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In vitro antioxidant potential of leaf extracts of *Capparis zeylanica* Linn

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Abstract
The present study was aimed to investigate the antioxidant activity of *Capparis zeylanica*. L leaf extract has reported to possess strong activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging, superoxide radical-scavenging and Hydroxyl radical scavenging activity. The phytochemical screening of the plant has shown the presence of flavonoids, tannins and alkaloids. The result of these three methods were compared with a natural antioxidant ascorbic acid as a standard. The result of DPPH activity was showed at 160 µg/ml of aqueous, acetone, ethyl acetate, methanol extract and standard were 51.73%, 70.13%, 40.96%, 83.67% and 91.66% respectively and the IC_{50} Value of these above extract was found to be 132.03, 57.42, 40.55, 33.32 µg /ml and standard was 19.07 µg/ml. The superoxide radical-scavenging activity at 160 µg/ml of four-leaf extract and standard were 50.39%, 67.53%, 59.59%, 85.31% and standard was 96.10% respectively. The IC_{50} values of these free radical scavenging activity of aqueous, acetone, ethyl acetate, methanol extract of leaf was found to be 169µg, 59.52µg, 97.59µg and 27.35µg respectively and the standard was 21.01 µg. Similarly, Hydroxyl radical scavenging activity was showed at 160 µg/ml of aqueous, acetone, ethyl acetate, methanol extract and standard were 55.14%, 63.98%, 46.23%, 71.46% and 92.55% and IC_{50} value of above-mentioned extract and standard ascorbic was found to be 124.77, 94.21, 153.80, 66.74 and 44.37 respectively. Among these three-antioxidant activities of different extracts of Capparis zeylanica leaf, the methanol extract showed strong free radical scavenging activity when compared with other extracts.

Keywords: Antioxidant potential, leaf extracts, *Capparis zeylanica* Linn

1. Introduction
It is well known that reactive oxygen species (ROS), play a major role in the development of oxidative stress that can lead to many illnesses including cardiovascular diseases, diabetes, inflammation, degenerative diseases, cancer, anaemia, and ischemia [1]. The origin of disease is multifactorial in nature and is now being understood as a violation in the basic hom (o) eostatic balance phenomenon in the body. Pro-oxidant state that leads either due to the increased generation of the free radicals caused by extreme oxidative stress of the current life, or due to the poor scavenging in the body caused by reduction of the dietary antioxidants. So Many plants, particularly medicinal plants, have been extensively studied for their antioxidant activity in recent years. It is believed that an increased intake of food rich in natural antioxidants is associated with lower risks of degenerative diseases, particularly cardiovascular diseases and cancer [2]. Plants have developed an array of defence strategies (antioxidant systems) to manage oxidative stress. In these systems, there is a wide variety of antioxidants [e.g., ascorbic acid, gluthione, uric acid, tocopherol, carotenoids, and (poly) phenols, which are different in their composition, mechanism, and site of action [3]. Due to increasing safety concerns the consumption of synthetic antioxidants, exploitation of cheaper and safer sources of antioxidants from natural origins, and especially from plants, is of interest now a days. The major phytocompounds characterized antioxidant activity due to presence of polyphenols [4] I.e., adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and decomposing peroxides. The most common and widely distributed group of phenolic compounds is flavonoids. These are present in most plants and are considered to prevent free radicals associated damages by numerous ways including direct scavenging of free radicals and inhibition of enzymes involved in free radical production [5]. *Capparis zeylanica.* Linn. is also known as Indian caper plant and the family is Capparidaceae. It is a rigid, wiry and much-branched shrub and widely distributed in Bangladesh, India, Sri Lanka and Malaysia [6]. *Capparis zeylanica* Linn. is reported to possess anti-oxidant [7], antipyretic [8], analgesic [9],

anti-inflammatory, antimicrobial and immunostimulant activity [10]. This plant has been of interest to researchers because it is a medicinal plant employed in Indian traditional system of medicine. Root bark ten gram with one cup water crush and filter; this juice is narcotic, useful for relieving accidental pain [11]. The present study was conducted to investigate the antioxidant activity of *Capparis zeylanica.*

2. Materials and methods

2.1 Collection of plant

The collected plant identified as *Capparis zeylanica*. L botanically identified and authenticated with the help of floras, such as Flora of Presidency of Bombay [12], Flora of British India, Flora of presidency of Madras [13] and Flora of Karnataka [14] by Dr. S. SOOSAIRAJ, St. Joesph's College, Thiruchirappalli (Accession No: SJCBOT 2561). The herbarium specimen was prepared and deposited at Department of Botany and Microbiology, A.V.V.M Sri Pushpam College, Poondi, Thanjavur, Tamilnadu, India.

2.2. Preparation of extracts

The leaf samples were washed sensibly with water to remove dust and foreign materials. Then the washed leaves samples (200 gm) were dried under shade at temperature (25 C) for 7 days. After drying the leaf samples were ground into a powder form using a grinder for 30 s. The powdered material was subjected to successive Soxhlet extraction by various solvents namely Aqueous, Acetone, Ethyl acetate and Methanol were used. After that, extract was concentrated and stored at 4 °C until further use in the equipment [15].

2.3 Screening for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity

The antioxidant activity of the extract was measured with the DPPH method [16] with slight modifications. The DPPH solution was prepared freshly by dissolving 6 mg 1, 1-diphenyl-2-picrylhydrazyl in 50 mL methanol (about 0.3 mM). The extract (2.5 mL) with varying concentrations (10-160 µg /mL) and DPPH solution (2.5 mL) was mixed together in a test tube. The test tube was incubated for 20 minutes in the dark at room temperature. The decrease in absorbance was measured at 517 nm using a UV-VIS spectrophotometer. The percentage inhibition of radicals was calculated using the following formula:

DPPH inhibition percentage = $[(A_0 - A_1)/A_0] \times 100$

Where, A_0 - Absorbance of the control

A_1 - Absorbance of the plant extract/ ascorbic acid.

The half-maximal inhibitory concentration (IC_{50}) was reported as the amount of antioxidant essential to decrease the initial DPPH concentration by 50%. These all DPPH tests were performed in triplicate, and graphs were plotted using the average of three determinations.

2.4 Screening for superoxide radical-scavenging activity

Superoxide-scavenging activity of the plant extract was determined by which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitro blue tetrazolium [17]. Different concentrations of plant extract (0.1ml each) and 0.1 ml of 6 µM ethylenediamine tetra acetic acid (EDTA) containing NaCN, 0.1 ml of 50 µM nitro blue tetrazolium, 0.05 ml of 2 µM riboflavin were moved to a test tube, and final volume was made up to 3 ml using phosphate buffer. Then the evaluated tubes were uniformly illuminated with bright light (40 Watt) for 15 minutes and thereafter the optical densities were measured at 560 nm. 0.1 ml of control was prepared in the place of plant extract. The superoxide radical-scavenging percentage inhibition was evaluated by comparing the absorbance values of standard control and experimental tubes.

2.6 Hydroxyl radical scavenging activity

The scavenging activity of the extract on hydroxyl radical was measured according to a previously described method [18]. In 1.5 mL of each diluted extract, 60 µL of FeCl3 (1 mmol/L), 90 µL of 1,10-phenanthroline (1 mmol/L), 2.4 mL of 0.2 mol/L phosphate buffer, pH 7.8 and 150 µL of H2O2 (0.17 mol/L) were added respectively. The mixture was then homogenized and incubated at room temperature for 5 min. The absorbance was read at 560 nm against the blank. The percentage of the hydroxyl radical scavenging activity of each extract was calculated from the equation below:

Percentage of hydroxyl radical scavenging activity= [(OD control-OD sample)/OD control] X 100. Where OD is the optical density. The extract concentration providing 50% inhibition (IC_{50}) was calculated and obtained by interpolation from linear regression analysis.

2.7. Calculation of 50% inhibition concentration

The optical density obtained with each concentration of the extract/ ascorbic acid was plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the 50% inhibition concentration of extract/ ascorbic acid (Vit-C) and result expressed mean ± standard deviation [19].

Table 1: DPPH Free radical scavenging activity of leaf

Extracts		Concentration (µg/ml)					IC_{50}
		10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	160 µg/ml	
Aqueous	Inhibition %	14.58	23.95	30.20	46.87	51.04	132.03
		15.62	25.00	31.25	46.87	52.08	
		15.62	26.04	31.25	47.91	52.08	
	Average	15.27 ± 0.60	24.99± 1.04	30.90 ± 0.60	47.21 ± 0.60	51.73± 0.60	
Acetone	Inhibition %	28.12	41.66	50.00	62.50	69.79	57.42
		30.20	41.66	50.00	62.50	69.79	
		31.12	43.75	51.04	63.54	70.83	
	Average	29.81 ± 1.53	42.35± 1.20	50.34 ± 0.60	62.84 ± 0.60	70.13± 0.60	
Ethyl acetate	Inhibition %	11.45	18.75	29.16	35.41	40.62	40.55
		12.50	19.79	29.16	35.41	40.62	
		13.54	20.83	30.20	35.41	41.66	
	Average	12.48± 1. 04	19.79± 1.04	29.50 ± 0.60	35.41 + 0.0	40.96± 0.60	
Methanol	Inhibition %	33.33	46.87	54.16	69.79	83.33	33.32
		34.37	47.91	55.20	71.87	83.33	

		35.41	48.95	57.29	72.91	84.37	
	Average	34.37 ± 1.04	47.91± 1.04	55.55± 1.59	71.52 ± 1.58	83.67± 0.60	
Standard	Inhibition %	36.45	48.95	57.29	78.12	90.62	
		36.45	48.95	58.33	79.16	91.66	
		37.50	50.00	59.37	80.20	92.70	19.07
	Average	36.80+0.60	49.30+0.60	58.33 + 1.04	79.16 ± 1.04	91.66± 1.04	
Blank	0.96						

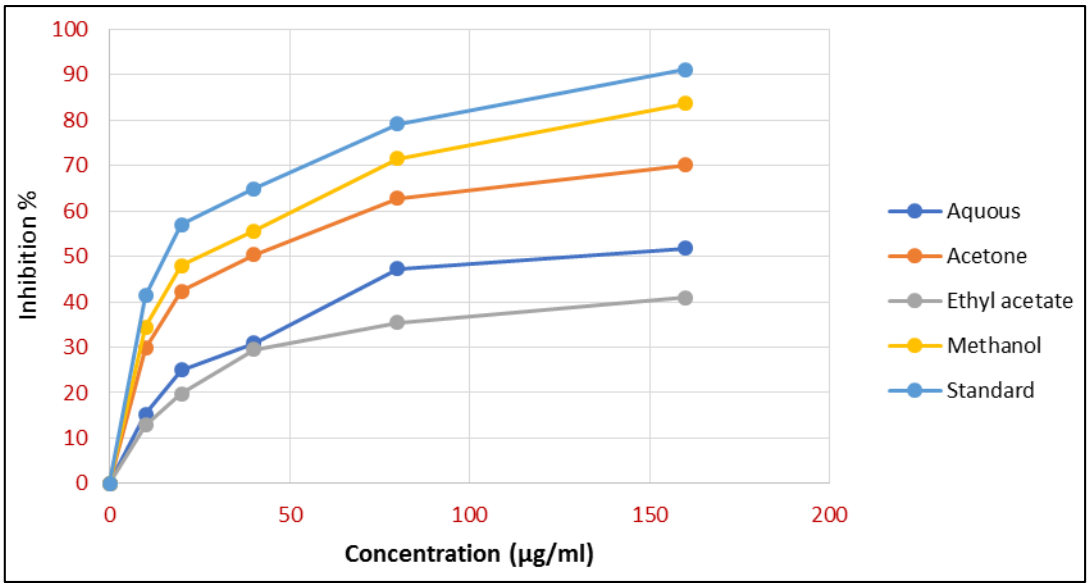

Fig 1: DPPH Free radical scavenging activity of leaf extracts

Table 2: Superoxide free radical scavenging activity of Leaf extracts

Extracts		**Concentration (µg/ml)**					**IC_{50}**
		20 µg/ml	**40 µg/ml**	**60 µg/ml**	**80 µg/ml**	**100 µg/ml**	
Aqueous	Inhibition %	16.19	25.00	34.52	40.23	49.76	
		16.19	26.90	35.47	42.14	49.76	
		17.14	27.85	35.47	43.09	51.66	169.36
	Average	16.50±0.54	26.58±1.18	35.15±0.44	41.82±1.18	50.39±0.89	
Acetone	Inhibition %	27.61	45.00	50.71	58.33	65.95	
		28.57	45.00	51.66	60.23	67.85	
		29.52	45.95	51.66	61.19	68.80	59.52
	Average	28.56±0.95	45.31±0.44	51.34±0.44	59.91±1.18	67.53±1.18	
Ethyl acetate	Inhibition %	19.04	32.61	39.28	53.57	59.28	
		20.00	33.57	39.28	55.47	59.28	
		21.90	34.52	40.23	56.42	60.23	97.59
	Average	20.31±0.45	33.56±0.77	39.59±0.44	55.15±0.18	59.59±0.44	
Methanol	Inhibition %	44.76	61.19	73.57	79.28	84.04	
		46.66	61.19	75.47	79.28	85.00	
		47.61	63.09	76.42	79.28	86.90	27.35
	Average	32.69±1.45	43.48±1.44	65.39±1.45	79.28 ± 0.0	85.31±1.18	
Standard	Inhibition %	41.90	64.04	75.47	82.14	95.47	
		42.85	65.00	77.38	82.14	95.47	
		44.76	65.00	77.38	84.04	97.38	21.01
	Average	35.55±0.55	47.61±0.95	65.39±1.45	82.77±1.09	96.10±0.90	
Blank	1.05						

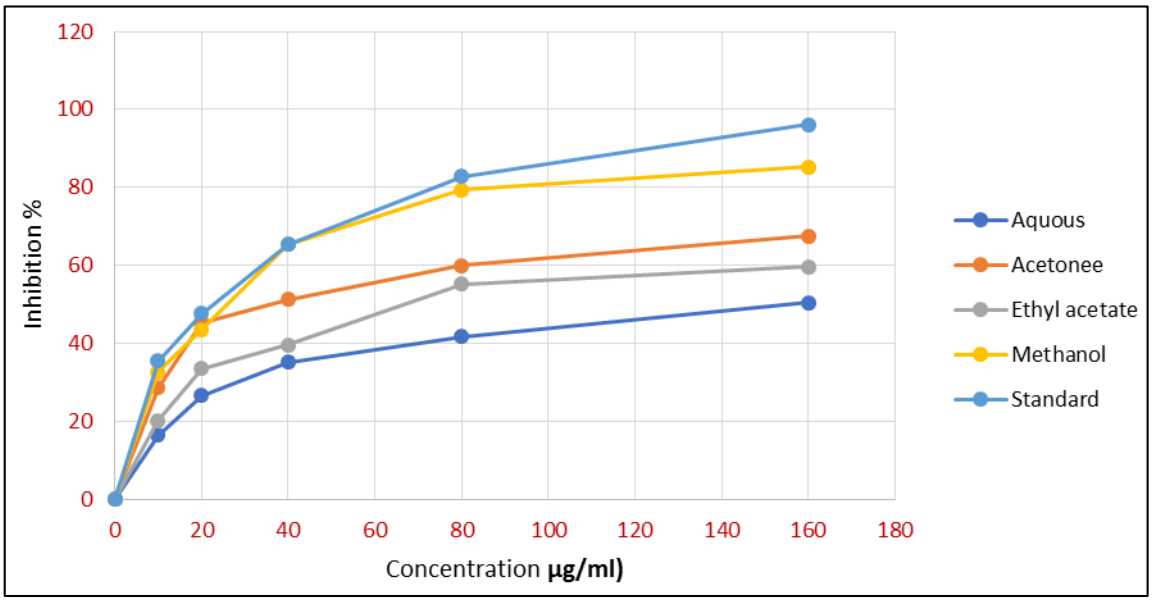

Fig 2: Superoxide free radical scavenging activity of Leaf extracts

Table 3: Hydroxyl radical scavenging activity of leaf extracts

Extracts		Concentration (µg / ml)					IC_{50}
		10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	160 µg/ml	
Aqueous	Inhibition %	15.30	24.53	32.69	40.85	54.12	124.77
		17.34	25.55	33.71	40.85	55.14	
		18.36	26.57	34.73	42.89	56.16	
	Average	17.00±1.55	25.55±1.02	33.71±1.02	41.53±1.17	55.14±1.02	
Acetone	Inhibition %	19.38	29.63	44.93	52.02	63.30	94.21
		21.42	30.65	45.95	53.10	64.32	
		23.46	31.67	45.95	54.12	64.32	
	Average	21.42±2.04	30.65± 1.02	45.61±0.58	53.08±1.05	63.98± 0.58	
Ethyl acetate	Inhibition %	11.22	16.36	23.51	37.37	45.95	153.80
		12.24	16.36	23.51	38.81	45.95	
		13.26	17.38	23.51	39.83	46.97	
	Average	12.24±1.02	16.70± 0.58	23.51 ± 0.0	38.67±1.23	46.29± 0.58	
Methanol	Inhibition %	31.63	41.87	54.12	65.34	70.44	66.74
		32.65	42.89	55.14	65.34	71.46	
		34.69	43.91	57.18	67.38	72.48	
	Average	32.99±1.55	42.89± 1.02	55.48±1.55	66.02±1.17	71.46± 1.02	
Standard	Inhibition %	37.75	46.97	61.26	77.59	91.87	44.37
		38.77	49.02	61.26	78.61	92.89	
		40.81	50.04	62.28	78.61	92.89	
	Average	39.11±1.55	48.67 ±1.56	61.60±0.58	78.27±0.58	92.55± 0.58	
Blank	0.98						

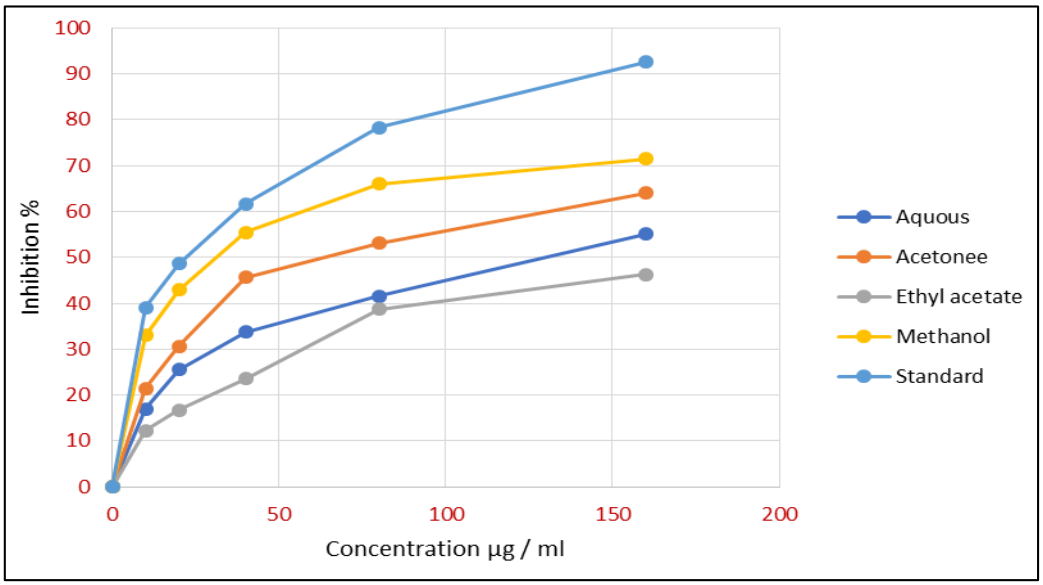

Fig 3: Hydroxyl radical scavenging activity of leaf extracts

Result

In vitro free radical scavenging activity

Medicinal plant has rich in secondary metabolites, including phenol, flavonoids, terpenoids and carotenoids. These compounds have highest activity of antioxidant due to their redox properties. The methanolic extract of leaf of *Capparis zeylanica* had strong activity against all the free radicals tested.

1. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity

The evaluation of antioxidant activities of aqueous, acetone, ethyl acetate, methanol extract of leaf was denoted in fig.1. all the extract showed different activity of DPHH radical scavenging activity over the range of 10 – 160 µg / ml concentration were used. The highest average was 51.73% at 160 µg / ml in aqueous, 70.13% at a concentration of 160 µg / ml in acetone, 40.96% at a concentration of 160 µg / ml in ethyl acetate and methanol extract had 83.67% at 160 µg / ml, while that of ascorbic acid was 91.66% at a concentration of 160 µg / ml and the IC_{50} Value of aqueous, acetone, ethyl acetate, methanol extract was found to be 132.03, 57.42, 40.55, 33.32 µg /ml respectively. The methanol extracts exhibited strongest DPPH radials scavenging activity compared to the other extracts. The mean IC_{50} value of ascorbic acid was found to be 19.07 µg. The results were given in Table-1 and fig.-1.

Several authors reported DPPH scavenging activity of different parts of *Caparis spinosa* [20] reported that methanolic extract of *Capparis* species showed highest activity in DPPH assay. Similarly, the IC_{50} value of *Cappris spinosa* [21] had low was 0.32 mg/ml in methanol extract reported.

2. Screening for superoxide radical-scavenging activity

In the present study, the effect of *C. zeylanica* leaf extract to interact with the superoxide radical was measured as a function of its inhibitory effect on NBT reduction caused by these radicals. It is clear from the figure 2 that all the extracts of leaf of *C. zeylanica* react directly with the superoxide radicals in a dose dependents manner (10-160 µg/ml). Among all the three extracts tested, methanol extract showed maximum scavenging response i.e. 85.31% at160µg/ml followed by acetone extract i.e. 67.53%, ethyl acetate extracts i.e. 59.59% and aqueous extract 50.39%. The standard ascorbic acid was 96.10%. The mean IC_{50} values for superoxide radical of aqueous, acetone, ethyl acetate, methanol extract of leaf was found to be 169µg, 59.52µg, 97.59µg and 27.35µg respectively. The mean IC_{50} value of ascorbic acid was found to be 21.01 µg. The results were given in Table-2 and fig.-2.

Similarly, the other test plants showed percentage inhibition of superoxide radicals according to concentrations. These results are in agree with the finding of [22] *Capparis decidua***.**

3. Hydroxyl radical scavenging activity

The hydroxyl scavenging activity of the extracts expressed as percentage of inhibition increased proportionally with the concentration of the extracts (Figure 3). The strong activity was recorded for all the extracts at 160 µg/mL of hydroxyl scavenged. The strong inhibition percentage was 55.14% in aqueous, 63.98% in acetone, 46.23% in ethyl acetate, 71.46% in methanolic extracts and 92.55% was recorded in the standard ascorbic acid at a concentration of 160 µg / ml. The IC_{50} value of aqueous, acetone, ethyl acetate, methanol extract and standard ascorbic was found to be 124.77, 94.21, 153.80, 66.74 and 44.37 respectively. The methanol extracts exhibited strongest Hydroxyl radical scavenging activity compared to the other extracts. The results were given in Table-3 and fig.-3. Previously the Hydroxyl radical scavenging activity methanolic extract *Kedrostis foetisissima* showed strong activity [23].

Conclusion

The free radicals are causing several diseases in human especially cardiovascular diseases and cancer. The plant develops defence mechanism against free radicals. The result of these three *in vitro* antioxidant model reveals that the leaf powder extracts of Capparis zeylanica L. had significant antioxidant activity. The *Capparis Zeylanica* leaf methanolic extract showed a strong antioxidant activity by inhibiting DPPH, superoxide radical-scavenging and Hydroxyl radical scavenging activities when compared with the standard ascorbic acid. In addition, the *Capparis Zeylanica* leaf was found to contain a noticeable amount of total phenols and flavonoids, which play a major role in controlling oxidation. The results of this study show that the

Capparis Zeylanica leaf can be used as an easily accessible source of natural antioxidant. Hence further studies are required to evaluate the *in vitro* antioxidant potential of these extracts in various animal models.

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