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IN VITRO ANTI-INFLAMMATORY ACTIVITY OF *MIRABILIS JALAPA* FLOWER EXTRACTS

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Abstract:

Natural products have been a valuable source of drug regimens that form the corner stone for modern pharmaceutical care. *Mirabilis jalapa* is the beneficial medicinal plant which is traditionally used for treatment of many GIT diseases, including dysentery, diarrhoea, muscular pain and abdominal pain. Besides this it also exhibits activities like antiviral, antimicrobial, antimalarial, anthelmintic, antioxidant and many others. This study was carried out to assess the anti-inflammatory activity of aqueous and alcoholic extracts of flowers of *Mirabilis jalapa* using *in vitro* models like bovine serum albumin denaturation, egg albumin denaturation and HRBC membrane stabilization method. The extracts were evaluated at concentration range of 50µg/ml - 300µg/ml. Both the extracts exhibited dose dependent inhibition of protein denaturation. The highest percentage inhibition observed in ethanolic extract is 70.14%, 61.66% and 55.23% by BSA, egg albumin denaturation method and HRBC membrane stabilization method respectively. Similarly the maximum percentage inhibition observed in aqueous extract was 72.33%, 73% and 59.35%. The results procured by the present investigation revealed that the extract have significant activity when compared to a standard drug.

Key words: Bovine serum albumin, anti-inflammatory, *Mirabilis jalapa,* HRBC membrane stabilization.

Introduction

Inflammation is the defense mechanism caused during infection or injury to the living tissue. It initiates healing process by eliminating the injurious agents and tissue debris. Inflammation may be caused by physical agents, microbes, chemical agents and impairment in immune responses (Chippada *et al.,* 2011). It is mainly associated with redness (rubor), heat (calor), swelling (tumour) and pain (dolor). Inflammation leads to increase in permeability of blood vessels, dilation of blood vessels, loss of function and accumulation of fluid in the injured area. Bradykinin, serotonin and prostaglandins also induce inflammation. Anti-inflammatory agents also reduce pain and body temperature which is seen in inflammatory conditions. Various diseases associated with inflammation are asthma, tuberculosis, peptic ulcer, periodontics, rheumatoid arthritis, crohn's disease, atherosclerosis, sinusitis and active hepatitis (Hansson, G.K., 2005).

Mirabilis jalapa is a evergreen herb which grows up to 2-2.5 metres tall. The roots are tuberous. It is termed as a weed which grows in the crop fields. The flowers usually bloom in the late afternoon or in the evening that is between 4pm to 8pm hence it is commonly called as 4 'o' clock plant. The word mirabilis is derived from Latin meaning wonderful. They are also called as 'Marvel of Peru'. Flowers are obtained in different colours like yellow, red, pink and white. Flower gives good fragrance and it remains throughout night. *Mirabilis jalapa* plant was used as emetic and purgative (Sumitra, P., 2014). It also acts as an antiviral, antimicrobial, antimalarial, anthelmintic and antioxidant agent (Oladunmoye, 2012). It was used as antidiabetic in china for centuries. Root and leaf infusions were used in the treatment of inflammation of skin as topically. The extract was found to have *invitro* antioxidant property (Rozina, R., 2016).

The research carried out on the medicinal herbs in last few decades has revealed the use of herbal anti-inflammatory agents in the traditional medicinal system (Chattopadhyay, 2010). Non-steroidal anti-inflammatory drugs (NSAIDs) are used worldwide to treat inflammation and inflammation-related diseases. However, various side effects such as damage to the gastric mucosa, platelet dysfunction, sodium and water retention etc. are the major drawback for the prolonged use of these drugs. Recent

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studies have also indicated that these drugs when used repeatedly may cause cardiovascular diseases, hypertension and nephrotoxicity. The major research in the recent years is focussed on the discovery of new anti-inflammatory drugs having more potency and less risk factor (Shaik, R.U., 2016). The study on the natural product may act as a driving force to achieve this goal. In the present study the flowers of the plant *Mirablis jalapa* was screened for anti- inflammatory activity using *in vitro* models like BSA method, egg albumin denaturation method and human red blood cells membrane stabilization method.

Materials and Methods

Collection of plant material

The flora of *Mirabilis jalapa* were gathered from in and around Mangalore, Dakshina Kannada district, Mangalore. The flowers were cleaned, shade dried and powdered. The powdered flower material was subjected to extraction.

Preparation of extracts

The coarsely powdered flower was subjected to extraction. The extraction was carried out by two methods which include cold maceration and infusion. Two different solvents were used that is ethanol and water. Cold maceration method was carried out using ethanol as a solvent. The powder was macerated with ethanol for 7 days and filtered. The filtrate was then evaporated in the water bath. Infusion method was carried out by boiling the solvent and placing the flower powder in the solvent for specified time. The solvent used for infusion was water. The extract was filtered and evaporated. The crude extract was then stored in the desiccator for further use.

Preliminary phytochemical analysis

The preliminary phytochemical analysis was done by using simple chemical tests. The standard procedures were followed to evaluate the secondary phytoconstituents like alkaloids, glycosides, steroids, tannins, flavonoids, resins, carbohydrates, triterpenoids present in the flower (Ekwueme, F.N., 2015).

Evaluation of *In vitro* ant-inflammatory activity

• Bovine serum albumin method:

0.45ml of 1% Bovine serum albumin solution and 1ml of various quantity of sample extract was added. pH of the above solution was maintained at 6.3 by using 1N HCl. The reaction mixture was incubated for 20min at 37°C and heated for 5min at 57°C. The above solution was cooled and 2.5ml of phosphate buffer was incorporated. The absorbance of the mixture was found out at 660nm (Sangeetha, M., 2013). Percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \times 100$$

• Egg albumin denaturation method

2.8ml of phosphate buffer, 2ml of test extract (of various concentration) and 0.2ml of egg albumin were mixed together. This reaction mixture was then incubated for 15min at 37°C. Further the mixture was heated for 5min at 70°C. The reaction mixture was then cooled and the absorbance was taken at 660nm (Fernandes, J., 2017).

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \times 100$$

Human Red blood cells membrane stabilization method (Leelaprakash, G., 2011):

• Collection of human blood

The fresh human blood was collected and transferred to the centrifuge tube. 10ml of human blood has been centrifuged at 3000 rpm for 10 min. It was washed 3 times after centrifugation, with equal volume of normal saline. The volume of blood obtained was assessed and 10% v/v suspension was prepared using standard saline. The above solution was utilized for the further study.

• Heat induced haemolysis

1ml test solution of different concentration (6.25-100) and 1ml of 10% RBC solution was mixed together and incubated in water bath at 56°C for 30 minutes. For control instead of test solution saline solution was added. The solutions were centrifuged at 2500 rpm for 5 min after cooling. Supernatant absorbance was measured at 560nm. The % inhibition of lysis was calculated using following formula:

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \times 100$$

Where $Abs_{control}$ is the absorbance of control and Abs_{test} is the absorbance of test extract.

Results and Discussion

Use of herbs as medicines is the oldest remedies

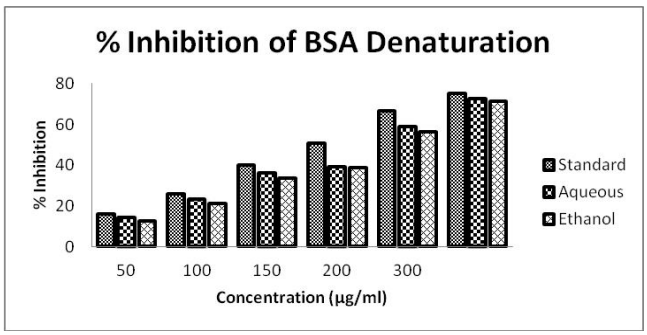

Fig. 1: Comparison of % Inhibition of BSA denaturation by *Mirabilis jalapa* flower extracts (aqueous and ethanolic) and standard drug.

Table 1: Effect of aqueous and ethanolic *Mirabilis jalapa* flower extracts on bovine serum albumin denaturation.

S. No.	Conc. of sample (µg/ml)	% Inhibition on BSA denaturation ± SEM		
		Standard	Aqueous extract	Ethanol extract
1	50	16.26±0.34	14.65±0.53	12.65±0.34
2	100	25.90±0.34	23.29±0.53	21.28±0.20
3	150	40.160±0.2	36.14±0.34	33.73±0.34
4	200	51.00±0.53	39.15±0.34	38.75±0.20
5	250	66.86±0.34	59.03±0.34	56.62±0.34
6	300	75.30±0.34	72.89±0.34	71.28±0.53

known to human beings. Herbal medicine indicates the use of any of the plant parts to cure or prevent diseases. India has a long history and strong base for Ayurveda. Herbal medical system is termed as foundation for the modern pharmaceutical drug regimen. Earlier when there was no allopathic medicines plant derived medicines were used for treatment. The limitations of the English medicines were slowly revealed in last two decades which led to increase in demand for herbal drugs (Singh, A., 2007).

Recent studies have also indicated that NSAIDs when used repeatedly may cause cardiovascular diseases, hypertension and nephrotoxicity. The preliminary phytochemical study of the *Mirabilis jalapa* flower extracts revealed the existance of flavonoids, triterpenoids, steroids, resins and reducing sugars. Studies have indicated that phytoconstituents such as flavonoids, xanthones and triterpenoids are responsible for anti-inflammatory behaviour utilizing various mechanisms. Such phytoconstituents need to be isolated and tested to reduce the pathologies associated with the inflammatory diseases and also to minimize the use of synthetic drugs (Lalrinzuali, K., 2016).

• BSA Denaturation method

Protein denaturation is one of the main causes of inflammation in some of the diseases like arthritis,

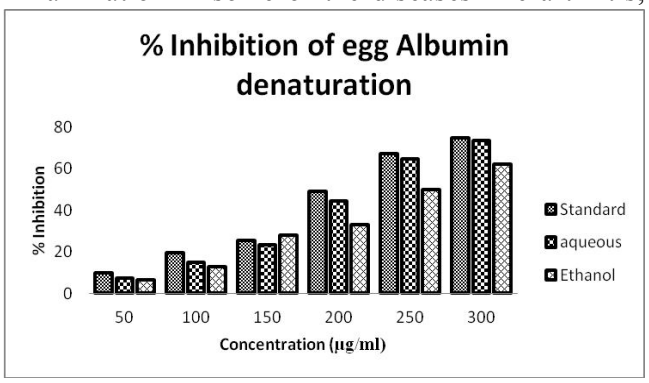

Fig. 2: Comparison of % Inhibition egg albumin denaturation by *Mirabilis jalapa* flower extracts (aqueous and ethanolic) and standard drug

Table 2: Effect of aqueous and ethanolic *Mirabilis jalapa* flower extracts on egg albumin denaturation method.

S. No.	Conc. of sample (µg/ml)	% Inhibition on egg albumin denaturation		
		Standard	Aqueous extract	Ethanol extract
1	50	9.49±0.09	7.10±0.09	6.39±0.064
2	100	19.46±0.19	14.91±0.064	12.70±0.098
3	150	25.44±0.12	23.31±0.064	27.69±0.064
4	200	48.69±0.09	44.35±0.037	32.95±0.064
5	250	67.11±0.16	64.42±0.134	49.77±0.064
6	300	74.66±0.12	73.20±0.064	61.99±0.064

diabetes, cancer etc. protein denaturation occurs due to the production of auto antigens (Sangeetha, G., 2016). The plant extract which inhibits the protein denaturation is said to have good anti-inflammatory activity. In BSA method both aqueous and ethanol extract showed good activity however the inhibition percentage of protein denaturation of aqueous extract was slightly higher than ethanol extract. The maximum percentage inhibition of the aqueous extract was 72.89%, ethanolic extract was 71.28% while the % inhibition of standard was found to be 75.30%. The IC50 values of standard, aqueous extract and ethanolic extract were found to be 141.65, 164.83 and 172.37 respectively.

• Egg albumin denaturation method

Similar result was observed in the egg albumin denaturation method. The highest % inhibition was 73.20% which was obtained by aqueous extract while the highest % inhibition of standard was found to be 74.66. The IC50 values of standard, aqueous and ethanolic extracts were found to be 157.67, 167.38 and 205.20 respectively.

• HRBC membrane stabilization method

Lysosomes consist of constituents of activated neutrophil the release of which may cause further inflammation. Human erythrocyte membrane mimics the

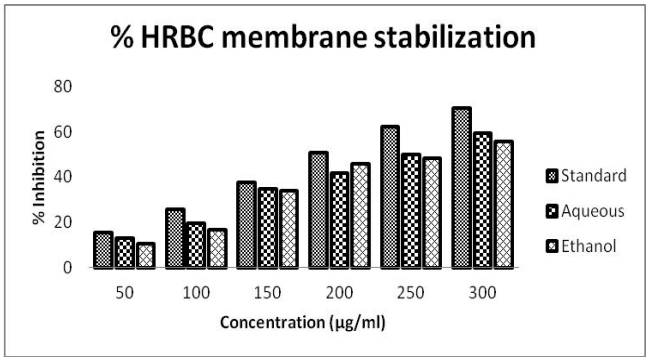

Fig. 3: Comparison of % HRBC membrane stabilization by *Mirabilis jalapa* flower extracts (aqueous and ethanolic) and standard drug.

Table 3: Effect of aqueous and ethanolic *Mirabilis jalapa* flower extracts on HRBC membrane stabilization.

S. No.	Conc. of sample (µg/ml)	% Membrane stabilization (% Inhibition of lysis)		
		Standard	Aqueous extract	Ethanol extract
1	50	15.45±0.024	13.04±0.041	10.86±0.041
2	100	26.08±0.041	19.56±0.024	16.66±0.041
3	150	37.92±0.024	34.78±0.041	34.05±0.041
4	200	50.72±0.041	41.78±0.041	46.13±0.024
5	250	62.56±0.024	50.00±0.041	48.30±0.63
6	300	70.53±0.024	59.66±0.041	55.79±0.041

lysosomal membrane (Leelaprakash, G., 2011). Most of the anti- inflammatory drugs reduce inflammation either by inhibiting the protein denaturation, release of lysosomal constituents or by stabilizing lysosomal membrane. In this method the percentage of human red blood cell membrane stabilization was measured. The aqueous and ethanol extract both exhibited effective membrane stabilization. The maximum stabilization was observed to be 59.42% and that of standard was 70.53%. The IC50 values were found to be 151.93, 196.42 and 202.53 of standard, aqueous and ethanol extracts respectively. The membrane stabilization was also concentration dependent.

Conclusion

Anti-inflammatory activity of the flowers of Mirabilis jalapa was studied using standard *in-vitro* methods like BSA denaturation method, egg albumin denaturation method and HRBC membrane stabilization method. Ethanolic and aqueous extract of Mirabilis jalapa showed notable increase in activity with increase in the concentration. The results achieved in the present investigation indicate that compared to Diclofenac which was used as a standard drug, the floral extract has considerable activity. These activities may be due to the presence of flavonoids, triterpenoids, steroids and tannins. Isolation of these phytoconstituents and further study may help in identifying the actual compound responsible for the above mentioned activity.

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