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TRYPSIN AND PROTEIN DENATURATION INHIBITORY ACTIVITY OF DIFFERENT FRACTIONATION AND ISOLATED COMPOUND OF LEAF AND ROOT OF *JUSTICIA GENDARUSSA*

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Justicia gendarussa, Anti-arthritic, Protein denaturation, anti-inflammatory activity, trypsin inhibitory action

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ABSTRACT: Rheumatoid arthritis is a major ailment among autoimmune disorders. A large number of herbal extracts were used for the treatment of various types of rheumatoid disorder. The leaf and root of *Justicia gendarussa* belonging to Acanthaceae family, commonly known as Nilinirgundi is traditionally used for chronic rheumatism. The present study deals with in-vitro anti-arthritic activity in pharmacological models were studied such as inhibition of protein denaturation and trypsin (proteinase) inhibitory activity. Different fractions and isolated compound from methanolic extract of leaf and root of *J.gendarussa* with different concentrations (10,100,1000µg/ml) were studied and results were compared with standard drug Indomethacin. The methanolic extract of leaf and root of *J. gendarussa* showed dose dependent activity which was found comparable to that of standard drug Indomethacin. Isolated compound-La of leaf of *J. gendarussa* inhibited the activity of trypsin with IC_{50} values 34.85 µg/ml as compared to standard drug Indomethacin with IC_{50} value 11.57 µg/ml. Compound-La of leaf of *J. gendarussa* showed protein denaturation inhibition at the IC_{50} value of 24.74 µg/ml as compared to the standard drug Indomethacin with IC_{50} value 19.41 µg/ml.

INTRODUCTION: Rheumatoid arthritis is a form of arthritis that causes pain swelling, stiffness, and loss of function in joints. It is a chronic condition with multiple conditions with multiple causes and defects the people in their most active period of life. The deformities that may develop due to the chronic forms stand as the greatest crippler of mankind [1, 2]. Bone composed primarily of type I collagen, invading synovium causes erosion of contiguous bone via release of prostaglandins and proteases by synovial cells.

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Proteases or proteinases are the proteolytic enzymes which play a vital role in the normal physiological functions of cells e.g. protein maturation, digestion, blood coagulation, control of blood pressure, immune response, etc. A variety of diseases such as cancer, pulmonary emphysema, muscular dystrophy, arthritis, pancreatitis, etc. are associated with the excessive activity of proteases. The role of proteases in diseases therefore provides targets for the possible treatment of a wide range of diseases by protease inhibitors as therapeutic agents from natural sources [3].

It has traditionally been believed that only the human collagenases (matrix metalloproteinase-1, -8, and -13) are capable of initiating the degradation of collagens. Here, we show that human trypsin-2 is also capable of cleaving the triple helix of human cartilage collagen type I [4]. A large number of

herbal extracts are used for the treatment of various types of arthritis. Methanolic extract of leaf [5] and root [6] of *Justicia gendarusa* was found to effective in *in-vivo* anti-inflammatory activities. The alcoholic extract of leaf of *J.gendarussa* was reported for its antiarthritic effect [7].

The leaf of *J. gendarussa* contains alkaloids, flavonoids, saturated steroidal saponins or triterpenoid saponins, amino acids, aromatic amines and potassium salts. The leaf also contain 2-amino benzyl alcohol, 2(2'-amino benzyl amino) benzyl alcohol and their respective 0-methyl ethers [8, 9] friedelin, lupeol while β-sitosterol is present in leaf and root of *J.gendarussa* [10] .

Hence, the present study was aimed to isolate bioactive compound from leaf and root of *J.gendarussa* and to determine antiarthritic effect by using in-vitro pharmacological models by inhibition of protein denaturation and proteinase enzyme activity. Anti-denaturation study was performed by using bovine serum albumin [BSA]. BSA assay eliminates the use of live specimen as far as possible in the drug development process.

When BSA is heated it undergoes denaturation and express antigens associated with type-III hypersensitivity reaction and which related to disease such as serum sickness, golmeralonephritis, rheumatoid arthritis and system lupus erythematosus. Thus, the assay was applied for the discovery of those drugs which can stabilize the protein from denaturation process, several non steroidal anti-inflammatory drugs such as indomethacin, ibufenac. Indomethacin, salicylic acid were used to prevent denaturation of BSA at pathological pH 6.2 to 6.5 [11].

MATERIALS AND METHODS:

Plant materials:

The root and leaf of *Justicia gendarussa* belonging to the family *Acanthaceae* were collected from fully grown flowering plants of Nili nirgundi (*J. gendarussa*) from Anand farm and nursery, Gandhinagar, Gujarat, India in month of January, 2009. The plant was authenticated by a taxonomist Prof. S.K. Patel, Department of Botany, School of Science, Gandhinagar, Gujarat, India. A voucher specimen (PH/509/001) was deposited at the Department of Pharmacognosy K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India. It was further authenticated by comparing their morphological and microscopical characters with reported literature [12-15].

Preparation of extracts and fractions:

The leaf and root powder of *J.gendarussa* (500g) was fractionated by solvent extraction method by soxhlet extractor using petroleum ether, methanol and water successively. These extracts were screened for the presence of phytoconstituents like steroids, triterpenods, alkaloids, saponins, phenolics, flavanoids, etc. It was reported that methanolic fraction shows significant anti-arthritic activity. Therefore, methanolic fraction of leaf and root was further fractionated by toluene, acetone and water.

All fractions of methanolic extract of leaf and root of *J. gendarussa* were standardized using Thin layer chromatography (TLC) for their chemical profile. All fractions and compounds from leaf and root of *J.gendarussa* were studied for its inhibitory action on Protein denaturation and Proteinase enzyme activity.

TLC study of fractions and isolated compound of *J.gendarussa:*

TLC was developed for the fingerprinting of methanolic extracts of leaf and root of *J. gendarussa* with the help of micro liter syringe, the standard or sample solutions of appropriate volume were applied on TLC plate using semiautomatic spotter (Camag Linomat V). The plates were prewashed by methanol and activated at 110^0C for 5 min prior to chromatography. The samples of methanolic extract of leaf and root of *J. gendarussa* were spotted triplicate in the form of bands width 6 mm with a Camag 100 µl syringe on silica gel precoated aluminum plate 60 F_{254}, using sample applicator. A constant application rate of 0.1 l/s was employed and space between two bands was 5 mm.

The slit dimension was kept at 5 mm× 0.45 mm and 10 mm/sec scanning speed was employed. The monochromator bandwidth was set at 20 mm, each track was scanned thrice and baseline correction was used. For methanolic extract of leaf n-butanol: formic acid (4.5:0.5) and for methanolic extract of

root n-butanol: methanol: water: formic acid (2.5:1:1:0.5) was used as mobile phase. Linear ascending development was carried out in 20 cm ×10 cm twin trough glass chamber saturated with the mobile phase.

The optimized chamber saturation time for mobile phase was 30 min at room temperature (25^0C ±2) at relative humidity of 60% ±5. The length of chromatogram run was 8 cm. Subsequent to the development; TLC plates were dried in current of air with the help of air dryer in wooden chamber with adequate ventilation. The flow rate in laboratory was maintained unidirectional.

The resolved bands on TLC plates was observed under UV light at 254 nm and 366 nm and after derivatization with anisaldehyde sulfuric acid reagent followed by heating at 110°C for 5 min. The plate was scanned using Camag 3 TLC scanner and WinCATS software, and chromatogram was recorded at 254 nm.

Physicochemical study of isolated compound:
Isolated compound from toluene fraction of leaf of *J. gendarussa* was screened for solubility and chemical nature.

***In-vitro* assay:**
Trypsin (Proteinase) inhibitory action:
The reaction mixtures (2.0 ml) contained 0.06 mg trypsin, 1.0 ml. 25 mM tris-HCl buffer (pH 7.4) and 1.0 ml of different fractions of leaf and root of *J. gendarussa* (10,100 and 1000 µg/ml of final volume for extracts and 10, 25 and 50 µg/ml for compounds). The mixtures were incubated at 37°C for 5 minutes then 1.0 ml of 0.8% (w/v) casein was added.

The mixtures were incubated for an additional 20 minutes. Then add 2.0 ml of 70% (v/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged and absorbance of the supernatant was read at 280 nm against buffer as blank[16-18].

The percentage of inhibition was calculated as follows.

$$\% \text{ inhibiton} = \frac{\text{Absorbance (control)- Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

Inhibition of protein denaturation:
The reaction mixture (0.5ml) consisted of 0.45 ml of bovine serum albumin (5% aqueous solution) and 0.05ml of *Justicia gendarusa* extract (10,100 and 1000 µg/ml of final volume for leaf and root extracts and 10, 25 and 50 µg/ml for isolated compounds). The pH was adjusted at 6.3 using a small amount of 1N HCl. The sample were incubated at 37°C for 20 min and then heated at 57°C for 3 min after cooling the sample, add 2.5ml of phosphate buffer solution in each test tube. Turbidity was measured at 600 nm for control tests 0.05 ml distilled water was used instead of extracts while product control tests lacked bovine serum albumin[19, 20].

The percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{O.D (control) -O.D of (test)}}{\text{O.D (control)}} \times 100$$

Where, O.D. = Optical Density.

The control represents 100% protein denaturation and the results were compared with standard drug indomethacin.

Statistical analysis:
Each experiment was run in triplicate. Results were reported as mean ± SEM

RESULTS:
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 while steroids, triterpenoids, saponins, carbohydrates and phenolics were present in the root of *J. gendarussa* (**Figure 1**). These are useful for quality evaluation and standardization of *J. gendarussa*.

TLC analysis of Fractions: Based on our TLC analysis observation, it was recognized that the methanolic fraction of leaf having one major compound at R_f 0.28 with 47.39 relative % area. The active toluene fraction also having the same compound which, resolved at the same R_f which showed strong quenching zone at 254nm as shown in **Figure 2** and orange color band with dragendorff's reagent , white to yellow band with

anisaldehyde sulphuric acid reagent and with Folin's phenol reagent as shown in **Figure 2** . The isolated compound was soluble in water, methanol, toluene and n-butanol. It get crystallize as flower shaped needle like crystals in methanol, toluene and n-butanol. Chemically this compound reacts with dragendorff's and ninhydrin reagent that proved it is nitrogenous and basic in nature and amino acid like compounds. It was melted at 225^0C temperature and absorbed in UV at 229 nm. It was not affected by acid hydrolysis, because it was resolved on TLC plate at same R_f and at same wavelength 229 nm with strong quenching.

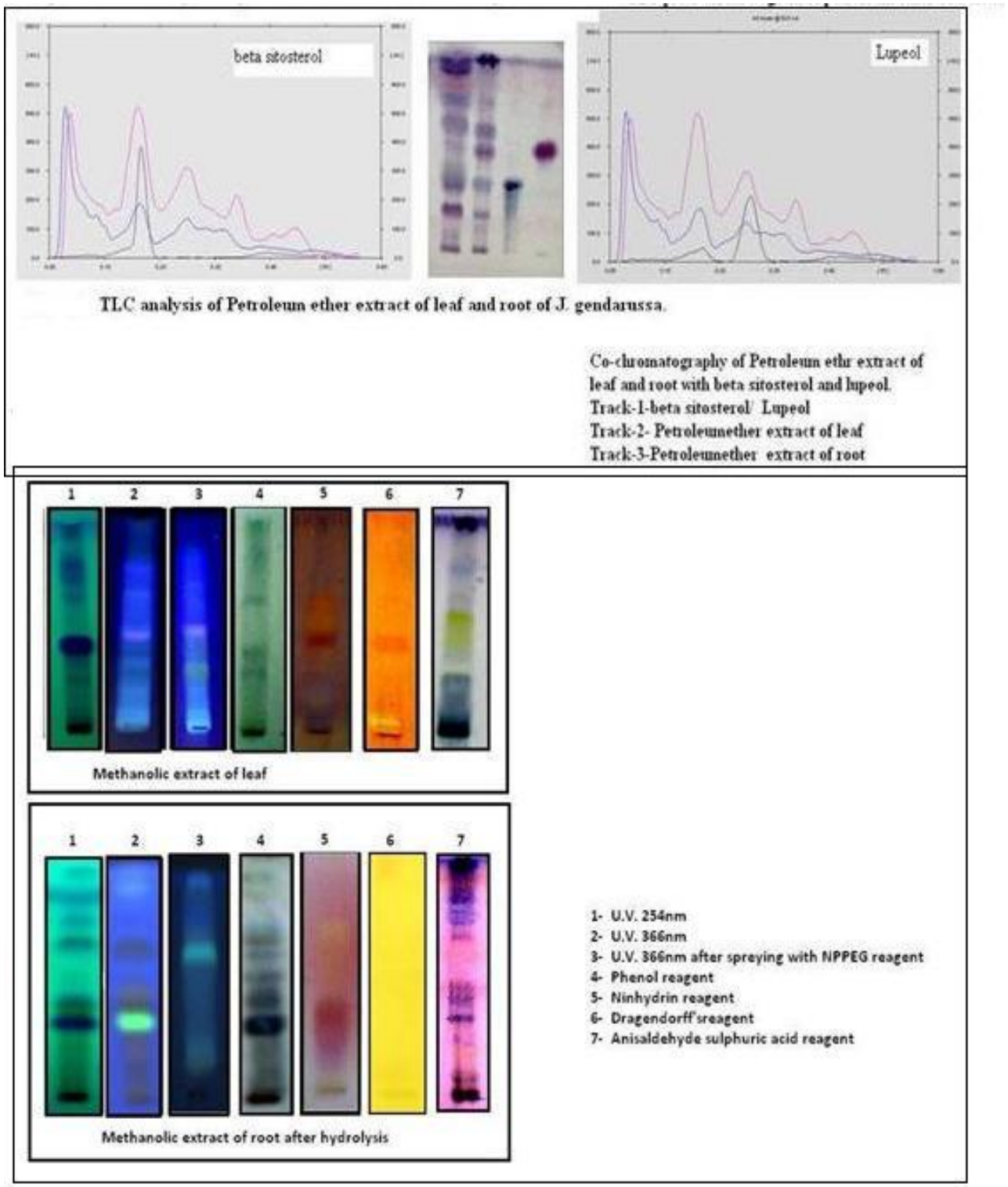

FIGURE 1: TLC ANALYSIS OF DIFFERENT EXTRACTS OF LEAF AND ROOT OF *J.GENDARUSSA*

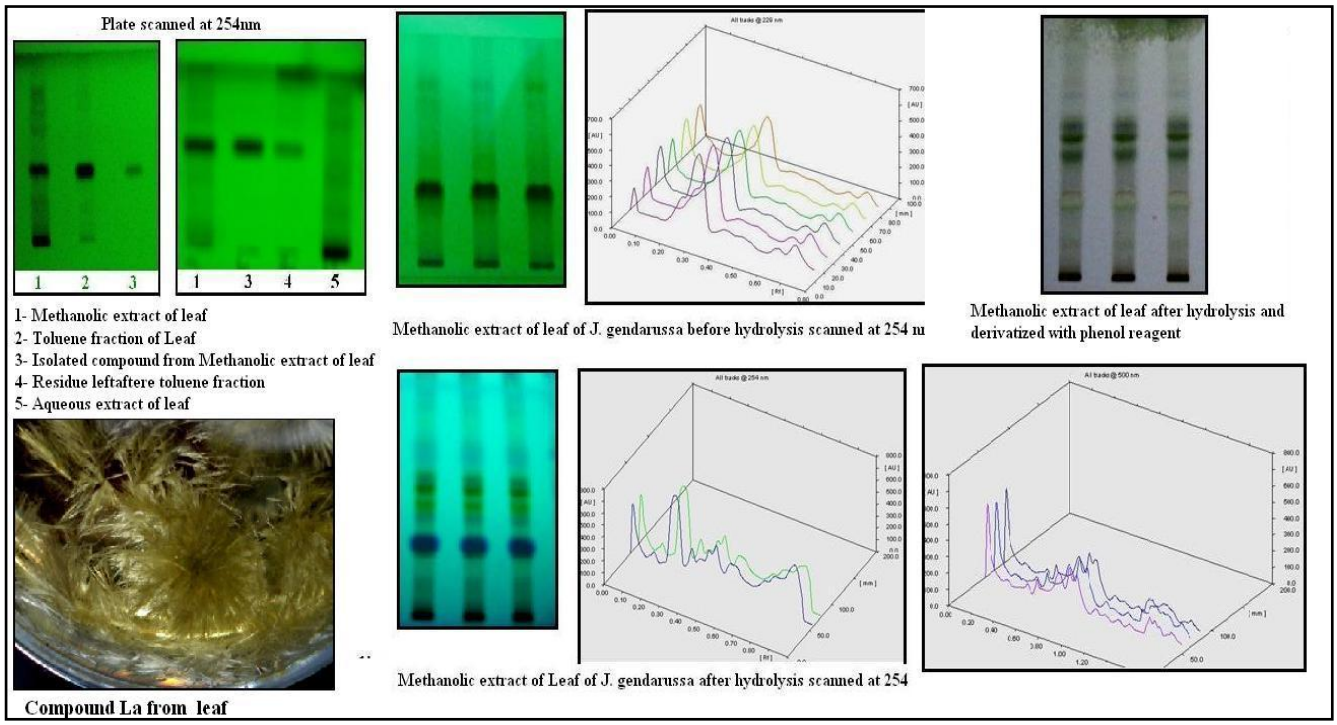

FIGURE 2: TLC ANALYSIS OF METHANOLIC EXTRACT AND FRACTIONS OF LEAF OF *J. GENDARUSSA*

Acetone insoluble fraction of root and its isolated compound [Ra] resolved at R_f 0.45 with strong fluorescence and bluish green band with anisaldehyde sulphuric acid reagent as shown in **Figure 3**. This compound was unstable at normal temperature and soluble in water and methanol. It was affected by acid hydrolysis as it showed fluorescence band at 366nm after hydrolysis while before hydrolysis it was not observed. That proved the compound Ra was glycosidic in nature which hydrolyze in acidic medium to liberate aglycone with strong fluorescence at 366nm.

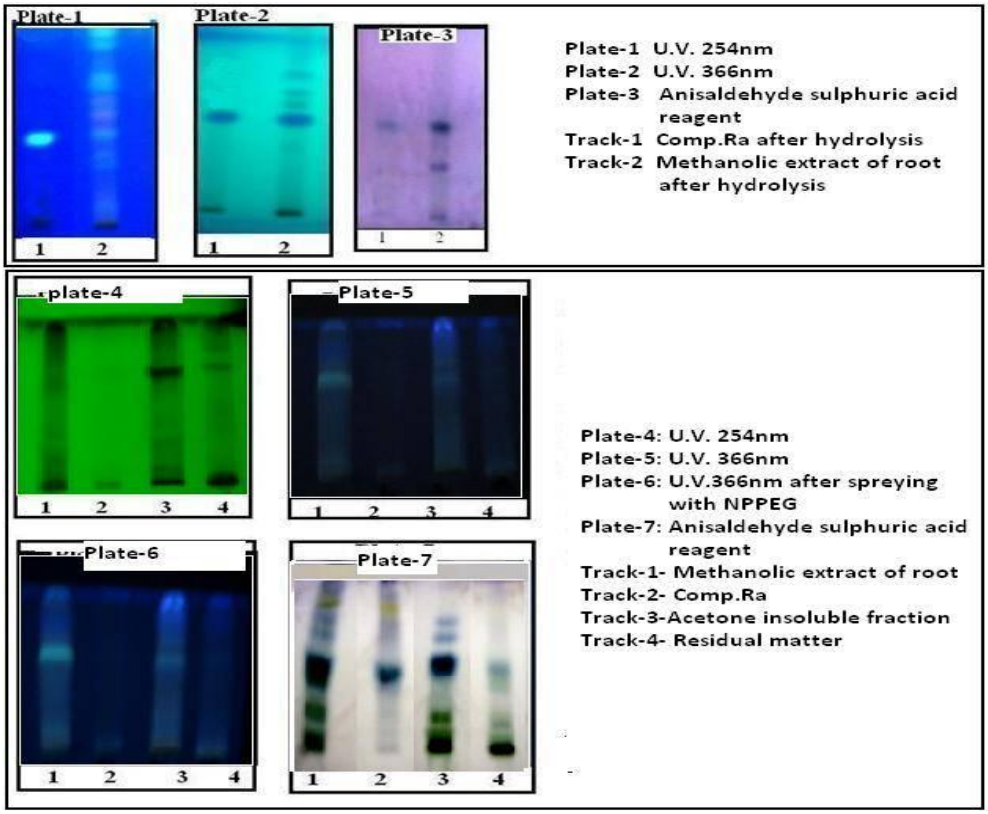

FIG. 3: TLC ANALYSIS OF METHANOLIC EXTRACT AND FRACTIONS OF ROOT OF *J. GENDARUSSA*

Trypsin inhibitory assay [Protease inhibitor assay]:

Trypsin inhibitory assay showed that the toluene soluble fraction of leaf, isolated compound-La of leaf and compound Ra of root of *J. gendarussa* inhibited the activity of trypsin with IC_{50} values 34.85 µg/ml, 13.43µg/ml and 17.15^{T} µg/ml respectively as compared to standard drug Indomethacin 11.57 µg/ml (**Table 1**).

The isolated compound may be non-specific inhibitor of a wide range of proteases. However, an earlier report showed that human trypsin is activated in certain forms of rheumatoid arthritis. Therefore, the trypsin inhibitory activity of the isolated compound may contribute to its chondroprotective activity. This point is reinforced by reports stating that all four classes of proteases, namely, the zinc MMPs, serine proteases, cysteine proteases and the disinterring containing metalloproteinases with thrombospondin motifs (ADAMTS) proteases contribute to the degradation of cartilage matrix, bone resorption and inflammation in chronic arthritis and rheumatism.

TABLE 1: EFFECT OF DIFFERENT FRACTIONS OF LEAF AND ROOT OF *JUSTICIA GENDARUSSA* ON TRYPSIN INHIBITORY ASSAY [PROTEINASE INHIBITORY ACTIVITY]

Test Extracts	IC_{50} µg/ml
L1- Methanolic extract of leaf	0209.01
L2-Toluene soluble fraction of leaf	0034.85
L3-Toluene insoluble fraction	0950.13
L4- Acetone soluble fraction	1292.20
R1-Methanolic extract of root	0054.71
R2- Acetone soluble fraction of root	0340.74
R3- Ether soluble fraction of root	0657.85
Compound La	0013.43
Compound Lb	0025.86
Acetone insoluble fraction of root / Compound Ra	0017.15
Compound Rb	0029.48
Indomethacin	0011.57

Protein denaturation inhibitory assay

Protein denaturation inhibitory assay showed that the methanolic extracts, toluene fraction, compound La of leaf and methanolic extract, compound-Ra of root of *J. gendarussa* are capable of controlling the production of auto antigen and thereby it inhibit the denaturation of proteins. Based on our finding, it was proved that all different fractions having one common compound as shown in Figure. 49 that may be compound La.

Protein denaturation inhibitory assay showed that the IC_{50} value of compound La of leaf and compound-Ra of root of *J. gendarussa* 24.74 and 25.75 µg/ml as compared with standard drug Indomethacin 19.41 µg/ml (**Table 2**).

TABLE 2: EFFECT OF DIFFERENT FRACTIONS OF LEAF AND ROOT OF *JUSTICIA GENDARUSSA* ON INHIBITION OF PROTEIN DENATURATION

Test Extracts	IC_{50} µg/ml
L1- Methanolic extract of leaf	0180.24
L2-Toluene soluble fraction of leaf	0153.25
L3-Toluene insoluble fraction	Inactive
L4- Acetone soluble fraction	Inactive
R1-Methanolic extract of root	0135.80
R2- Acetone soluble fraction of root	1246.00
R3- Ether soluble fraction of root	5024.65
Compound La	0024.74
Compound Lb	0030.16
Compound Ra	0025.75
Compound Rb	0035.23
Indomethacin	0019.41

DISCUSSION: Trypsin inhibitory assay:

Proteases are also secreted from synovial fibroblasts as the pannus invades contiguous bone and cartilage. The proteases act enzymatically to degrade the collagen and proteoglycan matrix of bone and cartilage. This destructive effect is further compounded by IL1 (and TNF) which suppresses synthesis of these matrix molecules. Thus, IL1 provides a "double insult" to connective tissue by both promoting its degradation by inducing synthesis of proteases and preventing its repair by suppressing synthesis of collagen and proteoglycans [21].

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinases which carry in their lysosomal granules many neutral serine proteinases. It was previously reported that leucocyte proteinases play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors. Isolated compound.La of methanolic fraction of leaf of *Justicia gendarussa* exhibited significant anti-proteinase activity.

Our results showed that isolated compound-La of leaf and compound Ra of root of *J. gendarussa* inhibited the activity of trypsin with IC_{50} values

34.85µg/ml, 13.43µg/ml and 17.15µg/ml respectively as compared to standard drug Indomethacin 11.57 µg/ml. This was suggested that these isolated compounds may be specific inhibitors of proteases. As per the earlier report showed that human trypsin is activated in certain forms of rheumatoid arthritis [4]. Therefore, the trypsin inhibitory activity of the isolated compound may contribute to its chondroprotective activity. This point is reinforced by reports stating that all four classes of proteases [22], namely, the zinc MMPs, serine proteases, cysteine proteases and the disintegrin containing metalloproteinases with thrombospondin motifs (ADAMTS) proteases[23] contribute to the degradation of cartilage matrix, bone resorption and inflammation in chronic arthritis and rheumatism.

Inhibition of protein denaturation:

From the result of the present study, it showed that methanolic extracts, toluene fraction and compound La of leaf of *Justicia gendarussa* were capable of controlling the production of auto antigen and thereby it inhibit the denaturation of proteins. The result was compared with the standard drug indomethacin. Based on our finding, it was proved that all different fractions having one common compound, that may be compound La. Therefore, it can be said that effect may be because of this common compound (Comp.La). While methanolic extract and compound Ra of root of *Justicia gendarussa* were capable of controlling the production of auto antigen and thereby it inhibit the denaturation of proteins and its effect was compared with the standard drug indomethacin. Protein denaturation inhibitory assay showed that the IC_{50} value of compound-La of leaf and compound-Ra of root of *J. gendarussa* 24.74 and 25.75 µg/ml as compared with standard drug Indomethacin 19.41 µg/ml.

Most of the investigators have reported that denaturation of the protein is one of the cause of rheumatoid arthritis [24]. Production of auto-antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins [25]. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. From the results of present study it can be stated that Compound La from methanolic extract of leaf of *Justicia gendarussa* is capable of controlling the production of auto-antigens due to in vivo denaturation of proteins in rheumatic diseases.

Hence, our finding justifies the usefulness of *Justicia gendarussa* for the management and treatment of inflammation associated diseases like arthritis. Based on our results, obtained in the present studies, it can be concluded that compound isolated form methanolic extract of leaf of *Justicia gendarussa* possess significant in- vitro anti-arthritic activity which is comparable to synthetic anti-inflammatory agents.

CONCLUSION: The study shows that compounds isolated from leaf and root of *J. gendarussa* having better inhibitory effect on trypsin and good inhibitory effect on protein denaturation. Based on that mechanism these compounds may be active in arthritic condition. So, these findings may helpful to find new lead molecule as antiarthritic drug.

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REFERENCES:

1. Maruotti N, Cantatore FP, Crivellato E, Vacca A, Ribatti D. Macrophages in rheumatoid arthritis. Histol Histopathol. 2007; 22(5): 581–586.
2. Muller-Ladner U, Pap T, Gay RE, Neidhart M, Gay S. Mechanisms of disease: the molecular and cellular basis of joint destruction in rheumatoid arthritis. Nat Clin Pract Rheumato., 2005; 1(2): 102–110.
3. Atta-ur- Rahman, Chaudhary, MI, Thomsan WJ. Bioassay Techniques For Drug Development. Harwood academic publishers. Australia, 2005; pp. 130-133.
4. Stenman M, Ainola M, Valmu L, Bjartell A, Guofeng M, Stenman UH. Trypsin-2 degrades type 2 collagen and is expressed and activated in mesenchymally transformed rheumatoid arthritis synovitis tissue. Am J Pathol, 2005; 167: 1119–1124.
5. Jaijesh P, Srinivasan K, Kaitheri B, Kumar P. Comparing the anti-arthritic activities of the plants Justicia gendarussa Burm F and Withania somnifera Linn. Int J Green Pharm, 2009; 3(4): 281-284.
6. Kavitha SK, Viji V, Kripa K, Helen A. Protective effect of Justicia gendarussa Burm.f. On carrageenan-induced inflammation. J Nat Med, 2010; 65(3-4): 471-479.
7. Jaijesh Paval, Srinivasan KK, Bhagath Kumar P, Sreejith Govinda., Raju S K, Sareesh NN, Sudheer M. Aanti-arthritic potential of the plant Justicia gendarussa Burm. Clinics, 2009; 64(4): 357-360.
8. Wahi SP, Wahi AK, Kapoor R. Chemical study of the leaf of J. gendarussa Burm. J Res Ind Med, 1974; 9(1): 65-66.
9. Chakravarty A, Dustidar P, Pratim G, Prakashi S. Study on Indian Medicinal Plants, part-67. In: Simple aromatic

amines from J. gendarussa. Carbon-13 NMR Spectra of the bases and their analogs. Tetrahedron, 1982; 38(12):1797-1802.
10. Govindachary TR, Jadav SJ, Joshi BS, Kamat VN. Chemical investigation of some Indian plants, part -4. Indian J Chem. 1969; 7: 308.
11. Shravan kumar N, Kishore G, Siva kumar G, Sindhu ES. In vitro anti-inflammatory and anti-arthritic activity of Leaves of Physalis angulatal. Int J Pharm Ind Res, 2011; 1: 3-4.
12. Mehrotra BN, Kandu BC. Pharmacognostic study of leaf and tender shoot of Justicia gndarussa Burm. J Sci Ind Res, 1962; 21: 55-60.
13. Wahi SP, Wahi AK, Kapoor R. Pharmacognostic study on Justicia gendarussa Burm. Res Indian Med, 1974; 9(4): 31-39.
14. Patel S, Kapadia N, Shah B, Shah M. Botanical identification and phytochemical screening of Justicia gendarussa Leaf. Int J Pharm Sci Rev Res, 2011; 9(2): 12-19.
15. Patel S, Zaveri M. Priliminary pharmacognostical study of root of Justicia gendarussa Burm. Asian J Trad Med, 2011; 6(2): 61-72.
16. Kunitz, M. Crystalline soybean trypsin inhibitor-ii-General properties. J Gen physiol, 1947; 30 (4): 291-310.
17. Zhibo Ga., Rona R, Marquardt HX. Protease and Protease Inhibitor Assays Using Biotinylated Casein Coated on a Solid Phase. Anal Biochem, 1999; 268(1): 151–156.
18. Atta-ur- Rahman, Chaudhary MI, Thomsan WJ. The enzyme assay- proteinase assay. Bioassay Techniques for Drug Development, Harwood Acedemic Publisher, Australia, 2005; pp. 130-133.
19. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins especially with some biologically active proteins. J Pharm Pharmacol, 1968; 20: 169-173.
20. Williams L, Connar AO, Latore L, Dennis O, Ringer S, Whittaker JA, Conard J. In-vitro antiarthritic acitivity. West India Med J, 2008; 57(4): 327.
21. Van der Woude D, Houwing-Duistermaat JJ, Toes RE. Quantitative heritability of anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis. Arthritis Room, 2009; 60: 916–923.
22. Evans, C.H. The role of proteinases in cartilage destruction. Agents Actions Suppl. 1991; 32: 135–152.
23. Porter S, Clark IM, Kevorkian L, Edwards DR. The ADAMTS metalloproteinases. Biochem J, 2005; 386: 15–27.
24. Brown JH, Mackey HK. Inhibition of heat-induced denaturation of serum proteins by mixtures of non-steroidal anti-inflammatory agents and amino acids. Proc Soc Exp Biol Med, 1968; 128: 225-228.
25. Grant NH, Alburn HE, Kryzanauskas C. Stabilisation of serum albumin by anti-inflammatory drugs. Biochem Pharmacol, 1970; 19: 715-722.

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