



ISSN: 2391-2371
Research Paper

Evaluation of *In vitro* anti inflammatory activity of *Murraya koenigii*

Abhishek Tandon^{*1}, Dr. Sampat Nehra², Dr. Vikram Kumar³

¹Biotech Scholar, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur-303002, Rajasthan, India

²Research Scientist, Birla Institute of Scientific Research, Jaipur-302001, Rajasthan, India

³Asst. Professor, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur-303002, Rajasthan, India

ABSTRACT

The leaf extract of *Murraya koenigii* were assessed for In vitro Anti Inflammatory activity by HRBC membrane stabilization method and Denaturation of protein inhibition method. The presence of flavanoids and carbazole alkaloids has been reported earlier in *M.koenigii*. Since the flavanoids and carbazole alkaloids have remarkable anti-inflammatory activity, so the present work aims at evaluating the anti-inflammatory activity of *M.koenigii*. Different concentrations of extract were compared against standard Diclofenac sodium. Maximum stabilization in our study is 69.15 % at 1000 µg/ml and maximum inhibition is 85.35% at 800 µg/ml. Therefore, our studies support the use of *M.koenigii* in treating inflammation.

Key words: *Murraya koenigii*, HRBC, Protein Denaturation, Diclofenac Sodium, Anti-inflammatory.

INTRODUCTION

Murraya koenigii, commonly known as *curry leaf* or *kari patta* in Indian dialects, belonging to Family Rutaceae which represent more than 150 genera and 1600 species. [1] *Murraya Koenigii* is a highly values plant for its characteristic aroma and medicinal value. It is an important export commodity from India as it fetches good foreign revenue. A number of chemical constituents from every part of the

plant have been extracted. The most important chemical constituents responsible for its intense characteristic aroma are P-gurjunene, P-caryophyllene, P-elemene and O-phellandrene. The plant is rich source of carbazole alkaloids. [2] Bioactive coumarins, acridine alkaloids and carbazole alkaloids from family Rutaceae were reviewed by Ito. [3]

M. koenigii is widely used in Indian cookery for centuries and have a versatile role to play in traditional medicine. The plant is credited with tonic and stomachic properties. Bark and roots are used as stimulant and externally to cure eruptions and bites of poisonous animals. Green leaves are eaten raw for cure of dysentery, diarrhoea and for checking vomiting. Leaves and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation, itching and are useful in leucoderma and blood disorders. [4,5]

***Corresponding Author:**

Abhishek Tandon

Biotech Scholar

Amity Institute of Biotechnology,

Amity University Rajasthan,

Jaipur, Rajasthan, India

E.Mail: abhishek-tandon@hotmail.com

Article Received on: 21-09-2014

Revised on: 29-09-2014

Published on: 30-09-2014

Several systematic scientific studies are also being conducted regarding the efficacy of whole plant or its parts in different extract forms for the treatment of different diseases including hypoglycaemic, Ant-diabetic, Wound healing. [6-8]

Inflammation is a physiologic series of responses generated by the host in response to infection or other insults. Inflammation can have rapid onset and last a short period of time (acute inflammation), or it can persist due to a continuous stimulus or injury (chronic inflammation). The initial events of inflammation are derived from vascular reactions at the site of injury. Vascular changes are important for the induction of the response and are characterized by redness, heat, and swelling, usually accompanied by pain and loss of function, and collectively represents the "cardinal signs" of inflammation. These signs of inflammation are the result of vasodilatation and increased vascular permeability, leading to exudation of fluid and plasma proteins and recruitment of leukocytes to the site of injury. [9]

MATERIALS AND METHODS

Extract Preparation

Samples were collected and shade dried for 4 weeks until they show consistent weight. The dried parts were later grinded to powder. The dried parts were used for ethanolic extract using Soxhlet Apparatus. The extracts were filtered using Whatmann's No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator. Powdered extract was stored in air tight container for further use. [10]

In Vitro Anti-Inflammatory Activity

(a) HRBC Membrane Stabilizing Method

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It was incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (500 µg/ml) was used as reference standard and a control was prepared by omitting the extracts and using distilled water. Product control lacks the red blood cells. [11-14]

The percentage of HRBC membrane stabilization or protection was calculated by using the following Formula,

$$\text{percentage stabilization} = 100 - \frac{\text{O.D. of test} - \text{O.D. of product control}}{\text{O.D. of control}} * 100$$

(b) Inhibition of Protein Denaturation method

The reaction mixture (2ml) will be containing 0.06mg/ml trypsin, 1ml 20mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100µg/ml, 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml). The mixture will be incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein will be added. The mixture will be incubated for an additional 20 min. 2 ml of 70% Perchloric acid will be added to terminate the reaction. Cloudy suspension

will be centrifuged at 2500rpm for 5 mins. [15] The absorbance of the supernatant will be read at 210 nm against buffer as blank and product control lacked casein. The percentage Denaturation of Protein inhibition activity will be calculated. The percentage inhibition of protein denaturation was calculated as follows-

$$\text{percentage inhibition} = 100 - \frac{\text{O.D. of test} - \text{O.D. of product control}}{\text{O.D. of control}} * 100$$

STATISTICAL ANALYSIS

Statistical Analysis was done by one way ANOVA following TURKEY test.

RESULTS

From the result of present study, the leaf extract of *M. koenigii* was subjected to In vitro anti-inflammatory activity in various concentrations i.e. 100, 200, 400, 600, 800, 1000 µg/ml and the percentage stabilization of different extracts by HRBC membrane stabilization method is shown in Table No.1. The extract demonstrated a significant (P<0.001) anti-inflammatory activity at all the doses tested compared to control. The percentage membrane stabilization shows increase with the increase in concentration of the extract.

The percentage inhibition of different extracts by Denaturation of Protein Inhibition action method is shown in Table No.6. The percentage inhibition is 33.27 at 100 µg/ml; 50.01 at 200 µg/ml; 63.56 at 400 µg/ml; 76.92 at 600 µg/ml and 85.35 at 800 µg/ml. The percentage inhibition shows increment with the increase in extract concentration. The extract concentration from 100 µg/ml to 600 µg/ml demonstrated extremely significant (P<0.001) anti-inflammatory activity at the doses tested compared to control. The concentration 800 µg/ml showed a significant (P<0.01) anti-

inflammatory activity at the dose tested compared to control.

DISCUSSION

Murraya koenigii contains a number of chemical constituents that interact in a complex way to elicit their pharmacodynamic response. A number of active constituents responsible for the medicinal properties have been isolated and characterized. This plant has been reported to have anti-oxidative, cytotoxic, antimicrobial, antibacterial, diabetes,[16] anti ulcer, positive inotropic and cholesterol reducing activities has been reported with the presence of flavanoids and carbazole alkaloids which has a remarkable anti-inflammatory activity. Therefore different concentrations of its extract have been taken for assessing the in vitro anti-inflammatory activity.

Leaf of *Murraya koenigii* exhibited membrane stabilization effect by inhibiting hypo tonicity induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. [17]

Denaturation of protein is one of the causes of lipodystrophy, hyperlipidaemia, diabetes mellitus type 2, kidney stones and rheumatoid arthritis that are documented. [18] Agents that can prevent denaturation of protein inhibition therefore, would be worthwhile for anti-inflammatory drug development. From the present study, it can be stated that the extract of *M.koenigii* is capable of controlling the denaturation of protein and thereby it inhibit

the denaturation of protein and its effect was compared with the standard drug.

CONCLUSION

The In vitro study on leaf of *M.koenigii* showed the presence of significant anti inflammatory activity. The activity is due to the presence of flavanoids and carbazole alkaloids. The future aspects can include the production and commercialization of drug.

ACKNOWLEDGEMENT

We thank the Department of Biotechnology, Amity University Rajasthan and most importantly the HOD Prof. (Dr.) A.N. Pathak. Also we like to thank Dr.Sampat Nehra (Scientist, BISR, Jaipur) and my family B.D.Tandon, Sandhya Tandon and Aishwarya Tandon and my friends.

REFERENCES

1. Satyavati GV, Gupta AK, Tendon N. Medicinal Plants of India, Vol-2, Indian council of medical research, New Delhi India, 1987, 289-299.
2. Kumar VS, Sharma A, Tiwari R, Kumar S. *Murraya koenigii* (curry leaf): a review. J Med Arom Plant Sci. 1999; 21(4): 1139-1141.
3. Ito C. Studies on Medicinal Resources of Rutaceous Plants and Development to Pharmaceutical Chemistry, Natural Med. 2000; 54: 117-122.
4. Nadkarni KM, Indian Materia Medica, Edition 3, Vol. I, Popular Prakashan, Mumbai, 1976, 196.
5. Kirtikar KR, Basu BD. Indian Medicinal Plants, Edition 2, Vol. I, Oriental Enterprises, Uttarchal Pradesh, 1981, 473.
6. Kumar V, Bandyopadhyay A, Sharma V. Wound Healing Activity of *Murraya Koenigii* in High Fat Diet And Streptozotocin Treated Type-2 Diabetic Rats. International Journal of Research in Pharmacy and Science; 2011,1(2),128-140.
7. Kumar V, Bandyopadhyay A, Sharma V. Investigation of Effect of *Murraya Koenigii* on Biophysical and Biochemical Parameters of Wound in Diabetic Hyperlipidemic Wistar Rats. International Journal of Pharmaceutical Sciences and Research. 2012; 3(6): 1839-1845.
8. Kumar V, Bandyopadhyay A, Sharma V, Suthar S. Comparative Study of Hypoglycemic and Hypolipidemic Potency of *Murraya Koenigii* for Wound Healing Activity in Type-2 Diabetic Rats. 2012; 2(2): 150-161.
9. Kumar V., Kumar GS, Gupta M, Mohanty J., Gupta A.; Anti-Inflammatory Activity of Methanolic Extract of *Ocimum Gratissimum* (Labiata) on Experimental Animals; International journal of pharmaceutical innovations. 2011; 1(2): 76-81.
10. Thakur R; Study of antioxidant, antibacterial and anti-inflammatory activity of cinnamon (*Cinamomum tamala*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*); American Journal of Life Sciences. 2013; 1(6): 273-277.
11. Ejebe DE, Siminialayi IM, Emudainowho JOT, Ofesi U, Morka L. Analgesic and anti inflammatory activities of the ethanol extract of the leaves of *Helianthus annuus* in wistar rats. Asian Pac J Trop Med 2010; 3(5): 341-347.
12. Kumar V, Bhat Z.A., Kumar D, Bohra P, Sheela S. In-Vitro Anti-Inflammatory Activity Of leaf extracts Of *Basella alba linn.*; International Journal of Drug Development & Research. 2011; 3(2): 176-179.

13. Faimum D A., Sudaroli M., Salman M I. In Vitro anti- inflammatory activity of *Vitex leucoxydon Linn.* leaves by HRBC membrane stabilization. International Journal Of Pharmacy & Life Sciences. 2013; 4(1): 2278-2281.
14. Selvi VS, Bhaskar A; Characterization of Anti-Inflammatory Activities and Antinociceptive Effects of Papaverine from *Sauropus androgynus (L.) Merr;* Global Journal of Pharmacology. 2012; 6 (3): 186-192.
15. Leelaprakash G and Dass SM. *In vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare.* *Int J Drug Develop & Res.* 2011; 3(1):189-196.
16. Pandey J. *Murraya koenigii* (L.) Spreng. ameliorates insulin resistance in dexamethasone-treated mice by enhancing peripheral insulin sensitivity. Journal of the Science of Food and Agriculture. 2014; 94(11):2282-2288.
17. Mittal S, Dixit PK, Gautam RK Gupta MM. In Vitro Anti-Inflammatory activity of hydroalcoholic extract of *Asparagus racemosus* roots. International Journal of Research in Pharmaceutical Science. 2013; 4(2): 203-206.
18. Fantry LE. "Protease Inhibitor-associated diabetes mellitus: A potential cause of morbidity and mortality". Journal of acquired immunodeficiency syndromes. 1999; 32(3):2434.

ANNEXURE

Table-1: Effect of different concentrations of *M.koenigii* leaf extract on membrane stabilization

Concentration ($\mu\text{g/ml}$)	% stabilization by ethanol extract	% stabilization by Diclofenac sodium at 500 $\mu\text{g/ml}$
100	32.45	
200	41.87	
400	43.71	73.69
600	55.16	
800	61.11	
1000	69.15	

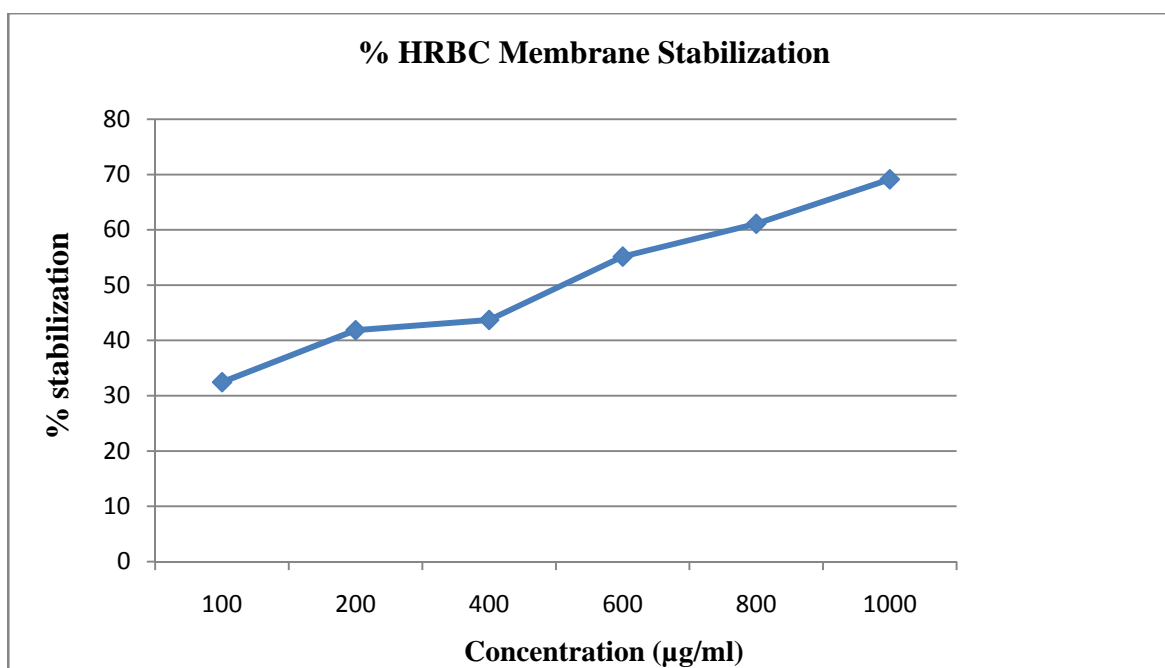
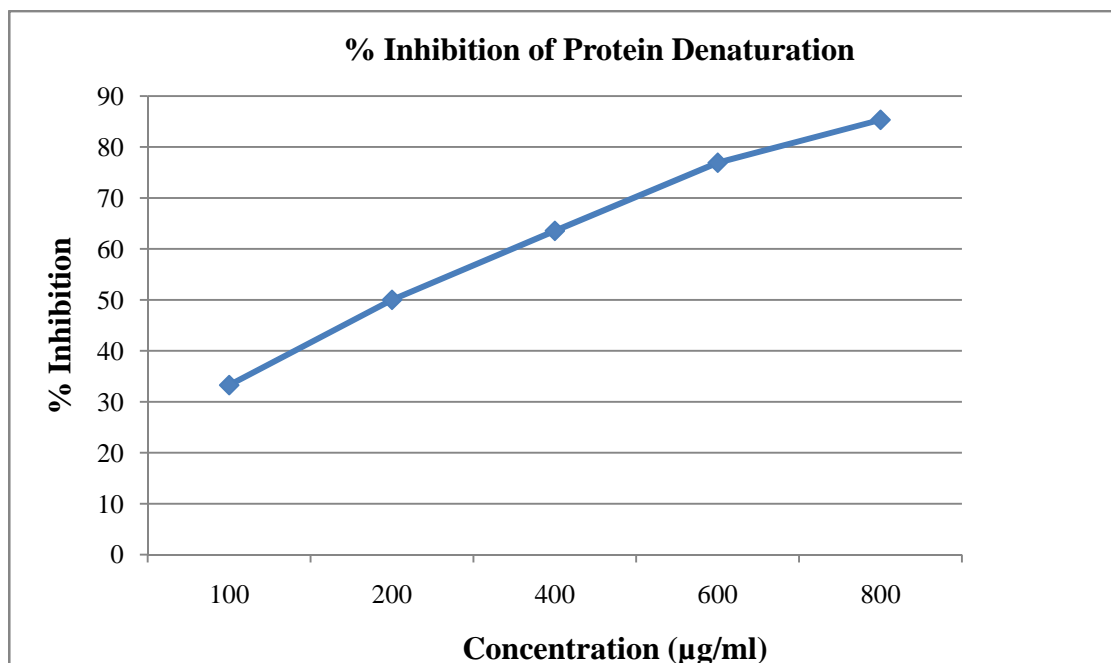
**Figure-1:** Effect of *M.koenigii* leaf extract on membrane stabilization.

Table-2: Effect of different concentrations of *M.koenigii* leaf extract on inhibition of protein denaturation.

Concentration ($\mu\text{g/ml}$)	% inhibition by ethanol extract	% inhibition by Diclofenac sodium at 500 $\mu\text{g/ml}$
100	33.27	
200	50.01	
400	63.56	87.18
600	76.92	
800	85.35	

**Figure-2:** Effect of *M.koenigii* leaf extract on inhibition of protein denaturation.