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PHYTOCHEMICAL SCREENING AND DETERMINATION OF IN VITRO ANTI-ARTHRITIC ACTIVITY OF THE LEAVES EXTRACT OF ACACIA FARNESIANA

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ABSTRACT

Rheumatoid arthritis is a systemic disease affecting the entire body, affecting joints like hands, feet, wrists, knees, and ankles. It affects 1% of people globally and can be influenced by hereditary and environmental factors. Symptoms include fatigue, joint tenderness, redness, swelling, joint pain, numbness, weight loss, and stiffness. RA impairs mobility, lowers health-related quality of life, and increases mortality. In ayurvedic medicine, Acacia farnesiana is a valuable medicinal plant that is used for many therapeutic purposes. The trunk, leaves, and fruits of this plant have been widely used. The aim of this study is to investigate in vitro anti-arthritic activity and phytochemical screening of fresh leaf extracts of Acacia farnesiana. The anti-arthritic activity of leaves of Acacia farnesiana was evaluated by using four extraction methods, including maceration, sonication, reflux, and soxhlation. It was assessed in terms of a protein denaturation assay. The highest IC50 S.D. value by using the maceration method was AFE50 102 g/ml. Sonication also showed highest yield in Hydro alcoholic

(AfE50) at 14.5%. On the other hand, Soxhlation method showed the highest yield at 28.5% using ethanolic (AfE), while reflux extraction showed the highest yield in Hydroalcoholic (AfE50) at 27%. Among all crude extracts, the macerated AFE50 possesses the most potent anti-arthritic activity. Tthe preliminary phytochemical analysis of AfE50 was carried out using standard methods to identify various phytochemicals. Alkaloids, flavonoids, phenols, terpenoids, steroids, tannins, and carbohydrates were present in AfE50; however, saponins, proteins, amino acids, anthraquinone glycosides, and starch were not detected in AfE50 extract.

Key words: Anti-arthritic Activity, Acacia farnesiana, Extraction, Protein Denaturation Assay, Phytochemical screening, Sonication, Soxhlation, Ethanolic, Hydro Alcoholic.

Introduction

An autoimmune reaction may result in rheumatoid arthritis, a chronic heterogeneous inflammatory disease that is characterized by inflammation of synovial joints, destruction of synovial membranes, and reduction in joint spacing, leading to considerable loss of functioning and mobility. It can manifest at any age, and women are more likely than men to get it. [1, 2] RA is a systemic disease that affects the whole body, most commonly the joints of hands, feet, wrists, knees, and ankles. There are various signs and symptoms of RA, including fatigue, joint tenderness, redness, swelling, and joint pain. Besides that, the person is also suffering from numbness and tingling, weight loss, and stiffness of joints. [3] Both hereditary and environmental factors may be risk factors for RA. Articular degeneration and comorbidities are common symptoms of RA that can interfere with various bodily systems, including bone health, psychological functioning, and metabolic pathways. [4] In addition to impairing mobility and joints, RA lowers HRQOL (health-related quality of life) and increases mortality. The burden of RA on health is very high. Based on estimates of work disability and efficiency related to arthritis, indirect costs are estimated to be four times higher than direct healthcare utilization, making arthritis one of the most expensive illnesses. In the USA, medical costs for arthritis and lost productivity account for about 303.5 billion dollars annually. [5–7].

About 5 out of every 1000 people have RA, which can worsen and cause severe joint destruction and disability [8]. Over the past two decades, significant advancements have been made in our knowledge of the pathophysiology of RA, ideal outcome metrics, and effective treatment strategies, such as the recognition of the importance of early diagnosis and RA treatment. The field of rheumatology is still relatively new in Pakistan. Only a small number of hospitals offer rheumatology clinical setups that are supervised by board-certified rheumatologists. There aren't many studies on Pakistani citizens that are readily available. These studies shed important light on the facts surrounding autoimmune diseases, such as RA. In Pakistan, the incidence of RA is roughly 5.5%. A Karachi study found that the proportion of RA cases among 4900 study participants was 12.9%, indicating that RA prevalence is fairly common in females. [9–11].

Reducing RA-related disability, managing inflammation, and relieving pain are the main objectives of RA therapy. Presently, RA is treated with available allopathic medications, which include corticosteroids, hydrocortisone, betamethazone, dexamethazone, and methotrexate, as well as non-steroidal anti-inflammatory drugs (NSAIDs) like diclofenac sodium, ibuprofen, mefanamic acid, and piroxicam and disease-modifying anti-rheumatic drugs (DMARDs) of cyclosporine, methotrexate, azothioprine, and sulfasalizine. These medications do, however, have a few very negative side effects. NSAID use for extended periods of time can result in renal impairment and hepatic dysfunction, which puts patients at risk for cardiovascular problems. It can also cause gastritis, belching, gastric ulceration, and bleeding [12]. In order to treat RA, it was necessary to discover new therapeutic options from natural sources that had greater therapeutic potential and fewer side effects.
Acacia Farnesiana plant has been found to have various pharmacological activities, including bronchodilator and anti-inflammatory properties. [13] Its glycosidal fraction has been reported to have

anti-inflammatory properties. The ethanolic extract of A. farnesiana has also been shown to have antifungal and urinary tract infection properties. [14] The plant also has antimicrobial, anti-diabetic, and anti-inflammatory properties. [15,16]. Studies have shown that the ethanolic extract of *A. farnesiana* has demonstrated the anti-malarial property. [17] Additionally, it has been studied the anti-oxidant, anti-inflammatory, and cytotoxicity potentials of the ethanolic extract of *A. farnesiana.* [18] This study evaluates the anti-arthritic activity of A. farnesiana using the Complete Freund's adjuvant (CFA) arthritis model. The plant's stem and root have been used to determine its anti-bacterial, anti-inflammatory, and antioxidant properties. Phytochemical screening reveals various compounds found in various parts of the plant, including tyramine, kaempferol,sitosterol, benzaldehyde, eugenol, p-cresol, methyl salicylate, anisaldehyde, coumarine, ellagicacid, gallic acid, methyl eugenol, diterpenes, triterpenes, and flavonoids.

MATERIAL AND METHODS

COLLECTION OF PLANT MATERIAL

The leaves of the plant Acacia farnesiana were collected in the month of August2022 and were authenticated by Taxonomist, Institute of Plant Sciences, University of Sindh Jamshoro, Pakistan.

PREPARATION OF PLANT MATERIAL

Just after collection, Fresh leaves of Acacia farnesiana were washed thoroughly with distilled water and were dried in an oven at temperature of 40˚C for 2-3 days. Dried material was ground mechanically into a powder.

PREPARATION OF THE LEAVES EXTRACT

20gm of the dried powder material was subjected to extraction by using 250ml of different solvents including Ethanol, Hydro alcohol and water. Four extraction methods were used including Maceration (at room temperature for 36 hours), Sonication (at 40˚C for 20 minutes), Reflux (at 70˚C for 6 hours) and Soxhlation (at 65-70˚C for 16 hours). The extract was filtered and subjected to dryness by vacuum rotary evaporator in order to obtain crude extract. Yield value of dried extract was calculated in w/w %.

EFFECT OF PROTEIN DENATURATION (EGG ALBUMIN DENATURATION ASSAY)

To analyze in vitro anti-arthritic activity of leaves extract of Acacia farnesiana plant, protein Denaturation test was performed. The test was conducted by using 0.2ml of fresh hen's egg white as a rich source of protein by mixing it with 2.8ml of phosphate buffer saline and with 2ml of various concentration of the extract or diclofenac sodium (25 and 100 µg/mL). The mixture was kept in an incubator for 15minutes at the temperature of 37 °C. After incubation it is heated at 70 °Con water bath for 5 minutes. The mixture then allowed to cool. After cooling turbidity was measured by spectrophotometer at 660 nm using the blank as a reference. By using the following formula the percent inhibition of protein denaturation was determined.

% Inhibition of Protein Denaturation = $[(Ab_{control} - Ab_{test}) / Ab_{control}] \times 100$

RESULTS

The maceration method showed the highest yield at 20% using hydro alcoholic (AfE50), similarly, Sonication also showed highest yield in Hydro alcoholic (AfE50) at 14.5%. On the other hand, Soxhlation method showed the highest yield at 28.5% using ethanolic (AfE), while reflux extraction showed the highest yield in Hydroalcoholic (AfE50) at 27%. Table-1 shows the percentage yield of *Acacia farnesiana* crude extracts using different methods.

Table 1: Yield (%) of Crude Leaves Extract of Acacia farnesiana

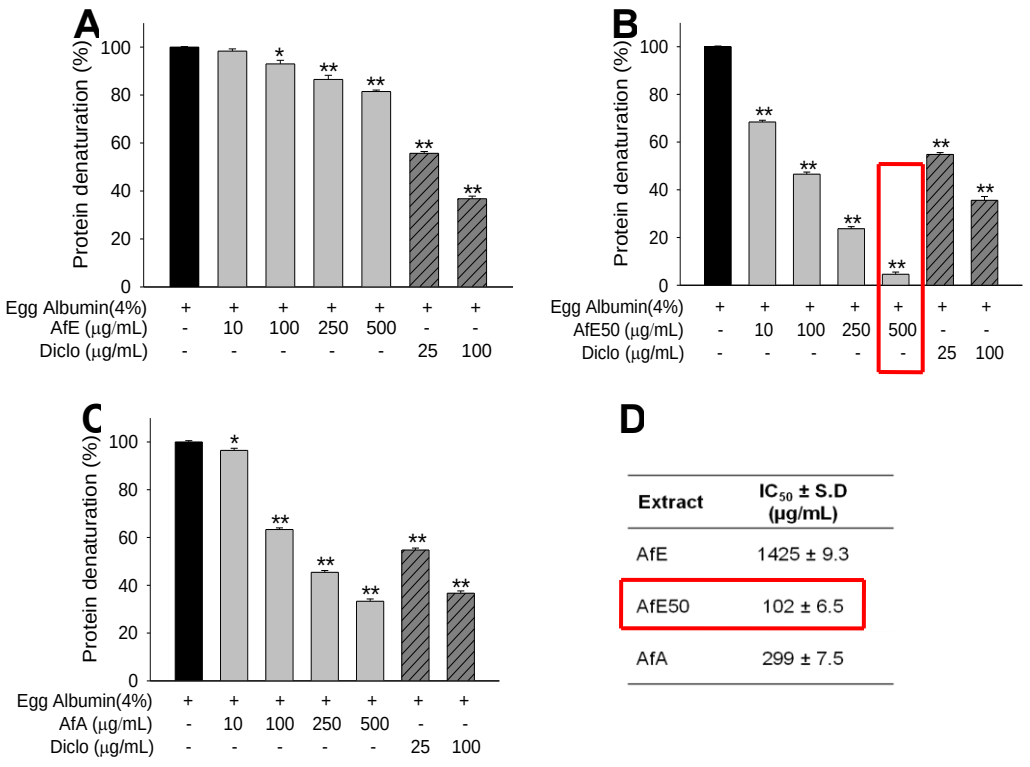
Methods	Extracts	Yield (%)
Maceration	Ethanolic (AfE)	7.5
	Hydro alcoholic (AfE50)	20.0
	Aqueous (AfA)	10.0
Sonication	Ethanolic (AfE)	6.7
	Hydro alcoholic (AfE50)	14.5
	Aqueous (AfA)	11.0
Reflux Extraction	Ethanolic (AfE)	22.6
	Hydro alcoholic (AfE50)	27.0
	Aqueous (AfA)	23.2
Soxhlation	Ethanolic (AfE)	28.5
	Hydro alcoholic (AfE50)	N/A
	Aqueous (AfA)	N/A

AfE= *A. farnesiana* ethanolic extract; AfE50= *A. farnesiana* hydro alcoholic extract and AfA= *A. farnesiana* aqueous extract; N/A= Not applicable.

IN VITRO ANTI-ARTHRITIC POTENTIAL OF ACACIA FARNESIANA MACERATED EXTRACTS

The macerated AfE, AfE50 & AfA 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 the macerated AfE50 extract is the most potent extract which has the minimum IC_{50} value102 ± 6.5µg/ml (Figure-1).

Figure-1: Protein denaturation inhibiting activity of various extracts of *A. farnesiana* by maceration method

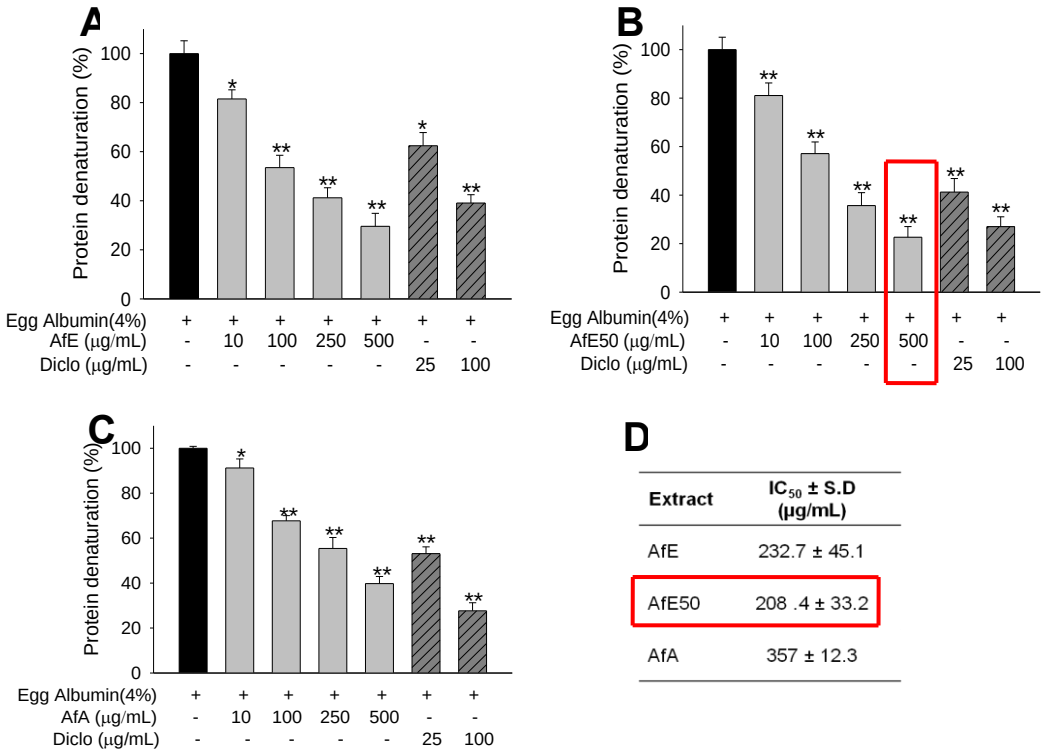


Data are shown as mean ± S.D (n=3). Significance of the data was analyzed by applying paired t-test. *p<0.05; **p<0.01 vs. control group. AfE= A. farnesiana ethanolic extract; AfE50= A. farnesiana hydro alcoholic extract; AfA= A. farnesiana aqueous extract; IC_{50}= Inhibitory concentration 50%; S.D= Standard deviation.

IN VITRO ANTI-ARTHRITIC POTENTIAL OF ACACIA FARNESIANA SONICATED EXTRACTS

The sonicated AfE, AfE50 &AfA 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 the macerated AfE50 extract is the most potent extract which has the minimum IC_{50} value AfE50 208.4 ±33.2µg/ml(Figure-2).

Figure-2: Protein denaturation inhibiting activity of various extracts of *A. farnesiana* by Sonication method



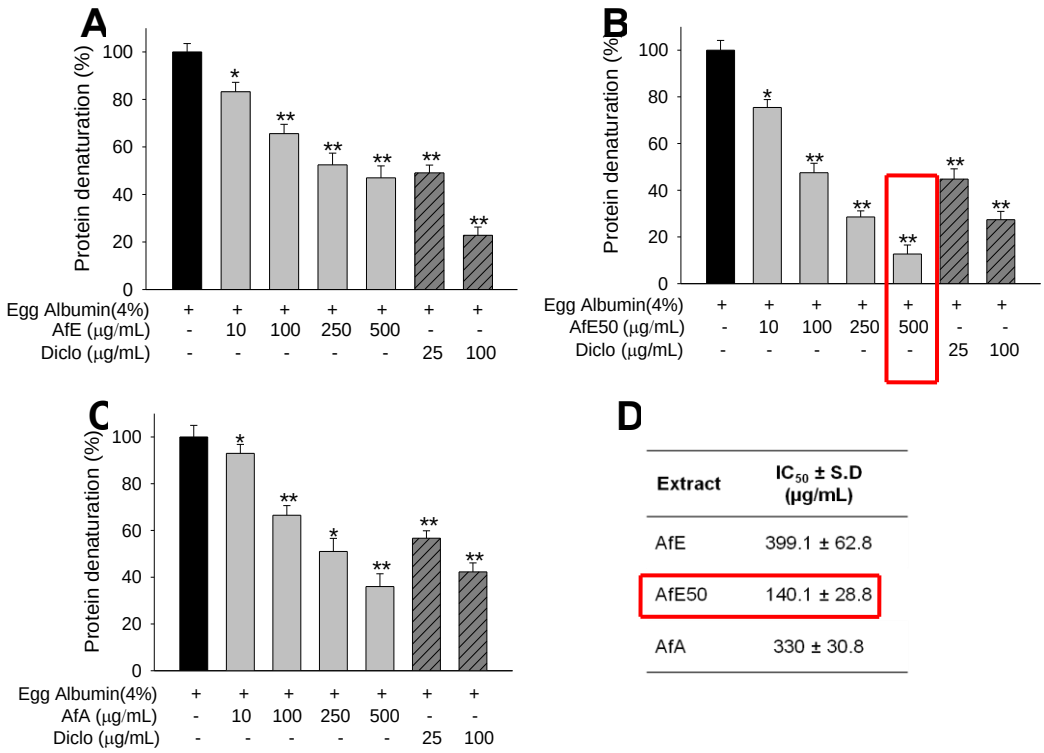
Extract	IC_{50} ± S.D (µg/mL)
AfE	232.7 ± 45.1
AfE50	208 .4 ± 33.2
AfA	357 ± 12.3

Data are shown as mean ± S.D (n=3). Significance of the data was analyzed by applying paired t-test. *p<0.05; **p<0.01 vs. control group. AfE= A. farnesiana ethanolic extract; AfE50= A. farnesiana hydro alcoholic extract and AfA= A. farnesiana aqueous extract; IC_{50}= Inhibitory concentration 50%; S.D= Standard deviation.

IN VITRO ANTI-ARTHRITIC POTENTIAL OF ACACIA FARNESIANA REFLUXED EXTRACTS

The Refluxed AfE, AfE50 &AfA 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 the macerated AfE50 extract is the most potent extract which has the minimum IC_{50} value AfE50 140.1 ± 28.8µg/ml (Figure-3).

Figure - 3: Protein denaturation inhibiting activity of various extracts of *A. farnesiana* made by using reflux method



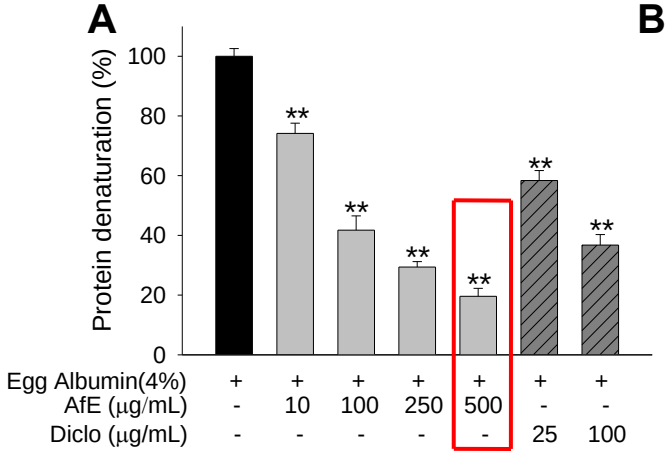
Extract	IC_{50} ± S.D (µg/mL)
AfE	399.1 ± 62.8
AfE50	140.1 ± 28.8
AfA	330 ± 30.8

Data are shown as mean ± S.D (n=3). Significance of the data was analyzed by applying paired t-test. *p<0.05; **p<0.01 vs. control group. AfE= A. farnesiana ethanolic extract; AfE50= A. farnesiana hydro alcoholic extract; AfA= A. farnesiana aqueous extract; IC_{50}= Inhibitory concentration 50% and S.D= Standard deviation.

IN VITRO ANTI-ARTHRITIC POTENTIAL OF ACACIA FARNESIANA SOXHLATED EXTRACTS

The Soxhlated AfE extract produced the inhibiting activity by reducing protein denaturation significantly in a concentration-dependent manner, having 125.3 µg/mL IC50 value (Figure-4).

Figure -4: Protein denaturation inhibiting activity of ethanolic extract of *A. farnesiana* made by using Soxhlation method



Extract	IC_{50} ± S.D (µg/mL)
AfE	125.3 ± 14.5

Data are shown as mean ± S.D (n=3). Significance of the data was analyzed by applying paired t-test. *p<0.05; **p<0.01 vs. control group. AfE= A. farnesiana ethanolic extract; IC_{50}= Inhibitory concentration 50%; S.D= Standard deviation.

CHEMICAL CONSTITUENTS SCREENING

Macerated crude leaves extract of acacia farnesianaAfE50 was subjected to qualitative tests for the identification of various phytochemical constituents.

Table 2: Preliminary Phytochemical Screening of Acacia farnesiana macerated hydro alcoholic extract (AfE50)

S. No.	Test	Results
1	Alkaloids	+
2	Flavonoids	+
3	Phenols	+
4	Saponins	-
5	Terpenoids	+
6	Proteins	-
7	Amino acids	-
8	Steroids	+
9	Anthraquinone glycosides	-
10	Tannins	+
11	Starch	-
12	Carbohydrates	+

DISCUSSION

A reasonable and significant anti-arthritic activity can be seen through various inhibitory concentration values of protein denaturation in an extract made from fresh Acacia farnesiana leaves, depending on the type of solvent, extraction technique, and drug dose.

Amongst all solvents, soxhlation method showed the highest yield 28.5% using ethanolic (AfE) extract. On the other hand, hydro alcoholic extract contained the highest yields by means of the reflux extraction method, followed by maceration and sonication methods. Reflux extraction produced a yield of 27%, while the maceration and sonication methods yielded 20% and 14.5%. Using the protein denaturation assay, the in vitro effect of extracts were investigated. Protein denaturation was considerably reduced by all extracts, However, among all the extracts protein denaturation effectively inhibited by the macerated AfE50 extract in concentration-dependent and significant manner. Qualitative tests were also performed on the macerated crude leaves extract of Acacia farnesiana AfE50 to identify various phytochemical constituents, which were identified as alkaloids, flavonoids, phenols, terpenoids, steroids, tannins, and carbohydrates and the compound that were not identified includes saponins, proteins, amino acids, anthraquinone glycosides, and starch.

As it was supposed, from different extraction methods and solvents, almost all have given good yields of anti-arthritic activity, with few having potent and significant values. Different results have been seen by comparing the anti-arthritic activity of extracts through the protein denaturation method. In which extracts were utilized to inhibit the protein denaturation. From these results it has been observed that by selecting and utilizing the most appropriate method of extraction with a suitable solvent we can get the best results and can have a reasonable anti-arthritic activity of this plant extract. By looking at the output values of these techniques utilized, it has been seen that amongst all, the soxhlation method with ethanol solvent has given the highest yield of anti-arthritic activity, which can be further utilized for getting even better results. On the other hand, regarding the selection of solvent for extraction of this plant, hydroalcoholic has given the best yields. Findings of this study contribute the

best understanding of how different extraction techniques and solvents of different natures can yield different activities of any plant extract. This also helps that for getting the best therapeutic output of any crude drug, what the techniques and resources we should implement.

Different studies have been done before on multiple plants regarding evaluation of anti-arthritic activity, like V. Nair et al.'s evaluation of "anti-arthritic and disease-modifying activity of Terminalia chebula Retz. in experimental models by using the complete Freund's adjuvant (CFA) model. Results indicated that the anti-arthritic activity of TCHE was at least in part due to its modulatory effect on pro-inflammatory cytokine expression in the synovium. [19]

The anti-arthritic and anti-inflammatory effects of Bungarus fasciatus venom using in vitro and in vivo assays (bovine serum protein denaturation method and egg albumin denaturation method) have been reported by Susmita Ghosh et al. The findings of this study showed that Bungarus fasciatus venom possesses significant in vitro and in vivo anti-arthritic and anti-inflammatory activities and may serve as a natural biomedical remedial agent against inflammatory conditions. [20]

Kumari et al. reported that the extract of Rhizophoramucronata demonstrated significant in vitro anti-arthritic potential using three different methods, such as a membrane stabilization assay, a bovine serum albumin denaturation assay, and an egg albumin assay. [21]

As compared to these studies, our study has been done on the plant Acacia Fernesiana, and its antiarhritic activity has been evaluated by only the in vitro method by using the protein denaturation method, and its extract has been obtained by multiple types of solvents and extraction methods.

Other reports from different studies have revealed that the Acacia Fernesiana plant also exhibits different pharmacological activities like anti-malaria and anti-fungal, and also its roots have activity against urinary tract infections. [14, 17, 22]. One of the studies by C. Trived et al. has reported the bronchodilator and anti-inflammatory potential of the glycosidal fraction of A. farnesiana [13]. But in our study, instead of fractions of only one constituent, whole plant extract has been evaluated for its anti-arthritic activity. Another report by Meckes et al. revealed the anti-inflammatory property of the methanolic extract of this plant in a carrageenan-induced edema model [23]. In our study, extract obtained from three different solvents has been evaluated for anti-arthritic activity by the protein denaturation method. Additionally, it has been studied the anti-oxidant, anti-inflammatory, and cytotoxicity potentials of the ethanolic extract of A. farnesiana [18]. But in our study, all three types of ethanolic, aqueous, and hydroalcoholic extracts of fresh leaves have been evaluated in vitro. Further, the anti-inflammatory activity of A. farnesiana on LPS-induced macrophages has also been reported. The anti-inflammatory activity of one of the constituents of this plant named diosmetin was studied in the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear edema model and found that diosmetin has significant anti-inflamatory activity. In another study, it was also found to inhibit the inflammation in the ear of the mouse edema in the TPA inflammation model. (34,35) Additionally, diosmetin has also been reported to moderately reduce pro-inflammatory IL-6 or TNF-alpha secretion. [24].

As compared to this, our study has not deeply gone through the evaluation of activity of individual constituents and has not used multiple models. It was previously reported that several parts of this plant, including the stem and roots, were used to determine antibacterial, anti-inflammatory, and anti-oxidant activity [25]. But our study has focused only on the fresh leaves part of the plant. Also, the proteins obtained from the seeds of this plant have been reported to exhibit anti-inflammatory and anti-nociceptive effects [26]. However, the study related to the anti-arthritic activity of this plant has not been reported previously. Hence, in the present study, we used the Complete Freund’s adjuvant (CFA) arthritis model to evaluate the anti-arthritic activity of A. farnesiana. Phytochemical screening

shows that various chemical compounds have been found from several parts of A. farnesiana, including tyramine, kaempferol, sitosterol, benzaldehyde, eugenol, p-cresol, methyl salicylate, anisaldehyde, isorhemnetin-3-rutinoside, coumarine, ellagic acid, gallic acid, methyl eugenol, terpineols, salicylic acid and benzoic acid, diterpenes, triterpenes, and some flavonoids. The outstanding point of this study is that it has used multiple solvents and different extraction methods on the fresh leaves of this plant and has evaluated anti-arthritic activity through the CFA model in vitro. This study contributes to the better understanding of the chemical constituents of this plant and its anti-arthritic activity. If an appropriate method of extraction with a compatible and feasible solvent is used, maximum therapeutic benefits can be obtained from this plant in the field of rheumatology, and the drawbacks of adverse effects of allopathic therapy can be minimized. The limitations of this study include that it has not gone through an in vivo study, and a quantitative study has not been done regarding the phytochemicals present. Also, it is only limited to the leaves parts of the plant. These gaps of this study have affected in-depth understanding of what types of chemical constituents and at which quantity they possess anti-arthritic activity. So we suggest that further studies can be conducted on these gaps to reach more facts about this plant.

CONCLUSION

In vitro experimental analysis of this study proved that macerated crude leaves extract AfE50 of Acacia farnesiana plant possesses potent anti-arthritic activity. Similarly, the Preliminary Phytochemical screening of macerated crude leaves extract AfE50 of Acacia farnesiana plant identify the presence of various phytochemical compounds such as alkaloids, terpenoids, steroids, tannins and carbohydrates.

Abbreviation List:

RA = Rheumatoid Arthritis
IC50 = Inhibitory Concentration 50%
Af = Acacia Farnesiana
AfE = Acacia Farnesiana Ethanolic
Af E50 = Acacia Farnesiana Hydro Alcoholic
AfA = Acacia Farnesiana Acquous
S.D. = Standard Deviation
N /A = Not Applicable

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