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Phytochemical constituents of some plants used by the tribals of Dakshin Dinajpur district for medicinal purposes

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ABSTRACT

Various phytochemical constituents including alkaloids, phenols, and ascorbic acids were assessed and compared in the four medicinal plants of different families. Four plants used locally for different medicinal purposes were selected for a comparison of their phytochemical constituents. The plants investigated were *Clerodendrum viscosum*, *Moringa oleifera*, *Cinnamomum tamala* and *Scoparia dulcis*. All theses plants were found to contain the active principles including alkaloids, tannin, saponin, terpenoid, flavonoid, cardic glycoside. Steroid was present in C. *viscosum, C. tamala, and M. oleifera* but absent in *S. dulcis.* The assay for the quantitative determination of phenols and ascorbic acids revealed that *C. tamala* contained the highest amount of phenol and ascorbic acids where as *C. viscosum* contained the least amount.

Keywords: phytochemical constituents, medicinal plants, active principles, quantitative determination, spectrophotometric.

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INTRODUCTION

From the very beginning of human evolution, man has turned to plants for all his uses- whether it be food, clothing or as medicines to combat illnesses. Plants are valuable sources of new natural products as these are non narcotic, have no side effect and easily available at affordable cost. The drugs of traditional system have been the starting points of the discovery of many important modern drugs. This leads to the investigation of plants and to undertake general biological screening programs of the plants not only in India but all over the world. So, random screening of plant materials in search of biodynamic compounds is necessary.

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 (1). Plant derived natural products such as flavonoids, terpenoids, and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidants. Considering the importance of investigations on medicinal plants, the present investigation was undertaken to determine the fundamental scientific bases for the use of some medicinal plants used by the tribal of Dakshin Dinajpur, West Bengal, by defining and quantifying the amount of crude phytochemical constituents present in these plants. The anti-diabetic and anti-oxidative properties of some of these plants were reported by us in earlier studies (2, 3).

MATERIALS AND METHODS

Collection and identification of plant materials

The leaves of the study plants were collected from the villages of Dakshin Dinajpur district. The sampling sites had hot, humid climate in summer when most of the sampling was done with a maximum of 29^0C. The plants were identified by the Botanical Survey of India, Kolkata and herbarium specimen of these plants were deposited to the North Bengal University herbarium.

Chemicals and Reagents

All chemicals and reagents used in the study were from Himedia Laboratories, Mumbai.

Extraction

Leaves of plants were air-dried and ground into uniform powder. The aqueous extract of each sample was prepared by soaking 100g of dried powder samples in 200 ml of distilled water for 12hour. The extracts were filtered using Whatman filter paper No 42 (125mm).

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 (4), Trease and Evans (5) and Harborne (6).

Test for Alkaloid

The extracts were dissolved separately in dilute hydrochloric acid and filtered. The filtrate was tested carefully with Mayer's reagent. Appearance of cream precipitate in response to the reagent indicates the presence of alkaloid.

Test for Flavonoid

Three methods were used to determine the presence of flavonoids in the plant sample (4, 6). Dilute ammonia solution (5ml.) was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_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 3min. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for Terpenoid (Salkowski test)

Five ml of each extract was mixed in 2ml of chloroform, and concentrated H_2SO_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 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear bellow the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for Steroid
Two ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Tannin
About 0.5 g of each dried powdered samples were boiled in 20 ml of water separately in test tubes and 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 each of the four powdered sample was boiled in 20ml of distilled water separately over a water bath and filtered. Each filtrate (10ml) was mixed with 5ml of distilled water and shaken vigorously for stable persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Quantification of Alkaloids
Quantification of alkaloid was carried out by the method of Harborne (6) with modifications. Five g of the leaves of the test plants were defatted with 100ml of diethyl ether using a soxhlet apparatus for 2h. after which they were transferred to a 250 ml beaker and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath. 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 was the alkaloid, which was dried and weighed.

Proteins
Soluble proteins were extracted from the leaves and estimated as suggested by Chakraborty and Pradhan (7). For the colorimetric assay, BSA was used as standard.

Carbohydrates
Carbohydrates were extracted in 90% ethanol from the fresh plant leaves of test plants and estimated as suggested by Sadasivam and Manickam (8). Total and reducing sugars were quantified using anthrone reagent and Nelson's arsenomolybdate reagent, respectively. Glucose was used as standard.

Phenols
The leaves of the test plants were dried at room temperature and then reduced to coarse powder. In order to prepare the extracts, 20g of the samples were extracted with n-hexane, after stirring for 2 days, and then the extraction solvent was evaporated *in vacuo* at 40^0C. The method used for the determination of total phenols using Folin-Ciocalteau reagent was adapted from McDonald *et al*. (9). Dried samples and standards were prepared in distilled water. Test solutions (samples and standards) of 0.5 ml were added to 4.0 ml of 1 M Na_2CO_3, 5 ml of Folin-Ciocalteau reagent (1:10, v/v) was added and the solutions allowed to stand at 45^0C for 15 min after which absorbance was measured at 750 nm. Gallic acid standard was used.

Ascorbic acid
Quantification of Ascorbic Acid was done as suggested by Chakraborty and Pradhan (7). The leaves of selected plants each were homogenized in a cold mortar placed on ice using 10ml of 6% trichloroacetic acid. To 4.0 ml of the extract, 2.0 ml 2% dinitrophenylhydrazine (in acidic medium) and 1 drop of 10% thiourea (in 70% ethanol)

were added. The mixture was then kept in a boiling water bath for 15 min. and after cooling at room temperature 5ml of 80% (v/v) H_2SO_4 was added to the mixture at 0^0C. The absorbance at 530nm was recorded. The concentrations of ascorbate were calculated from a standard curve plotted with known concentration of ascorbic acid.

Antioxidant activity

The spectrophotometric assay for the quantitative determination of antioxidant capacity (TAC) was carried out essentially as described by Prieto *et al*. (10). The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate/ Mo (V) complex at acidic pH. An aliquot of 0.1 ml of extracts (1mg/ml) was combined in an Ependroff tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95^0C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution was measured at 695 nm against a blank. TAC was determined by comparison with the α-tocopherol acetate standard calibration curve. The amount of TAC was expressed for extract samples in mM α-tocopherol acetate equivalent/ g dry mass.

RESULTS

Results of qualitative analysis presented in Table 1 showed that among the various phytochemical constituents, *C. tamala, Moringa oleifera* and *C. viscosum* were rich in alkaloid, tannin, saponin, steroid, terpenoid, flavonoid and cardiac glycoside. Alkaloid, tannin, saponin, terpenoid, flavonoid, and cardiac glycoside were present in *S. dulcis* but steroid was not detected in this case.

Results of the quantitative analysis (Table 2) revealed that *C. tamala* contained the highest percentage crude yield of alkaloid (4.92%) while *S. dulcis* contained the lowest (0.84%), whereas the other two had moderate yield.

Estimation of contents of proteins (Table 2) revealed that *M. oleifera* contained the highest amount of protein (55 mg/g tissue) among the four studied leaf extracts and *C. viscosum* contained the least amount (7.5 mg/g tissue). Similar results were also observed in case of carbohydrates where *M. oleifera* contained the highest amount of total sugar (40.5mg/g tissue) and *C. viscosum* contained the least amount (Fig 1).
The spectrophotometric assay for the quantitative determination of phenol revealed that *C.tamala* contained the highest amount (20.83mg/g tissue) of phenol and *C. viscosum* contained the least amount (2.32mg/g tissue) (Table 2).
Quantification of ascorbic acid revealed that *C. tamala* contained the highest amount (22.3mg/g tissue) followed by *M. oleifera* (Table 2).

The antioxidant capacities of the leaf extracts have been depicted in Fig 2.In this case also, *C.tamala* had the highest antioxidant activity, and *C.viscosum* had the least.

DISCUSSION

Phytochemical screening of *C. viscosum, C. tamala, M. oleifera* and *S. dulcis* revealed the presence of secondary metabolites including alkaloids, flavonoids, tannins, saponins, cardic glycosides and terpenoids. Saponins have been reported to possess good antihyperglycemic activity in recent

studies (11, 12).Steroid was detected in *C. viscosum, C. tamala, M. oleifera* but not in *S. dulcis.* Some of these compounds were shown to have anti-inflammatory, antibacterial, hepatoprotective, immuno-modulator, cardiovascular, diuretic, protozoocidal, fungicidal, molluscidal, cytotoxix,cytostatic, antitumor activities (13-18). Plant derived natural products such as flavonoids, terpenoids and steroids etc. have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity (19-21).

Table 1

Qualitative analysis of the phytochemical compounds of the selected plants

Plants	**Alkaloids**	**Tannin**	**Saponin**	**Steroid**	**Terpenoid**	**Flavonoid**	**Cardic Glycoside**
CV	+	+	+	+	+	+	+
MO	+	+	+	+	+	+	+
CT	+	+	+	+	+	+	+
SD	+	+	+	-	+	+	+

(CV = *Clerodendrum viscosum*, MO= *Moringa oleifera*, CT = *Cinnamomum tamala*, SD = *Scoparia dulcis*; + = Present, - = Absent)

Table 2

Alkaloids, protein, total phenols and ascorbic acid content of leaves of the study plants

Sample	**Alkaloid (% of alkaloid/g leaf tissue)**	**Protein (mg/g tissue)**	**Total phenol (mg/g tissue)**	**Ascorbic acid (mg/g tissue)**
CV	1.76 ± 0.08	10.83 ± 2.20	2.33 ± 0.36	7.22 ± 0.15
MO	2.56 ± 0.25	59.17 ± 2.20	6.25 ± 0.07	15.46 ± 0.14
CT	4.92 ± 0.04	23.33 ± 1.67	20.83 ± 0.11	22.30 ± 0.21
SD	0.84 ± 0.02	19.50 ± 0.29	4.31 ± 0.11	8.53 ± 0.11

Each value represents mean; ± = SE; (CV = *Clerodendrum viscosum*, MO= *Moringa oleifera*, CT = *Cinnamomum tamala*, SD = *Scoparia dulcis*)

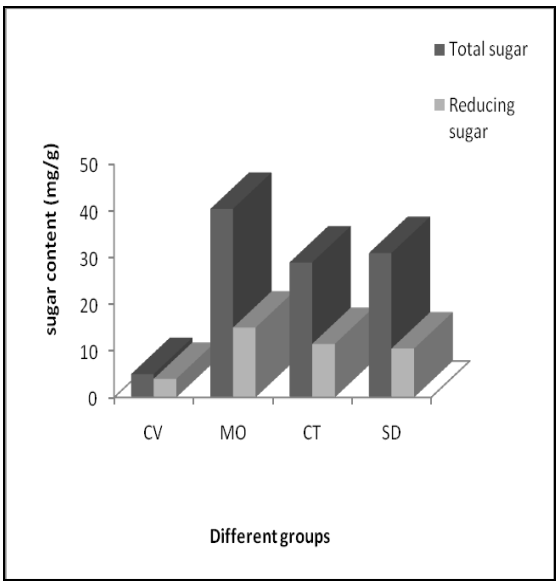

Fig. 1: Total and reducing sugar content of study plants.

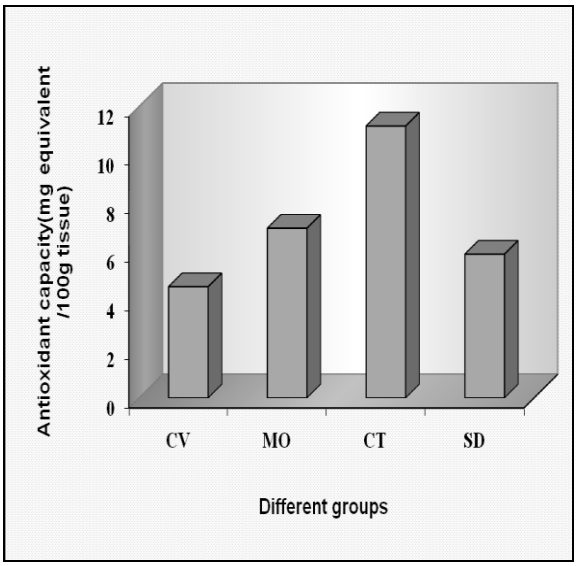

Fig. 2: Antioxidant capacity. CV= *Clerodendrum viscosum*, MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis*.

In our investigation, among the four studied plants highest amount of alkaloid was present in *C. tamala*, while *M. oleifera* contained maximum amounts of proteins and carbohydrates. The spectrophotometric assay for the quantitative determination of phenol revealed that *C. tamala* contained the highest amount of phenol while *C. viscosum* contained the least amount. The phenolic contents of some food and medicinal plants were determined by Bajpai *et al*. (22). The leaves, bark and fruits of *Terminalia arjuna*, *Terminalia bellerica*, *Terminalia chebula* and *Terminalia muelleri*, the leaves and fruits of *Phyllanthus emblica*, and the seeds of *Syzygium cumini* were found to have high total phenolic contents (72.0-167.2 mg/g). *C. tamala* also contained the highest amount of ascorbic acid as well as total antioxidant capacity.

It is clear from the results of the present study that while *M. oleifera* contained the highest amount of carbohydrates and proteins, *C. tamala* contained the highest amount of phenol, ascorbic acid and total antioxidant activity. 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.

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