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In vitro assessment of antioxidant and anticancer activities of *Capparis Zeylanica* L. leaf extracts against human breast cancer (MCF-7)

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Abstract

The preliminary phytochemical analysis of leaf extract of *Capparis zeylanica* L. revealed the presence of secondary metabolites such as Phenol, Alkaloids, Saponins, Anthroquinones, Flavonoids, Phlobatannins, Protein, Steroids, Tannins and Terpenoids. The anti-oxidant activity has been studied after the phytochemical analysis of the leaf extract by using DPPH and Superoxide radical scavenging assay. Based on the studies on phytochemical activity and anti-oxidant activity by using various leaf extracts such as Aqueous, Acetone, Ethyl acetate and Methanol extract, we put forward methanol extract for the anti-cancer studies due to its enhanced activities. Different concentrations of methanol leaf extract were exposed to MCF-7 cancer cell lines. Three replicate samples OD value were measured at 570nm and the IC_{50} value of the sample found at 132.3µg/ml. Increased concentration of the leaf extracts increased the mortality which denoted the decreased cell viability of the cancer cells. The lowest cell viability of MCF-7 cancer cell found in 500µg/ml.

Keywords: *Capparis zeylanica*, anti-oxidant, DPPH, Superoxide, MCF-7

Introduction

Medicinal plants are often used for healthcare in both developed and developing countries. According to World Health Organization (WHO), about 80% of the world population are using herbs and other traditional medicines for their primary healthcare and herbal products due to poverty and lack of access to modern medicine [1, 2]. Medicinal plants usage has gotten a lot of attention for the past 20-40 years and traditional use in various part of India. In the recent years number of reports on the usage of medicinal plant in traditional healing by either tribal peoples or indigenous communities is increasing in India [3, 4]. The medicinal plants have been used as herbal medicine for their healing properties since ancient times and some bioactive compounds within these plants are responsible for pharmacological value [5]. These phytochemicals are mostly secondary metabolites constantly synthesized by the plant for defensive purposes [6]. Phytochemicals are natural and non-nutritive bioactive compounds that act as protective agents against external stress and pathogenic attack. The secondary metabolites are crucial for plant defences (eg. As an antioxidant or antimicrobial agent) which has enabled plants to survive. Based on their biosynthetic origin, phytochemicals are categorized in to phenolics, alkaloids, flavonoids, steroids, terpenes, saponins, etc. [7]. Normal cells may be transformed into cancerous one due to the rapid mutations or abnormalities occurring in the genetic material, the accumulation of such cells paves the way to the formation of cancer initiation stage. Then, the proliferative stage which exhibits rapid multiplication of those cells resulting in the formation of tumour. Anti-cancerous studies help to prevent cancer or delays the onset of cancer, progression from precancerous lesion or recurrence after treatment, as an alternative to treatment of cancer cases after clinical symptoms have appeared. Therefore, ultimate goal of cancer prevention is preferably to live without cancer or with cancer without suffering from symptoms until the natural termination of life [8].

Materials and Methods

Collection and authentication of medicinal plant

The collected plant *Capparis zeylanica* L. were botanically identified and authenticated with the following floras: Flora of Presidency of Bombay, Flora of British India, Flora of presidency of Madras and Flora of Karnataka [9,10] by Dr. S. SOOSAIRAJ, St. Joesph's College, Tiruchirappalli (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, Tamil Nadu, India

Preparation of plant extracts

To remove dust and other debris, the leaf samples were sensibly washed with water. Washed leaves (200 gm) were shade dried for 7 days at 25°C. After drying the leaf samples were ground into a powder form using a grinder for 30 seconds and subjected to successive Soxhlet extraction by various solvents namely Aqueous, Acetone, Ethyl acetate and Methanol. Concentrated extracts were stored at 4 °C until further use [11].

Phytochemical screening

For phytochemical screening, plant extracts with different solvents were used to identify the major phytochemical

constitution [12] such as Phenol, Tannins, Alkaloids, Flavonoids, Antroquinones, Terpenoids, Phlobatannins, Protein, Saponins and Steroids [13]. Further, methanolic extracts of *C. zeylanica* were selected for further studies based on the qualitative analysis from four extracts.

Determination of antioxidant activity by using vitro methods

DPPH Radical Scavenging Assay

The antioxidant assay of *Capparis zeylanica* leaf extracts were used against DPPH radical was determined by UV spectrophotometry at 517 nm. This activity was measured according to the method previously performed [14]. Five different concentrations (10µg, 20µg, 40µg, 80µg and 160µg) of the four different solvents of plant extracts were prepared. Ascorbic acid was used as a standard. 1 ml from each extract, 3ml of each solvent were mixed by 0.5 ml of 1.0 mM DPPH in methanol and allowed to react at room temperature for 30 minutes. Same amount of solvent and DPPH to prepare the blank solution which is a control. The sample were prepared in triplicate value for each analysis and the mean value of the absorbance was noted. The DPPH radical scavenging was calculated by the following formula:

DPPH inhibition percentage = [(A0-A1)/A0] x 100

Where, A0 - Absorbance of the control, Al - Absorbance of the plant extract/ascorbic acid. The inhibitory concentration (IC_{50}) of the plant extract was reported as amount of antioxidant required to reduce the initial DPPH concentration by 50%. Triplicate test was performed and graphs were plotted using the average of three determinations.

Superoxide Radical Scavenging Assay

The superoxide radical scavenging activity is performed through modified method [15]. Superoxide radicals were generated in a PMS-NADH system by oxidation of NADH and examined through reduction of Nitro blue tetrazolium. In this study a test tube with different concentration of plant extract were mixed with 0.1ml of EDTA containing NaCl, 0.1ml of NBT, 0.05ml of riboflavin and the volume was made up to 3 ml of phosphate buffer (100 mM, pH 7.8). The reaction started by adding 1 ml of PMS solution (60mM) to the mixture. The reaction mixture was incubated at 25C for 15 minutes, and the absorbance was measured at 560 nm against the corresponding blank solution. Vitamin-C was used as the standard control.

Based on the absorbance values of the scavenging activity the percentage were calculated Scavenging percentage = [(A0 - Al)/ A0] × 100

Where A0 is control absorbance, Al is plant extract/ ascorbic acid absorbance.

***In vitro* Anti-cancerous activity: MTT assay**

MTT (3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) assay was based on the ability of a mitochondrial dehydrogenase enzyme of viable cells to cleave the tertrazolium rings of the pale yellow MTT and form a dark blue colored formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilization of cells by the addition of detergents (DMSO) results in the liberation of crystals which are solubilized. The number of surviving cells is directly proportional to the level of formazan product created. The color can be quantified using a multi-well plate reader [16].

DMEM medium, Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and 3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide (5 mg/ml) were from Sigma, (USA), 1X PBS was from Himedia, (India). 96 well tissue culture plate and wash beaker were from Tarson (India). MCF-7 (Human breast carcinoma cells) cell line were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 u/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37ºC.

The *Capparis zeylanica* L. leaf extract was tested for *In vitro* cytotoxicity by MTT assay. The cultured MCF-7 cells were harvested by trypsinization, pooled in a 15 ml tube and plated at a density of 1×10^5 cells/ml cells/well (200 µL) into 96-well tissue culture plate in DMEM medium containing 10% FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the sample in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. After the incubation period, MTT (20 µL of 5 mg/ml) was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under aninverted microscope. Finally, the medium together with MTT (220 µL) were aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a micro plate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC_{50} value was calculated using GraphPad Prism 6.0 software (USA).

Results

Qualitative Phytochemical Analysis

The preliminary phytochemical analysis of leaf methanol extract of *Capparis zeylanica* revealed the presence of secondary metabolites such as Phenol, Alkaloids, Saponins, Anthroquinones, Flavonoids, Phlobatannins, Protein, Steroids, Tannins and Terpenoids. In this present study, the *C. zeylanica* leaf are subjected to qualitative and quantitative analysis of various phytocompounds (Table 1).

Table 1: Qualitative Phytochemical analysis of *C. zeylanica*

Phytochemicals	Leaf Extracts			
	Aqueous	Acetone	Ethyl acetate	Methanol
Alkaloids	+	-	+	+
Anthraquinones	-	+	-	-
Flavonoids	+	-	-	+
Phenol	-	-	+	+
Phlobatannins	-	-	-	-
Protein	-	-	-	-
Saponins	+	-	-	+
Steroids	-	+	+	-
Tannins	-	-	+	+
Terpenoids	-	-	-	+

DPPH radical scavenging activity

The evaluation of antioxidant activities of aqueous, acetone, ethyl acetate, methanol extract of leaf was denoted in figure 1. All the extracts showed different activity of DPHH

radical scavenging activity over the range of 10-160µg/ml concentration was used. The highest average was detected in methanol extract (83.67 ± 0.60), followed by acetone extract (70.13 ± 0.60), aqueous extract (51.73 ± 0.60) extract and ethyl acetate extract (40.96 ± 0.60) at 160µg/ml, The IC_{50} value of *C. zeylanica* leaf aqueous, acetone, ethyl acetate, methanol extracts were found to be 132.03µg/ml, 57.42µg/ml, 40.55µg/ml, 33.32µg/ml. The methanol extracts of leaf exhibited strongest DPPH radials scavenging activity.

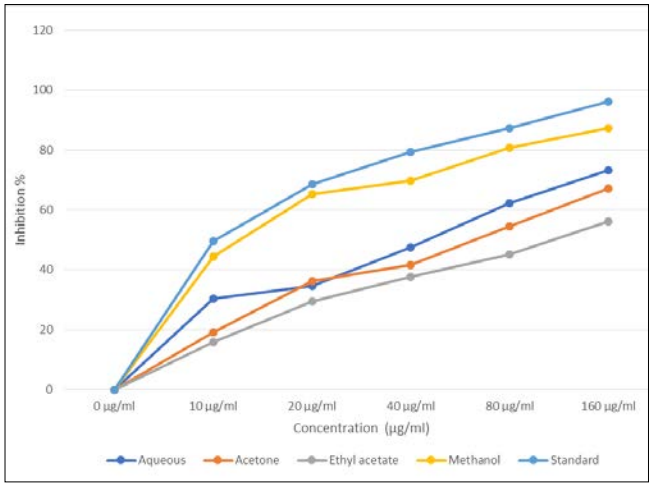

Fig 1: DPPH Free radical scavenging activity of *C. zeylanica* leaf

Screening for superoxide radical-scavenging activity

The effect of *C. zeylanica* leaf aqueous, acetone, ethyl acetate and methanol extracts to interact with the superoxide radical was measured as a function of its inhibitory effect on NBT reduction caused by these radicals were studied all extracts of leaf of *C. zeylanica* reacts directly with the superoxide radicals in a dose dependents manner (10-160 µg/ml concentration). The highest inhibition of superoxide radical scavenging activity was recorded at 160 µg/ml concentration in all extracts. Among the various tested extracts, maximum scavenging response was observed in methanol extracts (85.31 ± 1.18) followed by acetone (67.53±1.18), ethyl acetate (59.59 ± 0.44) and aqueous extract (50.39 ± 0.89). The standard ascorbic acid was 96.10 ± 0.90. The IC_{50} mean values for superoxide radical scavenging activity of aqueous, acetone, ethyl acetate, methanol extract of leaf was found as 169 µg/ml, 59.52 µg/ml, 97.59 µg/ml and 27.35 µg/ml respectively.

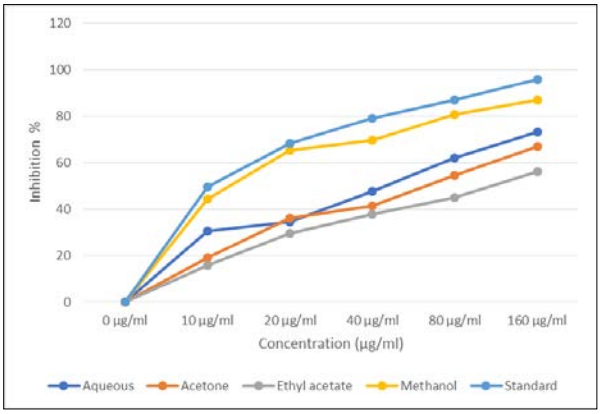

Fig 2: Superoxide free radical scavenging activity of *C. zeylanica* leaf extracts

***In vitro* Anti-cancerous activity: MTT assay**

Different concentrations of leaf extracts of *C. zeylanica* were exposed to MCF-7 (Human breast carcinoma cells) cancer cell lines. Three replicate samples OD at 570nm were measured and the mean values were recorded (Figure 3). The IC_{50} value of leaf extract sample found at 132.3µg/ml. Increased concentration of the leaf extracts increased the mortality which denoted the decreased cell viability of the cancer cells (figure 4 & 5). The lowest cell viability found in 500µg/ml as 6.77%.

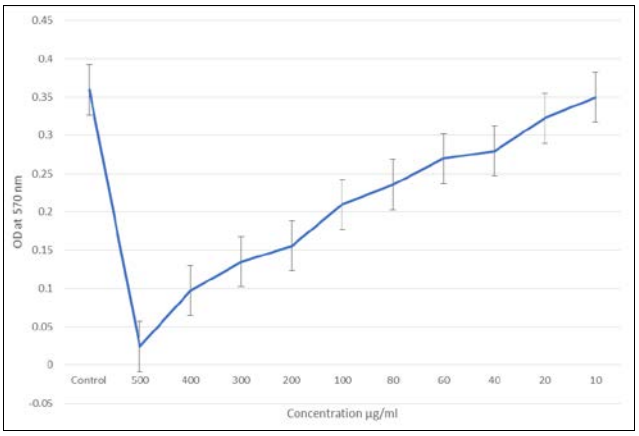

Fig 3: Average optical density of various concentrations of leaf extracts of *C. zeylanica*

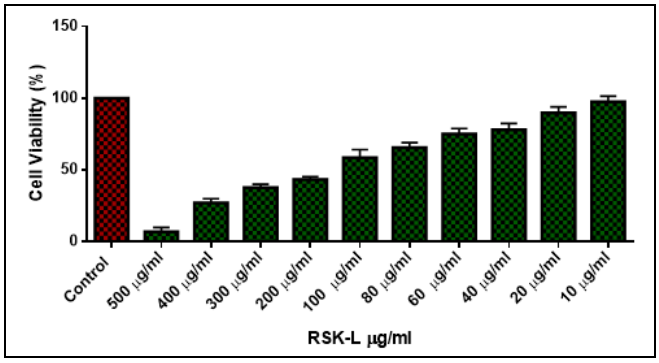

Fig 4: Cell viability of MCF-7 human breast cancer cell line treated with different concentrations of leaf extracts of *C. zeylanica*

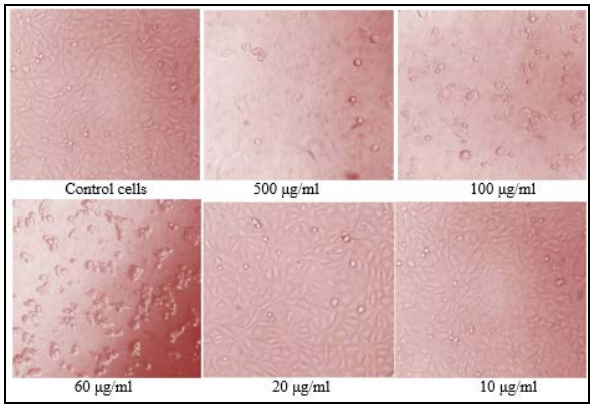

Fig 5: Cell line cultures of control and various concentrations of leaf extracts of *C. zeylanica*

Discussion

In this study we have demonstrated the potent phytochemical, antioxidant and cytotoxicity activities of leaves extracts of *Capparis zeylanica*. Various researches reported that methanol extracts were the effective solvent

for Phytochemical anslysis of Capparis [17] reported methanolic and aqueous extracts of *Faidherbia albida* [18] reported various alcoholic and aqueous extracts of *H. radicata* [19] reported chloroform extract of C. sinensis, methanol extract of T. arjuna and petroleum ether extract of *P. longifolia*. In our study, *Capparis zeylanica* L. leaf extract was obtained by four different solvents such as aqueous, acetone, ethyl acetate and methanol.

Preliminary (qualitative) phytochemical analysis revealed the presence Alkaloids, Phenol, Anthroquinones, Flavonoids, Protein, Saponins, Steroids, Tannins, Phlobatannins and Terpenoids in leaf extract of *C. zeylanica* L. Quantitative Phytochemical analysis revealed methanolic leaf extract showed high contents of phytochemicals than other solvent extracts. Similar pattern of phytochemcial results were reported [20]. DPPH radical scavenging antioxidant activity of *C. zeylanica* leaf extracts (aqueous, acetone, ethyl acetate and methanol) were analysed. The IC_{50} values were observed at 132.03µg/ml, 57.42µg/ml, 40.55µg/ml, 33.32µg/ml for aqueous, acetone, ethyl acetate, methanolic leaf extracts respectively. Methanolic extract showed high DPPH activity as 83.67±0.60µg/ml, followed by acetone extract as 70.13±0.60 µg/ml, and aqueous extract as 51.73± 0.60µg/ml and finally ethyl acetate extract as 40.96± 0.60. The IC_{50} values for superoxide radical scavenging activity of aqueous, acetone, ethyl acetate and methanol extract of *C. zeylanica* leaf was found as 169µg/ml, 59.52µg/ml, 97.59µg/ml and 27.35µg/ml respectively. Methanolic leaf extract showed maximum scavenging response (85.31±1.18µg/ml) followed by acetone extract (67.53± 1.18µg/ml), ethyl acetate extracts (59.59±0.44µg/ml) and aqueous extract (50.39±0.89µg/ml). Similarly, the other test plants showed percentage inhibition of superoxide radicals according to concentrations. These results are in agreed with the findings of *Capparis decidua*. Similarly several author reported that DPPH assay of different *C.spinosa* [21] reported that methanolic extract of caper showed the strong activities in DPPH assay. Many studies have confirmed that the group of phytochemical compounds has various potential mode of anticancer activity [22]. MTT assay of the leaf extracts results showed decreased cell viability in MCF-7 human breast cancer cells, which denoted the increased mortality of the cancer cells. The lowest cell viability found in 500µg/ml as 6.77%. Similarly [23] carried out the screening of the extracts by measuring their invitro antiproliferative properties against several common human cancer cell lines. Some plant extracts exhibited antiproliferative activity toward all the cancer cell lines.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgments

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