Preliminary phytochemical screening of some Indian Medicinal Plants

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Abstract

Alkaloids, tannins, saponins, steroid, terpenoid, flavonoids, phenolic compounds and cardic glycoside distribution in five medicinal plants belonging to different families were assessed and compared. The medicinal plants investigated were *Asteracantha longifolia (L.) Nees, Psassiflora edulis Sims, Berberis tinctoria Lesch, Sphaeranthus indicus Linn.* and *Solanum trilobatum Linn*. All the plants were found to contain Phenols, Cardiac glycosides, Steroids, Saponins and Tannin except for the absence of flavonoids and Terpenoids in *A.longifolia (L.) Nees* and Alkaloids in*, P. edulis Sims, A.longifolia (L.) Nees, B. tinctoria Lesch and S. indicus Linn.* 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 India.

Introduction

Infectious diseases are an important health hazard all over the world, both in developing and developed countries (Sasikumar *et al*., 2003).Even though pharmacological industries have produced a number of new antibiotics in the last three decades resistance to these drugs by microorganisms has increased (Nascimento *et al*.,2000). There is an urgent need to alternative medicine for ailments. The increasing interest on traditional ethono medicine may lead to discovery of novel therapeutic agents. Medicinal plants are finding their way into pharmaceuticals, neutraceuticals, cosmetics and food supplements. The World Health Organization (WHO) estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care needs (Mohanasundari *et al*., 2007). 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 (Dhandapani and Sabna .,2008). *Asteracantha longifolia (L.) Nees, Psassiflora edulis Sims, Berberis tinctoria Lesch, Sphaeranthus indicus Linn.* and *Solanum trilobatum Linn*. are used in traditional medicine of 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.

Material and Methods

Asteracantha longifolia (L.) Nees, Psassiflora edulis Sims, Berberis tinctoria Lesch, Sphaeranthus indicus Linn. and *Solanum trilobatum Linn*. are the plant samples were used in the present investigation. Fresh leaves of *Asteracantha longifolia (L.) Nees, Psassiflora edulis Sims, Sphaeranthus indicus Linn., Solanum trilobatum Linn. and roots of Berberis tinctoria Lesch* were collected from Western Ghats, Nilgris & Coimbatore. The plant samples were authenticated by Botanical Survey of India (Southern circle), Coimbatore, Tamilnadu.

Preparation of extracts

The collected samples were shade dried under room temperature for 7 days and then milled into coarse powder by a mechanical grinder. The methanol extract of each sample was prepared by soaking 10g dried powder samples in 100ml of methanol for 24 hrs. The extracts were filtered and evaporated under reduced pressure.

Phytochemical Screening

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

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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 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 H2S04. 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 steroids

Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H2S04. 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 H2S04 (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 terpenoid

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

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 amyl alcohol 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 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 hr 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 pipette out into a test tube and mixed with 2 ml of 0.1 M FeCl3 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 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hr 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 curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols *etc*. The successive extracts of root, and leaves of all the five plants have revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, sterols, and tannins (Table 1).Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. The phytochemical analysis of the five medicinal plants investigated is tabulated in Tables 1 and 2. Tannins, steroids, saponins , Phenol and cardiac glycosides were present in all the plants. Flavonoids and terpenoids were absent in *S. indicus Linn. and A.longifolia (L.) Nees* respectively. Only *S.trilobatum Linn.* showed the presence of alkaloids (Table 1). Quantitative estimation of the percentage crude chemical constituents in these medicinal plants studied is summarized in Table 2. *P.edulis Sims* contained the highest percentage crude yield of tannin (14.15%), while *B.tinctoria* Lesch *contained* the lowest yield of saponin (0.08 %) but the highest yield of phenol (10%).

Discussion

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube., *et al*., 2008). The phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the plants studied showed that the leaves and roots were rich in alkaloids, phenol and tannins. The presence of phenolic compounds in the plants indicates that these plants may be anti-microbial agent. This agreed with the findings of Ofokansi *et al*. (2005). Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes. Apart from tannin and phenolic compounds, other secondary metabolite constituents of all the five plants detected include the alkaloids, saponin and flavonoids. Flavonoids, on the other hand are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity (Salah *et al*., 1995; Del-Rio *et al*., 1997 & Okwu, 2004). Saponin has the property of precipitating and coagulating red blood cells (Sodipo *et al*., 2000 & Okwu, 2004). Therefore, the data generated from these experiments have provided the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments. However, there is need to further carry out advanced hyphenated spectroscopic studies in order to elucidate the structure of these compounds. Furthermore, this data may be handy in probing of biochemistry of this plant in the future.

Table: 1. Qualitative analysis of the phytochemicals of the medicinal plants.

Plants	Alkaloids	Cardiac glycoside	Flavonoid	Terpenoid	Phenol	Steroids	Saponin	Tannin
B. tinctoria	-	+	+	+	+	+	+	+
S. indicus	-	+	+	-	+	+	+	+
P. edulis	-	+	+	+	+	+	+	+
A. longifolia	-	+	-	-	+	+	+	+
S. trilobatum	+	+	+	+	+	+	+	+

Table: 2. Percentage of crude alkaloids, phenols, tannin, flavonoids, and saponin

Plants	Alkaloids	Phenol	Flavonoid	Tannin	Saponin
B. tinctoria	0.28 ± 0.12	10.2 ± 0.12	1.86 ± 0.31	0.40 ± 0.11	0.34 ± 0.20
S. indicus	0.31 ± 0.22	0.42 ± 0.19	0.10 ± 0.11	0.60 ± 0.22	5.08 ± 0.2
P. edulis	5.63 ± 0.20	12.85 ± 0.28	0.46± 0.3	14.15 ± 0.30	0.08 ± 0.11
A. longifolia	1.14 ± 0.14	0.16 ± 0.10	0.24 ± 0.19	4.92 ± 0.18	0.68 ± 0.12
S. trilobatum	0.46 ± 0.10	4.30 ± 0.20	0.21± 0.12	0.15 ± 0.20	6.23 ± 0.20

Reference

1. Agoha RC 1974. Medicinal Plants of Nigeia. Offset Drakkerij.Faculfcitder Wiskunde in Naturwetenschappen, the Netharlands p 41 and 33.
2. Boham BA, Kocipai-Abyazan R 1974. Flavonoids and condensed tannins from leaves of *Hawaiian vaccinium vaticulatum* and *V. calycinium.* Pacific Sci. 48: 458-463.
3. C.Mohanasundari, D.Natarajan, K.Srinivasan, S.Umamaheswari and A.Ramachandran.,2007.Antibacterial properties of Passiflora foetida L.- a common exotic medicinal plant.African journal of Biotechnology,6 (23),pp: 2650 - 2653
4. Del-Rio A, Obdululio BG, Casfillo J, Marin FG,Ortuno A .,1997. Uses and Properties of citrus flavonoids. J. Agric Food Chem. 45:4505- 4515.
5. Gislene G.F.Nascimento,Juliana Locatelli,Paulo C.Freitas,Giuliana L.Silva.,2000.Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria.Brazilian Journal of Microbiology,31 pp: 247-256.
6. Harborne JB 1973. *Phytochemical methods*, London. Chapman and Hall, Ltd. pp. 49-188.
7. J.M.Sasikumar,M.Remya, and K.Janardhanan.,2003.Antibacterial activity of ethnomedical plants for nilgiri biosphere and western ghate.Asian Jr.Microbiol.Env.Sci.,5 (2) , pp: 183-185
8. Ncube N. S.1, Afolayan A. J.2 and Okoh A. I.,2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology 7 (12), pp. 1797-1806.08
9. OKwu DE .,2004. Phytochemicals and vitamin content of indigenous spices of Southeastern Nigeria. J. Sustain. Agric. Environ. 6(1): 30- 37.
10. Ofokansi KC, Esimone CO, Anele CK 2005. Evaluation of the in Vitro combined anti bacterial effects of the leaf extracts of *Bryophyllum pinnatum*.(Fam: crassulaceae) and *Ocimum gratissium* (Fam: Labiate). Plant Prod. Res. J. 9: 23-27.
11. Obdoni BO, Ochuko PO 2001. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Global J. Pure Appl. Sci. 8 b:203-208.
12. R.Dhandapani and B.Sabna. 2008. Phytochemical constituents of some Indian medicinal plants. Ancient Science of Life. XXXVII (4), pp:1-7
13. Sofowara A 1993. Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. p. 289.
14. Sodipo OA, Akiniyi JA, Ogunbamosu JU 2000. Studies on certain Characteristics of extracts of bark of pansinystalia macruceras (K schemp) pierre Exbeille. Global J. Pure Appl. Sci. 6: 83-87.

15. Salah N, Miller NJ, Pagange G, Tijburg L, Bolwell GP, Rice E, Evans C 1995. Polyphenolic flavonoils as seavenger of aqueous phase radicals as chain breaking antioxidant. Arch Biochem. Broph. 2: 339-346.
16. Sodipo OA, Akiniyi JA, Ogunbamosu JU 2000. Studies on certain Characteristics of extracts of bark of pansinystalia macruceras (K schemp) pierre Exbeille. Global J. Pure Appl. Sci. 6: 83-87.
17. Trease GE, Evans WC 1989. Pharmacognsy. 11th edn. Brailliar Tiridel Can. Macmillian publishers.
18. Van-Burden TP, Robinson WC 1981. Formation of complexes between protein and Tannin acid. J. Agric. Food Chem. 1: 77.