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EVALUATION OF ANTI-ARTHRITIC ACTIVITY OF *SIMAROUBA GLAUCA* LEAVES EXTRACT

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ABSTRACT

The Ethanolic *Simarouba glauca* leaves extract was evaluated for its anti-arthritic activity. A preliminary phyto-chemical study of the extract for the presence of different phyto-constituents was carried out. *In-vitro* anti- arthritic activity of the Ethanolic *Simarouba glauca leaves* extract has been done by on Bovine serum albumin protein denaturation method and the inhibition of protein denaturation were evaluated in this study. The effect was compared with the standard drug Diclofenac sodium. The Ethanolic *Simarouba glauca leaves extract* significantly restored the altered arthritic activity when compared with standard values. It concludes that *Simarouba glauca* leaves posses anti-arthritic activity.

KEYWORDS: Anti-arthritic activity, *Simarouba glauca*, Bovine serum albumin Protein, Phyto-constituents, Diclofenac sodium.

INTRODUCTION

Herbal medicines are a valuable and precious gift from nature. They were existing even before human beings appearance on the earth. Wherever we are born we have around us

shrubs, herbs, and plants useful to us.[1] It is gratifying to note that in India, the importance and relevance of herbal system (Ayurveda, Unani, Sidda) is increasingly being realized for the past few decades. It is apparent that, sleeping giant of ayurveda is finally waking up.[2] Food, shelter and clothing are three important necessities of life and are supplied to human by plant kingdom. In olden days, people collected information on herbs methodically, scientifically and developed well-defined herbal pharmacopoeias.[3]

India is a gold mine of well-recorded and well practiced knowledge of traditional herbal medicine. Herbal medicine is still the mainstay of about 75–80% of the world population, in the developing countries, for primary health care because of better compatibility and cultural acceptability with the human body and lesser side effects.[4] Novel chemical compounds synthesis from plant active constituents, which are of potential use in medicine and other useful application. Herbal remedies are popular for diseases used by a vast majority of the world's population.[5] According to WHO, 80% of population rely on plants derived medicine for their health care.[6] Natural remedies from plants are considered to be risk-free and effective. The demand for plant based food supplements, pharmaceuticals, medicines, health products and cosmetics etc, are increasing in developed and developing countries, due to growing recognition that, the natural products are having less side effects, non-toxic and easily available at affordable prices. Herbal plants having many pharmacologically active compounds like tannins, flavonoids, steroids, alkaloids, phenols, glycosides and fixed oils, which are stored in their specific parts of root, leaves,flowers, bark, seeds, fruits, etc.[7]

Men and animals distinguished between those plants which are poisonous and those which are not and gradually developed knowledge of naturally occurring drugs transcended orally, latter in written form as baked clay tablets, papyri, parchments manuscript herbals, printed herbals, Pharmacopoeias, other works and recently by computerized information retrieval systems. Modern isolation techniques and Pharmacological screening procedures, have helped plant drugs to modern medicine had purified substances rather than galenical preparations. In recent years, there has been an immense interest in the herbal and homeopathic systems of medicine, which rely on plant sources.[8]

In the developing world, the trend has been changing from synthetic to natural products. The use of medicinal plants was compiled in Ayurveda, which listed more than 8000 herbal remedies. India is one of the world's twelve leading biodiversity centers with the presence of over 45,000 different plant species. Of these, about 15,000-20,000 plants have well medicinal

properties of which only about 7000-7500 are being used by traditional practitioners. The Siddha system of medicine uses around 600, Amchi 600, Unani 700, Ayurveda 700 and modern medicine about 30 plant species.[9]

The ancient civilizations of Indians, Chinese and North Africans provide written evidence for the use of natural sources for curing various diseases. The earliest known written document is a 4000 year old Sumerian clay tablet that records remedies for various illnesses. For instance, mandrake was prescribed for pain relief, turmeric possesses blood clotting properties, roots of the endive plant were used for treatment of gall bladder disorders, and raw garlic was prescribed for circulatory disorders. These are still being used in several countries as alternative medicines.[10] In view of progress of western medicine not only new synthetic drugs but also herbal drugs, have to fulfill the international requirements like safety, quality and efficacy. Herbal drugs have advantage of being available for patients in geographical area.[11]

The shrubs, herbs and trees of yester years are even used today, nearly 50% of all medicines currently prescribed are derived from members of plants kingdom. A large number of plants constantly screened for their possible pharmacological benefits (for their hypoglycaemic, anti inflammatory, amoebicidal, hypotensive, anti-fertility, antibiotic, cytotoxic and anti-parkinsonism properties).[12] Hebalists around world known of many plants with arthritic properties. Whether it be Bogbean (*Menyanthes trifoliata*), Nettles (*Urtica dioica*), or kalahari desert herb (*Harpagophytum procumbens*), they all work. Such remedies are under intense investigations as anti arthritic agents.[13]

Health and well-being has been a subject of man's primary concern since time immemorial. Herbal medicines are in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. A detailed investigation and documentation of plants used in health traditions and pharmacological evaluation of these plants and their taxonomical relatives can lead to the development of invaluable plant based drugs for many dreaded diseases.[14]

MATERIAL AND METHOD

Table 1: List of Chemicals.

Chemicals	Source
Dragendraff's reagent	Spectrum Reagents and Chemicals Pvt. Ltd
Mayer's reagent	NICE Chemicals Pvt. Ltd. Cochin
Wagner's reagent	NICE Chemicals Pvt. Ltd. Cochin
Hager's reagent	NICE Chemicals Pvt. Ltd. Cochin
Sodium hydroxide	NICE Chemicals Pvt. Ltd. Cochin
Sulphuric acid	Spectrum Reagents and Chemicals Pvt. Ltd
Ferric Chloride	NICE Chemicals Pvt. Ltd. Cochin
Hydrochloric acid	NICE Chemicals Pvt. Ltd. Cochin
Paraffin liquid light	Sd fine- chem limited
Bovine serum albumin	Sd fine- chem limited
Croton oil	Allin exporters
Dimethyl sulphoxide	Sd fine –chem limited
Dextramethasone cream	Glaxosmithkline pharmaceuticals limited

METHODOLOGY

Collection of plant material

Leaves of Simarouba gauca was collected from the local area of mid land of Idukki in Kearala state. The plant was identified, confirmed and authenticated by Botanist and Head of the Department of Botany, Bharathinagara. The leaves were collected and shade dried under room temperature. The dried material was pulverized separately into coarse powder by mechanical grinder. The coarse powder was subjected to hot continuous extraction with ethanol in a Soxhlet extractor. The extract was subjected for phytochemical analysis and anti-arthritic activity.

Extraction

Continuous hot extraction process - 1 kg of dry coarse powder of the leaves of *Simarouba glauca* was extracted with ethanol by using hot continuous Soxhlet apparatus. The extraction was continued until the solvent in the thimble become clear. After complete extraction, the extract was filtered and solvent was distilled off. The extract was concentrated for drying in desiccators over anhydrous calcium chloride. A dark green residue was obtained.

Qualitative phytochemical screening

The following tests were carried out on the extract to detect the presence of various phytoconstituents in the extract of *Simarouba glauca* leaves.

A. Tests for Alkaloids

a. Mayer's test: (Potassium mercuric iodide solution)

To the extract solution, added few drops of Mayer's reagent, creamy white precipitate was produced.

b. Dragendroff's test: (Potassium bismuth iodide solution)

To the extract solution, added few drops of Dragendroff's reagent, reddish brownprecipitate was produced.

c. Wagner's test: (Solution of Iodine in Potassium iodide)

To the extract solution, added few drops of Wagner's reagent, reddish brown precipitatewas produced.

d. Hager's Test: (Saturated solution of Picric acid)

To the extract solution, added few drops of Hager's reagent, yellow precipitate was produced.

B. Tests for Carbohydrates

a. Molisch's test

Treated the ethanol extract solution with few drops of alcoholic α-napthol. To this added 0.2 ml of Conc. H_2SO_4 slowly through the sides of the test tube, purple to violet coloured ring appeared at the junction.

b. Benedict's test

Treated the extract solution with few drops of Benedict's reagent (alkaline solutioncontaining cupric citrate complex) and upon boiling on water bath, reddish brown precipitate formed, (reducing sugars are present).

c. Barfoed's test

General test for monosaccharide's

Heated the test tube containing 1 ml reagent and 1 ml of extract solution in a beaker containing boiling water, if red cuprous oxide is formed within two minutes, a monosaccharide is present. Disaccharides on prolonged heating undergo hydrolysis to monosaccharide.

d. Selwinoff's test

HCl reacts with ketose sugar to form derivative of furfuraldehyde, which gives red coloured compound when linked with resorcinol. Added extract solution to about 5 ml of reagent and boiled. Fructose gives red colour within half minute. This test were sensitive to 5.5mol/lit, if glucose is absent. If glucose is present it is less sensitive and on additionof large amount of glucose it gives similar colour.

e. Fehling's test

Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and NaOH in distilled water) reagents are mixed along with few drops of extract solution, boiled, a brick red precipitate of cuprous oxide forms.

f. Tollen's test

To extract solution, added 2 ml of Tollen's reagent, a silver mirror were obtained inside the wall of the test tube, indicates the presence of aldose sugar.

g. Bromine water test

It gets decolorized by aldose but not by the ketose, because bromine water oxidizes selectively the aldehyde group to carboxylic group, giving raise to general class of compounds called aldonic acid.

C. Tests for Proteins & Amino acids

a. Millon's TestT

To extract solution, added 2 ml of Millon's reagent (Mercuric nitrate in nitric acidcontaining traces of nitrous acid) white precipitate appears, which turns red colour upongentle heating.

b. Ninhydrin Test

Extract solution were boiled with 0.2% solution of Ninhydrin solution (Indane 1, 2, 3- trione hydrate), produces violet colour.

D. Tests for Steroids and Triterpenoids

a. Libermann-Burchard test

Extract solution mixed with few drops of acetic anhydride, boiled and cooled, conc. H_2SO_4 was added from the side of the test tube, a brown ring at the junction of two layers and the upper layer turns green indicates the presence of sterols and formation of deep red colour indicates the presence of triterpenoids.

b. Salkowski's test

Dissolveed the extract in chloroform with few drops of Conc. H_2SO_4, shake well and allow to stand for some time, red colour appears in the lower layer indicates the presence of sterols and formation of yellow coloured in lower layer indicates the presence of triterpenoids.

E. Test for Diterpens

a. Copper acetate test

Extract solution mixed with H_2O and copper acetate solution, formation of emerald green colour indicates the presence of diterpens.

F. Tests for Glycosides

a. Test I: Extract 200 mg of the drug by warming in a test tube with 5 mL of dil. (10%) H_2SO_4 on a water bath at 100^0C for two minutes, centrifuge or filter, pipette out supernatant or filtrate. Neutralize the acid, extract with 5% solution of NaOH. Added 0.1 mL of Fehling's solution A and B until alkaline (test with pH paper) and heated on a water bath for 2 min. Note the quantity of red precipitate formed and compare with that formed in Test II.

b. Test II: Extract 200 mg of the extract solution using 5 mL of NaOH and boiled on water bath. After boiling added equal volume of water to the volume of NaOH used in the above test. Added 0.1 mL of Fehling's A and B until alkaline (red litmus changes to blue) and heated on water bath for two minutes. Note the quantity of the red precipitate formed. Compare the precipitates of Test II with Test I. If the precipitate in Test-I is greater than in Test-II, then Glycoside may be present. Since Test II represents the amount of free reducing sugar already present in the crude extract, whereas Test-I represents the Glycoside after acid hydrolysis.

c. Coumarin glycoside

Place small amount of sample in a test tube and cover the test tube with filter paper moisteric with NaOH solution. Placed test tube on a water bath and paper exposed to UV, formation of Green fluorescence indicates the presence of coumarin glycoside.

G. Test for Saponin glycoside

Froth formation test

a. 2ml of solution of extract with water in a test tube and shake well, formation of froth (foam) indicates the presence of saponin glycoside.

b. 2ml of solution of extract with water in a test tube and shake well and kept aside for10 mintues, formation of froth (foam) indicates the presence of saponin glycoside.

H. Tests for Tannins

a. Gelatin test

Extract solution with 1% gelatin solution containing 10% sodium chloride gives white precipitate.

b. Ferric chloride test

Extract solution with gives blue-green colour with $FeCl_3$.

c. Vanillin Hydrochloride test

Extract solution, when treated with few drops of Vanillin Hydrochloride reagent gives purple red colour.

d. Alkaline reagent test

Extract solution with sodium hydroxide solution gives yellow to red precipitate withinshort time.

I. Tests for flavanoids

Lead acetate test

Extract solution with lead acetate and formation of yellow colour indicates the presence of flavanoid.

a. Shinoda Test (Magnesium Hydrochloride reduction test)

To the extract solution added few fragments of magnesium ribbon and HCl drop wise, crimson red appears after few minutes.

b. Zinc-Hydrochloride reduction test

To the extract solution, added a mixture of zinc dust and Conc. HCl. It gives red colour after few minutes.

c. Alkaline reagent test

To the extract solution, added few drops of NaOH solution, formation of an intense yellow colour that turns to colourless on addition of few drops of dil. acetic acid indicates the presence of flavonoids.

Experimental Animals

Albino rat (Wistar strain) of either sex weighing between 100-170 g were used for this study obtained from animal house facility, Bharathi college, Bharathinagara. The animals were acclimatized for 12 days under laboratory conditions.

The animals were fed with standard commercially available rat pellet diet. Water was allowed *ad libitum* under strict hygienic conditions. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) (Approval no: **BCP/IAEC/PCOL/01**) of Bharathi college, Bharathinagara. Studies were performed in accordance with the CPCSEA guidelines.

PHARMACOLOGICAL ACTIVITY

Experimental design

Evaluation of anti-arthritic activity by inhibition of protein denaturation method

The test solution was prepared by taking 0.45ml of bovine serum albumin (BSA- 5 % w/v aqueous solution) and 0.05 ml of ethanol extract of *Simarouba glauca* leaves in various concentration (10-1000 µg/ml). The test control solution was prepared by adding 0.45ml of BSA and 0.05ml of DMSO. Product control consists of 0.45ml of distilled water and 0.05ml of ethanol extract of *Simarouba glauca* leaves in various concentrations (10-1000 µg/ml). Standard solution consists of 0.45ml of BSA and 0.05ml of Diclofenac sodium solution in various concentrations (10-1000 µg/ml). The pH of the above solutions is to be adjusted to 6.3 using 1N HCl and incubated at 37^0C for 20 minutes. Then the resultant solutions were heated at 57^0C for 3 minutes. The solutions were cooled. 2.5 ml of phosphate buffer was added and the absorbance of resultant mixtures were read at 660 nm.

The percentage inhibition of protein denaturation was calculated as follows.

Percent inhibition=100-[OD of test solution–OD of product control]/ OD of test control × 100

The control represents 100% protein denaturation. The results are to be compared with the standard Diclofenac sodium.[15]

Statistical analysis

The data were expressed as Mean ± S.E.M.

RESULTS

Extraction by hot continuous Soxhlet process, the percentage yield is described in Table-2.

Table 2: Physical characteristics of Ethanolic leaves extracts of *Simarouba glauca.*

Solvent	% Dry wt ingms	Colour	Odour	Consistency
Ethanol	7.80	Dark green	Characteristic	Sticky mass

Table 3: Phytochemical screening data of crude ethanolic leaves extract of *Simarouba glauca.*

Tests	Ethanolic extract of *Simarouba glauca* leaves
Alkaloids	-ve
Carbohydrates	+ve
Saponins	+ve
Terpenoids	+ve
Flavanoids	+ve
Proteins	-ve
Glycosides	+ve

+ve indicates present and –ve indicates absent

Table 4: *In-vitro* anti-arthritic activity data of ethanolic leaves extract of *Simarouba glauca.*

Conc.(µg/ml) Standard & Test	Absorbance		Productcontrol		% inhibition	
	Standard	Test	Standard	Test	Standard	Test
10	0.75	0.84	0.02	0.02	26.27 ± 0.0115	17.18 ± 0.0404
50	0.58	0.80	0.019	0.019	43.34 ± 0.0288	21.12 ± 0.0202
100	0.46	0.66	0.018	0.018	55.36 ± 0.0692	35.16± 0.0750
200	0.37	0.56	0.017	0.017	64.35± 0.0288	45.16± 0.0288
400	0.30	0.48	0.016	0.016	71.32 ± 0.0519	53.14± 0.0173
800	0.24	0.37	0.015	0.015	77.28± 0.0346	64.15 ± 0.0519
1000	0.17	0.26	0.001	0.001	84.95± 0.0230	73.84± 0.0346
Control	0.99		-		-	

The experiments were done in triplicate for concordant values.

The *in-vitro* anti- arthritic activity of the ethanolic leaves extract of *Simarouba glauca* has been done by Bovine serum protein denaturation method and results are compared with standard. The results are summarized in table-4 at different concentration (10 to1000 µg/ml). The percentage inhibition of protein denaturation was calculated and found for test at 10 to 1000 µg/ml showed 17.18 ±0.0404, 21.12 ±0.0202, 35.16±0.0750, 45.16±0.0288, 53.14±0.0173, 64.15± 0.0519, 73.84±0.0346. Standard Diclofenac sodium (10 to 1000µg/ml) showed 26.27±0.0115, 43.34±0.0288, 55.36±0.0692, 64.35±0.0288, 71.32±

0.0519, 77.28±0.0346, 84.95±0.0230 % inhibition of protein denaturation respectively.

Simarouba glauca leaves showed significant activity at various concentrations. Significant activity was seen at the concentration 73.84% at 1000 µg/ml when compared with the standard reference drug Diclofenac sodium. The production of auto antigen in certain arthritic disease may be due to denaturation of protein. From the results of the present study it can be assumed that the plant extract is capable of controlling the production of auto antigen and inhibits denaturation of protein in arthritic conditions.

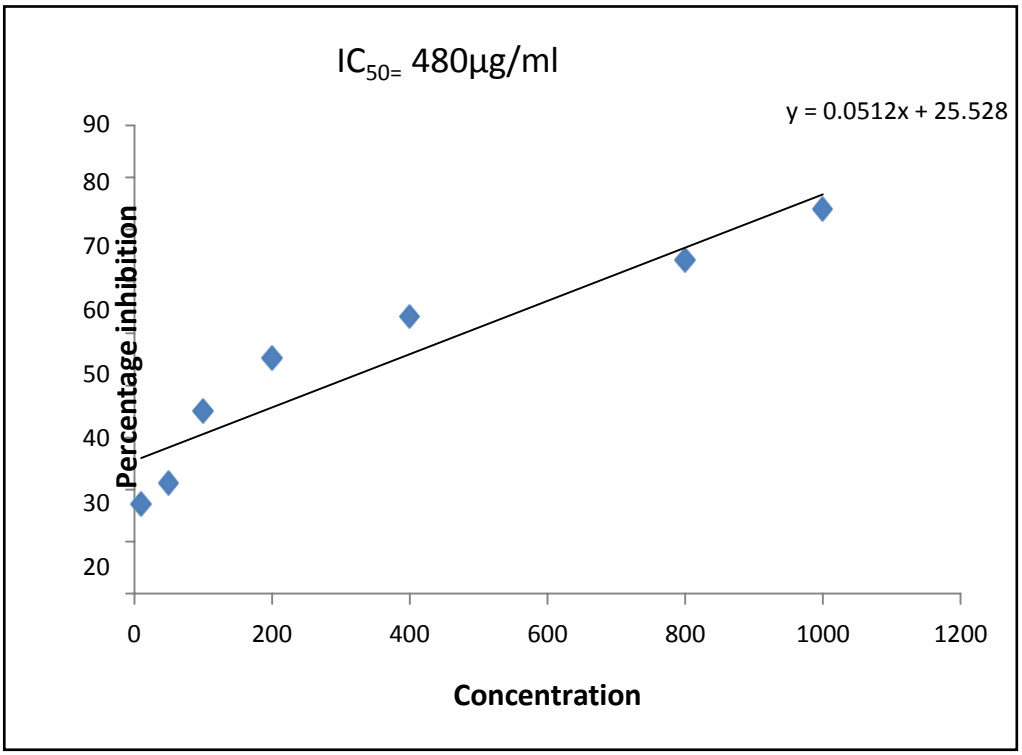

Fig- 9: **The IC_{50} value of test by using bovine seum albumin method.**

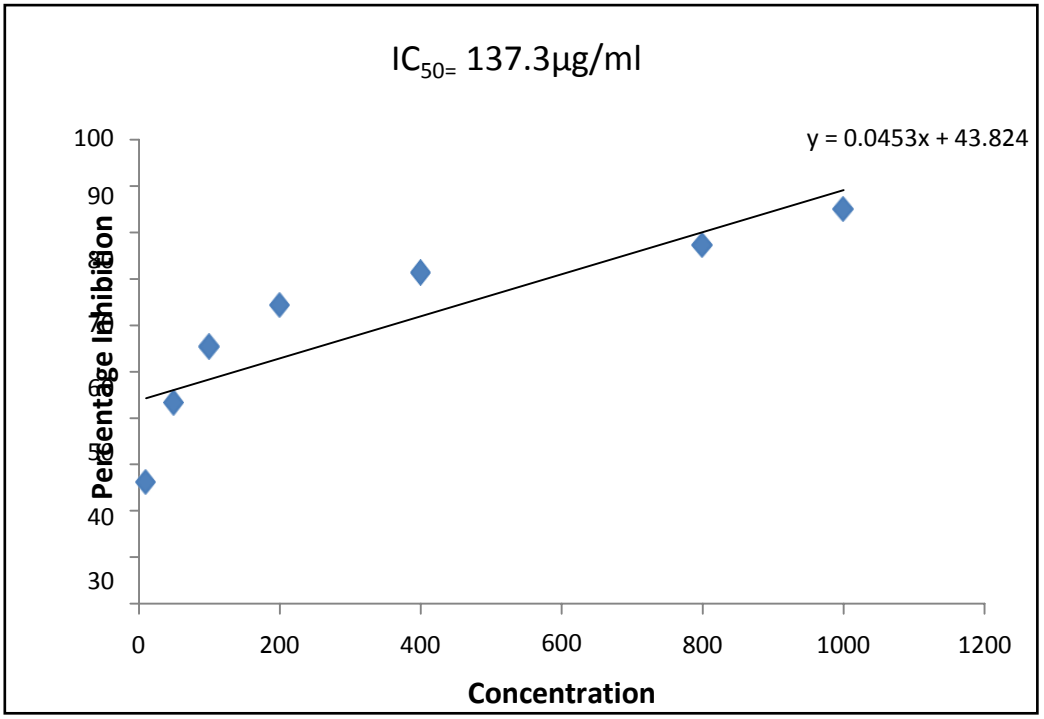

Fig-10: The IC_{50} value of standard byusing bovine serum albumin method.

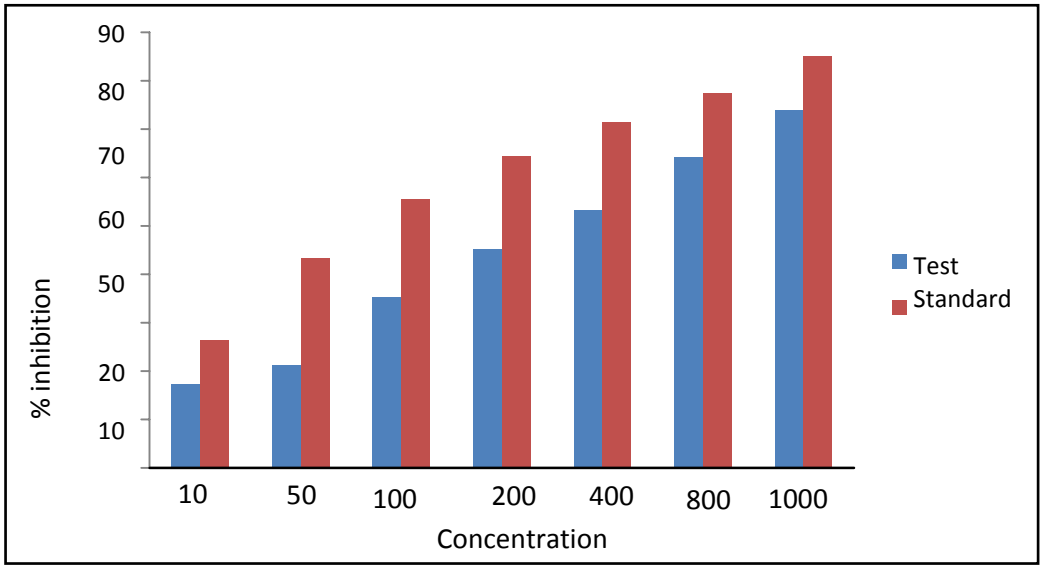

Fig-11: Comparison between *in-vitro* anti-arthritic potential of test and standard by using bovine serum albumin denaturation method.

DISCUSSION

Most of the investigatory have reported denaturation of protein is one of the cause of rheumatoid arthritics.[16] Production of auto antigens in certain rheumatic diseases may be due to *in-vitro* denaturation of proteins. Mechanism of action of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding.

Arthritics, the common human autoimmune disease is characterized by chronic inflammation in joints followed by pannus formation with infiltered lymphocytes and fibrinoid joints of synovial membrane with concomitant destruction of cartilage and bone. Exact etiological mechanism is not known but cytokines play a role in pathogenesis of arthritics. T-cells cause direct impact on TNF-α, IL-1 and IL-6 and TNF-α plays a critical role in pathogenesis of arthritic and β-cells also play an important role through cell interaction with T-cells, dendritic cells, synovial nerves like cells (SNLC) and fibroblasts, leukotrienes were also seen.[17,18]

The protein denaturation is a process in which protein lose their tertiary and secondary structure by application of external stress or compound such as strong acid or base a concentration inorganic salt, an organic solvent or heat. Most biological protein lose their biological function when denatured.[19] Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. *In-vitro* anti arthritic activity was done for the extracts of *Simarouba glauca* by using bovine serum albumin method. The ethanolic extract fabricates significant activity at 73.84±0.0346 at 1000µg/ml by inhibition of protein denaturation and its effect was compared with the standard drug Diclofenac sodium. In present study, the ethanolic extract is capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease.

CONCLUSION

The traditional plant taken for this research proposal was carried out with experiment for anti-arthritic activity. The causative factor may be exogenous (Infections) or endogenous. *In-vitro* studies demonstrated suppression of arthritis. The ethanolic extracts of the leaves of *Simarouba glauca* contains some novel, phytoconstituents which are responsible for the anti-arthritic activity. Hence proper isolation of the active principles might help in the findings of a new lead molecule in anti-arthritic drug research.

- From the preliminary screening, the extract of *Simarouba glauca* leaves showed the presence of carbohydrates, flavanoids, tannins, saponins terpenoids and glycosides. These constituents were might be a reason for anti arthritic activity.
- Arthritis is a chronic inflammatory disease, morphological triad affecting synovial cartilage and bone. In present study, the ethanolic extract was capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease. Hence at this point it is concluded that the ethanolic extract of *Simarouba glauca* leaves possess significant anti-arthritic activity.

Finally, it is concluded that the ethanolic extract of *Simarouba glauca* leaves possess significant anti-arthritic activity. This may be due to presence of carbohydrates, flavonoids, glycosides, saponin, tannins and diterpenoids and triterpenoids, thus it proves the traditional information of the plant is scientifically validated and confirmed by anti-arthritic activity.

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