WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 5, Issue 01, 1382-1389. Research Article ISSN 2277– 7105

COLLAGENASE INHIBITORY ACTIVITY AND PHYTOCHEMICAL PROFILE OF LEAF OF *JUSTICIA GENDARUSSA*

S. S. Patel* and M. N. Zaveri

India.

Article Received on
16 Nov 2015,

Revised on 09 Dec 2015,
Accepted on 31 Dec 2015

***Correspondence for Author**
S. S. Patel
India.

ABSTRACT

Ethnomedicinally *Justicia gendarussa* is used for treatment of chronic rheumatism. The alcoholic extract of leaf was reported for its anti-inflammatory and antiarthritic activity. This research is aim to find collagenase inhibitory fraction. The leaf was fractionated based on polarity. Methanolic extract and its fractions of leaf of *J. gendarussa* were screened phytochemically. Based on phytochemical screening the resultant fractions were evaluated for their collagenase (Matrix metalloproteinases) inhibitory activity at 100, 250, 500 and 1000 µg /ml concentration. Among the tested extracts, toluene fraction of leaf of *J. gendarussa* showed maximum Matrix metalloproteinases inhibition with IC_{50} value 44.75µg /ml. Further, the phytochemical analysis of isolated compounds indicated the alkaloids and saponins in leaf of *J. gendarussa respectively*. The Matrix metalloproteinases inhibitory activity may be because of high content of alkaloids and saponins. Based on Matrix metalloproteinases inhibitory activity, it could be suggested that the toluene fraction of *J. gendarussa* might have an influence on arthritis.

KEYWORDS: Matrix metalloproteinases, Collagenase, Toluene fraction, Leaf.

INTRODUCTION

The collagen, the major component of the cartilage, has been thought to undergo only minimal turnover under normal circumstances but is known to be actively destroyed in rheumatoid and septic arthritis. Joint cartilage is composed of a type II collagen-based fibrillar network complexed to proteoglycans. Type II collagen consists of 3 identical α chains arranged in a triple helix that form fibrils. Within this fibrillar meshwork resides the large aggregating proteoglycan aggrecan. In arthritis type II collagen is extensively cleaved and destroyed by the activity of collagenases, which results in loss of type II collagen. [1-3]

Different collagenases, namely matrix metalloproteinases (MMP)-1, MMP-8 and MMP-13, can cleave type II collagen. In synovial fluid, MMP-8 is stored as latent proenzyme in polymorphonuclear neutrophils.[2] The loss of the collagen components may be a function of articular cartilage collagenase that leads to the arthritic condition.[4] The discovery and development of compounds with collagenase inhibitory activity is therefore an effective method for preventing arthritis.

Ethnomedicinally the fresh leaves of *J. gendarussa (Acanthaceae*) are pounded into a paste, warmed and rubbed or applied onto the affected area which is then bandaged to ease muscle pains, broken/fractured bone, muscle sprains and cuts or added to coconut oil to treat boils.[5] The leaves are used in Vietnamese folk medicine as a poultice to treat rheumatism and arthritis. In Sri Lanka traditional medicine, the leaf of *J. gendarussa* are used as an analgesic to treat hemiplegia, rheumatism, arthritis, headache and earache.[6] It is used in India for the treatment of chronic rheumatism, cephalalgia, cough, and bronchitis. In Brazil, it is used to treat pains, fever and for the treatment of diseases of magical-religious origin.[6] The methanolic extract of leaf is reported to have effect in inflammation[7] –while methanolic extract of leaf have proven antiarthritic activity.[8] Therefore, we hypothesized that *J.gendarussa* may inhibit collagenase (matrix metalloproteinases) enzymes, which destroy type –II collagen.

MATERIALS AND METHODS

Chemicals

Collagen (Sigma–Aldrich Chemical Laboratories), Collagenase Type-II (Dutt enterprise, Nadiad), O- Phenanthrolin (Yucca, Mumbai), UV spectrophotometer (Shimadzu-1800), Micropipette (DK Scientific Technologies). All the other chemicals and reagents used in this study were of analytical grade.

Collection of plant material

J. gendarussa was authenticated by a taxonomist Dr. S.K. Patel, Head of the Department of Botany, School of Science, Gandhinagar, Gujarat, India. A voucher specimen (PH/08/001 and PH/509/008) was deposited at the Department of Pharmacognosy, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India. It was also authenticated by comparing their morphological and microscopical characters with reported literature.[10-13]

Preparation of extracts

The leaves were coarsely powdered to 60 # and stored in air tight container which was used for the present work. The powder (500 gm) of leaf of *J.gendarussa* was subsequently extracted in a Soxhlet extraction unit (Borosil, Mumbai) using petroleum ether, methanol and water to get non-polar, semipolar and polar constituents. The resulting extracts were stored in vials and used for *In-vitro* assay.

These three extracts were standardized by studying their phytochemicals by chemical test[12-13] and also by Thin Layer Chromatography (TLC) before and after hydrolysis with 2 M Hydrochloric acid (HCl). According to the chemical constituents present in all extracts, finally methanolic and water extract of leaf was selected for *In-vitro* screening using collagenase inhibitory assay. As a result of this study methanolic extract was selected for further fractionation to Toluene, chloroform, Ethyl acetate, N- butanol and residue. These all fractions of methanolic extract of leaf were evaluated for its collagenase inhibitory action.

TLC study of Fractions

TLC was developed for fingerprinting of above prepared extracts of leaf of *J. gendarussa* with Camag HPTLC System. The standard solutions of 10 µl volume were applied on TLC plate. The samples of all extract of leaf of *J. gendarussa* were spotted. All plates were run in the mobile phase as mentioned in Table 1. The resolved bands on TLC plates were observed under UV light at 254 nm and 366 nm and after derivatization with different reagent.

Collagenase (Matrix metalloproteinases) inhibitory assay

Collagenase causes hydrolysis of protein to form amino acids, which reacts with ninhydrin reagent and it, gives purplish blue color. Collagenase inhibitory activity was carried out in eppendorfs tubes, 500 µl of different fractions of leaf of *J. gendarussa* (100, 250, 500 and 1000 µg/ml) solutions were serially diluted with 500 µl of tris buffer and 10 µl collagenase enzyme (0.125 %). All the tubes were mixed well and incubated at 37˚C for 3.5 hours. Then 2.5 mg collagen was added to all the tubes, the tubes were incubated for equilibration at 37˚C for 1.5 min. After 1.5 min, tubes were centrifuged at 5,000 rpm and the supernatant was taken for colour development. 20 µl of the supernatant was taken for colour development in a 96 well plate and to this 200 µl of ninhydrin colour reagent was added and the plate was placed over boiling water bath for 20 min for the reaction to take place, after completion of the reaction the plate was cooled to room temperature. 50 µl of the reaction mixture was again taken in to another 96-welI plate and to this 200 µl of n-butanol was added. The solution was

mixed well and the absorbance was read at 570 nm. The percentage inhibition was calculated by using mean control absorbance value. Control samples were run without drug and blank samples were run without drug and enzyme. O-phenanthroline (a known zinc chelator), is an inhibitor of metalloproteinases. It is used for validation of assay in concentration range from 200 -2000 ng.[14-15]

Statistical analysis

The results were presented as mean ± SEM of three determinations. The IC_{50} concentration was calculated by Finney software.

RESULTS AND DISCUSSION

Phytochemical study of extracts

Preliminary phytochemical tests and TLC study indicated the presence of phytoconstituents such as phenolics, carbohydrates, flavonoids (flavanone), steroids, carotenoids, alkaloids and triterpenoids in the leaf of *J. gendarussa* (Figure 1). These are useful for quality evaluation and standardization of *J. gendarussa*. The leaf of *J. gendarussa* was found to be rich in carotenoids (7.88 % w/w), phenolics (2.21 % w/w), alkaloids (1.62 % w/w), flavonoids (2.03 % w/w) and saponins (foaming index- 125. Wide ranges of phytoconstituents were responsible for anti-arthritic activity which includes alkaloids, glycosides, tannins, phenolics, anthocyanins, sterols and triterpenoids. These phytoconstituents present in *J. gendarussa* exert desired pharmacological effect on body and thus act as natural anti-arthritic agents.[16] Based on phytochemical screening, extraction of the leaf of *J. gendarussa* was performed as per the polarity of the different solvents. They were extracted in petroleum ether (steroids), semipolar solvent like methanol (other alkaloids, flavanoids, saponins and phenolics) while in polar solvent such as water (carbohydrates). Based on above profile probably active extract may be methanolic extract of leaf of *J. gendarussa*. For further confirmation of chemical profile of petroleum ether, methanolic and aqueous extract of leaf of *J. gendarussa*, they were screened by TLC study. TLC study of petroleum ether extract of leaf of *J.gendarussa* shows presence of lupeol and β-sitosterol and other steroidal and triterpenoid constituents (Figure 1). Lupeol and β-sitosterol showed anti-inflammatory action in acute and chronic inflammation in rats and mice. Methanolic extract of leaf of *J.gendarussa* shows the phenolic and alkaloidal constituents and also shows same pattern of constituents at 254 nm and 366 nm before and after hydrolysis of material.

From the result of collagenase inhibitory action methanolic extract of leaf shows better protection against collagen type –II at IC_{50} value 52.63 µg/ml. Therefore, methanolic extract was selected for further fractionation. Methanolic extract of leaf having phenolics glycosides and alkaloids that's why it was first fractionated with toluene, chloroform, ethyl acetate and n-butanol.

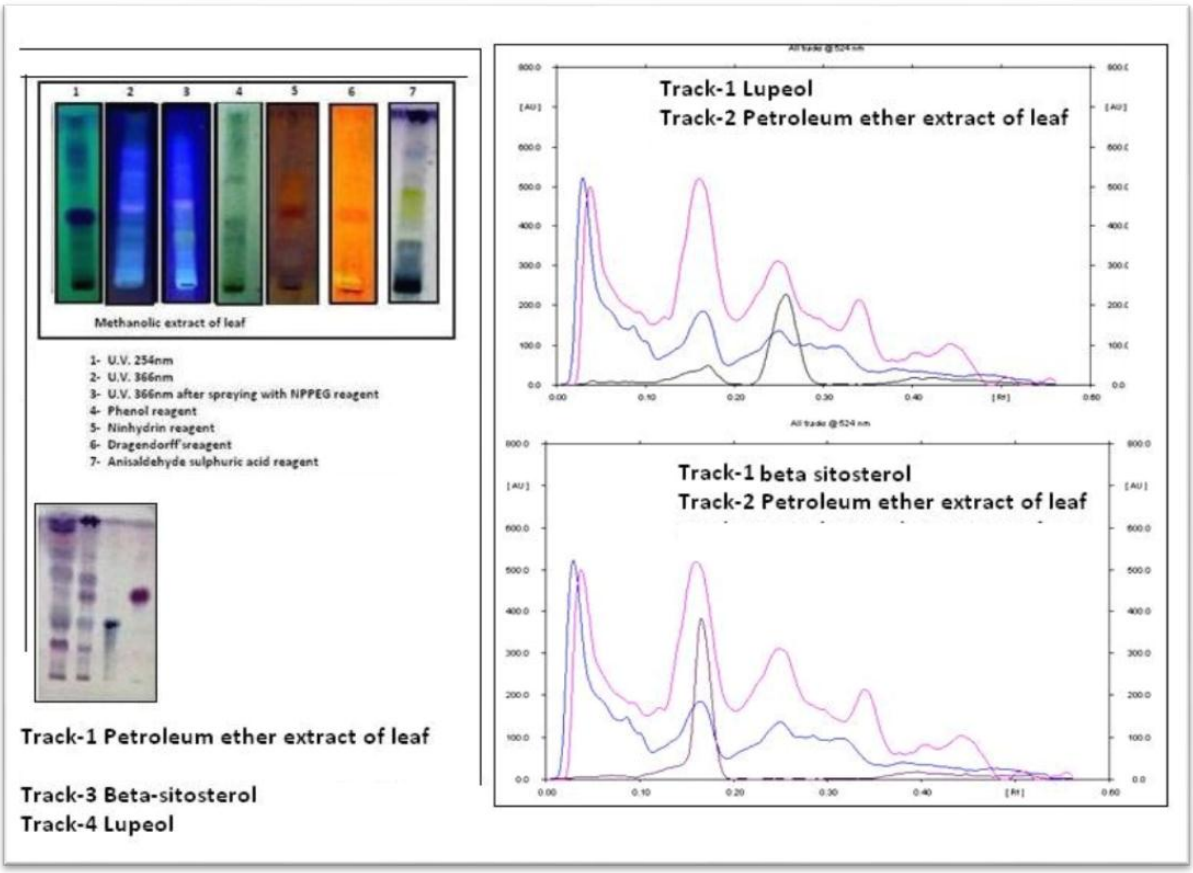

Figure 1. HPTLC analysis of leaf of *Justicia gendarussa*

Collagenase inhibitory assay

The result shows that methanolic extract and toluene fraction of leaf showed maximum enzyme inhibition at **IC_{50}** value 52.63 µg/ml and 44.75 µg/ml (Table 1). The collagenase inhibitory activity was gradually increased from methanolic extract to toluene soluble fraction which showed purity and potency leaf of *J. gendarussa.* Number of alkaloids were isolated from Justicia genus which have anti-inflammatory action.[17] The Wide ranges of phytoconstituents were responsible for anti-arthritic activity includes alkaloids, glycosides, tannins, phenolics, anthocyanins, sterols, triterpenoids *etc.* [16] like, the drugs nimesulide [18] and doxycycline [19] inhibited collagenase in cartilage derived from osteoarthritis patients *in-vitro* [20]; quinic acid esters from *Pluchea indica* with collagenase, MMP-2 and MMP-9

inhibitory activities [21]; green tea polyphenols show gelatinase inhibitory activity. [22, 23] Because of the alkaloidal nature, it showed cytotoxic action and collagenase inhibitory action, it can shows inhibitory effect on arthritis.

Table 1 Collagenase inhibitory activity of extracts and fractions of leaf of *J.gendarussa*

TEST EXTRACTS	IC $_{50}$ µg/ml
Methanolic extract	0052.63
Toluene fraction	0044.75
Chloroform fraction	0272.76
Ethyl acetate fraction	1564.41
n-Butanol fraction	0349.87
Residue	0311.19
Standard – O- Phenanthroline	0007.24

CONCLUSION

This finding may be physiologically significant, since this enzyme can be activated in arthritis. The toluene fraction of leaf of *J.gendarussa* is a highly potent inhibitor of collagenase. Such a level of potency of toluene fraction from *J.gendarussa* (IC_{50} value 44.75 µg /ml) has pharmaceutical significance for drug development programs. In summary, inhibition of collagenase activity by the isolated compound may be an important mechanism underlying its chondroprotective effects.

ACKNOWLEDGEMENT

We are very thankful to GUJCOST for financial assistance for a period of two years as a Minor Research Project scheme in the year starting May-2010.

REFERENCES

1. Billinghurst RC, Dahlberg M, Ionescu A, Reiner C, Bourne P, Rorabeck P, et al. Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. *Journal of Clinical Investigation.*, 1997; 99: 1535-1545.
2. Sorsa T, Konttinen YT, Lindy O, Ritchlin C, Saari H, Suomalainen K, Eklund KK. Collagenase in synovitis of rheumatoid arthritis. Semin. *Arthritis and Rheumatisum*, 1992; 22(1): 44-53.
3. Michael G, Ehrlich H, Mankin J, Hardin J, Richard W, Charles C, Gloria V. Collagenase and Collagenase Inhibitors in Osteoarthritic and Normal Human Cartilage. *The Journal of Clinical Investigation*, 1977; 59: 226-233.

4. ANONYMOUS. The Wealth of India: Raw Materials, Council of Scientific and Industrial Research, New Delhi, 1959; 5(H-K): 312.
5. Kirtikar CR, Basu, BD. Indian Medicinal Plants, F. L. M. Basu, Allahabad, 1935; 2: 1861.
6. De Albuquerque UP, Monterio JM, Ramos MA, Amorim ELC. Medicinal and magic plants from a public market in northeastern northeastern Brazil. *Journal of Ethnopharmacology*, 2007; 110: 176-191.
7. Kavitha SK, Viji V, Kripa K, Helen A. Protective effect of *Justicia gendarussa* Burm.f. on carrageenan-induced inflammation. *Journal of Natural Medicine*, 2010; 65(3-4): 471-479.
8. Paval J, Srinivasan KK, Bhagath KP, Govindan S, Raju SK, Sareesh NN, Sudheer M. Anti-arthritic potential of the plant *Justicia gendarussa* Burm. *Clinics.*, 2009; 64(4): 357-360.
9. Paval J, Srinivasan KK, Bhagath KP, Govindan S, Raju SK, Sareesh NN, Sudheer M. Comparing the anti-arthritic activities of the plants *Justicia gendarussa Burm F* and *Withania somnifera Linn. International Journal of Green Pharmarmacy*, 2009; 3: 281- 284.
10. Mehrotra BN, Kandu BC. Pharmacognostic study of leaf and tender shoot of *Justicia gndarussa Burm*. *Journal of Scientific and Industrial Research*, 1962; 21(c): 55-60.
11. Wahi SP, Wahi AK, Kapoor R. Pharmacognostic study on *Justicia gendarussa Burm*. *Research In Indian Medicine*, 1974; 9(4): 31-39.
12. Patel S, Zaveri M. Priliminary pharmacognostical study of root of Justicia gendarussa Burm. *Asian Journal of Traditional medicine,* 2011; 6(2): 61-72.
13. Patel S, Kapadia N, Shah B, Shah M. Botanical identification and phytochemical screening of *Justicia gendarussa* Leaf. *International Journal of Pharmaceutical Science Review and Research*., 2011; 9(2): 12-19.
14. Sumantran VN, Kulkarni AA, Harsulkar AA, Koppikar SJ, Chandwaskar R, et al. Hyaluronidase and collagenase inhibitory activities of the herbal formulation Triphala guggulu. *Journal of Bioscience*, 2007; 32(4): 755–761.
15. Herbal composition for the prevention of wrinkles and skin disorders method of preparing the same and uses thereof. United States Patent Application 20100040709 Kind Code: A1
16. Arya V, Gupta V, Kaur R. A review on plant having anti-arthritic potential. International Journal of Pharmaceutical Science Review And Research., 2011; 7(2): 131-136.

17. Geone MC, Antonio FC, Alcantara. Chemical constituents and biological activities of species of Justicia - a review. Revista brasileria de farmacognosy, 2011; 22(1): 12-18.
18. Pelletier JP, Martel-Pelletier J. Effects of nimesulide and naproxen on the degradation and metrixmetalloprotease synthesis of human osteaoarthritis cartilage. *Drug, 1993;* 46: 134-139.
19. Smith GN, Yu LP, Brandt KD, Capello WN. Oral administration of doxycycline reducescollagenase and gelatinase activities in extracts of human osteoarthritic cartilage.. *Journal of Rheumatology*, 1998; 25: 532-535.
20. Tanaka N, Sakahashi H, Sato F, Hirose K, Ishima TIshi S. Intra-articular injection of high moleculer weight hyaluronan after arthrosclerosis as treatment for rheumatoid knees with with joint effusion. Rheumatology International., 2002; 22(4): 151–154.
21. Takashi O, Emiko Y, Takashi K, Srisomporn P, Thaworn K, et al. Quinic acid esters from Pluchea indica with collagenase, MMP-2 and MMP-9 inhibitory activities. Phytotherapy research., 2008; 22(2): 264-266.
22. Demeule M, Brossard M, Page M. Matrix metalloproteinase inhibition by green teacatechins. *Biochemistry Biophysics Acta*, 2000; 1478: 51–60.
23. Patel, SS and Zaveri, MN. Cytotoxic activity investigation of bioactive compounds from *Justicia gendarussa* using a brine shrimp lethality assay. *Asian Journal of Traditional medicine*, 2012; 7(3): 1-7.