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Studies on antioxidant activity of *Cissampelos pareira* L. with different solvents

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Abstract
The present investigation of antioxidant activity with three methods such as hydrogen peroxide scavenging assay, DPPH assay and Thiobarbituric acid were performed. The *Cissampelos pareira* leaf with aqueous extract antioxidant results represented when compared with acetone extract of thiobarbituric acit was maximum antioxidant results represented when campared with methods with higher concentration was excellent activities recorded respectively. The effect of *Cissampelos pareira* leaf with aqueous extract by various methods were performed. The hydrogen peroxide scavenging assay was better antioxidant activities than that of the other methods. In this technique 500 µg/mL concentration was extraordinary all binding of molecules were determined with ascorbic acid as a standard for the activity. The ethanolic leaf extract of antioxidant properties of thiobarbituric acid with higher concentration found to be exhibited when compared with other two methods respectively. The methanolic leaf extract of *Cissampelos pareira* with hydrogen peroxide scavenging assay (H_2O_2) maximum antioxidant properties were observed at higher concentration then the DPPH assay were analysed. However the antioxidant properties of *Cissampelos pareira* leaf extract of aqueous solvents was excellent results represented. The *Cissampelos pareira* leaf phytochemicals for more suitable in antioxidant activities.

Keywords: *Cissampelos pareira*, antioxidant, scavenge free radicals and aliments

Introduction
Cissampelos pareira is perennial climbing shrubs 2 to 5 m high commonly known as "Patha" in Ayurveda. It belongs to family Menispermaceae and has significant importance for the treatment of urinary problems, fever and skin infection (Jain *et al*., 2015) [8]. Antioxidants contain free radicals and properties of breaking chain reactions. The most effective part of their defence mechanism and reduce the action of free radicals which cause stress of oxidation (Pourmorad *et al*., 2006) [11]. Antioxidants are reported to play a central role in preventing oxidative stress (Ali *et al*., 2008; Mazhar *et al*., 2015) [4, 9]. Since the ancient times, all over the world traditional people depended upon medicinal plants having less toxicity and cost effectiveness due to their biological properties, against various viral infections and ailments caused by oxidative stress (Ajaib *et al*., 2017) [2].
The antioxidant effect was evaluated by using 1, 1-diphenyl-2picrylhydrazyl (DPPH) radical scavenging assay, total phenolic and total flavonoid content. The stem extract of plant displayed good antioxidant potential as compared to the leaf extract. The percentage of DPPH free radical scavenging activity was maximum of the stem extract. The maximum result of antioxidant activity was observed by total phenolic content. The antioxidant potential was expressed as the amount of the extract needed to decrease 50% of the initial concentration of the free radical. The plant leaf extracts were found to be active antioxidants in the concentration range of scavenge free radical. (Ebrahimzadeha *et al*.,2010) [7].

Materials and Methods
Collection of plant
The fresh, healthy plant materials of *C. pareira* leaf collected from the Mannargudi regions of Thiruvarur Dt, Tamilnadu, India. The leaves were washed thoroughly under running tap water then with distilled water and shade dried at room temperature and remove the moisture completely. The dried leaves are then homogenized into fine powder using a mixer grinder and stored in airtight containers for further study

Sample preparation
Ten gm of the dried powder of leaves of *Cissampelos pareira* were taken separately in labelled airtight bottles and 50 ml of solvents such as acetone, aqueous, methanol and ethanol were individually added and prepared.

Hydrogen peroxide (H_2O_2) radical scavenging activity assay (Singh and Singh 2008) [13]
Solution of 0.2 M potassium dihydrogen phosphate and 0.2 M sodium hydroxide solutions were prepared as per the Indian Pharmacopoeia 1996 standards. 50 ml potassium dihydrogen phosphate solution was placed in a 200 ml volumetric flask and 39.1 ml of 0.2Msodium hydroxide solution was added and finally volume was made up to 200

ml with distilled water to prepare phosphate buffer (pH-7.4). 50 ml of phosphate buffer solution was added to equal amount of hydrogen peroxide and generate the free radicals and solution was kept aside at room temperature for 5min to complete the reaction. Extracts (1 ml) in distilled water were added to 0.6 ml hydrogen peroxide solution and the absorbance was measured at 230 nm in a spectrophotometer against a blank solution containing phosphate buffer solution without hydrogen peroxide. The percentage of scavenging of H_2O_2 of extract was measured. The ability to scavenge the H_2O_2 radical was calculated using the following equation:

H_2O_2 scavenging activity (%) = (A0 – A1) /A0 ×100

Where
A0 is the absorbance of the control and A1 is the absorbance in the presence of extract sample. A standard of ascorbic acid was run using same concentrations as that of extract. The antioxidant activity of the sample was expressed as IC50 value was defined as concentration (mg/ml) of sample that inhibited the formation of H_2O_2 radicals by 50%.

DPPH assay (Brand-Williams *et al.*, 1995)
The antioxidant activity of the *Cissampelos pareira* on the basis of the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method described with slight modification. The following concentrations of aqueous and methanol extract were prepared 100, 200, 300, 400 and 500 µg/mL. All the solutions were prepared with methanol. Five ml of each solution prepared and concentration was mixed with 0.5mL of 1ml DPPH solution. Experiment was done in triplicate. The test tubes were incubated for 30 min at room temperature and the absorbance measured at 517nm. Lower the absorbance of the reaction mixture indicated higher free radical scavenging activity. Vitamin C (0.1 mg/ml) was used as a standard and the same concentrations were prepared as the test solutions. The different in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical.

% Scavenged [DPPH] = [(AC – AS)/AC] x 100.

Thiobarbituric acid (TBA) assay (Alam Zeb and Fareed Ullah 2016) [3]
Preparation of TBA Reagent. The standard solution of 4.0 mm of TBA was prepared in glacial acetic acid. For this purpose, 57.66 mg of TBA was dissolved in 100 mL of glacial acetic acid. Samples of *Cissampelos pareira* plant leaf extract with 100% glacial acetic acid (AA) and 50% glacial acetic acid with water (AW). The extract of leaf sample (1 mL) was mixed with 1 mL TBA reagent and the above procedure was repeated five times (n = 5). The TBARS was calculated using the formula as μM/g of the sample:

$$\text{TBARS } (\mu\text{M/g}) = (\text{Ac} \times V) / W, \quad (1)$$

Where
Ac is the amount determined from the calibration curve and W is the weight of the sample taken while V is volume in mL or dilution factor of the total leaf extract prepared.

Results and discussion
The present investigation suggested that the antioxidant activity of acetone, aqueous, ethanol and methnol of leaf of *C. pareira.* The antoxidant activity was evaluated by using Hydrogen peroxide (H_2O_2) radical scavenging activity assay, DPPH radical scavenging activity and Thiobarbituric acid (TBA) assay. Several methods were used for analysis of the antioxidant compounds. The result obtained by H_2O_2 hydrogen peroxide radical scavenging activity had recognized that the aqueous extract of leaf showed maximum radical scavenging potential 42.3±0.04 µg/ml and lowest value of 17.3±1.12 µg/ml in acetone extract. According to the results, established in the investigation, it was found that all of the test plant extracts showed notable scavenging activities against DPPH model in a concentration dependent manner, that is, the higher the concentration, the highest scavenging potential (Table-1-4). The leaf with methanol extract value of 37.2±0.16 µg/ml and lowest value of 19.5±0.21 µg/ml in acetone. The TBA assay were carried out from the aqueous, acetone, ethanol and methanol extracts of leaf. The IC_{50} value of TBA aqueous extracts of leaf 35.3±0.03 µg/ml and lowest value of 20.4 ±0.12 µg/ml in aqueous extract.
Seetharaman *et al.* (2018) [12] were also reported that methanolic extracts of *C. pareira* leaf exhibited highest free radical scavenging activity. The antioxidant assays were carried out from the methanolic extracts of leaf, stem, root and callus (Uthpala and Raveesha 2019) [14]. The IC50 value of DPPH (325.62 µg/ml) and ABTS (55.17 µg/ml) radical activity was found to be maximum in methanolic extract of stem compared to other extracts. The extracts exhibited concentration dependent hydrogen peroxide scavenging activities. The scavenging efficiency increased as follows: CPW < CPA < CPH < CPE. CPE was most efficient with the lowest IC50 value, 75.79±1.63 µg/ml (Mir Zahoor Gul *et al* 2016). Therefore, *C. pareira* extracts quench hydrogen peroxide which can be due to the existence of phenolic groups that exhibited proton donating ability thereby, neutralizing it into water. The CPE scavenged as much as 80.7% of DPPH radicals at a concentration of 50 µg/mL (Amresh *et al* 2007) The antioxidant activities of H_2O_2, DPPH and TBA assays leaf of *C. pareira* was maximum and over all performed aqueous extract compared to other extracts. (Table: 1-4)

Table 1: Analysis of antioxidant activity of *Cissampelos pareira* L. leaf with acetone extract by various methods

Different concentration of leaves extract (µg/ml)	Standard (ascorbic acid)	Hydrogen peroxide scavenging (H_2O_2) assay (%) activity	Standard (ascorbic acid)	DPPH assay (%) activity	Standard (fericthocayante)	Thiobarbutaric acid
100	15.4±0.05	17.3±1.12	10.3±0.04	19.5±0.21	12.4±0.10	20.5±0.16
200	13.5±0.10	21.4±0.15	14.0±0.11	23.5±0.10	15.3±1.14	25.3±0.09
300	16.3±0.15	24.6±1.12	16.2±0.14	27.3±1.14	16.4±0.13	27.6±1.12
400	17.2±0.12	29.5±0.14	18.3±0.13	29.4±1.12	19.4±1.13	29.4±0.10
500	18.4±0.14	32.3±1.10	21.4±0.21	30.2±1.11	21.4±0.16	32.5±0.23

Standard deviation ±error

Table 2: Analysis of antioxidant activity of *Cissampelos pareira* L. leaf with aqueous extract by various methods

Different concentration of leaves extract (µg/ml)	Standard (ascorbic acid)	Hydrogen peroxide scavenging (H_2O_2) assay (%) activity	Standard (ascorbic acid)	DPPH assay (%) activity	Standard (fericthо cayante)	Thiobarbutaric acid
100	18.2±0.14	24.9±0.03	14.4±0.04	19.8±0.13	10.3±0.01	20.4±0.05
200	21.1±1.03	28.5±1.05	18.8±0.10	20.7±0.16	14.5±0.03	24.3±0.03
300	27.4±1.02	32.3±0.03	21.3±0.06	21.8±1.09	13.2±0.06	26.7±0.04
400	32.7±0.25	35.4±1.04	23.2±0.10	24.5±0.04	15.4±0.13	30.4±0.03
500	39.8±0.03	42.3±0.04	25.7±0.04	28.7±0.05	17.8±0.03	35.3±0.03

Standard deviation ±error

Table 3: Analysis of antioxidant activity of *Cissampelos pareira*,L. leaf with ethanol extract by various methods

Different concentration of leaves extract (µg/ml)	Standard (ascorbic acid)	Hydrogen peroxide scavenging (H_2O_2) assay (%) activity	Standard (ascorbic acid)	DPPH assay (%) activity	Standard (fericthо cayante)	Thiobarbutaric acid
100	13.2±0.12	19.2±0.14	12.0±0.14	20.4±0.14	10.2±0.12	22.4±0.12
200	15.4±0.12	21.3±0.21	15.5±0.13	22.3±0.12	13.4±0.15	25.2±0.19
300	16.2±0.10	23.0±0.15	16.2±1.13	25.6±1.06	15.3±0.16	28.4±0.12
400	14.1±0.15	25.3±0.10	19.2±0.21	28.4±1.13	18.3±1.21	30.2±0.11
500	14.5±0.10	34.5±1.12	22.3±0.19	32.2±0.11	23.5±0.13	33.4±0.20

Standard deviation ±error

Table 4: Analysis of antioxidant activity of *Cissampelos pareira* L. leaf with methanol extract by various method

Different concentration of leaves extract (µg/ml)	Standard (ascorbic acid)	Hydrogen peroxide scavenging (H_2O_2) assay (%) activity	Standard (ascorbic acid)	DPPH assay (%) activity	Standard (fericthо cayante)	Thiobarbutaric acid
100	13.3±0.04	22.4±0.14	11.4±0.10	23.4±0.05	13.2±0.13	19.3±0.05
200	18.5±0.03	24.2±0.13	15.2±0.16	25.2±0.04	15.3±0.15	22.4±0.07
300	21.6±0.02	27.3±0.21	19.3±1.14	28.3±0.12	17.3±0.17	24.6±0.06
400	24.3±0.05	32.4±0.14	21.3±0.10	30.4±0.08	20.4±0.13	28.3±0.03
500	27.5±0.03	35.2±0.19	24.2±0.12	37.2±0.16	22.3±0.14	32.5±0.04

Standard deviation ± error

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