

## Antioxidant potential of *Trichosanthes dioica* Roxb. Extract

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**ABSTRACT:** *Trichosanthes dioica* Roxb. have been used since ancient times in the traditional medicinal systems like, Ayurveda, Siddha, Chinese and many other system of medicines in the treatment of various ailments. The fruits have been used topically in various conditions like, sprains, swellings, wounds and bruises. 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. The discovery of inflammatory inhibitors from natural origin will present an opportunity for a medicinal chemist to design novel, structurally diverse selective inflammatory inhibitors. The present study was undertaken based on the ethno medical background of the plants such as *Trichosanthes Dioica* Roxb. leaves part of these plants were selected for anti oxidant activity. Methanol extract of *Trichosanthes Dioica* Roxb. leaves extract at 400 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

**Keywords:** *Trichosanthes Dioica* Roxb., Methanolic extracts, antioxidant, Free radicals

**Introduction:** Free radicals generated in aerobic metabolism are involved in a series of regulatory processes such as cell proliferation, apoptosis, and gene expression. When generated in excess, free radicals can counteract the defense capability of the antioxidant system, impairing the essential biomolecules in the cell by oxidizing membrane lipids, cell proteins, carbohydrates, DNA, and enzymes. 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]. An over-production of these reactive species can occur, due to oxidative stress brought about by the imbalance of the bodily antioxidant defense system and free radical formation. These reactive species can react with biomolecules, causing cellular injury and death. This may lead to the development of chronic diseases such as cancers and those that involve the cardio- and cerebrovascular systems. The consumption of fruits and vegetables containing antioxidants has been

found to offer protection against these diseases. Dietary antioxidants can augment cellular defences and help to prevent oxidative damage to cellular components.

Besides playing an important role in physiological systems, antioxidants have been used in the food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats. These components in foods are readily oxidised by molecular oxygen and is a major cause of quality deterioration, nutritional losses, off-flavour development and discolouration. 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]. The search for antioxidants from natural sources has received much attention and efforts have been put into identify compounds that can act as suitable antioxidants to replace synthetic ones. In addition, these naturally-occurring antioxidants can be formulated to give nutraceuticals that can help to prevent oxidative damage from occurring in the body. In this investigation, water was used as the extraction solvent to extract the hydrophilic antioxidants present in the plants. For use in foods, plant extracts made with water are nutritionally more relevant and would have obvious advantages in relation to certification and safety [8-9]. The present investigation were done to evaluate antioxidant potential of *Trichosanthes Dioica Roxb.* leaves and fruit extract.

**Material and Methods:** *Trichosanthes Dioica Roxb.* leaves / fruit 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 extractor and extracted with petroleum ether (non polar solvent) for complete extraction, extract filtered and solvent was removed with the help of rotatory evaporator to get petroleum ether extract. The exhausted *Trichosanthes Dioica Roxb.* leaves / fruit powder was dried in air to remove traces of petroleum ether than again 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, filter and dried to acquire aqueous extract. Percent yield of the extracts obtained after removing the solvents was calculated. The completion of the extraction was confirmed by evaporating a few drops of extract from the thimble on watch glass to observe that no residue remained after evaporation of the solvent. The consistency, color, appearance of the extracts and their percentage yield were noted.

**Antioxidant Study by DPPH Free Radical Scavenging Assay:** 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-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 516nm. Extract (1 ml) in various concentrations (50, 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,

$$\text{Percentage inhibition} = [(\text{Control} \times \text{Test}) / \text{Control}] \times 100$$

Then, the concentration of the test compounds required for the 50% reduction in absorbance (IC50) 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 (50,100,150,200& 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 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 (IC50) was calculated using the linear regression analysis.

Percent inhibition was calculated using equation,

$$\text{Percentage inhibition} = \left[ \frac{(\text{Control Test})}{\text{Control}} \right] \times 100$$

### Result And Discussion

**in-vitro antioxidant activity by dpph free radical scavenging assay:** 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 petroleum ether, chloroform, ethyl acetate, methanol and water extract of *Trichosanthes Dioica Roxb.* leaves / fruit at five concentrations (50, 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 / fruit 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**). The petroleum ether, chloroform, ethyl acetate, methanol and water extract of *Trichosanthes Dioica Roxb.* leaves / fruit at concentrations (50, 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 / fruit extract at 250 µg/ml concentration displayed, 58.02±3.16 % inhibition for DPPH free radical inhibition and 52.13±1.12 % inhibition of nitric oxide inhibition (**Table 2**).

**In-vitro studies:** in-vitro anti-oxidant screenings of extract of *Trichosanthes Dioica Roxb.* leaves / fruit 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 petroleum ether, chloroform, ethyl acetate, methanol and water extract at different concentrations showed considerable in vitro antioxidant activity. Methanol extract of *Trichosanthes Dioica Roxb.* leaves showed significant in-vitro antioxidant activity than petroleum ether, chloroform, ethyl acetate, and water extract.

**Acute Toxicity Studies:** Plants extracts when orally administered in the dose range of 5-2000 mg/kg mice 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. So, the extracts were safe for administered up to the dose of 2000 mg/kg.

**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
Pet ether extract	50	18.91±2.11	18.11±3.11
	100	29.16±2.14	25.11±3.21
	150	33.64±2.34	28.12±2.12
	200	39.02±2.37	37.09±2.11

	250	46.02±2.11	44.02±2.01
Chloroform extract	50	23.98±2.37	23.14±2.12
	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	50	28.98±3.31	29.21±2.11
	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	50	29.98±3.01	27.11±2.11
	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	50	27.98±3.21	25.11±2.07
	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 *Trichosanthes Dioica Roxb.* fruit extracts on *in vitro* antioxidant activity by DPPH radical scavenging assay**

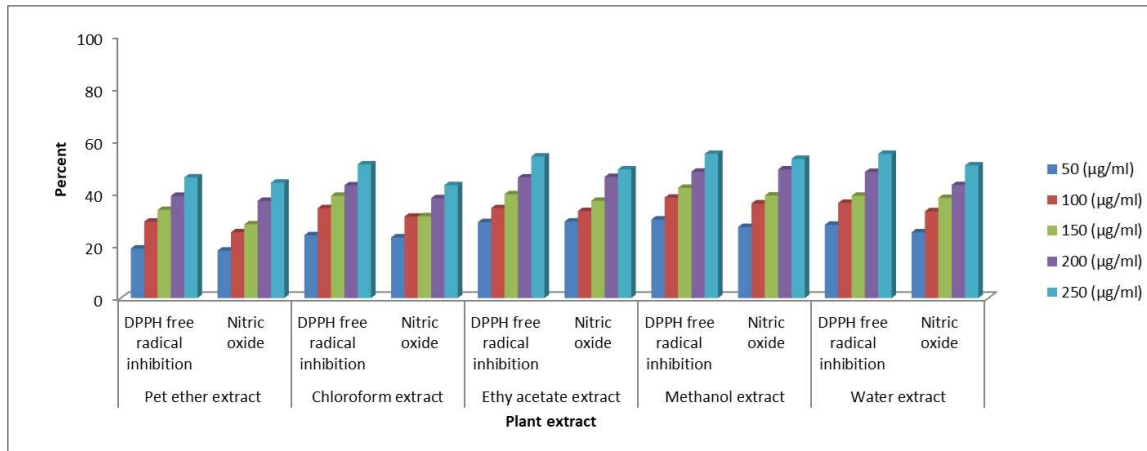
Plant extract	Concentration (µg/ml)	DPPH free radical inhibition	Nitric oxide
Pet ether extract	50	17.98±1.21	11.11±1.13
	100	23.36±2.14	21.11±1.31
	150	32.64±2.24	27.21±1.24
	200	38.02±2.17	35.13±1.21
	250	43.02±3.20	40.21±2.17

Chloroform extract	50	23.98±2.30	19.21±2.11
	100	28.36±2.24	27.16±1.14
	150	34.64±2.04	33.11±1.08
	200	41.02±2.23	38.21±1.52
	250	49.02±2.11	46.21±2.21
Ethy acetate extract	50	23.08±1.81	21.03±1.21
	100	33.06±2.11	30.11±1.31
	150	36.64±2.04	35.03±2.34
	200	44.02±2.07	42.12±2.27
	250	51.02±2.91	45.21±2.02
Methanol extract	50	27.12±2.01	22.22±2.03
	100	36.06±2.16	31.12±2.06
	150	39.14±2.15	33.12±2.22
	200	44.02±2.29	42.12±2.13
	250	55.02±2.36	51.03±1.22
Water extract	50	23.98±2.89	22.11±2.02
	100	32.36±2.94	31.13±2.86
	150	39.64±3.54	38.19±2.33
	200	44.02±3.87	43.09±2.03
	250	50.02±3.21	49.21±2.34

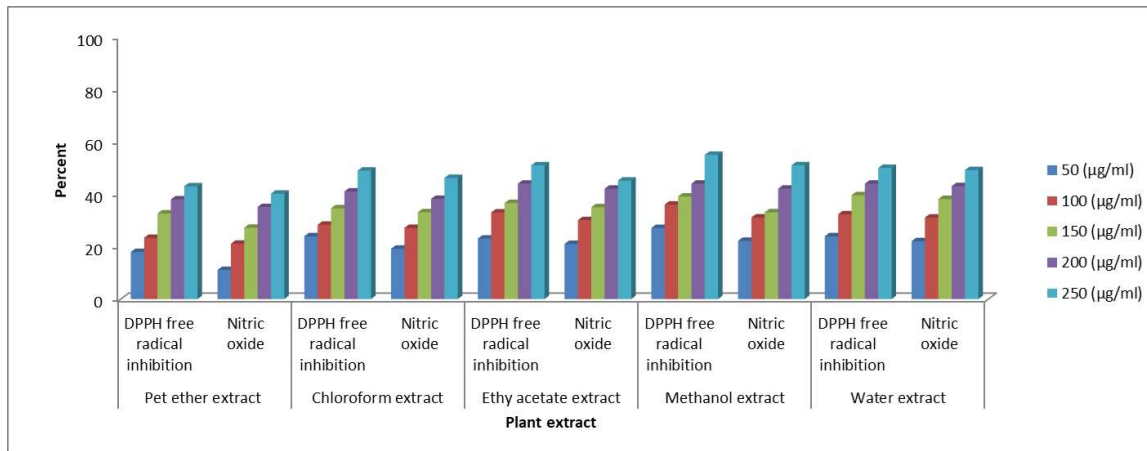
Values are Mean ± SEM, n=3.

**Table 3: Effect of Ascorbic acid on *in vitro* antioxidant activity by DPPH radical scavenging assay**

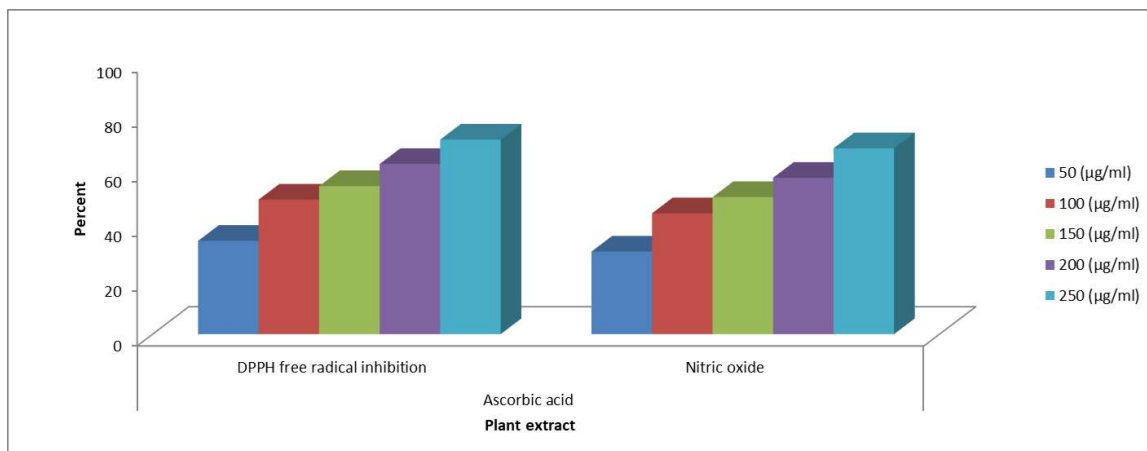
Standard solution	Concentration (µg/ml)	DPPH free radical inhibition	Nitric oxide
Ascorbic acid	10	34.14±2.03	30.21±2.99
	20	49.21±2.33	44.21±2.16
	30	54.17±2.14	50.13±2.18
	40	62.25±2.21	57.21±2.04
	50	71.18±2.17	68.01±2.22



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



**Figure 2: Effect of *Trichosanthes Dioica Roxb.* fruit extracts on *in vitro* antioxidant activity by DPPH radical scavenging assay**



**Figure 3: DPPH radical scavenging assay standard (Ascorbic acid)**

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