Pharmacognosy Research, 2023; 15(1):184-205.
www.phcogres.com

Original Article

Pharmacological Evaluation of Different Extracts of *Asparagus officinalis (Asparagaceae)* as an Analgesic, Anti-Inflammatory and Anti-arthritic Agent in Rats

Satish Kumar[1], Pratima Srivastava[2], Sumeet Gupta[1,*], Meenakshi Dhanawat[3], Sapna Rani[1], Basiru Olaitan Ajiboye[4], Rupesh K Gautam [5]

[1]M.M. College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Haryana, INDIA.
[2]Aragen Life Sciences Private Limited, Hyderabad, Telangana, INDIA
[3]Amity Institute of Pharmacy, Amity University, Maneswar, Gurugram, Haryana, INDIA.
[4]Phytomedicine and Molecular Research , Federal University Oye-Ekiti, NIGERIA.
[5]Indore institute of Pharmacy, IIST campus, Indore, Madhya Pradesh, INDIA.

ABSTRACT

Background: To treat the joint pain arial part of *Asparagus officinalis* (asparagus) has historically been used. However, its efficacy for rheumatoid arthritis has not been pharmaceutically evaluated. We explore the phytochemical analysis anti-inflammatory, analgesic and anti-arthritic activity of petroleum ether, ethanol and aqueous extracts of *Asparagus officinalis* aerial part. **Materials and Methods:** Tail- flick method was used to evaluate the analgesic activity anti-inflammatory activity was carried out using paw oedema induced with carrageenan and CFA induced arthritic model was used to evaluate the potential in anti- arthritic activity in rats. The Petroleum ether, ethanolic and aqueous extracts were dosed orally in three divided doses (75, 150 and 300 mg/kg). For anti- inflammatory and analgesic activity diclofenac sodium at 10 mg/kg was used as standard, whereas in anti-arthritic model prednisolone at 5 mg/kg and methotrexate at 0.5 mg/kg were used as standard. One-way ANOVA followed by Dunnett's multiple range test were used to analyse statistical significance between means. **Results:** The results revealed a dose-controlled anti-inflammatory, anti-arthritic effect with different extracts whereas at some extent analgesic activity was observed. Four compounds were present and confirmed by LCMS/MS. The CFA model's findings showed improved defence against arthritic lesions and changes in body weight. Additionally, *Asparagus officinalis* significantly improved rheumatoid factor, changed WBCs, and favourably altered radiographic and histological alterations. **Conclusion:** The findings indicate that *Asparagus officinalis* is a strong anti-arthritic and anti-inflammatory compound that may be suggested for the treatment of both chronic and acute inflammation.

Keywords: Carrageenan, Chemical constituents, LCMS/MS, Rheumatoid arthritis, Sparrow grass.

Correspondence:
Dr. Sumeet Gupta
Professor, Department of Pharmacology, M.M. College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana-133207, Haryana, INDIA.
Email id: sumeetgupta25@gmail.com

Received: 19-Oct-2022 ; **Revised:** 26-Nov-2022 ; **Accepted:** 05-Dec-2022

INTRODUCTION

Pain and Inflammation are both prominent symptoms of Rheumatoid arthritis (RA) which is a significant worry marked by non-specific joint inflammation, articular tissue loss, and joint abnormalities. As the condition advances, the likelihood of bone degradation and cartilage breakdown increases, resulting in significant impairment.[1] An immune system response known as inflammation may be brought on by a variety of causes, such as infections, damaged cells, and toxins.[2] The creation of prostaglandins is triggered by infections (germs) such as viruses, fungi, bacteria, and illnesses, medical disorders, and external traumas such scratches or injury from foreign objects, chemicals, or radiation,[3] interleukins (IL- 1 and IL-6) and tumour necrosis factor (TNF-α) are examples of inflammatory cytokines that have been demonstrated to have a significant role in inflammation and joint destruction that occurs throughout the progression of arthritis.[4,5] Articular cartilage and the subchondral bones are degraded as a result inflammation induce in synovial membranes. Despite substantial research, the specific cause of RA is unknown. Recent research suggests that increased oxidative stress due to free radical production may be a key factor in the progression of RA.[6] Cyclooxygenase isoenzyme (COX) plays a major role in development of arthritis apart from cytokines (IL-1, IL-6, TNF-α, etc.)[7,8] Excessive cytokines (pro-inflammatory) can form a positive bridge between RA fibroblasts and macrophage-like synovial cells. Inflammatory cytokine inhibition is the most common molecular target in the treatment of RA.[9]

DOI: 10.5530/097484900333

Publishing Partner : EManuscript Tech. [www.emanuscript.in]

Non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids have been widely employed in the treatment of RA.[10] These analgesic medicines, are reported to relieve only 50% of the pain particularly NSAIDs, that too roughly 30% of people in most cases.[11] However, due to serious adverse effects shows in heart, gastrointestinal tract and kidney function, this drug is now lost their major role as first-line treatment in response to current findings.[12] In recent decades, new categorized drugs are available for this treatment such as glucocorticoids and immunosuppressant are effective anti-inflammatory medications, but they have their serious adverse effects that make long-term use difficult. As a result, we are now depending upon herbal treatment which may show lesser adverse effect and may better safety profile as compared to existing one.[13] Scientists are always in search of better alternate treatment. In that case Ayurveda is always preferred choice.

Through literature survey and endangered species existing in India especially in Southern Region we observed many plants which are still unexplored for various pharmacological activities.[14] They are traditionally use in sub local rural areas but scientific validation is not yet reported. The primary motivation for researching traditional plants is because many of the existing allopathic medications are made from plant material, and many of the structures employed in allopathy are comparable to plant constituents.

According to a recent WHO (World Health Organization) estimate, 80% of people globally rely on herbal remedies for some of their basic medical requirements. Approx 485 plant species belonging to 100 families are used for arthritis as traditional drug.[15,16] The Asparagaceae family includes Asparagus as a part. Mentioned in one of the earliest treatises of human knowledge, the Rig-Veda, was written between 4500 and 1600 BC.[17]

Added to this, very less work is available on *Asparagus officinalis* whereas other members of same class are reported for many pharmacological activities such as *Asparagus racemosus, Asparagus sprengeri* and *Asparagus acutifolius* are all members of the same genus.[18] *Asparagus officinalis* contains steroids, amino acids, saponins, fructans (Asparagosine and Asparagose), flavonoids and ferulic acid. Ethanolic and aqueous extracts of this plant have been use in Southern Interior region for treatment of many diseases but traditionally it is used to treat kidney and bladder stones, asthma, and cough.[19] *Asparagus officinalis* was found to have inhibitory effects on both cyclooxygenases-1 and -2, indicating that it possesses anti-inflammatory properties. Linoleic acid was one of the compound present in this plant and found active in various pharmacological activities[19] includes liver toxicity treatment,[20] anti-microbial,[21] anti-arthritic,[15] anti-oxytoxic, antiulcer, hypertensive and anticoagulant effects,[22] Ethanolic extract of *Asparagus* was reported anti-diabetic at dose of 50 mg/kg and 100 mg/kg,[23,24] Another study was also shown that 400 mg/kg of ethanolic extracts act as potential protective agent against oxidative stress in liver and kidney organs.[25] Based on our survey, we designed this study to access the pharmacological evaluation of different extracts of *Asparagus officinalis (*Asparagaceae*)* as an analgesic, anti-inflammatory and anti-arthritic activity in experimental rats.

MATERIALS AND METHODS

Plant material

Arial part of *Asparagus officinalis* was procured form botanical Garden of Southern region. The specimen plant was identified and authenticated by NISCAIR, New Delhi, India with reference number NISCAIR/RHMD/consult/-2021/3827-28. Fresh plant material was cleansed with tap water to remove dirt and dried for 10 days in the sun and shade before being crushed into a powder in an electric grinder.

Preparation of extracts

Powder of dried aerial plant part (2.5 kg) was added in the soxhlet's apparatus using Petroleum ether, Ethanol and water for successive extraction. The final traces of all three solvent extracts were removed using the dried vacuum method. All extracts were kept at temperature between 2- 4°C for further use.

Chemical and drugs

We bought carrageenan and Complete Freund's adjuvant from Sigma Chemicals (St Louis, USA). Ind. Swift Laboratories (Baddi, India) provided pure samples of prednisolone and diclofenac sodium, while MacLeod's Laboratory provided a gift sample of methotrexate (Mumbai, India). The study's other reagents and compounds were all of analytical grade.

Animals

Sprague dawley rats (130–150 gm) were utilised with the institutional animal ethics committee's previous consent (1125/PO/Rc/S/07/CPCSEA) with protocol no B-098. The animals were kept in normal housing with a 12:12 light-dark cycle and temperatures between 24 and 28°C. Standard feed pellet diet (Safe Diet procured from Samitek Instruments) were provided to animals and water was allowed *ad libitum.*

Drugs and dosage

75 mg/kg, 150 mg/kg, and 300 mg/kg of the extracts were administered orally as a suspension in 5 mL/kg of 1% carboxy methyl cellulose and 1.0% v/v tween 80. As an oral suspension, methotrexate (0.5 mg/kg *p.o.*) and prednisolone (5 mg/kg *p.o.*), were utilised as standards for anti- arthritic activity. Whereas diclofenac sodium (10 mg/kg *p.o.*) was used as standard in analgesic and anti-inflammatory activities.

Experimental Design

Experiment no-1 (Analgesic activity)

Rats were slit up into eleven different groups with 6 rats in each group and treated as given; Group (1): Control vehicle treated group received 1% carboxymethylcellulose, Group (2): received diclofenac sodium (10 mg/kg *p.o.*), Group (3, 4 and 5): received petroleum ether extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis,* Group (6, 7 and 8): received ethanolic extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis*, Group (9, 10 and 11): received aqueous extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis.*

Experiment no-2 (Anti-inflammatory activity)

Rats were split up into twelve groups (6 rats each) and treated as follows; Group (1): Control vehicle treated group received 1% carboxymethylcellulose, Group (2): received normal water Group (3): received diclofenac sodium (10 mg/kg *p.o.*), Group (4, 5 and 6): received petroleum ether extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis,* Group (7, 8 and 9): received ethanolic extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis*, Group (10, 11 and 12): received aqueous extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis.*

Experiment no-3 (Anti-arthritic activity)

Rats were split up into thirteen different groups (6 rats each) and treated as follows; Group (1): Vehicle treated control group received 1% carboxymethylcellulose, Group (2): received normal water Group (3 and 4): received diclofenac sodium (10 mg/kg *p.o.*) and methotrexate (0.5 mg/kg. *p.o*) respectively, Group (5, 6 and 7): received petroleum ether extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis,* Group (8, 9 and 10): received ethanolic extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis,* Group (11, 12 and 13): received aqueous extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis.*

Tail flick method (Analgesic activity)

Analgesiometer was used to measure the analgesic effect in rats. A nichrome wire within the instrument is heated to the necessary temperature and kept there by heat regulators. The current through the bare nichrome wire was maintained at a consistent strength (4 Amps). The rat was housed in a rat cage with only its tail sticking out. The centre of the tail was positioned on the platform such that it was barely above the hot wire but not touching it. The animal's sudden, recognisable flick or tail raising in response was used to measure the animal's latency (reaction time). To prevent any tissue injury to the animal, a cut-off duration of 10 sec was planned. On the seventh day following a one-week oral medication treatment, the reaction times for each group were tested at 30, 60, 90, and 120 min.[26]

Carrageenan induced paw oedema in rats (Acute- inflammation)

After one week of oral medication treatment, all groups except the control group received 0.1 ml of 1% w/v carrageenan injections into the rat paw. After a gap of one hour, two hours, three hours, four hours, and six hours, the paw oedema volume was measured using a digital plethysmometer (Model 7140, UGO Basile, Italy). Anti-inflammatory activity was determined by calculating the proportion of oedema that was inhibited compared to the control. The formula used to get the percentage inhibition of oedema was: oedema (% inhibition) = (A-B)/A X 100. where A denotes the volume of the paws in the control group and B denotes the volume of the paws in the group that received the test medication.[27]

Complete Freunds Adjuvant induced arthritis (Chronic inflammation)

By injecting 0.1ml (0.1% w/v) of deceased Mycobacterium TB bacteria homogenised in liquid paraffin into the left hind paw, arthritis was developed. On the fifth day, a second CFA booster was administered, while the control group received an equal volume of normal saline with minor modifications as per Dar *et al.* (2019). The drug treatment programme began on day one and lasted for 21 days. The paw volume was assessed using a digital plethysmometer on days 1, 7, 14 and 21. (Model 7140, UGO Basile, Italy). The proportion of oedema that was inhibited in comparison to the control was interpreted as an anti-inflammatory response. Methotrexate (0.5 mg/kg twice weekly) and prednisolone (5 mg/kg once daily) were employed as benchmark medications for comparison. The verneir calliper device was used to measure the ankle joint diameter on the 1st, 7th, 10th, 15th, and 20th days.[28]

Sample Collection

For the analysis of rheumatoid factor, blood samples were taken on the 21st day from the retro orbital plexus, and serum was obtained by centrifuging at 3500 rpm for 30 min at 4°C and stored at -80°C. Then, animals from each group were euthanized for an ankle joint histology study and an x-ray of the femur bone.

Extracts quantification using LC-MS/MS

Bioanalytical method

Preparation of quality control and calibration samples

Main stock solutions of Stigmasterol, β-sitosterol, Rutin, Telmisartan (Internal Standard for Rutin and Quercetin) and Metoprolol (Internal Standard for Stigmasterol, β-sitosterol) were made at 1 mg/mL concentration in methanol.

Sample preparation

Extracts were dissolved in methanol by looking at the solubility of Stigmasterol, β-sitosterol, and Rutin. And for Quercetin extracts were dissolved in DMSO. Prepared samples were diluted in acetonitrile containing internal standard + methanol + water. For LC-MS/MS (Liquid chromatography-tandem mass spectrometry) analysis, 10 µL were injected.

LC-MS/MS analysis

For Stigmasterol and β-sitosterol Q TRAP 6500+ was run in positive ion mode for the analysis. The LC system utilised was the Sciex Exion LC, which included a vacuum degasser, two isocratic pumps, an AD Multiplate Autosampler with a temperature control set to 4°C, and a thermostatic column oven with a (40°C) temperature control (Sciex Exion, AD column oven). Kinetex EVO, C_{18}, 5 µm particle size 50*4.6 mm stationary phase was utilised for the chromatography (Phenomenex, US). For the mobile phase, 0.1% formic acid in water served as the aqueous reservoir, while 0.1% formic acid in methanol served as the organic modifier. A common reverse phase gradient programme (time (min) / % B= 0.00/60, 3.0/99, 6.50/99, 7.00/99, 10.00/60 and 11.00/60) was used with run time of 11 min. Using

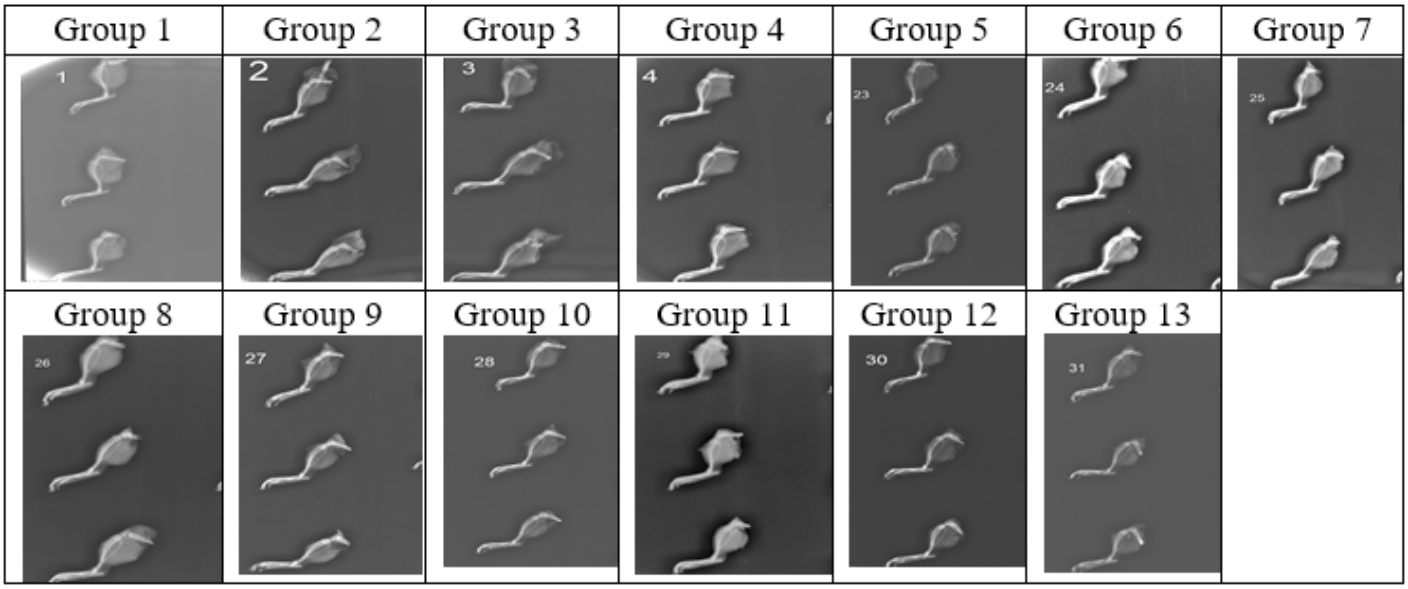

Figure 1: Effect of *Asparagus officinalis* extracts on arthritic joints in X-ray.

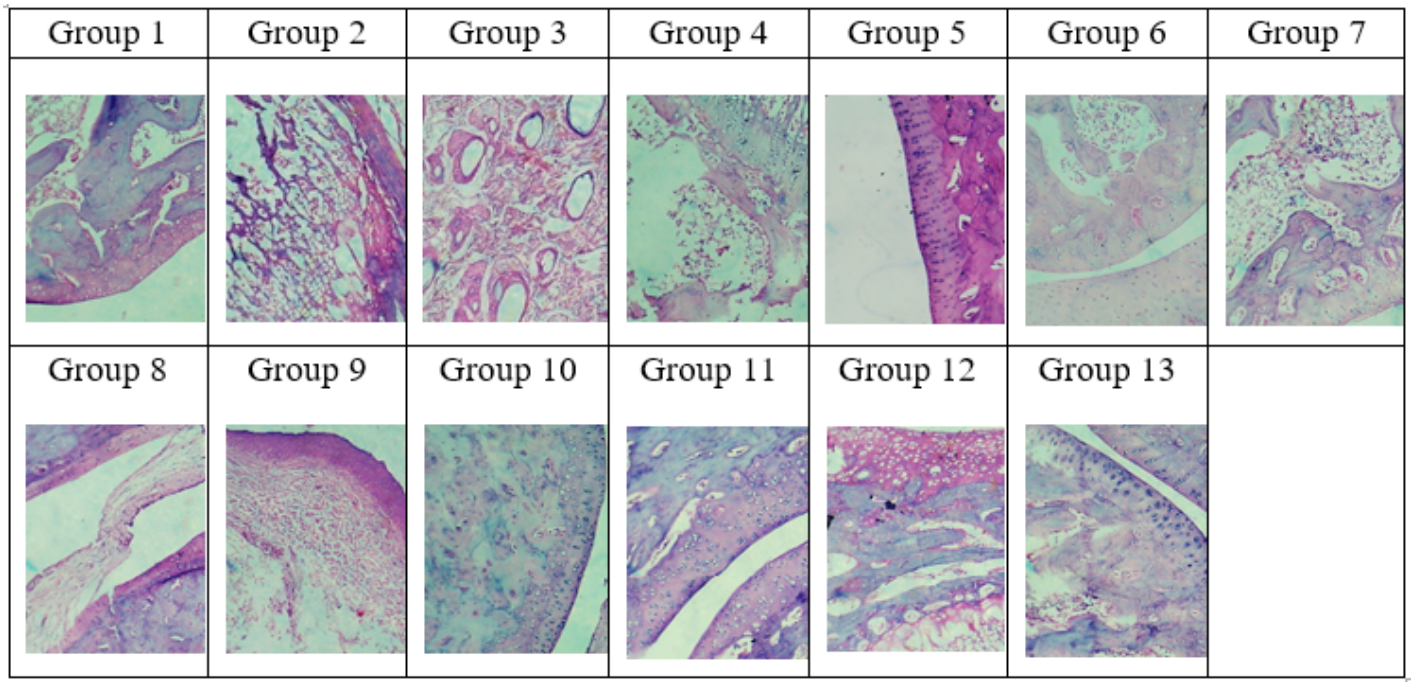

Figure 2: Histopathology of rat joints after treated with different drugs.

Analyst software version 1.6.3, data collecting and processing for quantification were carried out (AB SCIEX). By employing a Harvard infusion pump (Harvard Apparatus, Holliston, USA) linked directly to the mass spectrometer and infusing a 500 ng/mL solution in water: methanol (50:50 *v/v*) at 10 µL/minute flow rate, the mass spectrometric settings were tuned for the substances. By using a 0.9 mL/minute flow rate of mobile phase without a column, flow injection analysis (FIA) was used to optimised the flow-dependent source parameters. The following optimised settings were used to run the Turbo V source with the ESI (Electro spray ionisation) probe: Positive polarity, 30 pressure for the curtain gas, 70 psi for the nebulizer gas, 55 psi for the heater gas, 5500 volts for the ion spray, and 550°C for the source temperature. The mass spectrometry was run in the MRM mode, which fixes both the parent ion and the fragment ion. The optimal declustering potential (DP) and collision energy (CE) for the Stigmasterol parent and fragment ions were 100 V and 5 V, respectively. For metoprolol, the parent and fragment ions' *m/z* values were 268.10 and 116.3, with DP and CE, respectively, of 110 V and 25 V (Figure 3).

For Rutin and Quercetin TRIPLE QUQD 5500+ was used. MRM for LC-MS/MS operated in positive ion mode for Quercetin and negative ion mode for rutin analysis. The LC system utilised was the Sciex Exion LC, which included a vacuum degasser, two isocratic pumps, an AD Autosampler multiplate with a temperature control at 4°C, and a temperature control thermostatic column oven with at 40°C (AD column oven. Exion, Sciex). The 50×4.6 mm Kinetex EVO, C_{18} (Phenomenex, US) was used as stationary phase used for the chromatography with 5 µm particle size. The mobile phase consisted of 10mM Ammonium Acetae with 0.1% Formic acid in Milli Q as aqueous reservoir and (100%) methanol (organic modifier) at flow rate of 1 mL/minute was used. A programme with common reverse phase gradient (time (min) / % B= 0.00/5, 1/95, 2.50/95, 2.6/5 and 11.00/5) was used for Quercetin and % B= 0.00/60, 1/99, 2.50/99, 2.6/99 and 3/60) for Rutin with short run time of 3 min. Using Analyst software version 1.6.3, data collecting and processing for quantification were carried out (AB SCIEX). By employing a Harvard infusion pump (Harvard Apparatus, Holliston, USA) linked directly to the mass spectrometer and infusing a 500 ng/mL solution in water: methanol (50:50, v/v) at 10 µL/minute flow rate, the mass spectrometric settings were tuned for the substances. By using flow injection analysis (FIA) and a 1 mL/min flow rate of mobile phase without a column, flow dependant source characteristics were optimised. The Turbo V source with the ESI (Electro spray ionisation) probe was run for quercetin with

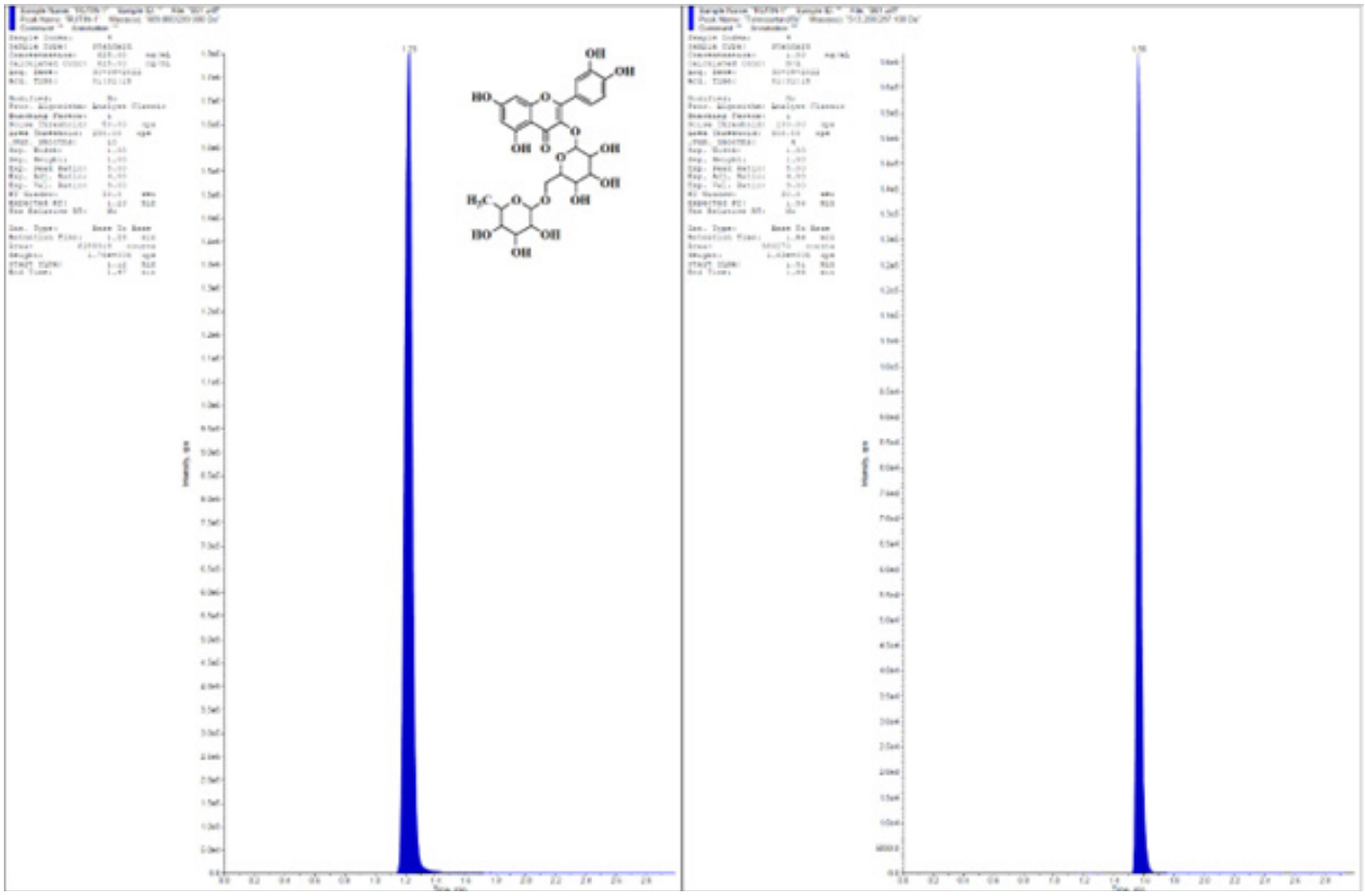

Figure 3a: LC-MS picture of Standard compound Rutin.

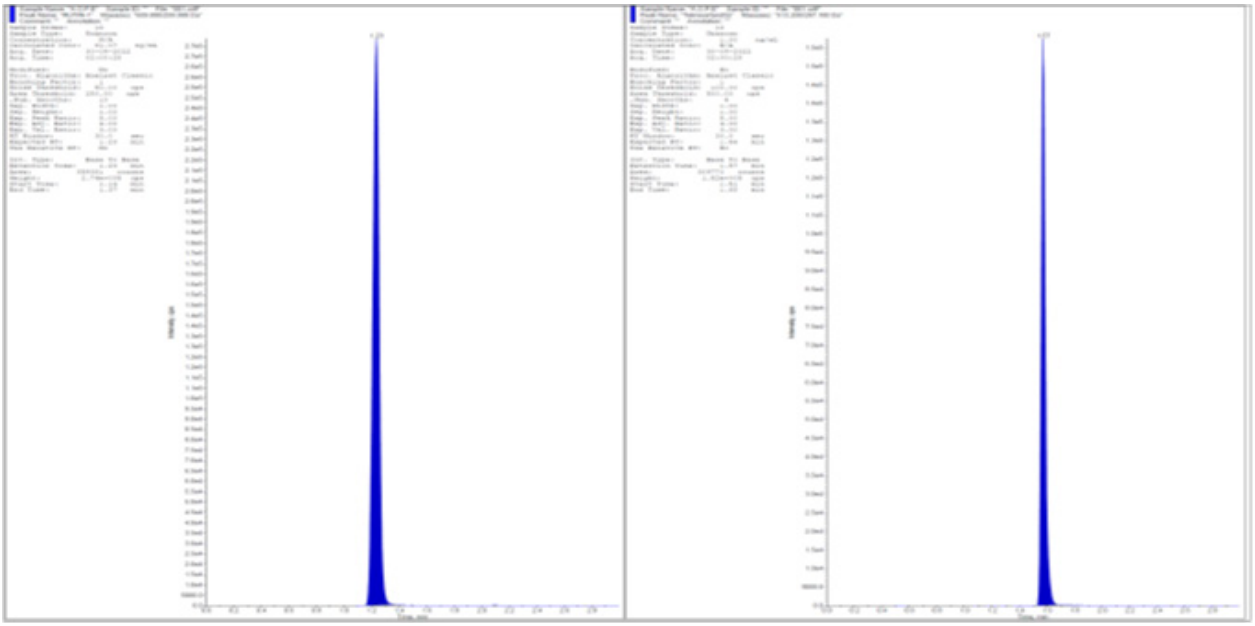

Figure 3b: LC-MS picture of Rutin in Petroleum ether extract of *Asparagus officinalis*.

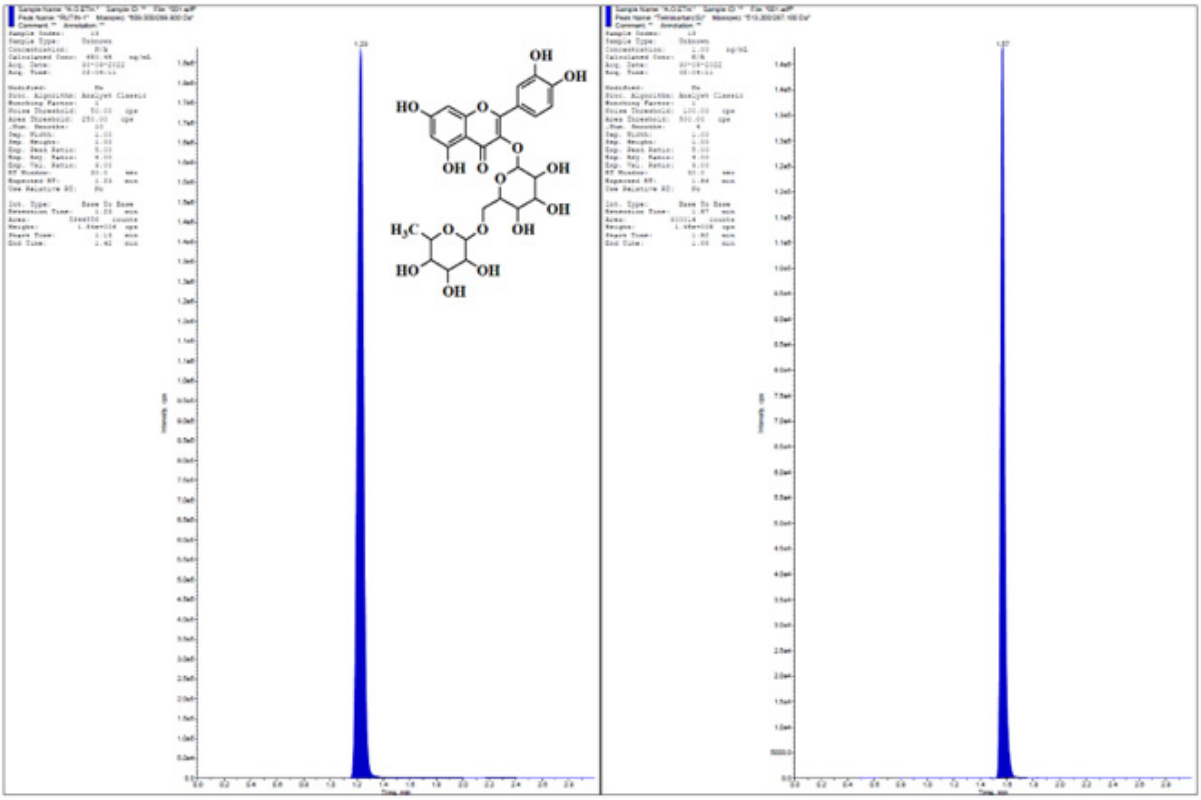

Figure 3c: LC-MS picture of Rutin in ethanolic extract of Asparagus officinalis.

the following optimised settings: Positive polarity, 45 pressure for the curtain gas, 60 psi for the heater gas, 55 psi for the nebulizer gas, 550°C for the source temperature and 5500 volts for the ion spray. The mass spectrometry was run in the MRM mode, which fixes both the parent ion and the fragment ion. The parent and fragment ions of quercetin were measured at *m/z* values of 303.20 and 229.00, respectively, with an ideal collision energy (CE) and declustering potential (DP) of 37 V and 77 V. The *m/z* values for parent and fragment ions for telmisartan were 515.30 and 276.00, with CE and DP of 50 V and 10 V, respectively.

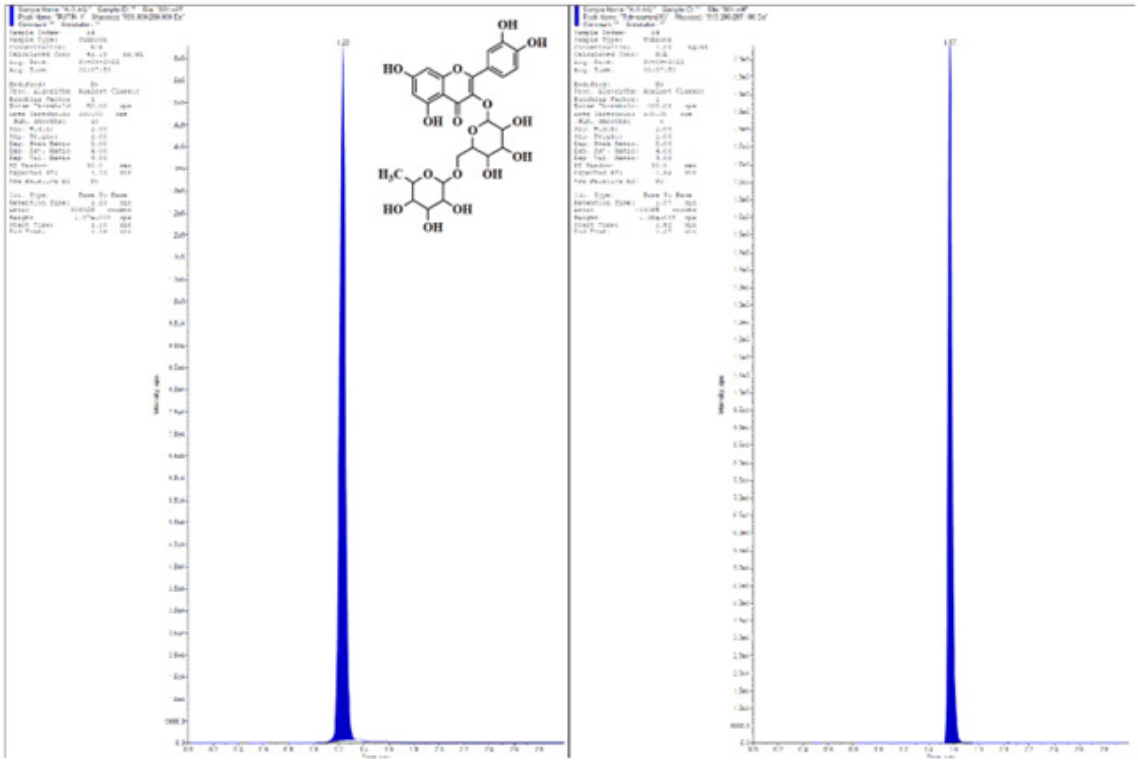

Figure 3d: LC-MS picture of Rutin in Aqueous extract of *Asparagus officinalis*.

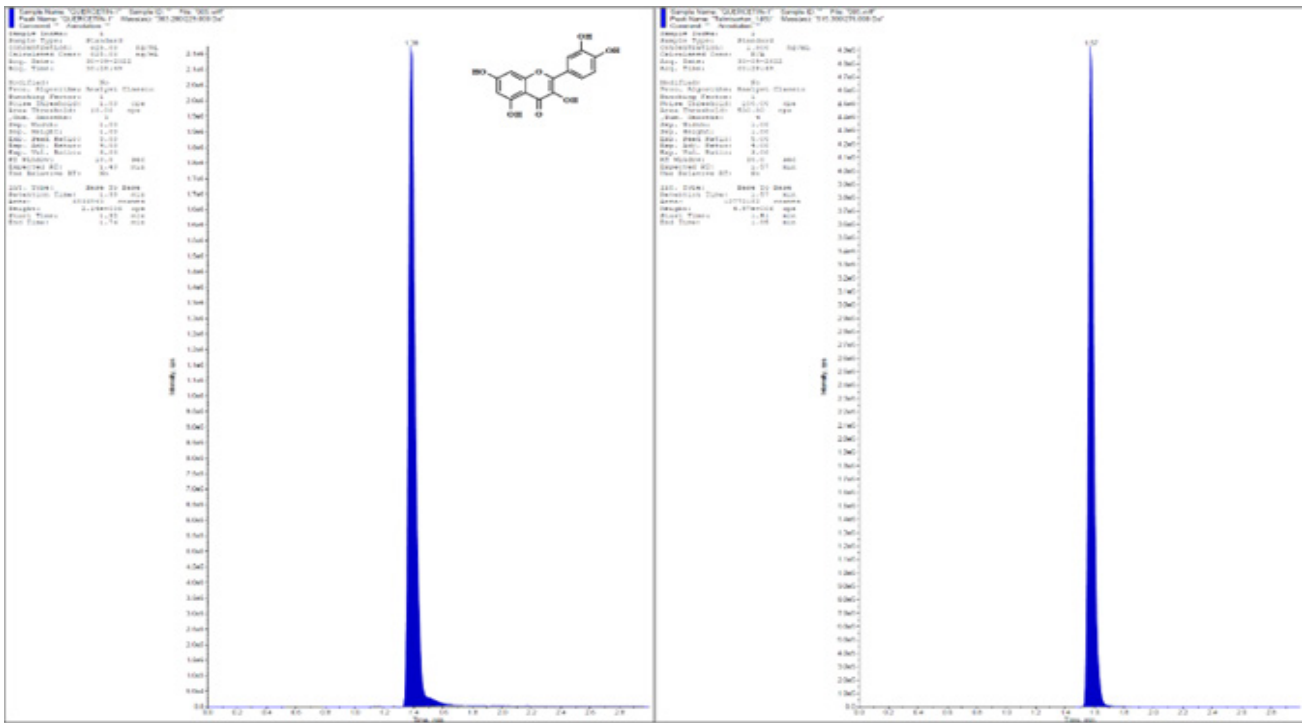

Figure 3e: LC-MS picture of Standard compound Quercetin.

The Turbo V source with the ESI (Electro spray ionisation) probe was used for Rutin with the following optimised settings: Positive polarity, 40 psi curtain gas, 550°C source temperature, 55 psi heater gas and 50 psi nebulizer gas. Ion spray voltage is -4500V. The mass spectrometry was run in the MRM mode, which fixes both the parent ion and the fragment ion. The Rutin parent and fragment ions employed had *m/z* values of 609.00 and 299,90, respectively, and collision energy (CE) and the ideal declustering

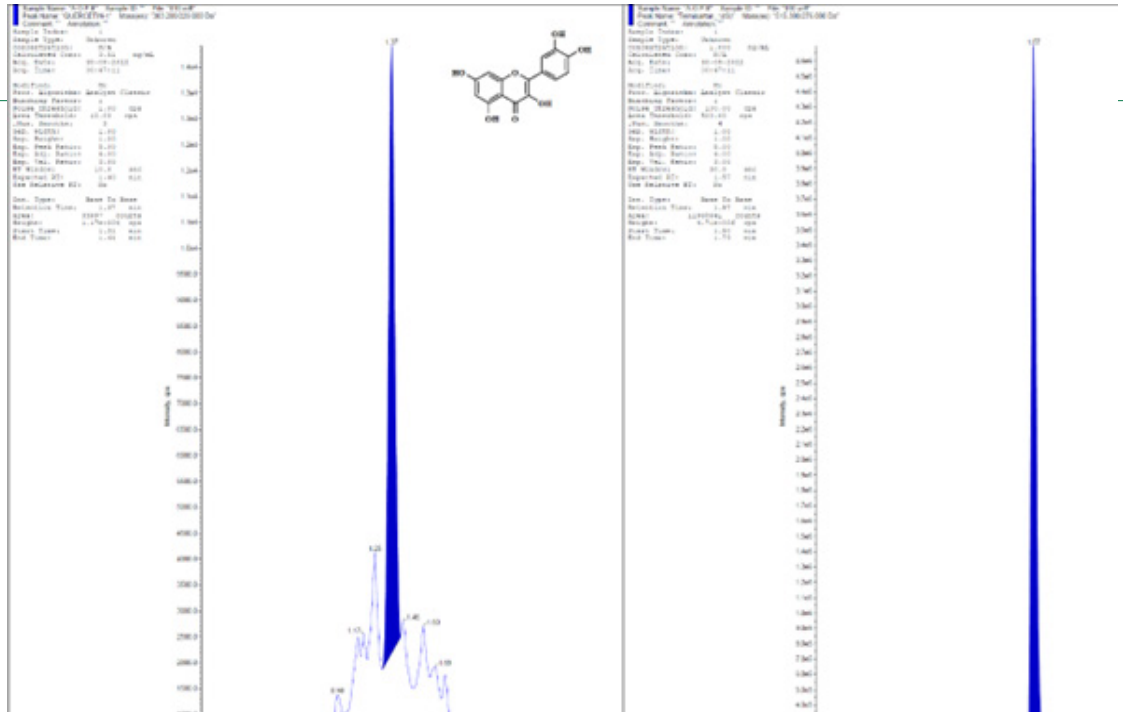

Figure 3f: LC-MS picture of Quercetin in Petroleum ether extract of *Asparagus officinalis*.

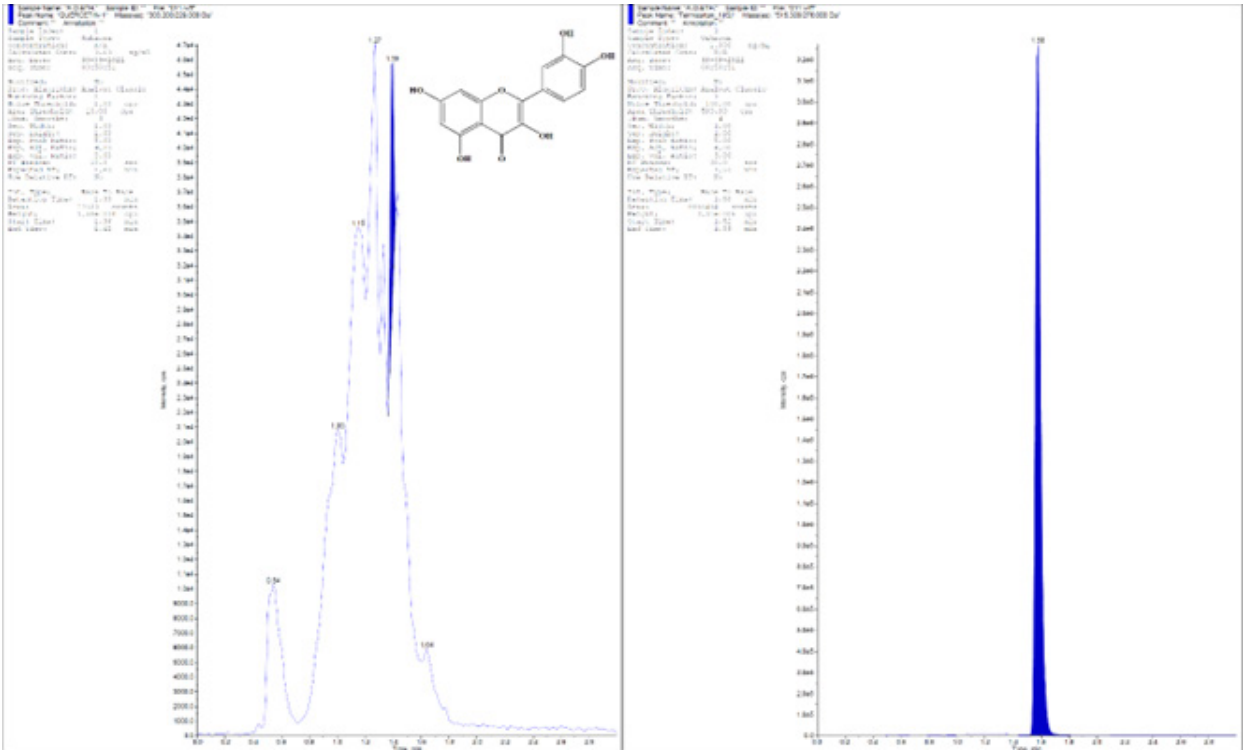

Figure 3g: LC-MS picture of Quercetin in Ethanolic extract of *Asparagus officinalis*.

potential (DP) were -49V and -122 V. For telmisartan, the parent and fragment ions' *m/z* values were 513.20 and 287.10, respectively, with CE and DP of -45 V and -110 V.

Statistically Analysis

Data were presented as mean standard deviation. Using a one-way ANOVA and Dunnett's multiple range test, the statistical analysis of the ankle joint diameter, paw oedema, % inhibition, and soft tissue thickness was conducted. a corresponding chance of error, $^{a}P<0.0001$, $^{b}P<0.001$, $^{c}P<0.05$ was considered statistically significant.

RESULTS

Qualitative Phytochemical Screening

The presence of flavonoids, terpenoids, alkaloids, tannins, sterols, proteins and carbohydrates content in extracts was confirmed by different test analysis.

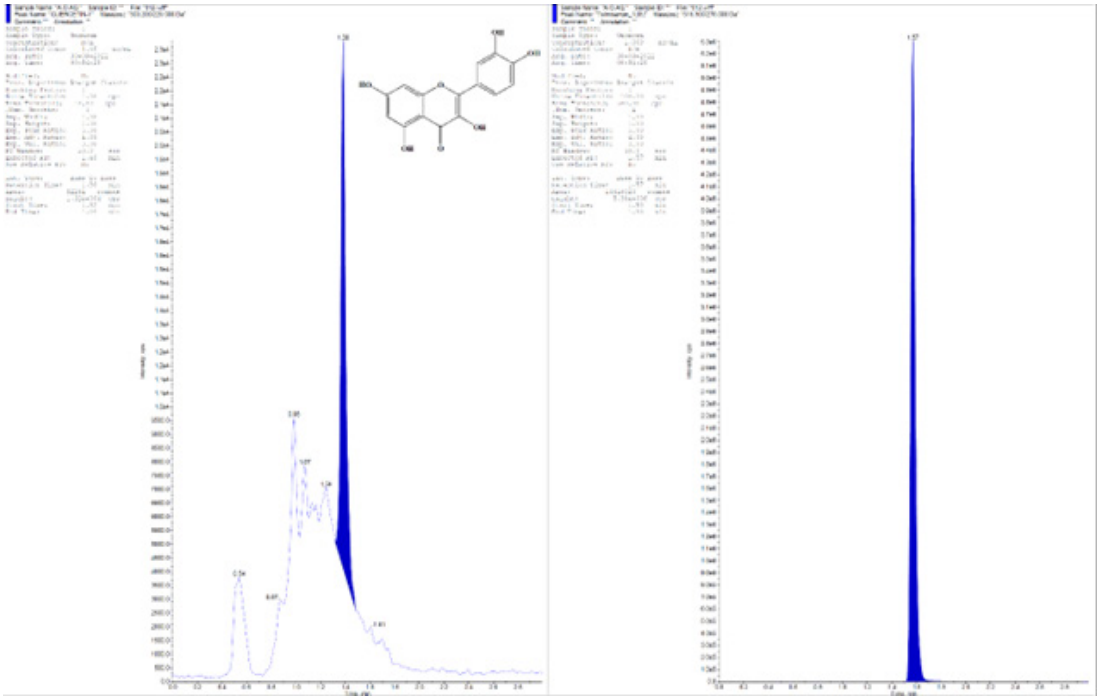

Figure 3h: LC-MS picture of Quercetin in aqueous extract of *Asparagus officinalis*.

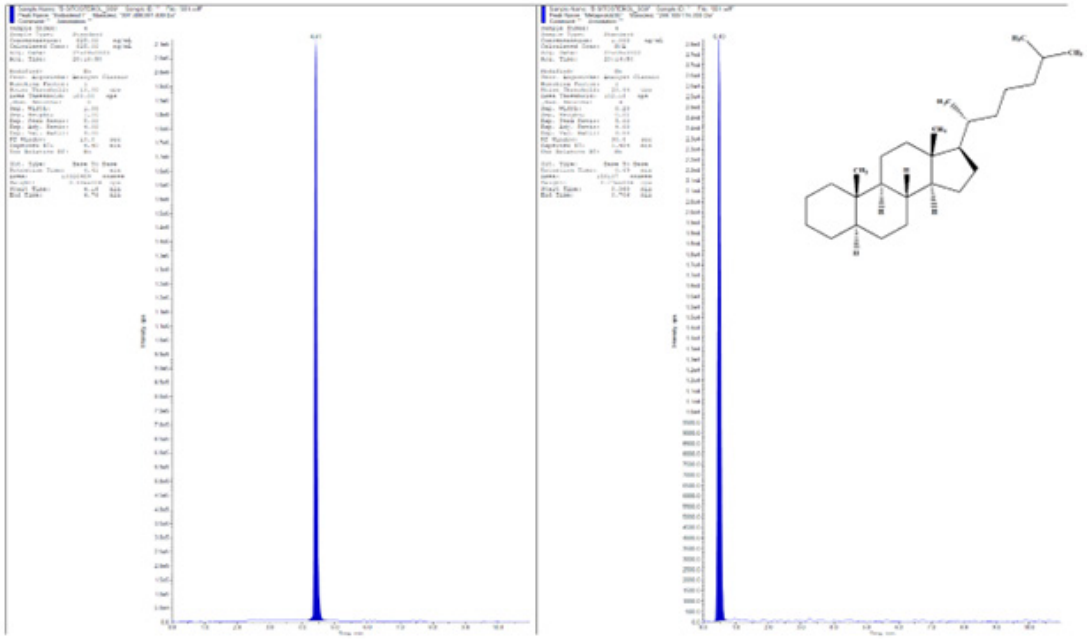

Figure 3i: LC-MS picture of Standard compound *β-sitosterol*.

Analgesic Activity

In this study, highly significant ($p<0.0001$) effect was found at 120 min with dose of 300 mg/kg of petroleum ether extract (83.49%), followed by aqueous extract (80.5%) and ethanolic extract (78.22%). Moderate analgesic effect noted with ethanolic extract, aqueous extract and petroleum ether extracts at 75 mg/kg and 150 mg/kg. Response time was increased 124% to 204% with diclofenac sodium (Table 1).

Anti-inflammatory activity

The results (Table 2) were found extremely statistical ($p<0.0001$) significant difference with all extracts at a higher dose from 2hr

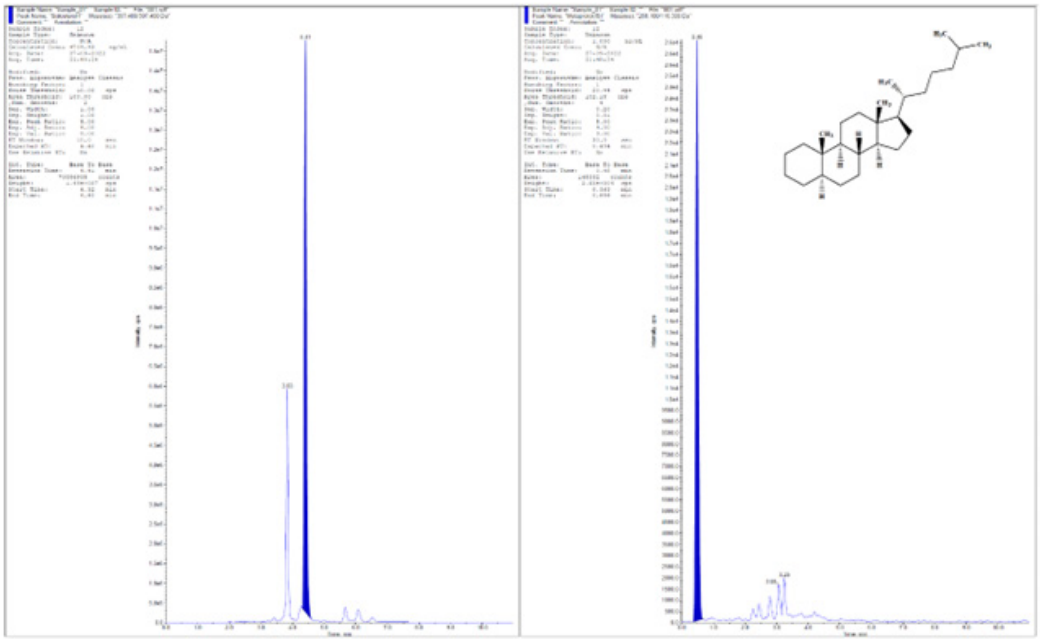

Figure 3j: LC-MS picture of β-sitosterol in pet ether extract of *Asparagus officinalis*.

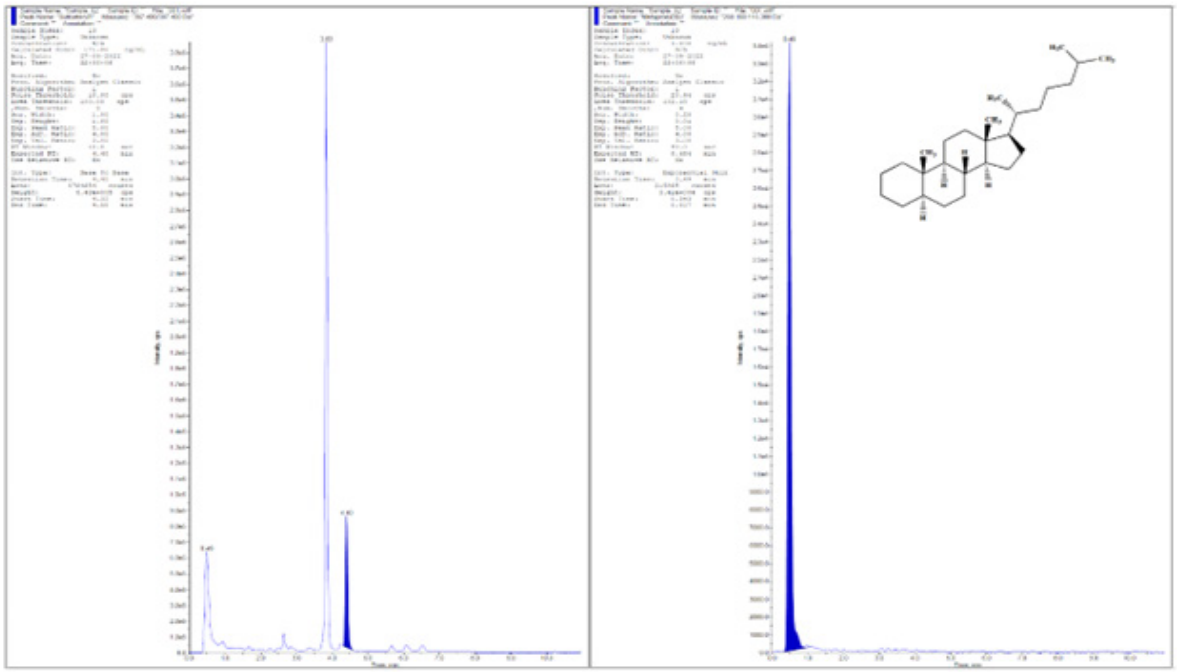

Figure 3k: LC-MS picture of β-sitosterol in ethanolic extract of *Asparagus officinalis*.

to 6 hr and less significant effect at lower doses also. Diclofenac sodium treated group showed 17.33% to 21.53% inhibition and petroleum ether extract was observed with the highest significant range of 13.14% to 22.95% inhibition followed by aqueous extract 9.90% to 20.74% inhibition and at last ethanolic extract was observed with a significant range of 11.17% to 20.46% inhibition.

Anti-arthritic Activity

Effect on body weight

Body weight of positive control group was decreased at different time intervals in comparison with normal control group. Higher dose of all three different extracts showed slightly changes in the body weights as compared to positive control group at 15th day

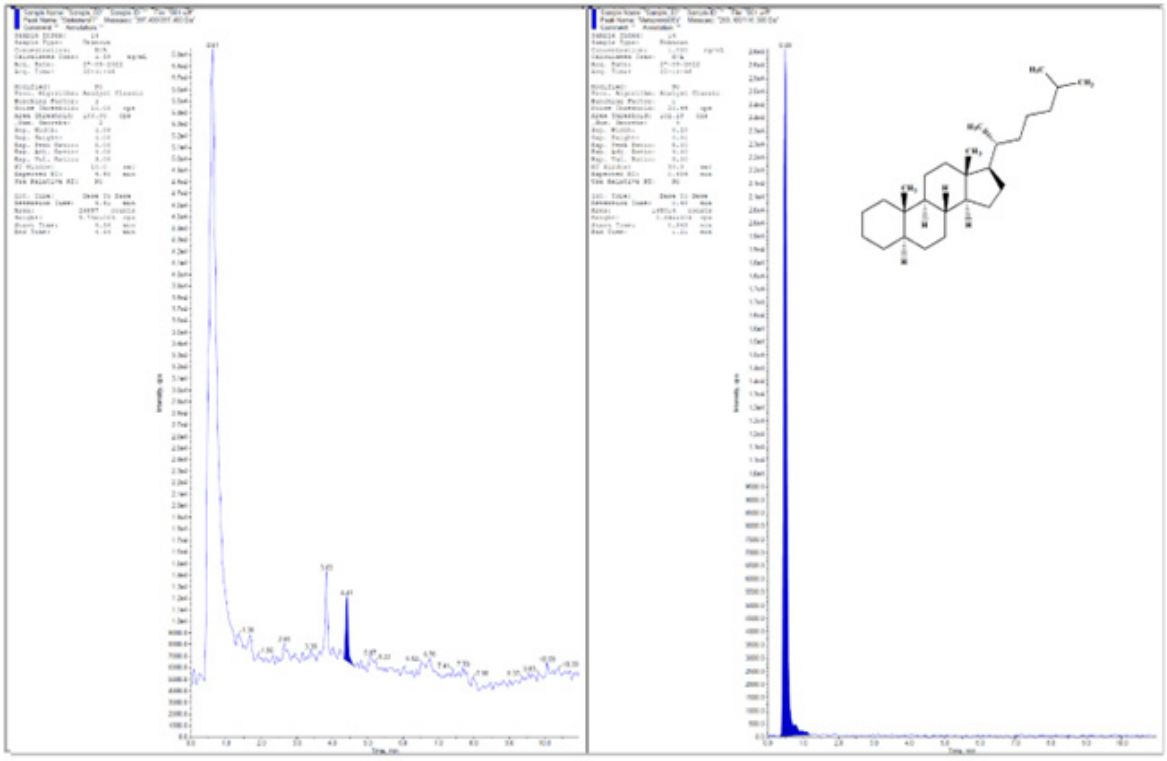

Figure 3l: LC-MS picture of β-sitosterol in aqueous extract of *Asparagus officinalis*.

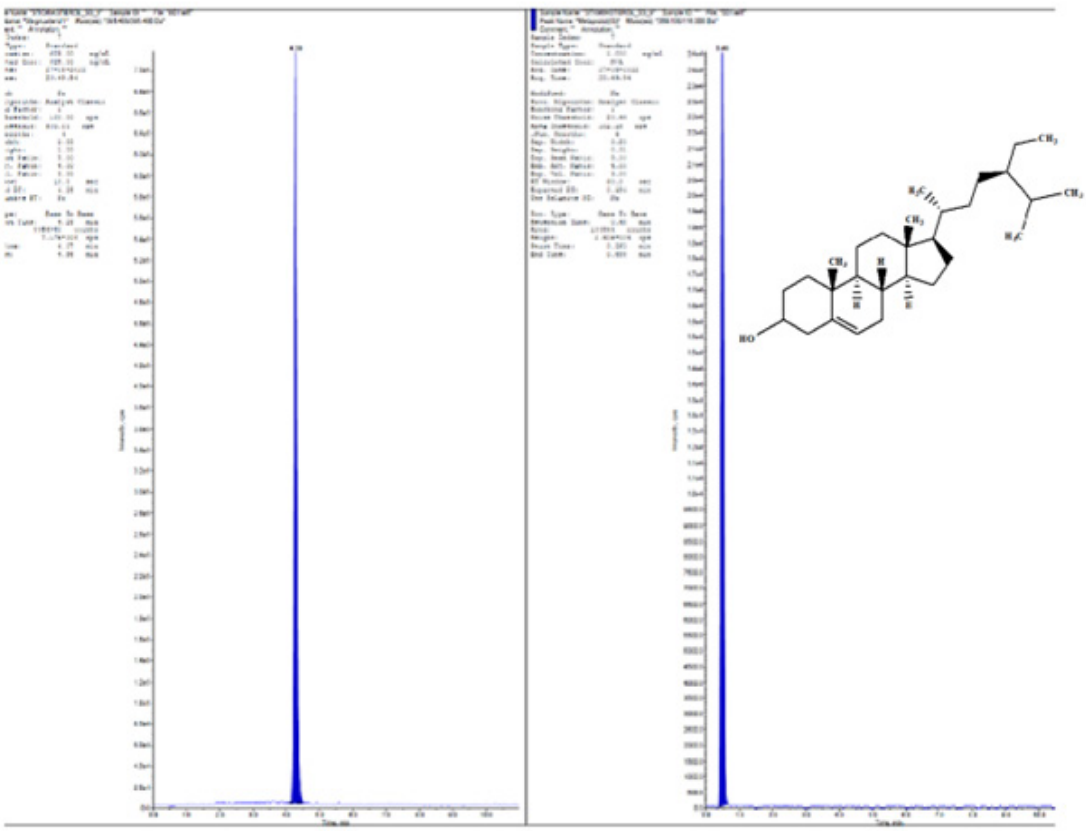

Figure 3m: Stigmasterol: Standard.

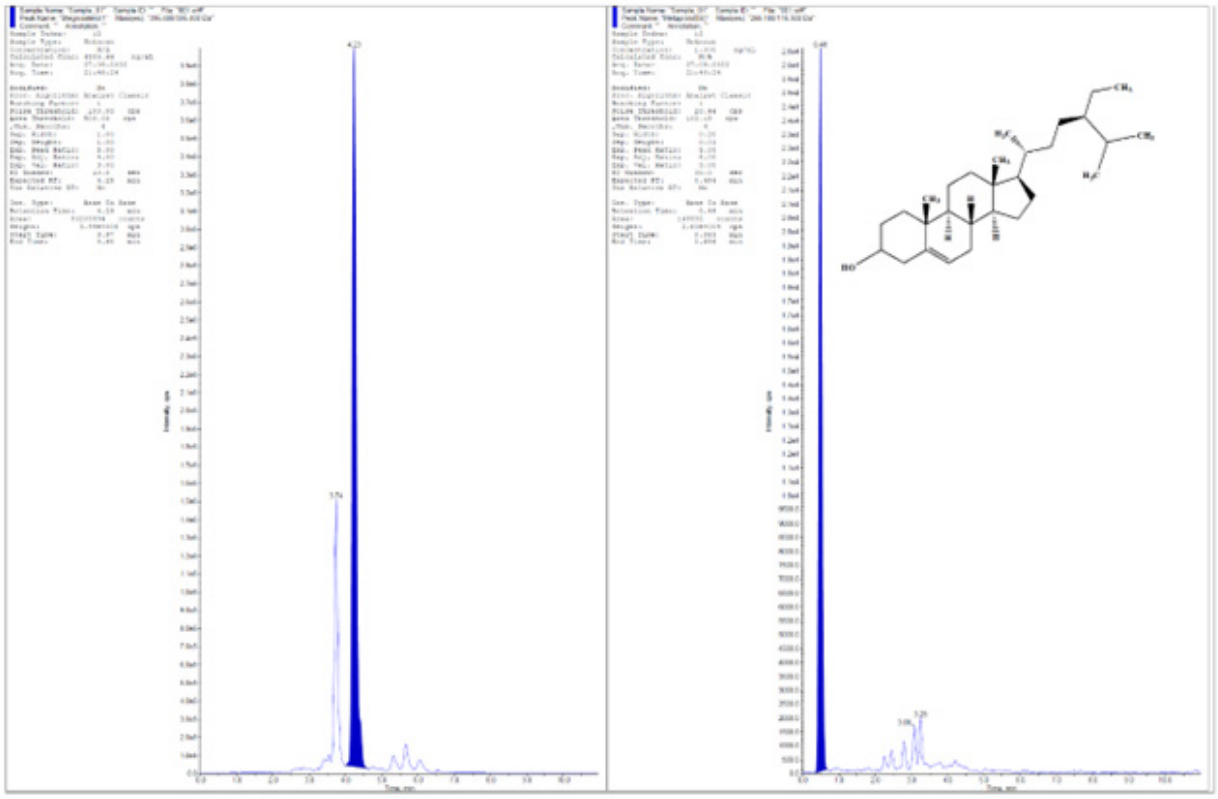

Figure 3n: Stigmasterol: *Asparagus officinalis*, petroleum ether extract.

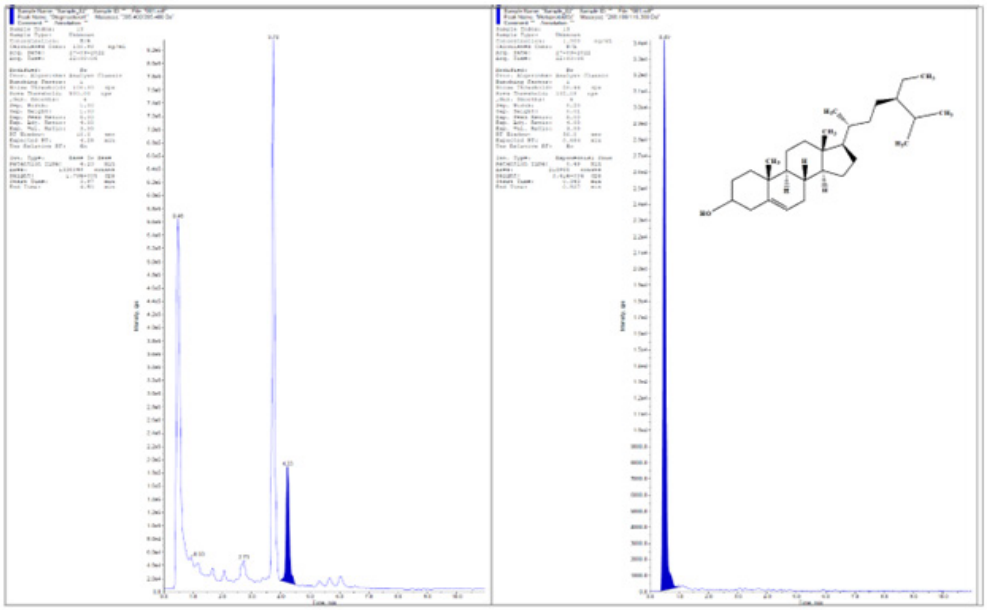

Figure 3o: Stigmasterol: *Asparagus officinalis*, ethanolic extract.

and 21st day but there was no statistical significant difference. Standard treated group (Methotrexate and Prednisolone) were able to keep the animal body weight in normal range. It was increased from 3.71% to 7.39% with petroleum ether extract and in aqueous extract treated group, it was noted from 0.79% to 14.76% (Table 3).

Effect on paw oedema

From 14th day and 21st day, the percentage of inflammation inhibition was found increase and statistically significant difference at all three doses of all three different extracts as compared with a positive control group. It was observed that

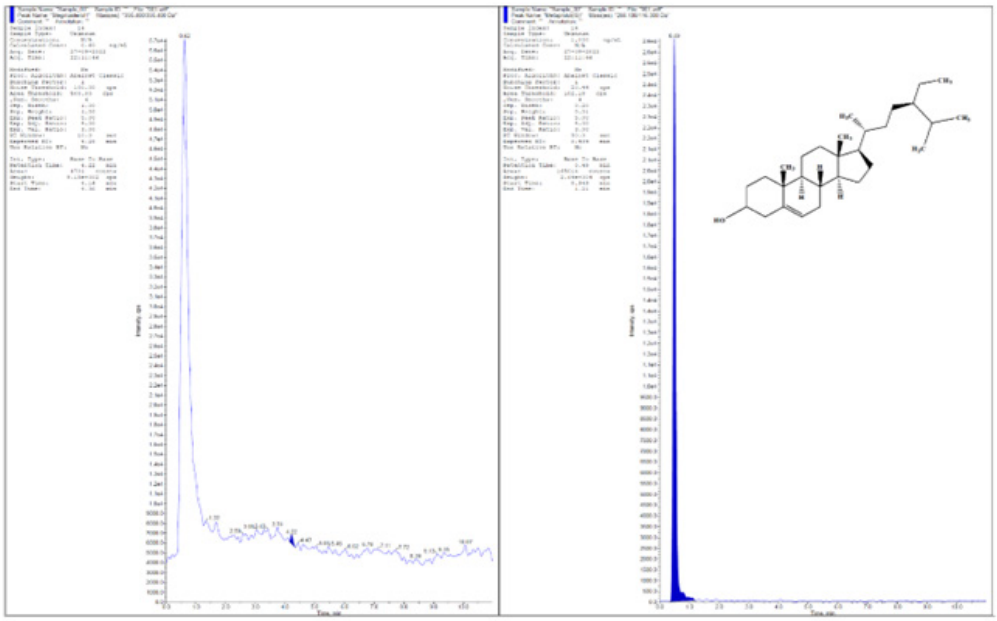

Figure 3p: Stigmasterol: *Asparagus officinalis*, aqueous extract.

Table 1: Effect of *Asparagus officinalis* extracts in analgesic activity on rats.

Groups ($n = 6$)	0 min (s)	After 30 min (s)	After 60 min (s)	After 90 min (s)	After 120 min (s)	After 180 min (s)
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	1.84 ± 0.35	1.76 ± 0.42	1.87 ± 0.38	1.85 ± 0.38	1.93 ± 0.24	1.62 ± 0.42
Group 2 (Diclofenac sodium, 10 mg/kg)	1.69 ± 0.18	1.84 ± 0.19 (4.74)	2.71 ± 0.52 [c] (45.05)	4.54 ± 0.37 [a] (145.76)	5.84 ± 0.44 [a] (203.29)	3.63 ± 0.42 [a] (124.97)
Group 3 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	1.73 ± 0.38	1.83 ± 0.32 (3.79)	1.86 ± 0.40 (-0.62)	2.09 ± 0.24 (13.18)	2.21 ± 0.41 (14.62)	1.91 ± 0.18 (18.27)
Group 4 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	1.66 ± 0.23	1.99 ± 0.18 (13.08)	2.13 ± 0.19 (14.18)	2.17 ± 0.49 (17.33)	2.51 ± 0.32 [c] (30.19)	2.60 ± 0.34 [a] (60.68)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	1.73 ± 0.25	1.73 ± 0.22 (-1.71)	1.96 ± 0.21 (4.91)	2.08 ± 0.19 (12.55)	2.54 ± 0.32 [c] (31.66)	2.96 ± 0.46 [a] (83.49)
Group 6 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	1.76 ± 0.23	1.79 ± 0.10 (1.80)	1.95 ± 0.38 (4.19)	2.20 ± 0.18 (19.13)	2.16 ± 0.24 (12.11)	2.23 ± 0.35 [c] (37.87)
Group 7 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	1.74 ± 0.34	1.91 ± 0.14 (8.34)	2.07 ± 0.41 (10.97)	2.40 ± 0.26 [c] (29.78)	2.29 ± 0.21 (18.60)	2.66 ± 0.37 [a] (64.60)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	1.77 ± 0.34	2.02 ± 0.16 (14.88)	2.19 ± 0.19 (16.95)	2.19 ± 0.25 (18.50)	2.35 ± 0.28 (21.80)	2.88 ± 0.26 [a] (78.22)
Group 9 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	1.70 ± 0.22	1.94 ± 0.19 (10.33)	1.97 ± 0.50 (5.26)	2.04 ± 0.27 (10.65)	2.17 ± 0.39 (17.82)	2.36 ± 0.27 [c] (46.23)
Group 10 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	1.67 ± 0.32	1.95 ± 0.24 (11.00)	2.00 ± 0.17 (6.87)	2.19 ± 0.18 (18.32)	2.27 ± 0.20 (17.99)	2.60 ± 0.23 [a] (61.20)
Group 11 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	1.74 ± 0.17	1.86 ± 0.14 (5.97)	2.08 ± 0.35 (11.42)	2.57 ± 0.41 [c] (39.08)	2.43 ± 0.28 (25.87)	2.92 ± 0.19 [a] (80.50)
F Value	0.1938	1.056	2.598	34.03	71.89	16.49
P Value	0.9960	0.4112	0.0117	<0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

Table 2: Effect of *Asparagus officinalis* extracts in Acute Inflammation (Using plethysmometer) activity on rats.

Groups (n = 6)	Pre-Induction hour	1st hour	2nd hour	3rd hour	4th hour	6th hour
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	0.690 ± 0.023 (-2.73)	0.695 ± 0.019 (1.65)	0.672 ± 0.015 [a] (23.24)	0.667 ± 0.022 (26.74)[a]	0.663 ± 0.023 (29.18)[a]	0.663 ± 0.019 [a] (23.90)
Group 2 (Carrageenan induced Inflammation Control)	0.672 ± 0.028	0.707 ± 0.022	0.875 ± 0.019	0.910 ± 0.011	0.937 ± 0.022	0.872 ± 0.028
Group 3 (Diclofenac sodium 10 mg/kg)	0.682 ± 0.023 (-1.49)	0.705 ± 0.018 (0.24)	0.723 ± 0.016 [a] (17.33)	0.752 ± 0.038 [a] (17.40)	0.735 ± 0.019 [a] (21.53)	0.702 ± 0.017 [a] (19.50)
Group 4 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	0.678 ± 0.033 (-0.99)	0.707 ± 0.010 (0.00)	0.732 ± 0.026 [a] (16.38)	0.768 ± 0.029 [a] (15.57)	0.753 ± 0.024 [a] (19.57)	0.737 ± 0.019 [a] (15.49)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	0.682 ± 0.029 (-1.49)	0.713 ± 0.020 (-0.94)	0.760 ± 0.011 [a] (13.14)	0.785 ± 0.019 [a] (13.74)	0.740 ± 0.023 [a] (21.00)	0.717 ± 0.016 [a] (17.78)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	0.675 ± 0.027 (-0.50)	0.715 ± 0.005 (-1.18)	0.742 ± 0.015 [a] (15.24)	0.798 ± 0.042 [a] (12.27)	0.722 ± 0.029 [a] (22.95)	0.733 ± 0.022 [a] (15.87)
Group 7 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	0.667 ± 0.027 (0.74)	0.702 ± 0.015 (0.71)	0.743 ± 0.024 [a] (15.05)	0.778 ± 0.033 [a] (14.47)	0.745 ± 0.018 [a] (20.46)	0.713 ± 0.022 [a] (18.16)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	0.678 ± 0.019 (-0.99)	0.715 ± 0.016 (-1.18)	0.740 ± 0.021 [a] (15.43)	0.788 ± 0.023 [a] (13.37)	0.748 ± 0.015 [a] (20.11)	0.725 ± 0.019 [a] (16.83)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	0.668 ± 0.029 (0.50)	0.713 ± 0.022 (-0.94)	0.757 ± 0.029 [a] (13.52)	0.808 ± 0.012 [a] (11.17)	0.752 ± 0.015 [a] (19.75)	0.732 ± 0.020 [a] (16.06)
Group 10 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	0.668 ± 0.025 (0.50)	0.713 ± 0.012 (-0.94)	0.755 ± 0.019 [a] (13.71)	0.793 ± 0.016 [a] (12.82)	0.773 ± 0.016 [a] (17.44)	0.715 ± 0.019 [a] (17.97)
Group 11 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	0.672 ± 0.031 (0.50)	0.718 ± 0.020 (-1.65)	0.740 ± 0.014 [a] (15.43)	0.753 ± 0.015 [a] (17.22)	0.743 ± 0.012 [a] (20.74)	0.702 ± 0.018 [a] (19.50)
Group 12 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	0.675 ± 0.029 (-0.50)	0.723 ± 0.018 (-2.36)	0.788 ± 0.017 [a] (9.90)	0.818 ± 0.017 [a] (10.07)	0.782 ± 0.019 [a] (16.55)	0.715 ± 0.015 [a] (17.97)
F Value	0.3819	1.186	35.30	29.37	60.09	37.85
P Value	0.9582	0.3162	<0.0001	<0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

aqueous, ethanolic and petroleum ether extracts have statistically high significant anti-arthritic effects from day 14th to 21st day at all doses. Methotrexate showed 28.33% inhibition of oedema, Prednisolone showed 32.18% inhibition of oedema. Aqueous extract was observed with highest significant range of 16.71% to 29.66% inhibition followed by ethanolic extract 16.19% to 26.34% followed by petroleum ether extract 11.41 to 23.95% inhibition (Table 4).

Effect on Paw diameter (Using Vernier caliper)

It was observed that aqueous, ethanolic and petroleum ether extracts have statistically high significant anti-arthritic effects from day 14th to 21st day at all doses. Methotrexate showed 30.78% inhibition of oedema, Prednisolone showed 32.97% inhibition of oedema. The aqueous extract was observed with highest significant (p<0.0001) range of 24.45 to 34.21% inhibition followed by ethanolic extract 22.19 to 29.91% followed by petroleum ether extract 18.69 to 26.87% inhibition (Table 5).

Effect on arthritis symptoms (Using Visual Scoring)

It was observed that aqueous, alcoholic and petroleum ether extracts have statistically high significant anti-arthritic effect from day 14 to day 21 at all doses. Visual scorings were given based on walking, inflammation, joint deformities and pain

Table 3: Effect of *Asparagus officinalis* extracts on body weights of Arthritic animals.

Groups (n = 6)	Day 1	Day 7	Day 15	Day 21
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	128.96±10.72 (-0.03)	179.67±10.99 (-5.46)	244.19±13.66 (4.25)	299.30±12.00 [c] (9.74)
Group 2 (CFA induced, Arthritic Control)	128.99±10.20	190.05±13.41	234.24±13.40	272.74±8.44
Group 3 (Methotrexate, 0.5 mg/kg)	129.10±9.75 (0.08)	181.75±12.26 (-4.37)	236.55±15.15 (0.98)	294.53±12.33 (7.99)
Group 4 (Prednisolone, 5 mg/kg)	129.43±9.06 (0.34)	182.93±9.67 (-3.75)	236.80±10.34 (1.09)	286.80±12.49 (5.16)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	129.70±6.97 (0.55)	189.53±7.84 (-0.27)	242.50±10.91 (3.53)	292.88±12.89 (7.39)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	129.71±7.00 (0.56)	188.57±8.57 (-0.77)	238.25±15.54 (1.71)	282.87±20.50 (3.71)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	129.68±6.96 (0.53)	187.54±10.12 (-1.32)	234.47±17.94 (0.10)	292.39±18.28 (7.21)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	129.75±6.83 (0.59)	189.76±11.09 (-0.15)	240.12±20.13 (2.51)	289.28±20.00 (6.07)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	129.77±6.83 (0.60)	192.10±5.46 (1.08)	243.53±6.39 (3.97)	299.04±9.20 [c] (9.65)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	129.73±6.89 (0.57)	189.80±8.50 (-0.13)	242.64±11.59 (3.59)	289.56±14.32 (6.17)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	129.76±6.84 (0.59)	177.73±11.82 (-6.48)	230.76±15.92 (1.48)	272.39±22.40 (0.13)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	129.84±6.95 (0.66)	199.14±6.05 (4.79)	254.41±10.06 [c] (8.61)	294.82±18.86 (8.10)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	129.90±6.97 (0.71)	190.37±8.10 (0.17)	240.08±13.13 (2.49)	312.99±11.16 [b] (14.76)
F Value	0.01032	2.070	1.109	2.957
P Value	>0.9999	0.0315	0.3687	0.0025

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

assessment using Grimace scale (0= Normal to 5= Severe). Methotrexate showed 51.85% recovery, Prednisolone showed 81.48% recovery. Aqueous extract was observed with highest significant (p<0.0001) range of 18.52 to 66.67% recovery followed by ethanolic extract 14.81 to 55.56% recovery followed by petroleum ether extract22.22 to 48.15% recovery of arthritic symptoms (Table 6).

Effect on Spleen weight

It is very common during arthritis spleen weight increases. It was observed that aqueous, alcoholic and petroleum ether extracts were able to statistically normalise the spleen weight which shows the anti-arthritic effect. Methotrexate showed 18.28% recovery, Prednisolone showed 43.38% recovery. Ethanolic extract was observed with highest significant(p<0.0001) range of 22.09 to 32.05% recovery followed by aqueous extract 17.37 to 26.94% recovery followed by Petroleum ether extract10.30 to 23.59% recovery of arthritic symptoms (Table 7).

Effect on RA Factor

An immune system protein called rheumatoid factor protein has the ability to assault the body's healthy tissues. The most typical association between elevated blood levels of rheumatoid factor and autoimmune illnesses. It was observed that aqueous, alcoholic and petroleum ether extracts were able to statistically decrease the RA factor value in arthritic animals. Methotrexate showed 38.43% recovery, Prednisolone showed 49.32% recovery. Ethanolic extract was observed with highest significant (p<0.0001) range of 30.11 to 63.99% recovery followed by aqueous extract 23.60 to 57.34% recovery followed by Petroleum ether extract 36.31 to 55.98% recovery of RA factor (Table 8).

Table 4: Effect of *Asparagus officinalis* extracts in Arthritis (Using plethysmometer) activity on rats.

Groups ($n = 6$)	1st Day	7th Day	14th Day	21st Day
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	0.74 ± 0.02	0.94 ± 0.02 [a] (49.09)	1.26 ± 0.06 [a] (48.11)	1.50 ± 0.09 [a] (40.41)
Group 2 (CFA induced, Arthritic Control)	0.75 ± 0.03	1.84 ± 0.03	2.42 ± 0.33	2.51 ± 0.26
Group 3 (Methotrexate, 0.5 mg/kg)	0.74 ± 0.05	1.84 ± 0.05 (0.09)	1.92 ± 0.17 [a] (20.65)	1.80 ± 0.16 [a] (28.33)
Group 4 (Prednisolone, 5 mg/kg)	0.73 ± 0.01	1.82 ± 0.10 (0.73)	1.77 ± 0.12 [a] (26.77)	1.70 ± 0.15 [a] (32.18)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	0.73 ± 0.02	1.82 ± 0.04 (1.13)	2.20 ± 0.07 (9.36)	2.23 ± 0.03 [c] (11.41)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	0.73 ± 0.02	1.84 ± 0.03 (0.00)	2.02 ± 0.08 [a] (16.66)	1.91 ± 0.08 [a] (23.95)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	0.74 ± 0.02	1.83 ± 0.02 (0.54)	1.98 ± 0.09 [a] (18.44)	1.92 ± 0.12 [a] (23.56)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	0.74 ± 0.02	1.82 ± 0.03 (1.18)	2.22 ± 0.16 (8.40)	2.08 ± 0.11 [a] (17.05)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	0.75 ± 0.02	1.84 ± 0.02 (0.09)	2.10 ± 0.11 [b] (13.42)	2.12 ± 0.19 [a] (16.19)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	0.74 ± 0.03	1.80 ± 0.03 (1.91)	1.87 ± 0.06 [a] (22.64)	1.85 ± 0.08 [a] (26.34)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	0.74 ± 0.02	1.83 ± 0.04 (0.36)	2.17 ± 0.07 [c] (10.25)	2.09 ± 0.10 [a] (16.72)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	0.73 ± 0.03	1.84 ± 0.04 (0.00)	2.03 ± 0.15 [a] (16.10)	1.94 ± 0.17 [a] (22.89)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	0.73 ± 0.02	1.83 ± 0.04 (0.27)	1.86 ± 0.09 [a] (23.33)	1.77 ± 0.09 [a] (29.66)
F Value	0.4824	204.9	24.41	20.68
P Value	0.9180	<0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

Effect on WBC's in Arthritic animals

As rheumatic arthritis is autoimmune diseases WBC's count increase is very common observation. The most common link between elevated WBC numbers is autoimmune disorders. It was observed that aqueous, alcohol and petroleum ether extracts were able to statistically decrease the WBCs value in arthritic animals. Methotrexate showed 43.32%, Prednisolone showed 65.21% decrease in WBC's count. The ethanol extract was observed with highest significant (p<0.0001) range of 52.07 to 77.19% decrease followed by petroleum ether extract41.94 to 68.43% recovery followed by aqueous extract 43.09 to 64.98% decrease in WBC's count (Table 9).

Effect on Joint Diameter in X-Ray

X-ray is good tool to see the deformities in bones and tissues around the same. It was observed that aqueous, alcohol and petroleum ether extracts were able to statistically cure the arthritis. Methotrexate showed 18.52%, Prednisolone showed 18.86% decrease joint diameter. Aqueous extract was observed with highest significant (p<0.0001) 8.13 to 22.36% decrease followed by ethanolic extract 13.53 to 18.34% decrease followed by petroleum ether extract 9.87 to 17.15% decrease joint diameter (Table 10, Figure 1).

Histopathological Study

A histological investigation of the joints after CFA injection is shown in Figure 2. The joint architecture appears normal in the non-arthritic control group; The synovial membrane, a sizable joint gap, and articular cartilage as well as bone are all destroyed in the arthritic control group. On the other hand, treatments using aqueous, ethanol, and petroleum ether extracts of *Asparagus officinalis* stopped the degeneration of the articular architecture in the treated animals. The healthy control knee joint underwent a histological investigation and showed no signs of bone erosion, inflammation, cartilage degeneration, or cellular infiltration. The

Table 5: Effect of *Asparagus officinalis* extracts in Arthritis (Using Vernier calliper) activity on rats.

Groups (n = 6)	1st Day (mm)	7th Day (mm)	10th Day (mm)	15th Day (mm)	20th Day (mm)
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	4.33 ± 0.23	4.45 ± 0.26 [a] (48.32)	5.15 ± 0.40 [a] (48.27)	5.37 ± 0.21 [a] (46.14)	5.54 ± 0. 13 [a] (43.82)
Group 2 (CFA induced, Arthritic Control)	4.29 ± 0.12	7.84 ± 0.87	9.95 ± 0.59	9.98 ± 0.27	9.86 ± 0.40
Group 3 (Methotrexate, 0.5 mg/kg)	4.31 ± 0.28	7.48 ± 0.44 (4.65)	8.14 ± 0.82 [a] (18.14)	7.22 ± 0.450 [a] (27.68)	6.83 ± 0.21 [a] (30.78)
Group 4 (Prednisolone, 5 mg/kg)	4.40 ± 0.26	7.55 ± 0.40 (3.76)	7.90 ± 0.24 [a] (20.58)	6.84 ± 0.41 [a] (31.44)	6.61 ± 0.50 [a] (32.97)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	4.17 ± 0.26	7.43 ± 0.33 (5.21)	8.75 ± 0.71 [c] (12.05)	8.24 ± 0.58 [a] (17.46)	8.02 ± 0.44 [a] (18.69)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	4.21 ± 0.07	7.41 ± 0.16 (5.46)	8.65 ± 0.27 [c] (13.06)	7.65 ± 0.58 [a] (23.35)	7.27 ± 0.27 [a] (26.25)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	4.27 ± 0.27	7.48 ± 0.16 (4.59)	8.80 ± 0.78 [c] (11.51)	7.52 ± 0.51 [a] (24.61)	7.21 ± 0.52 [a] (26.87)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	4.22 ± 0.09	7.51 ± 0.49 (4.23)	8.45 ± 0.90 [b] (15.02)	7.52 ± 0.64 [a] (24.66)	7.67 ± 0.42 [a] (22.19)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	4.21 ± 0.10	7.34 ± 0.37 (6.44)	8.86 ± 0.74 [c] (10.93)	7.35 ± 0.42 [a](26.34)	7.41 ± 0.48 [a] (24.84)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	4.22 ± 0.12	7.37 ± 0.32 (5.99)	8.05 ± 0.59 [a] (19.02)	7.11 ± 0.40 [a] (28.75)	6.91 ± 0.34 [a] (29.91)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	4.21 ± 0.15	7.55 ± 0.29 (3.72)	8.26 ± 0.40 [b] (16.98)	7.50 ± 0.39 [a] (24.79)	7.45 ± 0.34 [a](24.45)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	4.19 ± 0.17	7.68 ± 0.52 (2.13)	8.17 ± 0.50 [a] (17.86)	7.55 ± 0.42 [a] (24.29)	7.44 ± 0.59 [a](24.52)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	4.21 ± 0.14	7.43 ± 0.40 (5.27)	8.31 ± 0.63 [b](16.44)	7.44 ± 0.71 [a] (25.41)	6.49 ± 0.33 [a] (34.21)
F Value	0.7459	24.77	18.18	26.36	36.83
P Value	0.7018	<0.0001	0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

joint architecture of the animals in the arthritis control group displayed cellular infiltration, bone erosion, and cartilage loss; however, animals dosed with different plant extracts (75, 150, and 300 mg/kg), prednisolone (5 mg/kg) and methotrexate (0.5 mg/kg) demonstrated significant joint architecture protection by bone erosion reduction, cartilage destruction reduction, and decrease in cellular infiltration.

Extracts quantification using LC-MS/MS

All three extracts were analysed using LC-MS/MS. Results depicted the presence of rutin, quercetin, β-sitosterol, stigmasterol (Table 11, Figure 3). Highest concentration of rutin was present in the ethanol extract i.e. 650.45 µg/gm whereas in other two extracts showed less than 100ug/gm. In case of quercetin, highest concentration was found in aqueous extract than other two extracts. The concentration of β-sitosterol and stigmasterol were present in higher quantity in pet ether extract and very less in ethanolic extract. Both compounds were absent in aqueous extracts.

DISCUSSION

Arthritis is nothing but the presence of swelling, redness, pain or inflammation in a joint. The word "disease of the joints" in Greek is the origin of the word "arthritis." Acute or persistent joint inflammation is what's known as it, and discomfort and structural damage are frequently present alongside it. Arthralgia, or joint pain without inflammation, can be brought on by disease in the joint itself or in the nearby soft tissues, ligaments, and tendons. Subcutaneous nodules on the surfaces of the extensor muscles and soft tissue edoema around the affected joints are also observed.[29]

Table 6: Effect of *Asparagus officinalis* extracts in Arthritis (Using Arthritis Visual Score) activity on rats.

Groups ($n = 6$)	1st Day	5th Day	10th Day	15th Day	20th Day
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	0.0000	0.00 ± 0.00 [a] (100)	0.00 ± 0.00 [a] (100)	0.00 ± 0.00 [a] (100)	0.00 ± 0.00 [a] (100)
Group 2 (CFA induced, Arthritic Control)	0.0000	2.67 ± 0.52	4.50 ± 0.55	4.50 ± 0.55	4.50 ± 0.55
Group 3 (Methotrexate, 0.5 mg/kg)	0.0000	2.83 ± 0.41 (-6.25)	4.33 ± 0.52 (3.70)	2.67 ± 0.52 [a] (40.74)	2.17 ± 0.75 [a] (51.85)
Group 4 (Prednisolone, 5 mg/kg)	0.0000	2.67 ± 0.52 (0.00)	3.50 ± 0.55 [c] (22.22)	2.17 ± 0.41 [a] (51.85)	0.83 ± 0.75 [a] (81.48)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	0.0000	2.67 ± 0.52 (0.00)	4.67 ± 0.52 (-3.70)	4.17 ± 0.75 (7.41)	3.50 ± 0.55 (22.22)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	0.0000	2.83 ± 0.41 (-6.25)	4.67 ± 0.52 (-3.70)	3.33 ± 0.52 [c] (25.93)	2.67 ± 0.52 [a] (40.74)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	0.0000	3.00 ± 0.00 (-12.50)	4.67 ± 0.52 (-3.70)	2.83 ± 0.41 [a] (37.04)	2.33 ± 0.82 [a] (48.15)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	0.0000	2.67 ± 0.52 (0.00)	4.83 ± 0.41 (-7.40)	3.67 ± 0.82 (18.52)	3.83 ± 0.75 (14.81)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	0.0000	3.00 ± 0.00 (-12.50)	4.83 ± 0.41 (-7.40)	3.17 ± 0.41 [b] (29.63)	3.50 ± 0.55 (22.22)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	0.0000	3.00 ± 0.00 (-12.50)	4.67 ± 0.52 (-3.70)	2.33 ± 0.52 [a] (48.15)	2.00 ± 0.63 [a] (55.56)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	0.0000	2.83 ± 0.41 (-6.25)	4.83 ± 0.41 (-7.40)	3.17 ± 0.41 [b] (29.63)	3.67 ± 0.52 (18.52)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	0.0000	2.83 ± 0.41 (-6.25)	4.50 ± 0.55 (0.00)	3.33 ± 0.52 [c] (25.93)	2.83 ± 0.75 [b] (37.04)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	0.0000	3.00 ± 0.00 (-12.50)	4.67 ± 0.52 (-3.70)	2.50 ± 0.55 [a] (44.44)	1.50 ± 0.55 [a] (66.67)
F Value		28.27	44.18	26.71	25.19
P Value		<0.0001	<0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001,

Elevated WBC count represents the change in immunity. A minor to moderate rise in WBC counts is brought on by the production of the IL-Ib inflammatory response during arthritic circumstances. Granulocyte production and macrophage colony-stimulating factor both rise in response to IL-Ib.[1] Same was observed in our study arthritic control (14466 Cells/cumm) was observed with maximum count whereas it was significantly decreased in treated groups (4566.67 to 8400 Cells/cumm).

Rheumatoid factor (RF) was measured in serum collected on the terminal day indicates that as compare to arthritic control (22IU/mL) significant decrease was observed in treatment groups. An immunoglobulin molecule known as serum rheumatoid factor (RF) is regarded as a "non-self" molecule that has the ability to activate the immune system. A major indication of RA in the RA aetiology may be abnormal variations in the blood levels of RF and CRP.[30]

Paw oedema and joint swelling is an indicator of the anti-arthritis activity of various medicines. Determining foot swelling is a simple, sensitive, and rapid way to assess and assess the degree of inflammation.[1] With the help of plethysmometer and vernier calliper oedema and swelling was observed. Treatment groups were observed with significant decrease in swelling as compare to inflammation control group.

Same way X-ray and histopathology suggest the treatment as compare to arthritic group. Radiographic changes in the condition of arthritis are a useful diagnostic tool to get to know the severity of the arthritis. Soft tissue swelling is an early sign of radiology, but marked radiological changes such as bone erosion and narrowing of the joint space can only be observed during development.[31]

Immediately after the completion of experiment, the spleen was removed from the animal and the organ weight was measured. Animal organ weight was calculated. Spleen weight increase is very common symptom during arthritis.[30,32] Systemic autoimmune disorders are marked by an extremely high prevalence and destructiveness of splenic long-lived plasma

Table 7: Effect of *Asparagus officinalis* extracts in Arthritis Animal Spleen Weight

Groups ($n = 6$)	Spleen Weight (g)
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	0.73 ± 0.08 [c] (19.67)
Group 2 (CFA induced, Arthritic Control)	0.91 ± 0.08
Group 3 (Methotrexate, 0.5 mg/kg)	0.75 ± 0.09 [c] (18.28)
Group 4 (Prednisolone, 5 mg/kg)	0.52 ± 0.08 [a] (43.38)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	0.82 ± 0.09 (10.30)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	0.70 ± 0.14 [c] (23.59)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	0.72 ± 0.06 [c] (20.80)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	0.69 ± 0.11 [c] (25.08)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	0.71 ± 0.09 [c](22.09)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	0.62 ± 0.07 [a] (32.03)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	0.76 ± 0.13 (17.37)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	0.73 ± 0.10 [c] (20.20)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	0.67 ± 0.08 [b] (26.94)
F Value	5.702
P Value	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

Table 8: Effect of *Asparagus officinalis* extracts on RA Factor in Arthritis.

Groups ($n = 6$)	IU/mL
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	7.60 ± 1.15 [a] (65.51)
Group 2 (CFA induced, Arthritic Control)	22.03 ± 0.59
Group 3 (Methotrexate, 0.5 mg/kg)	13.57 ± 4.19 [b] (38.43)
Group 4 (Prednisolone, 5 mg/kg)	11.17 ± 5.35 [a] (49.32)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	14.03 ± 2.01 [c] (36.31)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	9.70 ± 3.05 [a] (55.98)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	13.63 ± 4.34 [b] (38.12)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	11.23 ± 2.06 [a] (49.02)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	15.40 ± 3.65 [c] (30.11)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	7.93 ± 2.67 [a] (63.99)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	16.83 ± 0.06 (23.60)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	12.93 ± 7.01 [b] (41.30)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	9.40 ± 1.81 [a] (57.34)
F Value	7.776
P Value	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

cells. The research has shown that spleen-derived CD11b+Gr-1+ myeloid cells (SDMCs) play a pathogenic role in the deposition of splenic long-lived plasma cells in the sanroque lupus-prone mouse and that SDMCs can be accumulate.[33]

Form qualitative phytochemical assays it is clear that *Asparagus officinalis* is reach in terpenoids and flavonoids. Various routes of action are already proven for mode of action against inflammation and arthritis by both flavonoids and terpenoids. Results of LC/MS proved that the extracts contain higher concentration of rutin, quercetin, β-sitosterol and stigmasterol and the protective effect may be due to the molecular action of these compounds in obstacle of rheumatoid arthritis.

The central effects of the extract in inducing anti- nociception were assessed using tail flick experiments. The tests can also be characterised by their proclivity to respond to pain stimuli delivered via various neural pathways. The tail flick method is thought to be a supraspinally organised reflex and requires higher brain activity. It looks like that flavones extracted from diverse plants, such as quercetin and rutin, produce considerable anti-nociceptive reactions. It's possible that flavonoids are responsible for the extract's analgesic action.[30] The excessive expression of TRPV1 can be inhibited by quercetin, which indicates that quercetin might relieve thermal hyperalgesia.[34]

In the case of rheumatoid arthritis, several inflammatory cytokines, such as TNF, IL6, JAK, and the TNFR1 receptor, were discovered to be actively engaged in the signalling resulting in the inflammatory response that causes the destruction of bones and adjacent tissues of the synovial joints. The inflammatory response that is involved in the course of rheumatoid arthritis can be directly inhibited by disrupting the signalling pathways involved

Table 9: Effect of *Asparagus officinalis* extracts on WBC's in Arthritis.

Groups ($n = 6$)	Cells/cumm
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	6066.67 ± 1795.36 [a] (58.06)
Group 2 (CFA induced, Arthritic Control)	14466.67 ± 750.56
Group 3 (Methotrexate, 0.5 mg/kg)	8200.00 ± 2271.56 [a] (43.32)
Group 4 (Prednisolone, 5 mg/kg)	5033.33 ± 702.38 [a] (65.21)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	8400.00 ± 3508.56 [a] (41.94)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	4566.67 ± 1553.49 [a] (68.43)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	5333.33 ± 1123.98 [a] (63.13)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	6933.33 ± 2064.78 [a] (52.07)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	6133.33 ± 680.69 [a] (57.60)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	3300.00 ± 173.21 [a] (77.19)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	8233.33 ± 862.17 [a] (43.09)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	5600.00 ± 1135.78 [a] (61.29)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	5066.67 ± 2369.25 [a] (64.98)
F Value	16.02
P Value	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

Table 10: Effect of *Asparagus officinalis* extracts on Arthritic Joints in X-Ray.

Groups ($n = 6$)	mm
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	6.29 ± 0.35 [a] (38.43)
Group 2 (CFA induced, Arthritic Control)	10.21 ± 0.64
Group 3 (Methotrexate, 0.5 mg/kg)	8.32 ± 0.68 [a] (18.52)
Group 4 (Prednisolone, 5 mg/kg)	8.29 ± 0.51 [a] (18.86)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	9.21 ± 0.44 [c] (9.87)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	8.54 ± 0.57 [a] (16.38)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	8.46 ± 0.20 [a] (17.15)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	9.73 ± 0.42 (4.72)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	8.83 ± 0.37 [a] (13.53)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	8.34 ± 0.40 [a] (18.34)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	9.38 ± 0.63 [c] (8.13)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	8.32 ± 0.31 [a] (18.51)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	7.93 ± 0.49 [a] (22.36)
F Value	23.75
P Value	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

Table 11: Extracts quantification using LC-MS/MS.

Extracts	Rutin (µg/g)	Quercetin (µg/g)	β-sitosterol (µg/g)	Stigmasterol (µg/g)
Asparagus officinalis, petroleum ether extract	91.07	3.51	941.986	860.932
Asparagus officinalis, ethanolic extract	650.45	3.63	34.36	26.196
Asparagus officinalis, aqueous extract	41.18	4.48	-	-

in the control of the inflammatory response, either by suppressing the aforementioned cytokines or by antagonisting the TNFR1 receptor. As a result, the TNFR1 receptor and the cytokines TNF, IL6, and JAK potentially serve as significant therapeutic targets for the therapy of rheumatoid arthritis.

Flavonoids have anti-inflammatory, analgesic, and antioxidant properties. These effects are associated with the suppression of NF-B-dependent pro-inflammatory cytokines, VEGF, ICAM-1, and STAT3, as well as the activation of the antioxidant transcription factor Nrf2.

The PI3K/Akt signalling pathway is thought to be a link between FLS cell proliferation and apoptosis. PI3K and Akt are highly expressed in RA-FLS cells and have an impact on FLS cell migration. Furthermore, many inflammatory cytokines, such as IL-17 and IL-21, can endorse FLS cell inflammatory proliferation by provoking and activating PI3K flavonoids, which can inhibit p-Akt expression in B cells.[35] Likewise, flavonoids can restrict the MAPK signalling pathway, thereby reducing RA symptoms.[36] In RA, NF-B and STAT are profoundly and sustainably activated, which flavonoids improve.[37] Triterpene decreases pro-inflammatory cytokines such IL-6, IL-1, TNF-α and increases the anti-inflammatory cytokine IL-10 in rats with arthritis caused by complete Freund's adjuvant (CFA).[38]

According to certain studies, the useful pharmacological effects of terpenes for arthritis are linked to the regulation of many intracellular signalling pathway proteins, including RANKL, the MAPK family, NFkB, PGE-2, COX-2, iNOS, matrix metalloproteinases, MPO, and c –FOS.[39] Angiogenesis is primarily mediated by VEGF/VEGFR2 signalling, which is a key target for anti-angiogenic therapies. When VEGF and VEGFR2 interact, VEGFR2 is phosphorylated on multiple tyrosine residues, triggering signalling cascades that promote EC proliferation, migration, survival, and permeability. Thus, VEGFR2 activation is a critical step in the angiogenesis process, and blocking VEGF/VEGFR2 signalling with VEGFR2 inhibitors can suppress angiogenic responses. β-Sitosterolis has already reported the improvement in RA by VEGF pathway.[40]

By lowering MMP-8 secretion and synthesis, Stigmasterol therapy is said to ameliorate joint-related pathology to levels close to normal. This drop in the levels of metalloproteinase MMP-8 and inflammatory mediators is most likely brought on by a decrease in the activity of the transcription factors MAPKs and NF-kBp65.[41]

CONCLUSION

According to the findings of this study, *Asparagus officinalis* is a plant that contains various chemical component groups that have anti-inflammatory, analgesic and anti-arthritic activities. *In vivo* tests have been done to assess these qualities. These findings support the plant's use in the conventional management of chronic inflammatory illnesses, and also suggest that it may be a candidate for the discovery of novel anti-inflammatory, analgesic and/or anti-arthritic compounds. We have identified the presence of rutin, quercitine, stigmasterol and β- sitosteroid. But during our analysis we observed many other molecular weight peaks which shows the presence of many more ingredient availability. To determine the precise mechanism of action of *Asparagus officinalis* on rheumatoid arthritis, more research can be done.

ACKNOWLEDGEMENT

We would like to acknowledge all authors for idea, execution and implementation. We would also appreciate management for giving the opportunity to writing this manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

LC-MS/MS: Liquid Chromatography with tandem mass spectrometry; **µL:** Microliter; **mL:** Millilitre; **ng:** Nanogram; **IL:** Interleukins; **TNF-α:** Tumour necrosis factor α; **COX:** Cyclooxygenase isoenzyme; **CFA:** Complete Freund's adjuvant; **kg:** Kilogram; **mg:** Milligram; **p.o.:** Per oral; **Amps:** Amperes; **DP:** Declustering potential; **CE:** Collision energy; **MRM:** Multiple reaction monitoring; **V:** Volt; **WBC's:** White blood cells; **gm:** Gram; **RF:** Rheumatoid factor; **IU:** International units; **SDMCs:** Spleen-derived CD11b+Gr-1+ myeloid cells; **TRPV:** Transient receptor potential cation channel subfamily V; **VEGF:** Vascular endothelial growth factor; **ICAM:** Intercellular Adhesion Molecule; **MAPK:** Mitogen-activated protein kinases; **RANKL:** Receptor activator of nuclear factor kappa-B ligand; **MPO:** Myeloperoxidase.

SUMMARY

Asparagus officinalis is one of the plant originate from region of South India. The plant has a potential of various pharmacological activities. Traditionally, this plant reported to treat inflammation. Extracts of *Asparagus officinalis* showed anti inflammatory and anti arthritic activity.

Authors' Contribution

All the authors contributed equally for the design of concept, execution of research work and drafting of the manuscript. SG and PS designed the concept, while the execution of studies was performed by SK. All SK, PS and SG equally contributed for the drafting and finalization of the manuscript.

Ethical Approval

This study was approved by the Deemed University Animal and Ethical Committee.

REFERENCES

1. Kshirsagar AD, Panchal PV, Harle UN, Nanda RK, Shaikh HM. Anti-inflammatory and antiarthritic activity of anthraquinone derivatives in rodents. Int J Inflam. 2014;2014:690596. doi: 10.1155/2014/690596, PMID 25610704.
2. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, *et al*. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018;9(6):7204-18. doi: 10.18632/oncotarget.23208, PMID 29467962.
3. Cologne. Germany: Institute for quality and efficiency in health care (IQWiG). p. 2006-; 2010. What is an inflammation? Inform Educ. Health.org; [updated Feb 22 2018] [internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279298.

4. Yuksel N. Meet Our Editorial Board Member. Curr Drug Deliv. 2017;14(1):1-. doi: 10.2174/1567201814014017010911533 2.
5. Yeom MJ, Lee HC, Kim GH, Lee HJ, Shim I, Oh SK, *et al*. Anti-arthritic effects of Ephedra sinica STAPF herb-acupuncture: Inhibition of lipopolysaccharide-induced inflammation and adjuvant-induced polyarthritis. J Pharmacol Sci. 2006;100(1):41-50. doi: 10.1254/jphs.fp0050637, PMID 16404132.
6. Peng S, Hu C, Liu X, Lei L, He G, Xiong C, *et al*. Rhoifolin regulates oxidative stress and proinflammatory cytokine levels in Freund's adjuvant-induced rheumatoid arthritis via inhibition of NF-κB. Braz J Med Biol Res. 2020;53(6):e9489. doi: 10.1590/1414-431x20209489. PMID 32401927, PMCID PMC7233197.
7. Marnett LJ, Rowlinson SW, Goodwin DC, Kalgutkar AS, Lanzo CA. Arachidonic acid oxygenation by COX-1 and COX-2. Mechanisms of catalysis and inhibition. J Biol Chem. 1999;274(33):22903-6. doi: 10.1074/jbc.274.33.22903, PMID 10438452.
8. Giménez-Bastida JA, Shibata T, Uchida K, Schneider C. Roles of 5-lipoxygenase and cyclooxygenase-2 in the biosynthesis of hemiketals E2 and D2 by activated human leukocytes. FASEB J. 2017;31(5):1867-78. doi: 10.1096/fj.201601136R, PMID 28096231.
9. Gou H, Ye J, Wang Y, Xu X, Shen Q, Xue J, *et al*. The anti-inflammatory activity of Toonaciliatin K against adjuvant arthritis. BioMed Res Int. 2017;2017:9436280. doi: 10.1155/2017/9436280, PMID 29181410.
10. Gupta S, Mehla K, Chauhan D, Nair A. Anti-inflammatory activity of leaves of Michelia champaca investigated on acute inflammation induced rats. 2010.
11. Fan SH, Ali NA, Basri DF. Evaluation of Analgesic Activity of the Methanol Extract from the Galls of Quercus infectoria (Olivier) in Rats. Evid Based Complement Alternat Med. 2014;2014:976764. doi: 10.1155/2014/976764. PMID 25254062.
12. Zhang L, Zhu M, Li M, Du Y, Duan S, Huang Y, *et al*. Ginsenoside Rg1 attenuates adjuvant-induced arthritis in rats via modulation of PPAR-γ/NF-κB signal pathway. Oncotarget. 2017;8(33):55384-93. doi: 10.18632/oncotarget.19526, PMID 28903427.
13. Toonen EJM, Laskewitz AJ, Van Dijk TH, Bleeker A, Grefhorst A, Schouten AE, *et al*. Glucose kinetics in the collagen-induced arthritis model: An all-in-one model to assess both efficacy and metabolic side effects of glucocorticoids. PLOS ONE. 2014;9(9):[e98684]. doi: 10.1371/journal.pone.0098684, PMID 25181348.
14. Kala CP, Dhyani PP, Sajwan BS. Developing the medicinal plants sector in northern India: Challenges and opportunities. J Ethnobiology Ethnomedicine. 2006;2(1):32. doi: 10.1186/1746-4269-2-32.
15. Choudhary M, Kumar V, Malhotra H, Singh S. Medicinal plants with potential anti-arthritic activity. J Intercult Ethnopharmacol. 2015;4(2):147-79. doi: 10.5455/jice.20150313021918, PMID 26401403.
16. Adhikari PP, Paul SB. History of Indian traditional medicine: A medical inheritance. Asian J Pharm Clin Res. 2018;11(1):421-6. doi: 10.22159/ajpcr.2018.v11i1.21893.
17. Tewari D. Traditional medicine an Indian Perspective: History and Outlook. Eur J Mol Clin Med. 2020;07:07.
18. Battu GR, Kumar BM. Anti-inflammatory activity of leaf extract of *Asparagus racemosus* Willd. Int J Chem Sci. 2010;8(2):1329-38.
19. Al-Snafi AE. Arabian medicinal plants with anti-inflammatory effects- plant based review (part 1). IOSR J Pharm. 2018;8(7):55-100.
20. Kim BY, Cui ZG, Lee SR, Kim SJ, Kang HK, Lee YK, *et al*. Effects of *Asparagus officinalis* extracts on liver cell toxicity and ethanol metabolism. J Food Sci. 2009;74(7):H204-8. doi: 10.1111/j.1750-3841.2009.01263.x, PMID 19895471.
21. Khorasani A, Sani W, Philip K, Tah RM, Rafat A. Antioxidant and antibacterial activities of ethanolic extracts of *Asparagus officinalis* cv. Mary Washington: Comparison of *in vivo* and *in vitro* grown plant bioactivities. Afr J Biotechnol. 2010;9(49):8460-6.
22. Minh NP, Them LT, Dang NC, Ho TP, Hieu NT, Luan LQ. Investigation of *Asparagus officinalis* Shoot size and drying affecting to herbal tea production. J Pharm Sci Res. 2019;11(2):267-72.
23. Hafizur RM, Kabir N, Chishti S. *Asparagus officinalis* extract controls blood glucose by improving insulin secretion and β-cell function in streptozotocin-induced type 2 diabetic rats. Br J Nutr. 2012;108(9):1586-95. doi: 10.1017/S0007114511007148, PMID 22221560.
24. Navya PE, Allenki V. Evaluation of *Asparagus officinalis* for anti- diabetic activity in rats. IJIPSR. 2017;5(07):1-9.
25. Poormoosavi SM, Najafzadehvarzi H, Behmanesh MA, Amirgholami R. Protective effects of *Asparagus officinalis* extract against bisphenol A- induced toxicity in Wistar rats. Toxicol Rep. 2018;5:427-33. doi: 10.1016/j.toxrep.2018.02.010, PMID 29854613, PMCID PMC5977383.
26. Lalan BK, Hiray RS, Ghongane BB. Evaluation of Analgesic and Anti-Inflammatory Activity of Extract of Holoptelea integrifolia and *Argyreia speciosa* in Animal Models. J Clin Diagn Res. 2015;9(7):FF01-4. doi: 10.7860/JCDR/2015/12059.6200. PMID 26393140.
27. Onasanwo SA, Fabiyi TD, Oluwole FS, Olaleye SB. Analgesic and anti-inflammatory properties of the leaf extracts of Anacardium occidentalis in the laboratory rodents. Niger J Physiol Sci. 2012;27(1):65-71. PMID 23235310.
28. Kumar S, Hitendra, Bhatia M, Garg LN, Gupta S. Acute and chronic inflammation studies of *Strobilanthes callosus* leaves extract on rat model. Inflammopharmacology. 2013;21(3):233-9. doi: 10.1007/s10787-012-0150-8, PMID 22983841.
29. Senthelal S, Li J, Ardeshirzadeh S, *et al*. Updated 2022 Jun 19. Arthritis [cited 6/12/2022]. In: StatPearls [internet]. Treasure Island, (FL): StatPearls Publishing; 2022 Jan-. Available from. Available from: https://www.ncbi.nlm.nih.gov/books/NBK518992 [cited 6/12/2022].
30. Mbiantcha M, Almas J, Shabana SU, Nida D, Aisha F. Anti-arthritic property of crude extracts of *Piptadeniastrum africanum* (*Mimosaceae*) in complete Freund's adjuvant-induced arthritis in rats. BMC Complement Altern Med. 2017;17(1):111. doi: 10.1186/s12906-017-1623-5, PMID 28202019.
31. Chinnasamy V, Subramaniyan V, Chandiran S, Kayarohanam S, Kanniyan DC, Velaga VSSR, *et al*. Antiarthritic activity of Achyranthes aspera on formaldehyde - induced arthritis in rats. Open Access Maced J Med Sci. 2019;7(17):2709-14. doi: 10.3889/oamjms.2019.559, PMID 31844425.
32. Cui X, Wang R, Bian P, Wu Q, Seshadri VDD, Liu L. Evaluation of antiarthritic activity of nimbolide against Freund's adjuvant induced arthritis in rats. Artif Cells Nanomed Biotechnol. 2019;47(1):3391-8. doi: 10.1080/21691401.2019.1649269, PMID 31394949.
33. Jang E, Cho S, Pyo S, Nam JW, Youn J. An inflammatory loop between spleen-derived myeloid cells and CD4+ T cells leads to accumulation of long-lived plasma cells that exacerbates lupus autoimmunity. Front Immunol. 2021;12:631472. doi: 10.3389/fimmu.2021.631472, PMID 33643317.
34. Gao W, Zan Y, Wang ZJ, Hu XY, Huang F. Quercetin ameliorates paclitaxel-induced neuropathic pain by stabilizing mast cells, and subsequently blocking PKCε-dependent activation of TRPV1. Acta Pharmacol Sin. 2016;37(9):1166-77. doi: 10.1038/aps.2016.58, PMID 27498772.
35. Feng FB, Qiu HY. Effects of artesunate on chondrocyte proliferation, apoptosis and autophagy through the PI3K/AKT/mTOR signaling pathway in rat models with rheumatoid arthritis. Biomed Pharmacother. 2018;102:1209-20. doi: 10.1016/j.biopha.2018.03.142, PMID 29710540.
36. Sun G, Xing C, Zeng L, Huang Y, Sun X, Liu Y. *Flemingia philippinensis* flavonoids relieve bone erosion and inflammatory mediators in CIA mice by downregulating NF-κB and MAPK pathways. Mediators Inflamm. 2019;2019:5790291. doi: 10.1155/2019/5790291, PMID 30906224, PMCID PMC6397971.
37. Bai L, Bai Y, Yang Y, Zhang W, Huang L, Ma R, *et al*. Baicalin alleviates collagen induced arthritis and suppresses TLR2/MYD88/NF κB p65 signaling in rats and HFLS RAs. Mol Med Rep. 2020;22(4):2833-41. doi: 10.3892/mmr.2020.11369, PMID 32945496.
38. Chen JY, Wu H, Li H, Hu SL, Dai MM, Chen J. Anti-inflammatory effects and pharmacokinetics study of *geniposide* on rats with adjuvant arthritis. Int Immunopharmacol. 2015;24(1):102-9. doi: 10.1016/j.intimp.2014.11.017, PMID 25434608.
39. Carvalho AMS, Heimfarth L, Santos KA, Guimarães AG, Picot L, Almeida JRGS, *et al*. Terpenes as possible drugs for the mitigation of arthritic symptoms – A systematic review. Phytomedicine. 2019;57:137-47. doi: 10.1016/j.phymed.2018.10.028, PMID 30668316.
40. Qian K, Zheng XX, Wang C, Huang WG, Liu XB, Xu SD, *et al*. β-sitosterol Inhibits Rheumatoid synovial angiogenesis through Suppressing VEGF Signaling Pathway. Front Pharmacol. 2021;12:816477. doi: 10.3389/fphar.2021.816477, PMID 35295740.
41. Ahmad Khan M, Sarwar AHMG, Rahat R, Ahmed RS, Umar S. Stigmasterol protects rats from collagen induced arthritis by inhibiting proinflammatory cytokines. Int Immunopharmacol. 2020;85:106642. doi: 10.1016/j.intimp.2020.106642, PMID 32470883