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EVALUATION OF *IN- VITRO* ANTIOXIDANT ACTIVITY OF ALCOHOLIC EXTRACT *OF CARDIOSPERMUM HALICACABUM* AERIAL PART.

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Abstract:

Many diseases are produced due to accumulation of free radicals in the body. The aim of the present study were to evaluate in vitro antioxidant activities. The extract was prepared by using pure ethanol solvent for Cardiospermum halicacabum aerial part. The in vitro antioxidant activity of Cardiospermum halicacabum aerial part extract was evaluated by using different methods like nitric oxide scavenging activity, free radical scavenging by DPPH method and scavenging of hydroxyl radical (Deoxyribose Method). Vitamin-E was used as standard. The tested plant extract in different concentration showed promising antioxidant and free radical scavenging activity, Concentration 200 µg showed best antioxidant activity compared to other concentration in nitric oxide activity. In DPPH method Concentration 400µg showed maximum antioxidant activity compared to other concentration. From the obtained results we could suggest that this plant extract can be used for antioxidant activity and suggest to go for In-vivo studies.

Key words: *Ethanolic plant extract, In vitro antioxidant activity, Nitric oxide, DPPH, Deoxyribose method.*

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INTRODUCTION:

Free radicals have been implicated in the causation of several problems like atherosclerosis, urolithiasis, ulcers, asthma, cancer, cardiovascular disease, cataract, diabetes, gastrointestinal inflammatory disease, liver disease, muscular degeneration, and other inflammatory process. Reactive oxygen species (ROS) are continuously produced during cell metabolism and under normal conditions; they are scavenged and converted to (Sen et al., 2010)[1] nonreactive species by different intra cellular enzymatic and non-enzymatic antioxidant system (Shao et al., 2008)[2]. Over production or an ineffective elimination of ROS may induce oxidative stress and cause damage to all types of bio molecules such as proteins, lipids and nucleic acids (Droge et al., 2011)[3] Antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and or activators of antioxidative defense enzyme system to suppress the radical damages in biological systems (Mur et al., 2011; Venkatesh et al., 2009)[4,5]. Antioxidants thus play an important role in the protection of human body against damage by reactive oxygen species (Peng et al., 2011; Ling et al., 2011)[6,7]. Therefore, inhibition of free radical-induced oxidative damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of these diseases. In recent years, it has been investigated that many plant species are serving as source of antioxidants and received therapeutic significance. Several anti-oxidants of plant origin are experimentally proved and used as effective protective agents against oxidative stress. They play an important role in major health problems such as cancer, cardio vascular diseases, rheumatoid arthritis, cataracts, Parkinson's disease, Alzheimer's disease and degenerative diseases associated with aging (Parietal., 2011; Battuetal., 2011)[8,9]. The herb *Cardiospermum halicacabum* aerial part contains alcohols, phenols, alkynes, flavonoids, alkanes and aliphatic esters are the major phytochemical components present in this plant. The herb also contains terpenoids, flavonoids, tannins, proteins, saponin, glycosides, carbohydrates, volatile esters, and fatty acids.

Due to presence of various active constituents in this plant an attempt has been made to evaluate the antioxidant property in various concentration of the plant extract, by *in vitro* antioxidant methods like Nitric oxide, DPPH, Deoxyribose method[10].

MATERIALS AND METHODS:

Collection of plant material:

The crude drug *Cardiospermum halicacabum was* collected from herbal garden (Moolika Vanam) aziz nagar, Hyderabad, Telangana, India. The plant was authenticated by Dr. Rafique, incharge of Moolika Vanam garden.

Preparation of extract:

The collected plant material *Cardiospermum halicacabum* aerial parts were washed twice with tap water and one time with distilled water finally allowed to dry under shade, and then coarsely powdered in a blender. The coarse powder (500 gm) was subjected to maceration for 72 hours, followed by exhaustive maceration for 48 hours by using 99.9% ethanol for *Cardiospermum halicacabum* aerial part. The solvents were decanted and filtered with filter paper and recovered by distillation at 60° C to 70° C. The extract was dried under desiccators[11].

Methodology:

The antioxidant activity of plant extract was proved by different In vitro methods which are given as follows -

a) Nitric oxide scavenging activity.
b) Free radical scavenging by DPPH method.
c) Scavenging of hydroxyl radical (deoxyribose method).

a) Nitric oxide scavenging activity procedure:

The nitric oxide scavenging activity of plant extract was determined according to the method (Greenet al.,1982)[12]. Aqueous solution of sodium nitro prusside spontaneously generates nitric oxide (NO) at physiological pH, which interacts with oxygen to produce nitrate ions and which was measured calorimetrically. 3 ml of reaction mixture containing 2 ml of sodium nitro preside (10 mM) in phosphate buffer saline (PBS) and1ml of various concentrations of the extracts were incubated at 37°C for 4 hours. Control without test compound was kept in an identical manner. After incubation 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and those of test compounds. Vitamin-E (10, 50, 100, 200, 400, 800, 1000 µg/ml) was used as standard. The percentage nitric oxide inhibition was calculated from the following formula. The results were tabulated in table 1and represented in graph no.1.

$$\% \text{ Nitric Oxide Inhibition} = \frac{\text{OD of control-OD of test}}{\text{OD of control}} \times 100$$

b) Free radical scavenging by DPPH method procedure[13]:

DPPH scavenging activity was measured by the spectrophotometric method. A stock solution of 25 mg of DPPH (150 µM) was prepared in100 mL of ethanol. To the 0.2 ml of extract of different concentrations, 3.8 ml of DPPH was added. Control without test compound was prepared in an identical manner. In case of blank, DPPH was replaced by ethanol. The reaction was allowed to be completed in the dark for about 20 minutes. Then the absorbance of test mixtures was read at 517 nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical. Vitamin-E (10, 50, 100, 200, 400, 800, 1000 µg/ml) was used as standard. The percentage DPPH inhibition was calculated from the following formula. The results are tabulated in table 2 and represented in graph no. 2.

$$\% \text{ DPPH Inhibition} = \frac{\text{OD of control-OD of test}}{\text{D of control}} \times 100$$

c) Scavenging of hydroxyl radical (deoxyribose method) procedure[14]:

To the reaction mixture containing deoxy ribose (3mM, 0.2ml), ferric chloride (0.1mM,0.2ml), Ethylene diamine tetra acetic acid sodium salt (EDTA) (0.1 mM, 0.2 ml), ascorbic acid (0.1 mM, 0.2 ml), and hydrogen peroxide (2 mM, 0.2 mM) in phosphate buffer (pH 7.4,20 mM), was added to 0.2 ml various concentrations of extracts or standard in DMSO to give a total volume 1.2 ml. the solutions were then incubated for 30 min at 37°C. After incubation, trichloro acetic acid (0.2ml,15%), and thio barbituric acid (0.2 ml, 1% w/v) in 0.25 N HCl were added. The reaction mixture was kept in a boiling water bath for 30min, cooled, and the absorbance was measured at 532 nm. The results are tabulated in Table no. 3 and represented in Graph no. 3.

RESULTS AND DISCUSSIONS:

a) Percentage inhibition by nitric oxide method:

Sample No.	Concentration(µg/ml)	% inhibition
plant ext.		
1.	10µg	47.47
2.	50µg	49.17
3.	100µg	55.51
4.	200µg	57.12
5.	400µg	55.85
6.	800µg	53.69
7.	1000µg	53.48
Standard Vit.E		
8.	10µg	44.0
9.	50µg	52.8
10.	100µg	56.9
11.	200µg	67.0
12.	400µg	74.0
13.	800µg	77.4
14.	1000µg	92.2

Table No. 1: Percentage inhibition by nitric oxide method.

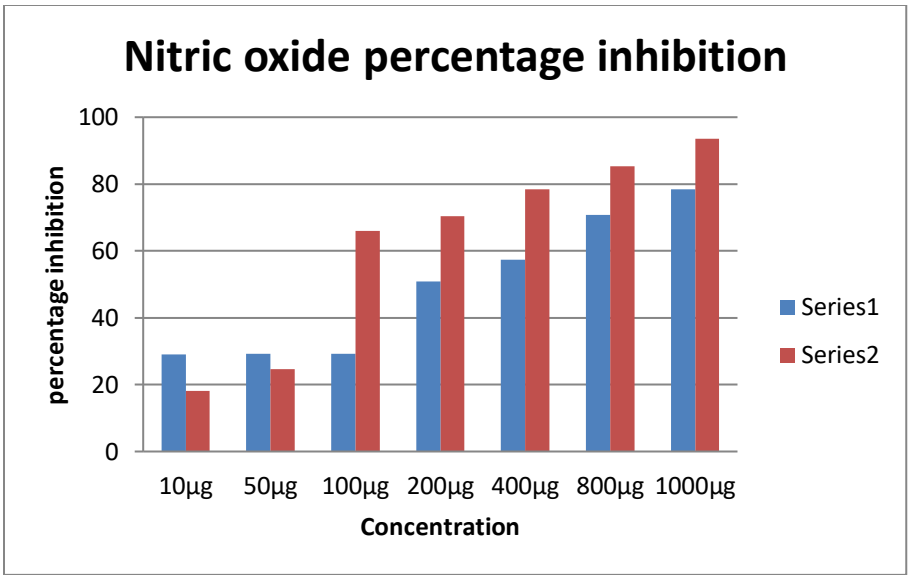

Figure 1: Nitric oxide percentage inhibition graph.

Discussion:

In the present study plant extract at different concentration were assessed for their free radical scavenging activity in an in vitro nitric oxide model. It was observed that % inhibition of plant extract scavenged free radical in a dose dependent manner. All the concentration showed significant increased or decreased in the nitric oxide percent inhibition. So the plant extract under investigation are potent inhibitor of nitric oxide.

b) Effect of different concentration of extract on DPPH percentage inhibition:

Sample No.	Concentration(µg/ml)	% inhibition
plant ext.		
1.	10µg	32.62
2.	50µg	50.41
3.	100µg	62.68
4.	200µg	78.57
5.	400µg	92.56
6.	800µg	90.39
7.	1000µg	87.97
Standard Vit.E		
8.	10µg	55.77
9.	50µg	65.46
10.	100µg	75.97
11.	200µg	81.91
12.	400µg	87.35
13.	800µg	95.75
14.	1000µg	98.96

Table No. 2: Percentage inhibition by DPPH method.

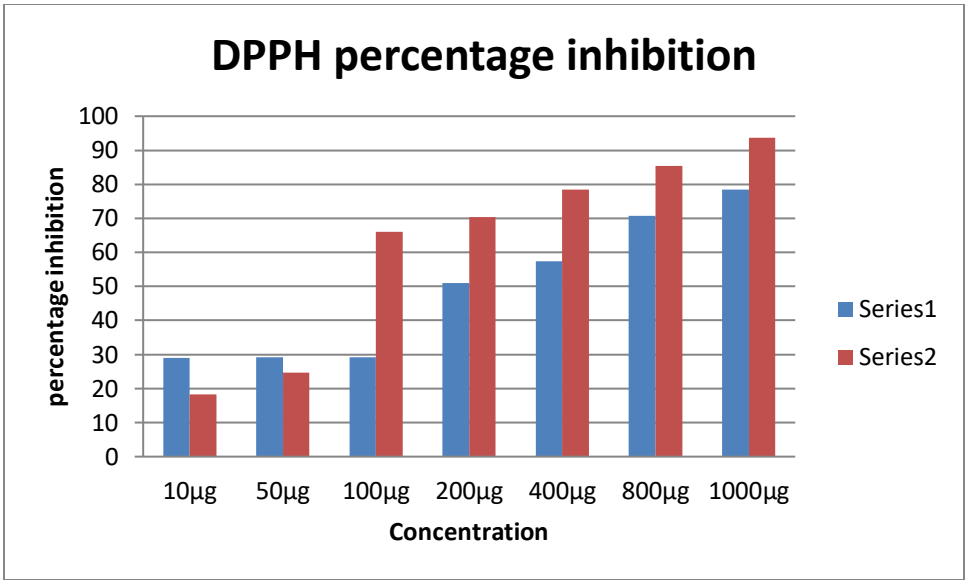

Figure 2: DPPH percentage inhibition graph

Discussion:

In the present study plant extract at different concentration were assessed for their free radical scavenging activity in an in vitro DPPH model. All the concentration showed change in the DPPH percent inhibition. It was observed that % inhibition of plant extract scavenged free radical in a dose dependent manner. All the concentration showed significant increased in the DPPH percent inhibition. So the plant extract under investigation are potent inhibitor of free radical.

c) Effect of different ratios of extracts on Hydroxyl radical percentage inhibition:

Sample No.	Concentration(µg/ml)	% inhibition
plant ext.		
1.	10µg	28.95
2.	50µg	29.21
3.	100µg	29.25
4.	200µg	50.95
5.	400µg	57.39
6.	800µg	70.72
7.	1000µg	78.38
Standard Vit.E		
8.	10µg	18.21
9.	50µg	24.60
10.	100µg	66.02
11.	200µg	70.30
12.	400µg	78.51
13.	800µg	85.40
14.	1000µg	93.62

Table No. 3: Percentage inhibition by hydroxyl radical method.

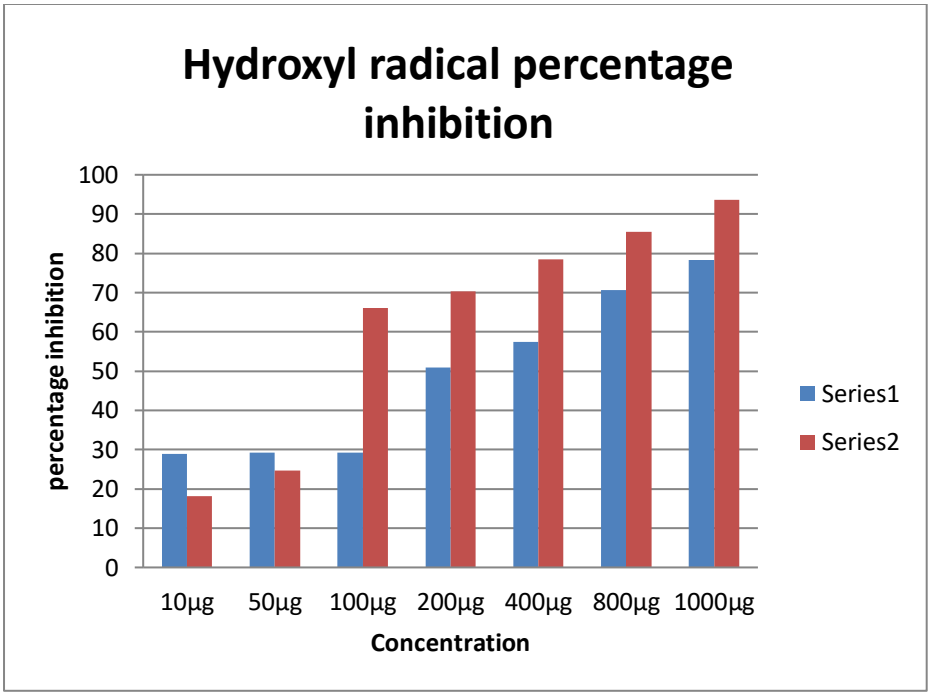

Figure 3: Hydroxyl radical percentage inhibition graph

Discussion:

In the present study plant extract at different concentration were assessed for their free radical scavenging activity in an in vitro hydroxyl radical method. All the concentration showed change in the percent inhibition. It was observed that % inhibition of plant extract scavenged free radical in a dose dependent manner. All the concentration showed significant increased in the Hydroxyl radical percentage inhibition in graph according to concentration. So the plant extract under investigation are potent inhibitor of free radical.

CONCLUSION:

The in-vitro antioxidant activity of plant extract results were clearly indicated in tabular column. That all the concentration of extract in different models was effective in scavenging free radicals and has the potential to be a powerful antioxidant activity.

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