

Invitro anti-arthritic, anti-inflammatory and anti-oxidant activity of Cissus quadrangularis linn

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ABSTRACT

The present investigation deals with the study of *in vitro* anti-arthritic activity by inhibition of protein denaturation method, anti- inflammatory by HRBC membrane stabilization method and anti-oxidant activity by DPPH method. *Cissus quadrangularis Linn* plant is a perennial tendril climber with quadrangular stem. It is used in the treatment of gout, syphilis, stomach ache, regularize menstrual cycle, antimicrobial activity and piles in Ayurvedic medicine and traditionally used for bone fracture. The air dried powder of *Cissus quadrangularis Linn* (aerial parts) were extracted using Soxhlet apparatus with ethanol as solvent. The extracts were concentrated under reduced pressure. The activities were carried out using the following concentration (31.2, 62.5, 125, 250, 500, 1000, 2000 μ g/ml). It has significant *in vitro* anti-arthritic, antioxidant and anti-inflammatory activity. The activity may be due to presence of chemical profile such as phenolic acid, flavonoid (leuteotin) and β -sitosterol. The results of the study have implications in the use of *Cissus quadrangularis Linn* as anti-arthritic, anti-inflammatory and antioxidant in several applications requiring these properties.

Keywords: *Cissus quadrangularis Linn*, Anti-arthritic, Anti-oxidant, Anti-inflammatory, DPPH, HRBC.

INTRODUCTION

1. Plant: *Cissus quadrangularis Linn* (Vitaceae) (Figure 1), aerial parts collected in July 2008 from medicinal garden in our campus which was authenticated by Dr P.Jayaraman, Director, PARC (botanist), West Tambaram, Chennai. Voucher specimens (No: PARC/2008/188) were deposited in our college herbarium for future reference.(Indian medicinal plants: Kiritikar K R, K M Nadkarni)

2. Uses in traditional medicine: The aerial parts of the plant were used for bone fracture, dyspepsia, indigestion, otorrhoea, epistaxis, scurvy, asthma and stomachic. The juice of the plant after pounding is used for otorrhoea. A bibliographical survey showed that there is no report on *invitro* anti-arthritic and anti-inflammatory activity.(C P Khare, The wealth of India). The present manuscript describes some of the *in vitro* activity of this plant using inhibition of protein denaturation, human red blood corpuscles membrane stabilization method and DPPH method.

3. Reported activity: Anti-fungal, wound healing, leighmanicidal, nitric oxide scavenging, anti-bacterial.

4. Prior isolated constituents: Flavonoid (leuteotin) and β -sitosterol. (Govindachari, 1968).

MATERIALS AND METHODS

Extraction: The *Cissus quadrangularis Linn* aerial parts were air dried and powdered (70g). It was extracted with ethanol using soxhlet apparatus for 48h. The solvent was concentrated under reduced pressure to get the crude

extract which is stored in desiccators for future use. (Yield: 3.5%).

Biological procedure:

Invitro anti-arthritic activity by inhibition of protein denaturation method: The Test solution (0.5 ml) consist of 0.45ml of Bovine serum albumin (5 % W/V aqueous solution) and 0 .05 ml of test solution (250 μ g/ml). Test control solution (0.5 ml) consist of 0.45ml of bovine serum albumin (5 % W/V aqueous solution) and 0.05 ml of distilled water. Product control (0.5 ml) consists of 0.45ml of distilled water and 0.05 ml of test solution (250 μ g/ml). Standard solution (0.5 ml) consists of 0.45ml of Bovine serum albumin (5 % w/v aqueous solution) and 0.05ml of Diclofenac sodium (250 μ g/ml). All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37° c for 20 min and the temperature was increased to keep the samples at 57° c for 3 min. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm. The control represents 100 % protein denaturation. The results were compared with Diclofenac sodium (250 μ g/ml). The percentage inhibition of protein denaturation of different concentration was given in table 1. (Sadique J, 1989).

Invitro anti-inflammatory activity by HRBC membrane stabilization method: The principle involved here is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture (1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml

hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with 0.5 ml of plant extracts of various concentrations (31.25, 62.5, 125, 250, 500, 1000, 2000 $\mu\text{g}/0.5\text{ml}$), standard drug diclofenac sodium (250, 500 1000, 2000 $\mu\text{g}/0.5\text{ml}$) and control [distilled water instead of hypo saline to produce 100 % haemolysis] were incubated at 37° C for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm. The percentage haemolysis produced in the presence of distilled water was taken as 100 % (Table 2). (Mizushima Y, 1968 & Ghandisan R)

Invitro antioxidant activity by DPPH method: The free radical scavenging activity of the ethanolic extract of *Cissus quadrangularis Linn* at different concentration were examined using DPPH radical. The reaction mixture consists of 0.1 μm DPPH in ethanol. The activities were carried out using the following concentration (31.2, 62.5, 125, 250, 500, 1000, 2000 $\mu\text{g}/\text{ml}$). The absorbance of the mixture was measured at 517nm after 20min incubating in dark condition (Table 3). The experiments were performed and the percentage of free radical scavenging was calculated using Quercetin as standard. (Agarwal R B, 2008 & Cotella A, 1996)

RESULTS AND DISSCUSION

Anti- arthritic: The ethanolic extract fabricates significant activity at 98.44% at 250 $\mu\text{g}/\text{ml}$ by inhibition of protein denaturation and its effect was compared with the standard drug Diclofenac sodium. The production of auto antigen in certain arthritic disease may be due to denaturation of protein. From the results of present study it can be stated that ethanolic extract capable of controlling the production of auto antigen and inhibit denaturation of protein in rheumatic disease.

Anti-inflammatory activity: A need arise for the progress of newer anti-inflammatory agents from natural source with potent activity and lesser side effects as substitutes for chemical therapeutics. The percentage protection of ethanolic extracts was 97.04% at 250 $\mu\text{g}/\text{ml}$. It possesses significant activity comparable with that of the standard Diclofenac sodium. *Cissus quadrangularis Linn* has significant anti-inflammatory activity may be due to presence of chemical profile such as flavonoid (leuteotin) and β -sitosterol. Further studies are necessary, to identify the active constituent(s) responsible for anti-inflammatory activity. Studies related to active constituents on lipid derived eicosanoids, enzyme expression (COX2, lipoxygenase) and cytokines are necessary to understand the mechanism of action in relation to the observed anti-inflammatory activity.

Anti-oxidant activity: Various disease circumstances are linked with free radicals when the generation of these species exceeds the levels of anti-oxidants mechanism. Herbal drugs containing free radical scavengers like phenolic, tannins and flavonoid are known for their therapeutic activity. Literature review showed the ethanolic extract of *Cissus quadrangularis Linn* has flavonoid (leuteotin) and β -sitosterol. The above reason prompted us to study free radical scavenging activity by DPPH method.

The method is based on the reduction of alcoholic DPPH solution in the presence of hydrogen donating anti-oxidant due to the formation of non-radical from DPPH-H by the reaction DPPH+AH \longrightarrow DPPH-H+A. The remaining DPPH is measured after certain time, corresponds inversely to radical scavenging activity of anti-oxidants. The ethanolic extract of *Cissus quadrangularis Linn* exhibits maximum inhibition of 47.52% at 250 $\mu\text{g}/\text{ml}$ when compared to standard Quercetin.

Table.1. *Invitro Anti-Arthritic Activity by Inhibition of Protein Denaturation Method*

Drug	Percentage Inhibition
Diclofenac sodium (250 $\mu\text{g}/\text{ml}$)	94.22 %
Ethanolic Extract Of <i>Cissus quadrangularis Linn</i> (250 $\mu\text{g}/\text{ml}$)	98.44%

Table.2. *Invitro anti-inflammatory activity by HRBC membrane stabilization method*

Concentration ($\mu\text{g}/\text{ml}$)	31.25	62.5	125	250	500	1000	2000
Diclofenac Sodium	-	-	-	82.74 %	88.39 %	90.19 %	99.94 %
<i>Cissus Quadrangularis Linn</i>	98.87%	98.68%	94.60%	97.04%	94.81%	84.90%	70.24%

Table.3.*Invitro antioxidant activity by DPPH method*

Concentration (μg/ml)	31.25	62.5	125	250	500	1000	2000
Quercetin	81.85%	87.23%	87.04%	87.76%	88.28%	88.56%	85.63%
<i>Cissus Quadrangularis Linn</i>	34.49%	45.09%	45.08%	47.52%	63.19%	74.89%	65.42%



Figure.1.*Cissus quadrangularis*

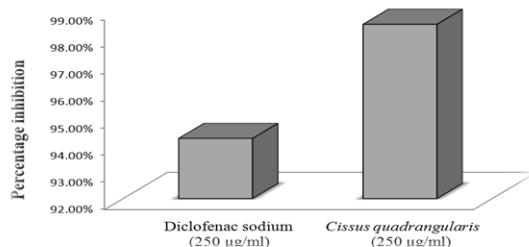


Figure.2.*Invitro Anti-Arthritic Activity by Inhibition of Protein Denaturation Method*

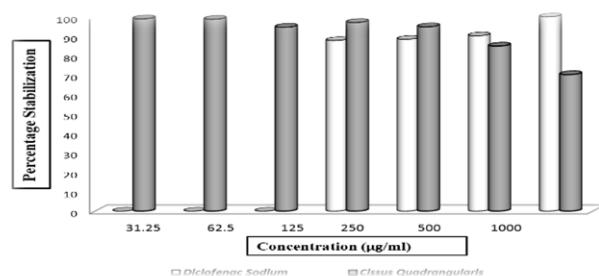


Figure.3.*Invitro anti-inflammatory activity by HRBC membrane stabilization method*

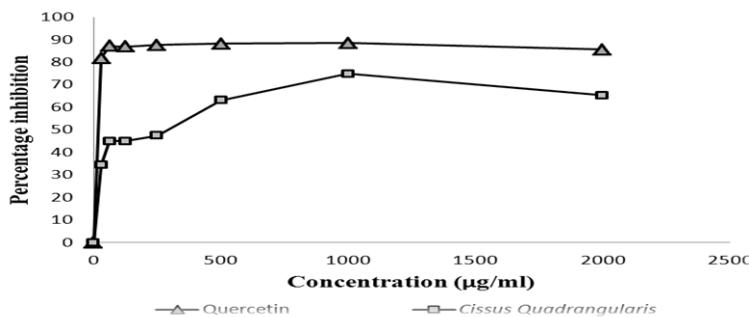


Figure.4.*Invitro antioxidant activity by DPPH method*

CONCLUSION

The *invitro* studies which was carried out by above mentioned methods bring out the fact that the ethanolic extracts possesses the anti-oxidant, anti-

inflammatory and anti-arthritis activity as similar to that of standard. *Cissus quadrangularis Linn* has significant activity may be due to presence of chemical profile such as flavonoid (leuteotin) and β -sitosterol. Further studies are necessary, to identify the active constituent(s)

responsible for the above activities (Gopinathan N, 2009).

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