Antioxidant potential of *Trichosanthes dioica* Roxb (fruits)

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Abstract

Objective: The study was undertaken to evaluate the antioxidant activity of fruits of *Trichosanthes dioica* (Cucurbitaceae) and compared with ascorbic acid (Standard).

Materials and Methods: Anti-oxidant activity of aqueous extract of *Trichosanthes dioica* (TSD) fruits was studied for its free radical scavenging property in different in vitro methods as 1, 1 diphenyl-2- picryl hydrazyl, nitric oxide, reducing power assay and hydrogen peroxide radical method. Different concentrations of aqueous extract of TSD were prepared and evaluated by standard methods.

Results: The IC_{50} values of aqueous extract of TSD were compared with ascorbic acid (Standard) and it was noted that, the extract showed significant concentration dependent free radical scavenging property in all the methods.

Conclusion: Results from the study showed that aqueous extract of TSD possess *in vitro* free radical scavenging activity. The findings could justify the inclusion of this plant in the management of antioxidant activity.

Keywords: *Trichosanthes dioica*, antioxidant, DPPH, Reducing power assay

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INTRODUCTION

Free radicals can be described as chemical species that have an unpaired electron and play very important role in human health and beneficial in combating against several diseases. These free radicals are highly unstable and when the amount of these free radicals exceed in the body, it can damage the cells and tissues and may involved in several diseases like cardiovascular disorders, lung damage, inflammation etc. Thus there is the need of antioxidant of natural origin because they can protect the human body from the diseases caused by free radicals (12).

Trichosanthes dioica (family- Cucurbitaceae) is a well known plant commonly called as Parwal in Hindi, Potol in Bengal, and Palwal in Punjab. The medicinal attributes *T. dioica* have been known since time immemorial. The plant is alternative, tonic, useful in obstinate fevers, boils etc. The roots are cathartics. The leaves are anthelmentic. The fruits are sweet, cardiotonic, appetizer and stomachic. The plant *T. dioica* possesses many medicinal properties including laxative, cooling, febrifuge, cathartic etc. (3–5).

However relevant experimental work has not yet been explored. Therefore, the aim of this study was to evaluate the antioxidant activity of aqueous extract of *Trichosanthes dioica* Roxb fruits.

MATERIAL AND METHODS

Plant material and extraction

Fresh unripe fruits of *Trichosanthes dioica* (2 kg) were purchased from the local market of Bhopal, India, in the month of August 2008. The plant was authenticated by Dr. A.S.Yadav, Professor, Government MVM College, Bhopal. The collected fruits were cut into small pieces and were shade dried. The dried pieces were pulverized into moderately coarse powder and stored in well closed container. The shade dried powder of TSD fruits (125g) were macerated with water for 72 hrs then concentrated and dried under reduced pressure to semisolid mass and

residue was obtained (16.96g yield w/w). The residue was stored in a desiccator.

Qualitative test analysis

Qualitative test analysis was performed to determine chemical constituents present in the dried aqueous extract of TSD. The extract was tested for the presence of various phytoconstituents viz. alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds and tannins (6–7).

DPPH scavenging activity

The procedure of Brand-Williams(8) has been adapted for evaluation of the free radical scavenging capacity of the aqueous extract. Different concentrations (05–45µg/ml) of aqueous extract of TSD were prepared in suitable solvent and 3ml of each solution was mixed with 1 ml of a 0.1mM DPPH solution. The decrease in absorbance was measured at 515 nm after 30 minutes of incubation period at room temperature using a UV Visible spectrophotometer 1700 (Shimadzu). The scavenging activity of sample extract was expressed as the inhibition of DPPH radical and calculated according to the following formula with as the control:

Scavenging Activity (%) = [(A control – A sample) / A control]*100, where $A_{control}$ (containing DPPH solution) and A_{sample} is the Absorbance with different dilutions of drug extract. Ascorbic acid was used as reference standard.

Reducing power assay

Reducing power of aqueous extract of TSD was estimated using the protocol reported by Oyaizu (9). Different concentrations of aqueous extract of TSD (5–25µg/ml) were prepared and 1ml of each solution was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.8) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 min. To this mixture, 2.5 ml of 10% trichloroacetic acid (TCA) was added and then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5ml) and $Fecl_3$(0.5 ml, 0.1%) was added and the absorbance was measured at 700 nm.

The percentage scavenging was calculated by using the formula

$(A_{control} - A_{sample})/ A_{control} \times 100$, where $A_{control}$ is the absorbance of solution without extract and A_{sample} is the Absorbance with different dilutions of drug extract. Ascorbic acid was used as reference standard.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was evaluated by the method of Gupta (10).1ml of Sodium nitroprusside (10mM) in phosphate-buffered saline (PBS) was mixed with 3.0 ml of different concentrations (20 – 120µg/ml) of the aqueous extract of TSD dissolved in water and incubated at 25°C for 180 min. The samples from the above were reacted with equal volume of Greiss reagent (1% sulphanilamide, 0.1% napthylethylenediamine hydrochloride and 3% of phosphoric acid). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm. The percentage scavenging was calculated by using the formula

$(A_{control} - A_{sample})/ A_{control} \times 100$, where $A_{control}$ is the absorbance of solution without extract and A_{sample} is the absorbance with different dilutions of drug extract. Ascorbic acid was used as reference standard.

Hydrogen peroxide radical scavenging activity

The ability of the aqueous extract of TSD to scavenge hydrogen peroxide was determined according to the method of Ruch (11). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a UV Visible spectrophotometer 1700. Then hydrogen peroxide solution (0.6 ml, 40 mM) was mixed to different concentrations (30 – 180µg/ml) of the extract dissolved in water. The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide.

The percentage scavenging was calculated by using the formula

$(A_{control} - A_{sample})/ A_{control} \times 100$, where $A_{control}$ is the absorbance of solution without extract and A_{sample} is the Absorbance with different dilutions of drug extract. Ascorbic acid was used as reference standard.

Statistical Analysis

Data are presented as the mean ± SEM of each triplicate test. The analysis was performed by using Dunnett vs. Control test and by ANOVA. $P<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemicals

The phytochemical screening of aqueous extract of TDS revealed the presence of alkaloids, carbohydrates,

glycosides, flavonoids, phenolic compounds and tannins.

DPPH radical scavenging activity

The results of DPPH radical scavenging activity of the aqueous extract of TSD with IC_{50} (% Inhibition) are shown in fig 1.1. The IC_{50} value of aqueous extract of TSD and standard (ascorbic acid) were found to be 33µg/ml, and 11µg/ml, respectively. The results showed a significant ($p < 0.01$) decrease in the concentration of DPPH radical due to the scavenging ability of aqueous extract as compared to standard (ascorbic acid).

Reducing power assay

The Reducing power of aqueous extract of TSD and ascorbic acid were shown in the fig 1.2. The IC_{50} value of aqueous extract of TSD and ascorbic acid (standard) were found to be 25µg/ml and 12µg/ml, respectively.

Nitric oxide scavenging activity

The nitric oxide scavenging activity of aqueous extract of TSD and ascorbic acid were shown in the fig 1.3 which illustrates the % inhibition of nitric oxide generation by aqueous extract of TSD. The IC_{50} value of aqueous extract and ascorbic acid (standard) were found to be 112µg/ml and 85µg/ml, respectively. The results indicate significant ($p<0.01$) decrease in the concentration of nitric oxide radical due to the scavenging ability of aqueous extract as compared to standard.

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide scavenging activity of aqueous extract of TSD and ascorbic acid were shown in the fig 1.4. It showed significant scavenging activity of hydroxyl radical generated from H_2O_2 system. The IC_{50}value for aqueous extract was 171µg/ml, whereas 167µg/ml was the value of ascorbic acid. The results indicated that aqueous extract of TSD possessed significant antioxidant activity ($p<0.01$).

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease.

Aqueous extract of TSD showed the presence of flavonoids and phenolic compounds. Since the antioxidant activities of these constituents from plant origin have already been established (12), 22 it can speculate that these constituents may be responsible for the observed antioxidant effects.

DPPH scavenging activity has been used by various researchers as a quick and reliable parameter to assess the *in vitro* antioxidant activity of crude plant extracts (13–14). A DPPH radical scavenging ability of the extract was significantly lower than those of ascorbic acid. It was evident that the extract did show the proton donating ability and could serve as free radical inhibitor or scavenger, acting possibly as primary antioxidants.

In the present study, the reductive capacity of the aqueous extract of TSD was compared with ascorbic

Fig 1.1 Effect of aqueous extract of Trichosanthes dioica on DPPH scavenging activity

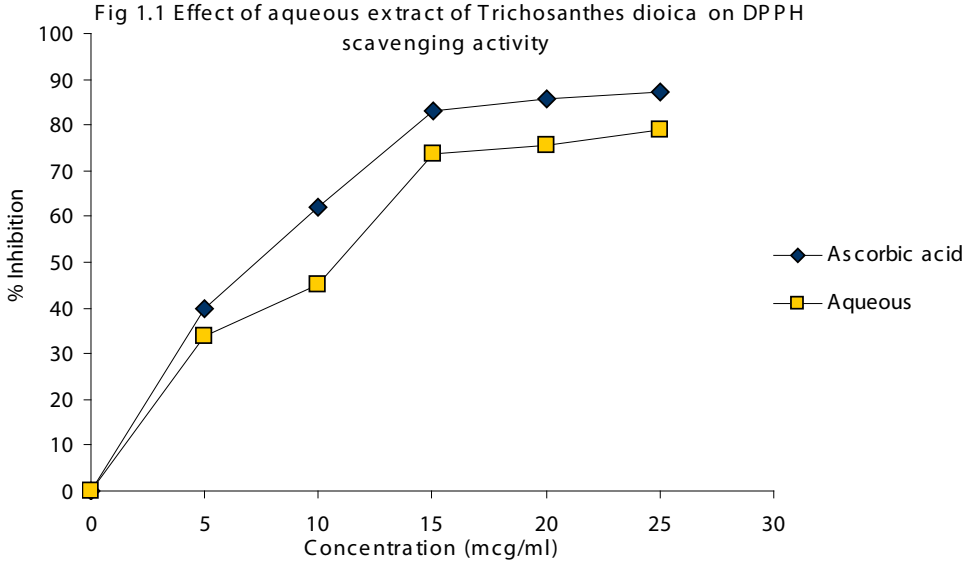


Fig 1.2 Effect of aqueous extract of Trichosanthes dioica on reducing power assay

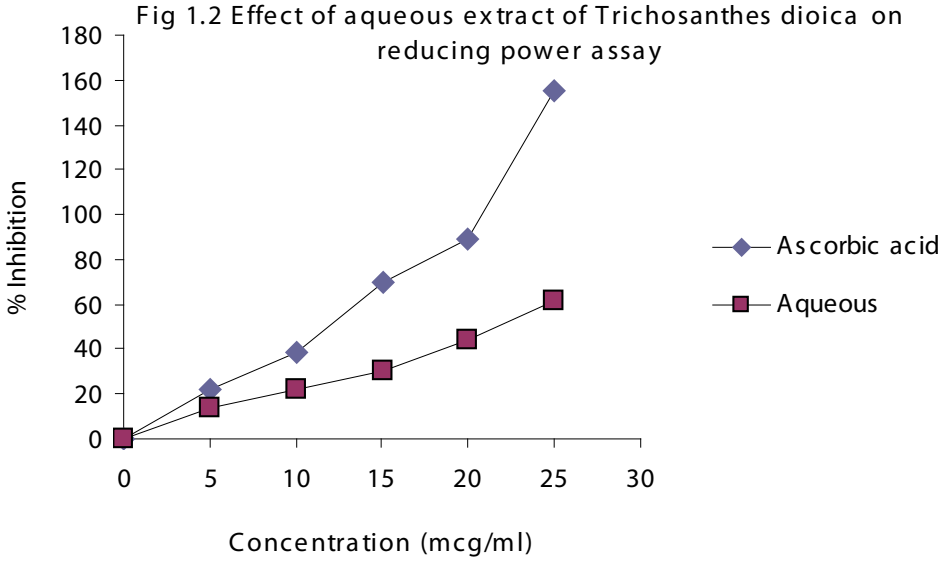
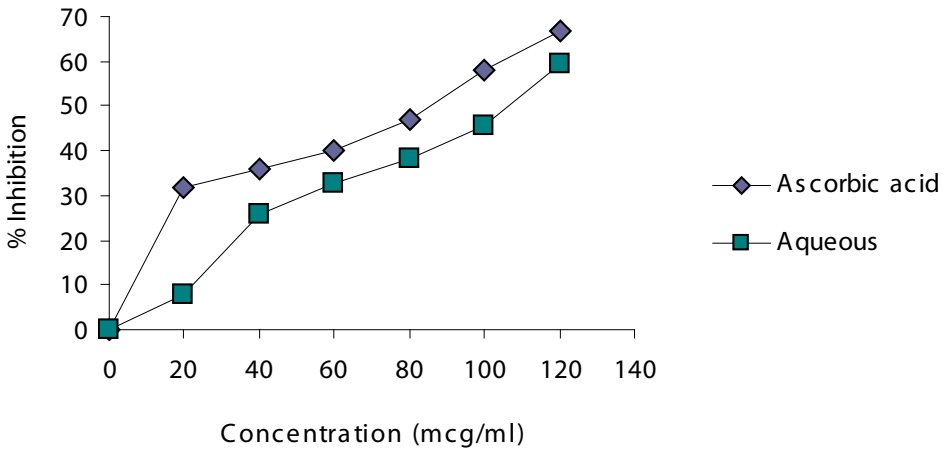


Fig 1.3 Effect of aqueous extract of Trichosanthes dioica on NO scavenging activity



acid (Standard). Significant antioxidant potential of any compound depends on its reducing capacity (15). The reducing capacity of the aqueous extract of TSD was found to be concentration dependent and showed significant potential.

Nitric oxide is a short-lived (half-life 3–30 s) colorless gas that is moderately soluble in water highly soluble in organic solvents (16). It is an important chemical mediator or essential bioregulatory molecule which is generated by neurons, endothelial cells etc and required for several physiological processes like immune response, neural signal transmission and control of blood pressure. Several diseases occur due to excess concentration of nitric oxide (17–18). Oxygen reacts with the excess nitric oxide to generate nitrites and anions which act as free radicals (19–20). In this study, the fruits of plant TSD compete with oxygen to react with nitric oxide and thus inhibit generation of anions.

Hydroxyl radicals are the major active species causing lipid oxidation and enormous biological and cellular damages (21). Its ability to produce active oxygen species is due to its ability to generate highly reactive hydroxyl

radical through the Fenton reaction (22). As the aqueous extract scavenged hydrogen peroxide radical similar to the standard (ascorbic acid) so it reflects that the plant extract could possibly inhibit the formation of hydroxyl radical.

CONCLUSION

In this study, the present results indicates that the aqueous extract of TSD possess antioxidant properties due to the presence of phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. This study has to some extent validated the medicinal value of the fruits of *Trichosanthes dioica*.

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