Original Research Article

Anti-oxidant and Anti-inflammatory activity of *Moringa oleifera* (Flowers)

ABSTRACT

Objectives: The aim of the present study was isolate the new compound from ethyl acetate extract of *Moringa oleifera flowers* and identify the anti oxidant and anti inflammatory activities.

Methods: Flavonoids, Biflavonoids are the major constituent of ethyl acetate extract of Moringa Oleifera flowers. The extraction and fractionation was carried out from solvents of ethanol, benzene, petroleum ether, diethyl ether and ethyl acetate. The 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.

Results: The results of the study, suggest that the sample isolated from the ethyl acetate fraction possesses anti-oxidant activity and anti-inflammatory activity. However, these effects need to be confirmed using in vivo models and clinical trials for its effective utilization as therapeutic agents.

Keywords: *Moringa oleifera*, Antioxidant activity, Anti-inflammatory activity, HRBC method, ABTS assay.

1. INTRODUCTION

The phytochemicals are extracted from all parts of the plant body, but the concentration of these components varies from part to part. Normally, parts known to contain the highest concentration of the principles are preferred to therapeutic purposes and it can either be the leaves, stems, barks, roots, bulks, rhizomes, woods, flowers, fruits or the seeds. The literature review revealed the antimicrobial, analgesic, antifertility and antibacterial, anti-inflammatory, anti-oxidant, purgative and hepatic protective activities of the plant body [1]. As oxidative stress plays a central role in liver pathologies and their progression, the use of antioxidants has been

proposed as therapeutic agents, as well as drug coadjuvants, to counteract liver damage [2]. Pain and inflammatory responses in the peripheral and central nervous systems take part in key roles in the growth and persistence of many pathological pain states [3]. A variety of natural compounds are able to alleviate pain targeting inflammation by reducing the synthesis of inflammatory mediators, or modulating inflammatory and nociceptive pathways [4].

Moringa oleifera Lam., a member of the Moringaceae family also known as Drumstick or Horseradish-tree, is home-grown to the sub- Himalayan regions of India, Pakistan, Bangladesh and Afghanistan. Due to the importance uses of *M. oleifera* in traditional medicine, many investigations have previously reported on pharmacological properties such as antifertility, anti-inflammatory, antispasmodic, and diuretic activities [5-7].

2. MATERIALS AND METHODS

2.1 Collection of Flowers

Fresh flowers of *Moringa oleifera* were collected from Karaikudi, Sivagangai (Dt), Tamil Nadu, India, during the month of April and identified by Dr.S.John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. AR003 dated: 05/04/2017). St.Joseph's College (Campus), Tiruchirappalli, Tamil Nadu, India.

2.2 Extraction and fractionation

Fresh flowers (3 kg) of *Moringa oleifera* collected were extracted with 90% ethanol. 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 give in any isolable compounds. Ethyl acetate fraction on concentration yielded a dry powder. The dried compound was dissolved in DMSO and were used for further studies..

3. IN VITRO ANTIOXIDANT ACTIVITY

3.1 DPPH Assay Method

The DPPH free radical is reduced to a corresponding hydrazine, when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donor

changes to yellow colour. It is a decoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490nm [8].

Reagents:

A. Preparation of 2,2-Diphenyl 1-picryl hydrazyl solution (DPPH, 100µM):

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.

B. Preparation of test solutions:

21 mg of ethyl acetate fraction compound was dissolved in distilled DMSO to get a solution of 21mg/ml concentration. This solution was serially diluted to prepare lower concentrations.

C. Preparation of standard solutions:

10mg of ascorbic acid was accurately weighed and dissolved in 1ml of Dimethyl sulfoxide (DMSO) to obtain 10mg/ml concentrations. These solutions were serially diluted with DMSO to prepare lower concentrations.

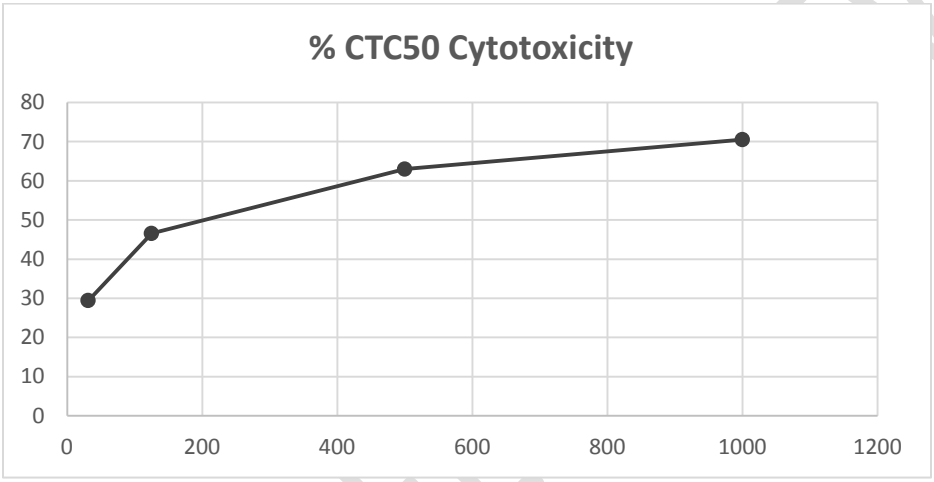
D. Procedure:

The antioxidant activity was carried out in a 96 well micro titre plate. To 200µl of DPPH solution, 10µl of each of the test sample or the standard solution was added separately in wells of the micro titre 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 37^0C for 30 minutes and the absorbance of each solution was measured at 490 nm, using a micro plate reader.

S. No	Concentration (µg/ml)	% CTC_{50} Cytotoxicity (µg/ml)	IC_{50} (µg/ml)

1	1000	70.54	369.4
2	500	63.01	
3	125	46.57	
4	31.25	29.45	

Table No. 1: Anti-oxidant activity of the compound isolated from the ethyl acetate fraction of flowers of Moringa oleifera by DPPH assay



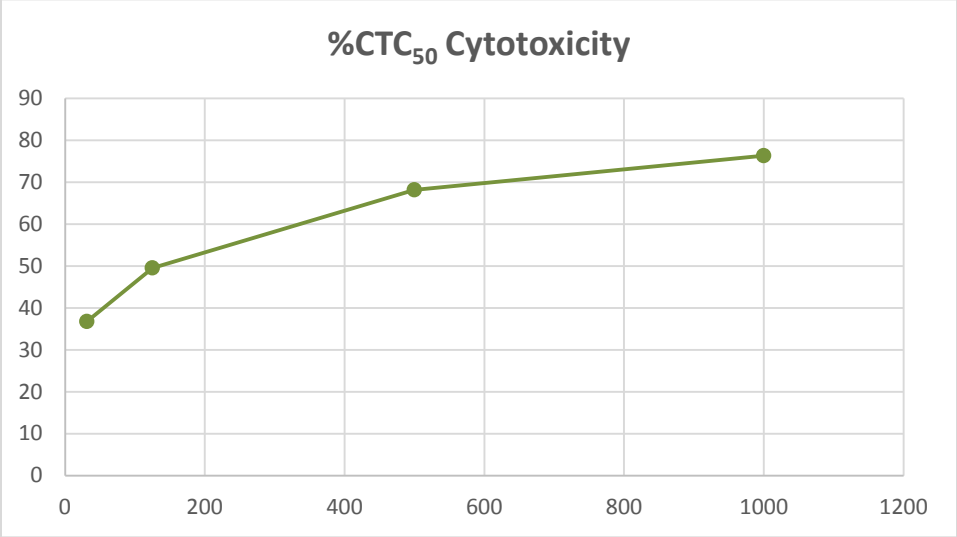
Graph No.1: Graphical representation of anti-oxidant activity of the compound isolated from the ethyl acetate fraction of flowers of Moringa oleifera by DPPH assay

3.2 ABTS radical scavenging activity:

ABTS radical scavenging activity was performed as described by Re *et al.* (1999) with a little 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 minutes. 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.

S. No	Concentration (µg/ml)	% CTC_{50} Cytotoxicity(µg/ml)	IC_{50} (µg/ml)
1	1000	76.34	211.9
2	500	68.19	
3	125	49.56	
4	31.25	36.78	

Table No. 2 : Anti-oxidant activity of the compound isolated from the ethyl acetate fraction of flowers of Moringa oleifera by ABTS assay.



Graph No.2: Graphical representation of radical scavenging activity of the compound isolated from the ethyl acetate fraction of flowers of Moringa oleifera by ABTS assay.

4. ANTI- INFLAMMATORY ACTIVITY

4.1 The human red blood cell (HRBC) membrane stabilization method

The anti-inflammatory studies (Gopalkrishnan *et al*., 2009; Sakat *et al*., 2010) was adopted with few modifications. The blood was collected from human volunteer who had not intake 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 prepared. 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 and incubated at 37^0C for 30 min then centrifuged at 3,000 rpm for 20 minutes. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 Jg/ml) was act as a reference standard and its 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 was calculated. [9,10]

Percentage of Protection (%) =
(100- OD of drug treated sample/OD of Control) X 100

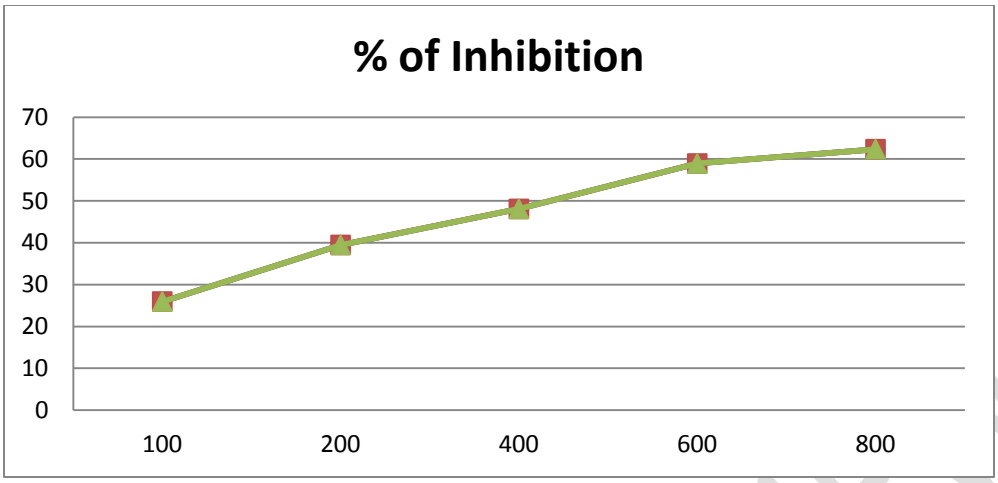
4.2 Albumin denaturation method

The method as prescribed (Sakat *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 mixtures were 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 used as a standard drug. The experiment was performed in triplicates and the mean value of the three was considered. Percentage inhibition of protein denaturation was calculated. [11,12]

Percentage of inhibition (%) =
(OD of Control- OD of Sample/ OD of Control) X 100

S. No	Concentration (µg/ml)	% of Inhibition Membrane Stabilization Mean ± S.E.M(S-I)
1	100	29.96 ± 0.41
2	200	39.48 ± 0.59
3	400	48.09 ± 0.61
4	600	58.93 ± 1.40
5	800	62.36 ± 1.86

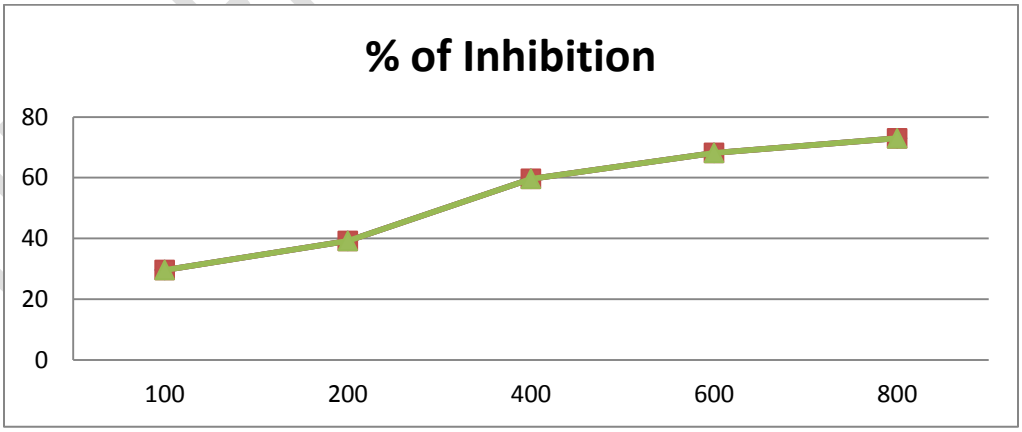
Table 3: The human red blood cell (HRBC) membrane Stabilization activity of the compound isolated from the ethyl acetate fraction of flowers of Moringa oleifera



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 Moringa oleifera

S. No	Concentration (µg/ml)	% of Inhibition Membrane Stabilization Mean ± S.E.M(S-I)
1	100	29.62 ± 0.58
2	200	39.18 ± 0.86
3	400	59.64 ± 0.94
4	600	68.17 ± 1.27
5	800	72.96 ± 1.49

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



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

5. RESULTS AND DISCUSSION

5.1 Anti-oxidant activity:

The compound isolated from the ethyl acetate fractions of *Moringa oleifera* flowers exhibited significant anti-oxidant activity when compared with DPPH assay. It is evidenced from the data presented in Table-1. The result showed the percentage of cytotoxicity for 1000 µg/ml as 70.54, for 500 µg/ml as 63.01, for 125 µg/ml 46.57, and for 31.25 µg/ml 29.45. 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 76.34, for 500 µg/ml as 68.19, for 125 µg/ml as 49.56, and for 31.25 µg/ml as 36.78.

5.2 Anti-inflammatory activity:

The compound isolated from the ethyl acetate fractions of *Moringa oleifera* flowers exhibited significant anti-inflammatory activity of the human red blood cell (HRBC) 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 29.96 ± 0.41, for 200 µg/ml as 39.48 ± 0.59, for 400 µg/ml as 48.09 ± 0.61, for 600 µg/ml as 58.93 ± 1.40, and for 800 µg/ml as 62.36 ± 1.86. The inhibition 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 29.62 ± 0.58, for 200 µg/ml 39.18 ± 0.86, for 400 µg/ml as 59.64 ± 0.94, for 600 µg/ml as 68.17 ± 1.27, and for 800 µg/ml as 72.96 ± 1.49. The anti-inflammatory effect of the compound isolated from ethyl acetate fraction (test sample) of *Moringa oleifera* may be due to presence of active constituent flavonoids. The results strongly suggest anti-inflammatory effects and anti-oxidant effects of the test sample by percentage of inhibitions, which are given in the Table 1,2,3,4.

6. CONCLUSION

In the present study, both DPPH assay and ABTS have showed a highest potential antioxidant activity and also the human red blood cell (HRBC) membrane stabilization activity of the test sample. It could be concluded that the compound isolated from the ethyl acetate fraction of flowers of *Moringa oleifera* is of phytopharmaceutical importance.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

7. REFERENCES

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