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IN-VITRO ANTI-INFLAMMATORY ACTIVITY AND QUALITATIVE BIOCHEMICAL ANALYSIS OF INGUVATHI URUNDAI - A SIDDHA HERBOMINERAL FORMULATION

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ABSTRACT
Siddha medicine is a traditional medical system providing preventive, promotive, curative, rejuvenative and rehabilitative healthcare. It deals with the many disease, it include 64 types of medicine (32 internal and 32 external medicine). **Background:** Inguvathi urundai is a siddha medicine which is mentioned in sarabendra vaithiya muraigal (Soolai, Moolam, Kustam, Pitha Roga Muraigal) indicated for Soolai Noi (Painful condition). **Aim:** The main aim of this study is to analyse the anti inflammatory activity by in-vitro method and bio chemical analysis of Inguvathi urundai (IU). **Methodology:** The present study was taken to evaluate the anti – inflammatory activity by using albumin denaturation assay. **Result:** The study result was concluded that Inguvathi urundai has significant Anti-inflammatory activity and Biochemical property.

KEYWORDS: *Vericose veins, Narambu Soolai, Soolai Noi, Inguvathi Urundai, Anti-inflammatory activity.*

1. INTRODUCTION

In Siddha literature, diseases are classified into 4448 types. Dhanvanthiri mentioned 13 types of soolai noi in Dhanvanthiri vaithiyam part – II, one among them is Narambu Soolai and the signs and symptoms of this disease is nearly correlated with varicose veins in modern medicine. Varicose veins are abnormally dilated, tortuous veins produced by prolonged, increased intraluminal pressure and loss of vessel wall support. The superficial veins of the upper and lower leg are main sites of involvement. Varicose dilation of veins renders the valves incompetent and leads to venous stasis, congestion, edema, pain and even thrombosis. Major risk factors are heredity, female sex, occupation that demands prolonged standing, immobility and obesity. Many number of medicines mentioned in Siddha literature for Soolai Noi. Herbo mineral medicines are potent and effective. This current study was carried out to prove the anti – inflammatory activity of Inguvathi urundai by invitro – assay and biochemical analysis.

2. MATERIALS AND METHODS

2.1 Drug selection:

The siddha herbo mineral formulation has taken from the classical siddha literature "Sarabendra Vaithiya Muraigal (Soolai, Moolam, Kustam, Pitha Roga Muraigal) (Page. No. 10 - 11)" for Soolai Noi.

2.2 Ingredients of inguvathi urundai:

1. Perungayam (Ferula asafoetida)
2. Induppu (Sodium chloride impura)
3. Chukku (Zingiber officinale)
4. Vasambu (Acorus calamus)
5. Korai kilangu (Cyperus rotundus)
6. Poondu (Allium sativum)
7. Kalarchi vithai (Caesalpinia bonduc)
8. Milagu (Piper nigrum)
9. Elumichai saaru (Citrus limon)

2.3 Collection of raw drugs:

The drugs were purchased from ASN herbal drug shop, Melapalayam, Tirunelveli.

2.4 Authentication of raw drugs:

The identification of herbomineral drugs are authenticated by faculties of Department of Gunapadam, Government siddha medical college and Hospital, Palayamkottai.

2.5 Method of purification:

All the raw drugs are purified as per the methods mentioned in Siddha literature.

2.6 Method of preparation

The above purified ingredients were powdered individually and mixed together and grind with lemon juice and make into pills (sundai alavu – 0.800gm).

2.7 Anti-inflammatory activity evaluation using albumin denaturation assay

Procedure:

In-vitro anti-inflammatory activity of the sample IU was studied using albumin denaturation technique. The reaction mixture consisting of bovine serum albumin (5% aqueous solution) and the test sample IU at varying concentration ranges from 100 to 500 µg/ml along with standard Diclofenac sodium at the concentration of 100 µg /ml of final volume. pH was adjusted by 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 buffer solution was added into each test tube. Turbidity developed was measured spectrophotometrically at 660 nm, for control distilled water was used instead of test sample while product control tests lacked bovine serum albumin. The experiment was performed in triplicate. The Percentage protection from denaturation is calculated by using the formula.

$$\left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}}\right] \times 100.$$

Statistical analysis

Results are expressed as Mean ± SD. The difference between experimental groups was compared by One-Way Analysis of Variance (ANOVA) followed by Dunnet Multiple comparison test.

2.8 Biochemical analysis of inguvathi urundai:

Preparation of the extract:

5 gms of the Inguvathi urundai was weighted accurately and placed in a 250 ml clean beaker then 50 ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100 ml volumetric flask and it is made to 100 ml with distilled water. This fluid is taken for analysis.

3. RESULTS

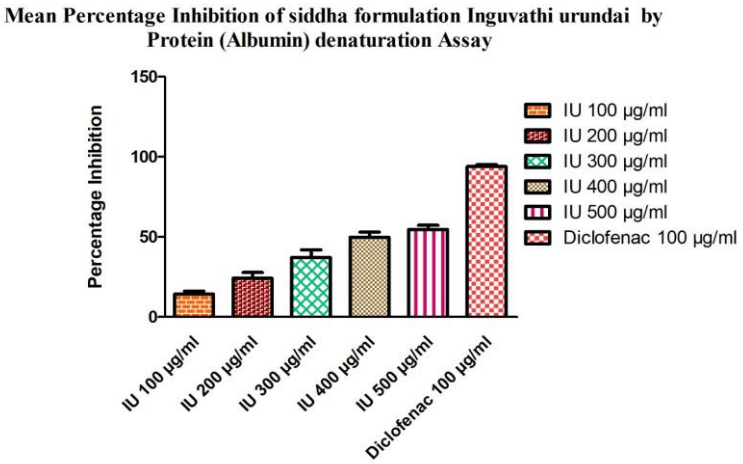
Anti-inflammatory activity evaluation using Albumin Denaturation Assay

Final RESULT

Concentration in µg/ml	Percentage Inhibition of protein denaturation
IU 100	14.34 ± 1.954
IU 200	24.29 ± 3.739
IU 300	37.21 ± 4.867
IU 400	49.82 ± 3.324
IU 500	54.79 ± 2.662
Diclofenac sodium (100 µg)	93.96 ± 2.149

Each value represents the mean ± SD. N=3

Percentage Inhibition of Protein Denaturation by IU and Standard



Biochemical analysis:

S. No.	Exprements	Observation	Inference
1	**Test for calcium:** 2ml of the above prepared extract taken in a clean test tube.to this add 2ml of 4% ammonium oxalate	No white precipitate is formed	Absence of calcium

	solution.		
2	**Test for sulphate:** 2ml of the extract is added to 5%barrium chloride solution.	No white precipitate is formed	Absence of sulphate
3	**Test for chloride:** The extract is treated with silver nitrate solution.	A white precipitate is formed	Indicates the presence of chloride
4	**Test for carbonate:** The substance is treated with concentrated hcl.	No brisk effervescence is formed	Absence of carbonate
5	**Test for starch:** The extract is added with weak iodine solution.	Blue colour is formed	Indicates the presence of starch
6	**Test for ferric iron:** The extract is acidified with glacial acetic acid and potassium ferro cyanide.	No blue colour is formed	Absence of ferric iron
7	**Test for ferrous iron:** The extract is treated with concentrated nitric acid ammonium thiocyanate solution.	Blood red colour is formed	Indicates the presence of ferrous iron
8	**Test for phosphate:** The extract is treated with ammonium molybdate and concentrated nitric acid**.**	Yellow precipitate is formed	Indicates the presence of phosphate
9	**Test for albumin:** The extract is treated with esbach's reagent**.**	No yellow precipitate is formed	Absence of albumin
10	**Test for tannic acid:** The extract is treated with ferric chloride.	No blue black precipitate is formed	Absence of tannic acid
11	**Test for unsaturation:** Potassium permanganate solution is added to the extract.	It gets decolourised	Indicates the presence of unsaturated compound
12	**Test for the reducing sugar:** 5ml of benedict'qualidative solution is taken in a test tube and allowed to boil for 2 minutes and add 8 to 10 drops of the extract and again boil it for 2 minutes	No colour change occurs	Absence of reducing sugar
13	**Test for amino acid:** One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% ninhydrin is sprayed over the same and dried it well.	Violet colour is formed	Indicates the presence of amino acid
14	**Test for zinc:** The extract is treated with potassium ferro cyanide.	No white precipitate is formed	Absence of zinc

4. DISCUSSION

Result Analysis of IU on response of albumin denaturation assay

The result obtained from the present study clearly indicates that the test drug IU was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 54.79 ± 2.662 % was observed at 500 µg/ml, when compare to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 93.96 ± 2.149 % at the concentration of 100 µg/ml.

Biochemical analysis reveals

IU contains chloride, starch, ferrous iron, phosphate, unsaturated compounds, amino acid.

5. CONCLUSION

From this study we concluded that INGUVATHI URUNDAI possess promising anti inflammatory

activity. So it is the most promising drug for Soolai Noi. This result can be further analysed invivo and more promising result may be expected in future.

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