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Anti-oxidant and Anti-inflammatory Activity of Ethyl Acetate Fraction of *Moringa oleifera* Flowers

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Authors' contributions

This work was carried out in collaboration among all authors. Author AR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MMS and DP managed the analyses of the study. Author DP managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To investigate the antioxidant and anti-inflammatory activities of ethyl acetate extract of *Moringa oleifera* flowers.
Place and Duration of Study: The research work was carried out at Research laboratory, Department of chemistry, Periyar E.V.R College, Trichy-23, between April 2017 and January 2018.
Methodology: Extraction and fractionation were carried out from the solvents of ethanol, benzene, petroleum ether, diethyl ether and ethyl acetate. The anti-inflammatory effect of the extract was investigated by HRBC membrane stabilization and Albumin denaturation methods. Anti-oxidant effect of the extract was determined by DPPH assay and ABTS method.
Results: The dry sample extracted from the ethyl acetate fraction of *Moringa oleifera* flowers possess highly anti-oxidant activity showed by the DPPH assay and ABTS method and also having

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anti-inflammatory activity is determined by human red blood cell (HRBC) membrane stabilization and Albumin denaturation methods. However, these effects need to be confirmed using *in vivo* models and clinical trials before its utilization as a therapeutic agent.

Conclusion: The present study was concluded that the dry sample of ethyl acetate fraction of *Moringa oleifera* flowers possesses effective anti-oxidant and anti-inflammatory activities.

Keywords: ABTS assay; anti-inflammatory activity; antioxidant activity; HRBC method; Moringa oleifera.

1. INTRODUCTION

Peoples may be exposed to endogenous free radicals sources like Ultraviolet radiation, cigarette and vehicles smoke, atmospheric pollutants, industrial effluent etc, in our day to day life [1]. Free radicals cause several metabolic disorders in cells in the human body. Some of the oxidants like reactive oxygen species reactive nitrogen species, hydroxyl radical and hydrogen peroxide may produce cancer, cardiovascular disease, and cataract, slow down the ageing in human beings. So we protect from these diseases intake of antioxidants from the external sources [2]. Antioxidants are substances that act against oxidants [3]. The high potential antioxidants such as vitamin C, vitamin E, β- Carotene, flavonoids and polyphenols are available in natural plants [4]. As oxidative strain is a vital role in liver pathologies and their progression, the use of antioxidants in natural remedies neutralize the liver damage [5].

Inflammation is a normal body response to a traumatic injury, irritation from harmful chemicals, or infection caused by microbial pathogens. It leads to painful conditions like rheumatoid arthritis, asthma, allergy, inflammatory bowel syndrome and atherosclerosis are caused by the presence of plasma and white blood cells in the affected area [6]. Pain and inflammatory responses in the peripheral and central nervous systems play key roles in the growth and persistence of many pathological pain states [7]. A variety of natural compounds can lighten the inflammation by reducing the formation of inflammatory mediators, or modulating inflammatory [8].

Moringa oleifera tree belongs to Moringaceae family [9]. It is widely cultivated species in home gardens and sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan [10] for food and medicines by the presence of vitamins, proteins and phenolic compounds in various parts [11]. The previous researches reported all the plant parts were contains different variety of biochemical compounds with anticarcinogenic [10], anti-inflammatory [12], antidiabetic [13], antioxidant [14], and antimicrobial effects [15]. However, it is known that phytochemical concentration varies from part to part of the plant. To the best of our knowledge, there have not been any phytochemical studies focusing on the flowers of *M. oleifera* [16]. Thus this study set out to investigate the antioxidant and anti-inflammatory activities of this plant part as a possible clinical therapeutic agent.

2. MATERIALS AND METHODS

2.1 Collection of Flowers

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

2.2 Extraction and Fractionation

The 3 kg freshly collected flowers 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°C) (6x250 ml), Peroxide free diethyl ether (4x250 ml) and ethyl acetate (8x250 ml). 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 samples were dissolved in DMSO and were used for further studies.

2.3 *In vitro* Antioxidant Activity

2.3.1 DPPH assay method

The DPPH free radical is reduced to corresponding hydrazine when it reacts with

hydrogen donors. The DPPH radical is purple and upon reaction with hydrogen donor changes to yellow colour [17]. It is a decolouration 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 490 nm [18].

2.3.2 Reagents

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

Preparation of test solutions: Twenty-one milligrams of dry sample extracted from ethyl acetate was dissolved in distilled DMSO to get a solution of 21 mg/ml concentration. This solution was serially diluted to prepare lower concentrations.

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

Procedure: The antioxidant activity was carried out in a 96 well microtitre plate. The final concentration of the dry sample extracted from ethyl acetate and standard solutions used 1000 μg/mL, 500 μg/mL, 125 μg/mL and 31.25 μg/mL were prepared. To 200μL of DPPH solution(violet colour), 10μL of each of the ethyl acetate fraction compound or the standard solution was added separately in wells of the microtitre plate. The plates were incubated at room temperature for 30 minutes. The absorbance of each solution was measured at 490 nm, using a microplate reader. The antioxidants present in the test sample scavenge the DPPH free radicals and turn violet into yellow colour.

2.3.3 ABTS free radical scavenging activity

ABTS free radical scavenging activity was carried out by 7.0 mM ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid] and 14.7 mM ammonium perdisulphate were dissolved in 5.0 ml demineralised water and stand 24 hrs at room temperature in the absence of sunlight, the blue-green ABTS radical is formed in the solution. The solution was diluted then its absorbance is 0.70±0.020 at 734 nm is noted spectrophotometer [19].

The different concentrations(1000 µg/ml, 500 µg/ml, 125 µg/ml, 31.25 µg/ml) of the dry sample extracted from ethyl acetate fractions of *Moringa oleifera* flowers is prepared using ethanol as a solvent. 20.0 µL of each sample solutions were added to 980.0 µL of ABTS radical solution and it was incubated in darkness for 10 minutes. The decrease in absorbance was noted at 734 nm. The anti-oxidant activity is determined by decolourization of the ABTS radical. 20.0 µL of ethanol containing a test tube was served as the control tube. Various concentrations of ascorbic acid (antioxidant) were used as the reference compound.

2.4 Anti-inflammatory Activity

2.4.1 HRBC membrane stabilization methodology

The anti-inflammatory activity of the dry sample extracted from ethyl acetate fractions of *Moringa oleifera* flowers was studied by HRBC stabilization method. The non-steroidal anti-inflammatory drugs are used to treat the inflammation [20]. The blood sample was collected from blood donors and confirmed who had not to intake any Non-steroidal anti-inflammatory drugs for 2 weeks earlier to the experiment. The collected blood was mixed with equal quantity of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride dissolved in water) and centrifuged at 3,000 rpm, then the packed cells washed with isosaline. 10% suspension was prepared for the method [21]. Various concentrations of dry sample extracted from ethyl acetate fractions of *Moringa oleifera* flowers were prepared in mg/ml using distilled water then added 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension in each concentration and incubated at 37°C for 30 minutes. The mixture was centrifuged at 3,000 rpm for 20 minutes. The supernatant solution containing haemoglobin was predicted spectrophotometrically at 560 nm. Diclofenac (100 Jg/ml) was performed as a standard. The experiments were carried out in three sets and their mean values were used to calculate the percentage (%) of HRBC membrane stabilization [22].

Percentage of Protection = (100-Optical density of drug-treated sample/Optical density of Control) X 100

2.4.2 Albumin denaturation methodology

The reaction mixture was containing dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flowers and 1% solution of bovine albumin fraction. A little amount of hydrochloric acid was added to the reaction mixture for adjusting the pH range [23]. The mixture was incubated at 37°C for twenty minutes. After the incubation process, the mixture was heated to 51°C for twenty minutes and cooled. The turbidity of the samples was measured spectrophotometrically at 660 nm. Diclofenac sodium was performed as a standard drug. The experiments were carried out in three sets and their mean values were used to calculate the inhibition percentage of protein denaturation was calculated [24,25,17].

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

3. RESULTS AND DISCUSSION

3.1 Anti-oxidant Activity: DPPH Assay

The antioxidant activity of the dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flowers exhibited using 2,2-Diphenyl 1-picryl hydrazyl radical assay. In the present study, the free radical scavenging potentials of the extracts at different concentrations were tested. This method determined the scavenging activity of DPPH radical. The activity of the sample is evidenced by the data presented in Table 1. The total antioxidant activity was increased with increasing concentrations of the dry sample extracted from ethyl acetate fraction is represented in Fig. 1. This result showed the IC_{50} value (369.4 µg/ml) of the compound.

3.2 Anti-oxidant Activity: ABTS Assay

ABTS radical assay also great technique for the investigation of antioxidant activity of antioxidants. The dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flower possesses effective antioxidant activity. It is evident from the data presented in Table 2. The result showed the percentage of cytotoxicity for the various concentration of dry sample isolated from the ethyl acetate fraction of flowers of Moringa oleifera. The total antioxidant activity was gradually increased with abnormal increasing the concentrations of the dry sample extracted from ethyl acetate fraction is represented in Fig. 2. The IC50 value of the assay was 211.9 µg/ml.

Table 1. Anti-oxidant activity of the dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flowers by DPPH assay

S. no	Concentration (µg/ml)	% 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	

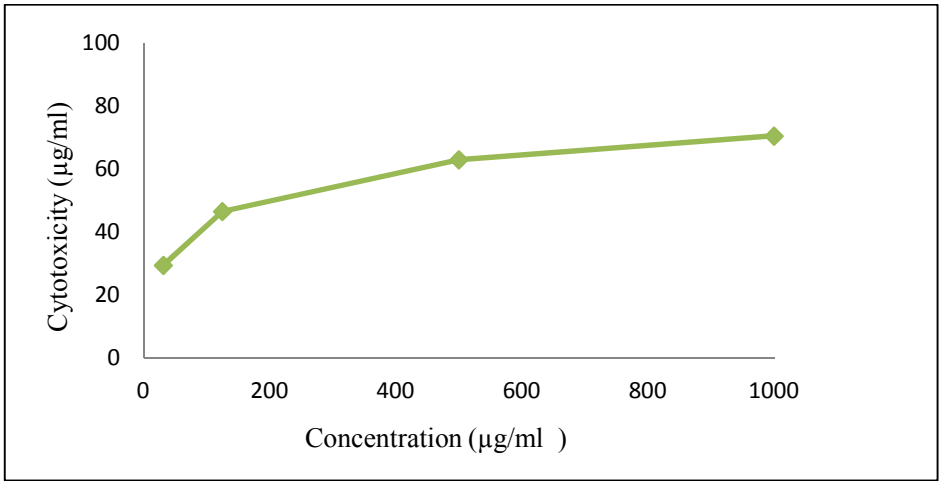

Fig. 1. Anti-oxidant activity of the dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flowers by DPPH assay

Table 2. Anti-oxidant activity of the dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flowers by ABTS assay

S. no	Concentration (µg/ml)	% 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	

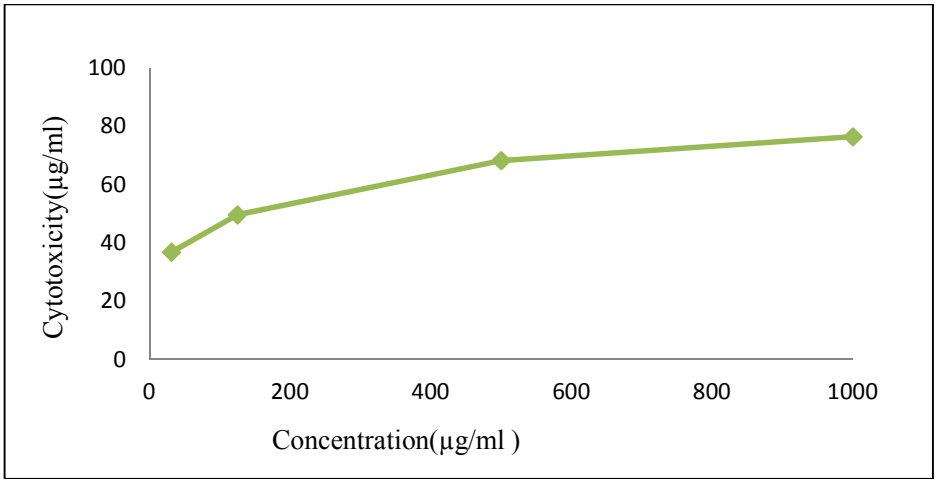

Fig. 2. ABTS radical scavenging activity of the dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flowers

Table 3. The human red blood cell membrane stabilization activity of the dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flowers

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

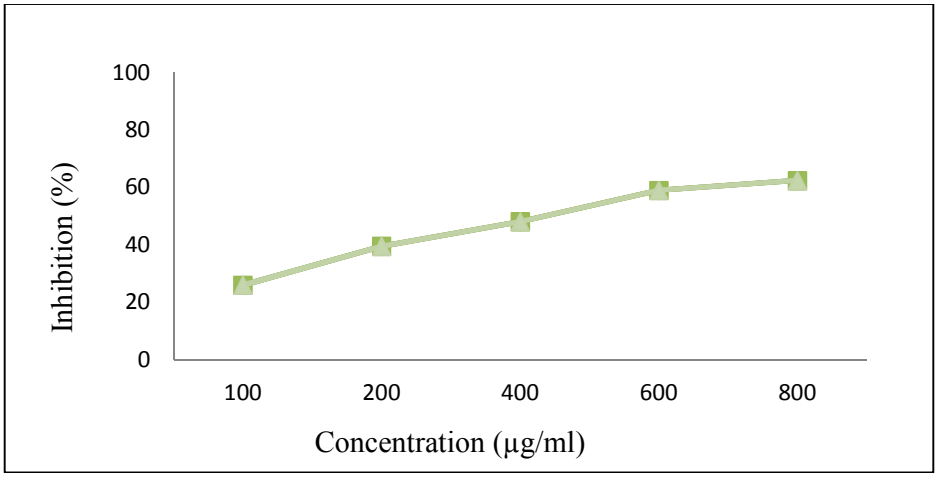

Fig. 3. The human red blood cell membrane stabilization activity of the dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flowers

Table 4. The inhibition of albumin denaturation activity of the dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flowers

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

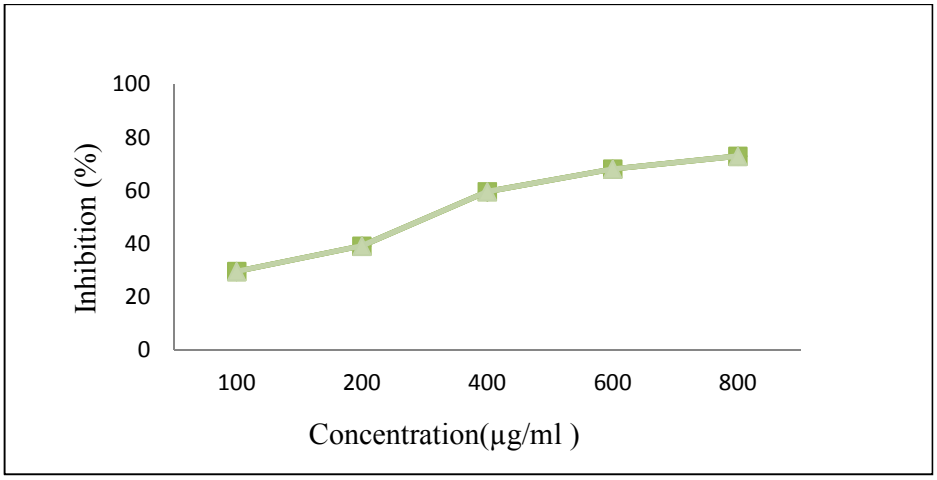

Fig. 4. The Inhibition of albumin denaturation activity of the dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flowers

3.3 Anti-inflammatory Activity of Human Red Blood Cell (HRBC) Membrane Stabilization Method

The dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flowers exhibited the significant anti-inflammatory activity of the human red blood cell (HRBC) membrane stabilization and the results are presented in Table 3. The results showed the percentage of inhibition in membrane stabilization gradually increased with the concentration of the dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flowers. The higher concentration of 800 µg/ml showed significant stabilization (62.36 ± 1.86) towards human red blood cell membrane was represented in Fig. 3.

3.4 Anti-inflammatory Activity of Albumin Denaturation Method

Inflammation is caused by denaturation of proteins. The anti-inflammatory activity was investigated by albumin denaturation method. The inhibition of albumin denaturation activity exhibited by the various concentration of the dry sample extracted from ethyl acetate fraction of *Moringa oleifera* flowers is given in Table 4. The results showed the effective inhibition in albumin denaturation. the inhibition activity was gradually increased with increasing the concentration of the test sample is represented in Fig. 4.

4. CONCLUSION

In the present study, both DPPH assay and ABTS have shown the 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 dry sample extracted from ethyl acetate fraction of flowers of *Moringa oleifera* is of phytopharmaceutical importance. The chemical compound present in the dry sample extracted from ethyl acetate fraction is ongoing in our research laboratory.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is 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 the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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