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PHARMACOLOGICAL SCREENING OF *ASPARAGUS RACEMOSUS* FOR ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY BY *EX-VIVO* METHOD

Dhananjay[1], Dr Himanshu B Sahoo[1]*

[1]RKDF College of Pharmacy, SRK University Bhopal, **rkdfcp95@gmail.com**

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Abstract:

Object*: The object of the present paper is* pharmacological screening of Asparagus Racemosus and evaluated for the antioxidant and anti-inflammatory potential by Ex-vivo method**.**

Materials and method*: The crude extract of* Asparagus Racemosus *was extracted out by the Soxhlet process. The quantitative chemical analysis was performed for the quantification and quality of active constituent. The antioxidant and anti-inflammatory activity of ethanolic extract of plant was determined using ex-vivo model. Antioxidant activity is assessed by the Hydrogen peroxide scavenging and reducing power assay method and Anti-inflammatory activity determined by the Protein denaturation assay.*

Result and Discussion*: The extract of* Asparagus Racemosus *was used for phytochemical constituent estimation and qualitative analysis. The Plant extract shown the sign of the alkaloid, saponin and tannins content. The ethanolic extract was found to be as tannin (15.12%), saponin (10.25%) and alkaloid (8.26%). The maximum hydrogen peroxide scavenging activity shown by ethanolic extract of Asparagus Racemosus was found to be 67.94 % at 400 µg/ml. In protein denaturation assay result stated that diclofenac sodium possesses maximum percentage of inhibition. The ethanolic extract of the plant Asparagus Racemosus possess significant inhibition activity at concentration 100, 200 and 400 µg/ml.*

Conclusion: *The ethanolic extract of Asparagus Racemosus showed dose dependent hydrogen peroxide scavenging activity. Reducing power of ethanolic extract of Asparagus Racemosus significantly increased with increasing concentration. The in-vitro anti-inflammatory potential of ethanolic extract of Asparagus Racemosus had shown concentration dependent inhibition of protein denaturation. The* plant Asparagus Racemosus has shown promising antioxidant and anti-inflammatory activity *without any side effects and will be beneficial for society and industry with standardization approaches.*

Keywords: *Asparagus Racemosus*, Soxhlet, antioxidant, anti-inflammatory, protein denaturation

Corresponding author:

Himanshu B Sahoo,

RKDF College of Pharmacy, SRK University Bhopal.

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INTRODUCTION:

Nutraceuticals have been increasingly utilized around the globe as pharmaceutical alternative collateral to modern western medicine. Over and above 300,000 seed plants, some 60% have been utilized for therapeutic interventions, mainly in South America, Africa, and Asia. Nutraceuticals have been reported to be beneficial in a multitude of ailments such as obesity, rheumatoid arthritis, diabetes mellitus, malaria, cancer, and nonetheless for neurological disorders affecting the central nervous system.[1]

Phytochemical compounds in plants are compounds delivered by plants having pharmacological or toxicological properties in man and animals. These non-nutrient (e.g., vitamins and minerals) chemical compounds are often denoted as phytochemicals or plant secondary metabolites.[2] In the modern days, investigation of the phytochemical compounds contained in the traditionally-acknowledged medicinal plants have established significant interest in drug research and developmental projects. Several advanced techniques are presently available, especially in separation, spectroscopic and bioassay procedures. The approach of natural product isolation mainly from plants has transferred from "old-fashioned approach" established on a straight forward-crude-extraction, to the current day, bioassay-guided isolation" technique.[3]

Oxidative stress can play an important role in the development of neuro-degradative disorders, such as Alzheimer's disease, and is implicated in the development of chronic diseases, such as cancer, arteriosclerosis and rheumatism.[4] Antioxidants are a group of compounds that neutralize or quench free radicals and other reactive oxygen species that are generated in the body, thereby reducing oxidative damage of cellular biomolecules such as lipids, proteins, and nucleic acids. [5] Plant constituents have been extensively studied for their antioxidant activity (AOC), and there is an increasing interest in natural antioxidants, namely phenols, present in medicinal and dietary plants that might help prevent oxidative stress.[6]

Development of new antimicrobial compounds against different microorganisms is becoming critically important, as infectious diseases are still a major threat to public health. Renewed interest in plant antimicrobials has emerged recently, probably due to the increasing development of drug resistance of human pathogenic organisms, as well as the appearance of undesirable side effects of certain antibiotics and the emergence of previously uncommon infections.[7]

Asparagus Racemosus, is an important medicinal plant of tropical and subtropical India. Its medicinal usage has been reported in the Indian and British Pharmacopoeias and in indigenous systems of medicine.[8] The genus Asparagus includes about 300 species around the world. The genus is considered to be medicinally important because of the presence of steroidal saponins and sapogenins in various parts of the plant. Out of the 22 species of Asparagus recorded in India.[9]

Asparagus Racemosus is the one most commonly used in traditional medicine. The main active constituents of *Asparagus Racemosus* are steroidal saponins (Shatavarins I–IV) that are present in the roots. Shatavarin IV is a glycoside of sarsasapogenin having two molecules of rhamnose and one molecule of glucose.[10] Other active compounds such as quercetin, rutin (2.5% dry basis) and hyperoside are found in the flowers and fruits; while diosgenin and quercetin-3 glucuronide are present in the leaves. A new isoflavone, 8-methoxy-5, 6, 4′-trihydroxyisoflavone-7-O-β-d-glucopyranoside was also reported from A. Racemosus. Beneficial effects of the root of A. *Racemosus* are suggested in nervous disorders, dyspepsia, diarrhoea, dysentry, tumors, inflammations, hyperdipsia, neuropathy, hepatopathy, cough, bronchitis, hyperacidity and certain infectious diseases.[11] The objective of research paper is pharmacological screening of *Asparagus Racemosus* and evaluated for the antioxidant and anti-inflammatory potential by *Ex-vivo* method**.**

MATERIALS AND METHODS:

The specimen of *Asparagus Racemosus* was authenticated by Plant Anatomy Research Centre, Chennai. The registration number for the specimen is PARC/2022/NP/2050 been preserved for future identification. The sample was shade dried so as to protect its chemical constituents and not to get degrade at high temp.

Extraction:

Preparation of extract:

The plant *Asparagus Racemosus* was dried in shade and powdered the mixture and extracted with ethanol as solvent using Soxhlet extraction for 10 hrs. and then centrifuged twice at 1500 rpm for 5 min. and then in a water bath evaporate to dryness. The powdered or semisolid content of extract was further utilized for the phytochemical and pharmacological evaluation.[12]

Phytochemical screening of active constituents:

The Plant extracts of plants was subject to the determination of the active phytoconstituents present in it. In that case the chemical test for alkaloid, glycoside, carbohydrate, tannins, steroid, triterpenoid, amino acid, protein, phenol, flavonoid, saponin was performed by taken the small amount of the extract of plants.[13]

Quantitative estimation of Phytochemicals:
Determination of the total amount of alkaloids:
The plant extracts were dissolved in ethanolic acetic acid, close the lid and let stand for 4 hours, filter and concentrate the extract 1/4 of the solution. All the solution was settled down by the addition of ammonium hydroxide and filter the residue.[14]

Estimation of the total amount of saponins:
Weigh 20 g of particles, inject 100 ml of ethanol, and then heat the sample in a warm water bath for 4 hours with constant stirring to about 55°C. The aggregates are filtered and the residue is removed. After adding 60 ml of n-butanol and extracting, repeat this purification step 3 times, the obtained n-butanol extract is washed with 10 ml of 5% sodium chloride aqueous solution, and the final solution is heated and evaporated in a water bath. The solvent evaporates after the inside of the sample is dried. Use the typical kiln weight and the saponin content of the material to be calculated as a percentage.[15]

Quantitative quantification of total tannins:
The content of tannins in the composition is estimated by the Toklai-Leventhal method, which mainly involves the oxidation titration of the sample leaching solution, and then similar determinations are carried out elsewhere, and then quantitative determinations are carried out for tannins. Boil 2 g of Extract sample in 200 ml of water for 2 hours, cool and make up the volume of the extract to 300 ml. Gelatin is used to separate tannin and non-tannin, In this Soak 25 gm of gelatin in salt water for 1 hour. The mixture is then heated to dissolve the gelatin. [16]

Quantitative total flavonoids:
10g extract was extracted with 100ml hydroalcoholic solution at 25˚C and solution was filtered to crucible and evaporate to dryness. Place in watch glass and weigh.

Pharmacological evaluation:
Preparation of extract:
The herbal powder extract was successfully extracted with ethanol as solvent and was extracted by Soxhlet extraction. The obtained dry extract is used for in-vitro antioxidant and anti-inflammatory activity.

***In-Vitro* Anti-arthritic activity:**
(A) Inhibition of protease activity:
The take a look at is predicated on proven fact that proteinase play a vital role within the repairing of tissue and cells, which will be broken throughout the inflammation. during this test trypsin is employed as protease enzyme and casein was used as substrate. Dissolve the optimized amount of trypsin (0.12 mg) in the buffer solution and add the test samples (100, 200, 300, and 400 µg/ml). Shake the contents for 5 min and incubate at 37°C for 10 min. Then, after adding 2 ml of 0.8% (w/v) casein, the mixture was incubated for another 20 min. Then, after incubating for 20 minutes, the reaction was stopped by adding 4.0 ml of 70% (v/v) perchloric acid. Add acid to stop the reaction. The appearance of the turbid suspension confirmed the termination of the reaction. Read the optical density (OD) of the supernatant at 280 nm with the control buffer. Use the following formula to calculate the inhibition percentage of all activity. [17]

$$\% \text{ Inhibition} = \frac{\text{O.D. of Control} - \text{O.D. of sample}}{\text{O.D. of Control}} \times 100$$

Evaluation of denaturation of protein:
Denaturation of protein is caused by inflammation and is the main cause of rheumatoid arthritis. The exact definition of protein denaturation is when the protein destroys its function and triggers an inflammatory response, the secondary and tertiary structure of the protein is lost. Protein denaturation is caused by heat, radiation, etc. During the analysis, protein denaturation is caused by heating. [32] Add 1 ml of test samples (100, 200, 300, and 400 µg/ml) to 9 ml of egg white protein. Keep the pH of the reaction at 6.5 by adding a small amount of hydrochloric acid (1N). The reaction mixture was stirred for 5 minutes and then kept at 37°C for 30 minutes. After 30 minutes, it was heated to 51°C for another 30 minutes. The reaction sample was then cooled to room temperature and the turbidity at 660 nm was measured. The experiment was performed in triplicate. Diclofenac sodium (200 µg/ml) was used as a standard. [18]

Membrane Stabilization Assay:
The principle of this assay is based on the stabilization of the membrane of red blood cells against membrane lysis induced by hypotension. Membrane stabilizers that reduce the release or effect

of mediators such as histamine, serotonin, prostaglandins, leukotrienes, etc. Add 4 ml of hypotonic saline to 2 ml of phosphate buffer. Add 2 ml test samples (100, 200, 300 and 400 µg/ml) to this solution, then add 1 ml rat red blood cells, 2 ml isotonic saline without red blood cells as a control, and incubate the mixture at 56°C For 30 minutes, cool the tube under running tap water for 20 minutes, centrifuge the mixture and apply it at an optical density of 560 nm. Diclofenac sodium (200 µg/ml) was used as a standard. [19]

RESULT AND DISCUSSION:

Phytochemical analysis:

Preliminary phytochemical screening was performed for ethanolic extract of *Asparagus Racemosus.* The *Asparagus Racemosus* has shown the presence of alkaloid, steroid, flavonoids, Gum and mucilage.

Quantitative estimation:

The *Asparagus Racemosus* was evaluated for the quantitative estimation of total alkaloid, saponin, and tannin content in aqueous ethanolic extract and date was represented in Table 1.

Table 1: Quantitative estimation of phytoconstituent

S. No.	Parameters	Percentage value in *Asparagus Racemosus*
1	Alkaloids	8.26±0.21
2	Saponins	10.25±0.13
3	Tannins	15.12±0.21

Pharmacological evaluation:

***In- Vitro* Antioxidant Activity:**

(a) Hydrogen peroxide scavenging:

The hydrogen peroxide scavenging activity of ethanolic extract of *Asparagus Racemosus* was determined. The percentage hydrogen peroxide scavenging ability of the test extract increased in a dose dependent manner and Ascorbic acid (References) (100µg/ml) exhibited 66.92% hydrogen peroxide scavenging activity. The maximum hydrogen peroxide scavenging activity shown by ethanolic extract of *Asparagus Racemosus* was found to be 67.94 % at 400 µg/ml. The hydrogen peroxide scavenging effect of ethanolic extract was shown in **Table 2 and Figure 1.**

Table 2: Hydrogen peroxide scavenging activity of ethanolic extract of *Asparagus Racemosus*

S. No.	Conc. (µg/ml)	Absorbance	Percent Inhibition
1.	Control	**0.780**±0.021	-
2.	25	0.712±0.052	8.97
3.	50	0.625±0.026	20.51
4.	100	0.421±0.024	46.15
5.	200	0.315±0.0035	60.25
6.	400	0.250±0.0032	67.94
7.	Ascorbic acid (100 µg/ml)	0.258±0.0022	66.92

(Values are Mean±S.E.M., where n=6) in each group, P< 0.05 *, P< 0.01 **(significant) compared with control.

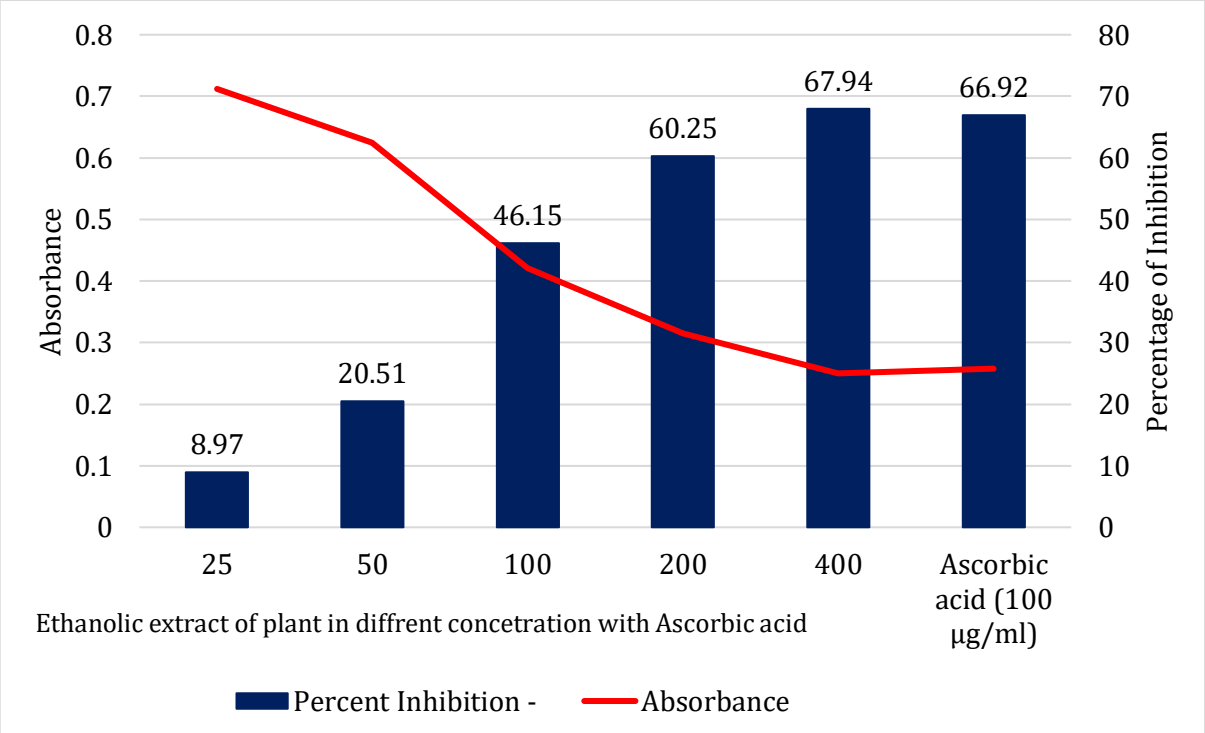

Figure 1: Hydrogen peroxide scavenging activity of ethanolic extract of *Asparagus Racemosus*.

(b) Reducing power assay:

Increase in absorbance of the extract indicates the reducing power of the test sample. Reducing power of ethanolic extract of *Asparagus Racemosus* increased with increasing concentration. Result data of reducing power assay was reported in Table 3 and represented in Fig. 2.

Table 3: Reducing power activity of ethanolic extract of *Asparagus Racemosus*

S. No.	Concentration (µg/ml)	Absorbance
1.	**Control**	0.450±0.22
2.	**25**	0.689±0.21
3.	**50**	0.78±0.15
4.	**100**	1.258±0.14
5.	**200**	1.358±0.32
6.	**400**	1.386±0.21

(Values are Mean±S.E.M., where n=6) in each group, P< 0.05 *, P< 0.01 ** (significant) compared with control. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

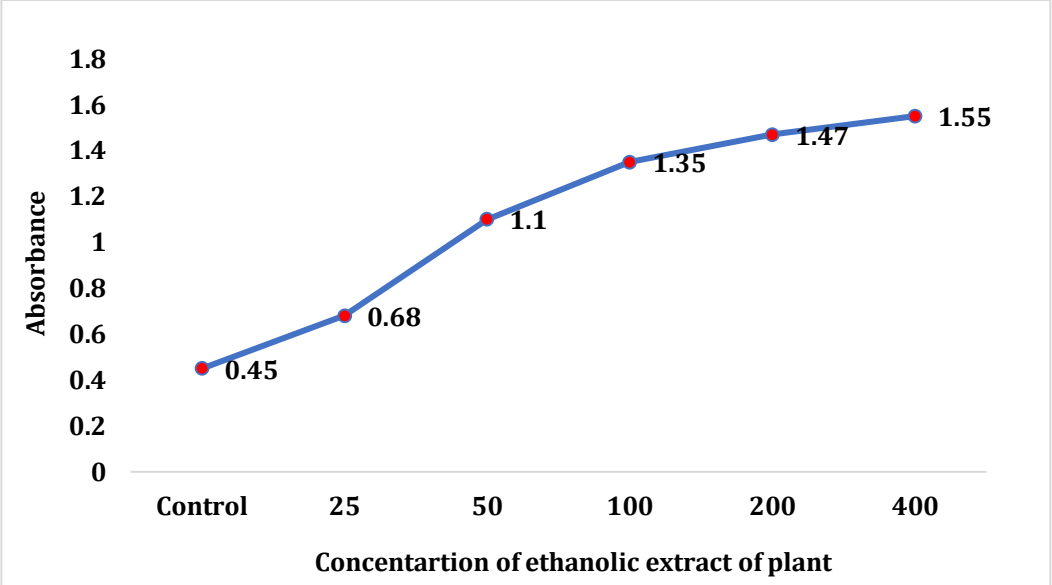

Figure 2: Reducing power assay of ethanolic extract of *Asparagus Racemosus*

***In-vitro* anti-inflammatory activity:**

(a) Protein denaturation:

As part of the evaluation of anti-inflammatory activity, ability of plant extract on protein denaturation was studied. It was effective in inhibiting heat induced protein denaturation. Diclofenac sodium a standard anti-inflammatory agent possesses maximum % inhibition. The ethanolic extract of the plant *Asparagus Racemosus* possess significant % inhibition activity at concentration 100, 200 and 400 µg/ml (Table 4). Effect of ethanolic extract *of Asparagus Racemosus* on protein denaturation represented in Fig. 3.

Table 4: Effect of ethanolic extract of *Asparagus Racemosus* on protein denaturation

S. No.	Concentration (µg/ml)	Absorbance	Percent of Inhibition
1.	Control	1.45	-
2.	25	1.36	6.20
3.	50	0.652	55.03
4.	100	0.345	76.20
5.	200	0.215	85.17
6.	400	0.112	92.27
7.	Diclofenac Sodium 100 µg/ml	0.098	93.24

(Values are Mean±S.E.M., where n=6) in each group, P< 0.05 *, P< 0.01** (significant) compared with control. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

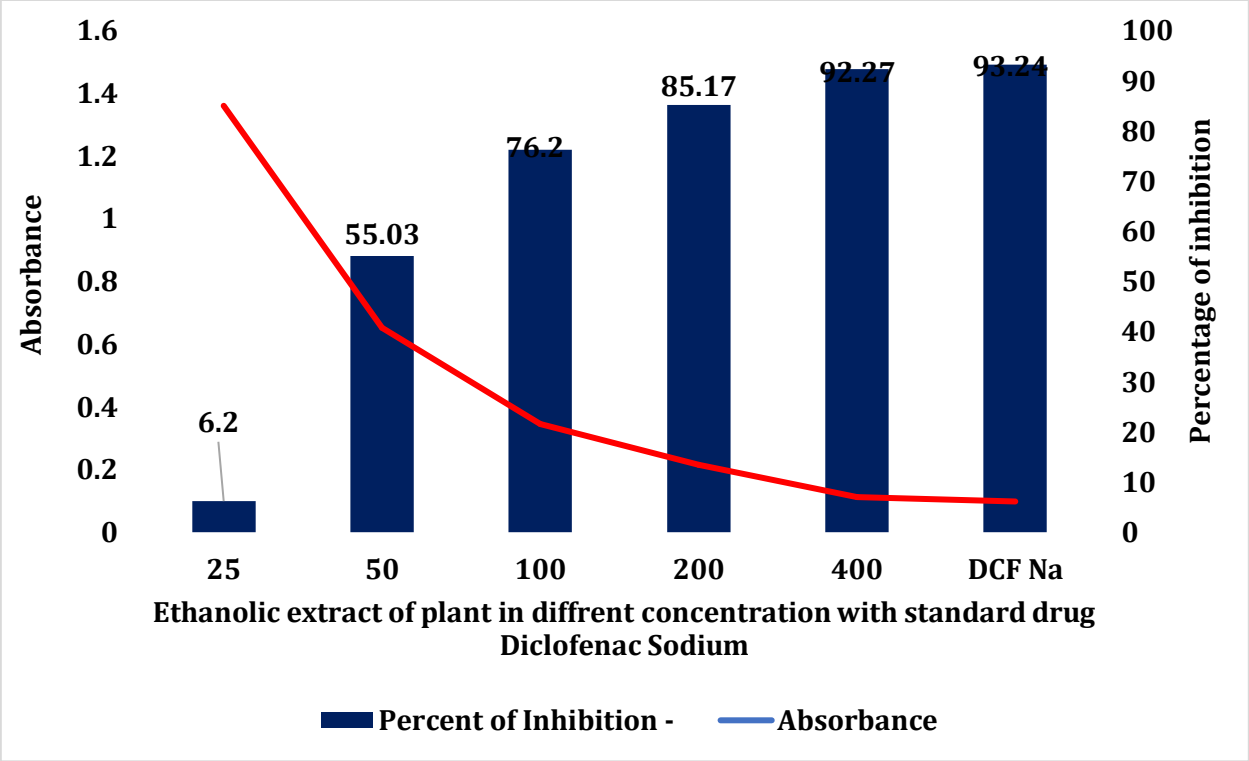

Figure 3: Effect of ethanolic extract *of Asparagus Racemosus* on protein denaturation.

DISCUSSION:

The ethanolic extract of *Asparagus Racemosus* were subjected to phytochemical screening. The result indicated that, rhizome extract shows the presence of carbohydrate, proteins, cardiac glycosides, flavonoid, tannins and phenol. Phytochemicals are natural bioactive compounds found in plants. They are mainly two groups, which are primary and secondary metabolites. Primary metabolites are sugars, amino acids, proteins and chlorophyll while secondary metabolites consist of alkaloids, terpenoids and phenolic compounds. The ethanolic extract of *Asparagus Racemosus* showed dose dependent hydrogen peroxide scavenging activity. Hydrogen peroxide (H_2O_2), a biologically relevant, non- radical oxidizing species, may be formed in tissue through oxidative processes. As a conclusion the ethanolic extract of the *Asparagus Racemosus* showed hydrogen peroxide activity as compared to standard ascorbic acid (100 µg/ml).

The reducing power of the extract, which may serve as a significant reflection of antioxidant activity, was determined using reducing power assay, where by the yellow color of the test solution changes to various shades of green and blue is depending upon the reducing power of each compound. The reducing power of the ethanolic extract of *Asparagus Racemosus* increased with increasing concentration. The ethanolic extract of the *Asparagus Racemosus* exhibited concentration dependent inhibition of protein denaturation.

CONCLUSION:

The study concluded that the *Asparagus Racemosus* plant extract possesses marked in vitro antioxidant and anti-inflammatory effect. Hydrogen peroxide scavenging ability of ethanolic extract of *Asparagus Racemosus* revealed that the extract scavenges the hydrogen peroxide. However, the hydrogen peroxide scavenging ability was low comparing to standard (ascorbic acid). Reducing power of ethanolic extract of *Asparagus Racemosus* significantly increased with increasing concentration. The *in-vitro* anti-inflammatory potential of ethanolic extract of *Asparagus Racemosus* had shown concentration dependent inhibition of protein denaturation.

The result of the investigation showed that the ethanolic extract of *Asparagus Racemosus* possess antioxidant and anti-inflammatory activity. Phytochemical analysis showed presence of alkaloid, saponin and tannins. The antioxidant and anti-inflammatory property showed by the plant may be because of these chemical moieties. The results obtained in the study supports the traditional and also demands further research and to isolate and characterize active principles responsible for

activity.

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