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Evaluation of biochemical contents, nutritional value, trace elements, SDS-PAGE and HPTLC profiling in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.)

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ABSTRACT

The present study was conducted to investigate the presence of biochemical contents, trace elements, nutritive value evaluation and determination of molecular weight of proteins by SDS-PAGE and phytochemicals detection by HPTLC in the leaves and 50% hydroethanolic leaf extracts of Ruellia tuberosa L. and Dipteracanthus patulus (Jacq.). The biochemical contents, trace elements, nutritive value were determined by different biochemical methods, trace elements presence was detected by using Atomic Absorption Spectroscopy (AAS), while the proteins and phytochemicals were detected by using SDS-PAGE and HPTLC. Ruellia tuberosa L. and Dipteracanthus patulus (Jacq.) leaves confirmed the presence of flavonoids, glycosides, phenols saponins and showed minimum amount of trace elements with moderate nutritive value. Vitamins (E, C), total phenolics, carotenoid content and nutritive value were found to be greater in the leaves of Ruellia tuberosa L. The protein bands obtained in the SDS-PAGE was found to be similar for both the plant leaves. Our findings suggest that leaves of Ruellia tuberosa L. and Dipteracanthus patulus (Jacq.) is endowed with antioxidant phytochemicals and moderate nutritive value could serve as a base for future drugs.

Keywords: *Ruellia tuberosa* L., *Dipteracanthus patulus* (Jacq.), Nutritive value.

INTRODUCTION

Plants have great importance due to their nutritive value and they are the major source of medicines which play an important role in the human history (1). Plants synthesize primary metabolites (proteins, fats, nucleic acids and carbohydrates) by simple substances such as water, carbon dioxide, nitrogen and a number of inorganic salts in small amounts. These primary metabolites are transformed into secondary metabolites (alkaloids, steroids, terpenoids, saponins, flavonoids etc.,) that are used as drugs (2).

All human beings require number of complex organic compounds as added (3) caloric requirements to meet the need for their muscular activities. Minerals and trace elements are chemical elements required by our bodies for numerous biological and physiological processes that are necessary for the maintenance of health (4). Plant materials form major portion of the diet and their nutritive value is important (5). In the present study the protein and HPTLC profile, biochemical, nutritional value and the trace element content in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) are investigated.

The plants belong to the family Acanthaceae (6). In folkloric medicine Ruellia or *Dipteracanthus patulus* (Jacq.) is used for curing eye sore (7). *Ruellia tuberosa* L. is another species of this Acanthaceae family. In folk medicine, this plant has been used as diuretic, antidiabetic, antipyretic, analgesic, antihypertensive, thirst quenching and antidotal agent (8,9). The plant is also used to treat urinary problems and high cholesterol levels and it is also used as anthelmintic and for oestrus induction (10).

EXPERIMENTAL SECTION

Plant material

Fresh leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) was collected from ABS (Altogether Botanical Species) Medicinal Plants Garden, Karipatti, Salem, Tamilnadu, India. The plant was identified by the Botanical Survey of India (BSI) Southern circle, Tamilnadu Agriculture University (TNAU) (No: BSI/SC/5/23/08-09/Tech-118-229).

Preparation of 50% hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.)

The fresh leaves collected, were shade dried for five days and crushed to coarse powder. The coarse powder thus obtained was cold macerated with 50% ethanol (1: 1, ethanol: water) and kept for 3 days at room temperature, with occasional stirring (11). The suspension was filtered through a fine muslin cloth and was evaporated to dryness at a low temperature (at 40º Celsius) under reduced pressure in a rotary evaporator. Dark brown colored crystals obtained were used for the HPTLC studies.

Biochemical estimations

Estimation of total carotenoids and lycopene was done by (12). Tocopherol and ascorbic acid was estimated by (13), (14). Estimation of phenol and tannin was done by (15), (16).

HPTLC

10µl of the test solution (plant sample) was applied as 10 mm band on 5x10 pre-coated silica gel 60 F254 Thin Layer Chromatography (TLC) plate of uniform thickness of 0.2 mm by using Linomat 5 system.The sample loaded TLC plate was kept in TLC twin trough developing chamber with respective mobile phase solvent for 15 minutes (for chamber saturation). The sample loaded plates were developed using respective mobile phase up to 80 mm.

The plates were removed and allowed to dry in air. The developed plates were dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber and plate images were captured in white and UV light at 254 and 366 nm. The plates were sprayed with respective spraying reagent and dried at 110^0C in hot air oven. The plates are photo-documented at UV 366 nm and white light using photo-documentation chamber.

Determination of nutritive value and trace elements

Estimation of ash and moisture content was done as per in (17). Crude fat and crude fiber was estimated by (18). To prepare the sample for mineral analysis, the washed and dried materials were ground to fine powder and used for dried ashing. One gram of sulphated ash was dissolved in 100ml of 5% HCl to obtain the solution ready for determination of mineral elements (Zn, Mn, Cu, Co, Fe) through Atomic Absorption Spectroscopy (AAS). Total protein was estimated by (19). Carbohydrate and nutritive value was calculated by the following formulae.

Percentage of carbohydrate was given by (20)

100 – (Percentage of ash + percentage moisture + percentage fat + percentage protein).

Nutritive value is finally determined by

Nutritive value = 4 x percentage of protein + 9 x percentage of fat + 4 x percentage carbohydrate.

SDS-PAGE

Total leaf protein was extracted by the acetone- TCA (trichloroacetic acid) precipitation method of (21) and the estimation of protein was done by the method of Lowry *et al.* (1951). The molecular weight of the protein was determined by Sodium Dodecyl Sulfate (SDS) polyacrylamide gel electrophoresis (PAGE). Samples (0.5 g) were homogenized with 2 ml of a buffer containing 50 mm Tris (hydroxymethyl) aminomethane (Tris)-Glycine (pH 8.3), 0.5 m sucrose, 50 mm EDTA, 0.1 m KCl, 2 mm PMSF and 0.1% (v/v) 2-mercaptoethanol in a chilled pestle and mortar at 4 ^{0}C. The homogenate was centrifuged in a refrigerated centrifuge at 14,000 x *g* for 10 min. Protein concentration in the supernatant samples was estimated according to the method of (22). Gels were made according to (23).

A 12.5% separating gel containing 375 mm Tris- HCl, pH 8.8, 0.1% (w/v) SDS, 0.05% (w/v) ammonium persulfate and 0.4 μl ml^{-1} TEMED was used for resolving the polypeptides whereas a 4% stacking gel containing 125 mm Tris-HCl, pH 6.8, 0.1% (w/v) SDS, 0.05% (w/v) ammonium persulfate and 0.5 μlml^{-1} TEMED was used to concentrate (stack) the polypeptides. The

electrophoresis running buffer consisted of 25 mm Tris, 192 mm glycine and 0.1% SDS, pH 8.3. Electrophoresis was accomplished at 35 mA for 4 h.

The gels were stained with 0.25% Coomassie Brilliant Blue R-250 (Sigma) in 50% (v/v) methanol and 10% (v/v) acetic acid for 2 h and destained with 50% (v/v) methanol and 10% (v/v) acetic acid until the background was clear.

RESULT

Table 1 shows the biochemical composition in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.). Ascorbic acid level was found to be 0.44 and 0.36 mg/g. Total phenol and tannin content was detected as 0.43, 0.56 and 10.0, 13.0 mg/g. The lycopene and carotenoid content was found to be 0.896, 0.486 and 0.046, 0.031 mg/g. The tocopherol levels in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) was found to be 0.187 and 0.160 mg/g respectively.

Table 1: Determination of antioxidant activity in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.).

S.No	Parameters	50 % hydroethanolic leaves extract of *Ruellia tuberosa* L. (mg/g)*	50% hydroethanolic leaves extract of *Dipteracanthus patulus* (Jacq.) (mg/g)*
1	Ascorbic acid	0.44	0.36
2	Total phenol	0.43	0.56
3	Tannin	10.0	13.0
4	Lycopene	0.896	0.486
5	Carotenoid	0.046	0.031
6	Tocopherol	0.187	0.160

**Values are mean of triplicates*.

Table 2 shows the nutritive value content of the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.). The ash and moisture content of the leaves were 6.2, 5.6 and 5.2, 3.6%. Crude fat and protein levels were found to be 1.32, 1.13 and 4.3, 4.9%. Total carbohydrate and crude fiber content was detected as 56.4, 62.8 and 2.7, 1.4%. The nutritive value of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) leaves were found to be 280.9 and 254.8% respectively.

Table 2: Evaluation of nutritive value in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.)

S.No	Parameters	*Ruellia tuberosa* L. (%)*	*Dipteracanthus patulus* (Jacq.) (%)*
1	Ash	6.2	5.6
2	Moisture content	5.2	3.6
3	Crude fat	1.32	1.13
4	Protein	4.3	4.9
5	Total carbohydrate	56.4	62.8
6	Crude fibre	2.7	1.4
7	Nutritive value	280.9	254.8

Values are mean of triplicates.

Presence of trace elements were detected in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) by AAS (Atomic Absorption Spectroscopy) in which cobalt and zinc levels were found to be 0.024, 0.01 and 0.35, 0.22 mg/g.

The copper and iron content was found to be 0.52, 0.50 and 2.11, 1.92 mg/g. Magnesium was found to be as major constituent in the leaves of the plants 3.10 and 3.06 mg/g respectively (Table 3). The preliminary phytochemical analysis in our laboratory confirmed the presence of secondary metabolites in 50% hydro ethanolic extract (24).

HPTLC profile (Fig. 2) of 50% hydro ethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.), gave four spots for phenols, six spots for saponin, four and five spots for glycosides, two spots for flavonoids at different Rf values. The absence of alkaloid was observed in both the plant extracts (Table 4).

Table 3: Determination of elements in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.)

S.No	Parameters	*Ruellia tuberosa* L. (mg/g)*	*Dipteracanthus patulus* (Jacq.) mg/g)*
1	Magnesium	3.06	3.10
2	Cobalt	0.01	0.024
3	Zinc	0.22	0.35
4	Copper	0.50	0.52
5	Iron	1.92	2.11

*Values are mean of triplicates.

Table 4: High performance thin layer chromatography (HPTLC) profile of 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.).

	Alkaloids		Phenols		Saponin		Glycosides		Flavonoids	
S.No	A	B	A	B	A	B	A	B	A	B
1	---	---	0.38	0.36	0.11	0.10	0.55	0.59	0.76	0.75
2	---	---	0.53	0.55	0.18	0.17	0.65	0.70	0.39	0.40
3	---	---	0.67	0.69	0.37	0.39	0.71	0.84	---	---
4	---	---	0.87	0.80	0.53	0.56	0.82	0.85	---	---
5	---	---	---	---	0.64	0.67	0.86	---	---	---
6	---	---	---	---	0.77	0.80	---	---	---	---

A- 50% Hydro-ethanolic leaf extract of Dipteracanthus patulus (Jacq.)
B- 50% Hydroethanolic leaf extract of Ruellia tuberosa L.

Electrophoretic leaf protein profiles of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) were presented in the Figure 2, Table 5. The lowest molecular weight protein was found to be as 11 and 64 KD and the highest molecular weight was 142 KD. The profile showed an identical number of bands with similar mobility.

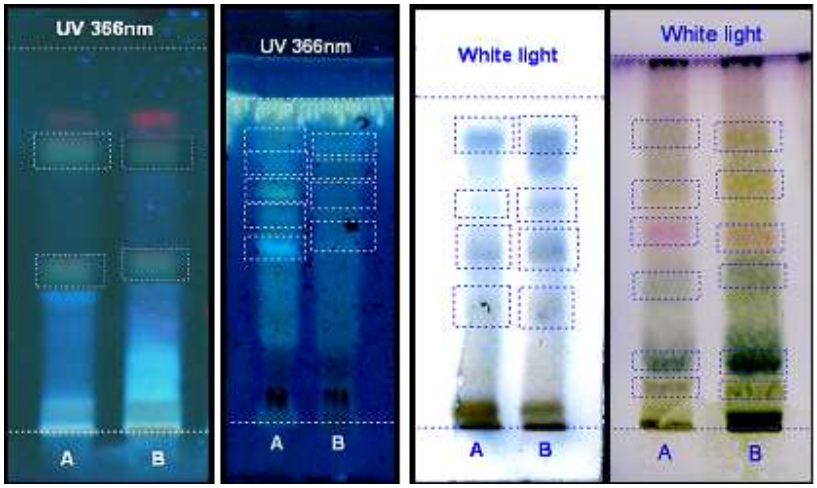

Figure 1: HPTLC profile of flavonoids, saponin, phenols and glycosidic compounds after derivatization

Table 5: SDS-PAGE profile for the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.)

S.No	*Ruellia tuberosa* L. (KD)	*Dipteracanthus patulus* (Jacq.) (KD)
1	11	64
2	115	115
3	125	125
4	142	142

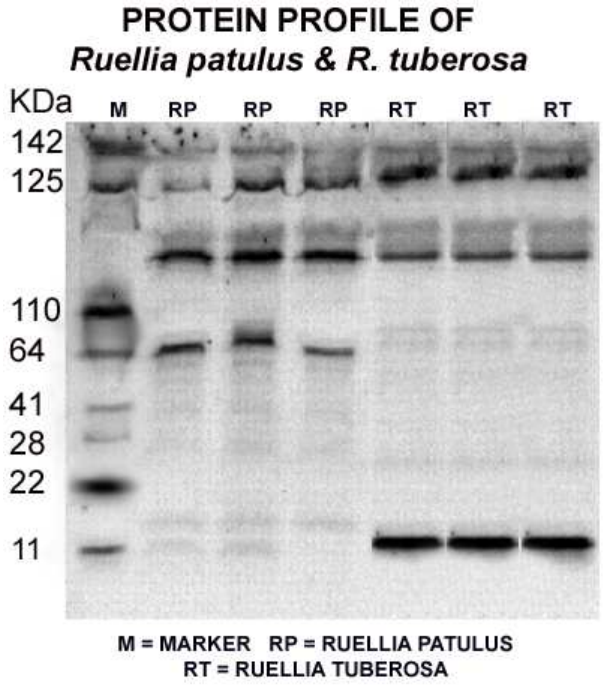

Figure 2: Protein profile of *Ruellia tuberosa* L. (RT) and *Dipteracanthus patulus* (Jacq.) or *Ruellia patulus* (RP) leaves

DISCUSSION

Phytochemicals are defined as bioactive non-nutrient plant compounds found in fruits, vegetables, grains and other plant foods that have been linked to reducing the risk of major chronic diseases (25). The Medicinal values of plants i.e. their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds produce a definite physiological action on the human body (26). Medicinal plants contain many antioxidants such as vitamin (A, C& K), carotenoids, flavonoids (flavones, isoflavones, flavonones, anthocyanidin, catechin and isocatechin), polyphenols (ellagic gallic acid and tannin). Several reports say that these compounds possess remarkable antitumor, antidiabetic and antioxidant activity (27, 28, 29).

Flavonoids are a group of naturally occurring polyphenolic compounds primarily from fruits and vegetables. They are one of the most numerous and wide spread groups of phenolic compounds seen in higher plants (30). Several studies have evaluated the cyto-toxic effect of saponins against tumor development. The active components in several herbal medicines that have been used as chemotherapeutic agents in Eastern countries were shown to be saponins. A Chinese herbal drug, '*Yunan Bai Yao'* has been used as a hemostatic agent and it is known to promote wound healing (31). The leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) possess significant amount of vitamin (C, K), carotenoids and phenols. The HPTLC profile shows the presence of secondary metabolites such as flavonoids, glycosides, phenols and saponin.

Iron sufficient in all studied medicinal plants, it make body tendons and ligaments, certain chemicals of brain are controlled by the presence or absence of iron, it is essential for the formation of hemoglobin, carry oxygen around the body (32). Cu was an important component of many enzyme systems such as cytochrome oxidase, lysyl oxidase and ceruloplasmin an iron oxidizing enzyme in blood (33). Cu deficiency has been associated with cardiac abnormalities in human and animal, cause's anemia and neutropenia (34). Zinc maintain various reactions of the body which help to construct and maintain DNA, required for the growth and repair of body tissues, important element of ligaments and tendons (35).

Mg plays important role in the formation and function of bones, muscles and prevents chronic disorders, high blood pressure and depression (34) also Mg plays important role in enzyme activity, deficiency interfere with transmission of nerve and muscle, impulses, causing irritability and nervousness, prevent heart diseases (36). Vitamin B12 exists in several forms and contains the mineral cobalt (37). Deficiency is characterized by megaloblastic anemia, fatigue, weakness, constipation, loss of appetite and weight loss (38). Neurological changes, such as numbness and tingling in the hands and feet, can also occur (39). Both the plants show minimum amount of elements and better nutritive value in the leaves.

HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. The 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) showed the presence of phytochemicals. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is most economical simple and extensively used biochemical

technique for the analysis of molecular weight of the proteins. The plant leaves showed similar protein bands in the SDS-PAGE electrophoresis.

It is concluded that both the plants were found to be having essential minerals with good nutritive value and secondary metabolites. Future goal is to isolate the secondary metabolites and to study their pharmacological activity.

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