Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 11 [7] June 2022 : 41-43

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

SHORT COMMUNICATION **OPEN ACCESS**

In Vitro Protein Denaturation Inhibition Assay of Ethanol Extracts of Stem Bark of *C. nurvala, C. tomentosa and C. decidua* For Potential Anti-Inflammatory Activity

Tolsarwad Ganesh S.*[1], Parul Mehta[2]
[1]School of Pharmacy, LNCT University, J K Town, Kolar Road, Bhopal, Madhya Pradesh- 462042
Correspondence Email: tolsarwadganesh988@gmail.com

ABSTRACT
The aim of the study was to investigate the ethanol extracts of stem bark of C. nurvala & C. tomentosa, Capparis decidua for its in vitro protein denaturation inhibition. In highest concentration of shows significant antiarthritic and membrane stabilizing activity compared with Diclofenac –Na. Further in depth studies on this plant can result in a costeffective herbal drug with anti-inflammatory activity contributing towards better healthcare of human society.
Keywords: *C. nurvala, C. tomentosa, C. deciduas, protein denaturation assay*

Received 14.03.2022 Revised 25.05.2022 Accepted 27.05.2022

INTRODUCTION

Medicinal plants are the nature's gift to human being to make disease free healthy life. It plays a vital role to preserve our health [1]. Capparidaceae family comprises various important medicinal properties distributed in tropical and subtropical India. In recent times, medicinal plants show tremendous potential and large number of evidences in health sector and due to that their research work is explored accordingly and more than 80% of the world population depends on plant-based medicines for their basic health care needs in under developed and developing countries [3].

Protein denaturation has been identified as the cause of inflammation in arthritis. Indications are that when living tissues are injured inflammation results. This is characterized by redness, pain, heat, swelling, as well as loss of function in the affected area. Disruption of electrostatic, hydrogen, hydrophobic and disulphide bonds in the protein structure occurs. In addition, a complex array of enzymes activation, mediator release, cell migration, tissue break down and repair occurs causing the protein to lose its molecular conformation and functions or become denatured [4]. It is therefore deduced that compounds which are able to prevent these changes and inhibit thermally or heat induced protein denaturation, have potential therapeutic anti-inflammatory activity [5].

The plants selected for present study contains various components which are responsible for anti-inflammatory activity.

MATERIAL AND METHODS

Collection of Plant

The Plant stem bark of *C. nurvala, C. tomentosa, C. decidua* were collected from local area, Sangli and Kolhapur districts. The plants were identified and authenticated by Dr. V. B. Awale, an approved Botanist, Dept. of Botany, Dr. Patangrao Kadam Mahavidyalaya, Sangli. A specimen voucher no. (LNCT/Bhopal/101 to 103).

Extraction

The plant materials were initially washed with tap water then with distilled water to remove soil, dust particles and other contaminants. They were dried at room temperature, crushed in grinder and sieved through mesh no 40 to obtain fine powder. The powders were then extracted by maceration for 7 days with 2-3 days interval using water and chloroform (16:1). All the extracts were concentrated and stored in well closed container [6].

Procedure

In vitro anti-inflammatory activity by Protein denaturation method [7]

The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 100 µL of sample. Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37°c ±2) in an incubator for 15 min and then heated at 70°c for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the different concentration was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula,

% inhibition = (Absorbance of control – Absorbance of test sample) / Absorbance of control

RESULT

Table no.1 *in vitro* anti-inflammatory activity of standard drug diclofenac sodium

	Concentration (µg/ml)	OD	% inhibition	IC50
Blank		0.69		
Standard Diclofenac sodium	100	0.37	46.37 ±0.80	15.06
	200	0.20	71.01 ±0.99	
	400	0.16	76.81 ±0.96	
	800	0.11	84.05 ±0.98	
	1000	0.08	88.40 ±0.86	
Capparis decidua	100	0.43	37.68 ±0.89	219.09
	200	0.34	50.72 ±0.76	
	400	0.21	69.56 ±0.98	
	800	0.20	71.01 ±0.89	
	1000	0.19	72.46 ±1.02	
C. nurvala	100	0.37	46.37 ±1.12	897.76
	200	0.33	52.17 ±0.99	
	400	0.26	62.31 ±0.99	
	800	0.22	68.11 ±0.98	
	1000	0.18	73.91 ±1.14	
C. tomentosa	100	0.44	36.23 ±1.96	>1000
	200	0.39	43.47 ±1.03	
	400	0.31	55.07 ±1.14	
	800	0.27	60.86 ±1.02	
	1000	0.20	71.01±0.91	

Values represent in the results are mean±SD of three replicates; linear regression analysis was used to calculate IC50 value

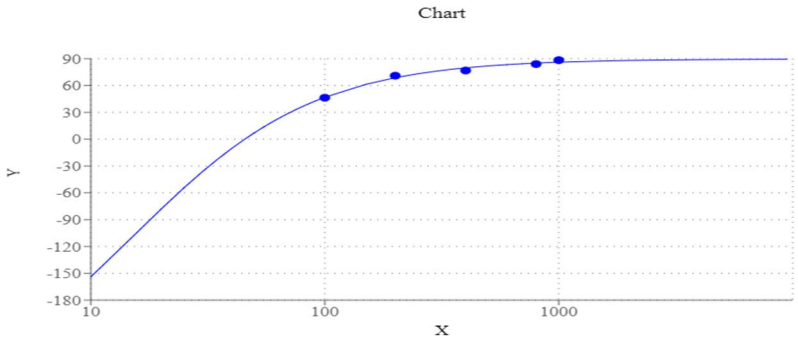

Fig IC_{50} value calculation of standard treated group

Statistical analysis

Data are reported as the mean=SD (standard deviation) and were analyzed statistically by the means of analysis of variance (ANOVA) followed by Students t-test. Values of $p<0.05$ are regarded as significant.

DISCUSSION

Denaturation of proteins is a well-documented cause of inflammation and rheumatoid arthritis. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation [1]. The inhibitory effects of different concentrations of *C. nurvala,C. tomentosa, and C. deciduas* on protein denaturation are summarized in Table 1 [2]. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by *C. nurvala,C. tomentosa, and C. deciduas* throughout the concentration range of 100 to 1000 µg/mL. Diclofenac sodium (at the concentration range of 100 to 1000 µg/mL) was used as reference drug which also exhibited

concentration dependent inhibition of protein denaturation. *C. nurvala, C. tomentosa, C. deciduas* extract at concentration of 1000 µg/ml and Diclofenac sodium at concentration of 200 µg/ml showed significant inhibition 72.46, 73.91, 71.01% and 71.01 ±0.99% respectively of protein denaturation when compared with control. This was further confirmed by comparing their IC50 values [6]. *Capparis decidua* possessed IC_{50} value 219.09µg/mL whereas that of diclofenac sodium was found to be 15 µg/mL.

CONCLUSION

The data of our studies suggests that ethanolic extracts of stem bark of *C. nurvala, C. tomentosa* and *C. decidua* showed significant anti-inflammatory activity in these models tested. Further studies involving the purification of the phyto-constituents of the plant and their further investigations may result in the development of a potent anti-inflammatory agent.

Conflict of Interest: No

REFERENCES

1. Elumalai, A., Eswaraiah, M. C., & Lakshmi, M. (2011). A short review on un-explored medicinal plant: Ecboliumviridie. *Int J Res Ayurveda Pharm*, *2*(5), 1539-1540.
2. Rajesh, P., Selvamani, P., Latha, S., Saraswathy, A., & Kannan, V. (2009). A review on chemical and medicobiological applications of capparidaceae family. *Pharmacognosy Reviews*, *3*(6), 378.
3. Kumar, B., Vijayakumar, M., Govindarajan, R., & Pushpangadan, P. (2007). Ethnopharmacological approaches to wound healing—exploring medicinal plants of India. *Journal of ethnopharmacology*, *114*(2), 103-113..
4. Dharsana, J. N., & Mathew, M. (2014). Preliminary sceening of Anti-inflammatory and Antioxidant activity of Morindaumbellata. *International Journal of Pharmacy & Life Sciences*, *5*(8).
5. Ruiz-Ruiz, J. C., Matus-Basto, A. J., Acereto-Escoffié, P., & Segura-Campos, M. R. (2017). Antioxidant and anti-inflammatory activities of phenolic compounds isolated from Melipona beecheii honey. *Food and Agricultural Immunology*, *28*(6), 1424-1437.
6. Murti Y, Sharma S, Mishra P. In-Vitro Cytotoxicity of Chromatographic Elutes of *Calotropis procera* (Ait.) R BR. leaves Against Human Hepatoma Cell Line (HEPG2). Indian Drugs. 2016 May;53(5):48-52.
7. Williams, L. A. D., O'connar, A., Latore, L., Dennis, O., Ringer, S., Whittaker, J. A., ... & Kraus, W. (2008). The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. *West Indian Medical Journal*, *57*(4). 90-98

CITATION OF THIS ARTICLE

Tolsarwad Ganesh S., Parul Mehta. *In Vitro* Protein Denaturation Inhibition Assay of Ethanol Extracts of Stem Bark of *C. nurvala, C. tomentosa and C. decidua* For Potential Anti-Inflammatory Activity. Bull. Env. Pharmacol. Life Sci., Vol 11[7] June 2022 : 41-43.