WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 8, 1693-1702. Research Article ISSN 2277– 7105

PHYTOCHEMICAL SCREENING AND IN VITRO ANTI ELASTASE, ANTI COLLAGENASE ACTIVITY OF *CISSUS QUADRANGULARIS LINN*.

Mrs. S. Shamina[1]*, Ms. Anjana[2] and Dr. S. Suja[3]

[1]Head and Associate Professor, Department of Biochemistry, Rathnavel Subramaniam College of Arts and Science, Coimbatore- 641402, Tamilnadu, India.

[2]II M. Sc Biochemistry, Department of Biochemistry, Rathnavel Subramaniam College of Arts and Science, Coimbatore- 641402, Tamilnadu, India.

[3]Professor and Head, Department of Biochemistry, Bharathiar University, Coimbatore- 641046, Tamilnadu, India.

Article Received on
05 June 2020,

Revised on 25 June 2020,
Accepted on 15 July 2020

DOI: 10.20959/wjpr20208-18197

***Corresponding Author**
Mrs. S. Shamina
Head and Associate Professor, Department of Biochemistry, Rathnavel Subramaniam College of Arts and Science, Coimbatore- 641402, Tamilnadu, India.

ABSTRACT

Cissus quadrangularis Linn. is considered to be one of the important herbal plants in traditional systems. It has medicinal properties with various activities like anti-inflammatory, antioxidant, free radical scavenging, and anti-osteoporosis. Soxhlet method was used for extraction of the medicinal plant using various solvents such as aqueous, ethanol, methanol, chloroform and petroleum ether. The preliminary phytochemical screening carried out to determine the various bioactive compounds present in the different solvents of the plant extracts. The methanolic extract showed the maximum bioactive compounds among the various solvent extracts. The plant extract was evaluated for anti collagenase and anti elastase activity. The plant extract was prepared in the concentration of 100-1000µg and the in vitro anti collagenase and anti elastase assay were evaluated, piroxicam was used as positive control. The anti collagenase assay the plant extract shows the percentage of inhibition as 17.94, 26.92, 50.64, 55.12, and 64.10%. Whereas, the anti elastase assay shows the percentage of inhibition as 20.12, 32.31, 45.12, 52.04 and 57.31% respectively. The results from the anti collagenase and anti elastase assay shows the plant extract has a vibrant ability of protecting the degradation of connective tissue proteins, it includes potential like anti-aging and anti-arthritic activity which can be approved with the

presence of the bioactive compounds like alkaloids, phenols, tannins from the phytochemical screening of the plant.

KEYWORDS: *Cissus quadrangularis*, anti-collangenase, anti-elastase, methanol, phytochemical screening, Soxhlet method.

INTRODUCTION

Cissus quadrangularis is a tender herbal plant belonging to Vitaceae family. It is fleshy and cactus in nature. It is also known in various common names like adamant creeper, square stalked vine, veldt grape, devil's backbone, asthisamharaka, hadjod, pirandai, sannalam, nalleru, vajravelli and mangara valli. It is a native plant in India, Bangladesh and Sri lanka.

The whole plant is used for various medicinal purposes. The stem of the plant is sectioned in quadrangular shape with leathery edges and reaches a height of 1.5meter. The leaves emerge from the nodes which are wide with 2 to 5cm. It has white, yellow or greenish flowers and its berries ripen to red colour (Ayesha Siddiqua et al 2017). The plant need hot or tropic region for growth, which is cultivated in coastal area, plains and wetlands. The stem of the plant is used for propogation (Sandip G Buddhadev et al, 2014).

Cissus quadrangularis is used as an alternative medicine for treating disorders like piles, anorexia, indigestion, chronic ulcers, otorrhoea, wound. The plant is considered of having the curative property of fracture healing and has an abundant source of calcium (Duraipandi Devipriya et al, 2017). In Ayurveda, cissus quadrangularis is known by the name bone setter, due to its potential to join bones. The plant also shows properties like anti-osteoporotic, anti-inflammatory and anti-microbial (Parvathi K et al, 2017).

The mammalian cells are encircled by connective tissues known as extracellular matrix (ECM) which are comprised of three classes of biomolecules: structural protein, specialized proteins and proteoglycans. The ECM is in charge for the protection of the organs and to provide elasticity. Collagen is one of the vital components in the ECM that are made up of 30 distinct polypeptide chains. They may be found in small portions in the tissues, but have essential roles in the physical properties of specific tissues. It provides the tensile strength of the skin. Elastin is a connective tissue protein responsible for the extensibility and elasticity of the tissues. They are not present all over as collagen but are present in large amounts in tissues that require these properties such as lungs, arterial blood vessels and some ligaments.

The skin, ear cartilage and other tissues have a smaller quantity of elastin (Victor W. Rodwell et al, 2018).

Collagenase is metalloproteinase enzyme which denatures the proteins present in the connective tissues. Collagenase cleaves the protein glycoside bond of the collagen fibre and also the synthetic peptides that contain the amino acid sequence which are blocked at any terminus. Elastase belongs to the chymotrypsin family of the proteases which primarily breaks the elastin fibres. These also have the ability to cleave the collagen, fibronectin and other proteins present in the extracellular membrane (Tamsyn SA Thring et al, 2009).

Collagenase and elastase are enzymes which are in charge for the breakdown of various components of the extracellular matrix that are collagen and elastin (Vinita D. Apraj et al, 2016).

The extracellular matrix is involved in various normal and pathologic processes like inflammatory responses and spread of cancer cells. Some reports state that the extracellular matrix components are involved in the disorders like rheumatoid arthritis, osteoarthritis and several other diseases due to the genetic disturbance caused in the synthesis of collagen and elastin fibres (Victor W. Rodwell et al, 2018). Skin aging is another major problem caused due to the over activation of extracellular matrix disruption enzymes, oxidative stress and DNA damage. The disruptive enzymes that are the collagenase and elastase interrupt the structural integrity which can only be by cured by inhibiting the activities of these enzymes from avoiding the formation of wrinkles. The inhibition of these activities can be done by the natural plants and in turn reduce the skin aging, arthritis, and other disorders caused due to the abnormal increase in the collagenase and elastase enzyme activity. (Rukiye Boran, 2018).

The present study determines the secondary metabolities present in the herbal plant along with it, it also evaluates the potential of the herbal plant in protecting the degradation of the proteins that are collagen and elastin present in tissues through the *in vitro* anti collagenase and anti elastase assay.

MATERIALS AND METHODS

Reagents and instruments used

The solvents such as ethanol, methanol, chloroform, petroleum ether and all the reagents used for the phytochemical screening were purchased from Himedia, India. The enzyme, substrate

and chemicals such as *Clostridium histolyticum* collagenase, N-(3-[2Furyl] acryloyl)-Leu-Gly-Pro-Ala [FALGPA], Tricine, Porcine pancreatic elastase (E.C. 3.4.21.36), N-Succinyl-Ala-Ala-Ala-p-nitroanilide [SANA], Tris HCl, Calcium chloride dehydrate were purchased from Sigma-Aldrich. The Readwell Touch- R070NIK, Automatic ELISA plate analyzer, Robonik, India was used for measurement of absorbance.

Collection of the plant

The fresh stem of *Cissus quadrangularis* was collected from Kalampalayam Coimbatore, Tamil Nadu during December and January, 2020. The plant was identified and confirmed by the Department of Botancy, Kongunadu Arts and Science College, Coimbatore.

Preparation of plant extract

The fresh stems of the plant were collected and prepared into thin slices and were shade dried. The thin slices of the stem were ground to a fine powder using a mixer grinder and stored in neat and clean plastic containers without any moisture content in it. The powdered material of the plant was extracted through Soxhlet method with aqueous, ethanol, methanol, chloroform and petroleum ether as solvents. 20grams of plant powder was taken in the Soxhlet apparatus and 200ml of each solvent were taken for the extraction procedure. The setup was maintained in 50-60^{o}C for 2 hours. Once the extract of the sample is prepared it is kept in boiling water bath under 70-80^{o}C for 15mins to evaporate away the solvent from the extract. The dried extract was stored in clean and sterile capped tubes in refrigerator for further use.

Preliminary phytochemical screening

The phytochemical screenings with aqueous, methanol, ethanol, chloroform and petroleum ether extracts of *Cissus quadrangularis* (stem) extract were done by modern method of Peach and Traycey (Peach et al, 1956) to identify the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpinoids, glycosides and phenols (Hatim M Y et al, 2019).

Anti collagenase assay

Collagenase inhibition assay was performed by the method described by Kim et al (Kim et al. 2004). Collagenase (0.8 units/ ml) from *Clostridium histolyticum* and FALGPA (2 mM) the synthetic substrate were used for assay. 25 µl of 50 mM Tricine buffer, 25 µl of test extract and 25 µl of 0.1 units of *Clostridium histolyticum* collagenase enzyme were added to the plate finally 50 µl of 2 mM FALGPA substrate was added and the collagenase activity was

measured immediately at 340 nm using a 96 well micro plate reader. Piroxicam was used as a positive control.

The percentage inhibition of anti collagenase assay is calculated by:

Enzyme inhibition activity (%) = $[(OD_{CONTROL} - OD_{SAMPLE}) / OD_{CONTROL}] \times 100$

Anti elastase assay

Elastase inhibition assay was performed by the method described by Lee et al (Lee et al. 1999). The assay was performed in 0.2 M Tris-HCl buffer at pH 8. 1mg/ml stock was prepared in 0.2M Tris-HCl buffer by dissolving the porcine pancreatic elastase (PE – E.C. 3.4.21.36). The substrate N-Succinyl-Ala-Ala-Ala-pnitroanilide (SANA) was dissolved in buffer (0.8 mM). The test extracts were incubated with the enzyme for 20 minutes before adding substrate to begin the reaction. 50 µl plant extract, 160 µl buffer, 20 µl enzyme and 20 µl substrate was the final reaction mixture. Positive control was piroxicam, while the negative controls were performed using Tris-HCl buffer. The absorbance was measured immediately at 410 nm, using a 96 well micro plate reader.

The percentage inhibition of anti elastase assay is calculated by:

Enzyme inhibition activity (%) = $[(OD_{CONTROL} - OD_{SAMPLE}) / OD_{CONTROL}] \times 100$

Calculation of IC_{50} value

The percentage of inhibition was subjected to calculate the inhibitory concentration of 50% (IC_{50}) value using the online tool, AAT Bioquest Calculator.

RESULT AND DISCUSSION

Preliminary phytochemical screening

The phytochemical screening with aqueous, ethanol, methanol, chloroform and petroleum ether extract of *Cissus quadrangularis* showed to possess secondary metabolites which are deposited in table.

Table No. 1: Phytochemical screening of *Cissus quadrangularis* (STEM).

S. no.	Test	Aqueous	Ethanol	Methanol	Petroleum ether	Chloroform
1	Alkaloids	-	-	++	-	-
2	Flavanoids	+	-	-	+	-
3	Tannins	-	+	+	-	-
4	Terpenoids	-	+	++	-	-
5	Saponins	-	-	+	-	-
6	Glycosides	-	-	++	-	-
7	Proteins	+	+	+	-	-
8	Carbohydrates	+	+	++	+	+
9	Phenols	-	+	++	-	+

Preliminary phytochemical screening of the *Cissus quadrangularis* stem extract was studied and it was found highest in methanolic extract when compared to the other prepared extracts. The phytochemical analysis with methanolic extract of *Cissus quadrangularis* showed the active presence of alkaloids, tannins, terpenoids, saponins, glycosides, proteins, carbohydrates and phenols. Hence, the methanolic extract of the stem was chosen for further research.

Anti collagenase assay

A family of proteolytic enzymes called Matrix metalloproteinases (MMPs) degrade various components of the ECM. One of the members of the MMP families is collagenase which is responsible for the degradation of collagen. Collagenase from the bacteria *Clostridium histolyticum* (ChC) is one of the few proteinases capable of degrading the triple‑helical region of native collagen under physiological conditions. In contrast, in *in vitro* conditions synthetic peptides are used as substrates (Vinita D. Apraj et al, 2016). The protective effect of plant extracts can be studied against collagenase enzyme using ChC and FALGPA substrate.

In the present study, collagenase inhibition activity was evaluated for the methanol extract of *Cissus quadrangularis* at varying concentrations from 100-1000µg. Piroxicam was used as a positive control.

Table no. 2: Inhibition of collagenase enzyme.

S. no.	Concentration (µg)	Standard % (Piroxicam)	Test (%)(methanol extract CQ)
1	100	23.71	17.94
2	200	33.97	26.92
3	400	52.56	50.64
4	800	68.58	55.12
5	1000	83.33	64.10

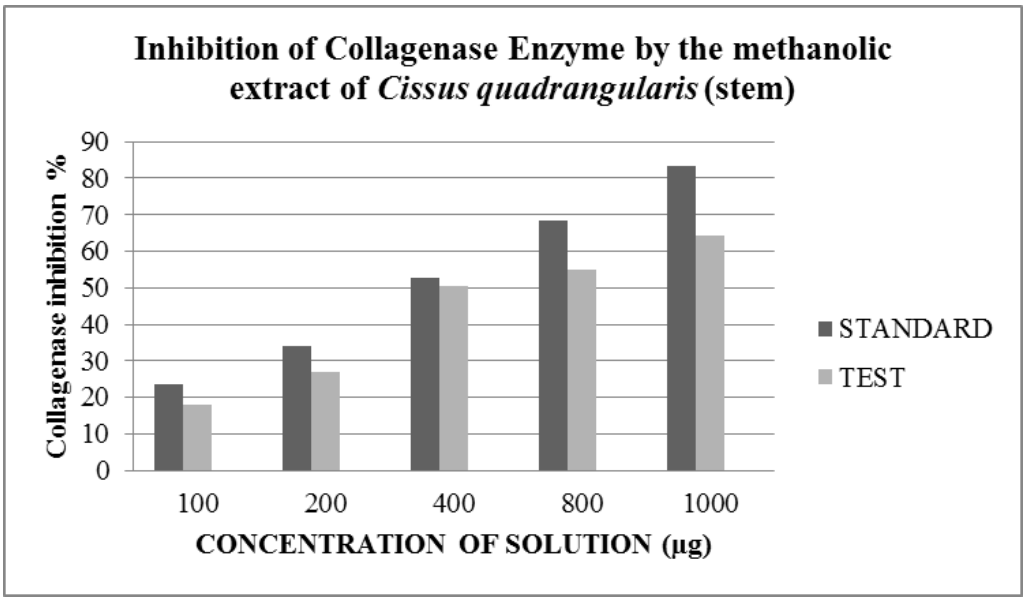

Fig. no. 1: Inhibition of collagenase enzyme by the methanolic extract of cissus quadrangularis (stem).

The inhibition of collagenase enzyme by the piroxicam and the methanol extract of Cissus quadrangularis results are deposited in table no.2. The results shows that piroxicam has an increasing inhibition of collagenase enzyme as the concentration level increases, that is the inhibition rate is 23.71% at 100µg, 33.97% at 200µg, 52.56% at 400µg, 68.58% at 800 µg and 83.33% at 1000µg. Both the positive control (piroxicam) and the plant extract exhibits utmost inhibition of the collagenase enzyme at a concentration of 1000 µg. The methanol extract of *Cissus quadrangularis* illustrate a similar increase in the inhibition of collagenase enzyme with the concentration hike that is 17.94% at 100µg, 26.92% at 200µg, 50.64% at 400µg, 55.12% at 800 µg and 64.12% at 1000µg. The inhibition concentration of 50% (IC_{50}) value calculated for piroxicam is 393.33 and 402.879 for the methanol extract of the plant.

Table no. 3: IC_{50} values of Anti-collagenase and Anti-elastase Assays.

Test performed	Ic_{50} value	
	Standard	Plant extract
Anti collagenase assay	393.33	402.879
Anti elastase assay	372.695	579.242

Anti elastase assay

The only enzyme which is capable of degrading elastin is Elastase. Inhibiting elastase enzyme can keep the elasticity and flexibility of skin (Vinita D. Apraj et al, 2016). In anti elastase assay, porcine pancreatic elastase was assayed using N-Succinyl-Ala-Ala-Ala-pnitroanilide (SANA) as a substrate. Piroxicam was used as a positive control.

Table no. 4: Inhibition of elastase enzyme.

S. no.	Concentration (µG)	Standard % (Piroxicam)	Test (%) (Methanol extract cq)
1	100	37.80	20.12
2	200	44.51	32.31
3	400	51.82	45.12
4	800	56.70	53.04
5	1000	67.07	57.31

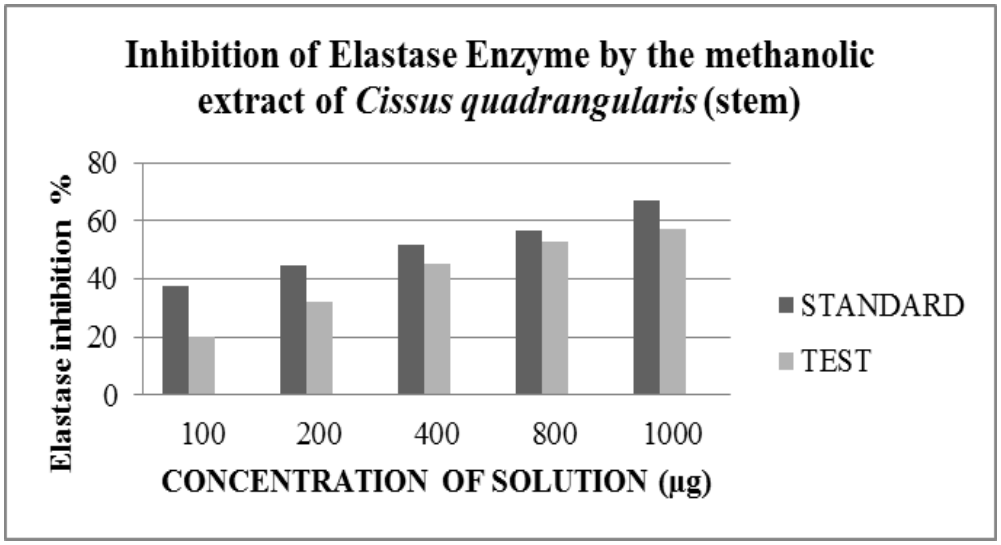

Fig. no. 2: Inhibition of elastase enzyme by the methanolic extract of cissus quadrangularis (stem).

The results from the anti elastase assay demonstrates that the positive control, piroxicam has an accelerating inhibition rate, 37.80% at 100µg, 44.51% at 200µg, 51.82% at 400µg, 56.70% at 800 µg and 67.07% at 1000µg with the increase in concentration level. Similarly, the methanol extract of *Cissus quadrangularis* exhibits raise in the inhibition of elastase enzyme as increase in its concentration that is 20.12% at 100µg, 32.31% at 200µg, 45.12% at

400µg, 53.04% at 800 µg and 57.31% at 1000µg. Piroxicam and the Cissus quadrangularis extract display peak inhibition of elastase enzyme at 1000 µg concentration. The inhibition concentration of 50% (IC_{50}) value calculated for the positive control was found to be 372.695 and 579.242 for the plant.

CONCLUSION

The herbal plant *Cissus quadrangularis* is reported of having anti-aging from former studies. The *in vitro* anti collagenase and anti elastase studies concluded that the plant extract has a potential effect of preventing the denaturation of connective tissue proteins. Therefore the plant can be used for anti-arthritic studies. Likewise *in vivo* anti-arthritic investigation is required for confirming the medicinal value of the plant.

REFERENCES

1. Ayesha Siddiqua and Sirisha Mittapally, A review on Cissus quadrangularis, The Pharma Innovation Journal, 2017; 6(7): 2277- 7695, 2349-8242, 329-334.
2. Sandip G Buddhadev , Mrs. Sheetal S. Buddhadev, A Review Update On Plant Cissus Quadrangularis L., An International Peer Reviewed Ayurved Journal, 2348; 1846, 2(4): 1-10.
3. Duraipandi Devipriya, Selvaraj Mohana Roopan, Cissus quadrangularis mediated ecofriendly synthesis of copper oxide nanoparticles and its antifungal studies against Aspergillus niger, Aspergillus flavus, Materials Science and Engineering C, 2017; 0928-4931: 38-44.
4. Parvathi K, Amit G. Krishnan, Anitha A, R. Jayakumar ∗, Manitha B. Nair, Poly(L-lactic acid) nanofibers containing Cissus quadrangularis induced osteogenic differentiation in vitro, International Journal of Biological Macromolecules, 2017; 8: 0141-8130.
5. Victor W. Rodwell, David A. Bender, Kathleen M. Botham, Peter J. Kennelly, P. Anthony Weil, Harper's Illustrated Biochemistry, McGraw-Hill Education, 2018; 31(50).
6. Vinita D. Apraj, Nancy S. Pandita, Evaluation of Skin Anti‑aging Potential of Citrus reticulata Blanco Peel, Pharmacognosy Research, 2016; 8(3): 160-168.
7. Tamsyn SA Thring, Pauline Hili and Declan P Naughton, Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants, BMC Complementary and Alternative Medicine, 2009; 9(27): 1472-6882, 1-11.
8. Rukiye Boran, Investigations of anti-aging potential of Hypericum origanifolium Willd. for skincare formulations, Industrial Crops & Products, 2018; 0926-6690, 290-295.

9. Peach K, Tracey M V, Modern Methods of Plant Analysis, Springer Verlag Berlin, 1956; 13: 25-125.
10. Hatim M Y and Makhawi M A, Phytochemical Screening of Leaves and Roots of Stylochiton Borumensis: A Medicinal Plant, Earth and Environment Science Research and Reviews, ISSN 2639-7455, 2019; 2(1): 1-5.
11. Kim YJ, Uyama H, Kobayashi S., Inhibition effects of (+)‐catechin‐aldehyde polycondensates on proteinases causing proteolytic degradation of extracellular matrix. Biochem Biophys Res Commun, 2004; 320: 256‐61.
12. K K Lee, J H Kim, J J Cho and J D choi, Inhibitory effects on elastase activity and their anti-inflammatory effects, International Journal of Cosmetic Science, ISSN 0142-5463, 1999; 71-82.