



PHARMACOCHEMICAL CHARACTERIZATION OF VARIOUS EXTRACTS OF LEAF AND FRUIT OF *Trichosanthes dioica* PLANT

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ABSTRACT

Aim: This study aims to investigate the presence of phytochemicals in various extracts of leaf and fruit of *Trichosanthes dioica*. **Methods:** In the present study the successive extraction of leaf and fruit of *Trichosanthes dioica* were carried out to investigate for its ash and extractive values and analysis for its active chemical ingredients by using conventional detection tests. **Results:** For qualitative and quantitative phytochemical analysis confirmed the presence of secondary metabolites in leaf and fruit of *Trichosanthes dioica*. **Conclusion:** The present investigation suggests that the presence of phytochemicals in ethanolic extracts of leaf and fruit of *Trichosanthes dioica* act as a source of remedial agent for treating various ailments.

KEY WORDS

Trichosanthes dioica, Phytochemicals, Ash value, Extractive value

INTRODUCTION

Medicinal plants have been used over the millennia for human welfare in the promotion of health. The World Health Organization (WHO) estimates that 80% of the people living in developing countries are almost completely dependent on the traditional medicine as therapeutic remedies for their primary health care needs. They have identified 3000 plants from the forests of India and other tropical countries which can be used as medicine. The therapeutic property of a plant depends upon the physiologically active chemical compounds present in the plant parts employed in preparation of a medicine. These chemicals are often referred to as "secondary metabolites" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids which are formed during the plants normal metabolic processes [1].

A continued search for medicinal plants during the last several centuries has given rise to a long list of plants which are of great use in the treatment of range of disorders including anxiety, arthritis, depression, high

blood pressure, insomnia, hormonal imbalances, migraines, skin problems such as eczema and have biological properties such as antioxidant activity, antimicrobial effect, anticancer property, modulation of detoxification enzymes and hormone metabolism [2,3,4]. The factors responsible for the continued and extensive use of herbal remedies in India are their effectiveness, easy availability, low cost, comparatively less toxic effects and shortage of practitioners of modern medicine in rural areas [5].

Trichosanthes dioica Roxb (family: *Cucurbitaceae*) is a dioecious perennial plant, grown throughout India and it is known as the pointed gourd. It is mainly cultivated as a vegetable crop. It has been used for overcoming problems like constipation, fever, skin infection and wounds. The fruits are used as a remedy for spermatorrhoea and also used for reducing body temperature and as a laxative [6]. The leaves are easily digestible and used for the preparation of syrup for convalescents and good for maintaining healthy heart and brain. The present study was carried out to

screening the preliminary phytochemicals in different extracts of leaf and fruit of *Trichosanthes dioica* Roxb.

MATERIALS AND METHODS

Collection and authentication of plant materials

Fresh unripe fruit and leaf of *Trichosanthes dioica* Roxb. (*T.dioica*) were collected from SKM Herbal Research Centre, Erode, Tamil Nadu, India. An herbarium for morphological studies was prepared, authenticated and a voucher specimen (No. VOCB 2307) was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu.

Pharmaco-chemical characterization

Evaluation of physicochemical parameters

Shade dried coarsely powdered crude drugs of leaf and fruit of *T.dioica* were subjected to various physico-

chemical parameters such as the percentage of loss on drying (LOD), total ash, water soluble ash, acid insoluble ash and sulphated ash were determined by the method [7].

Determination of extractive values

Coarsely chopped 4 gm of the air dried coarse powdered samples of leaf and fruit of *T.dioica* were subjected to maceration for 24 hrs in a closed flask using 100 ml of solvent (such as petroleum ether, benzene, chloroform, acetone, methanol, ethanol and distilled water) frequently shaken during the first 6 hrs and allowed to stand for 18 hrs. It was then filtered rapidly by using Whatmann No. 42 filter paper. The filtrate was then evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105°C and weighed. The Percentage of soluble extractive fraction (W/W) was calculated with reference to that of air dried drug [8].

Weight of flask with extract-Weight of empty flask

$$\text{Percentage of extractive value} = \frac{\text{Weight of flask with extract} - \text{Weight of empty flask}}{\text{Weight of sample}} \times 100$$

Preparation of plant extracts

Freshly collected leaf and fruit of *T.dioica* were washed with distilled water and the fruits were cut into small pieces. Both fruits and leaves were dried under shade for two weeks. The shade dried leaves and fruits were coarsely powdered separately. The powdered materials were kept in airtight containers until use.

Preliminary phytochemical screening in different extracts

About 500 gm of dried coarse powdered samples were weighed and subjected to 1250 ml of successive solvent extraction. The extraction was carried out with the following solvents in increasing order of polarity viz., petroleum ether, chloroform, methanol, ethanol followed by water in a Soxhlet extractor for 24 hrs. All the extracts were filtered through Whatmann No.41 filter paper separately and the extracts were concentrated in vacuum at 60°C using a rotary evaporator. To evaporate the remaining solvent, the extracts were kept in an oven at a temperature of 40-50°C for 8 hrs. Later the extracts were used for qualitative identification of various phytochemical constituents as per the standard procedures [9,10,11].

Quantification of phytochemicals and nutrients

Flavonoid was estimated by the method [12], total phenolic content [13], tannins [14], saponins [15] and alkaloids [16] were measured quantitatively. Vitamin C was estimated by the method [17], total carbohydrates and total protein were determined by the method [18,19].

Statistical analysis

The values are expressed as means of triplicate analysis of the samples (n=3) ± SD.

RESULTS

Pharmaco-chemical characterization

Evaluation of physicochemical parameters

The evaluation of physico-chemical constituents of the plant drug is an important parameter in detecting adulteration, substitution or improper handling of the drugs. The physico-chemical characters like moisture content, ash values and crude fiber content of the leaf and fruit of *T.dioica* powders were analyzed and depicted in **Table 1**.

TABLE 1: Moisture content and ash values of the powdered leaf and fruit of *T.dioica*

S.No.	Physical Evaluation	Values obtained (%) (W/W)	
		<i>T.dioica</i>	
		Leaf	Fruit
1.	Moisture content (LOD)	7.11	7.55
2.	Total ash	14.21±0.12	8.26±0.04
3.	Water soluble ash	5.14±0.11	3.50±0.02
4.	Acid insoluble ash	0.90±0.01	0.30±0.01
5.	Sulphated ash	12.86±0.21	10.91±0.17
6.	Crude fiber content (%)	35.67±1.12	40.60±0.56

Values are means of three independent analyses of the extract ± standard deviation (n = 3)

Moisture content was found to be less in the test samples. The amount of total ash, water soluble ash and sulphated ash were higher in leaf than the fruit of *T.dioica*. The crude fiber content of the leaf and fruit of *T.dioica* were found to be 35.67±1.12% and 40.60±0.56% respectively.

Determination of extractive values

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure.

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. These values also indicate the nature of the constituents present in a crude drug. The results of extractive values were tabulated in **Table – 2**.

The percentages of extractive values of crude drugs were higher in water and ethanol extracts in comparison to the other solvents. The extractive values increase as the polarity of the solvent increases.

TABLE 2: Extractive values of the powdered leaf and fruit of *T.dioica*

S.No.	Physical Evaluation	Values obtained (%) (W/W)	
		<i>T.dioica</i>	
		Leaf	Fruit
1.	Petroleum ether	4.36±0.03	4.58±0.06
2.	Benzene	4.21±0.03	3.78±0.04
3.	Chloroform	3.89±0.06	3.61±0.03
4.	Acetone	8.56±0.11	8.88±0.09
5.	Methanol	8.84±0.08	9.02±0.17
6.	Ethanol	9.38±0.10	10.26±0.07
7.	Water	10.36±0.13	11.32±0.11

Values are means of three independent analyses of the extract ± standard deviation (n = 3)

Preliminary phytochemical characterization

The distribution of different phytochemical components in petroleum ether, chloroform, methanol, ethanol and water extracts of powder of leaf and fruit of *T.dioica* were evaluated qualitatively and presented in **Table - 3**. From the Table - 3, it was evident that a wide range of active compounds like alkaloids, flavonoids, glycosides, phenols, tannins, steroids, saponins, anthraquinone, catechin, coumarin, quinine, sugar, terpenoids and

xanthoproteins were present in the methanol and ethanol extracts of both part of the plant. Whereas in other solvents like petroleum ether, chloroform and water, compounds like catechin, saponins, steroids, terpenoids and other biologically active compounds were present in either one or two of the test samples. Anthraquinone, flavonoid, tannin and xanthoprotein were absent in petroleum ether extract; alkaloids and anthraquinone were absent in chloroform extract.

Quantification of phytochemicals and nutrients

The quantitative analysis of phytochemicals and nutrients in the ethanolic extract of leaf and fruit of the investigated plant was given in **Table - 4**.

Among the studied *T.dioica* fruit was found to contain higher amount of saponins and alkaloids than the leaf.

Total flavonoid content was expressed as rutin equivalent. Total phenolics and tannin content were expressed as gallic acid equivalent and tannic acid equivalent respectively.

TABLE 3: Qualitative phytochemical screening of different extracts of leaf and fruit of *T.dioica*

Phytochemicals	Petroleum ether		Chloroform		Methanol		Ethanol		Water	
	TDL	TDF	TDL	TDF	TDL	TDF	TDL	TDF	TDL	TDF
Alkaloids	-	+	-	-	+	+	+	+	+	+
Anthraquinone	-	-	-	-	+	+	+	+	-	+
Catechin	+	+	-	+	+	+	+	+	-	-
Coumarin	-	+	-	+	+	+	+	+	+	+
Flavonoids	-	-	-	+	+	+	+	+	+	+
Glycosides	-	+	-	+	+	+	+	+	+	+
Phenols	+	-	+	-	+	+	+	+	+	+
Proteins & free amino acids	-	+	-	+	+	+	+	+	+	+
Quinones	+	+	-	-	-	+	+	-	+	+
Saponins	+	+	-	+	+	+	+	+	+	+
Steroids	-	+	-	+	+	+	+	+	+	-
Sugars	-	-	+	+	+	+	+	+	+	+
Tannins	-	-	-	+	+	+	+	+	+	-
Terpenoids	-	+	+	-	+	+	+	+	-	+
Xanthoprotiens	-	-	+	+	-	+	+	+	+	-
Fixed oil	-	+	-	+	+	+	+	-	-	-

Note: + = Present, - = Absent; TDL = *T.dioica* leaf and TDF = *T.dioica* fruit

TABLE 4: Quantitative analysis of phytochemicals and nutrients in ethanolic extract of leaf and fruit of *T.dioica*

S.No.	Parameters	<i>T.dioica</i>	
		Leaf	Fruit
1.	Phytochemicals		
	Flavonoids (mg RE/ gm extract)	36.1±2.02	48.2±1.06
	Total phenolics (mg GAE/ gm extract)	26.0±0.03	38.1±0.03
	Tannins (mg TAE/ gm extract)	38.26±3.81	65.72±7.10
	Saponins (%)	0.38±0.02	0.78±0.01
2.	Nutrients		
	Total carbohydrates (mg glucose/ gm extract)	225± 0.29	120± 0.89
	Total proteins (mg/ gm extract)	174±11.21	188±12.03
	Vitamin C (mg AAE/ gm extract)	52.26 ± 0.13	60.03±0.17

Values are mean of three independent analyses of the extract ± standard deviation (n = 3)

RE-Rutin equivalent;

GAE – Gallic acid equivalent;

TAE-Tannic acid equivalent,

AAE–Ascorbic acid equivalent

DISCUSSION

Medicinal plants are the richest bio-resource to produce drugs of traditional medicines, modern medicines, nutraceuticals, folk medicines, pharmaceutical

intermediates and chemical entities for synthetic drugs [20]. Medicinal plants maintain the health and vitality of individuals and cure various diseases, including diabetes mellitus without causing toxicity [21].

Pharmacochemical characterization

Physico-chemical properties

The percentage of physico-chemical properties in crude drugs is mentioned on air dried basis. Moisture facilitates the enzyme hydrolysis or growth of microbes which lead to deterioration of the drugs. Therefore, the loss on drying (LOD) of plant materials should be determined and the water content should also be controlled. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water. The test for loss on drying determines both water content and volatile matter present in the plant materials [22].

Moisture content

Loss on drying at 110°C is one of the major factors responsible for the deterioration of the drugs and formulations. In the present study, the percentage loss on drying for leaf and fruit of *T.dioica* is below 8% implying that the plant parts can be stored for a long period and will not easily be attacked by microbes. Low moisture content is always desirable for higher stability of drugs.

Ash values

The ash value determination is an important parameter to standardize the herbal drugs. The residue remaining after incineration of plant material is the ash content or ash value, which simply represents the amount of inorganic salts. The ash values obtained from the plant tissue is called 'physiological ash' as well as from extraneous matter is called 'non-physiological ash', which is the residue of the extraneous matter adhering to the plant surface. The determination of the physiological ash and non-physiological ash together is called the total ash determination. The total ash determination is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matters such as metallic salts and/or silica [23].

Total ash may vary within wide limits for specimen of genuine drugs due to the variable natural ash. In such cases, the ash obtained is treated with acid in which most of the natural ash is soluble leaving the silica as acid insoluble ash which represents most of the ash from the contaminating soil. Since the ash values are constant for a given drug, these values are also one of the diagnostic parameters of the drug [24].

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. These ash values are important pharmacognostic tool to standardize the crude drugs [25,26].

In the present study, appreciable amount of ash values implies that the plant has a higher organic content and fairly low inorganic content. The samples have more water-soluble ash than acid insoluble ash. It shows that a very small amount of the inorganic component is acid insoluble. These ash values are generally considered as the index of the purity as well as identity of the drug.

Crude fiber content

Crude fiber is the fraction of carbohydrate that remains after treatment with acid and alkali. In the present study, substantial amount of the crude fiber content were found in leaf and fruit of *T.dioica*. A high fiber diet has been proved to work better in controlling diabetes than the diet recommended by the ADA and may control blood sugar levels like oral diabetes drugs [27].

Extractive values

A successive extractive value reveals the solubility and polarity particulars of the metabolites in the crude drugs. Extractive values are useful for the evaluation of nature of the active phytoconstituents present in the drug especially when the constituents of a drug cannot be readily estimated by any other means [28].

The water soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material to the crude drug, adulteration or incorrect processing during drying or storage. The alcohol soluble extractive value also indicates the same purpose as the water soluble extractive value. The ether soluble extractive value signifies the presence of amounts of fats, lipids and some steroids present in the drug [29].

In the present study, the higher percentage of extractive values of crude drugs in water and ethanol extracts implies that water and ethanol are better solvents for extraction than petroleum ether and chloroform. The extractive percentage clearly indicates that the leaf and fruit of *T.dioica* are best for drug action and effects. The variation in the extractive values may be possible due to the presence of specific compound according to the solubility, soil condition, atmospheric condition and water content of the sample.

Preliminary phytochemical characterization

The phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening, chemo profiling and marker compound analysis [30]. Presence or absence of certain important bioactive compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation [31].

In India traditional communities like tribal and rural populations are frequently using the crude extracts of local plants for medicinal and other purposes. Crude extracts and medicines manufactured on the principles of natural compounds even by pharmaceutical companies, may lead to large scale exposure of humans to natural products. The first step towards this goal is the biological and phytochemical screening of plant extracts from traditional preparations used in popular medicine [32,33]. The phytochemicals are well known to have curative activity against several human problems such as diuretics, skin diseases [34], hyperglycemic and hyperlipemic disorders [35,36] and therefore could suggest the folk use of the medicinal plants.

In the present study, occurrence of wide range of active phytochemicals such as alkaloids, anthraquinone, catechins, coumarins, flavonoids, glycosides, phenols, quinones, saponins, steroids, sugars, tannins and xanthoproteins were seen in methanol and ethanol extracts when compared to other solvents indicated that these compounds were not disturbed during the preparation and storage in the former solvents.

Methanol and ethanol seems to possess high extraction capacity when compared to other solvents. Compared to methanol, ethanol showed stronger extraction capacity. It may be due to the nature of active constituents that are heat labile, but stable in the ethanol [37]. Ethanol is one of the good solvents in plant extractions which include low toxicity, easy evaporation at low heat, preservative action, inability to cause the extract to complex or dissociate. Hence the ethanol extracts of leaf and fruit of *T.dioica* were used for further investigation which includes identification of pharmacologically active chemical compounds and quantitative estimation of the leaf and fruit of *T.dioica*.

Quantification of phytochemicals and nutrients

Plants possess potent biochemicals and have been components of phytomedicine. Plant based natural constituents can be derived from any part of the plant. Systemic screening and quantification of these compounds with a purpose of discovering new bioactive compounds is a routine activity in many laboratories [38].

Phytochemicals or secondary metabolites

In the present examination, flavonoids, total phenolics and tannins were present in considerable quantity in leaf and fruit of plant extracts. These secondary metabolites act as major contributors to the antioxidant activity of fruits, vegetables and medicinal plants [39].

Flavonoids act as insulin secretagogues (mimetics) [40]. They were known to regenerate the damaged β -cells in the alloxan induced diabetic rats [41]. In plants, flavonoids serve as protectors against a wide variety of environmental stresses and are responsible for the radical scavenging effect while, in humans, flavonoids appear to function as "biological response modifiers". It has been demonstrated to have anti-inflammatory, anti-allergenic, anti-viral, anti-ageing and anti-carcinogenic activity [42,43,44,45] and protection against heart diseases through the inhibition of cyclooxygenase activities in platelets and macrophages [44].

The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. It inhibits lipid peroxidation by scavenging free radicals or by other mechanisms such as singlet oxygen quenching, metal ion chelation and lipoxygenase inhibition [46]. Epidemiological studies suggest that the consumption of flavonoid rich foods protects against human diseases associated with oxidative stress like neurodegenerative diseases, diabetes mellitus [47], platelet aggregation [48], cardiovascular diseases, cancer and osteoporosis [49,50,51].

Phenolic compounds have strong in vitro and in vivo antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions, chelate metals ions [50] and prevent oxidative damage of lipids and lipoproteins [51,52,53]. It acts as free radical terminators [54]. These compounds are very important plant constituents because their hydroxyl groups confer scavenging ability [55].

Many tannins containing drugs are used in medicine as astringent. They are used in the treatment of burns as

they precipitate the proteins of exposed tissues to form a protective covering [56]. They are also medically used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns and piles and as antidote [23].

Saponins, a group of natural products, reduces the uptake of certain nutrients including glucose and cholesterol at the gut through intra-luminal physico-chemical interactions and delay glucose transfer from the stomach to the small intestine [57]. Hence, it has been reported to have hypocholesterolemic effect and thus may aid lessening metabolic burden that would have been placed in the liver [58].

Nutrients (Total carbohydrates, total proteins and vitamin C)

The study revealed that the ethanolic extract of leaf and fruit of *T.dioica* possess good amount of vitamin C, carbohydrates and proteins. Vitamin C also exhibits antioxidant activity. Phytochemicals working together with nutrients and fibers may help to slow the ageing process and reduce the risk of many diseases, including cancer, heart disease, stroke, diabetes mellitus, high blood pressure, cataracts, osteoporosis and urinary tract infections [59].

CONCLUSION

On the basis of the above results, it can be concluded that the leaf and fruit of *Trichosanthes dioica* contains different bioactive compounds, which are responsible for preventing diseases along with protection from free radicals produced in the body systems due to various metabolic disorders, promoting health and serve as therapeutic agent for the treatment of various diseases.

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