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In vitro potential of plant stress hormone Methyl Jasmonate for anti arthritis, anti-inflammatory and free radical scavenging activity

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Abstract: Plant stress hormones are immerging new area of interest in the field of medicine and scientifically proved active moiety in broad spectrum of clinical applications. Current study was performed to screen the in vitro potential of Methyl Jasmonate for anti-arthritic activity using protein denaturation method, anti-inflammatory activity using human red blood cells membrane and free radical scavenging activity using DPPH method. Result advocate that percent inhibition of protein denaturation and membrane stabilization were 79% and 83% respectively in dose of 1000µg/ml. The IC_{50} value of methyl Jasmonate was found to be 270µg/ml and exhibits significant anti-oxidant property.
Keywords: Plant stress hormone, Methyl Jasmonate, anti arthritis, anti-inflammatory, free radical scavenging activity.

Introduction:

Plant stress hormones are responsible for activating the plants cellular response to diverse stress situations, including cell death. They are made in the cells of the plants that are faced with a massive scarcity of nutrients. Abscisic acid, Ethylene, Jasmonic acid and Salicylates are the most common PSHs that have shown positive health effects to humans.[1] Plant stress hormones are natural bioregulators in plant intracellular signaling and defense in response to injury or environmental stresses, such as ultraviolet radiation, osmotic shock and heat. [1.2]

Among the plant hormones, salicylic acid and its derivative aspirin are extensively studied as potential anti-cancer therapeutics and chemo preventive agents.[3] The Jasmonate family, a group of plant stress hormones consisting of Cis-jasmone, Jasmonic acid, and Methyl Jasmonate (MJ), are fatty acid-derived cyclopentanones that occur ubiquitously in the plant kingdom and regulate plant developmental processes and adaptation to environment.[4] In recent years, emerging evidence has shown the anticancer effects of naturally occurring Jasmonate and their synthetic derivatives [5, 6,7] previous studies have demonstrated that MJ exerts anti-tumor properties through down-regulating the expression of proliferating cell nuclear antigen, X-linked inhibitor of apoptosis protein, and survivin[8]

Arthritis is a painful and associated with several inflammations in joints. The exact mechanism of arthritis is still unknown several factors contribute for the inflammation and pain. The mechanism of

inflammation injury is attributed in part, to release of reactive oxygen species (ROS) from activated neutrophil and macrophages. Free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation. Cartilage degeneration also has greater impact to generate pain and inflammation. Some literatures advocated and state that arthritis is contributed due to denaturation of proteins in-vivo. In-vitro screening of Protein denaturation and membrane stabilization are considered as a significant index for Anti arthritic, anti inflammatory activity.[11, 12]

Experimental Details:

1. Screening of In vitro anti arthritic activity by Protein Denaturation Method

1. Test (MJ) solution (0.5ml): It consist of 0.05ml of test solution (MJ) of various concentrations (50-1000 µg/ml) and 0.45ml of Bovine serum albumin (5% aqueous solution)
2. Test control solution (0.5ml): It consists of 0.05ml of distilled water and 0.45ml of Bovine serum albumin (5% aqueous solution).
3. Product control (0.5ml): It consists of 0.05ml test solution (MJ) of various concentrations (50-1000µg/ml) and 0.45ml of distilled water.
4. Standard solution (0.5ml): It consists of 0.05ml of Diclofenac sodium (50-1000µg/ml) and 0.45ml of Bovine serum albumin (5%aqueous solution). PH was adjusted to 6.3 to all above solution by using 1N Hcl. All the sample solution was incubated at 37^{o}C for 20 minutes and the temperature was increased to 57 °C for 3 minutes. Allow the solution to cool for some time then add 2.5ml of Phosphate buffer to all above solution. The absorbance of the resulting solution is measured at 416 nm using UV visible spectrophotometer.[12,13] The Percentage inhibition of protein denaturation was calculated as per the given formula.

Percentage Inhibition of protein denaturation:

$$100 - (OD\ of\ Test\ Solution - OD\ of\ product\ control) \div (OD\ of\ test\ Control)$$

The control represents 100% protein denaturation. The results were compared with Diclofenac sodium (1000µg/ml) treated samples.

2. In Vitro screening of Anti inflammatory Effect on membrane stabilisation / Inhibition of membrane lysis

The principle involved here is stabilization of human red blood cell (HRBC) membrane by hypo tonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10% v/v] with 0.5 ml of MJ and standard drug diclofenac sodium of various concentrations (50, 100, 250, 500, 1000µg/ml) and control (distilled water instead of hypo saline to produce 100 % hemolysis) were incubated at 37oC for 30 min and centrifuged respectively. The haemoglobin content in the suspension was estimated using spectrophotometer at 560 nm. The percentage inhibition of membrane stabilization was calculated by using same formula given for protein denaturation. [11, 13]

3. Antioxidant Activity using DPPH Radical Scavenging Assay

DPPH scavenging activity was measured by the spectrophotometric method. A stock solution of 25 mg of DPPH (200µM) was prepared in 100 ml of ethanol, 0.05 ml of test compounds dissolved in ethanol were added at different concentrations (50,100,250,500,800,1000 µg/ml). An equal amount of ethanol was added to the control, the reaction was allowed to be completed in the dark for about 20 minutes. After 20 minutes the decrease in absorbance of test mixtures was read at 517 nm.[12, 13] The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical.

Percentage inhibition $= (Control - Test \div Control) \times 100$

Result and Discussion:

% inhibition of Protein Denaturation:

Arthritis is chronic, systemic inflammatory disorders that may affect many tissue and organs but mainly attack synovial joints. The production of auto antigen in certain arthritic disease may be due to in vivo denaturation of protein. In vitro percent inhibition of protein denaturation is one of the significant method to screen the anti arthritic activity. In-vitro anti arthritic activity of methyl Jasmonate were performed using various concentrations i.e. 50,100,250,500 and 1000µg/ml and % inhibition found were 45%, 53%, 65%, 71% and 79% represented in Table No.1

Table No:1: Effect of Methyl Jasmonate on protein denaturation and Membrane Stabilization.

Sample	Conc µg/ml	% Inhibition of protein Denaturation
Methyl Jasmonate	50	45.35
	100	53.25
	250	65.89
	500	71.05
	1000	79.19
Standard Dichlofenac Sodium	50	43.57
	100	60.44
	250	72.0
	500	78.22
	1000	87.15

Methyl Jasmonate show significant inhibition of protein denaturation in dose dependant manner.

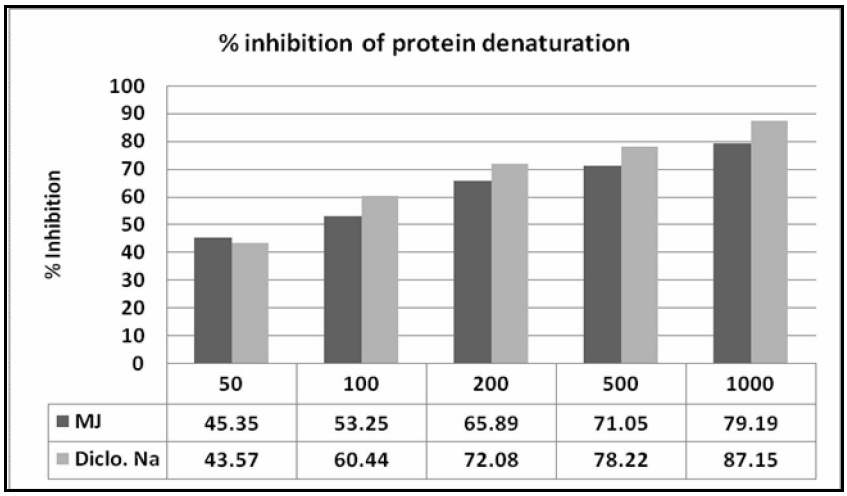


Figure No:1: Effect of Methyl Jasmonate on protein denaturation and Membrane Stabilization.

% Membrane Stabilization:

The inhibition of hypotonicity induced HRBC membrane lysis i.e, stabilisation of HRBC membrane was taken as a measure of the anti inflammatory activity. The percentage of membrane stabilisation for methyl jasmonate and Diclofenac sodium were done at 50, 100, 250, 500, 1000µg/ml. Methyl Jasmonate showed effective in inhibiting the hypotonicity induced hemolysis of HRBC at different concentrations (50-1000µg/ml) as shown in Table 2. It showed the maximum inhibition 83% at 1000µg/ml. With the increasing concentration the membrane hemolysis is decreased as shown in graph.

Table No 2 : Effect of Methyl Jasmonate on Membrane Stabilization.

Sample	Conc µg/ml	% of Membrane stabilisation
Test Sample MJ	50	50.89
	100	57.27
	250	68.11
	500	75.42
	1000	83.04
Standard Dichlofenac Sodium	50	58.10
	100	62.57
	250	67.76
	500	78.11
	1000	83.83

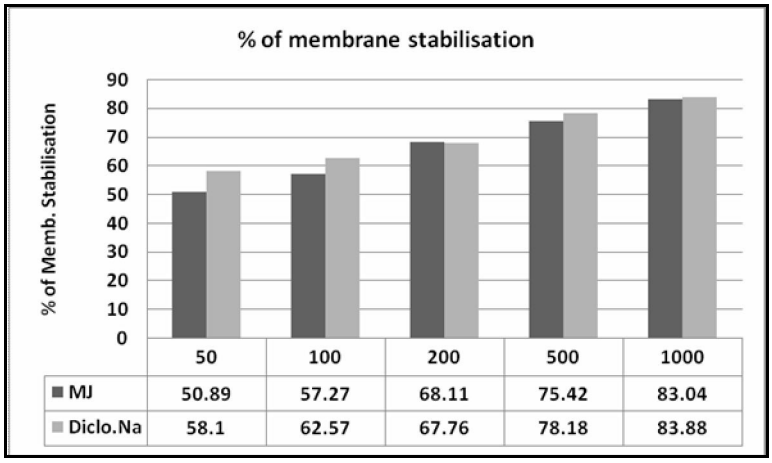
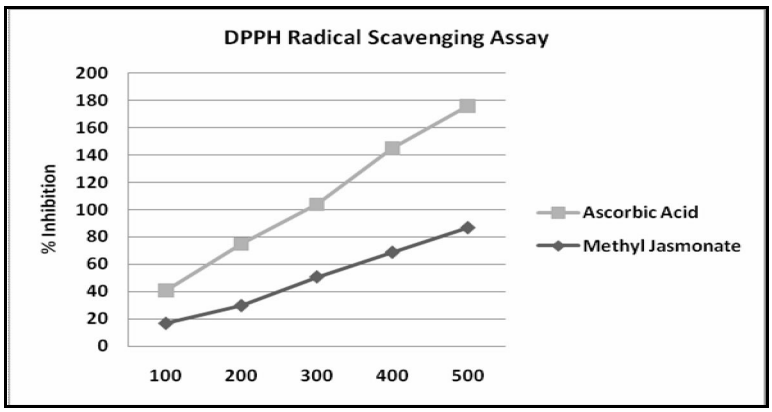


Fig No 2: Effect of Methyl Jasmonate on Membrane Stabilization.

Antioxidant Activity using DPPH Radical Scavenging Assay:

The free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation. The age related arthritis is also associated with degeneration of bone cartilage and generation of free radicals that destroy bone cartilage leads to severe inflammation and unbearable pain. Considering this basis methyl Jasmonate anti oxidant activity was performed by using DPPH method. MJ exhibits significant free radical scavenging activity and IC50 were 270µg/ml represented in Figure No.3.



IC 50of Ascorbic acid 125µg/ml
IC50 of Methyl Jasmonate 270µg/ml
Fig No.3: Free radical scavenging activity of Methyl Jasmonate

Conclusions

From present study it can be concluded that plant stress hormone methyl Jasmonate possess significant anti arthritic, anti-inflammatory and free radical scavenging activity.

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