Research Article

DETERMINATION OF ANTI-ARTHRITIC ACTIVITY OF THE LEAVES EXTRACTS OF PROSOPIS JULIFLORA (SW) DC BY USING IN VITRO METHOD

Nargis Saharan[1], Muhammad Akram Khatri[2], Geeta Kumari[2], Fozia Panhwar[2], Shahbibi Syed[2], Muhammad Ali Ghoto[1], Rabia Mangi[1]

[1]Department of Pharmacy Practice, University of Sindh, Pakistan, [2]Department of Pharmacology, Faculty of Pharmacy, University of Sindh, Jamshoro, Pakistan

Correspondence:
Nargis Saharan
Department of pharmacy practice, university of Sindh, Pakistan
Email:nargissaharan@yahoo.com

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ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune inflammatory condition with an unknown aetiology that causes cartilage and bone to erode. The ineffectiveness and side effects of the current therapeutic agents, such as glucocorticoids, disease-modifying anti-rheumatic drugs, and non-steroidal anti-inflammatory drugs (NSAIDs), highlight the need for finding new agents with more significant therapeutic potential and fewer side effects. The plant Prosopis Juliflora, also known as mesquite and a member of the Fabaceae family, has been used as a general remedy for a variety of ailments, including catarrh, diarrhoea, dysentery, wound healing, flu, measles, hoarseness, infection, and sore throat. The anti-arthritic effect of P. juliflora leaves of plant was subjected to four extraction methods: maceration, sonication, soxhalation and reflux extraction. In vitro study was conducted by using a protein denaturation assay. The IC50 values of macerated, PJE, PJE50 and PJA extracts were 355.02, 302.8, and 230.1 µg/mL, respectively. The IC50 values of the sonicated crude extracts, i.e., PJE, PJE50 and PJA, were calculated as; 3792.2, 1129.4 & 44.91 µg/mL, respectively. The IC50 values of refluxed crude extracts PJE, PJE50 and PJA were calculated as; 250.35, 337.80 & 130.36 µg/mL, respectively. The IC50 value of soxhlated crude extract PJE was determined as 160.50 µg/mL. The result shows that all the extracts possessed protein denaturation-inhibiting activity. Among all crude extracts, the PJA extract made by the sonication method is the most potent with the lowest IC50 value.

Key Words: Rheumatoid arthritis, Prosopis juliflora, Leaves, in vitro, anti-arthritic, protein denaturation

INTRODUCTION

Worldwide, arthritis affects millions of people, severely limiting their ability to get on with their daily lives and contributing to musculoskeletal imbalances (1, 2). It is a term frequently used by health care professionals to describe the progressive inflammatory condition in one or more joints brought on by various factors, such as traumatic, rheumatic, and degenerative concerns that can result in muscle stiffness and restricted physical movement(3). Rheumatoid arthritis is a degenerative disease affecting people of all ages, races, gender, and geographical regions (4, 5). The patient's clinical symptoms can range from mild pain and swelling to severe forms like total or partial joint immobility, muscular atrophy, and contractures (2). Non-steroidal anti-inflammatory drugs (NSAIDs) are typically administered to these patients as a first line of treatment as part of the medication regimen, but their prolonged use can led to some potential side effects, including gastroduodenal diseases and renal insufficiency, which are most likely caused by cyclo-oxygenase inhibition

for a decrease in prostaglandin content. Due to side effects or disease progression, patients need second and even third lines of therapeutic options. Some other types of treatments available today other than NSAIDs, including corticosteroids and disease-modifying antirheumatic drugs (DMARDs), primarily focus on treating symptoms rather than the pathological causes, such as membrane stabilization, protein denaturation, etc. Additionally, using the aforementioned treatment options could result in severe liver damage and gastric bleeding(6, 7).

Therefore, researchers are looking into plants as a source of medicine to overcome all these problems and discover a safer yet equally effective therapeutic option. In our study, we have selected the plant Prosopis juliflora (SW) DC, also known as mesquite belonging to the family Fabaceae. It has been traditionally used to treat diarrhoea, catarrh, dysentery, hoarseness, measles, throat infection, and wound healing(6). It has also demonstrated antibacterial, antioxidant, antifungal, antitumor, and anthelmintic activities (8). The previous study showed that leaves of P. juliflora contain tannins, saponins, alkaloids, carbohydrates, flavonoids and cardiac glycosides(6, 9).

Thus this study was designed to identify the extraction method with the highest yield and to evaluate the protein denaturation potential of the extract as foundation work to explore its potential for the treatment of RA.

METHODS

This was a prospective study conducted at the Department of Pharmacy, University of Sindh, Jamshoro, Pakistan between June 2022 till January 2023. Sigma Aldrich Co. in St. Louis provided all the chemicals and reagents, and all substances/solvents were of an analytical grade.

Collection and Preparation of Extract

Leaves of P. juliflora (SW.) DC were collected from Sindh university colony, Jamshoro, Pakistan and taxonomical identification was done at the Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan (voucher no 20047). The prepared powdered leaves (15g) were extracted with water (75 ml), ethanol-water (75 ml) and ethanol (75 ml) for subjected to extraction through maceration, reflux, sonication and soxhlation. The extract was filtered and dried. The yield of obtained dried extract was calculated in % w/w.

Effect of Protein Denaturation (egg albumin denaturation assay)

In vitro effects were checked by using a mixture (5 ml) was used, which contained 0.2 ml of egg albumin, 2.8 ml of phosphate-buffered saline (PBS) with a pH of 6.4, and 2 ml of plant extract in different concentrations (7.5, 30, 120, and 480 ug/mL). A comparable volume of PBS and egg albumin was combined for the control. The mixture was then heated for 5 minutes at 70 °C after 15 minutes of incubation at 37 °C. Their absorbance was measured at 660 nm after cooling, using the blank as a reference(10).

The percentage of inhibition of protein denaturation was calculated by using the following formula;

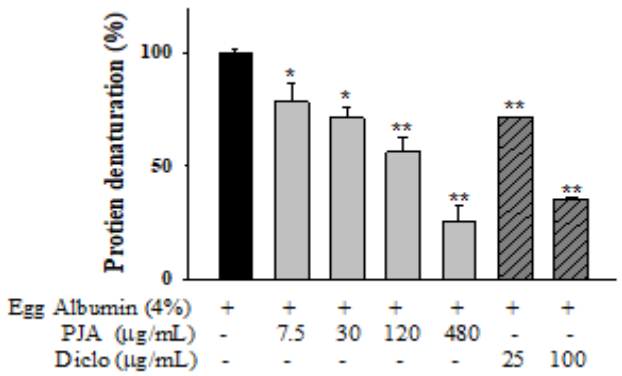
% inhibition= Abs control - Abs treated /Abs control ×100

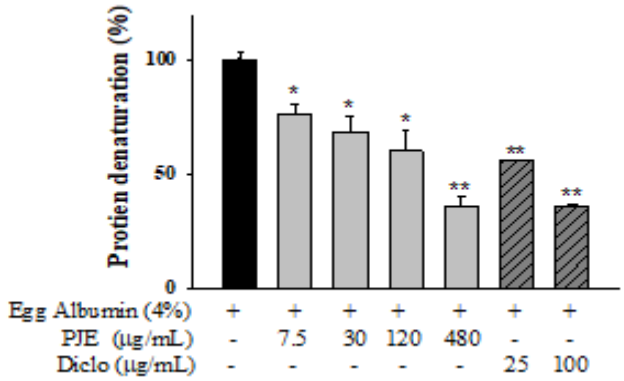
RESULTS

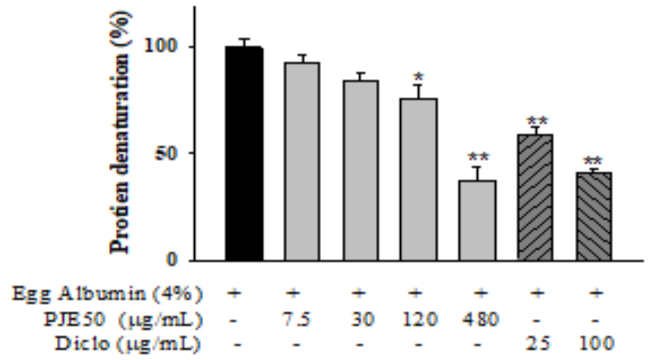
The soxhlation method showed the highest yield at 22.3% using ethanolic PJE, while reflux extraction showed the highest yield in aqueous PJA at 26.8%. Table 1 shows the percentage yield of P. juliflora crude extracts using different methods.

In vitro anti-arthritic effect of P. juliflora through macerated extracts

The macerated PJE, PJE50, and PJA extracts were used to study the in vitro impact through protein denaturation assay. All extracts lowered protein denaturation in a dose-dependent and substantial way, but PJA appears to be the most potent with the lowest IC50 value (230.1 ± 13.6µg/ml). Figure 1 presents a summary of the in vitro activity of all extracts.







Extracts	IC_{50} ± S.D (µg/mL)
PJA	230.1 ± 13.6
PJ50	302.8 ± 15.7
PJE	355.02 ± 57.04

The data is shown as mean S.D. (n=3). Paired t-test was used to examine the data's significance. Compared to the control group, *p0.05; **p0.01. PJE = ethanolic extract of P.juliflora; PJE50 = hydroalcoholic extract of P.juliflora; AfA = aqueous extract of P.juliflora; IC50 = inhibitory concentration of 50%; S.D = standard deviation

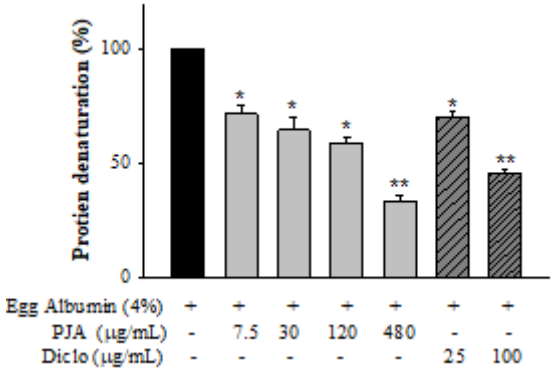
Figure 1: Protein denaturation inhibition of extracts of P. juliflora made by maceration

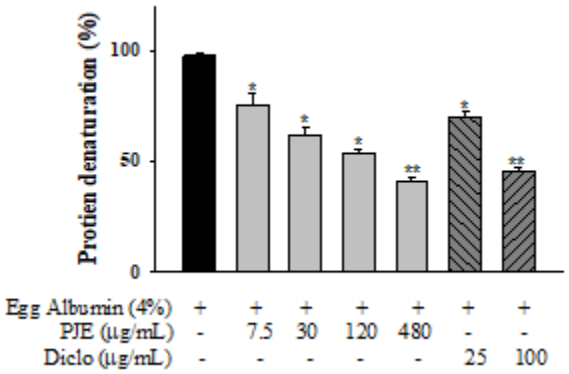
In vitro anti-arthritic effect of P. juliflora through reflux extraction

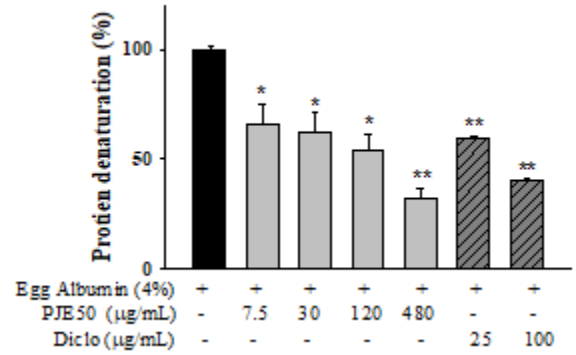
The refluxed PJE, PJE50, and PJA extracts were used to study in vitro impact through protein denaturation assay. Figure 2, demonstrates that all extracts lowered protein denaturation in a dose-dependent and substantial way, but among all extracts, PJA is most potent with the lowest IC50 value (130.36 ± 4.06 µg/ml).

In vitro anti-arthritic effect of p. juliflora through sonication

Using sonication method, the PJE, PJE50, and PJA extracts were used to study in vitro impact through protein denaturation assay. Figure 3, demonstrates that all extracts lowered protein denaturation in a dose-dependent and significant way, but among all extracts, PJA is most potent with the lowest IC50 value (44.91 ±15.69 µg/ml).



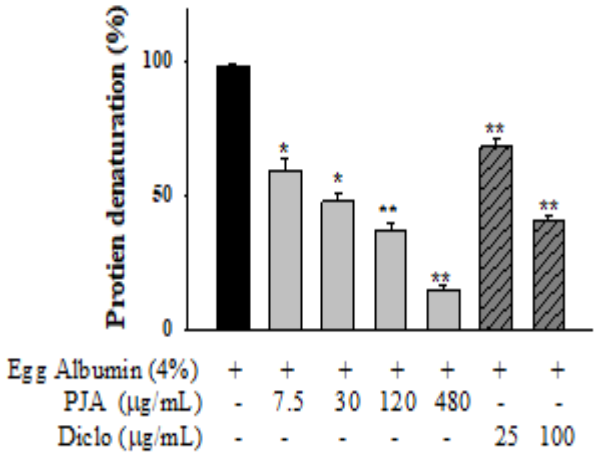


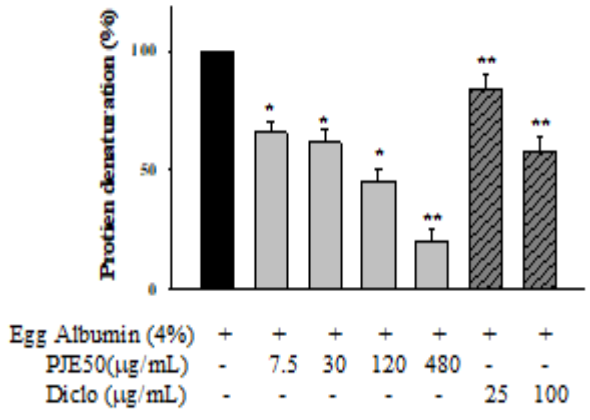


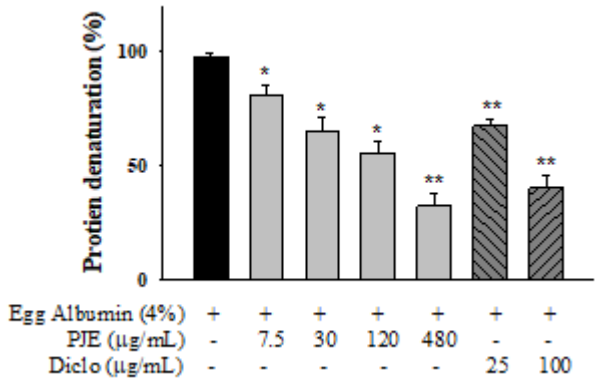
Extracts	IC_{50} ± S.D (µg/mL)
PJA	130.36 ± 4.06
PJ50	337.80 ± 22.25
PJE	250.35 ± 3.90

*The data were expressed as mean S.D. (n=3). A pairwise t-test was used to examine the data's significance. Compared to the control group, *p0.05; **p0.01. PJE stands for P. Juliflora ethanolic extract, PJE50 for P.juliflora hydro alcoholic extract, PJA stands for P.juliflora aqueous extract, IC50 for 50% inhibition, and S.D for standard deviation*

Figure 2: Protein denaturation inhibition of extracts of P. juliflora made by using the reflux method







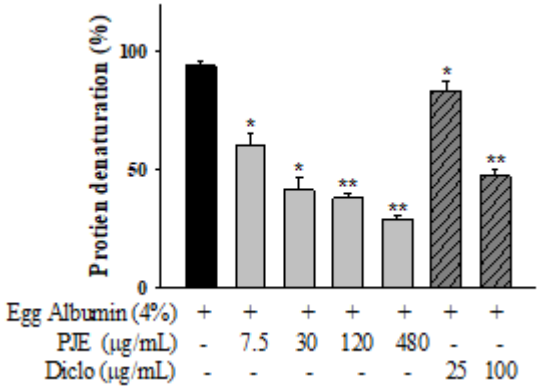
Extracts	IC_{50} ± S.D (µg/mL)
PJA	44.91 ±15.69
PJ50	1129.47 ± 28.37
PJE	3792.13 ± 96.92

*The data is expressed as mean S.D. (n=3). Paired t-test was used to examine the data's significance. Compared to the control group, *p0.05; **p0.01. PJE stands for P.juliflora ethanolic extract, PJE50 stands for P.juliflora hydroalcoholic extract, and PJA stands for P.juliflora for aqueous extract.*

Figure 3: Protein denaturation inhibition of extracts of prosopis juliflora made by using sonication method

In vitro anti-arthritic effect of p. juliflora through soxhlation

Soxhlation extract of the plant also shows protein denaturation inhibition in dose-dependent and significant way(160.50 ± 16.89 µg/ml). Figure 4 presents a comparison of different doses.



Extract	IC_{50} ± S.D (µg/mL)
PJE	160.50 ± 16.89

*The data is expressed as mean S.D. (n=3). Paired t-test was used to examine the data's significance. Compared to the control group, *p0.05; **p0.01. PJE stands for P. juliflora ethanolic extract, IC50 for 50% inhibition, and S.D for standard deviation*

Figure 4: Protein denaturation inhibition of extract of P. juliflora made by using soxhlation method

DISCUSSION

Rheumatoid Arthritis is a chronic illness that affects both adults and elderly people equally. Even though the prevalence rate of arthritis ranges from 0.3% to 1%, elderly women are predominantly affected. Low to middle-income countries show high rates of arthritis. Most therapeutic options, such as NSAIDs, Glucocorticoids, DMARDS and certain biological agents, demonstrate several side effects (4, 11). Even with highly harmful drug side effects, these drugs target the symptoms, but the

root cause of the disease remains untreated, whereas surgical treatments can result in post-operative complications. Prosopis juliflora is part of the family Fabaceae. It has been used traditionally for treating diarrhoea, catarrh, dysentery, hoarseness, measles, throat infection, and wound healing(12). It has also demonstrated antibacterial, antioxidant, antifungal, antitumor, and anthelmintic activities (5), but in vitro, anti-arthritic effect was not studied. The study shows that different crude leaves extract of the plant possesses potent anti -arthritic effects(13). Significant protein denaturation inhibition was determined through in vitro egg albumin denaturation assay. Among all crude extracts, aqueous extract by sonication method was found to be the most effective compared to ethanolic and hydroalcoholic extracts of this plant.

The plant has great potential to be used in novel drug development. Several plants are used for drug development of plant-based drugs. The plant extracts are natural compounds with relatively less side effects than synthetic molecules. The next line in our experiment is to test these extracts in cell lines and animal models so that these molecules can be taken to the next level of testing in the human body.

This study used standard methods for extraction and analysis and showed promising results. However, not testing these molecules in animal models is considered a limitation.

CONCLUSION

The current study proved that PJA extract of plant possesses potent anti-arthritic activity as determined by in vitro experimental models. Therefore, aqueous plant extract (sonication) may be considered for further determination of anti-arthritic effect of this plant by using in vivo studies.

Further studies on animal models and cell lines are recommended.

Conflict of interest:

All the authors declared no conflict of interest.

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