

Anti Arthritic Activity of *Cissus quadrangularis* L and *Justicia tranquebariensis* in the Treatment of Rheumatism

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ABSTRACT

Cissus quadrangularis and *Justicia tranquebariensis* are the species widely used in folk medicine to treat Rheumatoid arthritis and hasten the fracture healing process. The ethanolic plant extract of *Cissus quadrangularis* and *Justicia tranquebariensis* was investigated to evaluate its anti-arthritic activity by Freund's adjuvant induced arthritis model, the plant extracts significantly reduced the arthritis of affected joint when compared with the controlled rats. The present study revealed the potential of plant extract in the management of inflammation and arthritis confirming the folk core use of medicinal plants.

INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory, systemic autoimmune disease characterized by the development of a chronic inflammatory proliferation of the synovial linings of diarthrodial joints, which leads to aggressive cartilage destruction and progressive bony erosions. Untreated, rheumatoid arthritis often leads to progressive joint destruction, disability, and premature death¹. The cause of rheumatoid arthritis is unknown, but it is supposed to be triggered by the combination of genetic susceptibility and exposure to environmental factors².

Cissus quadrangularis L. is a rambling shrub syn. *Vitis quadrangularis* Wall, belongs to the family Vitaceae. It is commonly known as "Pirandai" (in Tamil). *Cissus quadrangularis* L. was grows almost everywhere in the plains of India. It is one of the valuable medicines in the Indian Traditional System of Medicine. The aerial parts of the plant are used in asthma, dog bite, insect bite, as alterative and stomachic, in scurvy, menorrhagia and digestive disorders, It is used as anti-inflammatory, and to promote wound - healing and cardiovascular activity, menstrual disorders, in epistaxis, and for its helicon bactericidal activity, and in hypotension. Fracture healing mechanism of the herb was unfolding. It contains 0.14% of amyryne delta triterpene, 0.1% of amyryne delta triterpene and of 0.0003% of triterpene in Thailand specimen. It also contains calcium oxalate, carotene, vitamin-C, sitosterols, tetra terpenoid, amyryns and an anabolic keto steroid, and 3-ketosteroid, acetylcholine. Therapeutic effect of medicinal plants depends upon their chemical constituents.

Justicia tranquebariensis is a small shrub, which is widely distributed in southern parts of India. Root is perennial, somewhat granulated, stem in young plants erect, in old ones uncertain, branches annual, small, stamling, round, a little downy, joined and swelled above the joints, from 1-2 ft long, leaves opposite, short, oval, round, entire, pretty smooth. *Justicia tranquebariensis* plant parts yielded several lignans, phytosterols, brassicasterol, campesterol, ergostadienol, stigmasterol, spinasterol, isofucostil and a sterol glycoside, betasitosterol- O -glycoside. The present investigation was undertaken with a view to subject the plant samples of *Cissus quadrangularis* and *Justicia tranquebariensis* to Phyto chemical and Antiarthritic activity.

Advances have been made in elucidating subsequent steps in the pathogenesis of this disease. A critical role for T cells in the pathogenesis of RA is suggested by the strong association between RA and certain human leukocyte antigen (HLA) heliotypes. Recent data suggest that the destruction of rheumatoid joints is initiated by complex cell-cell interaction between antigen presenting cells and CD4+T cells. However it is thought that these cell-cell interaction result in the activation of macrophages and induction of the inflammatory process, culminating in degradation and resorption of cartilage and bone. Pro inflammatory cytokines particularly TNF and interleukin 1(IL-1) are critical components of this process³.

Rheumatoid arthritis is predominantly a disease of the elderly, but children's can also be affected by the disease. Nearly 46 million U.S. adults were reported to be arthritic, among this prevalence rate a quarter million are children's

and 60% are women's. While the prevalence of rheumatoid arthritis in Indian adults was reported to be about 0.75%. This prevalence rate is higher than that reported from China, Indonesia, Philippines and rural Africa⁴.

The therapy for rheumatism includes medications with steroids, non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs) and immunosuppressant drugs. Unfortunately although these drugs have shown to improve signs and symptoms alter the natural history of the disease and improve quality of life but there is still no cure. In addition, these available therapies are associated with potential risks of death/ irreversible organ damage⁵.

MATERIAL AND METHODS

The aerial parts of *Cissus quadrangularis* and *Justicia tranquebariensis* were collected during flowering period from different places with different types of soil after the plant specimen was identified with the help of local Floras. Identity of specimen was further confirmed with the help of Herbarium sheets available in The Rapinat Herbarium, St. Joseph's College, Tiruchirapalli.

The air dried (shade) plant materials were powdered, which were subjected to determination of total ash, water-soluble ash, acid-insoluble ash, sulphated ash by the methods described in Indian Pharmacopoeia. The solubility percentage of powder in water, ethanol and 50% ethanol was also estimated. Qualitative phyto chemical analysis and Quantitative estimation of total alkaloid, total terpenoid, total glycoside, calcium salt and vitamin-C 42 was also determined⁶.

Extract Preparation

The collected plant materials were shade dried and coarsely powdered. About 1 kg of the powdered material was soaked in methanol for 48 h and extracted by soxhlet extraction. The extract was vacuum dried and was stored at -4°C until further use.

Photochemical Analysis

Qualitative and Quantitative Photochemical Evaluation

The plant extracts were subjected to qualitative tests for the identification of the phyto constituents present in it viz., alkaloids, carbohydrates, glycosides, phytosterols, fixed oils & fats, Phenolic compounds & tannins, proteins and free amino acids, gums & mucilages, flavonoids, lignin's and Saponins⁷.

Test for Alkaloids

A small portion of the solvent free Pet. Ether, hexane, alcohol and aqueous extracts were stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate may be tested carefully with various Alkaloidal reagents such as,

- a. Mayer's reagent- Cream precipitate
- b. Dragendorff's reagent- Orange brown precipitate
- c. Hager's reagent- Yellow precipitate
- d. Wagner's reagent- Reddish brown precipitate

Test for carbohydrates & glycosides

The minimum amount of extracts were dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates and glycosides.

a. Molisch's test

The filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube.

b. Fehling's test

The filtrate was treated with 1ml of Fehling's solution and heated. Orange precipitate was obtained shows the presence of carbohydrates. Another portion of the extracts was hydrolyzed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legal's, Borntrager's test to detect the presence of different glycosides.

c. Legal's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Purple colour in ammonical layer was observed.

Test for Phytosterol - Liebermann Burchard Test

1 gram of the extract was dissolved in few drops of dry acetic acid, 3ml of acetic anhydride was added followed by few drops of conc. sulphuric acid. Appearance of bluish green colour showed the presence of phytosterol.

Test for fixed oils and fats

A small quantity of the various extracts was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5 N alcoholic Potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

Test for Saponins

The extracts were diluted with 20ml of distilled water and it was agitated on a graduated cylinder for 15 min. The presence of Saponins was indicated by the formation of 1cm layer of foam.

Test for Tannins and Phenolic Compounds

Small quantities of various extracts were taken separately in water and tested for the presence of Phenolic compounds and tannins with

- i. Dilute ferric chloride solution 5%- violet colour,
- ii. 1% solution of gelatin containing 10% NaCl- white precipitate
- iii. 10% lead acetate solution- white precipitate

Test for Proteins and Free Amino Acid

Dissolve small quantities of various extracts in a few ml of water and treated with,

- i. Million's reagent - Appearance of red colour shows the presence of proteins and free amino acids.
- ii. Ninhydrin reagent - Appearance of purple colour shows the presence of proteins and free amino acids.
- iii. Biuret Test – Equal volume of 5% solution of Sodium hydroxide and 1% solution of Copper sulphate were added. Appearance of pink colour shows the presence of proteins and free amino acids.

Test for Gums and Mucilage's

About 10ml of various extracts were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

Test for Flavonoids

With aqueous sodium hydroxide solution, blue to violet colour (antho cyanins), yellow colour (flavones) and yellow to orange (flavonones).

- a. With concentrated sulphuric acid, yellowish orange colour (anthocyanins), yellow to orange colour (Flavones), orange to crimson (flavonones)
- b. Shinoda's Test: The extract is dissolved in alcohol, to that piece of magnesium followed by conc. Hydro choleric acid drop wise are added and heated. Appearance of magenta colour shows the presence of flavonoids.

Test for lignin

With alcoholic solution, Phloroglucinol and hydrochloric acid, appearance of red colour shows the presence of lignin.

Acute toxicity studies

Animals were fasted overnight; however water made available *ad libitum*, after which ethanolic extract was given at one of the four fixed-dose levels (5, 50, 300, and 2000 mg/kg) as per OECD-423 guidelines to five male and five female mice. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72hr. Animals treated with highest dose were kept observation for 14 days for further toxicity study .

Freund's adjuvant induced arthritis in rats

Albino rats Wister strain weighing between 150-200 g will be divided into 7 groups of 6 animals each with 3 animals housed in each cage. Except those in the control group rats in all other groups are injected with s.c 0.1 ml of Complete Freund's Adjuvant into the planter region of the left hind paw.

Group 1: Not administered Complete Freund's adjuvant (**Normal control**)

Group 2: Administered with only Complete Freund's adjuvant (**Arthritic control**)

Group 3: Administered with only Complete Freund's adjuvant + Indomethacin (**3mg/kg**)

Group 4: Administered with only Complete Freund's adjuvant + 100 mg Extract *Cissus quadrangularis*

Group 5: Administered with only Complete Freund's adjuvant + Extract 100mg *Justicia tranquebariensis*

Group 6: Administered with only Complete Freund's adjuvant + 200 mg Extract *Cissus quadrangularis*

Group 7: Administered with only Complete Freund's adjuvant + Extract 200mg *Justicia tranquebariensis*

The changes in paw volume were measured on various days up to 21 days following Freund's adjuvant injection^{9, 10, 11}. The extracts and indomethacin was administered orally for 14 days from the day of Freund's adjuvant injection. On 21st day rats are anaesthetized with ketamine and diazepam and various parameters estimated.

The following biochemical parameters were estimated

- Alkaline phosphatase
- Serum glutamic oxaloacetate transaminase
- Serum glutamic pyruvate transaminase
- Histo pathological parameters.

RESULTS AND DISCUSSIONS

Qualitative Phyto chemical analysis of 50% Ethanol extract revealed that all the biologically active compounds were present in the plant extracts. Quantitative Phyto chemical analysis of the two plant samples showed that total Alkaloidal Content was (0.022 – 0.024%) and Vitamin - C content was almost similar (0.11 – 0.18%). Greatest quantity of total glycosides (2.276 %) was found. Calcium salts (1.1990%), total Terpenoids (2.392%) were found to be higher. Earlier workers on both the plants reported physicochemical values which are of significant deviation from the values reported here. The difference might be due to the type of from which the specimen was collected. Further is in progress to know their activity profiles

Acute toxicity study

The dizziness was observed at higher dose of ethanolic extract and no death was reported. The highest dose chosen for the acute oral toxicity was 2000mg/kg, hence 1/10th of this dose 100mg/kg was taken as initial effective dose and next higher doses 200mg/kg was selected for the present study.

Biochemical estimation

The CFA (1%, 0.01ml) in arthritis control showed significant elevated level of SGOT, SGPT and ALP as compare to vehicle control. The extract of dose of 100/kg significantly decreased the level of SGOT and ALP and insignificant effect on SGPT level. However the standard indomethacin and extracts of 200mg/kg dose level has shown significant effect on preventing the level of SGOT, SGPT and ALP as compared to arthritis control.

Antiarthritic activity (Paw edema)

The challenge with CFA(1%, 0.1ml) showed significant increase in paw edema which has reached to peak and remained constant by the end of 2nd week in arthritis control as compared to vehicle control. Extract of dose of 100mg/kg has shown moderate effect on prevention of

paw edema, but the treatment with standard indomethacin and extracts dose of 200mg/kg has shown significant prevention of paw edema as compared to arthritis control. The plant extracts of *Cissus quadrangularis* and *Justicia tranquebariensis* possess Antiarthritic activity in Freund's adjuvant induced arthritis, the plant extracts significantly reduced the arthritis of affected joint when compared with the controlled rats. The cardinal science of chronic inflammatory reactions like redness, swelling, ophthalmia and immobility of affected joints were significantly less in drug treated animals than those of control. The present study revealed the potential of plant extract in the management of inflammation and arthritis confirming the folk core use of medicinal plants.

CONCLUSION

The present preclinical study had revealed the traditional use of *Cissus quadrangularis* and *Justicia tranquebariensis* in treatment of rheumatism. However the study also concludes that ethanolic extract of *Justicia tranquebariensis* dose of 200mg/kg exhibited significant anti-arthritis activity than the lower doses of 100mg/kg. These effects may probably attribute to presence of phytochemical such as alkaloid and flavonoids in *Cissus quadrangularis* and *Justicia tranquebariensis*. Because a lot of these secondary plant metabolites identified so far exhibit anti-arthritic properties. Hence one or more of these plant metabolites could be responsible for its anti-arthritic activity.

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Table 1: Analytical values of *Cissus quadrangularis* and *Justicia tranquebariensis* samples

Parameters	<i>Cissus quadrangularis</i> (aqe)	<i>Cissus quadrangularis</i> (ete)	<i>Justicia tranquebariensis</i> (aqe)	<i>Justicia tranquebariensis</i> (ete)
Total Ash (%)	13.0305	12.4559	12.7945	12.5315
Water soluble Ash (%)	5.5497	5.4509	5.3306	8.9063
Acidi-insoluble Ash (%)	1.0352	0.1608	0.7706	0.5499
Sulphated Ash (%)	22.4691	21.0741	21.9188	18.0807
Loss on Drying (%)	88.42	86.91	86.91	87.20
Extractives - water	12.60	12.62	11.90	12.78
Extractives-Ethanol	3.40	3.28	3.48	4.14
Extractives – 50% ethanol	13.82	13.74	13.48	13.44

Table 2: Quantitative Phyto-chemical estimation of *Cissus quadrangularis* and *Justicia tranquebariensis* samples

Compound	Colour & Physical Nature	<i>Cissus quadrangularis</i>	<i>Justicia tranquebariensis</i>
Total Alkaloids (%)	Greenish yellow oily semisolid	0.024	0.022
Total Terpenoids (%)	Dark Green oily semi solid	2.252	2.392
Total Glycosides (%)	Dark Brown oily semi solid	2.228	2.276
Vitamin-C (%)	-	0.1894	0.1139
Calcium Salt (%)	-	1.1990	0.5958

Table 3: Phyto chemical Screening of the *Cissus quadrangularis* and *Justicia tranquebariensis*

S.No.	Constituents	<i>Cissus quadrangularis</i> extract	<i>Justicia tranquebariensis</i> extract
1.	Alkaloids	+	+
2.	Carbohydrates	+	+
3.	Glycosides	+	+
4.	Phytosterols	+	+
5.	Saponins	+	+
6.	Fixed oil and fats	+	+
7.	Tannin	+	-
8.	Proteins	+	+
9.	Gums and mucilages	+	+
10.	Flavonoids	+	+
11.	Volatile oils	-	-
12.	Lignin	+	+

Table 4: Anti-arthritis activity of plant extracts of *Cissus quadrangularis* and *Justicia tranquebariensis* against Freund's adjuvant induced arthritis in rats

Treatment group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Vehicle Control	44.19±2.188	49.50±1.443	124.37±0.019
Arthritis Control	107.60±2.136	93.70±2.880	254.28±3.423
Standard Indomethacin	60.10±2.140***	72.48±2.092***	143.72±3.909***
ECQ 100mg/kg	99.00±2.582*	88.39±1.882	218.358±4.991***
EJT 100mg/kg	97.00±2.572*	85.39±1.862	216.358±4.000***
ECQ 200mg/kg	86.62±2.041***	84.86±2.092*	199.00±3.270***
EJT 200mg/kg	74.25±2.327***	79.55±2.777**	174.12±3.639***

Values expressed as mean ± SEM (n=6),

*P<0.05,**P<0.01,***P<0.001 as compared to arthritis Control.

Table 5: Anti-arthritis activity of plant extracts of *Cissus quadrangularis* and *Justicia tranquebariensis* against Freund's adjuvant induced arthritis in rats

Treatment Group	Day 7	Day 14	Day 21	Day 28
Arthritis Control	0.92±0.028	1.35±0.016	1.32±0.019	1.30±0.019
Standard Indomethacin	0.83±0.006*	0.90±0.011***	0.87±0.011***	0.85±0.015***
ECQ 100mg/kg	0.92±0.012	1.27±0.009**	1.20±0.014***	1.10±0.026***
EJT 100mg/kg	0.91±0.013	1.26±0.010**	1.19±0.015***	1.09±0.025***
ECQ 200mg/kg	0.91±0.016	1.12±0.009***	1.0±0.085**	0.91±0.070***
EJT 200mg/kg	0.87±0.015	1.0±0.072***	0.95±0.050**	0.91±0.050***

Values expressed as mean ± SEM (n=6)

*P<0.05,**P<0.01,***P<0.001 as compared to arthritis Control.

REFERENCES

- Stephen JM and William FG. Patho physiology of Disease: An Introduction to Clinical Medicine. 5th edition. McGraw-Hill publication; 2006.
- Ollier WE, Harrison B and Symmons D. What is the natural history of rheumatoid arthritis, Baillieres Best Pract Res Clin Rheumatol 2001; 15: 27-48.
- Robbins, Cotron, Vinay k, Abdul KA and Nelson F. Pathologic Basis of Disease. 7th edition, Elsevier publication. 2008;1306-1308.
- Irfan AK and Atiya K . Herbal therapy for arthritis. Ukaaz publications; 2008;15-72.
- Susan JL and Arthur K. Pharmacological treatment of established rheumatoid arthritis. Best Pract Res Clin Rheumatol. 2003;17:811-829.
- Harborne JB. Phyto chemical methods, Chapman and Hall, London. 1998;4-32.
- Kokate CK. Practical Pharmacognosy, 4th edn, 1994;107-135.
- Ecobichon DJ. The Basic of Toxicology Testing. 3rd edition. CRC press; 1997;43-86.
- Mythily Priya R, Shanthi P and Sachdanandam P. Therapeutic effect of Kalpamruthaa, a herbal preparation on adjuvant induced arthritis in wister rats. J Inflammopharmacology. 2008; 16:21-35.
- Jaijesh P, Srinivasan KK, Bhagath KP, Sreejith G, Raju SK and Sareesh NN. Anti- arthritic potential of the plants *Justicia gendarussa* Burm F. J Clinics. 2009;64:357- 362.
- Jaijesh P, Srinivasan KK, Bhagath KP, Sreejith G, Raju SK and Sareesh NN. Comparing the anti-arthritis activities of the plants *Justicia gendarussa* Burm F and *Withania Somnifera* Linn. Int J Green Pharm. 2009;3:281- 284.