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Research Paper

Trichosanthes dioica Roxb. leaves extract's potential as an Antioxidant property

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ABSTRACT

Since ancient times, *Trichosanthes dioica Roxb*. has been used to cure a variety of ailments in traditional medical systems such as Ayurveda, Siddha, Chinese, and many more. The leaves have been applied topically to a variety of ailments, including bruises, cuts, sprains, and swellings. In present work Phytochemical and pharmacological evaluation of various extract of *Trichosanthes dioica Roxb*. leaves was performed to proves as Anti-oxidant agent. In India, abundant precious plants are used in ayurveda as well as traditionally for the treatment of inflammation. A medicinal chemist will have the chance to create new, structurally varied selective inflammatory inhibitors with the discovery of inflammatory inhibitors derived from natural sources. The ethnomedical history of plants like *Trichosanthes dioica Roxb* served as the foundation for the current investigation. These plants' leaves were chosen for their antioxidant properties. Methanol extract of *Trichosanthes dioica Roxb*. leaves extract at 200 µg/ml concentrations displayed 55.1±0.33, maximum activity compared to other all extracts. Methanol extract of *Trichosanthes dioica Roxb.* leaves (TDL) was evaluated for nephroprotective activity.

INTRODUCTION

Numerous regulatory mechanisms, including gene expression, apoptosis, and cell proliferation, are impacted by free radicals produced during aerobic metabolism. Excessive production of free radicals can undermine the antioxidant system's defenses by oxidizing membrane lipids, cell proteins, carbohydrates, DNA, and enzymes, which damages vital biomolecules in the cell. Oxidative stress results in cytotoxic compounds occurrence (malonyl dialdehyde, 4-hydroxynonenal) and alters the oxidant-antioxidant balance (redox homeostasis) that characterizes normal cell functioning [1–4]. Because of the mismatch between the body's antioxidant defense system and the creation of free radicals, oxidative stress can

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lead to an excess of these reactive species. When these reactive species come into contact with biomolecules, they can harm or even kill cells. Chronic illnesses that affect the cardiovascular and cerebrovascular systems, as well as malignancies, may result from this antioxidant-rich fruits and vegetables have been shown to provide protection against certain illnesses. Antioxidants included in food can strengthen cellular defenses and shield cell components from oxidative damage. Antioxidants have been utilized in the food business to extend the shelf life of foods, particularly those high in polyunsaturated fats, in addition to their significant involvement in physiological systems. These food ingredients are easily oxidized by molecular oxygen and are a substantial contributor to nutritional losses, discoloration, off-flavor development, and quality degradation. The addition of synthetic antioxidants, such as propyl gallate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone has been widely used industrially to control lipid oxidation in foods. However, the use of these synthetic antioxidants has been questioned due to their potential health risks and toxicity [5-7]. Many efforts have been made to find chemicals that can function as appropriate antioxidants to replace synthetic ones, and significant focus has been paid to the search for antioxidants from natural sources. Furthermore, these naturally occurring antioxidants can be combined to create neutraceuticals that can aid in halting the body's oxidative damage. In this study, the hydrophilic antioxidants found in the plants were extracted using water as the extraction solvent. Water-based plant extracts are more nutritionally relevant for use in food and would clearly have benefits in terms of safety and certification.[8-9]. The present investigation were done to evaluate antioxidant potential of *Trichosanthes dioica Roxb*. leaves extract.

MATERIAL AND METHODS:

Trichosanthes dioica Roxb. leaves 5g the air-dried powdered was successively extracted with the following solvents of increasing polarity in a soxhlet apparatus. The dried *Trichosanthes dioica Roxb*. leaves powder was packed in soxhlet and extracted with chloroform, filtered and dried to get chloroform extract. The same process was repeated with ethyl acetate and methanol to get ethyl acetate and methanol extracts. After extraction with methanol stem powder were dried and macerated with hot water repeatedly, filtered and dried to acquire aqueous extract. Percent yield of the extracts obtained after removing the solvents was calculated. A few drops of extract from the thimble were evaporated on a watch glass to verify that the extraction was complete and that no residue was left behind after the solvent evaporated. The extracts' appearance, color, consistency, and yield percentage were recorded.

DPPH Free Radical Scavenging Assay for Antioxidant study:

The free radical scavenging capacity of extracts of selected plant parts was determined using DPPH assay method. The mechanism involved in the assay is the ability of phyto antioxidant molecules to quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH molecule) and convert them to a colourless (i.e.,2,2-diphenyl1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 516nm. Extract (1 ml) in various concentrations (100,150, 200 & 250 µg/ml) was added to 1ml of 0.1 mM solution of DPPH in methanol. After 30 minutes, absorbance was measured at 517 nm, using a spectrophotometer (SHIMADZU, UV 1800). A 0.1 mM solution of DPPH in methanol was used as blank, whereas ascorbic acid was used as a reference standard. All

tests were performed in triplicate. Percent inhibition was calculated using equation,

$$\text{Percentage inhibition} = \frac{\text{Control} \times \text{Test}}{\text{Control}} \times 100$$

Then, the concentration of the test compounds required for the 50% reduction in absorbance (IC_{50}) was calculated using the linear regression analysis. Antioxidant study by superoxide scavenging assay: The superoxide scavenging potential of extracts was assessed by the method [11]. This assay is based on the inhibition of the production of nitroblue tetrazolium formazon of the superoxide ion by the plant extracts and is measured spectrophotometrically at 490 nm. Extracts (1 ml) in various concentration (100,150,200& 250µg/ml) was added to EDTA (0.2ml), NBT (0.1ml), riboflavin (0.05 ml) and phosphate buffer (2.64 ml). The control tubes were also set up where dimethyl sulfoxide (DMSO) was added instead of the extracts. All the tubes were vortexed and the initial optical density was measured at 560 nm in a spectrophotometer. The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 490 nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity. All tests were performed in triplicate. Then, the concentration of the test compounds required for the 50% reduction in absorbance (IC_{50}) was calculated using the linear regression analysis. Percent inhibition was calculated using equation,

$$\text{Percentage inhibition} = \frac{\text{Control} \times \text{Test}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION:

DPPH free radical scavenging assay for *in-vitro* antioxidant activity:

The DPPH radical scavenging potential of plant extracts was concentration dependent. The potential decrease in the concentration of DPPH radical due to scavenging property for in vitro antioxidant activity of extract in *Trichosanthes dioica Roxb.* leaves extract showed significant free radical scavenging activity. The chloroform, ethyl acetate, methanol and Aqueous extract of *Trichosanthes dioica Roxb*. leaves at four concentrations (100, 150, 200, 250 µg/ml) used for DPPH free radical inhibition and nitric oxide inhibition assay were performed. In this study the methanol extract of *Trichosanthes dioica Roxb*. leaves extract at 250 µg/ml concentration displayed, 55.05±2.11 % inhibition for DPPH free radical inhibition and 48.12±2.03 % inhibition of nitric oxide inhibition (Table 1).

***In-vitro* studies:**

In-vitro anti-oxidant screenings of extract of *Trichosanthes dioica Roxb*. leaves were performed using various assay methods. Results of *in vitro* screening illustrate that methanolic extract of *Trichosanthes dioica Roxb*. leaves revealed highest activity than other extract. The chloroform, ethyl acetate, methanol and aqueous extract at different concentrations showed considerable *in vitro* antioxidant activity. Methanol extract of *Trichosanthes Dioica Roxb*. leaves showed significant *in-vitro* antioxidant activity than chloroform, ethyl acetate, and aqueous extract.

Acute Toxicity Studies:

Plants extracts when orally administered in the dose range of 50-2000 mg/kg animal did not produce any significant changes in the autonomic or behavioural response during the observation period. The body weight was not significantly altered. No mortality was observed up to 14 days of monitoring. and observed skin, fur, eye, respiratory, circulatory, nervous system, motor activity and behavioural patterns.

Table 1: Effect of *Trichosanthes dioica Roxb*. leaves extracts on in vitro antioxidant activity by DPPH radical scavenging assay

Plant extract	Concentration (µg/ml)	DPPH free radical inhibition	Nitric oxide
Chloroform extract	100	34.31±2.24	31.06±2.12
	150	39.04±2.34	31.21±2.34
	200	43.02±2.16	38.13±2.11
	250	51.02±3.15	43.11±3.13
Ethyl acetate extract	100	34.31±2.36	33.22±2.31
	150	39.64±2.24	37.11±2.21
	200	46.02±2.37	46.21±2.41
	250	54.02±2.81	49.11±2.12
Methanol extract	100	38.31±2.06	36.12±2.12
	150	42.04±2.16	39.13±2.46
	200	48.21±2.37	49.11±2.11
	250	55.02±3.21	53.11±2.03
Water extract	100	36.31±2.27	33.11±2.27
	150	39.04±3.64	38.21±2.28
	200	48.11±3.23	43.12±2.11
	250	55.02±3.15	50.62±2.12

Values are Mean ± SEM, n=3.

Table 2: Effect of Ascorbic acid on in vitro antioxidant activity by DPPH radical scavenging assay

Standard solution	Concentration (µg/ml)	DPPH free radical inhibition	Nitric oxide
Ascorbic acid	10	34.14±2.03	30.21±2.99
	20	49.21±2.33	44.21±2.16
	30	54.17±2.14	50.13±2.18
	40	62.25±2.21	57.21±2.04
	50	71.18±2.17	68.01±2.22

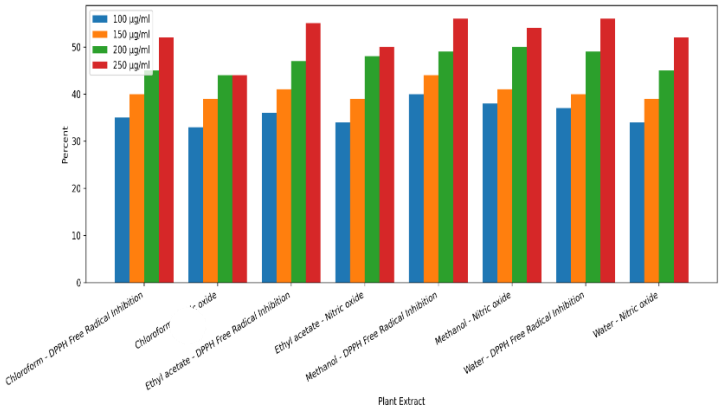

Figure 1: Effect of *Trichosanthes dioica Roxb*. leaves extracts on in vitro antioxidant activity by DPPH radical scavenging assay

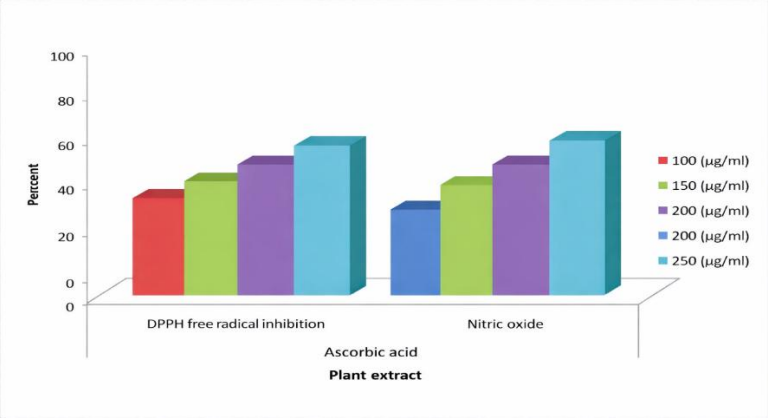

Figure 2: DPPH radical scavenging assay standard (Ascorbic acid)

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