International Journal of
Medicinal Chemistry & Analysis
www.ijmca.com
e ISSN 2249 - 7587
Print ISSN 2249 - 7595

IN VITRO STUDIES OF ANTIOXIDANT AND ANTIINFLAMMATION ACTIVITY OF *Argemone maxicana* L. FLOWER EXTRACT

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ABSTRACT

The aim of the study was to evaluate the *invitro* antioxidant, anti-inflammation and activity of methanolic extract of *Argemone mexicana* Linn flowers. The flowers extract were screened for major phytochemical using established procedures. Antioxidant activity was evaluated by free radical scavenging activity using total antioxidant, hydrogen peroxide, reducing power assay, nitric oxide scavenging activity. The results showed- that the flower extracts was a rich source of phytochemicals. Anti-inflammatory effect of methanolic extract of *Argemone mexicana* against the denaturation of protein was studied by HRBC membrane stabilization. The prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of the anti-inflammatory activity. All the result above suggested that *Argemone mexicana* is a potential candidate plant for future exploitation in medical properties.

Keywords: *Argemone mexicana* Linn, Antioxidants, Anti-inflammatory effect.

INTRODUCTION

Inflammation is the result of concerted participation of a large number of vasoactive at different stages and there are many targets for anti-inflammatory action [1]. Currently used anti-inflammatory drugs are associated with some severe side effect. Therefore, there is a need for development of potent anti-inflammation drugs with fewer side effect are in demand necessary. Plant based medicines, health products, cosmetics etc., Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in a normal physiological and metabolic process, approximately 5% of oxygen gets univalently reduced tooxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. Allthese radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of humanbody rendering each cell to face about 10,000 oxidative hits per second [2].

Argemone mexicana Linn. (Family-Papaveraceae) used in folk medicine to alleviate several ailments especially for its antibacterial [3], antimicrobial [4], antimalarial [5], Cytotoxic activity [6] and Hepatoprotective activity [7]. The plant contains alkaloids as berberine, protopine, sarguinarine, optisine, chelerytherine etc. The seed oil contains myristic, palmitic, oleic, linoleic acids etc. The yellow juice containing small quantities of berberine, potassium nitrate was identified among the salts naturally existing in the plant. Two aliphatic compounds; mexicanol and mexicanic acid have been isolated from leaves. Three isoquinoline alkaloids have been isolated as dihydropalmitine hydroxide; berberineand protopine, from the seeds. Oil contain up to 40% free glycerides of fatty acids.

MATERIALS AND METHODS

Plant Material

The flowers of *Argemone mexicana* Linn. were collected form Big Temple, Mannargudi. Flowers were

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washed several times with distilled water to remove the traces of impurities from the flower. The flowers were dried at room temperature and coarsely powdered. the plant was identified and authenticated by Dr. S. John Britto, Department of Botany, St. Josephs's college, Tiruchirappalli [Voucher number of the specimen: BK 001]. 20g of power was extracted in 100ml of methanol for 48 hours. The solvent was removed under the vacuum at temperature below 50ºC. A semi solid extract was obtained often complete elimination of alcohol under reduced pressure. The filtrate was concentrated in a lyophilizer. Both the extract was stored in a deep freezer until use.

Total Antioxidant Capacity

To 1ml of extract of different concentrations was treated with 1ml of reagent solution (0.6mm sulphuric acid, 28mm msodium phosphate and 4mm ammonium molybdate) in eppendorf tube. Capped tubes were incubated in thermal block at 95C for 90min. After cooling to room temperature, the absorbance was measured at 695nm against blank. The activity was compared with ascorbic acid standard by using the formula [8].

Scavenging of Nitric Oxide Radical

Nitric oxide was generated from sodium nitroprusside and measured by Griss reaction. Sodium nitroprusside (5mm) in standard phosphate buffer saline solution (0.25m, PH7.4) was incubated with extract dissolved in phosphate buffer saline (0.25m PH 7.4) and the tubes were incubated at 25ºC for 5hrs. Control experiments without test compounds but with equivalent amount of buffer were conducted in indentical manner. After 5hrs, 0.5ml of solution was removed and diluted with 0.5ml of Griss reagent. The absorbance of chromophore formed during diazotization of nitrite with sulphanilamide, and its subsequent coupling with napthylentylenediamine was read at 546nm. Ascobic acid was used as standard.

Reducing Power Assay

To 1ml of extract was mixed with phosphate buffer (2.5ml, 0.2M, PH 6.6) and potassium ferricyanide (2.5ml). The mixture was incubated at 50C for 20min. A portion (2.5ml) of trichloroactic acid (10%) was added to the mixture, which was the centrifuged at 3000 rpm for 20min. the upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride (0.5ml, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. The activity was compared with ascorbic acid standard.

Hydrogen peroxide scavenging activity

1ml of sample is mixed with 3ml of phosphate buffer 1ml of H_2O_2 and incubated for 10min at 37°C. After incubation the absorbance value of the reaction mixture was recorded at 230nm. The % of inhibition was calculated. The activity was compared with ascorbic acid standard by using the formula [9].

Table 1. Total Scavenging activity of *Argemone mexicana* L.

Concentration (µg/ml)	Total antioxidant activity				Hydrogen peroxide activity			
	Methanolic Extract		Standard (Ascorbic Acid)		Methanolic Extract		Standard (Ascorbic Acid)	
	% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)
100	28.42 ± 1.05	280	37.20± 1.33	240	31.20 ± 2.35	290	28.42 ± 1.32	270
200	35.84 ± 1.15		42.11 ± 2.21		39.1 ± 1.32		42.40 ± 2.23	
300	55.25 ± 2.22		58.10 ± 2.32		51.5 ± 2.21		55.62 ± 1.17	

Values are expressed as mean ± SD.

Table 2. Reducing power assay and Scavenging activity of *Argemone mexicana* L.

Concentration (µg/ml)	Reducing power assay				Scavenging activity			
	Methanolic Extract		Standard (Ascorbic Acid)		Methanolic Extract		Standard (Ascorbic Acid)	
	% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)
100	35.20 ± 1.21	250	35.25 ± 1.2	260	22.42 ± 1.12	280	23.21 ± 2.15	270
200	45.10 ± 2.2		46.3 ± 2.31		33.57 ± 2.21		35.11 ± 3.26	
300	62.20 ± 1.31		69.02 ± 1.17		54.61 ± 1.25		56.12 ± 1.32	

Values are expressed as mean ± SD.

Table 3. Anti- inflammation activity of *Argemone mexicana* L. flower extract

Concentration (µg/ml)	Methanolic Extract	Standard Drug
100	81%	87%
200	65%	75%
300	51%	66%

Values are expressed as mean ± SD.

RESULT AND DISCUSSION

The antioxidant activity of *Argemone mexicana* flower was evaluated by total antioxidant, hydrogen peroxide, reducing power assay, nitric oxide scavenging methods. *Argemone mexicana* flower showed a dose dependent scavenging activity and free radical inhibition of total antioxidant, hydrogen peroxide, reducing power assay, nitric oxide comparable to free radical scavenging activty of ascorbic acid (Table 1-2).

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membrane rapidly. Once inside the cell, H_2O_2 can probably react with fe^{2+} and possibly Cu^{2+} to form hydroxyl radical and this may the origin of many of its toxic effect. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is the allowed to accumulate. The decomposition of H_2O_2 by flower extract of *Argemone mexicana* may result from its antioxidant and free radical scavenging activity.

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc., and involved in the regulation of various physiological processes. Excess concentration of Nitric oxide was associated with several diseases [10]. Oxygen reacts with the excess nitric oxide to generate nitric and peroxynitric anions, which act as free radicals [9]. In the present study, the extract competes with oxygen to react with nitric oxide and it inhibits the generation of the anions.

The reducing capacity of compounds may serve as a significant indicator of its potential antioxidant activity. The present study revealed that reducing power of extract was significant. The study showed some compounds in the extract were electron donors and could stable products and terminal the radical chain reaction [11]. The results obtained in the present study indicate the *Argemone mexicana* flower extract exhibits free radical scavenging activity. The over antioxidant activity of *Argemone mexicana* flower extract might be attributed to its polyphenolic content and other phytochemical constituents [12]. The findings of the present study suggested the *Argemone mexicana* Linn flowers could be a potential source of natural antioxidant that could have greater important as therapeutic agent in preventing or slowing oxidative stress related degenerative disease.

In the present investigation the lysosomal enzymes released during inflammation products a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. Since HRBC membrane are similar to lysosomal membrane components the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs (Table-3).

The *Argemone mexicana* was exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bacterial enzymes and proteases which cause further tissue inflammation and damage. The mechanism behind the action of membrane stabilization will be worked out in future studies.

ACKNOWLEDGMENT

We express our gratitude to Dr. V. Divaharan, Secretary S.T.E.T Womens College, Mannargudi, for his support to the research work.

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