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RESEARCH ARTICLE

IN VITRO SCREENING OF ANTI-INFLAMMATORY POTENTIAL OF *MIRABILIS JALAPA LINN FLOWERS* AND ABELMOSCHUS *ESCULENTUS LEAVES*

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ABSTRACT

Background: Flowers of *Mirabilis jalapa* were used as traditional medicine in Asia, Europe, America and Africa as purgative, diuretic and wound healing. *Abelmoschus esculentus* (Leaves) were used to treat abdominal pain, diarrhoea, genitourinary and respiratory problems.
Objectives: The present study was to evaluate the anti-inflammatory potential of 50% hydro ethanol extracts of *Mirabilis jalapa* flowers and leaves of *Abelmoschus esculentus* through various *in vitro* assays.
Methods: The anti-inflammatory activity was demonstrated by inhibiting the heat induced albumin denaturation, membrane stabilization and protein denaturation activity.
Results: The results of the study revealed that *Mirabilis jalapa* flower extract exhibited increased anti-inflammatory activity at the concentration of 0.1g/ml than the leaves of *Abelmoschus esculentus* which is comparable to that of the standard Aspirin.
Conclusions: From the results, it is concluded that *Mirabilis jalapa* flowers were a source of high-value health promoting commodity which can be administered for its anti-inflammatory activity.

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INTRODUCTION

Inflammation is more concerned about the response of body to injury, which involves a complex array of enzyme activation, mediator release, and extravasations of fluid, cell migration, tissue breakdown and repair (Perianayagam *et al*., 2006; Vane and Bolting, 1995). Inflammation is divided into acute and chronic. Acute inflammation is the primary response of the body to injury and it is involved in vascular changes i.e. vasodilatation and migration of leukocytes. Chronic inflammation is prolonged inflammation characterized by progressive destruction and injured tissue is retrieved from the inflammatory process (Sharon and Elizabeth, 2003) and (Nathan, 2002). The nitric oxide synthase release and activity is demonstrated in both acute and chronic models of inflammation (Mederos, 1995) and their release might be the cause for osteoarthritis (Steven, 2008). The process of inflammatory response is mediated by a variety of signaling molecules produced by mast cells, macrophages, granulocytes, platelets, lymphocytes and complement activation factors. The erythrocyte membrane is similar to that of lysosomal membrane (Chou, 1997) and during inflammation lysosomal enzymes are produced that might lead to variety of disorders.

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The diseases due to inflammation include hepatitis, rheumatoid arthritis, asthma and colitis that are the major causes of death (Snehal, 2015). At present, there are many synthetic drugs available in the market but element of toxicity that these drugs entail, cannot be ruled out. Their prolonged use may cause severe adverse effects on chronic administration (Yesilada *et al*., 1997**).** Therefore, screening and development of drugs for their anti-inflammatory activity is more needed and there are many efforts for finding drugs against inflammation from medicinal plants (Srinivasan *et al*., 2011). *Mirabilis jalapa* belongs to the family *Nyctaginaceae* that may include 30 genus and 400 species widely distributed in tropical and subtropical -regions of the world. The flowers usually opens at four-0' clock therefore it is commonly known as "four-0' clock plant" (Levin and Raguso, 2001). Flower possesses flavonoids and anthocyanins (Sathish kumar and Eram fathima, 2017). The presence of oxymethyl anthraquinone, trigonelline, arabinose, galactose, beta-sitosterol in leaves has been reported. The flowers possess significant anti-arthritic activity (Augustine *et al*., 2013). *Abelmoschus esculentus* Linn belongs to the family of Malvacea. *Abelmoschus esculentus* (common okra) is most widely cultivated in south and east Asia, Africa and the southern USA (Onakpa, 2013). The ethanol extracts were screened for anti-viral activity and they showed anti-HBV (Hepatitis B Virus) activity (Lin-Lin *et al*., 2007).

The consumption has been described to reduce serum cholesterol, triacylglyceride and blood cholesterol (Ijeh *et al*., 2003). In ethiopia, the parts were used to treat menstrual pain and hypertension (Andullu and vardacharyulu, 2010). The purpose of the present study is to elucidate the Anti-inflammatory activity of *Mirabilis jalapa* flowers and *Abelmoschus esculentus* leaves.

MATERIALS AND METHODS

The flowers of *Mirabilis jalapa* and leaves of *Abelmoscus esculentus* in fresh condition without any defect was collected from Coimbatore, Tamil nadu. They were shade dried and powdered using a blender.

Preparation of extract

10 grams of dried powder was added with 100ml of 50% hydroethanol with occasional stirring for 3 days. At the completion of 3 days, it was filtered with the help of Muslin cloth and it was evaporated to dryness at low temperature in a rotary vacuum evaporator (<40ºC). Further the powder was stored in a refrigerator and it was used for analysis.

Assessment of *in vitro* anti-inflammatory activity

Inhibition of albumin denaturation

The anti-inflammatory activity of *Mirabilis jalapa* flowers and *Abelmoschus esculentus* leaves was studied by the method of Mizushima and Kobayashi, (1967) and Sakat *et al.,* (2010) followed with some minor modifications.

The reaction mixture includes 1ml of *Mirabilis jalapa* flowers and *Abelmoshcus esculentus* leaves and 1% aqueous solution of bovine albumin fraction with a pH 6.5. The extracts were incubated at 37°C for 20 minutes. After cooling the samples, the turbidity were measured spectrophotometrically at 660nm. The experiment was performed in triplicates. Percentage inhibition of protein denaturation was calculated as follows:

$$\text{The percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Membrane stabilization

Preparation of Red Blood cells (RBCs) suspension

RBCs suspensions was prepared according to the method of Sakat *et al.,* (2010) and Sadique *et al.,* (1989). The blood of healthy human volunteers was collected and was centrifuged at 3000 rpm for 10min. The pellet was washed three times with same volume of normal saline. The volume of blood was measured and reconstituted as a 10% v/v suspension with normal saline.

Heat induced haemolysis

Haemolysis of RBCs was performed with minor modifications of Shinde *et al*., (1999). The reaction mixture consisted of 1 ml of plant extracts and 1 ml of 10% RBCs suspension. 1ml of saline was taken as control. Diclofenac sodium was used as a standard. All the tubes were incubated in a water bath at 56°C for 30min. The tubes were cooled and centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. The Percentage inhibition of protease was calculated as follows:

$$\text{The percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Proteinase inhibition

The experiment was performed by the modified method of Sakat *et al*., (2010). The reaction mixture 2ml containing 0.06 mg trypsin, 1ml 20 Mm Tris Hcl buffer (PH 7.4) and 1ml of different concentration of plant extracts (62.5, 125, 250, 500, 1000µg). The reaction mixture was incubated at 37ºC for 5 mins and 1ml of 0.8% (W/V) casein was added. It was incubated at 37ºC for 20 mins followed by the addition of 70% perchloric acid to arrest the reaction. The cloudy suspension was centrifuged and the absorbance of supernatant was recorded at 210 nm.

Table 1. In vitro anti-inflammatory activity of *Mirabilis jalapa* (flowers) and *Abelmoschus esculentus* (Leaves) by human red blood cell (HRBC) membrane stabilization on varying concentrations

Concentration (µg/ml)	HRBC membrane Stabilization		
	Mirabilis jalapa	*Abelmoschus esculentus*	Diclofenac sodium
62.5	32.49±0.54	15.66±1.02	36.83±0.13
125	46.23±0.72	29.32±0.43	48.02±0.52
250	60.13±0.12	38.43±0.63	61.92±0.10
500	69.03±0.33	43.99±0.45	72.06±0.56
1000	80.83±0.11	52.76±0.70	83.63±0.62

The values are expressed as Mean ± SD. (n=3)

Table 2. In vitro anti-inflammatory activity of *Mirabilis jalapa* (flowers) and *Abelmoschus esculentus* (Leaves) by albumin denaturation and proteinase inhibition on varying concentrations

Concentration (µg/ml)	Albumin denaturation			Proteinase inhibition		
	Mirabilis jalapa	*Abelmoschus esculentus*	Aspirin	*Mirabilis jalapa*	*Abelmoschus esculentus*	Aspirin
62.5	24.73±0.49	14.27±0.68	35.33±0.23	19.73±0.21	17.48±0.60	22.08±0.35
125	28.53±0.67	29.32±0.03	41.62±0.24	31.61±1.27	21.56±0.14	32.06±0.36
250	47.13±0.52	33.43±0.83	56.45±0.67	32.08±0.96	32.68±1.32	37.87±0.53
500	63.03±0.50	40.99±0.32	68.55±0.54	46.65±0.64	46.39±0.89	48.37±0.60
1000	78.90±0.38	53.40±0.07	82.61±0.44	79.88±0.86	59.37±0.24	80.70±0.18

The values are expressed as Mean ± SD. (n=3)

The percentage inhibition of protease inhibition was calculated as follows:

$$\text{The percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs of control}} \times 100$$

Statistical analysis

The results are expressed as mean ± SD for triplicates.

RESULTS

The Anti-inflammatory activity of the *Mirabilis jalapa* flowers and *Abelmoschus esculentus* leaves extracts by heat induced membrane stabilization was depicted in Table 1. Maximum inhibition of 80.83% was observed for the hydroethanolic extracts of *Mirabilis jalapa* flowers than *Abelmoschus esculentus* which showed the inhibition of 52.76%. An inhibition of 83.63% was observed for standard Diclofenac sodium at a concentration of 0.1g/ml. The inhibition of albumin denaturation was shown in Table 2. An inhibition of albumin denaturation of 82.61% was observed for standard aspirin at a concentration of 0.1g/ml. *Mirabilis jalapa* showed maximum inhibition of 78.90% when compared to *Abelmoschus esculentus* of 53.40% at a concentration of 0.1 g/ml. The results of proteinase inhibition was depicted in table 2.The results confirmed that maximum proteinase inhibition was found in *Mirabilis jalapa* of 79.88% when compared to that of *Abelmoschus esculentus* 59.37% compared to that of standard Aspirin of 80.70% at the concentration of 0.1g/ml.

DISCUSSION

From the study it is clear that the anti-inflammatory activity of hydroethanolic extracts were concentration dependent, with increasing the concentration the activity is increased. The major mechanism of non-steroidal drugs are inhibiting the lysosomal enzymes and stabilizing the lysosomal membrane (Rajendran Vadivu, 2008). Moreover, it is evident that the deformability and cell volume of RBCs are closely related to the intercellular content of the calcium stabilisation of lysosomal membrane that plays an important role in limiting the inflammatory response (Gandhidasan *et al*., 1991).The flowers of *Mirabilis jalapa* showed maximum stabilization of RBC membrane which is analogous to lysosomal membrane. It is observed that during inflammation protein gets denatured by losing their secondary and tertiary structure by the application of acid, base, organic salt, organic solvent or by heat (Megha *et al*., 2013). *Mirabilis jalapa* extract possess maximum inhibition of albumin denaturation. Neutrophils are known to be a rich source of proteinase which carries in their lysosomal granules. Previous study revealed that leukocytes proteinase play vital role in the development of tissues damage during inflammation and protection was provided by proteinase inhibitors. *Mirabilis jalapa* showed maximum inhibition of proteinase when compared to that of *Abelmoschus esculentus*. So these results clearly indicated that *Mirabilis jalapa* flowers showed significant anti-inflammatory activity than *Abelmoschus esculentus* leaves.

Conclusion

The present investigation confirmed the Anti-inflammatory activity of 50% hydroethanolic extract of *Mirabilis jalapa* flowers and *Abelmoschus esculentus* leaves and maximum activity was found with *Mirabilis jalapa*. Further works on *in vivo* anti-inflammatory activity of *Mirabilis jalapa* flower extract needs to be assessed.

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Conflict of interest

There is no conflict of interests.

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