Birla College of Arts, Science & Commerce.

"Physico-chemical, Pharmacognostical evaluation and Cytotoxicity study of *Cassia angustifolia* (Vahl) leaves."

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By

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(PhD Scholar)

Department of Biotechnology

INTRODUCTION

- **Indian medicinal plants** are the essence of Ayurveda and Ayurvedic treatments.

- ***Cassia species*** (Family- **Caesalpiniaceae**)

  Medicinal properties :

  - Cathartic and Antibiotic properties.
  - Used for the treatment of Ulcers and Jaundice.
  - Used as a Laxative and Purgative.

It is also a rich source of Polyphenols, Glycosides, Flavonoids, Steroids and Anthracene derivatives.

Botanical Description

- *Commonly known as **'Senna'***
- Native of Saudi Arabia & Naturalized in **India**.
- Small Drought-resistant **S**hrub.

- **Leaves** are usually have 5-8 Leaflets, Axillary, Erect, Glabrous and Lanceolate in shape.
- **Flowers -** Big size & Yellow colored.
- **Pods** (1.4 to 0.8 in wide) –

Greenish brown Colour contain 5-7 obovate dark brown & smooth Seeds.

Different Parts of *Cassia angustifolia* (Vahl.)

1. Dry leaves
2. Pods (Green)
3. Dry Pods (Greenish Brown)
4. Flowers (Yellow)
5. Seeds (Green and Smooth)

Taxonomic Position

Botanical Name: ***Cassia angustifolia***

(***Senna alexandrina*** **Mill.)**

Common Name: **Senna leaves**

Category: Raw herbs

Ayurveda : **SVARÛAPATRI**

Systematic Classification
(Bentham and Hookers)

Division:	**Spermatophyta**
Sub Division:	**Angiosperms**
Class:	**Dicotyledonae**
Sub Class:	**Polypetalae**
Series:	**Calyciflorae**
Order:	**Resales**
Family:	**Leguminosae**
Subfamily:	**Caesalpineae**
Genus:	***Cassia***
Species:	***angustifolia***

Medicinal Uses of *Cassia angustifolia*

- As a *Febrifuge*: Splenic Enlargements, Anemia, Typhoid, Cholera.
- An Excellent Blood Purifier.

- ***Senna*** leaves - Traditionally used as :
  - Herbal Laxative to support regularity
  - Skin problems
  - Jaundice
  - Bronchitis
  - Liver disease
  - Splenomegaly

Aim: **Physico-chemical, Pharmacognostical evaluation and Cytotoxicity study of *Cassia angustifolia* (Vahl) leaves.**

Objectives:

1.) Physico-chemical Parameters:

i) **Foreign Matter.**

ii) **Total moisture content.**

iii) **Ash Values.**

iv) **Extractive Values.**

v) **Heavy Metal Analysis.**

vi) **pH**

2.) Pharmacognostic Characterization.

3.) Phytochemical analysis.

4.) Cytotoxicity assay.

MATERIALS & METHODS

- **Plant Material Collection And Authentication.**

1.) Physico-chemical Determination.

(*Ayurvedic Pharmacopoeia of India* (API), Indian Pharmacopoeia, 2007; British Pharmacopoeia, 1980 & WHO, 2007)

i) Total moisture content (Loss on Drying).

ii) Ash Values: a) Total Ash
b) Acid Insoluble Ash
c) Water Soluble Ash
d) Sulphated Ash

iii) Extractive Values: a) Alcohol Soluble. b) Water Soluble.

iv) Heavy Metal Analysis (*S A I F Department of I I T* , Bombay)

v) pH

2.) Pharmacognostic Characterization.

(*American Herbal Pharmacopoeia,* AHP – Botanical Pharmacognosy)

i) Macroscopic ii) Microscopic

3.) Phytochemical analysis.

(Trease & Evans,1996; Harborne,1998; Wagner H. *et.al.*; 2009; Edeoga *et al.*, 2005)

a) Phytochemical Screening .

b) Thin layer Chromatography (TLC)

c) Total Alkaloid and Flavonoid content Quantification.

4.) Cytotoxicity Assay.

(Meyer, *et al.* 1982)

Brine Shrimp Lethality Bioassay.

[Brine Shrimp Eggs (San Francisco Origin, *Artemia* Cysts) were purchased from Artemia International LLC, USA.]

RESULTS

&

DISCUSSION

1.) Physico-chemical Determination

Sr. No.	Parameters	Test Sample Values	Reference Value (w/w)
1.	**Foreign Matter**	0.8 %	≤ 1.0 %
2.	**Total moisture content** (Loss on Drying)	0.87 ± 0.02 %	≤ 12 %
	Ash Values:		
3.	Total Ash	10.2 ± 0.05 %	≤ 14 %
4.	Acid Insoluble Ash	0.85 ± 0.002 %	≤ 2 %
5.	Water insoluble Ash	9.8 ± 0.04 %	7. 410 %
6.	Sulphated Ash	11.46 ± 0.004 %	7.5 – 9.9 %
	Extractive Values:		
7.	Alcohol Soluble	5.84 ± 0.72 %	> 5 %
8.	Water Soluble	17.28 ± 0.01 %	≥ 25 %
9.	**pH (5 % Solution)**	6.5 ± 0.5	6 ~ 7.5

1.) Physico-chemical Determination

Sr. No.	Heavy Metal Analysis	ppm
1.	**Mercury (Hg)**	< 0.01 ppm
2.	**Chromium (Cr)**	< 0.01 ppm
3.	**Arsenic (As)**	< 0.01 ppm
4.	**Cadmium (Cd)**	< 0.01 ppm
5.	**Lead (Pb)**	< 0.01 ppm
	Micronutrient	
6.	**Zinc (Zn)**	14.72 ppm

Heavy metal analysis was found to be less than 0.01 ppm i.e. in **permissible limit. Zinc,** an essential micronutrient for human being, was also found in permissible limit (14.72ppm) in the leaves.

2. a) Pharmacognostic Evaluation

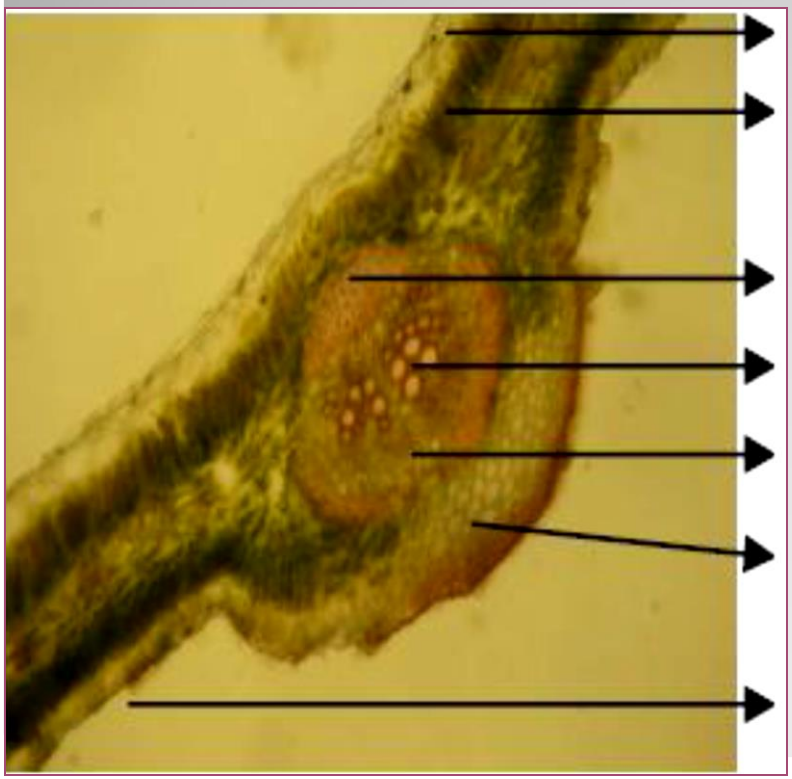
Leaves:

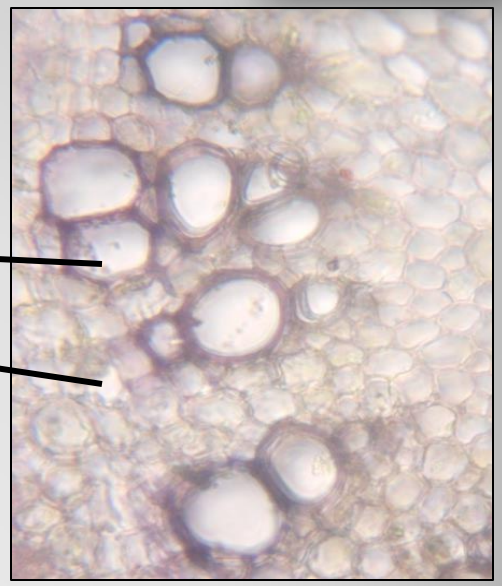
- 5-8 Leaflets
- Axillary
- Erect
- Glabrous
- Lanceolate in shape
- 20 mm to 50 mm long
- 7 mm to 20 mm wide

Macroscopic Evaluation

2. b) Pharmacognostic Evaluation

Microphotography



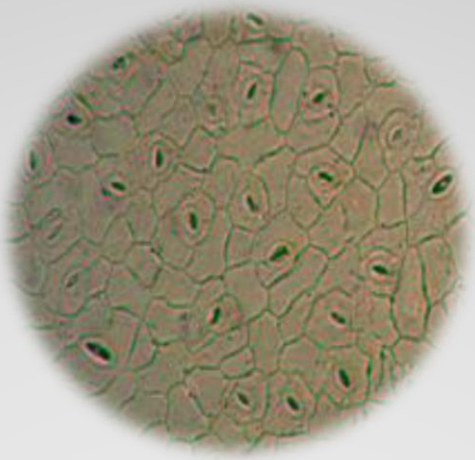


Microscopic Evaluation

2. b) Pharmacognostic Evaluation

Powder of leaves of
C. angustifolia

Stomata &
Epidermal cells
(abaxial : LM 40X)



Stomatal index:
14- 17.5 -20

Microphotography

Unicellular Trichome of
C. angustifolia

Oraganoleptic Characters : Green, Fine Powder, Slight Characteristic Odour.

Microscopic Evaluation

3. a) Phytochemical Screening in Different Solvents (Trease & Evans,1996; Harborne,1998)

Phtochemical Constiuents	P.E	CH	ACE	E.A	EtOH	Aqueous
Flavonoids	+	+	+	++	++	++
Alkaloids	++	+	++	+	++	++
Phenols	+	+	+	++	+++	+++
Glycosides	+	+	++	++	++	++
Saponin	+	+	++	+++	++	+
Cardiac Glycosides	+	++	+	++	++	+
Tanins	+	+	++	+++	+++	+++
Steroids	+	+	++	++	++	+
Terpenoids	+	+	+	++	++	+
Carbohydrates	-	-	+	+	++	+++
Xanthoproteins	+	+	+++	+	+++	+++

** *KeyNotes* – P.E (Petroleum Ether); CH (Chloroform); ACE (Acetone); E.A (EthylAcetate); EtOH (Ethanol)

3. b) Thin layer Chromatography (TLC)

Wagner H. *et.al.*; 2009

Sample – Hydro-alcohol Extracts
Sample Applicator – Linomat V (CAMAG)

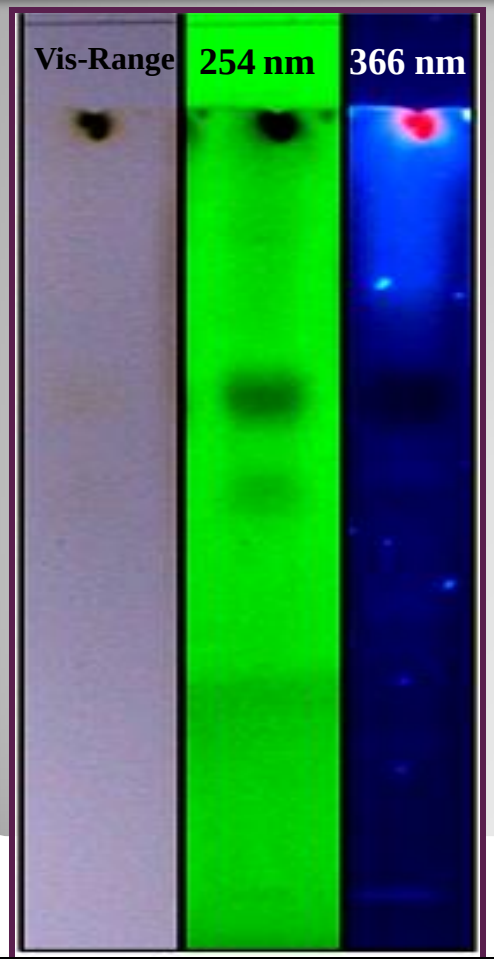
Sr. No.	Compounds	Solvent System used for TLC
1.	**Saponin**	EtOAc : HCO_2H : H_2O (8 : 1 : 1 v/v/v)
2.	**Tannins**	Toluene : EtOAc : HCO_2H : Methanol (3 : 3 : 0.8 : 0.2 v/v/v)
3.	**Alkaloids**	Diethylamine : Ether : Toluene (10 : 24 : 40 v/v/v)
4.	**Flavonoids**	EtOAc : HCO_2H : GAA : water (100 : 1.0 : 1.0 : 28 v/v/v)
5.	**Anthraquinone**	EtOAc : n-propanol : HCO_2H : water (10 : 10 : 2.5 : 4.5 v/v/v)

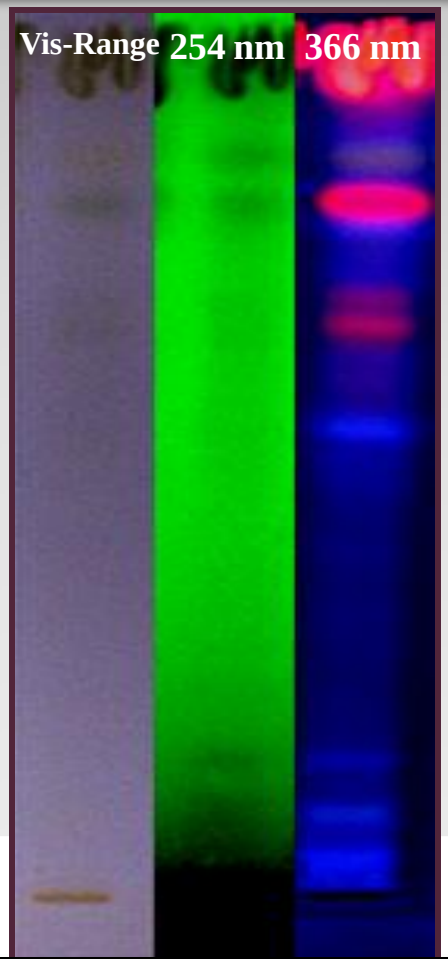
Note : EtOAc – Ethyl Acetic Acid;* HCO_2H *– Formic Acid; GAA – Glaciel Acetic Acid

3. b) Thin layer Chromatography (TLC)

Chromatographs

Saponin	Tannins	Alkaloids



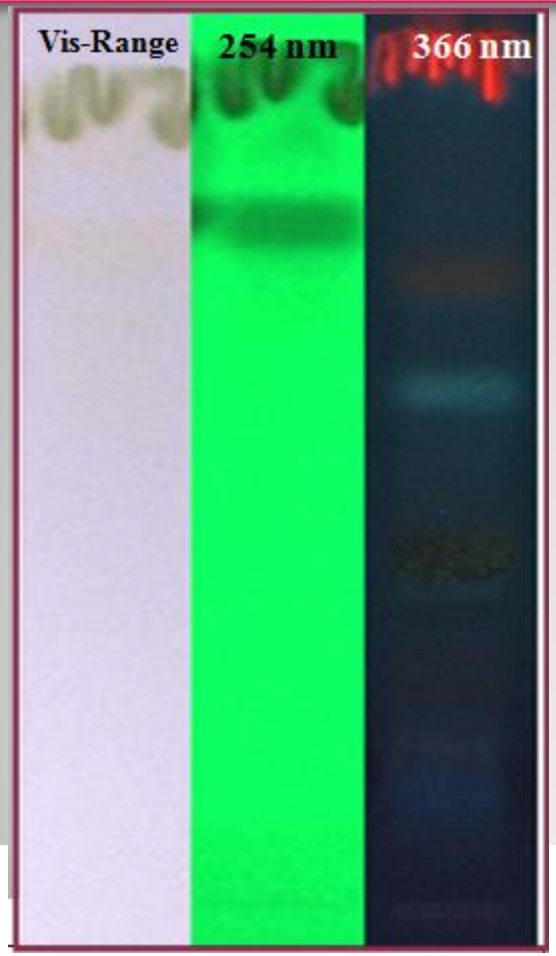


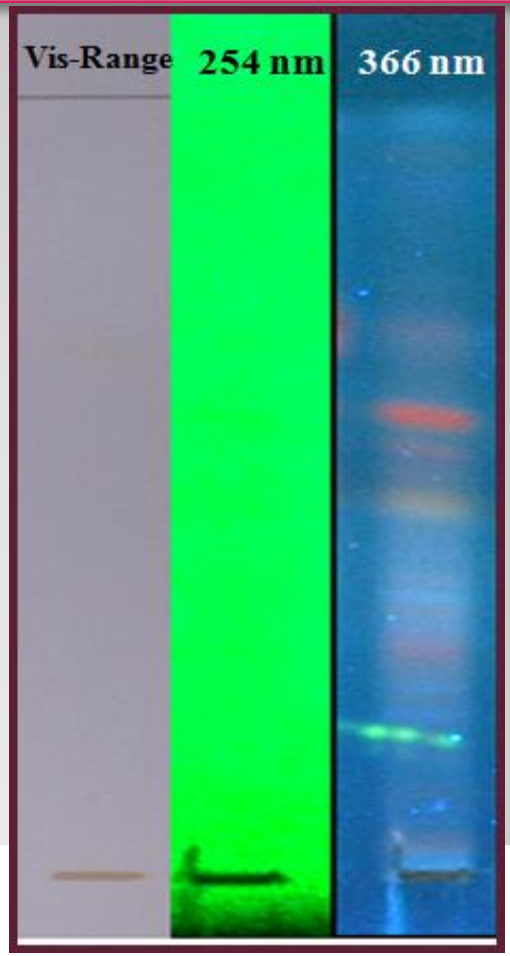
Hydro-alcohol Extracts Leaves of *C. angustifolia*

3. b) Thin layer Chromatography (TLC)

Chromatographs

Flavonoids	Anthraquinone





Hydro-alcohol Extracts Leaves of *C. angustifolia*

3. Phytochemical analysis

c) Total Alkaloid and Flavonoid content Quantification.

(Edeoga *et al.*, 2005)

Sample	Alkaloid	Flavonoids
Hydro – alcohol Extracts	119 mg/g	160 mg/g
Alcohol Extracts	89 mg/g	126.6 mg/g
Aqueous Extarcts	42.7 mg/g	85 mg/g

4.) Cyto-toxicity Assay.

Brine Shrimp Lethality Bioassay.

Samples		No. D.N	No. A.N	Mortality (%)
Negative Control	Aq Extract	30	0	100 %
	HA Extract	29	1	~97 %
Control	Aq Extract	15	15	50 %
	HA Extract	12	18	40 %
Positive Control	Aq Extract	0	30	~ 1 %
	HA Extract	0	30	~ 1 %
Aq Ext (100ppm)		20	10	~ 67 %
HA Ext (100ppm)		6	24	~ 20 %

This assay helped in evaluation to check toxicity effect in different extracts.

***Note -** No. – Number;*
D.N – Dead Nauplii ;
A.N – Alive Nauplii;
Aq Ext- Aqueous Extract;
HA Ext – Hydroalcohol Extracts

Conclusions

The results of the present study suggested :

- Foreign Matters and Ash contents evaluation – Quality and Purity of herbal medicine.
- Sulphated Ash – Determine inorganic impurities in an organic substance.
- Total Moisture Content – Measure the amount of water and volatile matters in a sample.
- The acid insoluble value – Refer ash which is insoluble in acids and usually has silicates.
- Extractive value study – Identification of adulterants in drugs.
- pH – Evaluation of crude drug w.r.t neutral solvent.
- Heavy Metal Analysis – Contamination Evaluation.
- Macroscopic and Microscopic – Fungal or Microbial contaimination.

- Phytochemical analysis – Qualitative screening of different Phyto-compound.
- Cytotoxicty Bioassay – Evaluation of toxicity effect in terms of mortality % in different extracts.

Summery

- Plant material contain **potent Phytoconstituents .**
- The data obtained in the present study adds to the existing knowledge of ***Cassia angustifolia* (Vahl.)** which may help in formulation of its standard drug and can be used to cure various diseases.

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Thank you


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Cytotoxicity Study

Formulae :

$$\% \text{ Mortality} = \frac{\text{Number of dead } \textit{Artemia nauplii}}{\text{Initial number of live } \textit{Artemia nauplii}} \times 100$$

Chapter 6

The Brine Shrimp Assay: Signs of Life, Sites of Value

If living beings are a classification, the plant is best able to express its limpid essence; but if they are a manifestation of life, the animal is better equipped to make its enigma perceptible.
—Foucault, *The Order of Things*.

February 8, 1997. Lab technician Mariana López stood in Dr. Rachel Mata's chemistry laboratory on the campus of Mexico's National Autonomous University (UNAM), holding a small glass flask up to the window and peering intently through her glasses at the clear liquid contained inside. Squinting against the Mexico City haze outside, Mariana was looking for signs of life. Twenty four hours previously, she had released ten tiny, recently hatched brine shrimp into this flask and several others like it, each containing different dilutions of a plant extract derived from UNAM ethnobotanists' field collections. It was her task now to conduct a body count in each vial. Spotting, chasing down, and siphoning the barely visible larvae into a pipette, she could make a fairly definite pronouncement of how many of the original *camaroncitos* (little shrimp) were still darting about and thus could be presumed alive, and how many had been rendered lifeless by their medium. (What some ethnographers of science might identify here as science-as-craft, Mariana identified to me as science-as-a-splitting-headache.) Shrimp mortality is here, as in many natural products chemistry labs across the world, translated into a preliminary indicator of plants' "bioactivity": the more dead shrimp in twenty-four hours, the more powerful the extract, and thus the more promising as a lead to a new drug or pesticide. This measure of potency has become increasingly common since the early 1980s, when the brine shrimp assay was first proposed by a group of chemists in the United States as a useful way to flag a plant's effects, broadly conceived, on life, broadly conceived (Meyer, et al. 1982).

Quantifcation of Alkaloids and Flavonoids

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Full Length Research Paper

Phytochemical constituents of some Nigerian medicinal plants

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Alkaloids, tannins, saponins, steroid, terpenoid, flavonoids, phlobatannin and cardic glycoside distribution in ten medicinal plants belonging to different families were assessed and compared. The medicinal plants investigated were *Cleome nutidosperma, Emilia coccinea, Euphorbia heterophylla, Physalis angulata, Richardia bransitensis, Scopania dulcis, Sida acuta, Spigelia anthelmia, Stachytarpheta cayennensis* and *Tridax procumbens*. All the plants were found to contain alkaloids, tannins and flavonoids except for the absence of tannins in *S. acuta* and flavonoids in *S. cayennsis* respectively. The significance of the plants in traditional medicine and the importance of the distribution of these chemical constituents were discussed with respect to the role of these plants in ethnomedicine in Nigeria.

Keywords: Medicinal plants, ethnomedicine, phytochemical constituents.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952). Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Okwu, 1999, 2001).

Cleome rutidosperma, Emilia coccinea, Euphrobia heterophylla, Physcalis bransiliensis, Scorparia dulcis, Richardia transiliensis, Sida acuta, Spigelia anthelmia, Stachytarpheta cayennensis and Tridxa procumbens are extensively used in herbal medicine in South Eastern Nigeria. Their various uses in traditional medicine are reviewed in Table 1. This study investigates the fundamental scientific bases for the use of some Nigeria medicinal plants by defining and quantifying the percentage of crude phytochemical constituents present in these plants.

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MATERIALS AND METHODS

Collection and identification of plant materials

The leaves and stems of the plants were collected from uncultivated farmlands located at Southern parts of Nigeria. All the ten plant samples were identified by the authors. The voucher specimens were deposited in the Biological Science laboratory of the Michael Okpara University of Agriculture, Umudike.

The plant samples were air-dried and ground into uniform powder using a Thomas- Willey milling machine. The aqueous extract of each sample was prepared by soaking 100 g of dried powdered samples in 200 ml of distilled water for 12 h. The extracts were filtered using Whatman filter paper No 42 (125 mm).

Phytochemical screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for phlobatannins: Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous

Table 2. Qualitative analysis of the phytochemicals of the medicinal plants.

Plants	Alkaloids	Tannin	Saponin	Steriod	Phlobatannin	Terpenoid	Flavonoid	Cardic glycoside
C. rutidosperma	+	+	+	-	-	-	+	+
E. coccinea	+	+	+	+	-	+	+	+
E. heterophylla	+	+	-	-	+	+	+	+
P. bransilensis	+	+	+	+	+	+	+	+
R. bransilensis	+	+	+	+	-	+	+	+
S. dulcis	+	+	+	-	+	+	+	+
S. acuta	+	-	-	-	-	-	+	+
S. anthelmia	+	+	+	+	+	-	+	+
S. cayennensis	+	+	+	-	+	-	+	+
T. procumbens	+	+	+	-	-	-	+	-

Presence of constituent
+ = - = Absence of constituent

Determination of total phenols by spectrophotometric method: The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505 nm.

Alkaloid determination using Harborne (1973) method: 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a waterbath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Tannin determination by Van-Burden and Robinson (1981) method: 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Saponin determination: The method used was that of Obadoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.

60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Flavonoid determination by the method of Bohm and Kocipai-Abyazan (1994): 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

RESULTS

The present study carried out on the plant samples revealed the presence of medicinally active constituents. The phytochemical characters of the ten medicinal plants investigated are summerized in Tables 2 and 3. Alkaloids, tannins, flavonoids and cardiac glycosides were present in all the plants. Tannins and cardic glycosides were absent in *S. cayennensis* and *T. procumbens*, respectively. Only *S. dulcus*, *E. heterophyila*, *P. angulata* and *E. coccinea* showed the presence of terpenoids (Table 2).

Quantitative estimation of the percentage crude chemical constituents in these medicinal plants studied is summarized in Table 3. *S. acuta* contained the highest percentage crude yield of alkaloids (1.04%), while *C. rutidosperma* contained the lowest yield of alkaloid (0.32%) but the highest yield of tannin (15.25%). Phenols were obtained in the plants but the yields recorded were minimal (0.20-0.04%).

DISCUSSION

The phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the plants studied showed that the leaves and stems were rich in alkaloids, flavonoids, tannins and saponins. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowara, 1993).

The absence of saponin in *S. dulcis* in the present study is in contrast with the opinion of Gill (1992) who