Nat. Volatiles & Essent. Oils, 2021; 8(5): 6410 - 6420

Assessment Of In Vitro Antioxidant And Xanthine Oxidase Inhibitory Potentials Of Ethanolic Stem Extract Of Cissus Quadrangularis

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ABSTRACT:

Aim: The present study was aimed to evaluate the in vitro antioxidant and xanthine oxidase inhibitory potentials of ethanolic stem extract of *Cissus quadrangularis.L.*

Background: *Cissus quadrangularis* is the commonest species, belonging to the Vitaceae, commonly referred to as "Hadjod'', a perennial plant. The stem of the *Cissus quadrangularis* was used to treat many diseases like piles, pain in joints, swelling out, scurry, asthma, disease of ear and nose bleeding. Xanthine oxidase is an enzyme catalysing the conversion of hypoxanthine to xanthine and xanthine to uric acid which get excreted by kidneys. Excessive production or inadequate excretion of uric acid results in hyperuricemia and gout.

Materials and methods: Ethanolic extract of *Cissus quadrangularis* were tested for its phytochemical constituents, *in vitro antioxidant*, xanthine oxidase inhibitory potential using standard protocols.Data were analysed by ANOVA and DUNCAN'S multiple range test to check the statically significance among the groups. The results with the $p<0.05$ level were considered to be statistically significant.

Results: The results indicate the rich existence of phytochemicals in the ethanolic stem extract of Cissus quadrangularis. The extract also showed potent antioxidant and xanthine oxidase inhibitory potential in a concentration dependent manner.

Conclusion: The study showed the potent in vitro antioxidant and antigout activity of *Cissus quadrangularis.* The presence of phytochemicals might have contributed to the beneficial properties of the extract.

Keywords: *Cissus quadrangularis,* phytochemical analysis, antioxidant activity, xanthine oxidase inhibitory potential, gout, Innovative technology, novel method.

INTRODUCTION:

The medicinal value of plants are attributed to the biological and pharmacological properties like anti bacterial, anti inflammatory, anti cytotoxicity, anti ulcer activity, analgesic, proteolytic, anti osteo porotic, mutagenic and genotoxic activity which makes the natural source of chemical compounds as drugs to cure diseases and thus making it as a great commercial value of plant (1). Primary metabolites in plants like proteins, lipids and starch are required for the growth. Secondary metabolites do not have any role in maintaining the life process of the plants. Apart from this they are essential for interaction with its surrounding environment for adaptation and defence (2). Free radicals are produced by oxidation, leading to chain reaction, and may damage cells and biological molecules. Antioxidants are the compounds richly found in vegetables, fruits, etc. which can prevent the oxidative damage caused by the free radicals. They can significantly delay or inhibit oxidation of an oxidizable substrate when present at low concentration as compared with those of the substrate (3).

Cissus quadrangularis is the commonest species, belonging to the *Vitaceae*, commonly referred to as "Hadjod'', perennial plant. The stem of the *Cissus quadrangularis* was used to treat piles, pain in joints, swelling out, scurry, asthma, disease of ear and nose bleeding. It is an ancient medicinal plant native to the warmer parts of India and Ceylon (4). It is also found in some parts of Srilanka, Malaya, Java and West Africa. Extract of *Cissus quadrangularis* have the properties such as the antibacterial activity antioxidant activity, antiulcer activity, analgesic and anti-inflammatory antiosteoporotic, proteolytic,

mutagenic and genotoxic activity. The juice of the stem is useful in scurvy and in irregular menstruation whereas the stem paste boiled in lime water is given in asthma. It is also used as a powerful stomachic(5).

Gout, a chronic rheumatologic illness characterized by hyperuricemia, arthritis, tophaceous deposits, and renal calculi, is associated with increased rates of cardiovascular and chronic kidney disease (6). Xanthine oxidase is an enzyme associated with the gout and hyperuricemia and is involved in the terminal steps of purine degradation. The enzyme catalyses the formation of xanthine and hypoxanthine to uric acid. Xanthine oxidase inhibitors play a major role in reducing serum urate levels in gout patients . Thus xanthine oxidase inhibitors play an important (7) role in the treatment of gout. (8),(9),(10),(11),(12),(13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24),(25),(26),(27).

MATERIALS AND METHODS:

1. Phytochemical Screening test

Test for phlobatannin

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

Test for Carbohydrates

Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

Test for Flavonoids

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

Test for Alkaloids

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

Test for Terpenoids

2 ml of sample along with 2ml of chloroform and 3ml of con. H_2SO_4 was added. Red color ppt obtained indicates the presence of terpenoids.

Test for proteins

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

Detection of saponins

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

Test for steroids

One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

2. DPPH free radical scavenging activity of *Cissus quadrangularis*

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of Hatano et al, (1989). DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0. 5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

DPPH radical scavenging (%) = Control OF-Sample OD X100

Control OD

3. In Vitro Xanthine Oxidase Inhibitory Activity of *Cissus quadrangularis*

In vitro Xanthine oxidase inhibitory of the extract was assessed as per the method of (Nguyen et al, 2004; Umamaheswari et al., 2007). Briefly, the assay mixture consisted of 1 ml of the fraction (0.1 to 0.5g/ml), 2.9 ml of phosphate buffer (pH 7.5) and 0.1 ml of xanthine oxidase enzyme solution (0.1 units/ml in phosphate buffer, pH 7.5), which was prepared immediately before use. After preincubation at 25◦C for 15 min, the reaction was initiated by the addition of 2 ml of the substrate solution (150 M xanthine in the same buffer). The assay mixture was incubated at 25◦C for 30 min. The reaction was then stopped by the addition of 1 ml of 1N hydrochloric acid and the absorbance was measured at 290 nm using a UV spectrophotometer. Allopurinol (0.1 to 0.5mg/ml), a known inhibitor of XO, was used as the positive control. One unit of XO is defined as the amount of enzyme required to produce 1 mmol of uric acid/min at 25◦C. XOI activity was expressed as the percentage inhibition of XO in the above assay system calculated as percentage of inhibition as follows.

Inhibitory activity (%) = (1 - As/Ac) x100 Where,
As – absorbance in presence of test substance, Ac – absorbance of control

4. Statistical analysis

The data were subjected to statistical analysis using one – way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the groups. In Duncan's test, significance was considered at the level of $p<0.05$.

RESULTS

Table. Qualitative phytochemical screening of ethanoic stem extract of *Cissus quadrangularis*

Phytochemical analysis of stem extract of *S.quadrangularis* showed the presence of protein, flavonoids,alkaloids, terpenoids and saponins whereas amino acids, steroids were found to be absent in the stem of *S.quadrangularis* (Table 1).

Phytochemical	***Cissus quadrangularis***
Protein	**+**
Amino Acids	**-**
Flavonoids	**+**
Alkaloids	**+**
Terpenoids	**+**
Steroids	**-**
Saponins	**+**

DPPH radicals scavenging potential of C.quadrangularis stem extract

Fig.1 represents the antioxidant potential of *Cissus quadrangularis* vs standard drug vitamin C.

Results of the present study showed that S.quadrangularis significantly increased the DPPH radical formation in a dose-dependent manner whose effect was interestingly near to that of the levels of standard vitamin C ($p<0.05$). This study clearly shows that *C.quadrangularis* has potential antioxidant properties by DPPH radical formation..

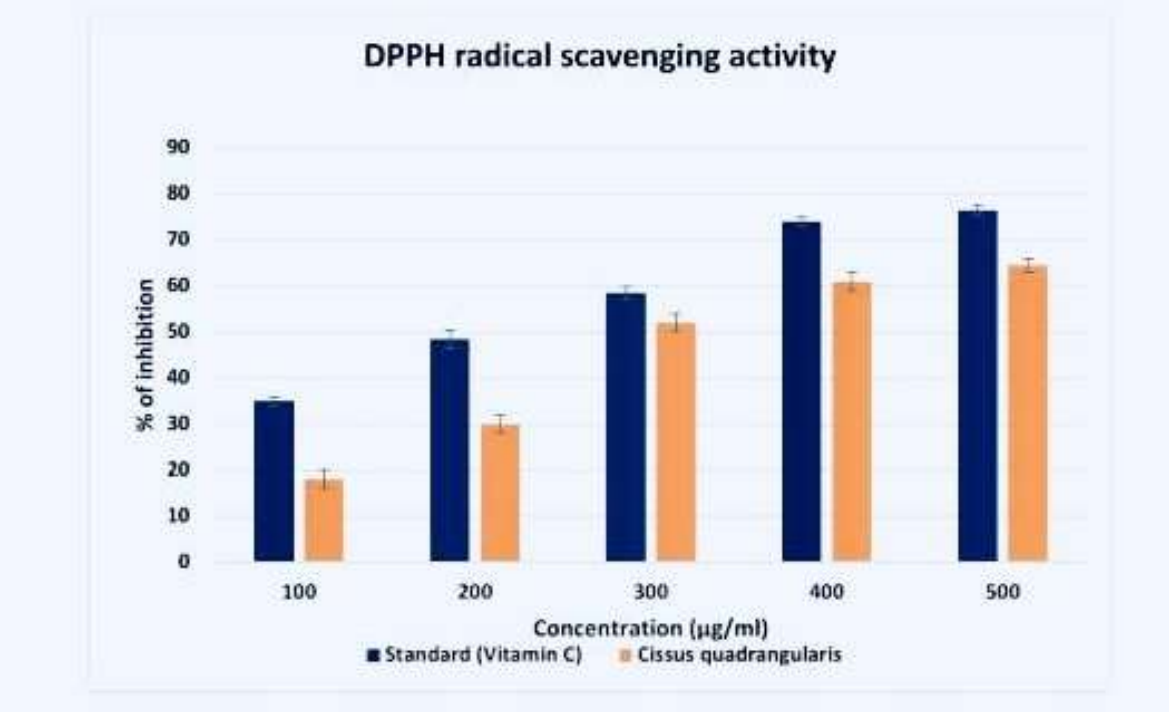

Figure1: DPPH radical scavenging activity of *Cissus quadrangularis* vs standard Vitamin C. X-axis represents increase in the concentration of C.quadrangularis stem extract while Y-axis represents % of inhibition. Each line Represents Mean ± SEM of 3 independent observations. Significance at $p < 0.05$.

Xanthine oxidase inhibitory potential of *C.quadrangularis* stem extract in comparison with allopurinol

Results of the present study showed that *S.quadrangularis* significantly increased the xanthine oxidase inhibitory activity in a dose-dependent manner (Fig.2) whose effect was interestingly near to that of the levels of standard vitamin C ($p<0.05$).

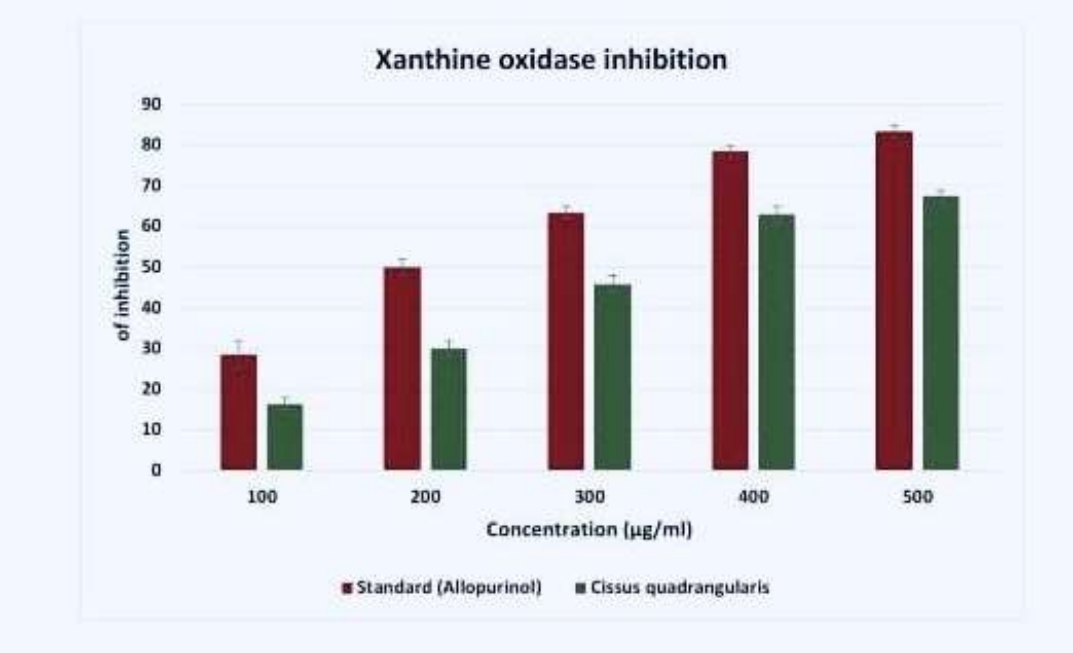

Figure2: Xanthine oxidase inhibitory activity of *Cissus quadrangularis* vs standard Allopurinol.

X-axis represents increase in the concentration of *C.quadrangularis* stem extract while Y-axis represents % of inhibition. Each line Represents Mean ± SEM of 3 independent observations. Significance at $p < 0.05$.

DISCUSSION

Phytochemical screening results revealed that the ethanolic stem extract of *Cissus quadrangularis* showed the presence of flavonoids, terpenoids, saponins and alkaloids. Previous studies on *Cissus quadrangularis* showed the presence of various constituents like vitamin c, flavonoids, triterpenoids, piceatannol, resveratrol, pallidol, perthenocissin and phytosterols. Out of this ascorbic acid, beta - sitosterol and ketosteroid were identified as major constituents of this plant (28). Several phytochemicals particularly polyphenols like phenolic acids, flavonoids, tannins, anthocyanins, well known for their the free radical scavenging and antioxidant activities (29). The analysis and characterisation of bio active compounds from plants is important to ascertain their medicinal value (30). Hence the result indicates that the presence of these phytochemicals in the extract might have contributed to its beneficial activities.

The ethanolic extract of *Cissus quadrangularis,* showed in vitro antioxidant activity in a concentration dependent manner as evident from the DPPH radical scavenging activity. Vitamin c is used as the standard for checking antioxidant activity. Free radicals are highly reactive molecules which are able to cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes, DNA, and proteins. Free radicals can originate endogenously from normal metabolic reactions or exogenously (31). Antioxidants capture the free radicals thereby delaying or preventing the damage to the cells and tissues of the living organisms. Antioxidants are also called reducing agents (32). Free radical scavenging activity of antioxidants is due to its H+ donating property. Hence the present study indicates that *Cissus quadrangularis* can prevent oxidative damages and related disorders due to its antioxidant property.

A dose dependent xanthine oxidase inhibitory activity was observed for the extract and the standard drug allopurinol. Xanthine oxidase serves as an important biological source of oxygen-derived free radicals and contributes to the oxidative damage of biomolecules (33). Increased activity of xanthine oxidase also is involved in the medical condition known as gout and hyperuricemia which leads to uric acid deposition in the joints leading to painful inflammation (7). Allopurinol is the only clinically used inhibitor of xanthine oxidase in the treatment of gout. However, many side effects such as hepatitis, nephropathy, and allergic reactions are associated with the usage of this drug (34). Thus search for a new therapeutic drug with higher activity and fewer side effects is increasing. *Cissus quadrangularis* extract can be evaluated in detail in developing drugs against gout and other disorders associated with xanthine oxidase activity.

CONCLUSION:

From the study it is evident that extracts of *Cissus quadrangularis* possess a significant antioxidant and xanthine oxidase inhibitory potential. Further research has to be done to validate the extract for drug formulation. More research needs to be done on natural herbal extracts to make it into a potential alternative for synthetic drugs which possess a lot of side effects.

ACKNOWLEDGEMENT

The authors would like to thank Saveetha Dental College and Hospitals, Saveetha Institute of medical and technical Sciences,Saveetha University for providing research laboratory facilities to carry out the study.

SOURCE OF FUNDING

The present study was supported by the following agencies.

- Saveetha Institute of Medical and Technical Sciences (SIMATS)
- Saveetha Dental College
- Saveetha University
- International association of Lions club, Madurai.

STATEMENT OF CONFLICT OF INTEREST

The author declares that there is no conflict of interest in the present study.

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