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Phytochemical analysis and *in vivo* toxicity evaluation of green vegetable *Tetrastigma angustifolia (Roxb.)* leaves

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Abstract

T. angustifolia (Vitaceae) is a Green vegetable and valuable ethnomedicinal plant in India and inflorescence has been reported for the treatment of antioxidant in Indian literatures. The present study intended with various phytochemical, physicochemical screening and toxicity studies were carried out on the leaves of the *T. angustifolia*. Phytochemical evaluation of extracts of revealed that the presence of flavonoids, glycosides, alkaloids, proteins and phenolic compounds. Physicochemical properties including total ash, water soluble ash, acid insoluble ash and loss on drying were determined. The toxicity studies were performed as acute oral toxicity determined the highest dose of 5000 mg/kg body weight 2 weeks. The results of the various phytochemical, physicochemical and toxicity tests indicated that the plant to be rich in various biologically active compounds which could serve as potential source of the crude drugs and in addition the plant is not toxic to the experimental model.

Key words : *Tetrastigma angustifolia*, phytochemical screening, physicochemical evaluation, Toxicity studies.

INTRODUCTION

India has rich treasure of variety of medicinal plants which are used in traditional herbal therapy. Folk remedies, which use whole plant or part of plants, related directly or indirectly as pharmaceutical preparation of modern medicine. Phytochemicals showing biological activity have had immense utility as pharmaceuticals and pharmacological actions. In recent years, because of their easy availability and cost effectiveness, there has been a dramatic rise in use of herbal drugs/preparations in the developed countries besides having desired pharmacological effectiveness with high level of safety and low toxicity profile [1]. Phytochemicals are naturally occurring in the medicinal plants and vegetables that have defense mechanism and protect from various diseases. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids, flavonied and phenolic compounds. Alkaloids and flavonied exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and antibacterial activities [2].

T. angustifolia belongs to family vitaceae which is one of the most dominant and diversified families of climbing plants distributed throughout the tropical forests including India. The family exhibit interesting geographical distribution patterns, some genera are strictly regional, some are endemic and some are worldwide [3]. Acevedo Rodrigues described throughout families of climbing plants and assessed their resemblances. Vitaceae is one of the primitive families of angiosperms and may evolve into Asclepidaceae [4]. Lambordi focused on the nomenclature system given to the Vitaceae members [5]. Wilson investigate the morphological and anatomical development in family Vitaceae. Their work reveals that some species of vitaceae shows important vegetative and reproductive differences among species [6]. In India, Chitubabu and Parthasarathy, Reddy and Parthasarthy, Rawal and Pangtey, Parthasarathy prepared the preliminary list, patterns of diversity and phenology of climbing plants from different ecological zones [7,8,9,10].

T. angustifolia is called as "Naltanga" in local language of North-East India. In North Eastern part of India, particularly Assam, the whole plant is used as a green vegetable and traditionally used to treatment of a variety of human disorders [11]. The present study on this plant was therefore undertaken to determine the quality of herbal plants, toxicity, physicochemical and phytochemical characterizations are required to be carried out for establishing their identity, purity, safety and quality standards for evaluating the plant material.

MATERIALS AND METHODS

Collection and extraction of plant material

Fresh leaves of *T. angustifolia* were collected in the month of March 2013 from the Dibrugarh forest, Dibrugarh district, Assam, India. The plant species was identified and authenticated by Botanical Survey of India, Eastern Regional Centre, Shillong, India, and a voucher specimen (BSI/ERC/ Tech./Plant Iden./2014/830) was deposited. The plant sample was shade dried for four days followed by drying in an oven preset at 45 for four days. The sample was powdered in a mixer grinder, sieved through 85 mesh (BSS) and stored in an air tight container. The powdered leaves were subjected to successive solvent extraction as follows:

About 100 gm of powder plants material was weighted & packed in a soxhlet apparatus for extraction (continuous hot percolation method). The solvents such as petroleum ether, chloroform, ethyl acetate, methanol and water were used for the study. The extraction was carried out at 60^{0C} for at least 72 h for every solvent. Each time before extracting with the next solvent, the powdered material was air dried first and then oven dried below 50°C. Each extract was concentrated by distilling off the solvent (in a rotary vacumm evaporator) and then evaporated to dryness on the waterbath.Each dried extract was collected and preserved in a desiccator for further experiment.

Reagent and chemicals

Chemicals of analytical grade were purchased from Merck Specialties Private Limited, Mumbai, Rankem, Mumbai and Himedia Labs., Mumbai.

Organoleptic characters of the leaves

T. angustifolia (leaves) were visually examined and organoleptic characters like colour, odour and taste were characterized.

Physicochemical analysis

The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value and moisture content were calculated as per WHO guideline.

Phytochemical screening

Preliminary phytochemical analysis of *T. angustifolia* (leaves) was carried out using all the five solvent extracts. Preliminary phytochemical analysis was carried out for flavonoids, phenols, alkaloids, carbohydrates, glycosides, fats and oils, steroids, tannins, proteins and amino acids [12, 13].

Fluorescence analysis

A small quantity of dried and finely powdered leaves sample was placed on a grease free microscopic slide and added 1-2 drops of freshly prepared solution like ferric chloride, glacial acetic acid, hydrochloric acid, iodine, nitric acid and sodium hydroxide etc, mixed by gentle tilting the slide and waited for 1-2 minutes and examined both in daylight and UV light (254 nm and 365 nm) [14, 15].

Thin layer chromatographic (TLC) analysis

For TLC analysis plate with Silica gel 60 F254 TLC (Merck, Germany), 7X6 cm was cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1µl of sample by using capillary at distance of 1 cm at 5 track, by the different solvent system methanol:chloroform:pet ether (2:1:2) and toluene:ethyl acetate:formic acid (4:5:1) as mobile phase. One dimensional ascending method was used for the development of plates as per standard protocol (Indian Pharmacopoeia 1996). The TLC plate was air-dried and spots were visualized under ultraviolet light (254 & 365 nm). The R_f values of the spots were also recorded.

Acute oral toxicity studies

The experiment was conducted on 18 healthy albino wistar rats (males and females) weight between 200 gm to 250 gm and aged 8 to 9 weeks obtained from the animal house, Dibrugarh University. The rats were distributed into two groups. The animals were used with the approval of the Institute Animal Ethics Committee (Approval no: IAEC/DU/50 Dt. 24.9.13, Regd. No. 1576/Go/a/11/ CPCSEA dated 17.02.2012) before starting the study and were conducted under the internationally accepted principles for laboratory animal use and care [16].

RESULTS

Organoleptic evaluation

The organoleptic study reveals that fresh leaves are dark green in colour, with characteristics odour and taste.

Physicochemical analysis

The results of physicochemical analyses lie within the acceptable limit which in turn ascertains the quality as well as purity of leaf drugs. These parameters are therefore some useful quality standards included in the standardization of *T. angustifolia* leaf drugs.

Phytochemical screening

Respective extracts were subjected to preliminary phytochemical screening and the results (Table 2) validated the presence of flavonoids, alkaloids, glycosides and phenolic compounds in leaves.

Fluorescence analysis

Fluorescence analysis of leaf powder was carried out after treating with several solvents. Fluorescence was observed at 254 and 365 nm comparing its change of colour in visible light. The observations are presented in Table 3 showing the variation in colour.

Thin layer chromatographic analysis

Table 4 reveal the TLC fingerprint profile of different extracts which depicts the number of spots obtained with their relative R_f values. TLC chromatograms depicted in figure 2 show certain distinct spots with their relative intensities.

Acute oral toxicity studies

The toxic effect of hydro-alcoholic extract of *T. angustifolia* leaves on the general behavioural and Body weight of rats are shown in Table 5 and Table 6.

Table 1: Physicochemical parameters

Parameter	% w/w±SEM*
Moisture content (%LOD)	16.35 ±0.32
Ash values	
Total ash	15.47±0.29
Acid insoluble	02.58±0.28
Water soluble	05.74±0.25
Extractive values	
Water soluble	25.49±0.37
Alcohol soluble	04.84±0.15

*Values are expressed as mean ± SEM of three replicate

Table 2: Phytochemical screening of various solvent extracts of *T. angustifolia* leaves

Constituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
Alkaloids	- -	- -	--	++	++
Glycosides	–+	++	++	+ +	++
Phenolic compounds and tannins	- -	--	+ +	++	++
Flavonoids	--	+ +	+ +	++	++
Carbohydrates	- -	--	++	+ +	++
Proteins	- -		--	++	++
Fats and oils	–+	++	- -	- -	--
Amino acids	- -	- -	- -	++	++
Steroids	–+	++	++	++	- -

'+ +' indicates presence '- -' indicates absenc

Biochemical Analysis

Table 7 shows the changes of biochemical parameters in the serum of rats induced by *hydro-alcoholic leaves extract of T. angustifolia*. In the rats, there are no significant changes for the serum levels of alanine aminotransferase and aspartate aminotransferase, alkaline phosphatase triglyceride, cholesterol ($P > 0.05$) after oral administration of hydro-alcoholic leaves extract of *T. angustifolia.*

DISCUSSION

The leaves of this plant were used by local people ethnomedically in the treatment of various ailments without any standardization. Standardization of a crude drug is a pre-requisite criterion for establishing its correct identity and various standard pharmacognostic parameters must be established for any crude drug to be included in an herbal pharmacopoeia. For establishing the correct identity of the source materials, macroscopic method is one of the simplest and cheapest methods to start with. The

Table 3: Fluorescence analysis of powdered leaves of *T. angustifolia*

Powdered drug/Treatment	Visible/Day light	Short UV (254 nm)	Long UV (365 nm)
Powder as such	Green	Dark Green	Dark violet
Powder + 1% Glacial acetic acid	Light yellow	Blackish brown	Violet
Powder + Dil. NH3	Blackish brown	Dark violet	Brown
Powder + Conc. HCl	Green	Dark Green	Brown
Powder + Conc. HNO_3	Brown	Dark Green	Dark Green
Powder + Conc. H_2SO_4	Bright green	Dark Green	Violet
Powder +Acetone + Methanol	Green	Dark violet	Dark Green
Powder + Methanol	Green	Brownish yellow	Greenish Violet
Powder + Picric Acid	Greenish Brown	Yellowish Brown	Yellowish Brown
Powder + NaOH Solution (20% w/v)	Light Green	Dark Green	Dark Green

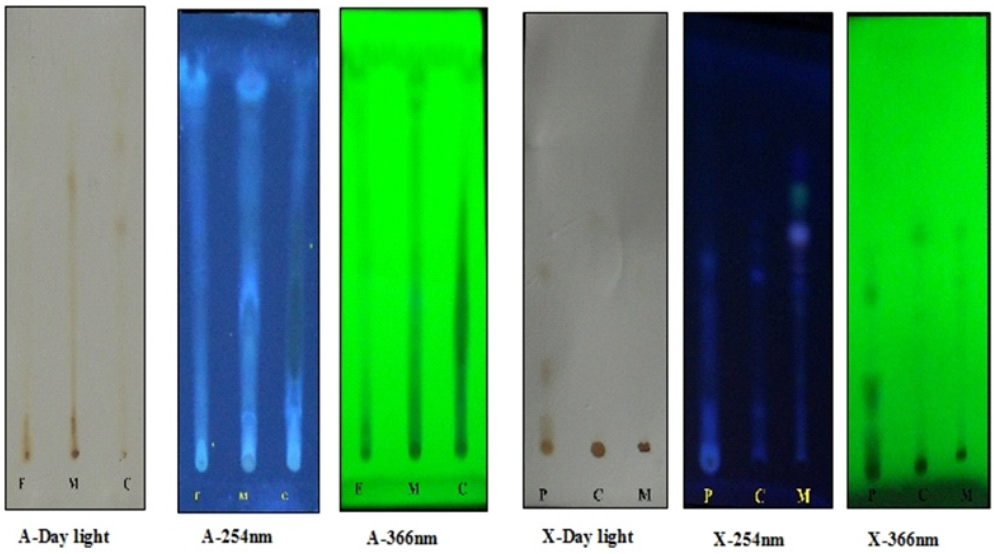

Figure 2: TLC Finger print profile of *T. angustifolia* under 254 nm (A-254 & X-254) and 366 nm (A-366 & X-366)

(**P** is Pet. Ether extract, **C** is Chloroform extract, **E** is Ethyl extract, **M** is Methanol Extract)
TLC Plate **A** was Developed with Toluene: Ethyl Acetate: Formic Acid (4:5:1)
and **X** was Developed with Methanol: Chloroform: Pet Ether (2:1:2)

phytochemical, physicochemical and toxicity studies standardization for leaves of *T. angustifolia* are carried out for the first time in this study.

The physicochemical standards, such as loss on drying, ash values, extractive values will be useful to identify the authenticity of the drug even from the crushed or powdered plant materials. The less moisture content of drugs could prevent the unwanted growth of bacteria, fungi or yeast etc., during storage. Moreover determination of moisture content is essential for the plant drugs because possible enzymatic deterioration of active principles could happen due to insufficient drying [17]. Acid insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the water soluble portion of the total ash [17, 18]. Ash values used to find out authenticity, quality and purity of drug and also these values are important quantitative standards. On the other hand, the water soluble extractive value of the drug was found to be 25.49±0.37 (%w/w) which indicates the presence of water soluble components such as sugar, acids and inorganic compounds etc.; and the alcohol soluble extractive value was found to be 04.84±0.15 (%w/w) which indicates the presence of polar constituents like phenols alkaloids steroids glycosides flavonoids. The extractive values are valuable to estimate the chemical constituents present in the crude drug and furthermore assist in evaluation of definite constituents soluble in a particular solvent.

Different phytochemical compounds such as carbohydrates, alkaloids, tannins, saponins, glycosides, flavonoids etc were

Table 4: Thin layer chromatographic analysis

Extract	No of spots (Plate A)	R_f values (Plate A)	No of spots (Plate X)	R_f values (Plate X)
Pet ether extract	--	---	3	0.22,0.27,0.44
Chloroform extract	3	0.21, 0.52, 0.57	- -	
Ethyl acetate	3	0.23, 0.55, 0.83,	6	0.22,0.43, 0.47.0.49,0.54, 0.82
Methanol	4	0.22, 0.49, 0.58,0.83	8	0.22,0.27,0.43, 0.47.0.49,0.54, 0.67,0.79

'- -' indicates absence

Table 5: Body weight of the animals during the treatment of hydro-alcoholic leaves extract of *T. angustifolia* during 14 days of treatment

Group	0 day	7 day	14 day
Control group	211.23 ± 2.22	213.65 ± 4.11	214.98 ± 1.45
Gr-I (2500 mg/kg body weight)	216.34 ± 3.43	216.79 ± 2.87	217.34 ±3.87
Gr-II (5000 mg/kg body weight)	219.65 ± 2.35	220.13 ± 5.87	221.43 ± 1.98

Table 6: General behavioral observations for control and treated groups

Observation	Control group		Test group	
	6 h	14 h	6 h	14 h
Tremors	Normal	Normal	Normal	Normal
Eyes and Skin	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal
Behavioural patterns	Normal	Normal	Normal	Normal
Lethargy and Sleep	Normal	Normal	Normal	Normal
Allergic reaction	Normal	Normal	Normal	Normal
Diarrhea	Normal	Normal	Normal	Normal
Mortality and Coma	Normal	Normal	Normal	Normal
Convulsion	Normal	Normal	Normal	Normal

Table 7: Changes of biochemical parameters in the serum of Rats induced by hydro-alcoholic leaves extract of *T. angustifolia.*

Groups	SGOT(U/L)	SGPT (U/L)	ALP (U/L)	Triglyceride (mg/dl)	Cholesterol (mg/dl)
Control group	57.65 ± 2.87	61.54 ± 3.87	122.21 ± 1.23	159.87 ± 4.33	212.54 ± 1.76
Gr-I 2500	56.43 ± 3.65*	62.62 ± 2.43	121.87 ± 3.44	160.32 ± 3.48*	211.59±2.98
Gr-II 5000	58.87 ± 2.35	64.23 ± 3.68	124. 54 ± 1.45	161.87 ± 5.23*	214.82 ± 4.33*

The data are expressed as the means ± SEM. *p < 0.05, **p < 0.01 vs control group.

found in the leaves of *T. angustifolia* in the preliminary phytochemical analysis. The ethyl acetate, methanol and aqueous extracts contain flavonoids, carbohydrates and phenolic compounds are present in all the solvent extracts except petroleum ether extract. The proteins are present in methanol and aqueous extracts only. Thus the preliminary screening tests used in the present study for the detection of the bioactive principles which will pave the way to the drug discovery and development

Fluorescence analysis is a very important and useful tool for the identification of different constituents present in natural products and the phenomenon may be due to a particular fluorescent substance or due to some fluorescent derivative formed after treatment with reagents. Powdered leaves were analyzed under ordinary light, short ultraviolet wavelength and long ultraviolet wavelength simultaneously after treatment with following organic and inorganic reagent like 50% H2SO4, 10% NaOH, 50% NHO3, FeCl3, distilled water, aniline, potassium hydroxide and chloroform**.** Under UV light many natural products (e.g., alkaloids like berberine) produces fluorescence. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by the application of different reagents [14, 19].

TLC fingerprint profile along with their recorded R_f values, can serve as reference standard for further research on the medicinal properties of the plant. Various phytochemicals gives different R_f values in different solvent system. This variation in R_f

values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The chromatograms (TLC) showing characteristic spots with their relative R_f values can be used for the identification of pure components present in the leaves of *T. angustifolia*. Eight spots having respective R_f values are found in the TLC plate X (methanolic extract) which may indicate the presence of various phytoconstituents like alkaloids, flavonoids, saponins, and tannins. Therefore, with the help of TLC fingerprinting the plant *T. angustifolia* can be identified as well as standardized[20,21].

In this study, the acute toxicity effect of the *T. angustifolia* leaf extract was evaluated as per OECD guidelines. The rats were observed for toxicity and mortality for 14 days. No toxic symptoms or mortality were observed in any animals, which lived up to 14 days after the administration of hydro-alcoholic extract at two dose level of 2500 or 5000 mg/kg body weight. The behavioural patterns of animals were also observed first 6 h and followed by 14 h after the administration and the animals in both vehicle-treated (saline) and extract-treated groups were normal and did not display significant changes in behavior, skin effects, breathing, impairment in food intake and water consumption, hair loss, postural abnormalities and Body weight. Moreover the normal levels of SGOT, SGPT, ALP, triglyceride and cholesterol signifies the safety profile *of T. angustifolia* leave extract.

CONCLUSION

The Toxicity profile and phytochemical, physiochemical analyses of the present study showed favorable effects for the standardization parameters of plant parts. This established a significant scope to develop a broad spectrum use of *T. angustifolia* in herbal medicine and as a base for the development of novel potent drugs and phytomedicine. Toxicity studies and chemical, physiochemical analyses reveal useful information which is of utmost important for the quality control of *T. angustifolia* leaves to be used as crude drugs. The documentation of standardized parameters therefore are an indispensable element in the development of herbal drugs from raw plant drugs (crude preparations), considering their desired therapeutic and safety profile. It is suggested that further work should be carried out to isolate, purify and possibly characterize the active constituents responsible for the activity of the plant.

Conflict of interest

The authors declare that they have no conflicts of interest.

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REFERENCES

1. Kadam PV, Deod RS, Shivatare, Yadav KY, Patil MJ. Pharmacognostic, phytochemical and physiochemical studies of *Mimusops Elengi* Linn stem bark (Sapotaceae). Der Pharm Lettr. 2012: 4: 607-613.

2. Annan K, Dickson RA, Amponsah IK, Jato J, Nooni IK. Pharmacognostic evaluation and physicochemical analysis of *Paullinia pinnata* L. (Sapindaceae). J Pharmacogn Phytochem. 2014: 2: 203-208.

3. Hui R, Min ML, Akiko S. Phylogenetic analysis of the grape family (Vitaceae) based on noncoding plastids sequences. Taxon 2011: 60, 629-637.

4. Acevedo PR: Smithsonian Institution, Washington, 2005. p. 268-273.

5. Lamberdi JA. Types of names in Ampelocissus and Cissus (Vitaceae) referring to taxa in the Crribean, Central and N. America. Taxon. 1997: 46: 423- 427.

6. Wilson TC, Gerrath J, Posluszny U. Morphological and anatomical development in Vitaceae Species: Vegetative and reproductive differences. Canadian Jr. of Botany: 2006: 84: 702-716.

7. Chittibabu C, Parthasarthy N. Liana diversity and host relationship in a tropical evergreen forest in the Indian Eastern Ghats. Ecological Research. 2001: 16: 519-529.

8. Reddy SM, Parthasarthy N. Liana diversity and distribution in four tropical dry evergreen forests on the Coromandal coast of South India. Biodiversity and Conservation. 2006: 12: 1609-1627.

9. Rawal RS, Pangtey YPS. Distribution and phenology of climbers of Kumaun in Central Himalaya, India. JSTOR. 1991: 97: 77-87.

10. Parthasarthy N, Seiwyn M, Udaykumar M. Tropical dry evergreen forest of peninsular India: Ecology and conservation. Trop conserv sci. 2008: 1: 89-110.

11. Begum SS, Gogoi R. Herbal recipe prepared during Bohag or Rongali Bihu in Assam. Indian J. of Trad. Knowledge. 2007: 6: 417-422.

12. Khandelwal KR: Nirali Prakashan, India, 2005. P.175-182.

13. Kokate CK, Purohi AP, Gokhale SB: Nirali Prakashan, India, 2007. P. 255-269.

14. Gupta MK, Sharma PK, Ansari SH, Lagarkha R. Pharmacognostical evaluation of Grewia asiatica fruits. Int J Plant Sci. 2006: 2: 249-251.

15. Kokashi CJ, Kokashi RJ,Sharma M. Fluorescence of powdered vegetable drugs in ultra- violet radiation. J Am Pharm Assoc. 1958: 47: 715-717.

16. Junejo JA, Mondal P, VermaV, Rudrapal M, Zaman K. Anti-Diabetic Assessment of the Hydro-Alcoholic Leaf Extracts of the Plant *Tetrastigma angustifolia* (Roxb.)a traditionally used North-Eastern Indian vegetable. B & P Journal.2014: 7: 635-644.

17. Sharan SB, Mondal P, Zaman K, Junajo JA, Verma VK. In vivo Anti-Diabetic Activity of the Methanolic and Aqueous Bark Extracts of the Plant *Emblica officinalis* Gaertn. A J Pharm Science. 2013: 6: 64-68.

18. Evans WC: Saunders Publication, USA, 2002. P.197-212

19. Ansari SH: Birla Publications Pvt. Ltd, India, 2006. P.199-223

20. Ravichandra D, Padma MP. Pharmacognostic and phytochemical investigation on leaves of Ficus hispida. Int J Pharm Pharm Sci. 2011: 131-134.

21. Junejo JA, Zaman K, Rudrapal M, Mondal P, Singh KD, Verma VK. Preliminary phytochemical and physicochemical

evaluation of *Carallia brachiata* (Lour.) Merr. Leaves. JAPS. 2014: 4: 123-127.