



Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR

www.japtronline.com

ISSN: 2348 – 0335

IN VITRO ANTI-OXIDATION ACTIVITY STUDIES OF THE WHOLE PLANT (EXCEPT FLOWER) OF THUNBERGIA COCCINEA

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Article Information

Received: 30th December 2019

Revised: 13th September 2020

Accepted: 3rd October 2020

Keywords

Thunbergia coccinea,
 antioxidant, DPPH, TBA,
 reducing power assay

ABSTRACT

An *in vitro* study of the methanolic extract of the whole plant (except flower) of *Thunbergia coccinea* was performed to check the anti-oxidant activity by different methods (Reducing power assay, TBA and DPPH scavenging assay) and before starting the study, the plant was extracted by Soxhlet apparatus using methanol as solvent and the marc was evaporated kept under desiccator. From the literature survey it was found that the plant contain flavonoids and tannins which are non-enzymatic antioxidants responsible for scavenging free radicals as reported and based on this, the study for antioxidant was carried out. The methanolic extract of the plant *Thunbergia coccinea* shows promising result in antioxidant studies when in comparison with the standard (Ascorbic acid). Thus further studies like *in vivo* evaluation can be performed in the mere future.

INTRODUCTION

Herbal medicines are having distinctive feature and characteristics which will continue to provide or generate new lead and active compounds or drugs in the field of medicines for treating different types of disease; it is different from synthetic medicine as they may contain more than one active compound and the main active compound is mainly unknown until further studies like isolation and characterisation are performed. About

50% of western drugs or lead molecule models are obtained from the plant materials [1].

Oxidation is a chemical reaction process that occurs in our body which is considered to be a natural and normal process that is necessary for our body but sometimes there are an imbalance in between the free radicals and antioxidation process. The free radicals are those radical which are produced due to oxidation; if the level of these free radicals are too high in the body and the

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antioxidant production is less, then this can lead to damage of vital molecules like DNA and proteins (this is called oxidative stress) which leads to diseases like cancer, diabetes and heart disease [2]. Oxidative stress is caused by some of the risk factors like pollution, obesity, smoking, drinking alcohol and exposure to certain chemicals and radiations. In the other hand antioxidants are the molecules that neutralised free radicals and prevent from further oxidation or damage in the body cells and tissues. Vitamin A, C and E which are found in fruits, vegetables and tea are main sources of free radical scavenging antioxidants (exogenous antioxidants) and antioxidants can also be found within the body itself (endogenous antioxidants) as the body cells can naturally produce antioxidants or “free-radical scavengers” like alpha lipoic acid, catalase, coenzyme Q10, superoxide dismutase and glutathione [3]. The endogenous antioxidants are more powerful than exogenous antioxidants, but as we grow older our body cannot produce enough endogenous antioxidants that can stop the chain reaction of the free radicals. It is due to this reason that antioxidant supplementation is important [4].

Thunbergia is a genus of flowering plant belonging to Acanthaceae family which is native and widely found in the tropical regions of Africa, Madagascar, Australia and South Asia. This species is found to be annual, perennial and shrub also many twinning climbers, as well as some shrubby types in this variable genus are seen [5]. Genus *Thunbergia* is having more than 100 species and *Thunbergia coccinea* (*T. coccinea*) is one of the species where our study is based on. *T. coccinea* is a climbing plant having a bright red flowers found in cluster. From the literature it has been found that the plant is having potential analgesic, antipyretic and anti-inflammatory agent [6] as well as properties. *T. coccinea* contains flavonoids and tannins which are non-enzymatic antioxidants that are able to scavenged free radicals and hence the study was carried out.

MATERIAL AND METHOD

Plant material and authentication

Whole plant of *T. coccinea* (except flower) was collected during the month of September-November from Mawlai Mawroh area, Shillong of East Khasi Hills District, Meghalaya. The plant species [BSI/ERC/Tech/2018/479] was identified and authenticated by the Botanical Survey of India (BSI), Shillong, Meghalaya as *Thunbergia coccinea* Wall belonging to Acanthaceae family.

Plant extraction [7]

Whole plant of *T. coccinea* (except flower) was rinsed and wash with running tap water and was left for shade drying till it becomes dry and was tray dried at a temperature not exceeding 40°C. After drying the crude drug was then ground into homogenous powder using a mechanical grinder, and then the powder was passed through 60 mesh sieve and stored at room temperature until extraction. About 80gm of powdered drug was loaded in the Soxhlet apparatus and successively extracted with 250ml of methanol taking 10% of fined powder. The marc obtained was filtered, concentrated under reduced pressure, fully dried and was finally stored in a desiccator till further study (extract marked as MTC).

In vitro antioxidant studies

➤ Reducing Power Assay Method (RP): [8]

1 ml of methanolic extract of the plant (MTC) (concentration of 100-400 µg/ml), standard solution and control sample (1 ml distilled water instead of sample solution) along with phosphate buffer solution (pH 6.6) of 2.5 ml and potassium ferricyanide (1%) of 2.5 ml was added. For 20 min the mixture was incubated at 50°C, after incubation the reaction mixture was rapidly cooled and 2.5 ml of trichloroacetic acid (10%) was mixed. For 10mins the mixture was centrifuged (3000 rpm). The supernatant from the mixture was taken about 2.5 ml, and distilled water of 2.5 ml and 0.5 ml of ferric chloride (0.1%) was added to it, then followed by mixing and was allowed to stand for 10 min. The absorbance was measured at 700nm.

➤ Thiobarbituric acid method (TBA): [9]

The test was performed according to the method of Kikuzaki and Nakatani (1993). The same samples prepared for FTC method were used. To the sample solution of 2ml, aqueous tri-chloro acetic acid (TCA) (20%) of 1ml and aqueous thiobarbituric acid (TBA) of 2ml solution were added and the final sample concentration was 0.02% w/v. Then the mixture was placed in a boiling water bath for 10 min. It was then cooled and centrifuged at 3000 rpm for 20 min. The supernatant was taken and the absorbance was measured at 532 nm. Based on the absorbance of the last day of the FTC assay was recorded for the antioxidant activity. Both FTC and TBA described antioxidant activity by percent inhibition.

$$\%Inhibition = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

➤ DPPH Assay: [10]

Total free radical scavenging capacity of the extract from different plant sample were estimated according to the previously reported method with slight modification using the stable DPPH radical, which has an absorption maximum at 515 nm. 2.4 mg DPPH in 100ml methanol was dissolved to make a solution of the radical. 3.995 ml of methanol DPPH was added to a test solution. For 30 min the mixture was shaken vigorously and kept at room temperature in the dark. Absorbance of the reaction mixture was measured at 517nm spectrophotometrically also absorbance of the blank was also measured (without antioxidant). The activity was performed in triplicate. Scavenging capability of DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenged (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

RESULTS AND DISCUSSION

Three different methods based on scavenging activity and reducing capacity of the plant extract was studied in this work. The ability to reduce the lipid peroxidation process by the compound or extract indicates that the compound that is present in the extract is an electron donor [11]. The result of the MTC extract shows the reducing ability with increase in the concentrations and this was also compared to the standard

(Ascorbic acid) and the graph was represented in Fig1 and reported in the Table1.

The inhibition of the production of carbonyl compounds which is degraded from the peroxides at a much later stage was evaluated by TBA method. From the study we came to know that the MTC extract caused a decrease in the carbonyl productions. Here also the scavenging activity of MTC and ascorbic acid at different concentrations were given in the Table2 and the IC50 values on TBA methods were found to be 88.959µg/ml and 54.766µg/ml for MTC and ascorbic acid respectively (Table3) and represented in Fig 2.

DPPH is known to have three aromatic rings which make the molecule more stable [12]. DPPH is considered as an appropriate method to evaluate the potential of the plant extract to scavenge the free radicals at a maximum absorbance of 517nm; so any substance that can scavenge DPPH will decrease the absorbance at this particular wavelength [12]. In this assay, the scavenging effect of MTC and Ascorbic acid at different concentrations were given in the Table2 and the IC50 values on DPPH radical were found to be 53.002µg/ml and 82.037µg/ml for MTC and Ascorbic acid respectively (Table3) and represented in Fig3. This shows that potent activity of the MTC to scavenge the DPPH radical.

Table1: Reducing power assay (RP) of MTC and Ascorbic acid. The values were expressed in terms of mean ± SEM (n=3).

Sample	Activity	0.2mg/ml	0.4mg/ml	0.6mg/ml	0.8mg/ml	1.0mg/ml
MTC	RP	0.545±0.12	0.678±0.13	0.711±0.15	0.798±0.08	0.941±0.1
Ascorbic acid	RP	1.012±0.13	1.324±0.21	1.388±0.1	1.573±0.31	1.802±0.02

Table2: TBA and DPPH radical scavenging activity of the MTC and Ascorbic acid. The values were expressed in terms of mean ± SEM (n=3).

Sample	Activity	20µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
MTC	TBA	11.3±0.02	31.41±0.09	38.33±0.01	43.27±0.06	52.95±0.02
	DPPH	20.54±0.03	31.47±0.03	38.51±0.10	40.08±0.03	43.11±0.05
Ascorbic acid	TBA	32.04±0.01	45.35±0.04	59.91±0.03	67.98±0.04	74.51±0.03
	DPPH	29.51±0.02	48.26±0.12	61.84±0.08	70.88±0.01	76.18±0.07

Table3: IC50 value of TBA and DPPH scavenging activity of MTC and Ascorbic acid

Activity	Sample	IC50
TBA	MTC	88.959
	Ascorbic acid	54.766
DPPH	MTC	82.037
	Ascorbic acid	53.002

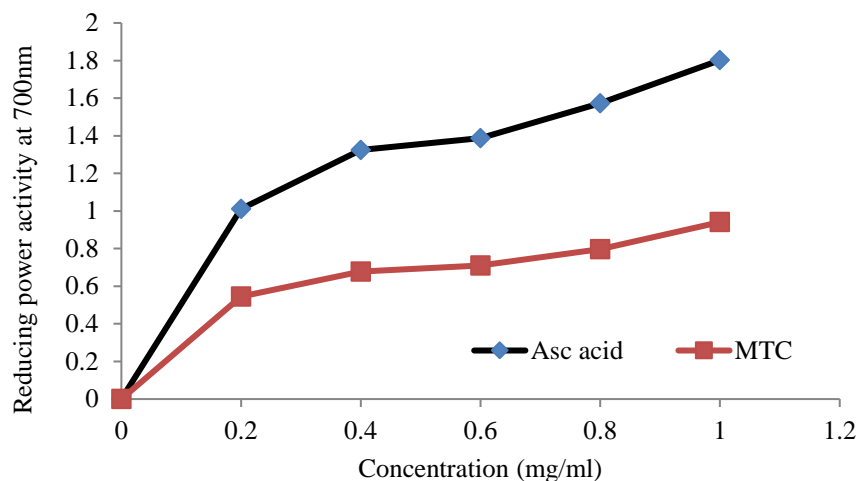


Fig1: Reducing power activity of MTC and Ascorbic acid. MTC shows the presence of compounds that have radical scavenging activity as to that of the standard.

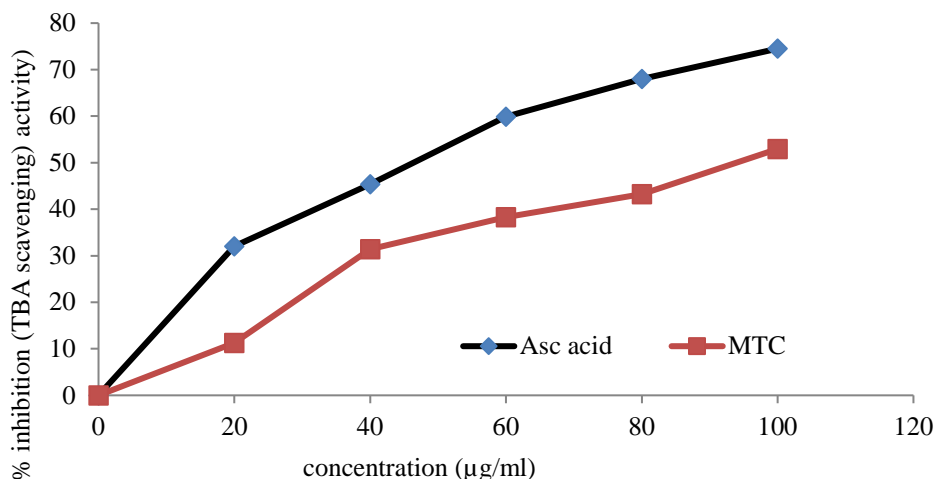


Fig2: TBA scavenging activity of MTC and Ascorbic acid. MTC shows scavenging activity as to that of the standard. IC₅₀ values on TBA methods were found to be 88.959µg/ml and 54.766µg/ml for MTC and ascorbic acid respectively.

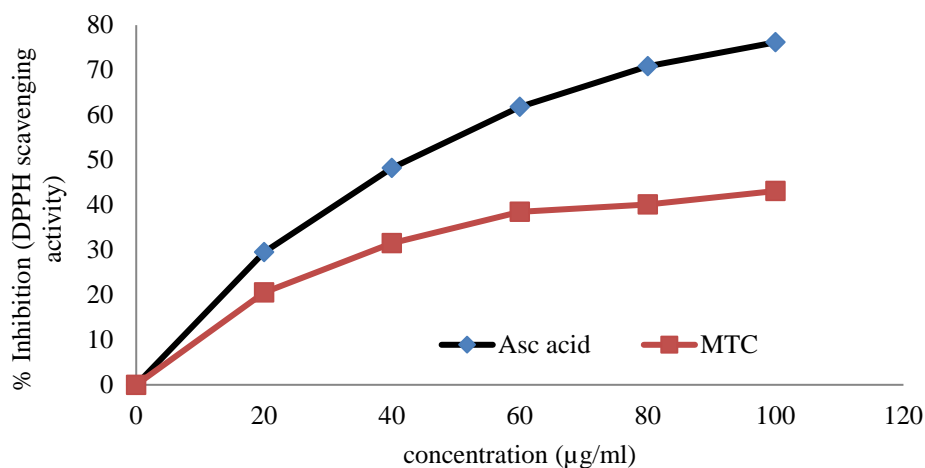


Fig3: DPPH scavenging activity of MTC and Ascorbic acid. MTC shows scavenging activity as to that of the standard. IC₅₀ values on DPPH radical were found to be 53.002µg/ml and 82.037µg/ml for MTC and Ascorbic acid respectively.

CONCLUSION

Apart from the analgesic, antipyretic, anti-diabetic and anti-inflammatory properties; the therapeutic approach on this study to show the antioxidation property had focus on the ability of the plant to scavenge DPPH and TBA and also the ability to reduce the lipid peroxidation process and the result shows that the IC50 of methanolic extract of *Thunbergia coccinea* by DPPH and TBA method is lower to that of the standard (Ascorbic acid) and also the reducing power of the methanolic extract of *Thunbergia coccinea* is lower to that of Ascorbic acid. However, research has yet to be carried out, like *in vivo* evaluation can be performed and these results provide evidence that the crude methanolic extract of *Thunbergia coccinea* is a potential source of natural antioxidants.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Damanbhalang Rynjah interpreted and contributed in drafting the manuscript and Dr. Sumit Das has contributed by performing the laboratory work and in recording of observations. Both the author analyzed the data work, read and approved the final manuscript submitted to the journal.

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