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Research Article

EVALUATION OF ANTIOXIDANT AND ANTICANCER ACTIVITIES OF *CIPADESSA BACCIFERA*

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ABSTRACT

Objective: This study was conducted to evaluate the antioxidant and anticancer activities of methanolic extract of *Cipadessa baccifera* leaves.

Methods: The total phenolic content in the extract was assessed by using Folin–Ciocalteu reagent. Antioxidant activity was assesses by different methods such as phosphomolybdate assay, ferric reducing power, 1,1-Diphenyl-2-picryl-hydrazyl, superoxide scavenging assay. Cytotoxic potential of the extract against Ehrlich Ascites carcinoma (EAC), colon carcinoma (HT-29), and breast adenocarcinoma (MCF-7) cell lines was evaluated by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay.

Results: The total phenolic content present in the methanolic extract of *C. baccifera* was found to be 338.38 mg GAL/L. In phosphomolybdate assay, *C. baccifera* extract showed good antioxidant activity (half maximal inhibitory concentration [IC_{50}]=0.42 mg/ml) which was comparable to the standard ferulic acid (IC_{50}=0.22 mg/ml). The extract showed better cytotoxicity against EAC (IC_{50}=4.22 mg/ml) and HT-29 cell lines (IC_{50}=1.86 mg/ml) compared the MCF-7 cell lines (IC_{50}=34.28 mg/ml).

Conclusion: The extract showed good radical scavenging activity and anticancer activity against EAC and HT-29 cell lines. However, the activity was less compared to the standard. Further analysis is required to confirm the *in vivo* activity of the extract. The extract showed negligible cytotoxic effect against MCF-7 cell lines.

Keywords: Anticancer, Antioxidant, Methanolic extract, *Cipadessa baccifera*, Radical scavenging, 1,1-Diphenyl-2-picryl-hydrazyl assay, Ehrlich Ascites carcinoma, HT-29, MCF-7.

INTRODUCTION

In the recent years, the use of antioxidants either in the form of crude extracts or chemical constituents has become of special interest in the scientific research due to their presumed safety and therapeutic value [1]. Reactive oxygen species and other free radicals promote tissue damage in living organisms and are known to be involved in many acute and chronic diseases, such as arteriosclerosis, heart diseases, diabetes, immunosuppression, cancer, and neurodegeneration, as well as in the aging process [2]. Antioxidants stabilize or deactivate free radicals before they attack targets in biological cells, and therefore, can enhance the immune defense and lower the risk of degenerative diseases and cancer [3]. Cancer is a major global health problem responsible for approximately 7.6 million deaths (approximately 13% of all deaths) worldwide, which is expected to rise to 13.1 million by 2030 [4]. Despite improvements in cancer therapy, still there is an unmet need for the evaluation of new anticancer drugs which are safe and effective compared to the current treatments. As the medicines derived from plant products are safer than their synthetic counterparts [5,6], the search for new plants with antioxidant and anticancer potential has ever since increased.

Cipadessa baccifera (Roth.) Miq. (Meliaceae) is a shrub mainly grows in the tropical areas of Asia [7,8]. It is one of the well-known traditional medicines in India for the treatment of rheumatoid arthritis, dysentery and pruritus [9], the paste of root, leaf and bark of this plant is applied topically cure psoriasis [10]. Its decoction has been utilized to treat dysentery, skin itches and malaria fevers by tribal community [11,12]. In recent years, the Meliaceae family, with nearly 1400 species, has grabbed attention as an important source of limonoids and tetranortriterpenoids with insecticidal and antifeedant activities [13]. Limenoids present in the Meliaceace family plants are also known as meliacins are of particular interest because of their abundance, structural diversity, and broad range of bioactivity, such as antifeedant, antimalarial, antimicrobial, cytotoxic, and growth-regulating activities, in contrast to those occurring in other families [14]. In the previous investigations five new mexicanolide-type tetranortriterpenoids, with six other known limonoids were isolated from the seeds of *C. baccifera* [15]. The cytotoxic activities of these isolates were also studied against human lung adenocarcinoma epithelial cell line (A549), breast adenocarcinoma (MCF-7), human cervical carcinoma cell line (ME-180), colon carcinoma (HT-29), mouse melanoma cell line (B-16), and renal cancer ACHN cancer cell lines using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. It was reported that four novel cipadessin-type limenoids present in the leaves and twigs of *C. baccifera* [16]. Antioxidant activity of ethanolic extract of this plant was analyzed by Kindo *et al*. [10]. The methanol and chloroform extracts of leaves were evaluated for antimicrobial, antioxidant, hemolytic, and thrombolytic activities [17].

This study was conducted to investigate the antioxidant and cytotoxic potential of the methanolic extract of *C. baccifera* against Ehrlich Ascites carcinoma (EAC), MCF-7, and HT-29 cell lines.

METHODS

Plant material collection and preparation of extract

The leaves of *C. baccifera* were collected from Puliarai village, Tirunelveli district, Tamil Nadu. Powdered sample (100 g) was subjected to cold extraction with 1000 ml of methanol. The solvent from the extract was removed by distillation, and the extract was lyophilized. The extract was re-suspended in ethanol at 10 mg/ml ratio and used for further analysis.

Analysis of total phenolic content

The total phenolic content was analyzed using Folin–Ciocalteu reagent method with some modifications to the method Singleton *et al*., 1999 [18]. The sample (50 µl opportunely diluted) is added to 250 µl of Folin–Ciocalteau reagent in a test tube and vortexed. Then, 4.7 ml of 2.2% sodium carbonate solution are added and the mixture is vortexed again. A blank is prepared with 50 µl of the sample solvent instead of the sample. The tubes are incubated at 40°C for 30 minutes in the dark. The absorbance is read at 750 nm against the blank using Spectrophotometer (Perkin-Elmer, Model). A calibration curve was prepared with standard ferulic acid (200-1600 mg/L, R^2=0.9978) and used to express the results as ferulic acid equivalents. The total phenolic content of the sample was then calculated and expressed on dry weight and fresh weight basis.

Antioxidant activity

Phosphomolybdate assay

The antioxidant activity of extracts was evaluated according to the method of Prieto *et al*. [19]. An aliquot of 100 µl of extract was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a screw-capped vial. The vials were closed and incubated in a water bath at 95°C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results expressed as an ascorbic acid equivalent antioxidant activity.

Ferric reducing power

The reducing power of extract was determined according to the method of Oyaizu [20]. Samples (2.5 ml) in phosphate buffer (2.5 ml, 0.2 M, pH 6.6) were added to potassium ferricyanide (2.5 ml, 1.0%) and the mixture was incubated at 50°C for 20 minutes. Trichloroacetic acid (2.5 ml, 10%) was added, and the mixture was centrifuged at 650 ×*g* for 10 minutes. The supernatant (5.0 ml) was mixed with ferric chloride (5.0 ml, 0.1%), and then the absorbance was read spectrophotometrically at 700 nm. Based on the absorbency value, the ferric reducing power of extract was expressed.

DPPH (1,1-Diphenyl-2-picryl-hydrazyl) radical scavenging activity

The DPPH radical scavenging activity was analyzed for each by following Sanchez-Moreno *et al*. method [21]. The extract (100 µl) was added to 3.9 ml of DPPH solution (0.025 g/L) and the reactants were incubated at 25°C for 30 minutes. Different concentrations of ferulic acid were used as a positive control and ethanol was used instead of extract in blank. The decrease in absorbance was measured at 515 nm using a spectrophotometer. The radical scavenging activity of tested samples was calculated and expressed on percentage basis.

Superoxide radical scavenging activity

The capacity of extracts to scavenge the superoxide anion radical was measured according to the method described by Zhishen *et al*. [22]. The reaction mixture was prepared using 3×10^{-6} M riboflavin, 1×10^{-2} M methionine, 1×10^{-4} M nitroblue tetrazolium chloride and 0.1 mM ethylenediaminetetraacetic acid in phosphate buffered saline (pH 7.4). For the analysis, 3.0 ml of the reaction mixture was taken with 100 µl of extract in closed tubes and illuminated for 40 minutes under fluorescent lamp (18 W). The absorbance was then read at 560 nm against the un-illuminated reaction mixture. Results were expressed as superoxide radical scavenging activity on percentage basis.

Anticancer activity

Cell lines

The anticancer activity of the extract was evaluated in EAC, HT-29, and MCF-7 cell lines. The EAC cells were obtained from Swiss mice after 15 days of cancer induction. The peritoneal fluid containing EAC cells were collected aseptically using a 5 ml syringe and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum. The HT-29 and MCF-7 cell lines were procured from National Centre For Cell Science, Pune and maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 1% non-essential amino acids, and 1% penicillin (5000 IU/ml)-streptomycin (5000 µl/ml) solution at 37°C under 5% CO_2 atmosphere. Initially, the cells were washed with phosphate buffered saline (PBS) for 2 times, centrifuged and the cell pellet was collected and re-suspended in DMEM medium and incubated at 37°C for 2 days in a CO_2 incubator.

MTT assay

The anticancer effect of the extract was analyzed by treating with EAC and HT-29 cells for 24 hrs and the cell viability was checked using MTT assay [23]. After arriving 60% of confluency, the cells were trypsinized and dispersed in 96 well plates with a cell count of 9000 cells per well and incubated for 24 hrs. Then, the extract and nanoparticles were added at different concentration and then again incubated for 24 hrs. At the end, the medium was discarded, cells are washed with PBS and 20 µl of MTT reagent was added in each well and incubated for 6 hrs at 37°C in a water bath. Then, 150 µl of acidic isopropanol was added and shaken for 30 minutes on a plate shaker under dark. The absorbance was measured at 540 nm and the percentage of cell viability was calculated and from that the anticancer effect was derived.

RESULTS AND DISCUSSION

The total phenolic content in the extract was assessed by using Folin–Ciocalteu reagent. Gallic acid was used as a standard, and a linear calibration curve was obtained with R^2=0.9948 (Y=0.0012X+0.1093) where, Y is absorbance at 750 nm and X is the concentration of extract. The total phenolic content present in the methanolic extract of *C. baccifera* was found to be 338.38 mg GAL/L. In the phosphomolybdate assay molybdenum (VI) is reduced to Mo (V) by an antioxidant, it forms a green colored complex at acidic pH in the presence of phosphorous with the absorption maxima at 695 nm. This assay evaluate the reducing or electron donating power of the antioxidant to molybdenum and the intensity of PMo(V) complex is proportional to the antioxidant power of the extract. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The results were expressed as ascorbic acid equivalents per mg of extract. In the phosphomolybdate assay, the percentage reducing power of *C. baccifera* (half maximal inhibitory concentration [IC_{50}]=0.42 mg/ml) was comparable to that of standard ferulic acid (IC_{50}=0.22 mg/ml) (Fig. 1).

In ferric reducing power assay, Fe (III) is reduced to Fe (II) by the antioxidant compound through electron transfer. The reduced Fe (II) forms the Pearl's blue complex, which can be measured at 700 nm. The ferric reducing power of extract is less (IC_{50}=1.88 mg/ml) compared to the standard ferulic acid (IC_{50}=0.32 mg/ml) (Fig. 2).

The evaluation of the antioxidant power by DPPH radical scavenging activity has been widely in use for different plant extracts. DPPH is a stable radical, methanolic solution of which has dark purple color with maximum absorption at 515 nm. Antioxidants can reduce DPPH through hydrogen transfer into its non-radical form (DPPH-H) and hence the absorption disappears at 515 nm. The decrease in absorbency at

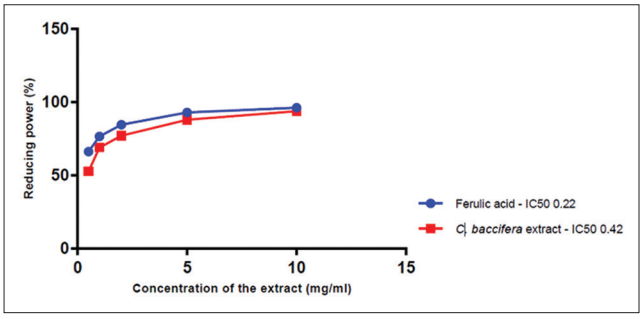

Fig. 1: Phosphomolybdate reducing power of methanolic extract of *Cipadessa baccifera*

515 nm may be due to the reaction between phytochemicals and DPPH, which indicates the antioxidant power. The radical scavenging potential of extract and ferulic acid was expressed in terms of % inhibition. The radical scavenging potential of the extract (IC_{50}=3.38 mg/ml) is less compared to that of standard (IC_{50}=0.91 mg/ml) (Fig. 3).

The superoxide radical scavenging activity of samples was investigated by generating superoxide through photo-induced reduction of riboflavin, which can generate superoxide radical in the presence of methionine. The generated superoxide radical reduce the nitro blue tetrazolium into purple color formazan, which was measured at 560 nm. In the presence of the antioxidant, the generated superoxide radicals were scavenged and hence, formation of purple color formazan is minimum or nil. Fig. 4 illustrated the superoxide radical scavenging of methanolic extract of *C. baccifera* and also the ferulic acid standard. The superoxide radical scavenging activity of ferulic acid was higher (IC_{50}=1.20 mg/ml) compared to standard (IC_{50}=4.91 mg/ml).

The cytotoxicity of the methanolic extract of *C. baccifera* was assessed against EAC, HT-29, and MCF-7 cell lines. The cancer cell lines were treated with different concentrations of methanolic extract *C. baccifera*. The extract was effective against EAC (IC_{50}=4.22 mg/ml) and HT-29 (IC_{50}=0.45 mg/ml) cell lines. However, the activity is less compared to the standard 5-fluorouracil (IC_{50}=0.45 mg/ml against EAC cell lines, and IC_{50}=1.86 mg/ml against HT-29 cell lines) (Figs. 5 and 6). The extract showed negligible cytotoxic effect against MCF-7 cell lines (IC_{50}=34.28 vs. 0.03 [extract vs. standard]).

In the earlier study methyl angolensate type and mexicanolide type limenoids with six other known limonoids were isolated from the seeds of *C. baccifera*. The cytotoxic activities these isolates were also studied against A549, MCF-7, ME-180, HT-29, B-16, ACHN cancer cell lines using MTT assay, and results indicated that compounds cipaferen H, granatumin E and Febrifugin displayed potent cytotoxic activity against B-16, ACHN cell lines [24]. The earlier investigations also concluded that an increased hydrogen peroxide (H_2O_2) scavenging activity was observed with the increasing concentration of the extract and the methanolic and chloroform extracts has bioactivity [17]. In this study, methanolic extract of *C. baccifera* showed good antioxidant activity in line with the earlier results and cytotoxic activities against EAC and HT-29 cell lines.

CONCLUSION

The methanolic extract *C. baccifera* showed good antioxidant potential and noticeable cytotoxic activity against EAC and HT-29 cell lines. The

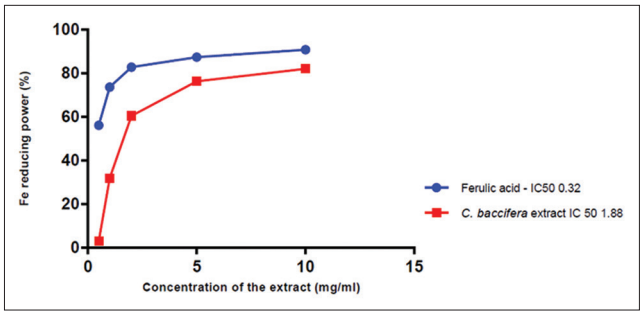

Fig. 2: Ferric reducing power of methanolic extract of *Cipadessa baccifera*

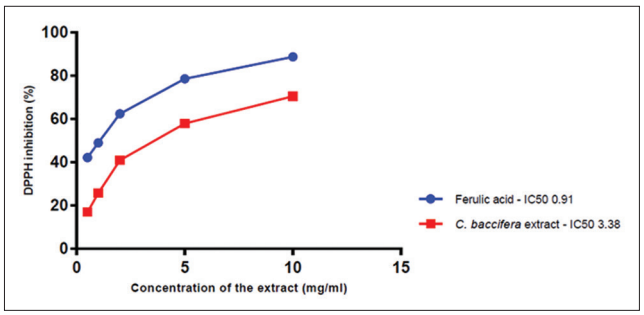

Fig. 3: 1,1-Diphenyl-2-picryl-hydrazyl radical scavenging activity of methanolic extract of *Cipadessa baccifera*

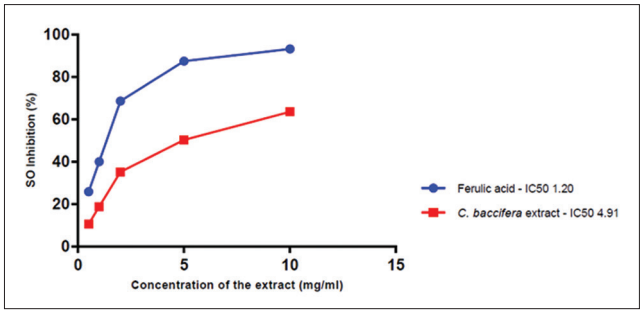

Fig. 4: Superoxide radical scavenging activity of methanolic extract of *Cipadessa baccifera*

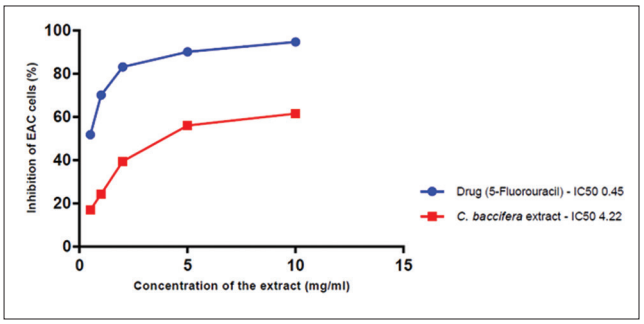

Fig. 5: Anticancer effect of methanolic extract of *Cipadessa baccifera* against Ehrlich Ascites carcinoma cell line

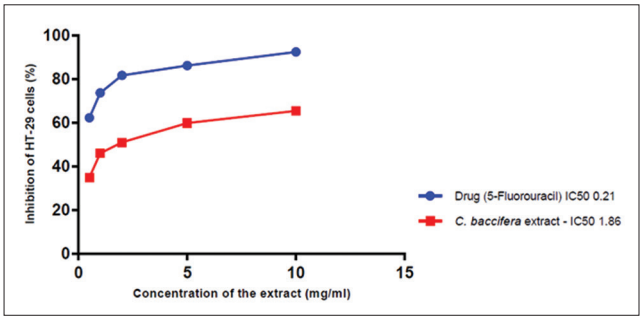

Fig. 6: Anticancer effect of methanolic extract of *Cipadessa baccifera* against HT-29 cell line

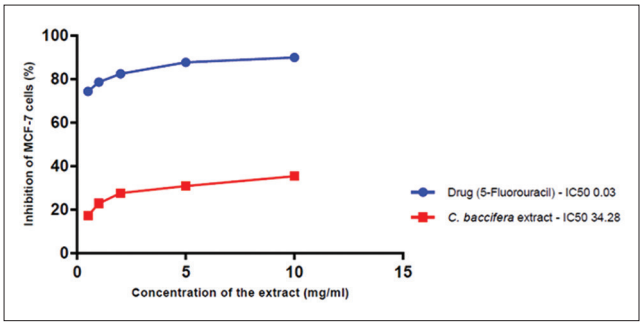

Fig. 7: Anticancer effect of methanolic extract of *Cipadessa baccifera* against MCF-7 cell line

extract showed negligible cytotoxic effect against MCF-7 cell lines. Further *in vivo* investigations are required to confirm the biological activity of the extract.

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