Research Article

Phytochemical Evaluation and Antioxidant Activity of Leaf and Tuber Extracts of *Theriophonum minutum* (Araceae)

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ABSTRACT

The ethnomedicinal plant, *Theriophonum minutum* (Willd.) Baill. (Araceae), was studied for the *in vitro* antioxidant activity of acetone extract of leaves and methanol extract of tubers through DPPH and Hydrogen peroxide scavenging activities, using ascorbic acid as the standard drug. The concentrations of extracts tested were 50, 100, 150, 200, and 250µg/ml. Both the extracts showed potent DPPH activity though the methanol extract of tubers showed greater potent Hydrogen peroxide scavenging activity than the acetone extract of leaves.

Keywords: Angiosperm, Aroid, tuber, leaves, DPPH and Hydrogen peroxide scavenging activities.

INTRODUCTION

The Araceae are a family of monocotyledonous flowering plants, known as the *Arum* family, with its members as *aroids*. The family comprises 105 genera and about 2550 species, mostly distributed in the tropics and subtropics[1]. *Theriophonum* Blume is small genus of seven species of tuber geophytes, confined to peninsular India and Sri Lanka[2]. The ethnomedicinal knowledge reveals that the tubers of *Theriophonum minutum* (Willd.) Baill. are boiled with tamarind leaves, washed and then cooked as vegetable by Konda reddis and Koyas of East Godavari district of Andhra Pradesh[3], and by the Koyas of Lankapalli Forest Reserve in Khammam district of Telangana State (present study). Tuber powder with honey is administered for gastrointestinal disorders, small intestinal injuries and stomach ache[4]. The Indian vernaculars of this species are *doda* and *tangya* in Maharashtra; the Koyas of Andhra Pradesh call it '*adavi champak*'. The leaves of *T. fischeri* Sivad. are ground with turmeric and applied topically to cure skin problems and wounds[5], and to treat rheumatic pains[6]. Its boiled tubers are consumed for a fortnight to cure piles[5] and relieved of body pains[7]. Hardly there is any phytochemical investigation into the plant parts of *Theriophonum minutum* but for the report of 13-phenyltridecanoic acid in the seed oil of *Theriophonum*[8]. Therefore, an attempt is made here, for the first time, to study the antioxidant activity of acetone extract of leaves and methanol extract of tubers of *Theriophonum minutum*.

MATERIALS AND METHODS

(a) Plant Material:

The whole plants *Theriophonum minutum* (Willd.) Baill. (synonyms: *Arum minutum* Willd., *Theriphonum wightii* Schott, *Typhonium minutum* (Willd.) Schott), a Monocot Angiosperm of Alismatales were personally gathered from the tropical dry deciduous forests of Sathupally (Kistaram village, *V.Ramabharathi 4615,* 2016-06-26) in Khammam district, Telangana State. The plant specimen was deposited in Plant Systematics Laboratory, KUW (Kakatiya University Herbarium, Warangal, India)[9], and it was identified by Prof. Vatsavaya S. Raju, of the same Institution.

The present study reports the phytochemical constituents of leaves and underground parts (tubers) of *Theriophonum minutum*. *In vitro* antioxidant activity of acetone extract of leaves and methanol extract of tubers were evaluated by studying DPPH and Hydrogen peroxide scavenging antioxidant activity, using ascorbic acid as a standard drug. The concentrations 50, 100, 150, 200 and 250µg/ml of acetone extract of leaves and methanol extract of tubers were screened.

Figure 1: Whole plant of *Theriophonum minutum*.

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(b) Preparation of Extracts:

The leaves (5g) and tubers (10g) were macerated at room temperature in a round bottom flask in acetone and methanol, respectively. The contents of the flask were shaken from time to time to ensure proper extraction. After seven days, the contents of the flask were filtered; the filtrate was concentrated to yield a yellow extract.

(c) Phytochemical Tests:

The following chemical tests were carried out to know the nature of compounds present in different extracts/ isolates[10,11].

Detection of Alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered. *Dragendroff's test***:** Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Detection of Carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.
Molisch's test: Filtrates were treated with two drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.
Detection of Glycosides: Extracts were hydrolysed with dil. HCl, and then tested for glycosides. (i) *Modified Borntrager's test***:** Extracts were treated with Ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution, and the formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides; and (ii) *Legal's test***:** Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide, and the formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of Saponins: *Froth test***:** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins.

Detection of Phytosterols: *Libermann Burchard's test***:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated Sulphuric acid was added, and the formation of brown ring at the junction indicates the presence of phytosterols.

Detection of Tannins: *Gelatin test:* To the extract tannins, 1% gelatin solution containing sodium chloride was added and the formation of white precipitate indicates the presence of tannins.

Detection of Flavonoids: *Lead acetate test:* Extracts were treated with few drops of lead acetate solution, and the formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of Proteins: *Xanthoproteic test***:** The extracts were treated with few drops of concentrated Nitric acid, and the formation of yellow colour indicates the presence of proteins.

Detection of Diterpenes: *Copper acetate test***:** Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution, and the formation of emerald green colour indicates the presence of diterpenes.

UV Spectroscopy: The UV Spectra and the absorption maxima (λmax) for the isolate were recorded in methanol using ELICO SL-210 double beam UV-Visible spectrometer
P^H Meter: The P^H meter used is ELICO NEPHLOMETER CL-52D LI 120 P^H to measure the phosphate buffer.

(d) Antioxidant activity:

(i) Materials: 1, 1- Diphenyl-2-picryl hydrazyl (DPPH) is a gift sample. Methanol, acetone, hexane, diethyl ether are collected from SD Fine chemicals.

(ii) Instruments: Absorbance was measured in ELICO UV-VIS-SPECTRO-PHOTOMETER. The pH of buffer was measured in pH meter.

(iii) Evaluation of Antioxidant activity:

(a) DPPH Radical Scavenging method[12]: Free radical scavenging activity of acetone extract of leaves and methanol extract of tubers of *Theriophonum minutum* were measured by DPPH. In brief, 0.1 mM solution of DPPH in methanol was prepared. This solution (1 ml) was added to 3 ml of different extracts in methanol at different concentration (50, 100, 150, 200 and 250 µg/ml). The mixture was shaken vigorously and allowed to stand at room temp for 30 min.; then, the absorbance was measured at 517 nm with spectrophotometer (UV-VIS ELICO). Reference standard compound being used was ascorbic acid. The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicates higher free radical activity. The percent DPPH scavenging effect was calculated by using following equation:

DPPH scavenging effect (%) or Percent inhibition = $A_0 - A_1 / A_0 \times 100$. Where, A_0 was the Absorbance of control reaction and A_1 was the Absorbance in presence of test or standard sample.

(b) Hydrogen Peroxide Scavenging Method[13]:

All the compounds and the standard were dissolved in DMSO as a solvent-stock solution (1mg/ml). From this, various concentrations (two fold dilutions) of 50, 100, 150, 200 and 250µg/ml of acetone leaf and methanol tuber extracts were prepared in different volumetric flasks. To each solution 2ml hydrogen peroxide was added and the volume was made to 10ml, with

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phosphate buffer saline (pH-7.4). A control solution was prepared with DMSO in phosphate buffer saline without the drug. The absorbance at 230nm was recorded using UV spectrophotometer against blank (Phosphate buffer saline). The standard drug used is Ascorbic acid. The % inhibition by Hydrogen peroxide scavenging activity was calculated using the following formula:

Percentage inhibition

$$= \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSSION

Phytochemicals

The phytochemical evaluation of acetone leaf extract of *T. minutum* showed the presence of diterpenes, proteins and tannins whereas the methanol extract of tubers revealed the presence of carbohydrates, diterpenes and proteins (Table 1).

Table 1: Phytochemical Screening

Extract	Solvent	Alkaloids	Diterpenes	Carbohydrates	Flavonoids	Phytosterols	Proteins	Tannins
Leaves	*Acetone*	–	+	–	–	–	+	+
Tuber	*Methanol*	–	+	+	–	–	+	–

+ present; – absent

Free radical scavenging activity by DPPH method:

Solution of various concentration 50, 100, 150, 200, 250µg/ml were added to 250 µg/ml DPPH in methanol extract of the tubers and it was kept at an ambient temperature for 20 minutes and the absorbance was then measured at 517nm. Positive control was kept whilst Ascorbic acid served as the standard drug. The results are presented in the Table 2 and Fig. 2.

Table 2: DPPH Activity of leaf and tuber extracts.

Con (µg/ml)	% Inhibition of DPPH	
	Acetone extract (Leaves)	Methanol extract (Tubers)
50	40.45	34.84
100	54.54	47.72
150	69.69	53.46
200	76.45	71.02
250	84.62	85.60

STD value: 97.72%

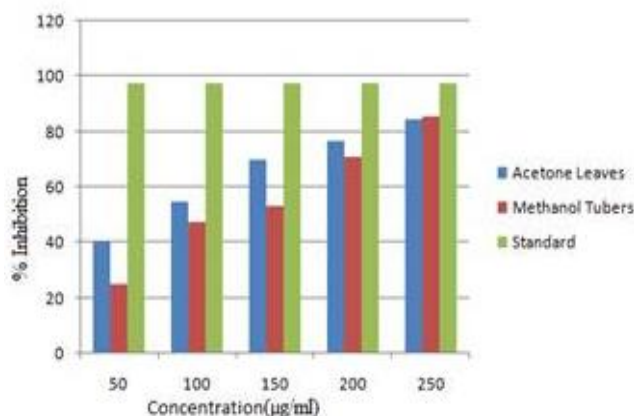

Figure 2: DPPH activity of acetone extract of leaves and methanol extract of tubers against the standard.

It is found that as the concentration of acetone extract of leaf and methanol extract of tubers (50, 100, 150, 200, 250µg/ml) increases, the % inhibition of DPPH activity also increased. Both the extracts showed potent activity (Fig. 3).

Hydrogen Peroxide Scavenging Method:

Solution of various concentration 50, 100, 150, 200, 250µg/ml were added to 250µg/ml of H_2O_2 in methanol and phosphate buffer (pH 7.4) of the tuber extracts kept at an ambient temperature for 30 minutes and the absorbance was measured at 230nm. Positive control was kept. Ascorbic acid is used as standard drug. The results are presented in Table 3 and Fig. 3.

Table 3: Hydrogen peroxide scavenging activity.

Con (µg/ml)	% Inhibition of H_2O_2	
	Leaf Acetone extract	Tuber Methanol extract
50	28.88	37.77
100	50.21	53.2
150	63.53	62.6
200	71.21	75.76
250	79.23	88.88

STD value: 94.04%

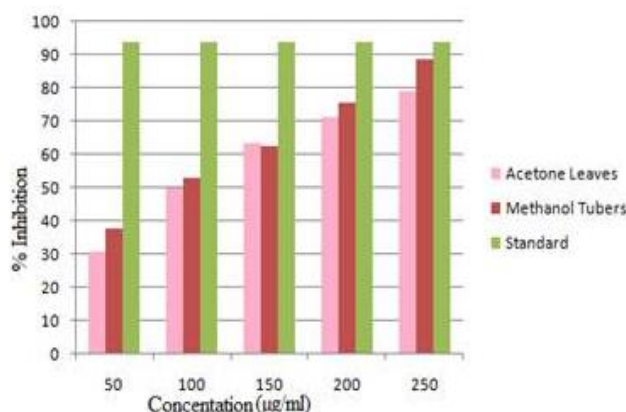

Figure 3: H_2O_2 Scavenging activity of acetone extract of leaves and methanol extract of tubers against the standard.

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It is found that as the concentration of acetone extract of leaves and methanol extract of tubers (50, 100, 150, 200, 250µg/ml) increased, the % inhibition Hydrogen peroxide scavenging activity also increased. Both the extracts showed potent activity, but relatively, methanol extract of tubers showed more potent activity (Fig. 3).

CONCLUSION

The study investigated the antioxidant activity of leaf and tuber extracts of *Theriophonum minutum*, a common ethnobotanical monsoonal herb of peninsular India and Sri Lanka. The literature review revealed no major study on its phytochemical constituents nor on its pharmacology activity. The preliminary phytochemical study of the acetone extract of leaf and methanol extract of tuber of *T. minutum* showed the presence of carbohydrates, diterpenes, proteins and tannins. Both the extracts showed potent DPPH activity but methanol extract of tubers showed better hydrogen peroxide scavenging activity than the acetone extract of leaves.

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