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In Vitro Antioxidant Study of Extracts of *Tinospora cordifolia* (Willd.) Hook & Thoms

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ABSTRACT

In the present investigation, an attempt has been made to investigate the invitro antioxidant potential of ethanolic and aqueous extract of *Tinospora cordifolia* (WILLD) HOOK & THOMS. The Nitric oxide assay method and Super oxide method has been performed at different doses (10-100µg). The results of the present study shows that the ethanolic extract of *Tinospora cordifolia* possess antioxidant activity through Nitric oxide and Super oxide scavenging activity. The prelimnary phytochemical investigation indicates the presence of flavanoids and flavono glycosides. The results are found to be significant when compare with the standard ascorbic acid. Further studies are required to determine the mechanism and isolation of active constituents involved in the antioxidant activity.

INTRODUCTION

Free radicals had been implicated in several human diseases e.g. atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, ageing, inflammatory response syndrome, respiratory diseases and cancer [1-4]. Many herbal plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species such as singlet oxygen, superoxide, hydroxyl radicals and peroxynitrite[5-6].

Tinospora cordifolia (WILLD)HOOK.F & THOMS pers belongs to the family the Menispermaceae[7]and a glabrous, succulent, woody climbing shrub nature to india simple alternating long petiole approximately 15cm.Rather succulent with long filform, fleshly, climbing in nature and found commonly India ,Bangladesh and Srilanka. The phytochemical studies revelved the presence of Alkaloids, Saponins, Carbohydrate, Glycoside, Flavonoids, Phenols,Coumarins[8].The plant traditionally used as anthelmintic, Anti-inflammatory, Anti-allergic, Anti-bacterial, Anti-diabetic, Anti-fertility, Anti-cancer, Hperlipidemic effects[9]. No systematic studies on antioxidant activity have seen reported on *Tinospora cordifolia*. Hence an effort has been made to establish the antioxidant activity.

MATERIALS AND METHODS

The stem part of *Tinospora cordifolia* was collected from Kanyakumari district in march 2017 and authenticated by Dr.V.CHELLADHURAI, Botanist, A voucher specimen of *Tinospora cordifolia* (SARPC/RXA/CC-0280) was deposited in the Department pharmacetiucal chemistry in S.A.Raja pharmacy college, Vadakangulam for future reference. The air dried stem parts of the plant material was dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh and then stored in an air tight and light resistant container for future use.

Preparation of extract

About 1 kg of coarsely powdered plant material was first extracted with ethanol for 72 hours. The extract was concentrated using rotary evaporator to get solid residue. The marc left was removed, dried and successively extracted with aqeous by hot percolation until complete extraction was effected. It was then concentrated under reduced pressure and finally dried in desiccators. All the extracts were used for Antioxidant studies.

Nitric oxide scavenging activity

Nitric oxide radical scavenging activity

Principle

Nitric oxide radical activity was done according to the method reported by Garrat *et al*., 1964. Nitric oxide ($NO^{\cdot}$) has been involved in a variety of biological functions, including neutral transmission, vascular homeostasis, anti-microbial and anti-tumor activities. Despite of the possible beneficial effects of $NO^{\cdot}$ its contribution to oxidative damage is also reported. This is due to the fact that $NO^{\cdot}$ can react with superoxide to form the peroxynitrite anion, which is a potential oxidant that can decompose to produce OH acid and $NO^{\cdot}$. This procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates $NO^{\cdot}$ Which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitric ions. Large amount of $NO^{\cdot}$ may lead to tissue damage (Ebrahimzadeh M.A.,2010).

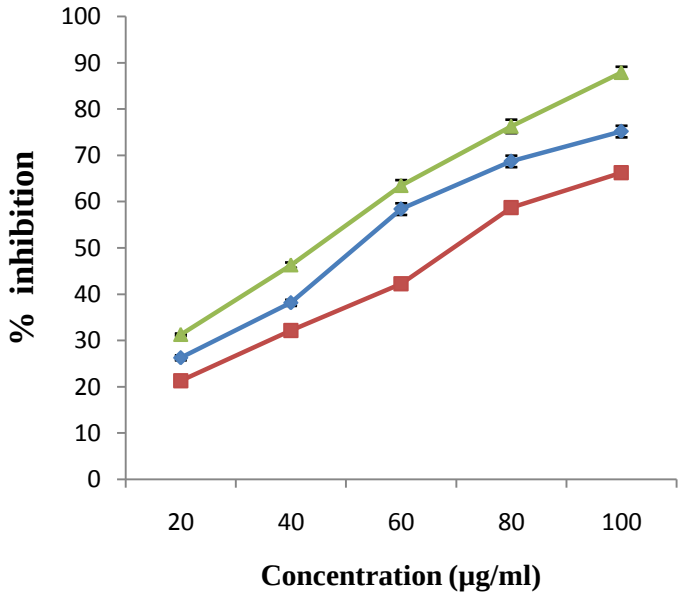
The chloroform and methanol extracts were subjected to nitric oxide scavenging activity. Sodium nitroprusside ($5mmolL^{-1}$) in phosphate buffered saline pH 7.4 was mixed with different concentration of the extract (20 to 100µg/ml) prepared in methanol and incubated at $25^{0}C$ for 30minutes. A control without the test compound, but an equivalent amount of methanol was taken after 30 minutes, 1.5ml of the incubated solution was removed and diluted with 1.5ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with N-l-naphthyl ethylene diamine dihydrochloride and incubated at the room temperature for 5 minutes.The absorbance of the mixture at 546nm with the spectrophotometer. Ascorbic acid was used as a standard[10] .The percentage inhibition was calculated by using the following formula

$$\% \text{ inhibition} = \frac{\text{Absorbance of control-Absorbance of the test}}{\text{Absorbance of control}} \times 100$$

Effects of various extracts of *Tinospora cordifolia* on Nitric oxide scavenging activity

Concenteration (µg/ml)	% of activity (±SEM)		
	Sample(Alcoholic extract) (µg/ml)	Sample(Aqueous extract) (µg/ml)	Standard (Ascorbic acid) (µg/ml)
20	26.28±0.54	21.26±0.36	31.27±0.26
40	38.16±0.65	32.16±0.51	46.31±0.57
60	58.41±1.26	42.26±0.68	63.47±1.21
80	68.72±1.24	58.72±1.07	76.28±1.46
100	75.17±1.25	66.27±1.25	87.91±1.26

All values are expressed as mean ±SEM for three determinations



Graph no:1 Effect of various extracts of *Tinospora cordifolia* by Nitric oxide Scavenging activity

Superoxide scavenging activity

Superoxide radical scavenging activity is generally based on the anion radical which is associated with PMSNADH system. The measurement of superoxide scavenging activity is based on method as described by Liu *et al*., with slight modifications. They are generated

within PMSNADH system by the oxidation of NADH and are assayed by the reduction of Nitro blue tetrazolium (NBT).PO_4 buffer(100µM,pH 7.4) containing 1 ml NBT(156µM) solution,1ml NADH(468µM) solution on and a sample solution of extract(20-100µg/ml) in methanol were mixed .

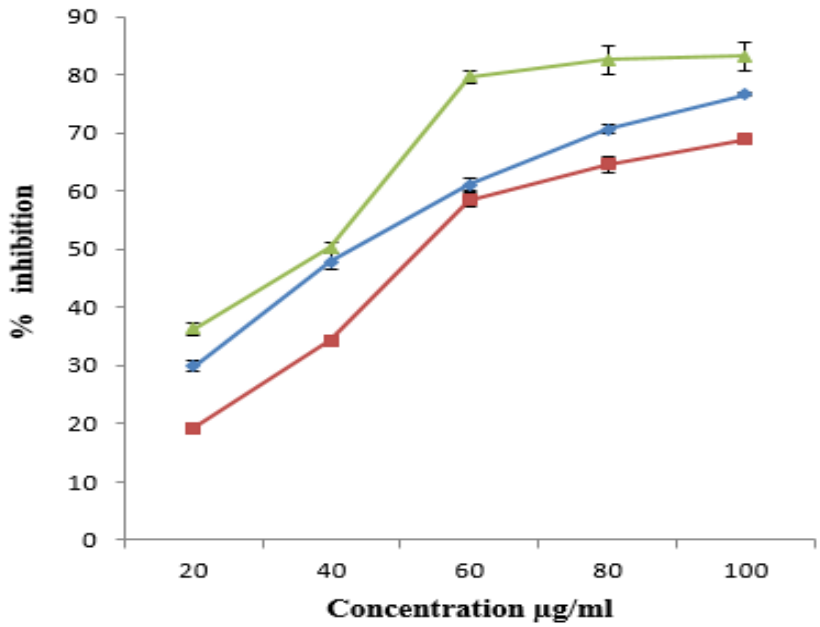
The chloroform and methanol extracts were subjected to superoxide free radical scavenging activity. The reaction was started when 0.1ml of phenazine methosulfate (PMS) solution (60µM) was added to the mixture. The reaction mixture was incubated at 25^0 C for 5 min, and the absorbance was read at 560nm against the corresponding blank samples. Ascorbic acid was used as a reference drug. Decreased absorbance of the reaction mixture indicated increased superoxide radical scavenging activity[11]. The Percentage inhibition was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control-Absorbance of the test}}{\text{Absorbance of control}} \text{X100}$$

Effects of various extracts of *Tinospora cordifolia* on Super oxide scavenging activity

Concentration (µg/ml)	**% of activity (±SEM)**		
	Sample (Alcoholic extract)(µg/ml)	**Sample (Aqeous extract)(µg/ml)**	**Standard(Ascorbic acid) (µg/ml)**
10	29.94 ± 1.03	19.26 ± 0.91	36.28 ± 1.04
20	47.90 ± 1.38	34.26 ± 0.96	50.27 ± 1.05
40	61.07 ± 1.03	58.45 ± 1.21	79.64 ± 1.03
80	70.65 ± 0.69	64.67 ± 1.38	82.63 ± 2.41
100	76.64 ± 0.34	68.86 ± 0.69	83.23 ± 2.41

All values are expressed as mean ±SEM for three determinations



Graph no:2 Effect of various extracts of *Tinospora cordifolia* by Superoxide radical scavenging activity

RESULTS AND DISCUSSION

The antioxidant activity was performed for both the alcoholic and aqueous extracts by nitric oxide scavenging activity, and superoxide free radical scavenging activity. The alcoholic extract showed maximum activity with inhibition 75.17% Nitric oxide scavenging radical and 76.64% Super oxide scavenging radical when compared to stand Ascorbic acid. The alcoholic extracts better antioxidant activity than aqueous extract for the all the two in vitro antioxidant activity screened.

CONCLUSION

Therefore the study may be concluded that, the stem portion of *Tinospora cordifolia* can be used for antioxidant and further anticancer activity studies, can be utilized therapeutically for the folklore medicinal claims or may be included as a main ingredient for anticancer herbal preparations after proper formulation.

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