WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Evaluation of *In-vitro* Anti-Rheumatic Arthritis Activity of Aqueous and Ethanolic Extract of *Caesalpinia Bonducella* Linn. Leaves

Rohit Jatav, Rekha Gour

Swami Vivekanand College of Pharmacy, Indore (M. P.), India
E- mail address - gourrekha5@gmail.com

ABSTRACT

The present study deals with the *in-vitro* anti-arthritic activity in ethanolic extracts and aqueous extract of leaf plant parts of *Caesalpinia bunducella*. The previous phytochemical analysis of ethanolic and aqueous extract of *Caesalpinia bunducella* has indicated the presence of several physiologically active phytochemicals such as phenols, flavonoids, triterpenoids, steroids, alkaloids etc. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, prompted us to check leaf part of *Caesalpinia bunducella* for *in-vitro* anti-arthritic activity by the inhibition of protein denaturation method and albumin denaturation method. The ethanolic extracts and aqueous extract of leaf part of *Caesalpinia bunducella* exhibited notable and remarkable anti-arthritic action. The inhibition of protein denaturation was found to be maximum in leaves extract (84.51% at a dose of 500µg/ml) and that of albumin denaturation was also found to be maximum in leaves extract (87.55% at a dose of 500µg/ml) as compare to other in vitro (leaves) plant parts. Therefore, our studies support the isolation and the use of active constituents from in vitro plant parts of *Caesalpinia bunducella* in treating rheumatism.

INTRODUCTION

Caesalpinia bonducella L. Bonduc nut and Nicker nut are members of the Caesalpiniaceae family and have been mentioned in folklore medicine and ancient Ayurvedic teachings. *C. bonducella* has been known to be used by Siddha practitioners in Malabar regions for psoriasis treatment. *C. Bonducella* is a big thorny shrub endemic to South India, Burma, and Ceylon, notably near the sea shore and up to 2500 feet in elevation in hilly regions. [3-4]

Herbal medicine has had considerable progress in the previous few decades. It is gaining popularity in both developing and developed countries because to its natural nature and the fact that it has fewer adverse effects. Herbal remedies provide a lot of drugs for the treatment of internal diseases which are considered to be stubborn and incurable by other system of medicines [5-7].

Arthritis is an autoimmune disorder characterized by pain, swelling, and inflexibility [6]. Rheumatoid joint inflammation influences more or less 1% of the populace around the world. Its etiology is still obscure [7]. However, advances in understanding the pathogenesis of the disease have raised the development of new therapeutics, with improved outcomes. Rheumatoid arthritis may quickly progress into a multisystem inflammation with irreversible joint destruction and increase the risk of humanity [8-10].

MATERIALS AND METHODS

Materials

Drugs and chemicals: All reagents procured were analytical grade.

Plant collection

Fresh leaves of *Caesalpinia bonducella* Linn was collected from Indore and authenticated by botanist. The leaves of *Caesalpinia bonducella* was dried and then crushed into fine powder using laboratory homogenizer, then stored for further use.

Preparation of plant extracts

The crude drugs were extracted with methanol *Caesalpinia bonducella* EECB and Aqueous *Caesalpinia bonducella* water AECB as a solvent using the Soxhlet apparatus for continuous hot extraction. To separate the solvent and residue, the extract was filtered and evaporated. The semisolid residue thus obtained was stored in desiccator until further use.

***In vitro* anti-arthritic activity**

Inhibition of Protein Denaturation (bovine serum method): Tissue protein denaturation is a well-known cause of inflammatory and arthritic disorders. Production of the autoantigen in certain arthritic diseases may be due to denaturation of protein *in vitro*. Agents that prevent protein denaturation may thus be useful in the development of anti-arthritic drugs. According to some studies, protein denaturation and macroglobulin production cause proteins to become antigenic, triggering an immune response and causing metabolic alterations in connective tissue, which eventually leads to rheumatoid arthritis.[10-14].

Methods

The following three solutions were used.

Test solution

0.5 ml of test solution consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of extracts in various concentrations (100, 200, 300, 400, and 500 µg/ml).

Test control solution

0.5 ml of test control solution consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of distilled water.

Standard solution

0.5 ml of standard solution consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of Indomethacin solution (100 µg/ml).

The pH of the above solutions was adjusted to 6.5 using a small amount of 1N HCl. The samples were incubated at 37°C for 20 min and heated at 57°C for 3 min which were cooled, and 2.5 ml of phosphate buffer (pH 6.3) was added to it. Control represents 100% proteins. Their absorbance was measured at 660 nm using a pure blank after cooling. Indomethacin (standard drug) was utilized as a reference medication and was handled as such for absorbance determination. The following % inhibition of protein denaturation was calculated:

$$\text{Percentage inhibition} = \frac{\text{OD control - OD sample}}{\text{OD control}}$$

Inhibition of albumin denaturation (egg albumin) [15, 18]

Methodology

The following three solutions were used.

Test solution

5 ml of test solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffer saline and 2 ml of in various concentrations of extracts (100, 200, 300, 400, and 500 µg/ml).

Test control solution

5 ml of test control solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffered saline and 2 ml of distilled water.

Standard solution

A typical solution of 5 mL contains 0.2 mL of egg albumin and 2.8 mL of phosphate buffer saline and Indomethacin 100 µg/ml. The pH of the above solutions was adjusted to 6.4 using a small amount of 1N HCl. The samples were incubated at 37°C for 20 min and heated at 70°C for 5 min denaturations, and the results were compared with standard Indomethacin.

Their absorbance was measured at 660 nm using a pure blank after cooling. Indomethacin (standard drug) was utilized as a reference medication and was handled as such for absorbance determination. The following % inhibition of protein denaturation was calculated:

$$\text{Percentage inhibition} = \frac{\text{OD control - OD sample}}{\text{OD control}}$$

RESULTS AND DISCUSSIONS

***In vitro* anti-arthritic activity**

Hence, the results of our study reveal that extracts of *Caesalpinia bonducella* were capable of controlling the production of autoantigens and inhibit denaturation of protein especially denaturation of albumin. The present studies indicate that extracts of *Caesalpinia bonducella* exhibit strong anti-arthritic property which could be a potential source of anti- arthritic property.

The inhibition of protein denaturation and albumin denaturation was studied to establish the mechanism of anti-arthritic activity of *Caesalpinia bonducella*. Therefore, our *in vitro* studies on the extract of *Caesalpinia bonducella* demonstrated the significant anti-arthritic activity. As a result, this mangrove plant has the potential to be a powerful natural anti-arthritic agent. The results showed that the extracts of *Caesalpinia bonducella* exhibited

anti-arthritic activities might be due to the presence of active principles such as polyphenolic content, triterpenoids, alkaloids, and flavonoids. From the results of the study, it can be concluded that the leaves extract of *Caesalpinia bonducella* possessed significant anti-arthritic property in EECB than compared to AECB. The present study revealed the potential of plant extract in the management of inflammation and arthritis confirming the folk core use of medicinal plants. However, by careful experimentation, one should try to find out extract more as having much better activity in the search of active candidate or chemical molecule that is primarily accountable for this activity. (Table 1)

Table 1: Anti-arthritic activity of *Caesalpinia bonducella* by bovine and egg serum albumin method

Effect of herbal extracts in different concentration	**(%) Inhibition by bovine serum method**	**(%) Inhibition by egg albumin method**
Control	----	----
Indomethacin 100 µg/ml	90.21	88.71
Aqueous extract of *Caesalpinia bonducella*		
100 µg/ml	26.67	15.22
200 µg/ml	39.66	19.53
300 µg/ml	46.52	28.42
400 µg/ml	62.00	50.74
500 µg/ml	84.51	82.25
Ethanol extract of *Caesalpinia bonducella*		
100 µg/ml	36.76	20.87
200 µg/ml	56.47	29.65
300 µg/ml	65.41	38.66
400 µg/ml	70.05	49.38
500 µg/ml	87.55	84.35

Statistical analysis

All the results were expressed as mean ± standard deviation, and all the grouped data were statistically evaluated with Graph Pad prism. One-way analysis of variance was used to evaluate hypotheses, followed by the least significant difference test.

CONCLUSION

This is the first comparative *in-vitro* study on anti-arthritic activities in leaves extract of *Caesalpinia bunducella*. The ethanolic extract and aqueous extract of the leaves of *Caesalpinia bunducella* showed maximum anti-arthritic activities as compared to standard Indomethacin solution. The plant contains many secondary metabolites e.g. flavonoids, sitosteroids, alkaloids, tri-terpenoids and phenolics. Hence proper isolation of the active principles might help in the findings of new lead compounds in the fields of anti-arthritic drug research. This established a significant scope to develop a broad spectrum use of *Caesalpinia bunducella* in herbal medicine and as a base for the development of novel potent drugs against arthritis.

REFERENCES

1. Manikandaselvi S, Vadivel V, Brindha P. Caesalpinia bonducella L. A nutraceutical plant. Journal of Chemical and Pharmaceutical Research. 2015;7(12):137-42.

2. Kannur DM, Paranjpe MP, Sonavane LV, Dongre PP, Khandelwal KR. Evaluation of Caesalpinia bonduc seed coat extract for anti-inflammatory and analgesic. J Adv Pharm Technol Res. 2012; 3(3):171-5.

3. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. New York: Springer Science+ Business Media. 2007; 107-8.

4. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products; Raw Materials and Industrial Products, Part II. In "C." B. N. Sastri. New Delhi, India: Council of Scientific and Industrial Research. 1950; 2:1951.

5. P Amudha, N Pushpa Bharathi, V Vanitha**, "***Caesalpinia bonducella* – A review on pharmacological and phytochemical activity of seeds", International Journal of Pharma and Bio Sciences, 2016 Oct, 7(4): (B), pg. no. 674 – 680.

6. Shukla Shruti, Mehta A, Mehta P, Vyas S P, Shivaprasad H N, "In vivo immunomodulatory activities of the aqueous extract of bonduc nut *Caesalpinia bonducella* seeds", Pharmaceutical Biology taylor and francisco, January 2010; 48(2), pg. no. 227-230.

7. Manikandaselvi S, Vadivel V, Brindha P, "Studies on Nutraceutical Properties of *Caesalpinia bonducella* L.: An Indian Traditional Medicinal Plant", Research Journal of Medicinal Plant, January 2016, 10 (2), pg. no. 127.139.

8. J Karthi, M Thamizhmozhi, C Saravanan, K Ayas Ahamed, K Niruban Chakravarthy, "In vitro anthelmintic activity of leaves extracts of *Caesalpinia bonducella*(L)", Scholars Research Library, 2011: 3 (4), pg. no. 317-319.

9. Sanjay Jain, Rakesh Barik, Nidhi Yadav and Shivpal Singh, "Evaluation of antitussive activity of leaves of *Caesalpinia bonducella* F. in experimentally induced cough in mice." International Journal of Pharmaceutical Sciences and Research, 2013; Vol. 4(1), pg. no. 415-418.

10. Srikanth N, Elumalai A, Eswaraiah MC, Veldi N. An updated review on anti-arthritic medicinal plant. Int J Pharm Sci Rev Res 2012; 2:11-5.

11. Montecucco F, Mach F. Common inflammatory mediators orchestrate pathophysiological processes in rheumatoid arthritis and atherosclerosis. Rheumatology 2009; 48:11-2.

12. Wang M, Li K, Nie Y, Wei Y, Li X. A network study of Chinese medicine xuesaitong injection to elucidate a complex mode of action with multicompound, multitarget, and multipathway. Evid Based Complement Alternat Med 2012; 12:1-10.

13. Kumari CS, Yasmin1 N, Raffiq M, Hussain N, Babuselvam M. *In vitro* anti-inflammatory and anti-arthritic property of *Rhizopora mucronata* leaves. Int J Pharm Sci Res 2016;6:482-5.

14. Lavanya R, Maheshwari SU, Harish G, Raj JB, Kamali S, Hemamalani D. Investigation of *in vitro* anti-inflammatory, anti-platelet and anti-arthritic activities in the leaves of *Anisomeles malabarica* Linn. Res J. Pharm. Biol. Chem. Sci. 2010; 1:746-52.

15. Srividya S, Sridevi G. Anti-arthritic and anti-inflammatory activity of ethanolic leaf extract of *Ormocarpum sennoides*. Int. J. Pharm. Pharm. Sci. 2016; 8:117-21.

16. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J. Pharm. Pharmacol. 1968; 20:169-73.

17. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of anti- inflammatory effect of ashwagandha: A preliminary study *in vitro*. Pharmacogn J, 2012; 4:47-9.

18. Sangeetha M, Kousalya K, Lavanya R, Sowmya C, Chamundeeswari D, Maheswara UC. *In vitro* anti-inflammatory and anti-arthritic activity of leaves of *Clerodendron inerme*. Res J Pharm Biol Chem Sci 2011; 2:822-7.

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.