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RESEARCH ARTICLE

Phytochemical Analysis and *Invitro* Antiarthritic Activities of Whole Plant of *Hybanthus enneaspermus*

S. Umamaheswari*, K. S. Sridevi Sangeetha, K. Sindhuja, R. Subash, J. Shanmugavalli, D. Sharmila

Department of Pharmacology, Faculty of Pharmacy, Sri Ramachandra University, Porur, India.

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ABSTRACT

The aim of the present study is to phytochemical analysis and invitro antiarthritic activities of whole plant of *Hybanthus enneaspermus*. The ethanolic extract of the plant was subjected to qualitative analysis for the presence of phytochemical substances. The invitro anti arthritic activity was determined by measuring the percentage of inhibition of protein denaturation. The phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, terpenoids, saponins, steroids and reducing sugars. The antiarthritic activity was determined by protein inhibition assay. The percentage protection was found to be 64.6 % for extract and 91.2% (Diclofenac sodium). The IC_{50} value for the extract was found to 512.6 µg/ml and for the standard it was found to be 172.82 µg/ml.

KEYWORDS

Hybanthus Enneaspermus, Anti Arthritic, Phytochemical

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease which is characterised by joint inflammation, synovial proliferation and destruction of articular cartilage which results in swollen, warm and painful joints.[1,2] Rheumatoid arthritis primarily affects the lining of joints, but can also affect other parts of the body in more than 15-25% of the individuals.[3]

The worldwide prevalence of RA is believed to be 0.4-1.3%.[4,5] In the year 2005, 1.5 million US adults of age 18 years and more have been affected by RA of which 70% are women. An average of 4% women and 3% men are estimated to have life time risk of rheumatoid arthritis. In the year 1995-2007, 41 of every 100,000 people were diagnosed with RA every year.[6] In 2010, about 49,000 deaths were reported due to RA.

***Address for Correspondence:**
S. Umamaheswari,
Professor,
Faculty of Pharmacy,
Sri Ramachandra University, Porur, India.
E-Mail Id: umadhilipcologist@gmail.com

In India over 180 million people are affected by this.[7] Rheumatoid arthritis has no complete cure but the symptoms can be treated and the progress of the disease can be slowed down with the help of drugs. Mild and early cases are still mostly treated with NSAIDs while disease modifying antirheumatic drugs is currently being recommended. However these drugs are associated with side effects like increased chest infection in case of immunosuppressant like methotrexate, retinal damage in case of chloroquines, neutropenia by sulfasalazine etc. Because of such side effects and toxicity the usage of these drugs are limited during pregnancy, breast feeding, liver diseases, leucopenia and many other physiological conditions. Hence, we are in a need to find an alternative medicine in the treatment of RA with less side effects and toxicity.

The objective of the present work is study the in-vitro anti-arthritic activity of whole plant of *Hybanthus enneaspermus*. It is a traditional

medicinal herb distributed in tropical and subtropical regions of the world.

Common name: spade flower, pink ladies slipper

Family: violaceae[8]

Vernacular names:

- Hindi – ratanpurush
- Tamil – orithalthaamarai
- Malayalam – orithamatai
- Telugu – ratanpurusha
- Sanskrit – rathnapurusha

Traditional Uses of the Plant

The plant is mainly cultivated as medicinal plant and been used in prevention of various diseases. In general the plant is used in conditions like diarrhoea, cholera, gonorrhoea, sterility and also as diuretics, demulcent tonic etc. Each part of the plant is believed to have medicinal values for the treatment of various ailments.[9-11]

- **Root sandals** are used in bowel complaints of children and as diuretic
- **Leaves and tender stalk** as demulcent, decoction, cooling liniment for headache or electuary.
- **Fruits** are used to treat scorpion sting.

The plant has been reported for aphrodisiac activity, hepatoprotective activity, anti-convulsant activity, anti-diabetic activity, antioxidant activity, anti microbial activity, antispasmodial activity.[12-14]

So far *invitro* anti-arthritic activity has not yet been performed in this plant and hence our work is to evaluate its potency as an anti-arthritic agent.

Plant Collection

The whole plant of *Hybanthus enneaspermus* were collected from Sengottai, Thirunelveli, Tamilnadu, India in the month of August 2015. The plant was identified and authenticated by Mr. V. Chelladurai, Retired research officer-botany, C.C.R.A.S.Govt. of India, Thirunelveli. The collected plant material was found to be free from disease and also free from contamination of other plants.

Plant Extraction

The whole plant of *Hybanthus enneaspermus* was air dried and powdered. The powdered crude plant was cold macerated for 3 days with absolute ethanol, with occasional stirring. At the end of third day the suspension was filtered and was evaporated using Rotary Evaporator under reduced pressure (< 40°c). Dark brown colour extract thus obtained was stored in an air tight dessicator for further analysis.

Qualitative Analysis of Phytochemical Substance[15-18]

The ethanolic extract of the plant was subjected to qualitative analysis and the following phytochemical substances were detected using standard qualitative procedures.

Test for Alkaloids

A test tube containing 2 ml of the ethanolic extract was treated with few drops of Dragendroff's reagent and the colour developed was observed. A reddish brown colour formed confirms the presence of alkaloids.

Test for Tannin

To 5ml of extract, few drops of ferric chloride was added and the formation of greenish black or dark blue colour confirmed the presence of tannins.

Test for Terpenoids

Salwoski test: 1 ml of extract in test tube was added with one bit tin and thionyl chloride. Appearance of pink colour in the test tube confirmed the presence terpenoids.

Test for Phenols

Ferric chloride test: Few drops of 10% aqueous ferric chloride was added to the extract and. The appearance of blue or green colour showed the presence of phenols.

Test for Saponins

5 ml of the ethanolic extract with 1 ml of water was shaken vigorously. The froth formed was added with olive oil. The emulsion formed indicated the presence of saponins.

Test for Steroids

Liberman burchard test: 1 ml of extract was added with each 1 ml of glacial acetic acid and acetic anhydride and 2 drops of concentrated sulphuric acid. The solutions becomes red, then blue and finally bluish green, confirming the presence of steroids.

Test for flavonoids

Shinoda test: To 2ml of extract, few magnesium turnings and few drop of concentrated HCl was added. Red colour formed indicates the presence of flavones.

Test for Reducing Sugars

To small fraction of extract equal volume of Fehling's solution was added and shaken vigorously. A brick red precipitate formed confirms the presence of reducing sugars.

Test for glycosides

1ml of extract was added with 10ml of 50% sulphuric acid. This mixture is heated in water bath for 5mins. 5ml of both Fehling's A and B solution was added and boiled. Formation of brick red colour indicated the presence of glycosides.

***In vitro* Anti-Arthritic Activity[19]**

The *in vitro* anti-arthritic activity of the ethanolic extract of whole plant of *Hybanthus enneaspermus* was studied using Bovine Serum protein denaturation method.[20]

Requirements

1. **Test solution:** (0.5ml) consists of 0.45ml, 5%w/v aqueous solution of bovine serum albumin and 0.05ml of various concentration of plant extract.
2. **Test control solution** (0.5ml) consists of 0.05ml distilled water and 0.45 ml of bovine serum albumin.
3. **Product control:** (0.5ml) consists of 0.45ml distilled water and 0.05 ml of test solution of various concentration.
4. **Standard solution:** (0.5ml) 0.45 ml, 5%w/v aqueous solution of bovine serum albumin and 0.05 ml of Diclofenac sodium (standard).

Method

Step1: various concentration (50, 100, 200, 400, 800, 1000 mcg/ml) of test extract and standard drug were prepared.

Step2: all the above solutions were adjusted to pH 6.3 with small amount of 1N HCl.

Step 3: the samples were incubated for 20 mins at 37°c and heated for 3 mins at 57°c.

Step 4: 2.5 ml of phosphate buffer was added to the above solutions after cooling and the absorbance was measured at 416nm using UV – visible spectrophotometer.

The percentage inhibition of protein denaturation can be calculated using the following formula

$$\text{Percentage inhibition} = \frac{(\text{OD of test solution} - \text{OD of Product control}) \times 100}{\text{OD of test control}}$$

The control represents 100% protein denaturation. The results were compared with the standard Diclofenac sodium.

RESULTS AND DISCUSSION

The ethanolic extract of whole plant of *Hybanthus enneaspermus* was found to contain phytoconstituents like alkaloids, flavonoids, tannins, terpenoids, saponins, steroids, reducing sugars.

Table 1: Ethanolic extract of whole plant of *Hybanthus enneaspermus* was found to contain phytoconstituents

Particulars	Ethanolic Extract
Alkaloids	++
Tannins	++

Terpenoids	++
Phenols	-
Saponins	++
Steroids	++
Flavonoids	++
Reducing sugars	++
Glycosides	-

In inhibition of protein denaturation assay it was found that the ethanolic extract of whole plant of *Hybanthus enneaspermus* at a concentration of 100µg/ml produced a percentage inhibition of 34.2% and in 200µg/ml it was increased to 41.6%. Likewise at 400µg/ml it was 49.7%, which further increased to 58.6% at 800µg/ml and in 1000µg/ml concentration it showed maximum inhibition of 64.6% activity.

Table 2: Effects of ethanolic extract of whole plant of *Hybanthus enneaspermus* on protein denaturation

Concentration (µg/ml)	% Inhibition	
	Ethanolic extract	Diclofenac sodium
100	34.2	40.6
200	41.6	54.1
400	49.7	68.7
800	58.6	72.9
1000	64.6	91.2

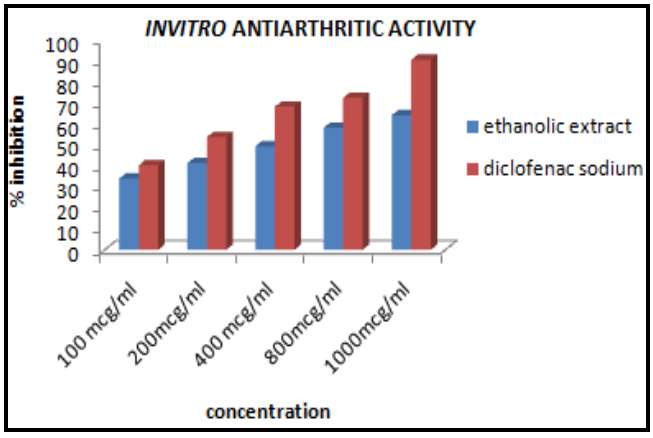

Figure 2: Effect of ethanolic extract on protein denaturation

The mechanism of protein denaturation probably involves alteration in the electrostatic, hydrogen, hydrophobic and disulphide bonding. In our present study, ethanolic extract of whole plant of *Hybanthus enneaspermus* inhibited protein denaturation and the maximum percentage of inhibition was observed as 64.6% at 1000µg/ml against the standard diclofenac sodium which was 91.2%. The IC_{50} value for the extract was found to 512.6 µg/ml and for the standard it was found to be 172.82 µg/ml.

From the results it can be emphasised that the ethanolic extract of the whole plant of *Hybanthus enneaspermus* was capable of controlling the production of protein denaturation.

CONCLUSION

The ethanolic extract obtained from whole plant of *Hybanthus enneaspermus* showed significant invitro anti-arthritic activity.

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