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Anti-oxidant and Anti-inflammatory activity of *Tabebuiarosea* (Flowers)

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A B S T R A C T

The aim of this study is to investigate the anti-inflammatory and anti-oxidant activities of the sample isolated from the ethyl acetate fraction of flowers of *Tabebuiarosea*. Anti-inflammatory activity of the sample was determined by HRBC membrane stabilization and Albumin denaturation methods. Anti-oxidant activity of the sample was determined by DPPH assay and ABTS method. The results of the study suggest that the sample isolated from the ethyl acetate fraction possesses anti-oxidant and anti-inflammatory activity.

Keywords: *Tabebuiarosea*, Antioxidant activity, Anti-inflammatory activity, HRBC method, Albumin denaturation, DPPH, ABTS assay.

A R T I C L E I N F O

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1. Introduction

The term inflammation originates from Lat: 'Inflammare' meaning 'to burn'. Inflammation is a homeostatic phenomenon, which is regarded as a wholesome response to irritant. The irritant provokes one after another the mechanism that combats damage and inflammation subsides when it is no longer needed. The clinical signs that inflammation evoke are heat, redness, swelling and loss of function [1]. 'Anti-inflammatory agent' is a drug that inhibits any facet of inflammation of an experimentally induced nature or as a part of clinical syndrome

[2].Tabebuiaare native to tropical rain forests throughout Central and South America. The herbal products obtained from the barkof tabebuia trees are called “taheebo”, “lapacho”. Taheebo is reported to be an astringent, anti-inflammatory, antibacterial, antifungal, diuretic, andlaxative [3-7].Tabebuiarosea (Bertol.) DC. Commonlyknown as “Pink Trumpet Tree” can grow up to 15meter and well known for its beautiful flowers. The timber is widely used for general construction andcarpentry in many European countries. The fruits are green, long and bean pod like with a length of 20-40 cm (8-16 inch). The fruits turn dark brown when ripe and contain flat, heart-shaped seeds with tiny wings. The graceful beauty is a treat for the eyes, but the tree has medical uses as well. Tea made from the leaves and bark is known to have a fever-reducing effect [8].

2. Experimental

Collection of Flowers

Fresh flowers of Tabebuiarosea were collected from Jail Corner, Trichydistrict, Tamil Nadu, India, during the month of May and identified byDr.S.JohnBritto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. SS 001 dated: 06/11/2015). St.Joseph’sCollege(Campus),Trichy, Tamil Nadu, India.

Extraction and fractionation

Fresh flower (1kg) of Tabebuiarosea collected at Jail corner, Trichy district, Tamil Nadu, India were extracted with 90% ethanol (5x500ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80^{0}C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). Petroleum ether fraction and diethyl ether fraction did not yield any isolable material. Ethyl acetate fraction on concentration yielded a dry powder which was dissolved in DMSO to get various concentrations and were used for further study.

***In Vitro* Antioxidant Activity**

DPPH Assay Method

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. TheDPPH radical is purple in colour and upon reaction with hydrogen donor changes to yellow colour. It is adecoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol ormethanol and the decrease in absorbance was measured at 490nm [9].

Reagents:

2,2-Diphenyl 1-picryl hydrazyl solution (DPPH, 100 «M): 22mg of DPPH was accurately weighed and dissolved in 100ml of methanol. From this stock solution, 18ml was taken and diluted to 100ml using methanol to obtain 100µM DPPH solution.

Preparation of test solutions:

21 mg of the solid obtained from ethyl acetate fraction was dissolved in distilled DMSO to obtain a solution of 21mg/ml concentration. This solution was serially diluted to obtain lower concentrations.

Preparation of standard solutions:

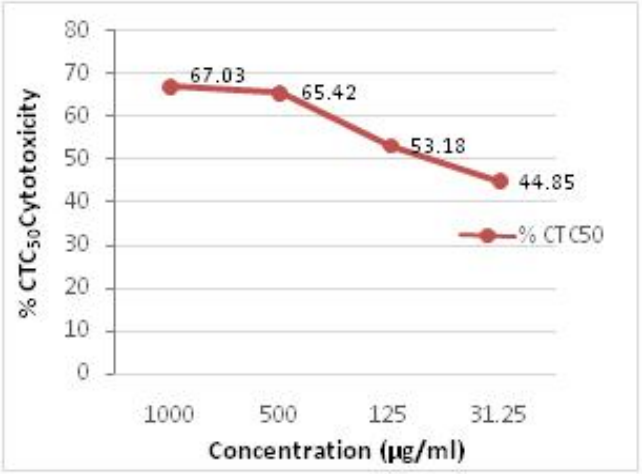
10mg each of ascorbic acid and rutin were weighed separately and dissolved in 1ml of Dimethyl sulfoxide (DMSO) to get 10mg/ml concentrations. These solutions were serially diluted with DMSO to get lower concentrations.

Procedure:

The assay was carried out in a 96 well microtitre plate. To 200µl of DPPH solution, 10µl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 1000, 500, 125 and 31.25 µg/ml. The plates were incubated at 37^o C for 30 min and the absorbance of each solution was measured at 490 nm, using a micro plate reader.

Table 1: DPPH assay activity of the compound isolated from the ethyl acetate fraction of flowers of Tabebuiarosea

S.No	Concentration (µg/ml)	% CTC_{50} Cytotoxicity (µg/ml)	CTC_{50} (µg/ml)
1	1000	67.03	104.23
2	500	65.42	
3	125	53.18	
4	31.25	44.85	



Graph 1: Graphical representation of DPPH activity of the compound isolated from the ethyl acetate fraction of flowers of Tabebuiarosea.

Evaluation of Total Antioxidant Capacity of the Extract

The total antioxidant capacity was determined by phosphor molybdenum method and is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex, which has the maximal absorption at 695nm.

Preparation of test and standard solutions

Weighed accurately 55mg of the sample and the standard, ascorbic acid and dissolved in 5ml of DMSO. The lower dilutions were made serially with DMSO.

Procedure

An aliquot of 0.1ml of the sample solution containing a reducing species in DMSO was combined in an Eppendorff tube with 1ml of reagent solution (0.6mM Sulphuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate). The tubes were capped and incubated in water bath at 95 °C for 90min. The samples were cooled to room temperature, and the absorbance of each solution was

measured at 695nm. The total antioxidant capacity was expressed as mM equivalent of ascorbic acid.

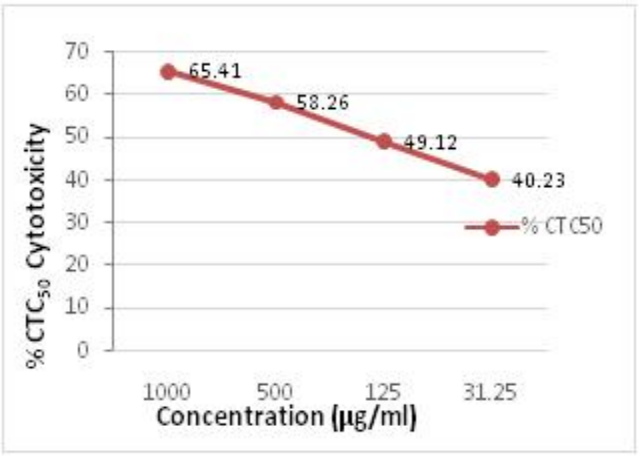
Total antioxidant activity = 343.8µg/ml

ABTS radical scavenging activity:

ABTS radical scavenging activity was performed as described by Re *et al.* (1999) with a slight modification. 7.0 mM ABTS in 14.7 mM ammonium peroxo-disulphate was prepared in 5.0 ml distilled water. The mixture was allowed to stand at room temperature for 24 hours. The resulting blue green ABTS radical solution was further diluted such that its absorbance is 0.70 ± 0.020 at 734 nm. Various concentrations of the sample solution (in ethanol) (20.0 µl) were added to 980.0 µl of ABTS radical solution and the mixture was incubated in darkness for 10 min. The decrease in absorbance was read at 734 nm. A test tube containing 20.0 µl of ethanol processed as described above was served as the control tube. Different concentrations of ascorbic acid were used as reference compound.

Table 2: ABTS assay activity of the compound isolated from the ethyl acetate fraction of flowers of Tabebuiarosea.

S.No	Concentration (µg/ml)	% CTC_{50} Cytotoxicity (µg/ml)	CTC_{50} (µg/ml)
1	1000	65.41	150.30
2	500	58.26	
3	125	49.12	
4	31.25	40.23	



Graph 2: Graphical representation of ABTS radical scavenging activity of the compound isolated from the ethyl acetate fraction of flowers ofTabebuiarosea.

Anti- Inflammatory Activity

The human red blood cell (HRBC) membrane stabilization method: The method as prescribed (Gopalkrishnan*et al.*, 2009; Saka*et al.*, 2010) was adopted with some modifications. The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10 % suspension was made. Various concentrations of test drug were prepared in mg/ml using distilled water and to each concentration, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was I ncubated at 37^0C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 Jg/ml) was used as reference standard and a control was prepared by omitting the test drug. The experiments were performed in triplicates and mean values of the three were considered. The percentage (%) of HRBC membrane stabilization or protection was calculated [10-11].

Percentage of Protection (%) =

(100- OD of drug treated sample/OD of Control) X 100

Albumin denaturation method

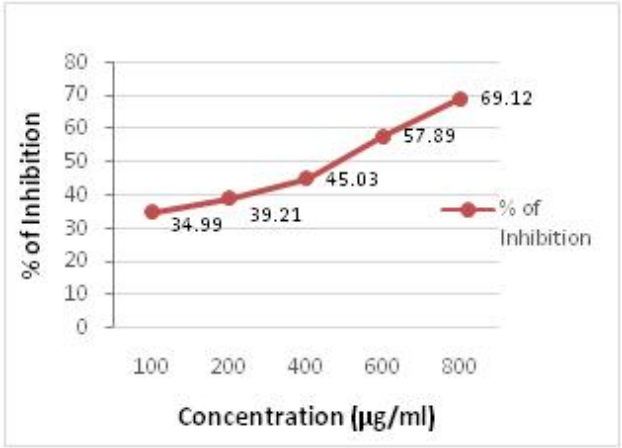
The method as prescribed (Saka*et al.*, 2010) was followed with some modifications. The reaction mixture was consisting of test sample and 1% solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of HCl. The mixtureswere incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates and the mean value of the three was considered. Percent inhibition of protein denaturation was calculated as follows [12-13].

Percentage of inhibition (%) =

(OD of Control- OD of Sample/ OD of Control) X 100

Table 3: The human red blood cell (HRBC) membrane Stabilization activity of the compound isolated from the ethyl acetate fraction of flowers of Tabebuiarosea

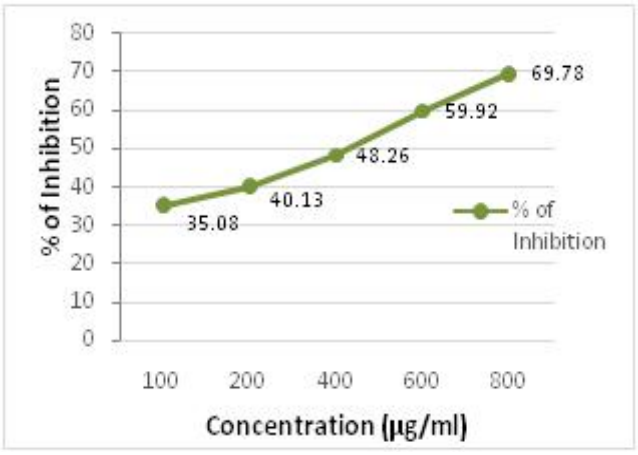
S.No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean ± S.E.M(S-I)
1	100	34.99 ± 0.17
2	200	39.21 ± 0.52
3	400	45.03 ± 1.73
4	600	57.89 ± 1.86
5	800	69.12 ± 1.34



Graph 3: Graphical representation of human red blood cell (HRBC) membrane Stabilization activity of the compound isolated from the ethyl acetate fraction of flowers of Tabebuiarosea

Table 4: The Inhibition of Albumin Denaturation activity of the compound isolated from the ethyl acetate fraction of flowers of Tabebuiarosea

S.No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean ± S.E.M(S-I)
1	100	35.08 ± 0.17
2	200	40.13 ± 0.25
3	400	48.26 ± 0.73
4	600	59.92 ± 0.39
5	800	69.78 ± 0.14



Graph 4: Graphical representation of Inhibition of Albumin Denaturation activity of the compound isolated from the ethyl acetate fraction of flowers of Tabebuiarosea

3. Results and Discussion

Anti-oxidant activity:

The compound isolated from the ethyl acetate fractions of Tabebuiarosea flowers exhibited significant anti-oxidant activity when compared with DPPH assay. It is evident from the data presented in Table-1 that the sample possessed DPPH assay activity. The result showed the percentage of cytotoxicity for 1000 µg/ml as 67.03%, 500 µg/ml as 65.42%, 125 µg/ml 53.18%, and for 31.25 µg/ml 44.85%. When compared with ABTS assay activity, it is evident from the data presented in Table-2 that the sample possesses ABTS assay activity. The result showed the percentage of cytotoxicity for 1000 µg/ml as 65.41%, 500 µg/ml as 58.26%, 125 µg/ml as 49.12%, and for 31.25 µg/ml as 40.23%. Total antioxidant activity of the sample is 343.8 µg/ml.

Anti-inflammatory activity:

The compound isolated from the ethyl acetate fractions of Tabebuiarosea flowers exhibited significant anti-inflammatory activity of the human red blood cell (HBRC) membrane stabilization and the results are presented in Table-3. The result showed the percentage of inhibition in membrane stabilization for 100 µg/ml as 34.99 ± 0.17 %, 200 µg/ml as 39.21 ± 0.52 %, 400 µg/ml as 45.03 ± 1.73 % , 600 µg/ml as 57.89 ± 1.86 % , and for 600 µg/ml as 69.12 ± 1.34 %. The inhibitions of Albumin denaturation activity exhibited by the compound are given in Table-4. The results showed the percentage of inhibition in membrane stabilization for 100 µg/ml as 35.08 ± 0.17%, 200 µg/ml as 40.13 ± 0.25 % , 300 µg/ml as 48.26 ± 0.73 % , 600 µg/ml as 59.92 ± 0.39 %, and for 800 µg/ml as 69.78 ± 0.14. The anti-inflammatory effect of the compound isolated from ethyl acetate fraction (test sample) of Tabebuiarosea may be due to presence of active constituent flavonoids. The results strongly suggest anti-inflammatory effects and anti-oxidant effects by percentage of inhibitions, which are explained in the Table-1,2,3,4.

4. Conclusion

The present study has confirmed that both DPPH assay and ABTS have showed a strong antioxidant activity and also the human red blood cell (HRBC) membrane stabilization. It could be concluded that the compound isolated from the ethyl acetate fraction of flowers of Tabebuiarosea of phyto-pharmaceutical importance. However, isolation of individual phytochemical constituents and subjecting it to biological testing will definitely give fruitful results.

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