

Estimation of in Vitro Antioxidant and Anti-Inflammatory Activity of Some Substituted Dihydropyrimidinones, Flowers of *Moringa Oleifera* & Seed Extraxct of *Zea Mays*

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Abstract: *Objective:* To evaluate and compare the antioxidant potential and anti-inflammatory activity of ethanolic extract of flowers of *Moringa oleifera* grown in Oman & The seed of *Zea Mays* commonly known as sweet corn. It is widely used as an ethnomedicine in India. *Method:* Flowers of *M. oleifera* were collected in the month of December and identified by a botanist. Alcoholic extract of the dry pulverized flowers of *M. oleifera* were obtained by cold maceration method. The ethanolic flowerextract was subjected to preliminary phytochemical screening as the reported methods. DPPH was used to determines in vitro antioxidant activity and It is prescribed for a wide variety . Preliminary phytochemical studies revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoides, steroids, and glycosides. The anti-inflammatory activity of *Zea Mays* ethanolic extrct was done by inhibition of protein denaturation. *Result:* Phytochemical analysis of extract showed presence of major classes of phytochemicals such as tannins, alkaloids, flavonoids, cardiac glycosides etc. *M. oleifera* flowers were found to contain 19.31 mg/g of gallic acid equivalent of total phenolics in dry extract but exhibited moderate antioxi-deant activity. The ethanolic extract of *Zea mays* was subjected to inviteo inhibition of protein denaturation in various concentration i.e. 100, 200, 400, 800, 1000 ug/ml. *Conclusions:* The result of our study suggest that flowers of *M. oleifera* possess potent anti-inflammatory activity and also good source of natural antioxidants. It is concluded that these studies of *Zea mays* seed is an good anti-inflammatory agent.

Keywords: Antioxidant, Anti-inflammatory activity DPPH, Total phenolic content , inhibition of protein denaturation

1. Introduction

Medicinal plants have been known for millennia and are considered as rich source of pharmaceutical agents for the prevention and treatment of diseases and ailments. According to WHO, More than 80% of the population within developing countries uses herbal and other traditional medicines to treat their common ailments^[1]Nature has bestowed Oman with an enormous wealth of medicinal plants which are widely used in traditional systems of medicine^[2].

Moringa oleifera Lam. Commaonly known as hourse-radish or drumstick tree in English, belongs to family Moringaceae. It is a small sized tree, which is native to South Asia, Africa and Arabia and used as traditional medicine in many tropical and subtropical countries^[3]. It is a dediduous tree growing rapidly even in poor soils, well adapted to droughts and able to reach up to 15 m in height. It is one of the 14 species of genus *Moringa*, which is native to India, Africa, Arabia, Southeast Asia, the Pacific and Caribbean islands, and South America^[4] The Flowers and the fruits appear twice each year, and seeds or cuttings are used to propagate the tree. Almost all the parts of *M. oleifera* are used for various ailments in the indigenous medicine of South Asia, including the treatment of diabetes, hypertension, inflammation and infectious diseases^[5]. Its leaves, ponds and flowers are generally consumed for nourishment.

The leaf extracts of *M. oleifera* have been reported to exhibit antioxidant activity due to abundant phenolic acids and flavonoids^[6]. The leaves as well as flowers, roots, gums and fruits are extensively used for treating inflammation^[7].

Flowes of *M. oleifera* are rich in calcium, potassium and antioxidants and are used in human diet, mainly in the Philippines^[8].

Inflammation is the means by which the body deals with insult and injury.Result may be caused: mechanically (eg. By pressure or foreign bodies) chemically (eg. By toxins, acidity, alkalinity) physically (eg. By temperature, by internal processes (eg.Uremia) and Fully understood communication between cellular and humoral elements^[9]. Inflammation rids the body of the foreign matter and disposes of damaged cells, and initiates wound healing inflammation is controlled by mast cells that are in close proximity to autonomic nerves. Mast cells are a constituents of connective tissues containing large granules that contain heparin, serotonin, bradykinin, and histamine. These substances are released from the mast cell in response to injury and infection, and by their degranulation, they control most of the processes of inflammation^[10]. Various molecule have been isolated from the plants which have been proven very effective in such condition. Drugs which are in use presently for the management if pain and inflammatory conditions are either narcotics.Eg. Opioids or Nannarcotics, salicylates and corticosteroids, hydrocortisone.

All of these drugs present well known side and toxic effects. It is well documented that these non steroidal anti-inflammatory drugs produce intestinal tract ulcers, and erosions of the stomach lining and intestinal tract in 30-50 percent of cases^[11].

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2. Materials and Method

Antioxidant

1) Plant material

Flowers of *M. oleifera* were collected from Muscat, Oman in the month of November and December 2012. The material was identified and authenticated by a botanist of Department of Natural Science, Oman Medical college. A voucher specimen (PHAR-425-13) was deposited at the herbarium unit of the pharmacy department for future reference. The flowers were detached from the inflorescence at the joint in the pedicel, and dried under shade. The dried samples were powdered and kept in air tight containers until use.

2) Drug and chemicals

Diclofenac sodium was a kind gift from National Pharmaceutical Industries LLC, Oman. 1, 1 - Diphenyl - 2 - picrylhydrazyl (DPPH) and gallic acid were purchased from Sigma - Aldrich USA. Folin - Ciocalteu reagent was obtained from Merck, Germany. All other chemicals used in the study were of analytical grade.

3) Extraction of the plant material

The dried powdered flowers (100g) were extracted by maceration with occasional shaking. The extract was filtered and the marc was re - extracted by the same process until plant materials were exhausted. The collected filtrates were pooled and evaporated to dryness under reduced pressure to yield the dry extracts (yield w/w: 8.69%) and was stored at 4°C until used.

4) Phytochemical screening of ethanolic extracts

The Freshly prepared crude of *M. oleifera* flowers were subjected to qualitative phytochemical analysis for the presence of various classes of active chemical constituents such as tannins, saponins, glycosides, flavonoids, alkaloids, terpenes and steroids etc. using standard procedures¹².

5) Determination of total phenolic content

The total phenolic content of the *M. oleifera* flower extract was determined by using folin - Ciocalteu reagent following a slightly modified method of Ainsworth¹³. Gallic acid was used as a reference standard for plotting calibration curve. A volume of 0.5 ml of the plant extract (100 ug/ml) was mixed with 2 ml of the folin - Ciocalteu reagent (diluted 1 : 10 with de-ionized water) and were neutralized with 4 ml of sodium carbonate solution (7.5%, W/w). The reaction mixture was incubated at room temperature for 30 min intermittent shaking for colour development. The absorbance of the resulting blue colour was measured at 765 nm using double beam UV - VIS spectrophotometer (UV Analyst - CT 8200). The content of total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg / g gallic acid equivalent (GAE) of dry extract.

6) Determination of antioxidant activity by DPPH-scavenging assay

The free radical scavenging activity of the flower extract of *M. oleifera* and of standard solution (ascorbic acid) were

investigated using 1, 1 - diphenyl - 2 - picrylhydrazyl (DPPH) radical scavenging method as reported in the literature¹⁴. The assay mixture contained 2 ml of 1.0 mmol / L DPPH radical solution prepared in methanol and 1 ml of standard or extract solution of different concentrations (10-500 ug/ml). The solution was rapidly mixed and incubated in dark at 37°C for 20 min. The decrease in absorbance of each solution was measured at 720 nm using UV/Vis spectrophotometer. Ascorbic acid, a well known antioxidant was used as positive control while DPPH radical solution with 1 ml ethanol was taken as blank.

The percentage of radical scavenging (%) was calculated by the following formula:

$$\% \text{ Free radical scavenging activity} = \frac{A_c - A_s}{A_c} \times 100$$

Where, A_c = Absorbance of control at 720 nm

A_s = Absorbance of sample

The concentration of sample required to scavenge 50% of the DPPH free radical (IC_{50}) was determined from the curve of percent inhibitions plotted against the respective concentration.

Anti-Inflammatory

- 1) Collection of plant materials:** The fresh seed of *zea mays* linn were collected from total shop, Mannargudi, Thiruvarur district, Tamilnadu, India. The collected material were cleaned, shade dried and coarsely powdered. The plant material was powdered and used for further studies.
- 2) Extraction :** The dried seeds are powdered using mixer grinder, 1000 gm of the powdered seeds was packed evenly in the Soxhlet extractor and subjected to extraction with ethanol. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator. The crude extract was used for further in-vitro anti-inflammatory activity.

Phytochemical screening of Qualitative analysis

Ethanolic extract of *zea mays* seeds are subjected to preliminary screening of Phytochemical constituents. The procedures were analyzed qualitatively by the method of Sofowora, 1993; Harbone, 1973¹⁵⁻¹⁶

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

The following procedure was followed by Saleem et al. (2011)¹¹³ for evaluating the percentage of inhibition of protein denaturation:

- 1) Control solution (50 ml)**
2 ml of egg albumin, 28 ml of phosphate buffer (pH 6.4) and 20 ml distilled water.
- 2) Standard drug (50 ml)**
2 ml of egg albumin, 28 ml of phosphate buffer and various concentration of standard drug (Asprin) concentration of 100, 200, 400, 800, and 1000 ug/ml.
- 3) Test solution (50 ml)**
2 ml of egg albumin, 28 ml of phosphate buffer and various concentration of plant extract (*Zea mays*

ethanolic seed extract) concentration of 100, 200, 400, 800, and 1000 ug/ml.

All the above solution were adjusted to pH using a small amount of 1N HCl. The samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes . After cooling the absorbance of the above solutions percentage inhibition of protein denaturation was calculated using the following formula¹¹⁴⁻¹¹⁵.

$$\text{Percentage inhibition} = [Vt / Vc - 1] \times 100$$

Where, Vt = Absorbance of test sample

Vc = Absorbance of control

3. Results

Antioxidant Activity

Experimental

Table 1: Ferric Reducing Antioxidant Power Assay Standard Observation - (Ascorbic acid) (720nm)

Concentration(ug/ml)	Absorbance 1	Absorbance 2	Absorbance 3
10ug/ml	0.002	0.006	0.061
20ug/ml	0.395	0.251	0.029
30ug/ml	0.941	0.775	0.499
40ug/ml	1.451	1.350	1.036
50ug/ml	2.000	2.000	2.000

Control Absorbance - 0.812 (720nm)

Sample Observation- (720nm)

$$\text{Formula- Scavenged(\%)} = (A_{\text{cont}} - (A_{\text{test}}) / (A_{\text{cont}}) \times 100$$

Table 2: (% Scavenged of DPPH free radical)

Sample name	Concentration	Absorbance	%Scavenged
4a (H)	a) 30ug/ml	0.121	72.1 %
	b) 50ug/ml	0.305	
4b (OCH ₃)	c) 30ug/ml	0.213	68.2 %
	d) 50ug/ml	0.504	
4c (NO ₂)	a) 30ug/ml	0.068	62.9%
	50ug/ml	0.463	
4d (Br)	e) 30ug/ml	0.171	17.1%
	f) 50ug/ml	0.629	
4e (Cl)	a) 30ug/ml	0.058	21.2%
	b) 50ug/ml	0.102	

Anti-inflammatory Activity

Experimental: Study report of in vitro anti-inflammatory activity

Table 1: % Inhibition of Protein Denaturation

% Inhibition of Protein Denaturation						
Concentration µg/ml	4a (H)	4b (OCH ₃)	4c (NO ₂)	4d (Br)	4e (Cl)	Standard (Diclofenac sodium)
50 µg/ml	50 %	52 %	56 %	58 %	54 %	71 %
100 µg/ml	57 %	63 %	78 %	75 %	71 %	79 %
200 µg/ml	61 %	69 %	85 %	86%	82 %	86 %
400 µg/ml	93 %	78 %	92 %	89%	93 %	89 %
800 µg/ml	96 %	93 %	98 %	97 %	96 %	96 %

Method used: Inhibition Of Albumin Denaturation

Formula used:

$$\% \text{ Inhibition} = (\text{absorbance of control} - \text{absorbance of sample}) / \text{absorbance of control} \times 100$$

Inhibition of Albumin denaturation method

The inhibitory effect of different concentration of Zea mays on protein denaturation as shown in below table. Zea mays L. at a concentration range of 100,200,400,800,1600 ug/ml and standard 100,200,400, 800,1600 ug/ml showed significant inhibition of denaturation of egg albumin in concentration dependent manner. Both membrane stabilization activity of Zea mays used in our study .

Table 2: (In Vitro Anti-Inflammatory activity of ethanolic extract of Zea mays and Aspirin of protein Denaturation Method)

Concentration µg/ml	Zea mays ethanolic extract % inhibition	Aspirin standard
100 µg/ml	54.51 ± 0.014	112.99 ± 0.022
200 µg/ml	114.15 ± 0.042	145.21 ± 0.036
400 µg/ml	234.81 ± 0.028	198.44 ± 0.022
800 µg/ml	380.11 ± 0.036	253.63 ± 0.036
1000 µg/ml	576.40 ± 0.223	320.29 ± 0.036

Values are expressed as Mean ± Standard deviation

4. Discussion

Antioxidant Activity

Medicinal plants since ancient time are lauded for their diverse pharmacological actions which could be attributed to the presence of secondary plant metabolites such as alkaloids, flavonoids, glycosides, tannins, steroids etc. Some of these plants are important source of natural antioxidants that have been shown to reduce the risk and progression of certain acute and chronic diseases such as cancer, heart diseases and stroke by scavenging free radicals which are implicated in the pathogenesis of many diseases¹⁷⁻¹⁸. The results of preliminary phytochemical screening confirmed the presence of various classes of secondary metabolites in the *M. oleifera* flower extract including poly phenols (tannins and flavonoids). Plant polyphenols, produced either from phenylalanine or from its precursor shikmic acid, are important dietary antioxidants because they possess an ideal structural chemistry for free radical scavenging activity. Numerous in-vitro studies have conclusively shown their antioxidant potential in protecting against many diseases¹⁹. The present study indicated that flowers of *M. oleifera* are rich in polyphenols (19.31 mg/g of GAE of dry extract), but their total phenolic content is found to be lesser than the previously reported result in leaves²⁰.

DPPH free radical scavenging activity is an easy and widely used method for testing invitro antioxidant activity of natural compounds or plant extracts²¹. DPPH is a stable free radical at room temperature, purple in color. Its reduction capability to accept an electron or a hydrogen radical from antioxidants is determined by measuring decrease in its absorbance values at 517 nm . DPPH radical scavenging activity of *M. oleifera* flower extracts was compared with standard ascorbic acid in this study. Although standard antioxidant had higher scavenging activity at all tested concentrations then the extract, the extract still showed good free radical scavenging activity. The free radical scavenging property of

M. oleifera may be one of the mechanisms by which this plant is effective as a traditional medicine. The consumption of effective as a traditional medicine. The consumption of the *M. Oleifera* flowers can be beneficial in preventing oxidative stress related degenerative diseases.

Anti-inflammatory Activity

Protein denaturation is a process in which protein lose their tertiary structure and secondary structure by application of external stress or compound such as strong acid or base a concentration inorganic salt, an organic solvent or heat most biological protein lose their biological function when denaturated. Denaturation of protein is a well documented cause of inflammation. As apart of the investigation on the mechanism of the anti-inflammatory activity, ability of plant extract to inhibit protein denaturation was studied.

Similarly,²² reported that methanol extract of *Murrayakoenigii* leaves produce significant Anti inflammatory activity in dose dependent manner in inhibition of protein denaturation. Padmanaban et al. (2012)²³ reported the inhibition of albumin denaturation of alcoholic extract showed significant inhibition of albumin denaturation.

5. Conclusion

Also the result of anti inflammatory studies showed that the inhibition of albumin denaturation at the highest concentration of 1000µg/ml and membrane stabilization at the highest concentration of 1600 µg/ml confirms the seed of *Zea mays L.* are potent inhibitor of acute and chronic inflammation.

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