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Preliminary Phytochemical Screening and *In Vitro* Antioxidant Activity of Hydroalcoholic Extract of *Tinospora Cordifolia*

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ABSTRACT

The aim of the present study was to determine qualitative and quantitative phytochemical and *in vitro* antioxidant activities of aerial part of *T. cordifolia* collected from Bhopal region of Madhya Pradesh. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenol and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by folins ciocalteau reagent method and aluminium chloride method respectively. The *in vitro* antioxidant activity of hydroalcoholic extract of the aerial part was assessed against DPPH assay method using standard protocols. Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenol, proteins, carbohydrates, saponins and diterpins. The total phenolic and flavonoids content of *T. cordifolia* aerial part of hydroalcoholic extract was 0.947 and 1.256 mg/100mg respectively. The activities of hydroalcoholic aerial part extract against DPPH assay method were concentration dependent with IC_{50} values of ascorbic acid and extract 17.68 and 79.20µg/ml respectively. These studies provided information for correct identification of this plant material. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

Key words: *Tinospora cordifolia,* Qualitative, Quantitative phytochemical, Antioxidant activity.

INTRODUCTION

India is the largest producer of medicinal herbs and appropriately called the Botanical garden of the world (Ahmedulla and Nayar, 1999). Since ancient times plants have been traditionally used in therapeutic practices for the treatment of different types of ailments (Balakumar et al., 2011; Md. Saleem et al., 2011; Pour and Sasidharan, 2011; Paulraj et al., 2011). There are a number of crude drugs

where the plant source has not yet been scientifically identified. A phytochemical is a natural bioactive compound found in plants foods that works with nutrients and dietary fibre to protect against diseases. Many researchers suggest that, phytochemical working together with nutrients found in fruits, vegetables and nuts. They can have complementary and overlapping mechanism of action in the body including antioxidant effect. The qualitative analysis of phytochemicals of a medicinal plant is reported as vital step in any kind of medicinal plant research. Screening of plants constituents accurately can be done by employing chromatographic techniques (Sneader, 2000).

Quantification usually employs the use of gravimetric and spectroscopic methods with several advanced approaches now available (Aguoru et al., 2014). Reactive Oxygen Species (ROS), such as hydrogen peroxide, super oxide anion and hydroxyl radical, capable of causing damage to DNA, have been associated with carcinogenesis, coronary heart disease and many other health problems related to advancing age (Beris, 1991). Antioxidant that scavenges these free radicals proves to be beneficial for these disorders as they prevent damage against cell proteins, lipids and carbohydrates (Marnett et al., 2000). Erythrocytes, which are the most abundant cells in the human body, possessing desirable physiological and morphological characteristics are exploited extensively in drug delivery (Hamidi and Taferzadeh, 2003). Oxidative damage to the erythrocyte membrane (lipid/ protein) may be implicated in haemolysis associated with some haemoglobinopathies, oxidative drugs, transition metal excess, radiation, and deficiencies in some erythrocyte antioxidant systems (KO et al., 1997). This assay is useful either for screening studies on various molecules and their metabolites, especially on one hand, molecule having an oxidizing or antioxidating activity or on the other hand, molecule having a long term action (Djeridane et al., 2007). Several herbal secondary metabolites such as flavonoid have been found to protect cells from oxidative damage (Kumar and Pandey, 2013). These compounds have been evidenced to stabilize RBC membrane by scavenging free radicals and reducing lipid peroxidation (Yu, 2001; Ebrahimzadeh et al., 2010).

Tinospora cordifolia (*T. cordifolia*), a shady climbing shrub belongs to the family *Manispermaceae,* is found in the tropical areas of India, Pakistan, Sri Lanka, Burma, Africa, Australia and China (Panchabhai et al., 2008). In the Hindi language, the plant is called *giloya,* which means a divine thing that prevents aging. The phytochemical constituents of *T. cordifolia* include aliphatic compound, alkaloids, steroids, glycosides, sesquiterpenoid, polysaccharides, different types of fatty acids and essential oils (Panchabhai et al., 2008; Patel and Mishra, 2011). Panchabhai et al. have provided a detailed summary of the pharmacological properties of *T. cordifolia* (Panchabhai et al., 2008). Experimental studies conducted on *T. cordifolia* have shown that it has significant therapeutic effects on diabetes and its associated complications, hepatotoxicity, different types of infections, gastrointestinal related complications and different types of cancers. Moreover**,** this plant extract has traditionally been used for the treatment of

fever (Panchabhai et al., 2008; Hussain et al., 2015). The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds and *in vitro* antioxidant activity of leaf of *T. cordifolia*.

Material and method

Plant material

Aerial parts of *T. cordifolia* were collected from local region of Bhopal in the month of Feb, 2018. Plant material (Aerial part) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvent used in this study were of analytical grade.

Defatting of plant material

The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

75gm of dried plant material were exhaustively extracted with hydroalcoholic solvent (ethanol: water: 80:20) using maceration method. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

Phytochemical screening of the extract

The extract of *T. cordifolia* was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, saponins, phenol, proteins and flavonoids (Khandelwal 2005; Kokate, 1994).

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso *et* al., (2011). A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso *et al.*, 2011. 1ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results

were expressed as quercetin equivalent (mg/100mg).

DPPH free radical scavenging assay

DPPH scavenging activity was measured by modified method (Olufunmiso *et al.*, 2011). DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC_{50}), IC_{50} was calculated based on the percentage of DPPH radicals scavenged. The lower the IC_{50} value, the higher is the antioxidant activity.

Results and discussions

The percentage yields of Pet ether and hydroalcoholic extract obtained from *T. cordifolia* are depicted in the Table 1. Preliminary phytochemical studies of the extract were done according to the published standard methods. These tests were broad in scope and used to determine the presence of alkaloids, flavonoids, phenol, proteins, carbohydrates, saponins and diterpins but Glycosides was absents in the extract Table 2. The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.042X+0.002$, $R^2= 0.999$,** where X is the gallic acid equivalent (GAE) and Y is the absorbance. The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.06X+0.019$, $R^2= 0.999$,** where X is the quercetin equivalent (QE) and Y is the absorbance. TPC of hydroalcoholic extract of *T. cordifolia* showed the content values of 0.947 and fallowed by TFC were 1.256 Table 3. Antioxidant activity of the samples was calculated through DPPH assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Ascorbic acid was taken as standard and the values were comparable with concentration ranging from 10 µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed Table 4 & Figure 1.

Table 1 % Yield of leaves of *T. cordifolia*

S. No.	Solvents	% Yield
1	Pet. ether	2.77
2.	Hydroalcoholic	6.21

Table 2 Phytochemical screening of extract of *T. cordifolia*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Mayer's Test Wagner's Test Hager's test	 -ve -ve +ve
2.	**Glycosides** Legal's test	 -ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve -ve
4.	**Phenol** Ferric Chloride Test	 +ve
5.	**Proteins** Xanthoproteic test	 +ve
6.	**Carbohydrates** Fehling's test	 +ve
7.	**Saponins** Froth Test	+ve
8.	**Diterpins** Copper acetate test	 +ve

Table 3 Total Phenol and total flavonoid content of *T. cordifolia* extract

S. No.	Extract	Total Phenol (mg/100mg)	Total flavonoid (mg/100mg)
1.	Hydroalcoholic *extract*	0.947	1.256

Table 4 % Inhibition of ascorbic acid and hydroalcoholic extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	44.65	30.14
2	20	48.62	34.63
3	40	65.34	42.87
4	60	69.65	47.51
5	80	77.41	50.74
6	100	84.13	52.67
IC_{50}		**17.68**	**79.20**

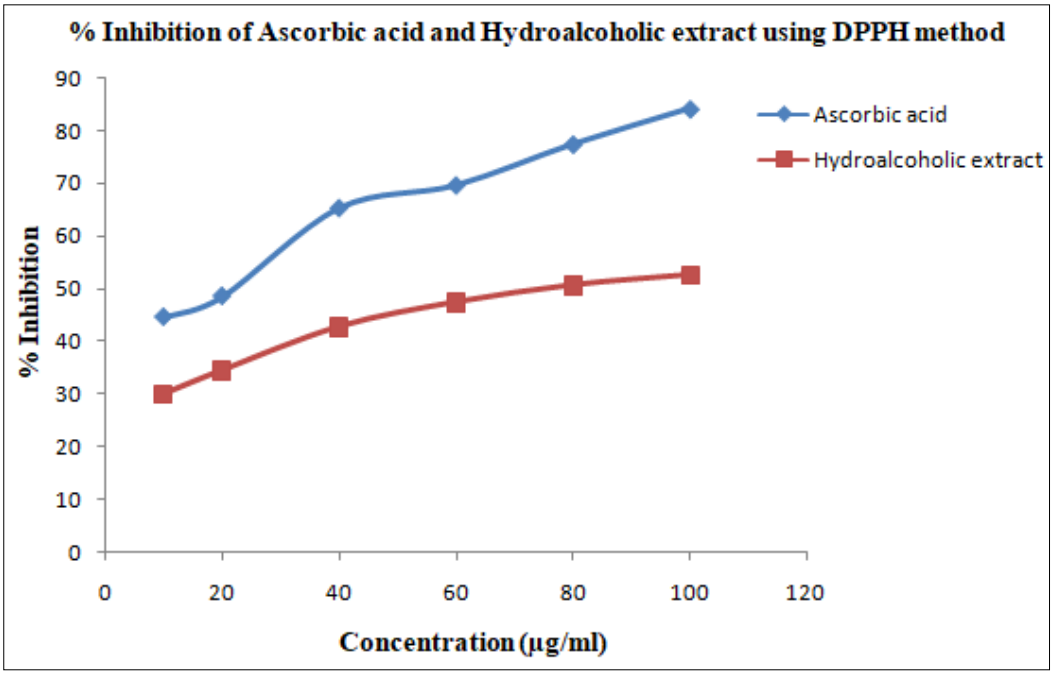

Figure 1 Graph of % Inhibition of ascorbic acid and hydroalcoholic extract using DPPH method

Conclusion

It can be concluded that from present investigation the phytochemical investigation gave valuable information about the different phytoconstituents present in the plant, which helps the future investigators concerning the selection of the particular extract for further investigation of isolating the active principle and also gave idea about different phytochemical have been found to possess a wide range of activities. The total phenolic and flavonoid content in hydroalcoholic leaves extract is further proved by in vitro antioxidant studies. Potential antioxidant activity has good correlations with the therapeutic use in the treatment of cardiovascular disorders. Further research to isolate individual compounds, their *in-vivo* antioxidant activities with different mechanism is needed.

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