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## **Antioxidant, Anti-inflammatory and Anti-pyretic Activities of *Trichosanthes dioica* Roxb. Fruits**

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### **ABSTRACT**

The present study was designed to investigate the antioxidant, anti-inflammatory and antipyretic potential of the methanolic extract along with its organic soluble fractions of the fruits of *Trichosanthes dioica* Roxb. Antioxidant potential of the extract/fractions was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) and NO (nitric oxide) scavenging assay method. Ethyl acetate fractions (EtOAc) showed highest scavenging activity in all the methods with IC<sub>50</sub> value of 12.32±0.16 and 5.38±0.07 µg mL<sup>-1</sup> for DPPH and NO assay method, respectively. In reducing power assay, EtOAc fraction also showed significant (p<0.001) activity. Further, the extract/fractions were studied for their anti-inflammatory (carrageenan induced paw edema in rats) and antipyretic (Brewer's yeast induced pyrexia) activities at a dose level of 100, 200 and 400 mg kg<sup>-1</sup> body weight for MeOH extract. Methanolic extract showed a dose dependent and significant (p<0.005, p<0.05) anti-inflammatory and antipyretic effect. Dichloromethane fraction (CH<sub>2</sub>Cl<sub>2</sub>) and Ethyl acetate fractions exhibited similar activity using a dose of 200 mg kg<sup>-1</sup> b.wt. in these models. The pharmacological activities of the (CH<sub>2</sub>Cl<sub>2</sub>) fraction were lesser than the MeOH extract and other fractions. In addition, total phenolic and flavonoid content and total antioxidant capacity were also determined. Altogether, these results suggest that the MeOH extract and its organic soluble fractions EtOAc could be used as a potential antioxidant, anti-inflammatory and antipyretic agent.

**Key words:** Free radical, inflammation, antipyretic, *Trichosanthes dioica*

### **INTRODUCTION**

Reactive Oxygen Species (ROS) are various forms of activated oxygen which include free radicals such as superoxide anion radicals (O<sub>2</sub><sup>•-</sup>) and hydroxyl radicals (OH<sup>•</sup>), as well as non free radicals species (H<sub>2</sub>O<sub>2</sub>) and the singlet oxygen (<sup>1</sup>O<sub>2</sub>) (Gulcin *et al.*, 2002a; Yildirim *et al.*, 2000). Also, excessive generation of ROS induced by various stimuli can easily initiate the peroxidation of

membrane lipids, leading to the accumulation of lipid peroxidation. The peroxidation products and their secondary oxidation products such as Malondialdehyde (MDA) and 4-hydroxynonenal can react with biological substrates such as protein, amines and deoxyribonucleic acid (Gulcin *et al.*, 2003), leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer (Kourounakis *et al.*, 1999; Gulcin *et al.*, 2002b). Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals, thermal and mechanical injuries (Oyedapo *et al.*, 2008). Various endogenous mediators like histamine, serotonin, bradykinin, prostaglandins etc., are most abundant in inflammatory cell and among them prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. Non Steroidal Anti-Inflammatory Drugs (NSAIDs) are the most clinically important medicine used for the treatment of inflammation related diseases like arthritis, asthma and cardiovascular disease (Conforti *et al.*, 2009). Having various and severe adverse effects like gastric lesions for NSAIDs, adverse cardiovascular thrombotic effects for selective cyclooxygenase-2 (COX-2) inhibitors (Chowdhury *et al.*, 2009), use of these drugs as anti-inflammatory agents have not been successful in all the cases. Moreover, ROS may also contribute to several chronic cutaneous inflammatory diseases like psoriasis, atopic dermatitis and contact dermatitis (Choi and Hwang, 2004). Therefore, compounds that have scavenging activities towards free radicals and/or suppressive activities on lipid peroxidation may be expected to have therapeutic potential for several inflammatory diseases.

Medicinal plants have the potential to provide compounds of novel and complex structures that are capable of interacting with biological systems, used in the treatment of diseases and for revitalizing body system in almost all ancient civilization. The research into plants with alleged folkloric use as anti-inflammatory agents with antioxidant properties should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs (Gupta *et al.*, 2006).

*Trichosanthes dioica* Roxb. (Cucurbitaceae) is a dioecious perennial herbaceous vegetable, commonly called as Potoi in Bengal. The medicinal attributes *T. dioica* have been known since time immemorial. The fruits are sweet, cardio tonic, appetizer and stomachic. It is a rich source of protein and vitamin A (Kumar *et al.*, 2003), Vitamin C and certain essential trace elements such as magnesium, potassium, copper, sulfur and chlorine which that seems an important role in human physiology (Kar *et al.*, 2003; Rai *et al.*, 2008). It has a number of medicinal properties and many reports are available regarding its role in the circulatory system, especially in lowering blood sugar and lipid profile (Sharma and Pant, 1988a, b; Sharma *et al.*, 1988, 1989). Direct intake of seeds of the plant was also found to be effective in the serum lipid profile of normal and mild-diabetic human subjects and rabbits (Sharma *et al.*, 1990; Sharma and Pant, 1988b). Seeds of the plant were also found to possess anti-fungal and anti-bacterial activity and are widely used in the treatment of acid dyspeptic disease (Harit and Rathee, 1996). The aqueous extract of fruits also showed *in vitro* antioxidant property (Shivhare *et al.*, 2009). The plant is alternative, tonic, useful in obstinate fevers, boils etc. (Bhargava *et al.*, 2008) and also have a wound healing (Shivhare *et al.*, 2010) and hepatoprotective (Ghaisas *et al.*, 2008) activity. The roots are cathartics. The leaves are anthelmintic (Bhattacharya *et al.*, 2009). The phytochemical investigation carried out in past with other species of *Trichosanthes* reports a number of different classes of compounds viz. flavonoids (Rahman and Moon, 2007), alkaloids, glycosides (Kanchanapoom *et al.*, 2002), terpenes and steroidal saponin (Saxena and Dave, 1995), from fruits and seeds of the plant could be responsible for their medicinal value.

In the previous study, the aqueous extract of the fruits of *Trichosanthes dioica* Roxb. was evaluated for its antioxidant activity (Shivhare *et al.*, 2009). Therefore, the present study was planned to evaluate the possible antioxidant, anti-inflammatory and antipyretic activities of methanolic extract along with its organic soluble fractions (dichloromethane and ethylacetate) of the fruits of *Trichosanthes dioica* in different experimental models.

## MATERIALS AND METHODS

**Plant materials:** The fruits of the *Trichosanthes dioica* were collected from the local market during the month of October 2008. The sample was identified by Mrs. Mahmuda Begum, Senior Scientific Officer, Bangladesh National Herbarium, Dhaka, where the voucher specimen has been deposited. Its Accession Number is 3489.

**Chemicals:** Ammonium molybdate, Folin-chiocaltu phenol reagent, were purchased from E. Merck (Germany). 1,1-diphenyl- 2-picryl-hydrazyl (DPPH), ascorbic acid, quercetin and potassium ferric cyanide, brewer's yeast and carraggenan, were purchased from Sigma Chemical Company (St. Louis, MO, USA). Paracetamol and Indomethacin were collected from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals and reagents were of analytical grade.

**Preparation of plant extract:** The fruits of the *Trichosanthes dioica* were dried in an oven at 37°C and then powdered with a mechanical grinder, passing through sieve #40 and stored in an air tight container. The dried powdered material (1.0 kg) was refluxed with MeOH for three hours. The total filtrate was concentrated to dryness, *in vacuo* at 40°C to render the MeOH extract (300 g). This extract was suspended in H<sub>2</sub>O and then successively partitioned with dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) and ethylacetate (EtOAc), to afford the CH<sub>2</sub>Cl<sub>2</sub> (120 g) and EtOAc (40 g), fractions along with a residue (120 g) present in aqueous phase.

**Animal:** Wistar rats (175-250 g) of both sexes were used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting of five animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh.

**Phytochemical screening:** The presence of various phytoconstituents such as alkaloids, carbohydrates, flavonoids, glycosides, proteins, resins, tannins and steroids were analyzed qualitatively using standard protocols (Ghani, 2003).

**Acute toxicity study:** Acute oral toxicity assay was performed in healthy nulliparous and non pregnant adult female Wistar rats (175-250 g) divided into different groups. The test was performed using increasing oral dose of the MeOH extract and different organic fractions in water (500, 1000, 1500 and 2000 mg kg<sup>-1</sup> body weight), in 20 mL kg<sup>-1</sup> volume to different test groups. Normal group received water. The rats were allowed to feed *ad libitum*, kept under regular observation for 48 h, for any mortality or behavioral changes.

### ***In vitro* antioxidant activity**

**The amount of phenolic compounds and flavonoids:** The total phenolic and flavonoid content of methanolic extract and several organic fractions were determined using Folin-Ciocalteu reagent (Yu *et al.*, 2002) and aluminium chloride colorimetric method (Chang *et al.*, 2002), respectively. The content of total phenolics in the extract and fractions of *T. dioica* was calculated from regression equation of the calibration curve ( $y = 0.013x + 0.127$ ,  $r^2 = 0.988$ ) and is expressed as Galic Acid Equivalents (GAE) and the flavonoid contents of the extract and fractions in terms of quercetin equivalent (the standard curve equation:  $y = 0.009x - 0.036$ ).

**Determination of total antioxidant capacity:** The antioxidant activity of the MeOH extract and several fractions were evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.* (1999). The assay is based on the reduction of Mo(VI)-Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. The antioxidant activity is expressed as the number of equivalents of ascorbic acid using the following formula:

$$C = (c \times V) / m$$

where, C is total antioxidant activity, mg g<sup>-1</sup> plant extract, in Ascorbic acid; c is the concentration of ascorbic acid established from the calibration curve, mg mL<sup>-1</sup>; V is the volume of extract, mL; m is the weight of pure plant extract, g.

### **Free radical scavenging activity measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH):**

The free radical scavenging activity of MeOH extract and other fractions, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al.* (2001). The percentage inhibition activity was calculated from  $[(A_0 - A_1) / A_0] \times 100$ , where  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance of the extract/standard. IC<sub>50</sub> value was calculated from the equation of line obtained by plotting a graph of concentration (µg mL<sup>-1</sup>) versus % inhibition.

**Nitric oxide radical scavenging assay:** The procedure is based on the method (Sreejayan and Rao, 1997) where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions.

**Reducing power activity:** The reducing power of *T. dioica* was determined according to the method previously described (Oyaizu, 1986). Increased absorbance of the reaction mixture indicated increased reducing power.

### **Anti-inflammatory activity**

**Carrageenan induced paw edema test in rats:** Male Wistar rats (175-250 g) were divided into four groups of five animals each. The test groups received 100, 200 and 400 mg kg<sup>-1</sup>, b.wt. (p.o.) of the extract. The reference group received indomethacin [10 mg kg<sup>-1</sup>, b.wt. (p.o.)] while the control group received 3 mL kg<sup>-1</sup> of 1% CMC. After 1 h, 0.1 mL, 1% w/v carrageenan suspension in normal saline was injected into the subplantar tissue of the right hind paw (Winter *et al.*, 1962).

The paw volume was measured at 1, 2 and 3 h after carrageenan injection using a micrometer screw gauge. The percentage inhibition of the inflammation was calculated from the formula: % inhibition =  $(1 - D_t/D_0) \times 100$ . Whereas  $D_0$  was the average inflammation (hind paw edema) of the control group of rats at a given time,  $D_t$  was the average inflammation of the drug treated (i.e., extract/fractions or reference indomethacin) rats at the same time (Gupta *et al.*, 2005).

**Antipyretic assay:** Fever was induced by injecting 15% suspension of Brewer's yeast (*Saccharomyces cerevisiae*), following a standard method (Loux *et al.*, 1972). A thermister probe was inserted 3-4 cm deep into the rectum, after fastened the tail, to record the basal rectal temperature. The animals were then given a subcutaneous injection of 10 mL kg<sup>-1</sup> of 15% w/v Brewer's yeast suspended in 0.5% w/v methylcellulose solution and at 19 h after yeast injection, the rectal temperature of the rats were recorded. Immediately the crude extract/fractions were administered at doses of 100, 200 and 400 mg kg<sup>-1</sup>, b.wt. Paracetamol was used as standard drug. Rectal temperature of all the rats was recorded at 19 h, immediately before extract/fractions or vehicle or paracetamol administration and again at 1 h interval upto 23 h, after yeast injection.

**Statistical analysis:** All values were expressed as the mean  $\pm$  standard error of three replicate experiments. The analysis was performed by using student's t test.  $p < 0.001$ ,  $p < 0.005$  and  $p < 0.05$  were considered to be statistically significant.

## RESULTS

**Phytochemical screening:** The results of various qualitative chemical tests for the detection of chemical constituents of *Trichosanthes dioica* is shown in the Table 1.

**Acute toxicity study:** The extract/fractions of *T. dioica* were safe up to a dose of 2000 mg kg<sup>-1</sup> (p.o.) b.wt. Behavior of the animals was closely observed for the first 3 h then at an interval of every 4h during the next 48 h. All extract/fractions did not cause mortality in mice and rats during 48 h observation but little behavioral changes, locomotor ataxia, diarrhea and weight loss were observed. Food and water intake had no significant difference among the group studied.

### *In vitro* antioxidant activity

**Total phenolic and flavonoid contents:** Table 2 represents the content of both groups in the extract and different organic soluble fractions. EtOAc fractions showed the highest total phenolic and flavonoid content and was found to be  $55.16 \pm 1.01$  mg g<sup>-1</sup> plant extract (in GAE) and  $94.62 \pm 0.72$  mg g<sup>-1</sup> plant extract (in quercetin equivalent), respectively.

Table 1: Result of chemical group tests of the Methanolic extract and its organic fractions of *Trichosanthes dioica* fruits

Phytoconstituents	Methanolic extract	CH <sub>2</sub> Cl <sub>2</sub> fractions	EtOAc fractions
Alkaloids	++	++	-
Amino acids	-	-	-
Carbohydrates	++	-	+
Flavonoids	++	-	+++
Glycosides	++	++	-
Proteins	-	-	-
Resins	-	-	-
Steroids	+	+++	-
Tannins	+	+	+++

+: Present, -: Absent, +++: Reaction intensity is high, ++: Reaction intensity is medium, +: Reaction intensity is normal

Table 2: Yield, total amount of plant phenolic compounds, flavonoids and total antioxidant capacity of methanolic extract and soluble organic fraction of *Trichosanthes dioica* fruits

Sample	Yield (%)	Total phenols mg g <sup>-1</sup> plant extract (in GAE) <sup>a</sup>	Total flavonoids mg g <sup>-1</sup> plant extract (in QA) <sup>b</sup>	Total antioxidant capacity mg g <sup>-1</sup> extract (in ASC) <sup>c</sup>
MeOH	30.00	15.94±0.12	29.98±0.32	197.24±0.69
CH <sub>2</sub> Cl <sub>2</sub>	40.00	3.85±0.19	1.24±0.41	3.36±1.01
EtOAc	13.33	94.62±0.02	55.16±0.23	279.48±0.45

<sup>a</sup>Gallic acid equivalents (GAE, mg g<sup>-1</sup> of each extract) for the total phenolic content. <sup>b</sup>Quercetin equivalents (mg g<sup>-1</sup> of each extract) for the total flavonoid content. <sup>c</sup>Ascorbic acid equivalents (mg g<sup>-1</sup> of each extract) for the total antioxidant capacity. The GAE, QA and ASC values are expressed as Means±SEM of triplicate experiments

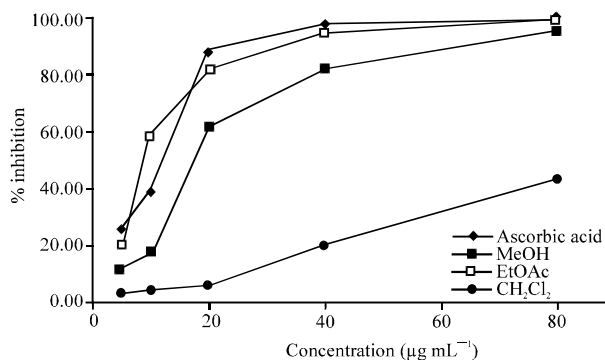


Fig. 1: Free radical scavenging activity of different concentrations of crude extract and different soluble fraction of *Trichosanthes dioica* and ascorbic acid by DPPH radicals

**Total antioxidant capacity:** Percentage yield of methanol extract and different organic fractions of *T. dioica* and their total antioxidant capacity are given in Table 2. Total antioxidant capacity of *T. dioica* is expressed as the number of equivalents of ascorbic acid. Total antioxidant capacity of EtOAc fractions showed the highest and was found to be 279.48±0.95 mg g<sup>-1</sup> equivalent of ascorbic acid.

**DPPH radical scavenging activity:** The percentage (%) scavenging of DPPH radical was found to be concentration dependent i.e. concentration of the extract/fractions between 5-80 µg mL<sup>-1</sup> greatly increasing the inhibition activity (Fig. 1). EtOAc fractions showed the highest DPPH scavenging activity with the IC<sub>50</sub> value of 12.32±0.16 µg mL<sup>-1</sup>, followed by MeOH extract with the IC<sub>50</sub> value of 17.37±0.22. EtOAc fractions showed the similar activity as standard ascorbic acid (IC<sub>50</sub> 12.30±0.11 µg mL<sup>-1</sup>).

**Nitric oxide (NO•) scavenging activity:** The percentage inhibition of nitric oxide production was illustrated in Fig. 2 and it is observed that scavenging of nitric oxide by the extract is also concentration dependent. It is also observed that all the extracts are likely to have the nitric oxide scavenging activity and statistically significant (p<0.001). The range of IC<sub>50</sub> values were 5.38±0.07 to 49.19±0.72 µg mL<sup>-1</sup>. EtOAc fraction's (IC<sub>50</sub> value 5.38±0.07 µg mL<sup>-1</sup>) showed 1.5 fold higher activity than the standard ascorbic acid (IC<sub>50</sub> value 8.22±0.22 µg mL<sup>-1</sup>).

**Reducing power ability:** For the measurement of the reductive ability, we investigated the Fe<sup>3+</sup> to Fe<sup>2+</sup> transformation in the presence of extract and organic fractions. Like the antioxidant activity,

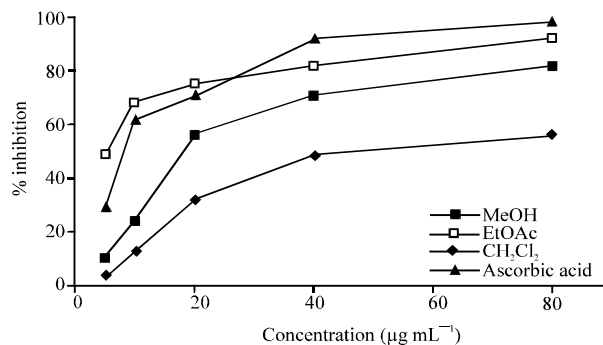


Fig. 2: Percentage inhibition of nitric oxide radical by different concentrations of crude extract and different soluble fraction of *Trichosanthes dioica* and ascorbic acid

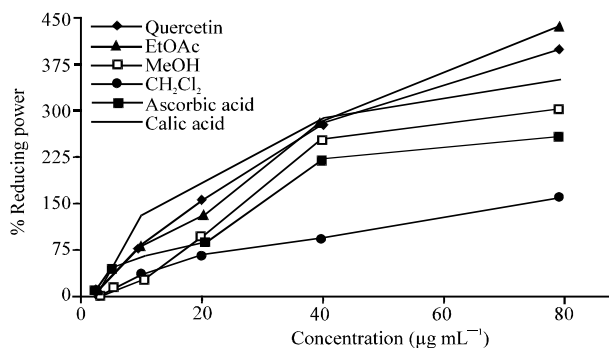


Fig. 3: Reducing power of MeOH extract and fractions of *Trichosanthes dioica* and quercetin by spectrophotometric detection of Fe<sup>3+</sup> to Fe<sup>2+</sup> transformation

the reducing power of *T. dioica* increased with increasing concentration of the sample. Figure 3 shows the reductive capabilities of the *T. dioica* compared with quercetin, galic acid and ascorbic acid. All *T. dioica* extract/fractions concentrations tested showed higher activities and these differences were statistically significant ( $p < 0.001$ ).

### Anti-inflammatory activity

**Carrageenan induced paw edema test:** To the carrageenan induced paw edema rats, the MeOH extract, at the dose of 100, 200 and 400 mg kg<sup>-1</sup>, b.wt. exerted a significant ( $p < 0.05$ ;  $p < 0.005$ ) and dose dependent inhibition on paw edema compared to the control group (Table 3). All of the fractions, the EtOAc and CH<sub>2</sub>Cl<sub>2</sub>, at the dose of 200 mg kg<sup>-1</sup>, also exhibited prominent anti inflammatory effect.

**Antipyretic activity:** In case of antipyretic assay, the MeOH extract, at the dose of 200 and 400 mg kg<sup>-1</sup>, b.wt., expressed significant ( $p < 0.005$ ) and dose dependent activity as compared to the control group (Table 4). EtOAc fractions, at the dose of 200 mg kg<sup>-1</sup>, also showed prominent antipyretic activity whereas, CH<sub>2</sub>Cl<sub>2</sub> extract showed insignificant activity.



Table 3: Effect of *Trichosanthes dioica* extract/fractions on carrageenan induced paw edema in rats

Group	Dose mg g <sup>-1</sup> , b.wt. (p.o)	Paw edema diameter in mm (% inhibition)			
		1 h	2 h	3 h	4 h
Control	1% CMC	11.98±0.25	13.38±0.16	14.58±0.65	11.38±0.12
Indomethacin	10	6.31±0.55*	5.31±0.37*	2.91±0.10*	2.69±0.17*
		(47.32)	(60.31)	(80.04)	(76.36)
MTD	100	11.25±0.25	10.25±0.17	8.25±0.17	8.03±0.02
		(6.09)	(23.39)	(43.41)	(29.43)
		200	9.99±0.10*	6.59±0.45*	4.39±0.29*
		(16.62)	(50.76)	(69.90)	(64.42)
	400	9.27±0.15**	5.27±0.45**	3.87±0.37**	3.47±0.25**
		(22.65)	(60.64)	(73.48)	(69.54)
CTD	200	11.61±0.55	11.05±0.33	10.53±0.20	10.03±0.02
		(3.02)	(17.41)	(27.75)	(11.86)
ETD	200	8.72±0.28**	5.28±0.23**	3.51±0.28**	3.43±0.28**
		(27.16)	(60.49)	(75.95)	(69.88)

The data represent the Mean±SEM (n = 5), \*p<0.005 and \*\*p< 0.001 by student's test for values between the sample and the vehicle treated group. MTD: Methanolic extract, CTD: Dichloromethane fraction, ETD: Ethylacetate fraction of *T. dioica*. Percentage inhibition indicated in parenthesis

Table 4: Effect of *Trichosanthes dioica* extract/fractions on Brewer's induced pyresia in rats

Group	Dose mg kg <sup>-1</sup> , b.wt. (p.o)	Rectal temperature (°C)						
		Before	1 h	2 h	3 h	4 h	5 h	
Control		36.15±0.03	37.87±0.05	37.84±0.05	37.78±0.05	37.73±0.03	37.71±0.02	
Paracetamol	100	36.01±0.04	36.20±0.04*	36.14±0.03*	36.08±0.03*	36.06±0.03*	36.04±0.02*	
MTD	100	36.28±0.06	37.78±0.05	37.73±0.05	37.49±0.03	37.48±0.03	37.46±0.03	
		200	36.18±0.05	37.73±0.05	37.51±0.04	36.58±0.13	36.54±0.11	36.52±0.11
		400	36.20±0.12	37.67±0.05	36.50±0.15	36.42±0.14*	36.39±0.12*	36.39±0.11*
CTD	200	36.20±0.21	37.87±0.05	37.84±0.05	37.78±0.05	37.72±0.04	37.70±0.01	
ETD	200	36.25±0.01	37.67±0.05	37.51±0.04	36.39±0.12*	36.38±0.11*	36.37±0.11*	

The data represent the Mean±SEM (n = 5), \*p<0.005 and by Student's test for values between the sample and the vehicle treated group. MTD: Methanolic extract, CTD: Dichloromethane fraction, ETD: Ethylacetate fraction of *T. dioica*

## DISCUSSION

Plant polyphenols, a diverse group of phenolic compounds (flavonols, flavonoids, tannic acid, anthocyanins, phenolic acid, etc.) possess an ideal structural chemistry for free radical scavenging activity (Bhandare *et al.*, 2010) and exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective and vasodilatory effects (Manach *et al.*, 2005; Middleton *et al.*, 2000; Puupponen-Pimia *et al.*, 2001; Samman, 1998). The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity (Heim *et al.*, 2002). Phenolic compounds could be a major determinant of antioxidant potentials of food (Parr and Bolwell, 2000) and could therefore be a natural source of antioxidants. Phenolic compounds are understood to induce the cellular antioxidant system; increase approximately 50% cellular glutathione concentration. Flavonoids are important in the modulation of  $\gamma$ -glutamylcysteine synthase in both cellular antioxidant defenses and detoxification of xenobiotics (Muchuweti *et al.*, 2007). The

highest amount of total phenolic and flavonoid content was found in EtOAc fractions that was  $55.1 \pm 1.01 \text{ mg g}^{-1}$  plant extract (in GAE) and  $94.62 \pm 0.72 \text{ mg g}^{-1}$  plant extract (in quercetin equivalent), respectively.

To determine the efficacy of natural antioxidants either as pure compounds or as plant extract, a great number of *in vitro* methods have been developed in which antioxidant compounds act by several mechanisms. The phosphomolybdenum method was based on the reduction of Mo(VI) to Mo(V) by the compounds having antioxidant property and is successfully used to quantify vitamin E in seeds (Prieto *et al.*, 1999). DPPH• is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Nakayama, 1994) and is usually used as a substrate to evaluate the antioxidant activity of a compound (Chang *et al.*, 2002). Based on the data obtained from this study, DPPH radical scavenging activity of EtOAc fractions ( $IC_{50} 12.32 \pm 0.16 \text{ } \mu\text{g mL}^{-1}$ ) of *T. dioica* was similar to the standard ( $IC_{50} 12.30 \pm 0.11 \text{ } \mu\text{g mL}^{-1}$ ). It was revealed that organic soluble fraction of *T. dioica* did show the proton donating ability and could serve as free radical inhibitor or scavenger. In fact, the radical scavenging capability of phenolic compounds are due to their hydrogen donating ability/number of hydroxyl groups present which in turn is closely related both to the chemical structure and spatial conformation, that can modify the reactivity of the molecules (Gorelik *et al.*, 2008). In the present study, this possibility is supported by the estimation of total polyphenols and flavonoids, (Loke *et al.*, 2008) which was found to be present in high concentration in the test extracts/fractions.

In the literature survey, only a few reports on the chemical composition of *Trichosanthes dioica* so far. In a previous study, a steroidal saponin, 24- $\alpha$ -ethyl-20-ene-7-hydro-stigmast-8  $\beta$ : 14  $\beta$ -di-3-O- $\beta$ -D-xylofuranoside (Saxena and Dave, 1995),  $\beta$ -Sitosterol, its glucosides and luteolin-7-glucoside have also been isolated from the leaves of *T. dioica* (Gopal and Ramchandra, 1979). In another phytochemical study, Rai *et al.* (2010) isolated flavonoids like 5-Hydroxy-2-(2,4-dihydroxy-5-methoxyphenyl)-7-methoxy-4H-chromen-4-one, 5,7-dihydroxy-2-(2-hydroxy-5-methoxyphenyl)-4H-chromen-4-one and 7-Hydroxy-4H-chromen-4-one from the seeds of *Trichosanthes dioica*. A direct correlation between antioxidant capacity and reducing power of certain plant extracts has been reported. The reducing properties are generally associated with the presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Duh *et al.*, 1999). MeOH extract and EtOAc fractions of *T. dioica* fruits showed significant and prominent activity. Our results suggest that the antioxidant activity of *T. dioica* might be attributed to the flavonoids which detected by phytochemical analysis in our study and also previously reported by Dixit and Kar (2009).

Carrageenan induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Antonio and Brito, 1998; Gupta *et al.*, 2006).

Since the extract/fractions significantly inhibited paw edema induced by carrageenan in the second phase and this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme. The high gastroprotective activity demonstrated by *Trichosanthes* sp. against ethanol or indomethacin induced gastric ulcers suggests that COX-2 inhibitory activity of *Trichosanthes* sp. may be greater

than the inhibitory effect on COX-1 (Arawwawala *et al.*, 2010a); thus an evaluation of inhibitory effects of *Trichosanthes dioica* extracts on COX-1 and COX-2 expression remains to be established. In addition, the release of several ROS (Koblyakov, 2001) and excessive Nitric Oxide (NO) due to the activation of neutrophils during tissue damage and inflammation (Annegowda *et al.*, 2010) which is responsible a variety of disease states like cerebral ischemia, atherosclerosis, cancers, migraine parkinsonism etc (Bhandare *et al.*, 2010). Srivastava *et al.* (2000) and Viana *et al.* (2003) suggested that tannic acid and polyphenols are potent inhibitors of NO synthase activity and NO production. As EtOAc fraction showed significant free radical as well as NO scavenging activity, so this can be responsible for the reduction of inflammation in the carrageenan induced paw edema in rats. Furthermore, from previous study (Arawwawala *et al.*, 2009, 2010b) it has observed that polyphenols (including phenolic compounds and flavonoids) rich *Trichosanthes cucumerina* possess antioxidant as well as anti-inflammatory activity. So polyphenols including flavonoids present in the *T. dioica* may induce the antioxidant properties as well as may also play a provital role in mediating the anti-inflammatory effects.

Fever may occur as a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection or other disease state (Rao *et al.*, 2002). Regulation of body temperature requires a delicate balance between the production and loss of heat and the hypothalamus regulates the set point at which body temperature is maintained. Therefore, the significant reduction in the brewer's yeast provoke elevated body temperature in the animals suggests antipyretic potential of the plant extract. Studies have shown that alkaloids have the ability to inhibit the synthesis of prostaglandin E<sub>2</sub> (Backhouse *et al.*, 1994), eventually reducing elevated body temperature in animal. Similarly, flavonoids have been implicated as an antipyretic agent by suppressing TNF- $\alpha$  (Chang *et al.*, 2007). The antipyretic properties of the MeOH extract of *T. dioica* could possibly be associated with the synergistic effect of flavonoids and alkaloid.

Phytochemical analysis showed that the extract contained alkaloids, glycosides, phenolic compounds, tannins, steroids and flavonoids. Flavonoids, tannins, phenolic compounds and glycosides have all been associated with various degrees of anti-inflammatory, antipyretic and antioxidant activities (Wang *et al.*, 2008). Selected phenolic compounds and flavonoids were shown to inhibit both the cyclooxygenase and 5-lipoxygenase pathways (Jothimanivannan *et al.*, 2010). Moreover, flavonoids also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation (Sawadogo *et al.*, 2006). Therefore, the anti-inflammatory, antipyretic and antioxidant effects observed in this study are perhaps due to the activity of one or more of the identified classes of compounds. Because an essential database on the chemical profile of *Trichosanthes dioica* fruit extract is established in which flavonoids and triterpenoids are the major constituents, this information should be considered for future purification of anti-inflammatory and antioxidant active compounds from this natural source. Our results indicate that *Trichosanthes dioica* fruits extract has both effective pronounced anti-inflammatory and strong antioxidant activity.

## CONCLUSION

In conclusion, the results of the present study, indicate that the MeOH extract and its various organic soluble fractions exhibits interesting antioxidant properties, as well as significant anti-inflammatory and antipyretic effect which may be due to the presence of phenolic compounds and flavonoids in the extract/fractions. Now our next aim is to explore the isolation and characterization of lead compound liable for aforementioned activity from this plant.

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