids, phenols, anthraquinones, glycosides, flavonoids and saponins. Previous phytochemical investigations on the seeds of *C. tora* have resulted in isolation of several anthraquinones such as chrysophanol, emodin, rhein and naphthopyrone derivatives (Yen *et al*., 1998; Duke, 2001).

Evaluation ofpharmacognostic and physicochemical properties of medicinal plants are essential to standardization and preparation of monographs.Moisture content is a quantitative measurement important to processing, preservation and storage of medicinal plants.Ash values of a medicinal plant givean idea of earthy matter or inorganic components andother impurities present with drug. Ash from medicinal plants is the sum total of the residue remaining after all the moisture has been removed as well as the organic material (such as fat, protein, carbohydrates, vitamins and organic acid) have been incinerated at a temperature between 450 - 600 ± 25°C. Extractive values show the degree to which chemical constituents present in the crude drug are soluble in either organic or aqueous solvents (African Pharmacopoeia, 1986; WHO, 1992).

Materials and method

Collection

Leaves of *C. tora* were collected from Suleja Local Government Area of Niger State, Nigeria in January, 2016. The plant specimen

was authenticated and a herbarium specimen was deposited at NIPRD Herbarium with Voucher number NIPRD/H/ 6735.

Chemicals, reagents and solvents

All chemicals, reagents and solvents used during the experimentation were of analytical grade.

Morphological Evaluation

Macroscopic studies which comprised of organoleptic characteristics *viz*. colour, odour, appearance, taste, shape, texture and fracture were carried out on the leaf as specified by WHO guidelines (African Pharmacopoeia, 1986; WHO, 1992; Wallis, 2005).

Microscopy

Microscopy was carried out on the adaxial and abaxial epidermal surfaces of the whole leaf and comminuteddried leaf. A quantity of the comminuted leaf of *C. tora* was cleared in chloral hydrate, mounted in dilute glycerol on a microscope slide and viewed at different magnifications. Leaf epidermal preparations were carried out using the method of Ugbabe and Ayodele, (2008). About $5mm^2$ – $1cm^2$ leaf fragments were obtained from the standard median portion of the whole leaf and macerated in concentrated nitric acid in a petri-dish for 24 hrs. The epidermises were separated after theappearance of air bubbles. The fragments were transferred into water in a petri-dish with a pair of forceps. The upper and lower epidermises were separated and cleaned using forceps and carmel hair brush. Each surface was transferred into 50% ethanol to harden and later stained with safranin O for 5 minutes. The excess stain was washed off in water and the sample mounted on a slide with glycerin.

Physicochemical Evaluation

Different physicochemical parameters such as moisture content, total, sulphated,acid-insoluble and water-soluble ashvalues and water and alcohol extractive values were determined following WHO guidelines (African Pharmacopoeia, 1986).

Chemomicroscopic studies

Chemomicroscopic studies of the comminuteddried leaf sample was carried out using various reagents and stains such as iodine, sulphuric acid (66 %), concentrated hydrochloric acid, ferric chloride, Sudan III, ruthenium red and phloroglucinolin HCl (1:1) to test for the presence of different metabolites(African Pharmacopoeia, 1986; Evans, 2009).

Quantitative microscopy

Quantitative examinations such as vein-islet number, vein-termination number, palisade ratio,stomatal number and stomatal index werecarried out using standard methods (African Pharmacopoeia, 1986)

Chromatographic fingerprinting

Analytical TLC was done on silica gel G60 F_{254}, 0.2mm layer and KC18 silica gel 60 Å, 200µm. The plates were developed, after spotting the ethanol$_{(70\ \%)}$ extract at the origin, using solvent system dichloromethane (CH_2Cl_2):methanol (CH_3OH) (7:3) and dichloromethane (CH_2Cl_2):methanol (CH_3OH):ammonia (NH_4OH) (5:4:1). Detection was done in daylight, under UV_{366} and with 10% aqueous H_2SO_4 spray reagent. Plates were dried at 100^oC after spraying. The different retardation factors (R_f) of each

spot were calculated (Nigeria Herbal Pharmacopoeia, 2008).

Photomicrography

Photomicrographs of different sections were taken at different magnifications(x100 and x400)using Leica CM E microscope with Digital Microscope Eyepiece attachment and Photo Explorer 8.0 SE Basic software.

Statistical analysis

The data obtained were expressed as mean ± SEM (standard error of mean), and n represents the number of replicates in an experiment.

Results

Table 1: Macroscopic characteristics of *C. Tora* leaf

Characters	Observation
Leaf colour	Light green (abaxial); Dark green (adaxial)
Odour	Characteristic
Texture	Papery
Leaf type	Simple
Leaf margin	Entire
Leaf apex	Obtuse
Leaf shape	Obovate
Leaf base	Cordate
Leaf venation	Pinnate
Leaf size: length Width	5.8cm - 6.5cm 2.7cm - 3.8cm

Table 2: Physicochemical evaluation of *C. tora* leaf (Dry matter)

Parameters	Result (% w/w)
Moisture content	4.6
Total Ash	7.9
Acid–insoluble ash	1.5
Water–soluble ash	6.8
Sulphated Ash	19.6
Alcohol-soluble extractive	9.5
Water-soluble extractive	24.1

Table 3: Quantitative microscopy of *C. tora* leaf

Parameters	(Range) Mean ± SEM
Stomatal number:abaxial surface *	(420-496) 445 ± 7.3
Stomatal number:adaxial surface	(396-435) 413.2 ± 4.1

Stomatal index:abaxial surface*	30.7 ± 0.0
Stomatal index:adaxial surface*	32.4 ± 0.0
Vein-islet number +	(12-26) 17.3 ± 1.7
Vein termination number +	(11-17) 14.2 ± 0.6
Palisade ratio +	17.1

***n=** 10, **+n** = 4

Table 4: Chemomicroscopic evaluation of *C. tora* leaf

Parameters	**Result**
Lignin	+
Mucilage	+
Cellulose	+
Tannins	+
Starch	+
Calcium oxalate crystals	+
Oils	+
Proteins	-

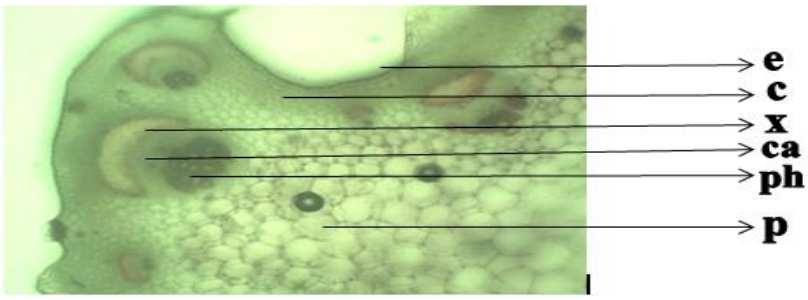

Figure 1: Transverse section of*C. tora* leaf showing (x 400): e- epidermis; c- collenchyma; x- xylem; p- pith parenchyma; ph- phloem; ca- cambium

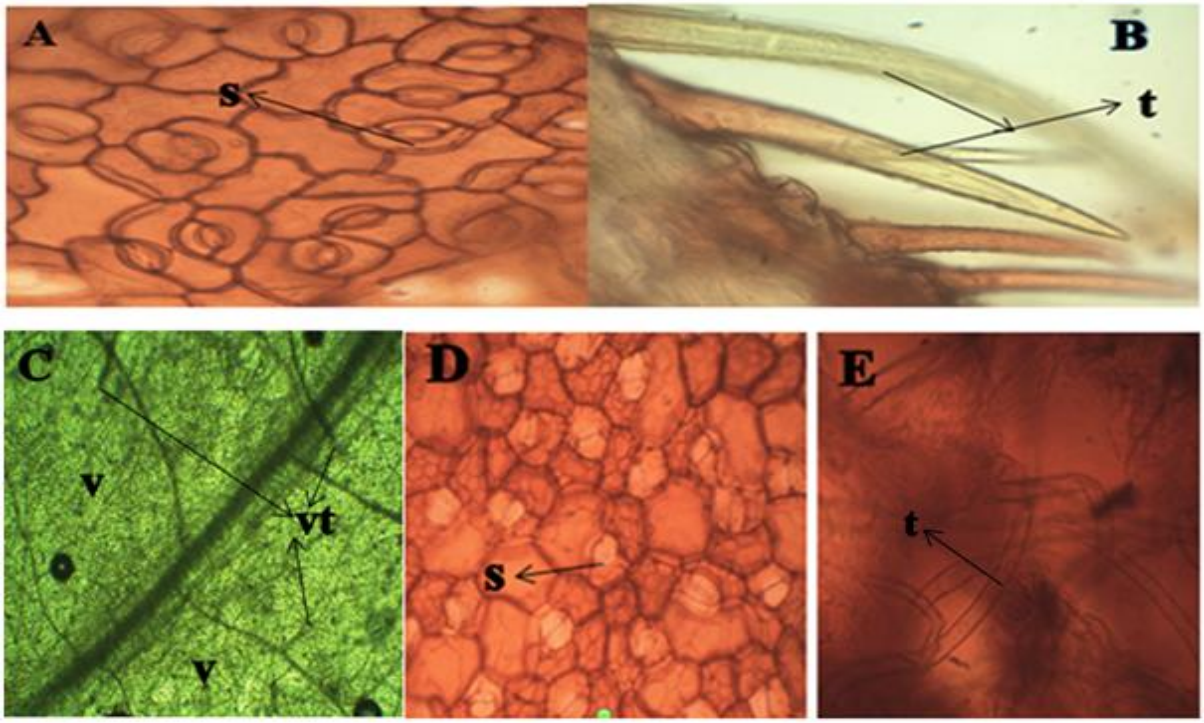

Figure 2: Epidermis of *C. tora*leaf(X400) showing: A) anomocytic stomata (**S**) on abaxial surface; B) unicellularuniseriatetrichomes on abaxial surface (**t**); C) Vein-islet (**v**) and vein-terminations (**vt**). D) Diacytic stomata (**s**) and palisade cells on adaxial surface E) unicellular uniseriatetrichomes on adaxial surface (**t**).

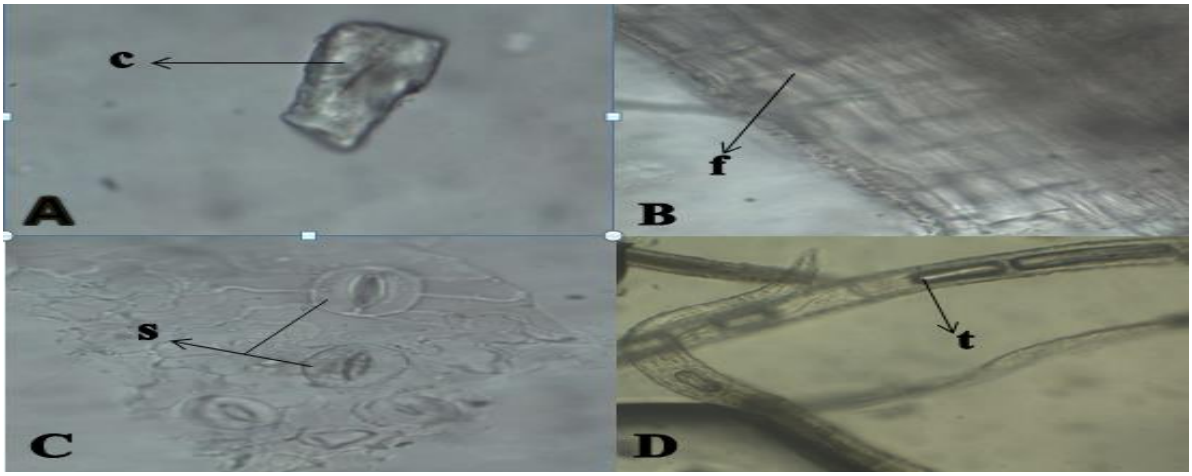

Figure 3: Microscopy of comminuteddried leaf (x400):**A)** calcium oxalate crystal (**c**) **B)** fibres (**f**)**C)** anomocyticstomata (**s**) and **D)** seriated unicellular trichomes (**t**)

Result of chromatographic fingerprinting

For chromatographic fingerprints of ethanol$_{(70\ \%)}$ extract on normal and reverse phase TLC. plates, major spots were observed as seen below (Tab. 5)

Table 5: Chromatographic fingerprinting of *C. tora* ethanol$_{(70\ \%)}$ leaf extract

Extract	R_f	Daylight	UV_{366}	10% v/v aqH_2SO_4
Normal phase TLC in CH_2Cl_2:CH_3OH (7:3)	0.92	green	red	brown
Normal phase TLC in CH_2Cl_2:CH_3OH:NH_4OH (5:4:1)	0.94	green	red	brown
Reverse phase TLCin CH_2Cl_2:CH_3OH (7:3).	0.76	light green	brown	brown
	0.85	green	red	brown
	0.90	brownish yellow	brown	brown
	0.94	brown	green	brown
Reverse phase TLC in CH_2Cl_2:CH_3OH:NH_4OH (5:4:1)	0.05	green	-	brown
	0.17	yellow	-	brown
	0.24	light brown	-	brown
	0.36	brown	-	brown
	0.88	brown	red	brown
	0.92	brown	red	brown
	0.96	deep brown	deep brown	brown

Discussion

It is important to set standards for all the parameters associated with pharmacognostic and physicochemical characters as these play key roles in determining the identity, purity and quality of a crude drug. These standards must be established for every crude drug to be included in a herbal pharmacopoeia.

Different morphological characters were observed on examination of the *C. tora* leaf. It is simple, entire and pinnately-veined (Tab. 1). Transverse section of the leaf across the midrib showed the presence of vascular bundles, parenchyma cells andwell-developed collenchyma cells (Fig.1).

Microscopy of *C. Tora* upper leaf surface showed polygonal epidermal walls, numerous trichomes and abundant diacytic stomata. Irregular wavy epidermal cells with numerous anomocytic stomata were observed on the lower epidermis (Fig.2). Unicellular seriatedtrichomes were present

on both surfaces. Vein-islets and vein-terminations were also observed (Fig. 2). The presence of numerous stomata on both surfaces of the leaf (amphistomatic) implied that transpiration occurs on both the abaxial (lower) and adaxial (upper) surfaces for photosynthesis and water loss (opening and closing of stomata). The adaxialepidermal surface also showed the presence of palisade cells, vein-islet and vein terminations (Fig. 2). Rakesh and Balaji, 2015 reported similar anatomical features present in leaf collected from India such as the presence of conical, unicellular, thick walled, covering trichomes, polygonal epidermal cells, calcium oxalate crystals and abundant stomata however paracytic stomata was observed.

Leaf constants such as stomatal number, stomatal index, palisade ratio, vein-islet number and veinlet termination number were measured. These parameters vary from plant species to plant species hence can be used in their identification (Tab. 3).

Chemomicroscopic evaluation of the comminuteddried leaf indicated the presence of lignin, tannins, cellulose, starch, oils, and prism-shaped calcium oxalate crystals. Proteins were absent (Tab. 4).

A higher extractive value was obtained for the aqueous solvent (24.1 %) than alcohol (9.5%), suggesting the possibility of the presence of more polar constituents (Tab. 2).

The moisture content observed for *C. tora*was4.6 % indicating a high shelf life of the crude drug. The results for ash analyses on dry mattershowed that total ash, acid-insoluble ash, water-soluble ash and sulphated ash were 7.9 %, 1.5 %, 6.8 % and 11.0 % respectively (Tab. 2). These values indicate low amounts of silica especially sand as well as siliceousearth in the sample. These results are indicative of low inorganic contents though the values are subject to factors such as the soil type, mining and construction activities around the area of cultivation. The various spots observed on TLC (Tab. 5) can be used as finger-prints in the identification of *C. tora*.

A review by Rakish and Balaji, 2015 on physicochemical properties of *C. tora* leaf showed similarities in samples collected from Nigeria and India. Similar moisture content, total as hand water soluble ash values was observed however samples from India gave a higher alcohol extractive value (Not less than 35 %) than water extractive value (Not more than 9 %). This suggests a variation in the quantity of constituents present in both countries (Tab. 2). Reports from Sushma and Sardana, 2014 on leaflets collected also from India also showed a few similarities however different macroscopic characters were observed ranging from differences in leaf size (smaller leaflets) to differences in morphological features such as leaf base (asymmetric), texture (smooth) and venation (reticulate).These macroscopic, microscopic and physicochemical property variations can be due to geographical/climatic differences.

Conclusion

The results from this study have provided information on the morphological, histological features and physicochemical parameters of the leaf of *Cassia tora* which

can be used for identification and quality control of the crude plant drug.

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