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Research Article

COMPARATIVE ANTIOXIDANT ASSAY OF *CISSUS QUDRANGULARIS* & *CARDIOSPERMUM HALICACABUM*

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ABSTRACT

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. 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 two plants *Cissus quadrangularis* and *Cardiospermum halicacabum* are taken in this assay. The analysis of antioxidant assay is done by two methods DPPH and FRAP and the mixtures of the ethanolic and methanolic extracts of both the plants were tested for antioxidant activity.

INTRODUCTION

Since ancient times, several societies have resorted to nature, mainly to plants as medical and health sources. Today, a great percentage of the world population, particularly in developing countries, uses plants for facing primary needs of medical assistance (Tene *et al.,* 2007). Human beings have used plants for medicinal purposes for centuries. It has been estimated that such use of medicinal plants possibly go back in time to around 3000 years. Traditional forms of medicine have existed and still exist in many countries. The various alternative medicinal systems of India (Ayurveda, Unani, and Siddha) uses more than 7500 plant species (Mukherjee *et al.,* 2006).

Documentation of these traditional medicinal systems is important as a number of important modern pharmaceuticals have been derived from plants used by indigenous people. Modern drugs like aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine are examples, which were originally discovered through observations of traditional cure methods of indigenous people (Gilani *et al.,* 2005).

The starting materials for about one-half of the medicines we use today come from natural sources. The future of higher plants as sources of medicinal agents for use in investigation, prevention, and treatment of diseases is also very promising. However, among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity, and 15% have been investigated phytochemically. 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. *Cissus quadrangularis* belongs to the family Vitaceae. It is commonly called as Bone setter used as common food item. The stem is used for the treatment of malaria, stomach ulcer, dyspepsia, eye, ear diseases, irregular menstruation, asthma, tumors, piles, cold, pains, fractures of bones, wounds and scurvy (Arbonier, 2000). The whole plant is considered to be edible while each part of the plant pharmacologically contributed to some activity. In Indian traditional medicine *Cissus quadrangularis* is used as a component of a plaster for treating swelling and bone fractures (Annie Shirwaikar *et al*., 2003). Phytochemical studies of *C. quadrangularis* found several phytochemical constituents such as ascorbic acid, carotene, anabolic steroidal substances, calcium, β-sitosterol, δ-amyrin, δ- amyrone, flavonoids, triterpenoids (Mehta *et al*, 2001) and various secondary metabolites.(Adesanya, *et al*, 1999). The stem of *Cissus quadrangularis* is also reputed in Ayurveda as alterative,

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anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases, in the treatment of irregular menstruation and asthma, in complaints of the back and spine. Scientific studies have revealed the Cissus extract to possess cardiotonic and androgenic property (Chopra *et al*., 1986).

The plant *Cardiospermum halicacabum* Linn. (Family: Sapindaceae) is a climbing plant widespread distributed in tropical and subtropical regions. *Cardiospermum halicacabum* also called as Ballon vine, is an annual or sometimes perennial herb, which has been used as diaphoretic, diuretic, emetic, emmenagogue, laxative, refrigerant, stomachic, and sudorific. (T. N. Kankanamalage *etal 2014)* The leaf extract was used to reduce the obesity and also used to reduce the rheumatic pain and swellings (B. S. S. Kapoor *etal 2013)* It is a small delicate, smooth, climber and the whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, and snakebites. (T. N. Kankanamalage *etal 2014*). A decoction of root is given for bleeding piles. The roots are used for nervous diseases.

MATERIALS AND METHODS

The plants *Cissus quadrangularis* and *Cardiospermum halicacabum* were collected, dried and pulverized. The methanol and ethanol extracts of the plant samples were obtained. The mixture of ethanol extracts of the plant and the mixture of methanol extracts of both the plants were also prepared. These samples were subjected to DPPH Assay and FRAP assay.

DPPH ASSAY: (Molyneux, 2004)

1,1-diphenyl-2-picrylhydrazyl) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centered at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Blois,1958) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present). Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is Z• + AH = ZH + A•

Where ZH is the reduced form and A• is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorised) by one molecule of the reductant.

Chemicals used

- 1,1 – diphenyl -2- picrylhydrazyl (DPPH)
- Dimethylsilphoxide (DMSO)
- BHT (standard)-1.6mg/ml in methanol
- Samples desired concentration from 1 mg /ml –max of 5mg / ml (in /DMSO)

Procedure

3.7 ml of absolute methanol was aliquoted in all test tubes and 3.8ml of absolute methanol was added to blank. 100µl of BHT was added to tube marked as standard and 100µl of respective samples to all other tubes marked as tests. 200µl of DPPH reagent was added to all the test tubes including blank. All the test tubes were incubated at room temperature in dark condition for 30 minutes. The asorbance of all samples was read at 517nm.

Protocol for DPPH Assay

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			

Ferric Ion Reduction FRAP) Assay Potential (: (Benzie and Strain, 1996)

FRAP assay is a simple novel method for assessing "Antioxidant power", Ferric to Ferrous ion reduction at low pH causes a coloured ferrous- tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm test reaction mixtures with those containing ferrous ion in Known concentration.

Preparation of FRAP Reagents

- Reagent A-Acetate Buffer (300mM, pH 3.6)
- 16 ml of glacial acetic acid was added to 3.1g of sodium acetate trihydrate; the solution was then made up to 1L using distilled water. The pH of the solution was checked using pH meter.
- Reagent B-TPTZ (2,4,6 tri[2 pyridyl] s-triazine)Solution 0.031g of TPTZ was added to 10ml of 40mM HCl.
- Reagent C-Ferric Chloride Solution
- 0.054g of ferric chloride was dissolved in 10ml of distilled water.
- Reagents B and C were freshly prepared every time when the assay was performed.

Preparation of FRAP Reagent

About 2.5ml of Reagent B and 2.5ml of Reagent C were added to 25ml of Reagent A to make 30ml of the FRAP reagent. This was placed in a 37ºC water bath for a minimum of 10 minutes. Standard-Ascorbic acid: 1.76 mg of Ascorbic acid was dissolved in 100 ml of distilled water.

FRAP Assay Procedure

About 1ml of distilled water and 80µl of test sample was pipetted into the standard 4ml plastic cuvette..600µl of incubated FRAP Reagent was added to the cuvette, which was briefly inverted to mix the solutions. The reagent blank was also prepared as described above but 80µl of distilled water was added instead of test sample. Change in absorbance at 593nm (as a result of the reduction of the Fe^{3+} -TPTZ complex at low pH) was recorded at exactly at 4 minutes using spectrophotometer. Each test sample dilution was tested in triplicate to allow a mean absorbance to be calculated.

RESULTS AND DISCUSSIONS

Table 1 The Antioxidant activity of given samples using DPPH Assay method

S.NO	SAMPLE	Concentration (µg/ml)	O.D	DPPH activity (%)
1.	*Cissus quadrangularis (Methanol extract)*	1000	0.179	71.89
2.	*Cissus quadrangularis* (Ethanol extract)	1000	0.185	70.95
3.	*Cardiospermum halicababum* (Methanol extract)	1000	0.294	53.84
4.	*Cardiospermum halicababum* (Ethanol extract)	1000	0.258	59.49

Blank O.D: 0.637

From the table-1, it can be seen that the methanolic extract of *Cissus quadrangularis* has the highest DPPH activity of 71.89% while its ethanolic extract has 70.95%. 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 *Cissus quadrangularis* has higher DPPH activity than *Cardiospermumhalicacabum*

Table 2 The Antioxidant activity of given samples using DPPH Assay method.

S.NO	SAMPLE	Concentration (µg/ml)	O.D	DPPH activity (%)
1.	*Cissus quadrangularis* + *Cardiospermum halicababum* Methanol extract	1000	0.293	44.20
2.	*Cissus quadrangularis* + *Cardiospermum halicababum* Ethanol extract	1000	0.297	43.53

Blank O.D: 0.526

From the table-2, the methanolic extract of the mixture recorded 44.20% DPPH activity while the ethanolic extract recorded 43.53%. Hence the methanolic extract of the mixture showed higher activity than the ethanolic extract. However, the activity of the mixture is lower than that of the individual extracts.

Table 3 The Antioxidant activity of given samples using FRAP Assay method

S.No	Name of the Sample	FRAP(µM)
1.	*Cissus quadrangularis* (Methanol extract)	2077.5
2.	*Cissus quadrangularis* (Ethanol extract)	2385
3.	*Cardiospermum halicababum* (Methanol extract)	3565
4.	*Cardiospermum halicababum* (Ethanol extract)	3857.5

From the FRAP assay method the Table-3 shows that the methanolic extract of *Cissus quadrangularis* yielded 2077.5 µM while the ethanolic extract yielded 2385 µM. For the methanolic extract of *Cardiospermum halicababum* 3565 µM was observed while 3857.5 µM was observed for the ethanol extract. Ethanolic extract of *Cardiospermum halicababum* exhibited highest FRAP value while that of the methanolic extract of the *Cissus quadrangularis* was the lowest. However, it can be seen that the FRAP value of *Cissus quadrangularis* was higher than that of *Cardiospermum halicababum.*

Table 4 The Antioxidant activity of given samples using FRAP Assay method

S.No	Name of the Sample	FRAP(µM)
1.	*Cissus quadrangularis* + *Cardiospermum halicababum* Methanol extract	2837.5
2.	*Cissus quadrangularis* + *Cardiospermum halicababum* Ethanol extract	6192.5

The Table-4 shows the methanolic extract of the mixture records 2837.5 µM while the ethanolic extract records 6192.5 µM of FRAP value. It can be seen that the ethanolic extract of the mixture is higher than their individual values.

Murthy *et al.,* (2003) studied the antioxidant activity of *Cissus quadrangularis* plant extract using various solvents. They observed that methanolic extract exhibited 19.8%. A much higher DPPH activity has been observed in this study. Chandra and Suhasini (2015) have studied the antioxidant activity of the ethanolic extract of the plant and observed 70% activity. The results in this study are similar.

Kumaran and Karunakaran (2006), found the antioxidant activity of the methanolic extract of *Cardiospermum halicababum* to be 57%. Huang *et al*., (2011), found that ethanol extract of *Cardiospermum halicababum* showed significant antioxidant activity. The same has been observed in this study.

In a one of its kind attempt, the mixtures of the ethanolic and methanolic extracts of both the plants were tested for antioxidant activity. Significant results were obtained from this study. Hence it can be concluded that both the ethanolic and methanolic extracts of the plants *Cissus quadrangularis, Cardiospermum halicababum* and their mixtures showed potential antioxidant activity. Through further research and validation, it can be used as a lead compound in the pharmacological industries.

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