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Evaluation of Phytochemical Analysis And Antiinflammatory Activity of Rhizome of Asparagus Racemosus (Ornamental)

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Abstract: *Medicinal plants have bioactive compounds which are used forthe treatment of various human diseases and also play an important role in healing.In the present investigation phytochemical analysis of the buffer extract of rhizomeof Asparagus racemosus showed the presence of flavonoids, phenols, tannins, glycosides and carbohydrates. The plant exhibited significant total phenolics, total flavonoids andascorbic acid content. The extract alsoshowed moderate antiinflammatory activity.The present study provides evidence that the antiinflammatory and antioxidant properties of buffer extract of Asparagus racemosus is due to medicinally important bioactive phytochemical constituents and this justifies the use of plant species as traditional medicine for treatment of various diseases.*

Keywords: *Asparagus racemosus, rhizome extract, phytochemical analysis, antiinflammatory activity.*

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I. Introduction

The therapeutic properties of plants could be based on their antioxidant, antimicrobial, antipyretic effects of the phytochemical constituents in them [1]. Phytochemicals are naturally occurring in the medicinal plants that exhibit defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are the primary constituents and the important secondary compounds are terpenoids, alkaloids,flavonoids and phenolic compounds [2].Medicinal plant is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, flavonoids, glycosides, saponins, resins, tannins, sesquiterpene, lactones and oils (essential and fixed) [3].

The evaluation of all the drugs is based on phytochemical and pharmacological approaches which leads to the drug discovery referred as natural product screening [4]. Flavonoids and Phenolic compounds are polyphenolic phytochemicals, known as antioxidants [5][6]. Alkaloids are used as anaesthetic agents and are found in medicinal plants [7]. Phenolic compounds have antiinflammatory, antiallergic, antibacterial, antiatherosclerotic, anticarcinogenic activity, antimutagenic, antitumor, antiviral effects [8].Polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans [9][10].

Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling, pain and loss of functions [11][12].It is a complex response that protects the host against tissue injury and microbial invasion. Prolonged inflammation contributes to the pathogenesis of many diseases such as asthma, arthritis, multiple sclerosis and even cancer [13][14]. Inflammation can be classified as acute and chronic based on the commencement time. Acute inflammation is the primary response of the body to injurious stimuli and is involved in vascular changes i.e. vasodilatation, increased capillary permeability and migration of leukocytes. Chronic inflammation is prolonged inflammation characterized by progressive destruction and retrieving of injured tissue from the inflammation [15][16].

Asparagus racemosus is a well-known medicinal plant in ayurvedicrasayana which prevent ageing, increase longevity, impart immunity, improve mental function and add vigor and vitality to the body and also used in nervous disorders, dyspepsia, tumors, inflammation, hyperdipsia, neuropathy and hepatopathy[17].It also has been reported to have potent adaptogenic activity [18] and antioxidant property [19].The whole plant is used in the treatment of diarrhea, rheumatism, diabetes and brain complaints. It is also used in management of behavioral disorder and minimal brain dysfunction [20].Thus; pharmacologically active plants should be investigated for better understanding of their properties, safety practices in addition to usefulness [21].

II. Materials And Methods

2.1Plant materials

Asparagus racemosus was obtained from Padma puram garden of Araku valley, Visakhapatnam district. Asparagus racemosus,belongs to familyAsparagaceae.The plant specimen was deposited in herbarium, Department of Botany, Andhra University. The voucher specimen number ofAsparagus racemosus is 22237.

The plant specimen was authenticated by Prof.S.B.Padal, Taxonomist, Department of Botany, Andhra University, Visakhapatnam. Rhizomes of Asparagus racemosus were used for evaluation of phytochemical analysisand antiinflammatoryactivity.

2.2Phytochemical evaluation (Qualitative methods)

2.2.1 Preparation of extract for phytochemical analysis

The plant rhizomes were washed, air dried, chopped into fine pieces and then homogenized with 0.1M Phosphate buffer, pH 7.6 at 4°C using rhizome to buffer ratio of 1:10 (w/v). The homogenate was filtered through cheese cloth and the filtrate was centrifuged at 6500 rpm for 20 min at 4°C in a refrigerated centrifuge (Remi C24 plus). The supernatant solution was used as buffer extract for the evaluation.

The preliminary tests for the detection of secondary metabolites were carried out with the above buffer extract.

2.2.2Phytochemical tests

Phytochemical tests were performed for the extract as per the standard methods[22][23]. Test for tannins To 1 ml of rhizome extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for alkaloids

To 2ml of rhizome extract, 2ml of concentrated hydrochloric acid was added. Then a few drops of Mayer's reagent were added. The presence of green color or white precipitate indicates the presence of alkaloids.

Test for glycosides

To 2ml of rhizome extract, 3ml of chloroform and 10% ammonia solution were added. Formation of pink color indicates the presence of glycosides.

Test for terpenoids

To 0.5ml of rhizome extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates the presence of terpenoids.

Test for flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for phenols

To 1ml of the extract, 2ml of distilled water, followed by a few drops of 10% ferric chloride was added. Formation of blue or green color indicates the presence of phenols.

Test for steroids and phytosteroids

To 1ml of rhizome extract equal volume of chloroform was added and subjected with a few drops of concentrated sulfuric acid, appearance of brown ring indicates the presence of steroids and appearance of the bluish brown ring indicates the presence of phytosteroids.

Test for carbohydrates

To 2 ml of rhizome extract, 1ml of Molisch's reagent and a few drops of concentrated sulfuric acid were added. The presence of purple or reddish color indicates the presence of carbohydrates.

Test for saponins

To 2 ml of rhizome extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15min lengthwise. Formation of a 1cm layer of foam indicates the presence of saponins.

Test for quinones

To 1ml of rhizome extract, 1ml of concentrated sulfuric acid was added. Formation of red color indicates presence of quinones.

Test for coumarins

To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates the presence of coumarins.

Test for phlobatannins

To 1ml of extract few drops of 2% HCl was added. Appearance of red color precipitate indicates the presence of phlobatannins.

Test for anthraquinones

To 1ml of extract, few drops of 10% ammonia solution were added; appearance of pink color precipitate indicates the presence of anthraquinones.

2.3Total phenolics, flavonoids and ascorbic acid content of rhizome extract

2.3.1 Analysis of total phenolics

The total phenolics were determined using the FolinCio-calteau reagent [24].

To 50 µl of the plant extract, 2.5ml of diluted FolinCio-calteau reagent and 2.0 ml of 7.5% (w/v) sodium carbonate were added and incubated at 45°C for 15 min. The absorbance was measured at 765 nm. The results were expressed as mg of gallic acid equivalents per gram weight.

2.3.2 Determination of total flavonoids

Total flavonoid content of extracts was determined according to a modified colorimetric method [25]. To 1ml of extract, 1ml of distilled water and 75µl of sodium nitrite were added. After 5 min, 75µl of 10% $AlCl_3$ solution was added. The mixture was allowed to stand for another 5min and then 0.5 ml of 1 M sodium hydroxide was added. The solution was mixed and kept for 15 min. The increase in absorbance was measured at 510 nm. The total flavonoid content was calculated using standard quercetin equivalents per gram tissue.

2.3.3Determination of total ascorbic acid

Ascorbic acid content was determined by the procedure given by Sadasivam[26].

To 5ml of ascorbic acid solution (1mg/ml), 10 ml of 4% oxalic acid was added and titrated against the dichlorophenol indophenol solution. The amount of dye consumed is equivalent to the amount of ascorbic acid consumed. Similarly, 5 ml of extract (1mg/ml) was titrated against the dye. The results were expressed as mg of Ascorbic acid/gm extract.

2.4Assessment of in vitro antiinflammatory activity

2.4.1 Membrane stabilization Assay

The human red blood cell (HRBC) membrane stabilization method has been used to study the antiinflammatory activity [27].HRBC or erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of human red blood cell membrane (HRBC) by hypotonicity induced membrane lysis can be taken as an in vitro measure of antiinflammatory activity of the drugs or plant extracts.

2.4.1.1 Preparation of Human Red Blood Cells (HRBC) Suspension

Blood was collected from the healthy volunteers and mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05 % citric acid and 0.42 % sodium chloride in distilled water).The blood was centrifuged at 3,000 rpm and packed cells were washed with isosaline (0.85 %, pH 7.2) and a suspension was made with isosaline(10 % v/v).

The principle involved here is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The assay mixture contains 1ml of 0.15 M phosphate buffer (pH 7.4), 2ml hyposaline (0.36 %), 0.5 ml HRBC suspension (10 % v/v) with 0.5 ml and 1 ml of 10% and 20% rhizome extracts. Diclofenac sodium was used as reference drug. In the control solution instead of hyposaline, 2 ml of distilled water was added. The mixture was incubated for 30 min at 37^{o}C and centrifuged. The hemoglobin content in the suspension was estimated using spectrophotometer (Systronics) at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis occured in the presence of distilled water as 100 %. The percentage of HRBC membrane stabilization or protection was calculated by using the formula

The percentage of hemolysis of HRBC membrane can be calculated as follows:

% Hemolysis = (Optical density of Test sample / Optical density of Control) X 100

The percentage of HRBC membrane stabilization can be calculated as follows:

% Protection = 100 – [(Optical density of test sample / Optical density of control) X100]

2.4.2 Heat induced hemolysis

Percent membrane stabilization was calculated by this method [28] [29].

The reaction mixture (2ml) consisted of 0.5 ml and 1 ml of 10% and 20% rhizome extracts respectively and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Diclofenac sodium was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56ºC for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples.

Percentage Inhibition (%I) =100−(Optical density of test– Optical density of product control)/ Optical density of positive control) × 100

2.4.3Inhibition of protein denaturation

The percentage inhibition of protein denaturation was calculated as follows [30].

The reaction mixture (0.5 ml) consisted of 0.45 ml of bovine serum albumin (5% aqueous solution) and 0.5 ml and 1ml of 10% and 20% rhizome extracts respectively, pH was adjusted at 6.3 using a small amount of 1N Hydrochloric acid. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the sample, 2.5 ml of phosphate bufferpH solution was added into each test tube. Turbidity was

measured spectrophotometrically at 600 nm for control tests,distilled water was used instead of extracts while product control tests lacked bovine serum albumin.
% of inhibition = 100 - (Optical density of test – Optical density of product control) x 100 /
Optical density of control.

III. Results

3.1 Phytochemical evaluation of rhizome extract

Preliminary phytochemical analysis of rhizome extract revealed thesignificant active phytochemical constituents flavonoids, phenols, tannins, glycosides and carbohydrates(Table1).

3.2Total phenolics, flavonoids and ascorbic acid content of rhizome extract

The total phenolic content of extract was14.46 ± 0.45GAE/gm extract, Total flavonoids 6.22 ±0.28 QuercetinEq/gm extract,Ascorbic acid 18.4±0.16mg/gm extract(Table 2).

3.3Antiinflammatory activity

3.3.1 Inhibition of hypotonicty induced hemolysis

The inflammation was done by hypotonicity induced membrane lysis. The antiinflammatory activity of the plant was measured on the basis of stabilization of HRBC (Human red blood cell) membrane in which the lysis was carried out by hypotonicity. The stabilization was determined against the plant extracts and compared with known antiinflammatory drug Diclofenac sodium. The results obtained from the extracts were compared with the known antiinflammatory drug. The resultsrevealed moderate potentialof the extract.

The rhizomeextracts (10% and 20%) were moderately effective in inhibiting the hypotonicity induced hemolysis to varying degree as shown in Fig.1. Of the two extracts, 20% extract of rhizome showed more inhibition of hypotonicity induced hemolysis. Diclofenac sodium, a standard drug showed the maximum inhibition, 89% at 100µg/ml. The stabilization against membrane induced hypotonicity was found to be concentration dependent, and the stabilization percentage in the extracts was found to be less than the reference compound (Diclofenac sodium).

3.3.2 Inhibition of Heat induced hemolysis

Stabilization of the RBC membrane was studied further to establish the mechanism of antiinflammatory action of rhizome extract.The extracts (10% and 20%) were moderately effective in inhibiting the heat induced hemolysis to varying degree as shown in Fig.2.Of the two extracts, 20% extract showed significant inhibition of heat induced hemolysis. Diclofenac sodium, standard drug showed the maximum inhibition, 85% at 100µg/ml.

3.3.3 Inhibition of protein denaturation

The extracts (10% and 20%) were effective in inhibiting heat induced albumin denaturation in varying degrees as shown in Fig.3. Of the two extracts, 20% extract of rhizomeshowed significant inhibition of heat induced protein denaturation. Diclofenac sodium, a standard antiinflammatory drug showed the maximum inhibition, 92% at the concentration of 100µg/ml.

IV. Discussion

The present study of phytochemical investigation in the rhizome extract ofAsparagus racemosus(ornamental)indicated the presence of phenols, flavonoids, glycosides, tannins and saponins which are substances reported to exert potent antiinflammatory and analgesic properties[31][32]. Phytochemicals were also reported from various other sources.Preliminary phytochemical analysis of the methanol extract of rhizome of Asparagus racemosus(wild) revealed the presence of alkaloid, amino acid, flavonoid, glycoside, phenol, steroid, tannin, and saponin[33].Similarly the chloroform, ethanol, methanol, ethyl acetate and water extract of A.racemosus(wild) confirmed the presence of steroid,saponins. Methanol extracts of dried bulbs of Asparagus acutifolius showed flavonoid, phenol, steroid, tannin, terpenoids and saponin.Phytochemical screening of ethanol and acetone crude extract of the roots of Asparagus racemosus revealed the presence of flavonoids, alkaloids, glycosides, phenolic compounds, tannins, saponins steroids and carbohydrates [34].This difference in phytochemical constituents is due to source variation and type of solvent used for extraction.

Many synthetic drugs reported to be used for the treatment of inflammatory disorders is of least interest at present due to their potential side effects, serious adverse effects and as they are found to be highly unsafe for humanbeings. Since the last few decades, herbal drugs have regained their popularity in treatment against several human ailments[35].Inflammation is a common manifestation of infectious diseases like leprosy, tuberculosis, syphilis, asthma, inflammatory bowel syndrome, nephritis, celiac diseases, autoimmune diseases etc. [36].Prolonged inflammation leads to the rheumatoid arthritis, atherosclerosis, hay fever, ischemic heart disease[37][38][39].

A number of studies have shown that flavonoids [40]and other plant compounds exhibit analgesic and antiinflammatory effects as a result of their membrane stabilizing ability in various experimental models[41].Steroids inhibit the activity of phospholipase A_2 and flavonoids are potent prostaglandin inhibitors as well as inhibitors of phosphodiesterases[42] .The production of free radicals, such as lipid peroxides and superoxides, are reported to be accountable for cell membrane destabilization [43]. Flavonoids and other phenolic compounds are reported to act as effective scavengers of free radicals [44]. Thus the flavonoids and other phenolic compounds in extracts of A.racemosus(ornamental)could be responsible for the observed membrane stabilizing effect in this study.

The anticancer[45], antiin flammatory [46] and nephro/ hepatoprotective properties [47] [48] of rhizomeextracts could well be due to their membrane stabilizing potential.

Denaturation of proteins is one of the causes of inflammation. Some researchers report that denaturation of protein is one of the reason for rheumatoid joint pain because of the generation of autoantigens in certain rheumatic diseases**.** It might be done to in vitro denaturation of proteins [49][50]. Denaturation is due to alteration of electrostatic force, hydrogen, hydrophobic and disulphide bonds. The antiinflammatory drug shows dose dependent ability to inhibit the thermally induced protein denaturation [51]. The antiinflammatory activity of extract may potentially hinder the arrival of lysosomal substances of neutrophils at the site of irritation. These neutrophil lysosomal constituents incorporate bactericidal chemicals and proteinases,bring an additional tissue irritation [52].

V. Conclusion

Asparagus racemosus(ornamental)is the source of the secondary metabolites i.e., alkaloids, flavonoids, phenols, tannins and reducing sugars. The antidiuretic, antiinflammatory, antianalgesic, anticancer, antiviral, antimalarial, antibacterial and antifungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. The phytochemical analysis has commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases. Thus the phytochemical properties and antiinflammatory activity of Asparagus racemosus identified in the investigation will be helpful in the treatment of different diseases.

Table1: Preliminary Phytochemical screening tests of rhizome extract of Asparagus racemosus

Secondary Metabolite	rhizome extract
Tannins	+
Alkaloids	-
Glycosides	+
Terpenoids	-
Flavanoids	+
Phenolics	+
Sterols	-
Carbohydrates	+
Quinones	-
Saponins	-
Coumarins	-
Phlobatannins	-
Anthraquinones	-

(+)Indicates respective constituent present and (-) Indicates absence of photochemical

Table 2 Total phenolics, flavonoids and ascorbic acid content of rhizome extract of Asparagus racemosus

Extract	Total phenolics GAE/gm extract	Total flavonoids QuercetinEq/gm extract	Ascorbic acid mg/gm extract
Rhizome extract	14.46 ± 0.45	6.22 ±0.28	18.4±0.16

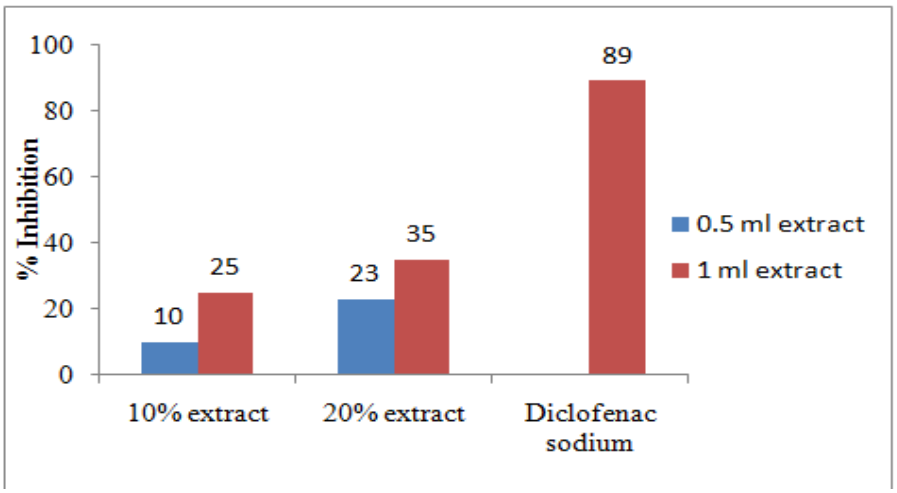

Figure.1 Effect of rhizome extract of Asparagus racemosus on membrane stabilization

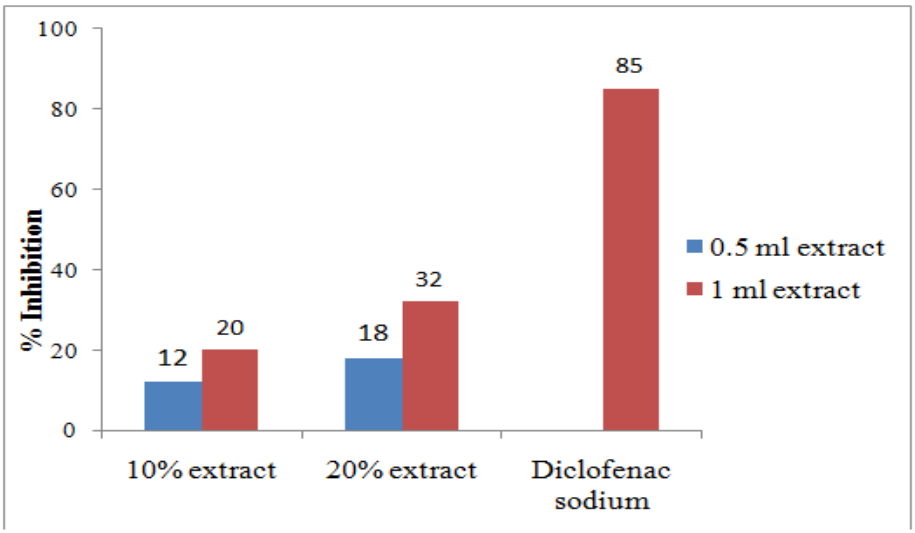

Figure.2 Effect of rhizome extract of Asparagus racemosus on heat induced hemolysis

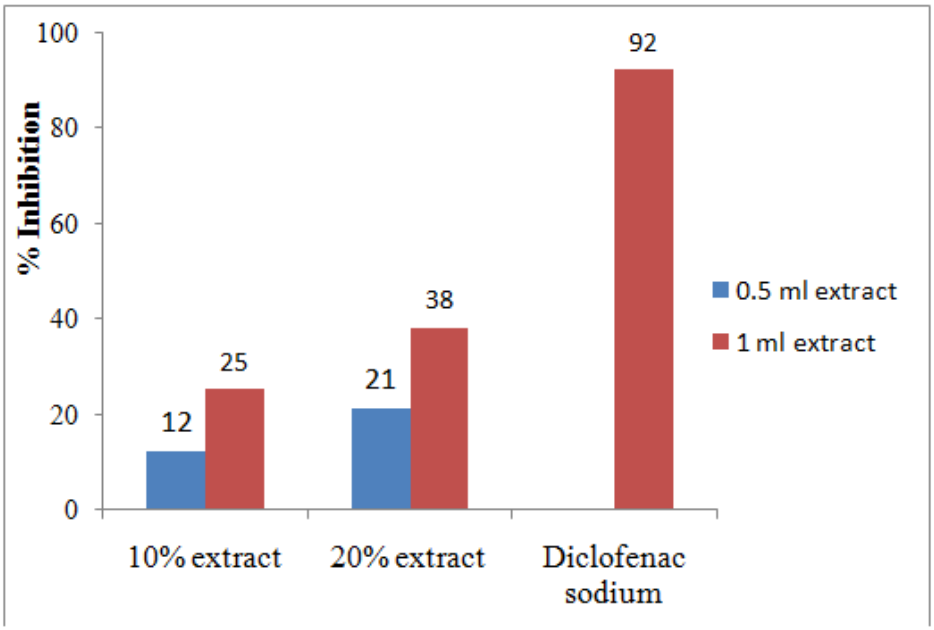

Figure.3 Effect of rhizome extract ofAsparagus racemosuson protein denaturation

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