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Antioxidant Status of Cardiospermum Halicacabum by DPPH and FRAP Method

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Abstract*: *Antioxidants are particles which can interrelate with free radicals safely and conclude the chain reaction and protect the major important macromolecules in our body. The free radicals are produced as a byproduct of metabolism which is exposed to the radiation and other pollution .The antioxidants fight against the free radical and prevent our body from the damage. The compounds like vitamin C and E,Carotenes, Flavinoids and also the antioxidants enzymes plays a major role in Scavenging of free radicals and protect the body from the Oxidant stress. Many medicinal herbs are having the antioxidant nature .The medicinal plant Cardiospermum halicacabum are taken in this assay which belongs to family Sapindaceae, also called Balloon Vine which can be used in traditional medicine specifically for the treatment of bone disorders like Rheumatoid Arthritis. The analysis of antioxidant assay is done by two methods DPPH and FRAP and the mixtures of the ethanolic and methanolic extracts of the plant were tested for antioxidant activity.

Index Terms*: *Antioxidant, Free radical, Ethanol- methanol Extract.

I. INTRODUCTION

Since ancient times, many societies mainly depend on nature and have resorted the wealth of natural plants and herbs and maintain the better health based on the natural way. Today, a large number of world people, particularly in emerging countries, usages plants products for their main needs of medical aid [1]. Humans have used plants for medical resolves for eras. It has been assessed that uses of medical plants perhaps go back in time to around 300 decades. Traditional procedures of treatment have occurred and quiet occur in several nations. The several alternate medical schemes of India (Siddha, Ayurveda, and Unani,) practices in excess of 8000 plant types [2]. Documents of these old medical schemes is main as an amount of significant current medicines have been gotten from plants utilized by ethnic individuals. Present day medicines like headache medicine, ephedrine, atropine, dioxin, quinine, morphine are models, which were initially found over perceptions of conventional fix strategies for indigenous individuals [3]. The antecedent for about one-half of the prescriptions we use these days are advanced from regular sources. The eventual fate of higher plants as antecedents for restorative operators for use in examination, counteractive action, and treatment of illnesses is additionally extremely encouraging and broadly utilized. In any case, among the evaluated 250,000-400,000 plant species, just 6% have been considered for natural movement, and 15% have been researched phyto-chemically. Hence there lies a need to investigate many more medicinal plants and validate their properties in order to use them as lead compounds in the pharmacological industries. The plant *Cardiospermum halicacabum* Linn belongs to personal Sapindaceae is a climbing plant generally circulated in subtropical and tropical districts. Cardiospermum halicacabum likewise called as Ballon vine , is a yearly or now and again perpetual herb, which has been utilized as emetic, diaphoretic, emmenagogue, diuretic, purgative, stomachic, sudorific and refrigerant [4]. The leaf extricate was utilized to lessen the stoutness and furthermore used to decrease the rheumatic agony and swellings [5]. It is a little sensitive, climber, smooth and the entire plant has been utilized for a few centuries in the action of ailment, firmness of snakebites and appendages. [6]. A decoction of origin is certain for draining heaps. The origins are utilized for anxious illnesses. They have a major role in many ailments.

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II. MATERIALS AND METHODS

The plant *Cardiospermum halicacabum* was collected, dried and pulverized and extracted by Soxhelt apparatus.The methanol and ethanol extracts of the plant samples were used for the assay. The mixture of ethanol extracts of the plant and the mixture of methanol extracts of the plants are prepared and analysed by FRAP Assay and DPPH assay.

A. DPPH ASSAY: (Molyneux, 2004)

α-diphenyl-β-picrylhydrazyl (DPPH) is afree radical searching technique offers the principal approach for assessing the cancer prevention agent capability of an exacerbate, a concentrate or other organic sources. This is the least complex method, 1-diphenyl-2-picrylhydrazyl, 1is described as a steady free fundamental by temperance of the delocalization of the extra electron over the particle as a whole. The delocalization additionally offers ascend to the profound violet shading, portrayed by a retention band in ethanol arrangement focused at around 520 nm. At the point when an answer of DPPH is blended with that of an element that can give a hydrogen iota, at that point this offers ascend to the diminished structure (Blois,1958) with the loss of this violet shading (in spite of the fact that there would be required to be a leftover light yellow shading from the picryl bunch still present).

Speaking to the DPPH radical by Z• and the benefactor atom by AH, the essential response is Z•+AH=ZH+A•
Where ZH is the decreased structure and A• is free radical created in this initial step.
This last extreme will at that point experience further responses which control the general stoi-chiometry, that is, the quantity of particles of DPPH diminished (de-colorised) by one atom of the reductant.

A.1. Chemicals used:

- 1,1 – diphenyl - 2-picrylhydrazyl (DPPH)
- Di-methyl sylph-oxide (DMSO)
- BHT (standard)-1.6mg/ml in methanol
- Trials wanted focus from 1 mg/ml–max of 5mg/ml (in/DMSO)

A.2.Procedure:

3.7ml of supreme methanol was taken in all trial cylinders and 3.8ml of outright methanol was added to clear. 100µl of BHT was added to tube set apart as standard and 100µl of particular examples to every single other cylinder set apart as tests. 200µl of DPPH reagent was included to all the test tubes counting clear. All the test tubes were brooded at room temperature in dim situation for half an hour. The absorbance of all examples was perused at 517nm alongside the clear.

Protocol for DPPH Assay: [7]

S.NO	REAGENTS	BLANK	STANDARD	TEST
1	Methanol	3.8ml	3.7ml	3.7ml
2	BHT	-	100µl	-
3	Sample	-	-	100µl
4	DPPH	200µl	200µl	200µl
Incubation at dark for 30 minutes				
O.D at 517 nm				

B. Ferric Ion Reduction FRAP) Assay Potential:[8]

FRAP examine is a basic and new technique for evaluating "Cell reinforcement control", Ferric to Ferrous particle decrease at low pH bases a shaded complex ferrous-tripyridyltriazine complex . FRAP ideals are acquired by contrasting the absorbance variation at 593 nm test response blends with those covering ferrous particle in Identified focus.

B.1. Preparation of FRAP Reagents

- Reagent An Acetate Buffer (300mM, pH 3.6)
  16 ml of frosty acidic corrosive was included to 3.1g of sodium acetic acid derivation tri-hydrate; the arrangement was then pretended to 1L utilizing refined water. The pH of the arrangement was checked utilizing pH meter.
- Substance B-TPTZ (2,4,6 tri[2 pyri-dyl] s-tri-azine)Solution 0.031g of TPTZ was added to 10ml of 40mM HCl.
- Substance C-Ferric Chloride Solution
- 0.054g of ferric chloride was broken down in 10ml of refined water.
- Substances B and C were crisply arranged every time when the test was achieved.

B.2 Preparation of FRAP Reagent:

About 2.5ml of Substance B and 2.5ml of Substance C were added to 25ml of Substance A to make 30ml of the FRAP substance. This was set in a 37ºC water shower for at least 10 mins.
Standard-Ascorbic corrosive: 1.76 mg of Ascorbic corrosive was disintegrated in 100 ml of refined water.

B.3 FRAP Assay Procedure

About 1ml of refined water and 80µl of test was pipette out into the standard 4ml plastic cuvette. 600µl of brooded FRAP Substance was added to the cuvette, which was quickly rearranged to blend the solutions. The reagent clear was additionally arranged as portrayed above yet 80µl of refined water was included rather than test. Variation in absorbance at 593nm (because of the decrease of the Fe3+ - TPTZ composite at low pH) was documented at precisely at 4 mins utilizing spectrophotometer. Each test weakening was tried in triplicate to enable a mean absorbance to be determined.

III. RESULTS AND DISCUSSIONS

Table.1 The Antioxidant activity using DPPH Assay technique

S.NO	EXAMPLE	Concentration (µg/ml)	O.D	DPPH Action in (%)
1.	*Cardiospermum halicababum* (Methanol extract)	1000	0.294	53.84
2.	*Cardiospermum halicababum* (Ethanol extract)	1000	0.258	59.49

Blank O.D: 0.637

From the table-1, it can be seen that the ethanolic extract of *Cardiospermum halicababum* has 59.49% DPPH activity while its methanolic activity has the lowest of 53.84%. Overall it can be seen that ethanolic extract of Cardiospermum halicacabum has higher DPPH activity than Methonolic extract.

Table.2 Antioxidant activity using FRAP Assay method

S.No	Name of the Sample	FRAP(µM)
1.	*Cardiospermum halicababum* (Methanol extract)	3565
2.	*Cardiospermum halicababum* (Ethanol extract)	3857.5

From the FRAP assay method the Table-2 shows that the Ethanolic excerpt of *Cardiospermum halicababum* exhibited highest FRAP value while that of the methanolic extract of the *Cardiospermum halicacabum* was the lowest.
From the table 1 and 2 it was confirmed that the Ethanol extract of *Cardiospermum halicababum* showed the maximum highest activity than the methonolic extract of both DPPH and FRAP method.

It is found the methanol extract activity of antioxidant status of *Cardiospermum halicababum* to be 57%. It is found that ethanolic extract of *Cardiospermum halicababum* showed significant antioxidant activity. The same has been observed in this study.

IV. CONCLUSION

In a one of its kind attempt, the mixtures of the ethanolic and methanolic extracts of Cardiospermum halicacabum was tested for antioxidant activity. Significant results were obtained from this study. Hence it can be concluded that both the methanol extract and ethanol excerpts of the *Cardiospermum halicababum* presented potential antioxidant action. Through further research and validation, it can be used as a lead compound in the pharmacological industries.

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