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Phytochemical constituents of some Indian medicinal plants

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Abstract

Alkaloids, tannins, saponins, steroid, terpenoid, flavonoids, phlobatannin and cardic glycoside distribution in seven medicinal plants belonging to different families were assessed and compared. The medicinal plants investigated were *Aegle marmelos, Cynodon dactylon, Eclipta prostrata, Moringa pterygosperma, Pongamia pinnata, Sida acuta and Tridax procumbens.* 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 India.

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). *Aegle marmelos, Cynodon dactylon, Eclipta prostrata, Moringa pterygosperma, Pongamia pinnata, Sida acuta and Tridax procumbens* are extensively used in herbal medicine in India. This study investigates the fundamental scientific bases for the use of some Indian medicinal plants by defining and quantifying the percentage of crude phytochemical constituents present in these plants.

Materials and Methods

Aegle marmelos, Cynodon dactylon, Eclipta prostrata, Moringa pterygo-sperma, Pongamia pinnata, Sida acuta and Tridax procumbens are the materials of the present investigation. Fresh leaves of *Aegle marmelos, Cynodon dactylon, Eclipta prostrata, Moringa pterygosperma, Sida acuta, Tridax procumbens* and stem barks of *Pongamia pinnata* were collected from Villamuthur, Perambalur District, Tamil Nadu, India [Latitude $11^0$23N: Longitude $78^0$88 E]. The study plants were identified with the help of available Indian literatures and the identities were verified with the help of Rapinaet Herbarium, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India.

Preparation of Powder

The collected fresh leaves of *A. marmelos, C. dactylon, E. prostrata, M. pterygosperma, S. acuta, T. procumbens* and stem barks of *P. pinnata* were shade dried at room temperature for 3 days and sun dried for 3 days and then milled into coarse powder by a mechanical grinder (Harborne, 1988).

Preparation of Aqueous Extract

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) (Rao et al. 1995).

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 Tannis

About 0.5 g of the dried powdered sample was boiled in 20ml 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 Phlotannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

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 5ml 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.

Test for Flavonoids

Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harborne, 1973). 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_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 10ml 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 Steroids

2ml 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)

5ml of each extracts was mixed in 2 ml of chloroform and concentrated H_2S0_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the interface

is formed to show positive results for the presence of terpenoids.

Test for Cardiac glycosides (Keller-Killani Test)

5ml 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 the thin layer.

Quantitative Determination of the Chemical Constituency

Preparation of Fat free Sample

2g of the sample were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 h.

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. 5ml of the extract was pipetted into a 50 ml flask, and then 10 ml of distilled water was added. 2 ml of ammounium 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. It 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 water bath to one-

quarter of the original volume. Concentrated ammonium hydroxide was added drop wise 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 mark. Then 5ml 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 55^0C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^0C. 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 water bath. 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 seven medicinal plants investigated are summarized in Tables 1 and 2. Flavonoids were present in all the plants. Alkaloids were absent in *C. dactylon* and *P. pinnata.* Tannin, saponin and phlobatannin were absent in *C. dactylon* and *S. acuta*. Steroid and terpenoid were present in all plants except *S. acuta* and *T. procumbens*. Only *A. marmelos*, *M. pterygosperma* and *S. acuta* showed the presence of cardic glycoside (Table 1).

Quantitative estimation of the percentage of crude chemical constituents in these medicinal plants studied is summarized in Table 2. *A. marmelos* contained the highest percentage crude yield of alkaloids (1.08%) while, *C. dactylon* and *P. pinnata* contained no alkaloids. The highest yield of tannin (15.26%) were contained in *A. mormelos*.

Discussion

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

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). Both *S. acuta, A. marmelos* 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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Table -1
QUALITATIVE ANALYSIS OF THE PHYTOCHEMICALS OF THE MEDICINAL PLANTS

PLANTS	ALKALOIDS	TANNIN	SAPONIN	STEROID	PHLOBATANNIN	TERPENOID	FLAVONOID	CARDIC GLYCOSIDE
A. marmelos	+	+	+	+	+	+	+	+
C. dactylon	-	-	-	+	-	+	+	-
E. prostrata	+	+	+	+	-	+	+	-
M. pterygosperma	+	+	+	+	+	+	+	+
P. pinnata	-	+	+	+	-	+	+	-
S. acuta	+	-	-	-	-	-	+	+
T. procumbens	+	+	+	-	-	-	+	-

(+) Presence

(-) Absence

Table -2

PERCENTAGE OF CRUDE ALKALOIDS, PHENOLS, TANNIN, FLAVONOIDS, AND SAPONIN ON THE MEDICINAL PLANTS INVESTIGATED

PLANTS	ALKALOIDS (%)	PHENOLS (%)	TANNIN (%)	FLAVONOID (%)	SAPONIN (%)
A. marmelos	1.08 ± 0.21	0.81 ± 0.10	15.26 ± 0.11	0.98 ± 0.10	2.62 ± 0.13
C. dactylon	0.00	0.14 ± 0.08	0.00	0.67 ± 0.13	0.00
E. prostrata	0.34 ± 1.11	0.00	11.86 ± 0.41	0.87 ± 0.16	1.71 ± 0.02
M. pterygosperma	0.92 ± 0.21	0.10 ± 0.20	12.41 ± 0.23	0.97 ± 0.18	3.26 ± 0.14
P. pinnata	0.00	0.00	6.24 ± 0.21	0.84 ± 0.14	1.12 ± 0.23
S. acuta	1.04 ± 0.20	0.08 ± 0.11	6.08 ± 0.23	0.98 ± 0.10	0.00
T. procumbens	0.58 ± 0.20	0.06 ± 0.33	7.45 ± 0.22	0.61 ± 0.01	1.70 ± 0.01

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