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*INVITRO*ANTIOXIDANT ACTIVITY OF *CISSUSQUADRANGULARIS* STEM EXTRACT

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ABSTRACT

Objectives: Free radicals are the important role in causing various kinds of diseases. Free radicals neutralized with the help of antioxidants, so the aim of this study is to find out the antioxidant potential of methanol extract of *Cissusquadrangularis*Linn stem (MECQ) and water extract of *Cissusquadrangularis*Linn (AECQ) by *in vitro* models. **Methods:** The plant material is collected, authenticated, dried, and grind into powder. The powdered plant material is subjected to successively Soxhlet extraction with a solvent of methanol and water. Both extracts of stems were taken and the extract is subjected to H_2O_2 assay and Nitric oxide assay by using standardized methods. **Results:** The results of all the assays proved that the methanol extract of *Cissusquadrangularis*Linn has an antioxidant potential on a dose-dependent manner. The highest percentage of inhibition (86.73%) is recorded at 100 µg/ml of methanol extract by H_2O_2 method and (91.66%) is recorded in nitrous oxide method, in water extract 100 µg/ml have a (81.26%) of scavenging activity in H202 method (61.26%) activity of nitrous oxide production. The results were compared with the antioxidant activity of standard ascorbic acid. **Conclusion:** The study proves that the methanol extract of *Cissus quadrangularis*Linn is a good source of antioxidants, and future medicated world choose this phytochemical constituent rich plant for their drug preparation after the deepness of research. In all the methods the plant leaves has been found to possess the antioxidant activity. which may be the responsible for various therapeutic properties. The current study showing that *Cissusquadrangularis*Linnis having high quantity of phytochemicals and a worthy source of

natural antioxidants. Using this kind of herbal medicine we can lead the life with harmless drug for harmful illness.

KEYWORDS: *Cissus quadrangularis* Linn, Hydrogen peroxide assay and Nitric oxide assay.

INTRODUCTION

The various living systems bear a rich biodiversity in nature. Since the ancient era before scientific knowledge would change plants performed myriad functions of the biosphere.Among which utilize of plants in curing illness is well documented. But after the advance technology and improved scientific knowledge changed plants as a source of therapeutic agents as they are able to offer, the purpose with lesser side effects that are frequently associated with synthetic antimicrobials.[1] It was estimated that the current global market for plant-derived drugs is worth more than 20 billion and the market keep on growing.[2]

The diversity of pathogenic bacteria is general and so is the variety of diseases caused by them. Despite the survival of many potent antimicrobial agents[3], multi-resistant pathogenic strains are continuously emerging, magnificent the need for a continuous search and development of new drugs.[4,5]

Most drugs hold many severe side effects. Many medicines of natural foundation had been used since long time without any severe adverse effects, however, a number of synthetic antioxidants and drugs are commercially available, natural products still substitute most of the chemical agents.[6] In the present study, solvent extracts like MECQ and AECQ. Were subjected for *in vitro* and antioxidant activity which may lead to the finding of most effective agent for the management of diseases and effective potential source of natural antioxidant that may help in preventing various oxidative stresses.

Cissus quadrangularis L. Is a succulent plant of the family *Vitaceae* commonly known as Asthisamhari found in tropical and subtropical xeric wood. It can be found throughout the hotter parts of India alongside hedges, neighboring countries like Pakistan, Bangladesh, Srilanka and Malaysia.[7] It can be cultivated in plains coastal areas, jungles and wastelands up to 500m elevation. The plant is propagated using cuttings. The stem juice of plant is used to treat scurvy, menstrual disorders, otorrhoea and epistaxis. The plant has been documented

in Ayurveda for the treatment of osteoarthritis, rheumatoid arthritis and osteoporosis5, 8. A paste of the stem is given in asthma, burns and wounds, bites of poisonous insects and for saddle sores of horses and camels. *Cissusquadrangularis* has been used by the common man in India for promotion of fracture healing and well known as "Hadjod". It is also known as *Vitisquadrangularis*Wall. It is a common perennial climber, which is distributed throughout India, particularly in tropical regions. The plant is commonly known as Vajravalli in Sanskrit, Hadjod in Hindi, Kandvelin Marathi, Haddjor in Punjabi, Hadbhanga in Oria, Vedhari in Gujrati, Perandi in Tamil, Nalleru in Telugu and Veldgrap, Edible Stemmed Vine in English.[8]

MATERIAL AND METHODS

Drugs and Chemicals

All reagents procured were analytical grade.

Plant collection

Fresh stem of *Cissus quadrangularis* Linn was collected from field of Thalavadi near erode and authenticated by Dr. M .PALANISAMY, Scientist D & Head office in charge, Southern Regional Centre, TNAU campus, Coimbatore. (BSI/SRC/5/23/2017/Tech–2844) .Voucher specimen (No: SSMCOP/106/25) has been deposited in the Department of Pharmacognosy, SSM College of Pharmacy, Jambai Tamilnadu, India.

The stem of *Cissus quadrangularis* Linn was dried and then crushed into fine powder by using laboratory Homogenizer then stored for further use.

Preparation of Plant Extracts

The crude drugs were extracted with methanol (MECQ) and water (AECQ) as a solvent by using the soxhlet apparatus for continuous hot extraction. The extracts was filtered and evaporated to separate solvent and residue. The semisolid residues thus obtained were stored in desiccator until further use.

TESTING FOR ANTI OXIDANT ACTIVITY

A. Nitrous oxide method

Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction (Green LC). Sodium nitroprusside (5 mM) in standard phosphate buffer saline solution (0.025 M, pH 7.4) was incubated with different concentrations (20-100 µg/mL) of the test extract

dissolved in phosphate buffer saline (0.025 M, pH 7.4) and the tubes were incubated at 25°C for 5 h. Control experiments were conducted in the identical manner using the equivalent amounts of buffer. After 5 h, 0.5 mL of the sample was diluted with 0.5 mL of Griess' reagent (1% sulphanilamide, 2 % O-phosphoric acid and 0.1 % napthylethylenediaminedihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with suphanilamide and its subsequent coupling with napthyl ethylene diamine was read at 546 nm. The experiments were repeated in triplicate. The percentage of sodium nitro prusside scavenging is calculated as follows:

% Scavenged = (A0 – A1 / A0) X 100

Where; A0 is the absorbance of control and A1 is the absorbance of test. Ascorbic acid, was used as a positive control.

B. Hydrogen peroxide method

Hydrogen peroxide radical scavenging (H_2O_2) assay

Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, food and beverages. It is widely used as a bleaching agent in the textile, paper and pulp industries. Human beings exposed to H_2O_2 indirectly via the environment are estimated as 0.28 mg/kg/day within take from leaf crops contributing most to this exposure. Hydrogen peroxide enters the human body through inhalation of vapor or mist and through eye or skin contact. In the body, H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\cdot}$) that can initiate lipid peroxidation and cause DNA damage. The ability of plant extracts to scavenge hydrogen peroxide is determined according to the following method.

H2O2 scavenging ability of extracts of *Cissus quadrangularis* is determined according to the method (20,40, 60,80, 100 µg/ml) is taken in different test tubes to which 1 ml of H2O2 is added. The tubes are incubated for 5 minutes at room temperature. After 5 minutes, 2 ml of potassium dichromate: Acetic acid reagent is added and the tubes are incubating for 10 minutes at room temperature. The absorbance value of the reaction mixture is recorded at 700 nm. Blank containing the phosphate buffer without the plant extract and a standard is also calculated as, follows:

% Scavenged (H2O2) = (A0 – A1 / A0) X 100

Where; A0 is the absorbance of control and A1 is the absorbance of test. Ascorbic acid, was used as a positive control.

RESULTS

In vitro antioxidant activity of methanol and aqueous extracts of *Cissusquadrangularis*

Table 1 shows the inhibition properties of the plant extract. The highest percentage of inhibition (86.73%) is recorded at 100 µg/ml of methanol extract by H2O2 method and (91.66%)is recorded in nitrous oxide method, in water extract 100 µg/ml have a (81.26%) of scavenging activity in H202 method (61.26%) activity of nitrous oxide production. The results were compared with the antioxidant activity of standard ascorbic acid.The results clearly show the increasing concentration of the methanol extract has a higher percentage of inhibition at the concentration of 100 µg/ml and the lower inhibition is noted at the concentration of 20µg/ml than compared to aqueous extract.

DISCUSSION

In this study, the methanol extract of *Cissus quadrangularis* has been evaluated related to antioxidant potentials. Various secondary metabolites are responsible for their therapeutic values which include phenol, flavonoids, sterols, alkaloids, and tannins. Based on this, the reducing power of the extracts determined by the electron transferring ability. The activity is depending on the transfer of Fe3+ ion into Fe2+ ion. The result obtained showed that the extract possessed antioxidant activity in a concentration dependent manner. The results clearly show the methanol extract of *Cissus quadrangularis*have the ability of transferring the Fe3+ into Fe2+, and it diminishes the oxidative hurt in the tissues than compared to aqueous extract.

Hydrogen peroxide has a capability to penetrate membranes present in the cells. If the hydrogen peroxide molecule is converted to hydroxyl radicals, it may damage the cells.[8] The phenolic compounds give the electrons and thus convert the hydroxyl ions into water.[10] The presence of the phenolic compounds of the methanol extract of *Cissus quadrangularis*may be the reason for its scavenging activity.

Nitric oxide is a free radical they have the potential to change the structural and functional activity of the many cellular membranes.[11] Flavonoid is a compound which has the scavenging activity to the oxygen derived free radicals.[12] The results prove that the extracthave inhibition activity of nitric oxide production. The inhibitory effect of the methanol extract*Cissusquadrangularis* of is may be due to the presence of flavonoids.

CONCLUSION

In the present study, we evaluated the *in vitro* antioxidant activity of crude methanol and water extracts of *Cissusquadrangularis*. The percentage of inhibition values clearly suggested that the therapeutic antioxidant potentials and also having significant scavenging and reducing power activities. So, further the studies are needed to the compound isolation, and to find that which compound is responsible for its scavenging activity. For this depth of research is needed and it will help to find new therapeutic drug related to oxidative hurt.

Effect of herbal extracts in different concentration		% Inhibition by Nitrous oxide method	% Inhibition by H_2O_2 method
Control			----------
Vit. C (20µg/ml)		96.87%	90.81%
AECQ	20 µg /ml	32.29%	27.55%
	40 µg /ml	39.58%	37.75%
	60 µg /ml	42.70%	51.68%
	80 µg /ml	65.62%	52.04%
	100 µg /ml	84.37%	61.26%
MECQ	20 µg /ml	43.75%	39.79%
	40 µg /ml	50.00 %	52.68%
	60 µg /ml	57.45%	61.26%
	80 µg /ml	87.50%	76.53%
	100 µg /ml	91.66%	86.73%

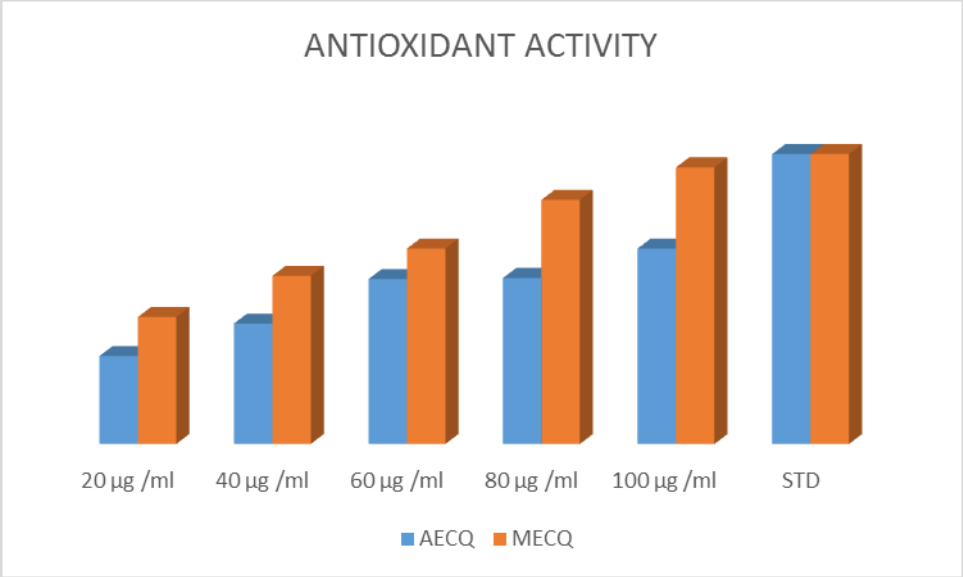

Figure 1: Anti oxidant activity of *Cissusquadrangularis*by nitrous oxide method.

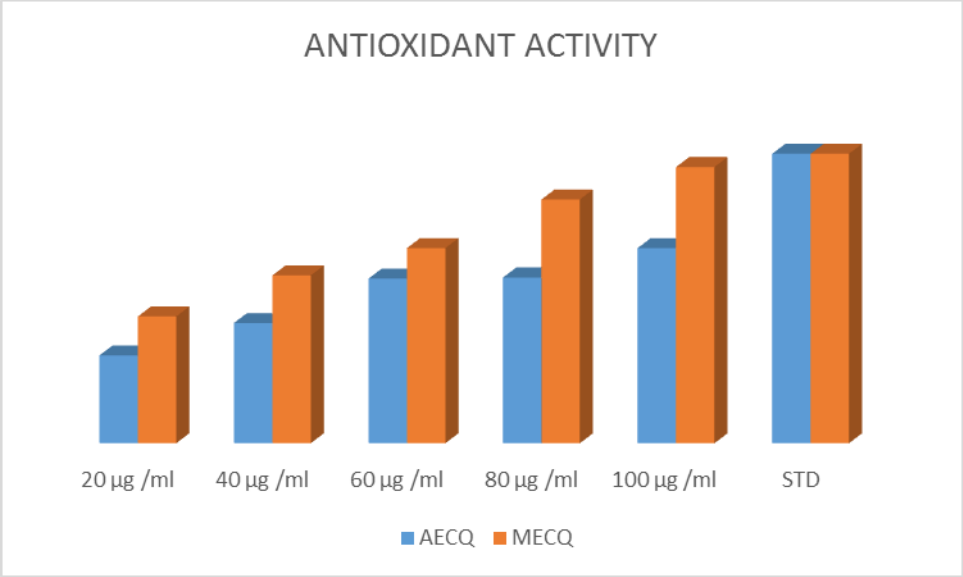

Figure 1: Anti oxidant activity of *Cissusquadrangularis*by Hydrogen peroxide method.

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