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In Vitro Anti-Inflammatory Evaluation of Aqueous Extract of *Asparagus racemes*

Eylin Purathur Paul[1], Feebarani John[1], Bindu Jose[1], Vidhya Mohanan Puthiyamadathil[2] and Nelson Pynadath Rumjit[2]

[1]Department of Zoology, Vimala college, Thrissur, Kerala, India
[2]Marian Centre for Advanced Research, St. Mary's College, Thrissur, Kerala 680020, India

**Corresponding Author*

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Abstract: *Asparagus racemosus*, found in Asia and Africa, is a significant medicinal herb which is also known by the names satavar, shatavari, or shatarnull. According to studies, shatavari root extracts do have anti-inflammatory properties. Root extract lowers myeloperoxidase activity, skin thickness, and the production of inflammatory cytokines. Histopathological analysis also revealed anti-inflammatory activity. In this study, an aqueous extract of *Asparagus racemosus* was produced using the decoction method, and its stability was assessed using an *in vitro* membrane stability experiment. The biological effect of *Asparagus racemosus* has been optimized. Moreover, compared the effects of an aqueous extract and an aspirin standard medication. The identification of the phytochemical components found in the racemosus aqueous extract and the bioactive fraction's characterization was performed by using GC-MS. Protein denaturation assay was also performed.

Keywords: Anti-inflammation, *Asparagus racemosus*, Aqueous extract, Decoction, GC-MS

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Introduction

Asparagus racemosus, commonly referred to as Satavar or Shatavari, is a member of the Asparagaceae family. As a general health tonic, it has been employed in traditional medical systems such as Ayurveda, Siddha, and Unani. *Asparagus* is the Greek term which means stalk or shoot. Though it is primarily grown in India, it is also found in tropical Africa, Java, Australia, Sri Lanka, and southern China. The primary bioactive components of the herb that confer its medical value are a class of steroidal saponins, sarsasapogenins, flavonoids, kaempferol, quercetin, rutin, and polyphenols (Ganie *et al*., 2024). The herbaceous plant's tuberous roots are frequently

utilized in the pharmaceutical and biotechnological industries for the production of various herbal treatments due to its exceptional potential (Hamdi *et al.*, 2024). Pharmacological characteristics like rejuvenator, antioxidant, immunomodulator, estrogenic, antistress, antidiabetic, antibacterial, antiviral, antifungal, anti-amoebic, anticancer, and gastroprotective, have been demonstrated for the root extracts (Tavanappanavar *et al*., 2024).

In the current study aqueous extract of *Asparagus racemosus* was prepared by using decoction method and evaluation through vitro membrane stability assay. Performed optimization of the biological action of *Asparagus racemosus*. Compared the action of standard drug (Aspirin) and aqueous extract. The determination of the phytochemical constituents, present in the aqueous extract of racemosus and characterization of bioactive fraction by employment of GC-MS. Also done protein denaturation assay.

Materials and Methods

Collection of plant materials:

Asparagus racemosus was collected from local areas of Thrissur. Plants were identified and authenticated by a botanist Dr. Sr. Meena K Cheruvathur, Department of Botany. St. Mary's College, Thrissur, Kerala, India. The samples were brought to the laboratory and were observed carefully for any kind of disease or infection and if found any, those parts were separated and not used for the experiment.

Preparation of plant extract:

Collected plants of *Asparagus racemosus* were washed thoroughly using tap water and again washed with distilled water. The washed materials were dried using hot air oven at 60°C and grinded into fine powder. 5 g of fine powder was boiled with 100 ml of deionized water for 15 min using water bath. The plant extract was filtered and collected separately (Waghulde *et al*., 2019).

Screening for Membrane stabilization assay (*Chowdhury et al., 2014*):

Using the *in vitro* HRBC membrane stabilization method, the anti-inflammatory efficacy of the aqueous extract of the *Asparagus racemosus* decoction was evaluated. Volunteers in good health gave blood. Then equal parts of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, 0.42% sodium chloride, and 100 ml of distilled water) were added to the drawn blood, the mixture was centrifuged using isosaline. An equal volume of the test medication in three separate concentrations -- 100, 200, and 300 μg/ml, was added to 1 ml of HRBC solution. Following a 30 min incubation period at 37°C, each assay combination was centrifuged. Using a spectrophotometer set at 560 nm, the amount of hemoglobin in the supernatant solution was calculated. The following formula was then used to determine the proportion of hemolysis:

Hemolysis percentage = (test/control) x 100

Therefore, the following equation can be used to compute the percentage of protection:

100 - (OD of test/OD of control × 100) = % of protection

Where, OD of control refers to the optical density or absorbance of the negative control, and OD of test refers to the optical density or absorbance of the test sample.

Optimizing the effect of limiting factors in membrane stability activity:

Various factors such as temperature, pH, and concentration were checked for its effect in maximizing membrane stability activity of *Asparagus racemosus*. The solution was prepared by adding varying concentration (0.2- 0.8 ml) of plant extract in distilled water. Influence of concentration was analysed from the obtained data. Similarly, the effect of pH was performed by varying the pH from 4 to 10 at a constant concentration (0.2 ml) of *Asparagus racemosus* aqueous extract. pH was maintained by three different buffers; acetate (3.6-5.6), phosphate (5.8-7.4), bicarbonate (9.2- 10.6). The effect of temperature was evaluated by incubating reaction mixture atvarious temperatures (3-73 °C).

Comparison of standard drug Aspirin with a queous extract of Asparagus racemosus:

The concentration showing the maximum activity from the screening was considered and compared with reference compound Aspirin.

Phytochemical analysis:

The leaf extract was evaluated by standard protocols (Abhimannue *et al*., 2022) to detect existence of various phyto-constituents like flavonoids, alkaloids, saponins, phenols (Bagewadi *et al*., 2019).

Characterization of Bioactive Fraction of plant extract:

The bioactive constitutes present in leaf extract was determined by Gas Chromatography (Agilent 6890 series) equipped with HP-5MS column mass spectrometer operated at initial column temperature of 30°C and heated up to 300°C at 10°C/5 min. Chromatographic were: flow of 1 ml/min of high purity helium carrier gas in split mode. The identification of the compounds in spectra was done based on retention time and integral area of peaks. The similarity of compounds matched with >70% are listed based on NIST library search.

Protein Denaturation Assay:

This was assayed based on a previously described method. Similarly, 5 ml of a reaction mixture consisting of egg albumin (0.2 ml), phosphate buffered saline pH 6.4 (2.8 ml.) and *Asparagus racemosus* extract or distilled water (control) (2ml) was prepared. Diclofenac sodium was used as the control. Two different concentrations of extract and diclofenac sodium (I ml, 250 µg/ml) were prepared. After that, each 5 ml of solution of extract and standard drug solution were added in their respective test tubes. The mixture was incubated at 57°C for 20 min and then heated at 70°C for 5 min. After cooling the absorbance of the solutions was measured at 660 nm against a blank solution (solution excluding the extract/standard). The percentage inhibition of protein denaturation was calculated using the formula as:

$$\text{Percentage (\%) of inhibition} = \frac{\text{100-AC -AT sample}}{\text{AC}} \text{ x 100}$$

Where AT is absorbance of test; AC is absorbance of control.

H_2O_2 Radical Scavenging Activity (*Tavanappanavar et al., 2024*):

0.05 g of potassium dichromate was dissolved in 1 ml of distilled water. To this 3 ml of acetic acid was added, and finally the solution was made up to 15 ml with distilled water. 0.2-1 ml of test sample (10 mg/10 ml) was taken in different test tubes to which 1 ml of H_2O_2 was added. The tubes were incubated for 5 min at room temperature. After 5 min 2 ml of potassium dichromate: acetic acid reagent was added and the tubes were incubating for 10 min at room temperature. The absorbance value of the reaction mixture was recorded at 700 nm. Blank containing the phosphate buffer without the plant extract.

Results and Discussion

Screening for membrane stabilization assay:

The blood sample collected was placed in incubator at room temperature and analyzed for activity within 24 h. From the results it was evident that the drug showed an activity percentage of 85.4% while for the aqueous plant extract of *Asparagus racemosus* it was 80.92%. This clearly indicated that the plant extract showed similar membrane stabilization activity. From previous research it was observed that at a concentration of 100 µg/ml methanolic extract of *Gardenia coronaria* had an anti-inflammatory activity of 67% while the positive control aspirin at l00 µg/ml had 62% activity (Table 1). Even the highest concentration of the extract at 300 µg/ml was able to stabilize the RBC membrane by 76% which was yet 14% greater than that of Aspirin when used in only l00 µg/ml (Chowdhury *et al*., 2014).

Phytochemical analysis:

The chemometric analysis showed the presence of secondary metabolites like terpenoids and

Table 1: Screening for aqueous extract of *Asparagus racemosus*

Aqueous extract	Percentage of membrane Stability (%)
Asparagus racemosus	64.61

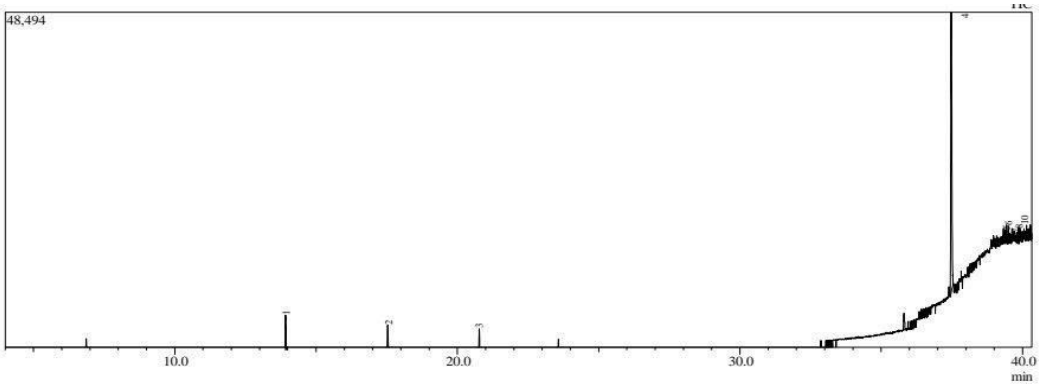

Fig. 1: GCMS analysis of *Asparagus racemosus.*

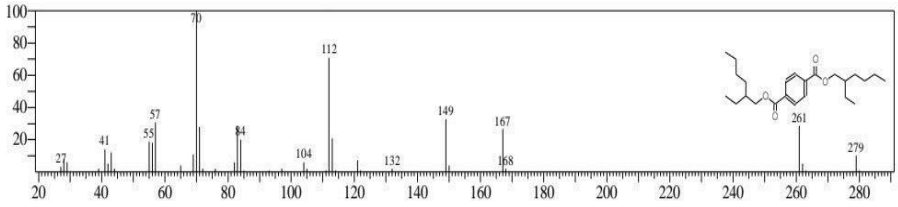

Fig. 2: GCMS analysis of compound 1,4- Benzenedicarboxylic acid, bis(2-ethylhexyl) ester.

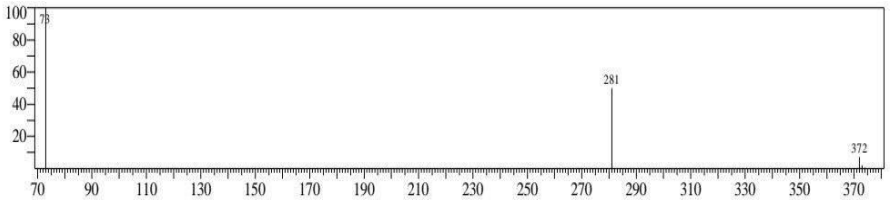

Fig. 3: GCMS analysis of compound 2-cyclobuten-1-one.

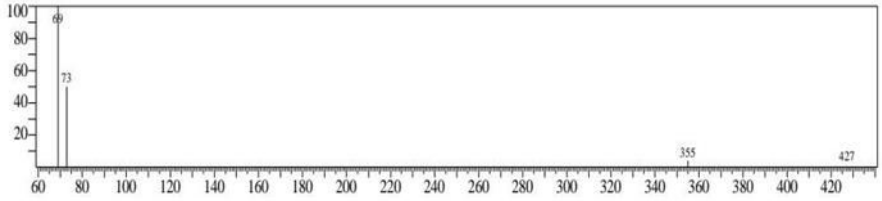

Fig. 4: GCMS analysis of compound phosphorous dibromide.

flavonoids etc. From GCMS data it was evident that the bioactive fragment was composed of 10 compounds (Fig. 1). From the chromatogram it was observed that the major compound was 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (Fig. 2). The molecular formula is $C_{24}H_{38}O_4$ and molecular weight was 390 D. The peak was found at retention time 37.475 min. The different compounds in the aqueous extract of *Asparagus racemosus* includes 2-cyclobuten-1-one (Fig. 3), Phosphonous dibromide (Fig. 4), 7-oxabicyclo [2.2.1] hept-2-ene (Fig. 5), 12-azabicyclo (9.2.1)

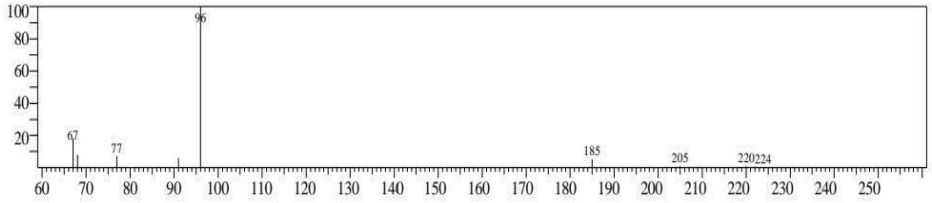

Fig. 5: GC MS analysis of compound 7- oxabicyclo [2.2.1]hept-2-ene.

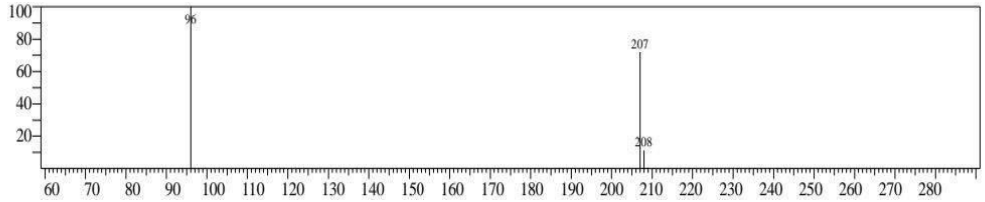

Fig. 6: GC MS analysis of compound 12- azabicyclo (9.2.1) tetradeca-1(14)-ene-13-one.

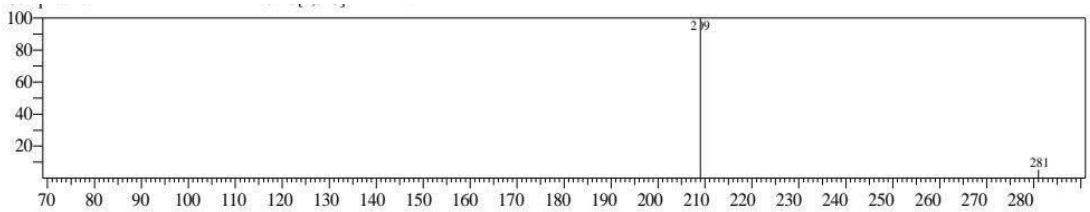

Fig. 7: GCMS analysis of compound N-butyl-(t-butyl-2-benzoylbenzamide).

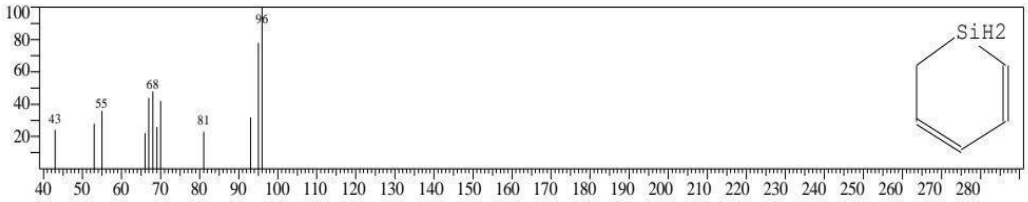

Fig. 8: GCMS analysis of compound 4-methyl-1H- pyrazolo[4,3-C]pyridine.

tetradeca-1(14)-ene-13-one (Fig. 6), n-(t-butyl)-2-benzoylbenzamide (Fig. 7), 4-methyl-1H-pyrazolo [4,3-c] pyridine (Fig. 8), 1-Silacyclo-2,4- hexadiene (Fig. 9), 1-Silacyclohexa-2,5-diene (Fig. 10), and Phosphonic acid (Fig. 11). Some of the compounds have antifugal, anti-inflammatory and anti-oxidant activities. Overall these phyto-constituents have been shown to possess different biological activities and industrial applications such as anti inflammation, anti-oxidant, anti-viral, etc. So the present study is expected to help in identifying the anti-inflammatory activity of aqueous extract of *Asparagus racemosus.*

GC-MS analysis of the aqueous extract of leaves of *Asparagus racemosus* revealed the presence of different acids, heterocyclic compounds, esters among others. This confirms the results on presence of the various secondary metabolite compounds detected by the qualitative procedures. These mass spectra are fingerprint of the compound which can be identified from the data library. Hence, the identified phyto components using GC-MS can be used as a pharmacognostical tool for the identification of key active compounds. Dhanya *et al*. (2024) suggests that the extract is a potent therapeutic

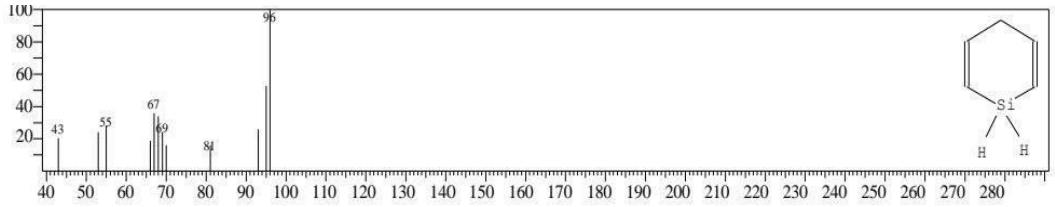

Fig. 9: GCMS analysis of compound 1-Silacyclohexa-2,4-hexadiene.

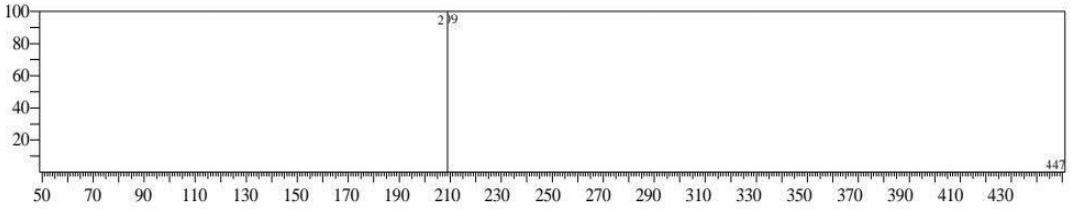

Fig. 10: GCMS analysis of compound 1-Silacyclohexa-2,5-diene.

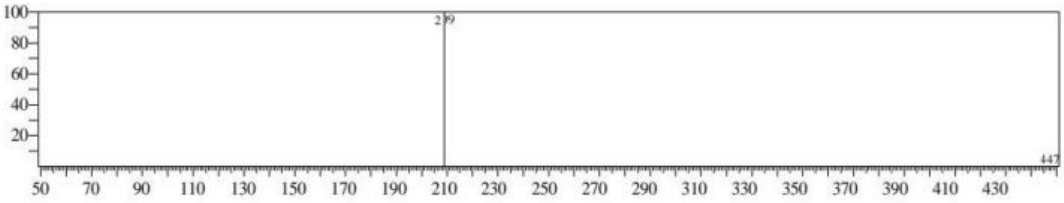

Fig. 11: GCMS analysis of compound Phosphonic acid.

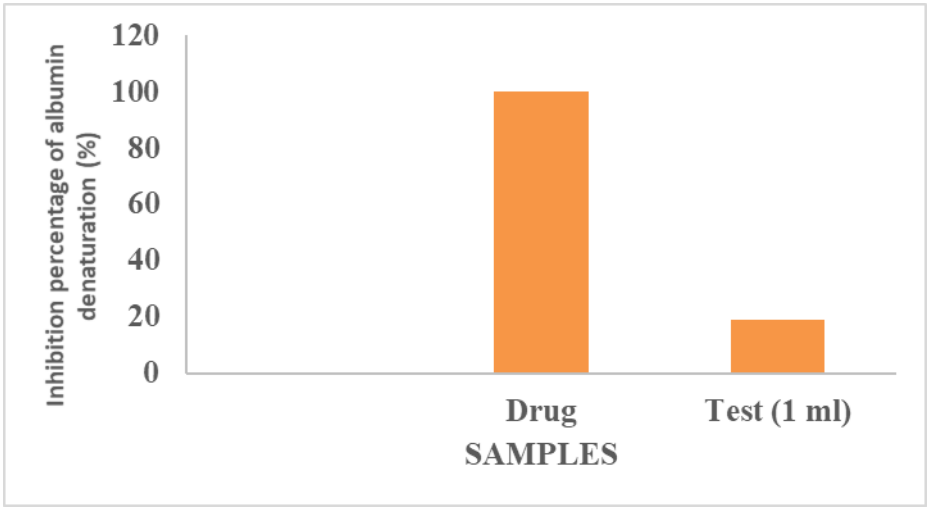

Fig. 12: The anti-inflammatory activity of the extract.

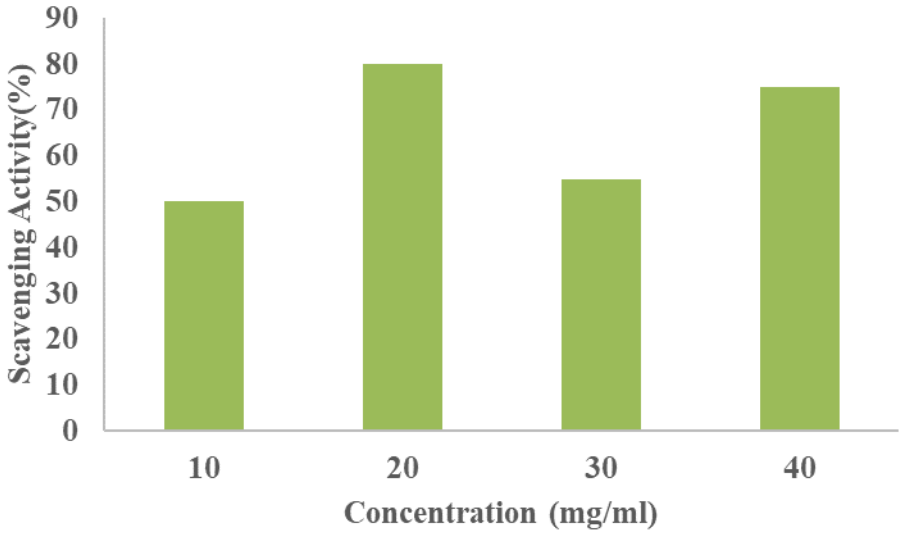

Fig. 13: Percentage of scavenging activity.

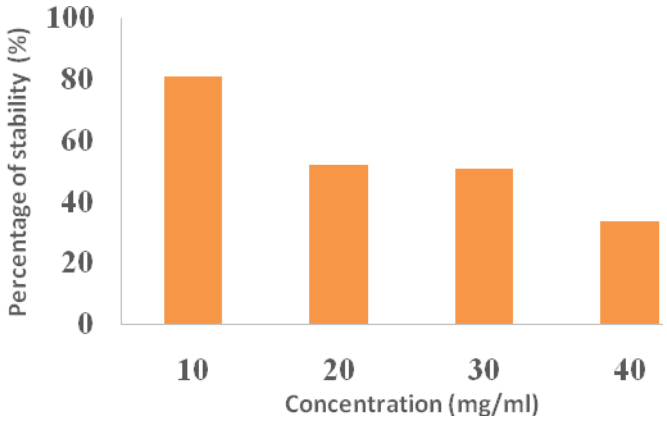

Fig. 14: Effect of extract on percentage stability at different concentration.

agent. It paves the way for the development of several treatment regimens based on this extract. In addition, further research is necessary to identify and purify the active compounds responsible for therapeutic activity, as well as the identified compounds. (Gavamukulya *et al*., 2015).

Inhibition of Albumin Denaturation:

500 ml of l% bovine serum albumin was added to *Asparagus racemosus* (500, 250, 100, 50 and 10 µg/ml) of test sample. This mixture was kept at room temperature for 10 min, followed by heating at 51°C for 20 min. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 mm. Acetyl salicylic acid was taken as a positive control. The experiment was carried out and per cent inhibition for protein denaturation was calculated using:

% Inhibition=100-((A1-A2)/Ai)*100)

Where Al is the absorbance of the control, A2 is the absorbance of the test sample and AO is the absorbance of the positive control.

A dose-response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run accurately.

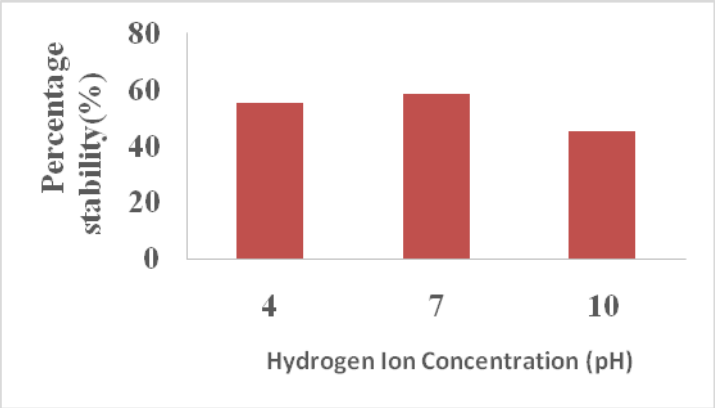

Fig. 15: Effect of extract on percentage stability at different pH.

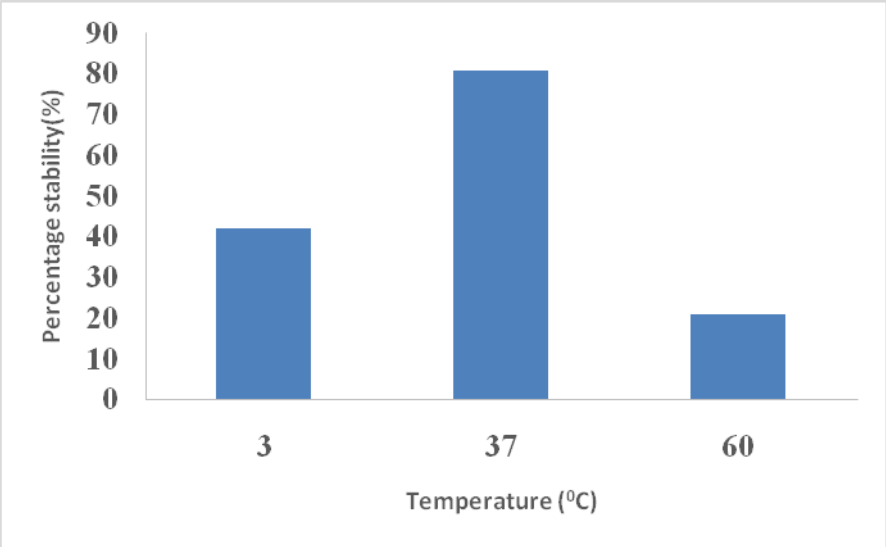

Fig. 16: Effect of extract on percentage stability at different temperature.

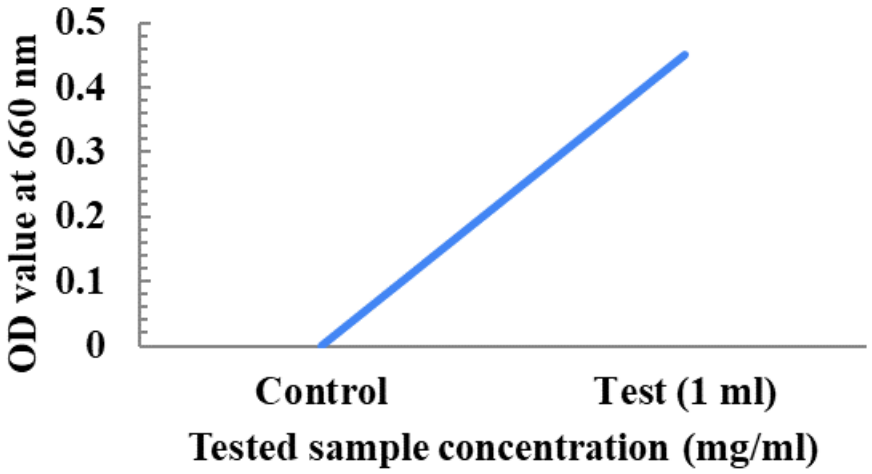

Fig.17: The OD inhibition of denaturation.

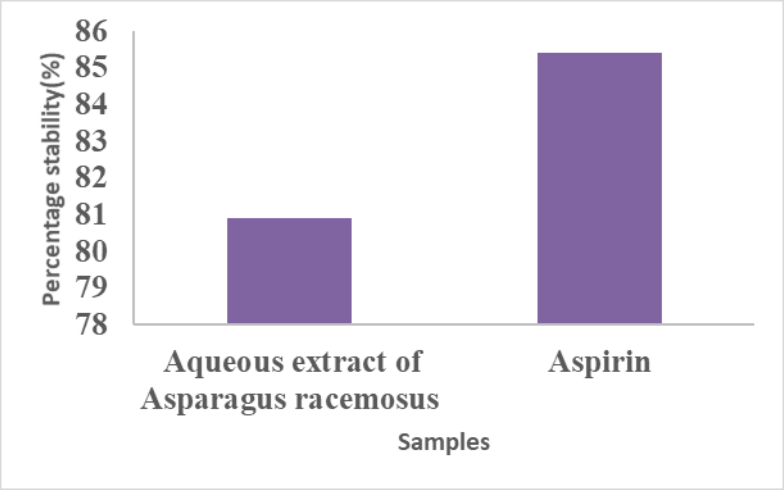

Fig. 18: Comparison of standard drug Aspirin with aqueous extract of *Asparagus racemosus.*

Evaluation of Anti-Inflammatory Efficacy:

The anti-inflammatory activity results showed that the extracts significantly decreased the rate of inhibition of protein denaturation.*Asparagus racemosus* extract exhibited significant proteinase activity at different concentration. It showed maximum inhibition 19% at 2.08 ml. IC_{50} value was found to be 2.08 ml (Fig. 12).

H_2O_2 Radical Scavenging Activity:

The hydrogen peroxide antioxidant assays are adopted in this study and the maximum radical scavenging activity was observed in 20 mg/ml of plant extract (Fig. 13).

Inflammation is a highly dynamic process, which can be characterized as the first protective response of body's immune system. Upon screening for membrane stabilization assay using aqueous extract of the plant it was clearly observed that the aqueous extract possessed stabilization activity. The optimum concentration of 0.2 ml showed the maximum stabilization activity of 80.96% (Fig. 14). The optimization of pH suggested that neutral pH was better i.e. showed 58.55% membrane stabilization (Fig. 15) while temperature of 37°C (Fig. 16) was optimized as it gave 80.96% membrane stability. From the results it was evident that the drug showed an activity percentage of 85.4% while the aqueous plant extract of *Asparagus racemosus* was 80.96%. This clearly indicated that the plant extract showed similar membrane stabilization activity. Phytochemical screening for aqueous extract of *Asparagus racemosus* indicated presence of various primary and secondary metabolites such as terpenoids and flavonoids. From the GCMS data it was evident that the bioactive fragment composed of 10 compounds. The different compounds in the aqueous extract of *Asparagus racemosus* include 2–cyclobuten–1-one, Phosphonous dibromide, 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, 7-oxabicyclo [2.2.1] hept-2-ene, 12-azabicyclo (9.2.1)tetradeca-1(14)-ene-13-one, n-(t-butyl)-2-benzoylbenzamide, 4-methyl-1H-pyrazolo[4,3-c] pyridine, 1-Silacyclo-2,4- hexadiene, 1-Silacyclohexa-2,5-diene, and phosphonic acid.

Denaturation of protein causes conditions of inflammation (Vadakkan *et al.*, 2020) so by inhibition of protein denaturation the inflammatory activity can be inhibited (Fig. 17). *Asparagus racemosus* shows anti-inflammatory activity with 80.92% of membrane stability. By performing *in vitro* analysis of Aspirin consuming person using aqueous extract of *Asparagus racemosus* the membrane stability was found to be

96.96%. From the results it can be concluded that if aqueous extract of *Asparagus racemosus*is given in addition to aspirin, higher membrane stabilization can be achieved as compared to aqueous extract or drug alone. Being a plant extract it would not cause any allergic reactions on consumption with an allopathic drug. This *in vitro* method was more time saving, flexible and convenientin other ways. The investigation suggested better ability of the aqueous leaves extract to resist the cell lysis in small concentration as compared to the standard drug Aspirin at 75 mg/ml, greater than Aspirin (Fig. 18). *Asparagus racemosus* ethanol extract exhibited significant anti proteinase activity at different concentrations. It showed maximum inhibition 19% at l ml. IC_{50} value was found to be 2.08 ml. From this study, it may be concluded that the aqueous extract of *Asparagus racemosus* has good membrane stability, hence is a good anti-inflammatory agent. Further, laboratory study and chemical isolation of this plant might confirm an effective drug molecule in pharmacologic aspects effectively, in both types of pharmaceutical arena.

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