



## Evaluation *In Vitro* Anti-arthritic Activity of Ethanolic Seed Extract of *Brassica juncea*

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### ABSTRACT

*Brassica juncea* belongs to family Brassicaceae (Mustard family), commonly known as Mustard. It is worldwide Cultivated plant, also known as oil- yielding crop or industrial crop. Seeds and leaves both are edible, seeds are used as spice. *Brassica juncea* were easily available and commonly used in our day-to-day life. *Brassica juncea* is extensively used for Anti arthritis and in the treatment of anti-helminthic, anti-convulsant, anti-cancer, anti-oxidant, anti-fungal, anti-viral, anti-hyperglycaemic, anti-inflammatory activity. The present study aimed at the *in vitro* study on anti-arthritis activity by using ethanolic extract on seeds of *Brassica juncea*. The study includes like Phytochemical Constituents & *in vitro* methods like Egg albumin protein denaturation method and Bovine serum albumin method. The various concentrations of the extract (100, 200, 400, 800, 1000 µg/ml) respectively. The activities were comparable with the standard drug Diclofenac. When the test concentration increases gradually % inhibition also increases by calculating the IC<sub>50</sub> values were observed in the anti-arthritis activity is observed.

**Keywords:** *Brassica juncea*, Diclofenac, denaturation, IC<sub>50</sub>.

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### INTRODUCTION

A commercially significant plant called *Brassica juncea* has long been valued in India for its therapeutic and nutritional properties. The second most popular source of protein meal in the world, behind soyabean, is mustard seed. Indian mustard leaves are useful in minimising the expense of meals for diabetic people who also have concomitant anxiety problems. Due to their non-toxic effects and pharmacological preparations, oils have been utilised in a variety of therapeutic compositions for years.

One of the most prevalent chronic inflammatory diseases and the leading cause of disability worldwide is arthritis. Arthritis and related disorders come in over 100 different varieties. The two most common types are osteoarthritis and rheumatoid arthritis. Synovial joints are primarily affected by joint disorders. One type of arthritis has different symptoms than another. Symptoms can vary from minor in some persons to severe in others. Common symptoms include joint swelling, pain, stiffness, warmth, redness, tenderness, loss of flexibility, limping, bone spurs, discomfort when standing or walking, and fatigue (feeling tired). (1,2,3).

Non-steroidal anti-inflammatory medicines (NSAIDs), corticosteroids, immunosuppressants, disease-modifying anti-rheumatic drugs (DMARD), and more recent biological agents like TNF- and monoclonal antibodies can all be used to treat arthritis. But one of their adverse effects is limiting. Hence, it is important to research complementary and alternative therapies that are effective, powerful, less toxic, and affordable [4].

A typical autoimmune condition known as rheumatoid arthritis is linked to progressive disability, systemic problems, early death, and socioeconomic expenses. Synovial inflammation and hyperplasia, also known as "swelling," autoantibody production (including rheumatoid factor and anti-citrullinated protein antibody, or ACPA), destruction of cartilage and bone (deformity), and systemic features such as cardiovascular, pulmonary, psychological, and skeletal disorders are all characteristics of rheumatoid arthritis [1, 3]. Rheumatoid arthritis has an issue that is partially caused by the body's natural defences. The immune

system stops defending the body and instead begins targeting specific bodily sections for an unidentified reason [4].

**Plant material**

Seed sample (50 gm) of species, *Brassica juncea* belonging to family *Brassicaceae* were collected from the local market of provisional stores in Balaji district (India). The seed samples were further identified and authenticated by

**Drugs and chemicals:**

Diclofenac, ethanol, phosphate buffer, distilled water

**Preparation of Ethanolic extract of *Brassica juncea*:**

The seeds of *Brassica juncea* was extracted by maceration process by using 50g of mustard seeds in 500ml of ethanol at room temperature for 24hrs then the extract should be concentrated were collected by filtration process through Whatman No.1 filter paper where the filtrate should be kept on rotary evaporator, then the concentrates were lyophilized and then stored at 4°C until used. The percentage yield of ethanolic extract of *Brassica juncea* was found be

**Phytochemical analysis:**

Preliminary phytochemical screening of *Brassica juncea* extract revealed the presence of Alkaloids, flavonoids, glycosides, anthracene glycosides, cardiac glycosides, O - C- glycosides, tannins, saponins (i) emulsion (ii) frothing test, proteins, and reducing sugar (Table 1).

**Table 1. Phytochemicals detected in ethanolic extract of *Brassica juncea* seeds**

phytochemicals	Results
Alkaloids	+ve
Flavonoids	+ve
Glycosides	+ve
Anthracene glycosides	-ve
Cardiac glycosides	+ve
O - and C - glycosides	+ve
Tannins	+ve
Saponins (i) Emulsion test (ii) Forthing test	+ve
Proteins	+ve
Reducing sugar	-ve

**Note: +ve = positive =present**

**-ve = Negative= Absent**

**Assessment of In vitro anti-arthritis activity**

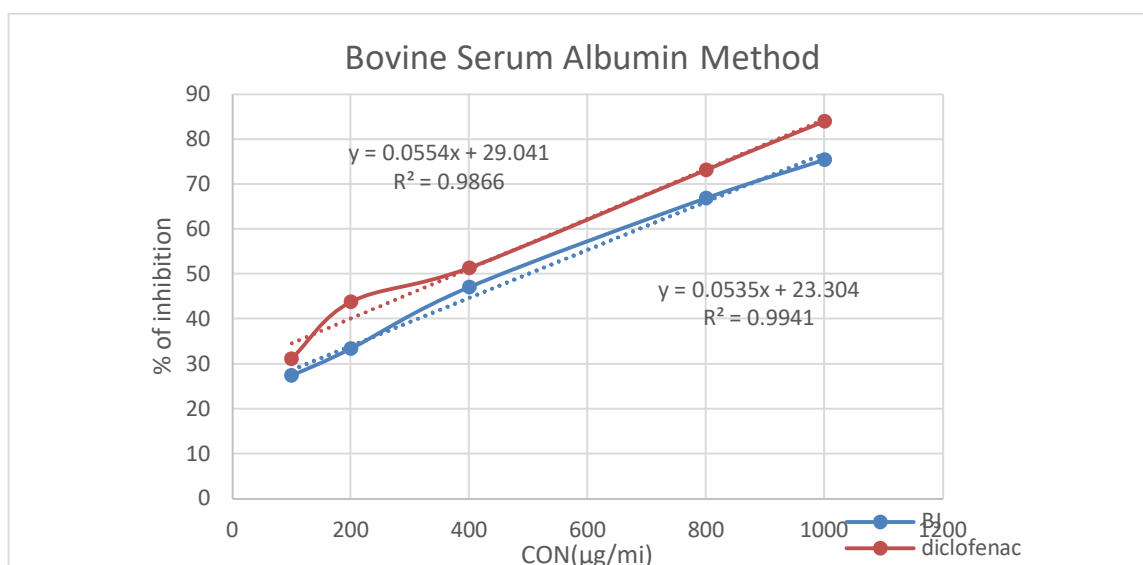
**Bovine serum albumin method:**

In this model assessment of anti-arthritis activity of ethanolic extract of the plant *Brassica juncea* was done for assessing the % inhibition of denaturation. The experiment was carried out by taking both bovine serum albumin and egg albumin. The reaction mixture 0.5ml consists of (0.45ml bovine serum albumin/egg albumin of 5% aqueous solution + 0.05ml of 100-500µg/ml of extract). The pH (6.3) was adjusted by using 1N HCl. Samples were incubated at 37°C for 20min and then heated at 57°C for 3min. After cooling the samples, 2.5ml of phosphate buffer saline (pH 6.3) was added to each tube. The pH of the buffer saline was adjusted to 6.3 using 1N HCl. Absorbance was measured spectrophotometrically at 660nm.

Percentage inhibition of protein denaturation was calculated using the formula

$$\% \text{Inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs control}} \times 100$$

IC<sub>50</sub> value of extract and standard were also calculated.



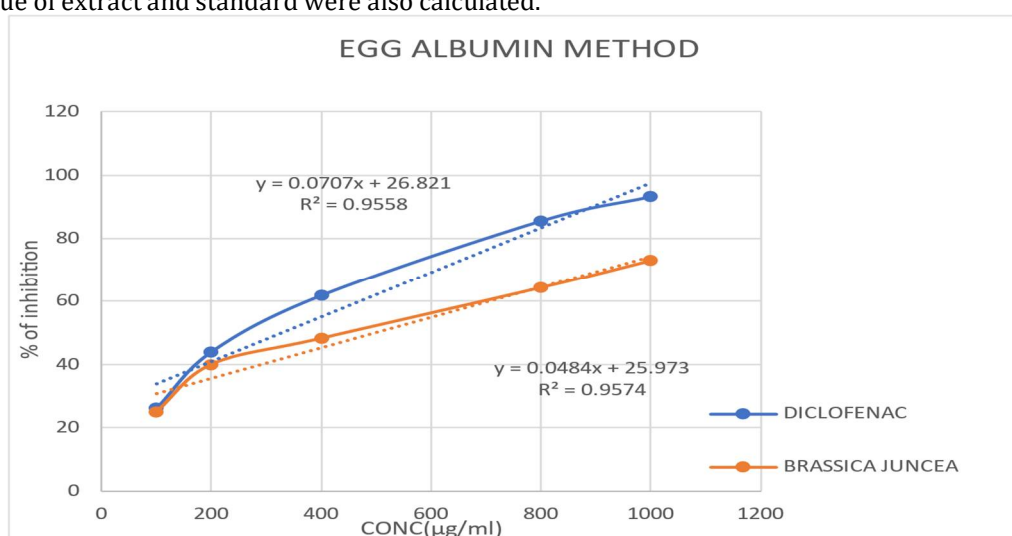
#### EGG ALBUMIN DENATURATION METHOD:

In vitro anti-arthritis activity of ESEBJ was performed by protein denaturation using egg albumin taking diclofenac sodium as standard drug. The method was carried out based on steps followed by [5]. The control solution (5 ml) contains of 0.2 ml of hen's egg albumin, 2.8 ml of freshly prepared phosphate buffer saline (pH 6.3), and 2 ml of distilled water. Standard solution (5 ml) contains of 0.2 ml of hen's egg albumin, 2.8 ml of freshly prepared phosphate buffer saline (pH 6.3), and 2 ml of various concentrations of diclofenac sodium (100, 200, 400, 800, and 1000 µg/ml). Test solution (5 ml) consists of 0.2 ml of hen's egg albumin, 2.8 ml of freshly prepared phosphate buffer saline (pH 6.3), and 2 ml of varying concentration of test extract (100, 200, 400, 800, and 1000 µg/ml). The pH of the all the samples was adjusted to 6.3 using 1 N HCl. The samples were incubated at room temperature for 15–30 min followed by heating at 70°C for 5 min. Then, the samples are cooled and the turbidity was measured spectrometrically at 660 nm. The control represents 100% protein denaturation. Each group consists of triplicates number of samples for statistical analysis. IC<sub>50</sub> value was determined for both standard and test treated groups. The percentage inhibition of protein denaturation was calculated as follows.

#### FORMULA:

$$\% \text{ inhibition of protein denaturation} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

IC<sub>50</sub> value of extract and standard were also calculated.



In vitro anti-arthritis activity of egg albumin method was carried out by taking the ESEBJ at various concentrations (100, 200, 400, 800, 1000 µg/ml). The higher % inhibition was shown at 73.08% at 1000 µg/ml concentration compared with standard drug (diclofenac sodium) at the higher % inhibition

was shown at 93.27% at 1000µg/ml concentration. IC50 values of ethanolic seed extract of *brassica juncea* was found to be 496.42µg/ml, while that of standard was found to be 327.85µg/ml.

*In Vitro* anti-arthritis activity of bovine serum albumin method was carried out by taking the ESEBJ at various concentrations (100, 200, 400, 800, 1000µg/ml). The higher % inhibition was shown at 75.47% at 1000µg/ml concentration compared with standard drug (diclofenac sodium) at the higher % inhibition was shown at 84.07µg/ml. IC50 values of ethanolic seed extract of *brassica juncea* was found to be 498.99µg/ml, while that of standard was found to be 378.32µg/ml.

Protein denaturation is one of the main causes for the inflammatory conditions and involves alteration in electrostatic, hydrogen, hydrophobic, and disulfide bonding [7]. ESEBJ found to be effective in inhibiting protein denaturation, suggesting that it has good anti-arthritis activity like diclofenac sodium [6]. *In vitro* anti-arthritis activity of seeds of *brassica juncea* may be due to the presence of sinapic acid is a phenolic acid that belongs to the family of hydroxycinnamic acids. Sinapic acid can reduce the severity of arthritis by suppressing the production of pro-inflammatory cytokines and other inflammatory mediators such as nitric oxide, interleukin-6 and tumor necrosis factor-alpha.

## CONCLUSION

Arthritis is the inflammation of the joints usually it can affect one joint or multiple joints. The safety and pharmacological profile of the available anti-arthritis drugs are not so promising which may leads to further complications. The seeds of the *Brassica juncea* showed potential anti-arthritis activity compared to standard drug (Diclofenac sodium). The anti- arthritis activity of seeds of *Brassica juncea* was due to the presence of alkaloids, flavonoids, glycosides, saponins, etc. Hence it could be beneficial and further pharmacological studies were required to find out the exact bioactive compound responsible for this activity and also to find out the mechanism for *in vitro* anti-arthritis activity.

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