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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 bransilensis, Scorparia dulcis, Richardia bransilensis, 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 distiilled 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 browrish 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 1. Review of the various medicinal uses of the study plants.

Species	Family	Traditional use	Reference
C. rutidosperma	Caparaceae	Leaves are edible.	Burkill, 1984
		Ear cure for inflammation. Anthelminthic and carminative.	Gill, 1992
			Gill, 1992
E. coccinea	Astcraccae	Treatment of fever and convulsions in children.	Agoha, 1981
		Treatment for ulcer, craw-craw, ringworm.	Burkill, 1984
E. heterophylla	Euphorbiaceae	Vegetable and latex used for insect bites.	Edeoga and Gomina, 2002
		Treatment for erysipelas, Treatment for cough, bronchial paroxymal asthma, hay fever, catarrh.	Holm et al., 1997
			Gill, 1992
P. bransilensis	Solanaccae	Treatment of malaria, toothaches liver ailment and rheumatism.	Gill, 1992
		Treatment of stomach disorders, vomiting, diarrhoea and asthma in children.	Gill, 1992
R. bransilensis	Rubiaceae	Cure for eczema, Treatment of boils.	Burkill, 1994
		Active cure against avine malaria	
S. dulcis	Scrophulaiaceae	Antiviral, inlibitory and antitumuor activity.	Hayashi et al, 1993
		Remedy for cough, chest pains and sore throet, treatment of gonorrhoea	Gill, 1992.
S. acuta	Malvaceae	Feed for livestock.	Egunjohi, 1969
		Stops bleeding, treatment of sores and wounds; antipyretic.	
S. anthelmia	Lonaniaceae	Worm excellar.	Gill, 1992
S. cyaenneusis	Verbanaceae	Remady for dysentery, syphilis, gonorrhoea and catarrahal conditions.	Gill, 1992
T. procumbeus	Asteraceae	Feed for livestock.	Egunjobi, 1969
		Stops bleeding.	Holms et al., 1997
		Treatment of diarrheca, malaria and stomachache.	Burcill, 1984

hydrochloric acid was taken as evidence for the presence of phlobatinins.

Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Teat for flavonoids: Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harbrone, 1973). 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2 $S0_4$. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids.

A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for steriods: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test): Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Quantitative determination of the chemical constituency

Preparation of fat free sample: 2 g of the sample were defatted with 100 ml of diethyl ether using a soxhlet apperatus for 2 h.

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.I 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 evoporation 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 cheracters 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

Table 3. Percentage of crude alkaloids, phenols, tannin, flavonoids, and saponin on the medicinal plants investigated.

Plants	Alkaloids (%)	Phenols (%)	Tannin (%)	Flavonoid (%)	Saponin (%)
C. rutidosperma	0.34±0.1	0.20±0.20	15.25±0.11	0.34±0.20	2.0±0.11
E. coccinea	0.92±0.22	0.81±0.10	11.85±0.31	0.96±0.10	2.30±0.20
E. heterophylla	0.86±0.31	0.10±0.11	12.46±0.22	0.74±0.11	0.00
P. bransilensis	0.40±0.11	0.80±0.22	13.15±0.30	0.15±0.20	3.92±0.11
R. bransilensis	0.45±0.20	0.14±0.23	12.13±0.11	0.56±0.33	1.12±0.22
S. dulcis	0.81±0.33	0.04±0.11	6.23±0.20	0.88±0.12	0.00
S. acuta	1.04±0.20	0.08±0.11	6.08±0.23	0.98±0.10	0.00
S. anthelmia	0.84±0.33	0.10±0.20	15.05±0.14	0.77±0.11	2.260.20
S. cayennensis	0.68±0.11	0.13±0.23	9.98±0.32	0.00	3.10±0.10
T. procumbens	0.58±0.20	0.06±0.33	7.45±0.22	0.61±0.01	1.70±0.01

noted that saponin is one of the active constituents. Also, the presence of saponin in *S. anthelmia* contradicts the observation of Taylor -smith (1966) who reported that saponin was absent in this taxon.

Steroids and phlobatannins were found to be present in all the plants. It has been found that some of these investigated plants contained steroidal compounds. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001). This may be the reason the leaves of *C. rutidosperma* are used as vegetable for expectant mothers or breast feeding mothers to ensure their hormonal balance, since steroidal structure could serve as potent starting material in synthesis of these hormones (Okwu, 2001).

The presence of terpenoids in *S. dulcis* has also been reported by other researchers, and this plant is widely used in herbal medicine (Hayashi et al., 1993). *E. heterophylla* contains tannins and alkaloids and this conforms with the report of Rahila et al. (1994) and Gill (1992). The latter also observed that some of the *Euphorbia* species including *E. heterophylla* are used as a purgative. They are also used in the treatment of cough, asthma and hay fever (Burkill, 1994; Gill, 1992).

Both *S. acuta* and *T. procumbens* possessed very high levels of alkaloids and flavonoids, and are employed in medicinal uses. They are also widely employed as livestock and poultry feed (Egunjobi, 1969).

The plants studied here can be seen as a potential source of useful drugs. Further studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. The antimicrobial activities of these plants for the treatments of the diseases as claimed by traditional healers are also being investigated.

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