MEDICINAL AND PHARMACOLOGICAL VALUES OF *CYANTHILLIUM CINEREUM* (POOVAMKURUNILLA) EXTRACTS: INVESTIGATING THE ANTIBACTERIAL AND ANTI-CANCER ACTIVITY IN MCF-7 BREAST CANCER CELL LINES

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ABSTRACT: *Ayurveda is an ancient medical treatise summarizing the art of healing and prolonging life free from harmful side effects. Many of the plants have recently been identified to possess remarkable antimicrobial, anticancer and various significant activities. Cyanthillium cinereum (Poovamkurunilla) is one such plant which has been traditionally known for its medicinal properties. Though only few properties of the Cyanthillium cinereum were explored and all aspects are yet to be exploited, the present research concentrates on the antibacterial and anti-cancer activity of the Cyanthillium cinereum extract. Cell apoptosis and MTT assay were carried out in MCF-7 breast cancer cell lines. The zone of inhibition of plant extract against Escherichia coli was found to be 21mm and 19mm against Staphylococcus aureus. The gram negative organism E. coli was found to be more sensitive than the gram positive organism S. aureus. The plant extract treated cells showed typical features of decrease in cells and cell death at the morphological level such as rounding off of cells, cell shrinkage, and detachment from the substrate. The plant extract exhibited significant reduction in cell viability as compared with control cells. Plant extract exerted dose dependent cytotoxic and cell viability effect. Cyanthillium cinereum plant extract possess a strong antibacterial and anti-cancer activity which can be used as a new promising drug and has a wide range of applications in pharmaceutical industry.*

Key Words:Cyanthillium cinereum, *antibacterial activity, anti-cancer activity, Cell apoptosis assay, MTT assay*

Introduction

Ayurveda is an ancient medical treatise summarizing the art of healing and prolonging life free from harmful side effects. Medicinal plants form the back bone of ayurvedic medicine and in the last few decades it has been subjected to very intense pharmacological studies, as highlighted by Packialakshmi (2010). Officially more than 3000 plants are recognized for their medicinal values. The medicinal value of these plants lies in a chemical substance known as phyotochemicals such as alkaloids, flavonoids and tannins. They are the precursors for the synthesis of complex chemical substances that produce a definite physiological action on the human body.

Many of the plants used for dye extraction are classified as medicinal and some of these have recently been shown to possess remarkable antimicrobial, anticancer and various significant activities, as stated by Manonmani et al. (2009). *Cyanthillium cinereum* (Poovamkurunilla) is one such plant which has been traditionally known for its medicinal properties. *Cyanthillium cinereum*, commonly known as little ironweed, is a common annual weed (Asteraceae) with a wide range of geographical distribution. The species is native to tropical Africa and to tropical Asia (India, Indochina, Indonesia, etc.) and has become naturalized in Australia, Mesoamerica, tropical South America, the West Indies, and the US State of Florida. *Cyanthillium cinereum* grows up to 120 cm (4 feet) tall. It produces flat-topped arrays of numerous flower heads, each with pinkish or purplish disc florets but no ray florets. The species can be confused with *Emilia sonchifolia*, but the flower bracts of the latter are much longer and vase-shaped. Cyanthillium cinereum has been used for smoking cessation in Thailand and other countries, and as relief for the common cold.

The plant has great medicinal value in diverse traditional usage in different nations, and also gets recognition in the *Ayurvedas*. The whole plant is used in decoction or infusion to treat fever. It provides remedy for spasms of the urinary bladder and strangury, and is often combined with quinine to treat malaria. Sesquiterpene lactones, which possess antimalarial activity, have been isolated from the

plant. *Cyanthillium cinereum* has therapeutic potentials against asthma, cancer, cholera, colic pain, cough, diarrhea, dysentery, impotency and night-blindness. The seeds are used as a source of alexipharmic and anthelmintic drugs, and as an alternative in leprosy and chronic skin diseases. *Cyanthillium cinereum*leaves have analgesic, antipyretic and anti-inflammatory effects. Paste of stem/bark is used to heal cuts, while flowers are traditionally used to treat conjunctivitis, arthritis and rheumatism. Root infusion is used as an antidote to scorpion sting and snake venom (Funk et al. 2007).

Though only few properties of the Cyanthillium cinereum were explored and all aspects are yet to be exploited, the present research concentrates on the antibacterial and anti-cancer activity of the Cyanthillium cinereum extract. The antibacterial activity was tested against Escherichia coli and Staphylococcus aureus. Cell apoptosis and MTT assay were carried out in MCF-7 breast cancer cell lines.

METHODOLOGY

Drying and Extraction of herbal extract

The collected leaves of *Cyanthillium cinereum* were shadow dried within a temperature range of 37-40ºC. The moisture content of the herb collected was reduced to less than 14% with proper drying since most of the herbs have moisture content of 60-80% and cannot be stored without drying. Proper drying has to be carried out otherwise important compounds may get contaminated. After drying, the grinding was carried out to break down the leaves of the plant into very small units ranging from coarse fragments to fine powder. Extraction refers to separating the desired material by physical or chemical means with the aid of a solvent. In the Soxhlet extraction method, finely ground sample - *Cyanthillium cinereum* powder was placed in a porous bag or "thimble" made from a strong filter paper or cellulose, which is placed, is in thimble chamber of the Soxhlet apparatus. Extraction solvent is heated in the bottom flask, vaporizes into the sample thimble, and condenses in the condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents is emptied into the bottom flask again and the process is continued. The extractor had been filled with solvent solution of ethanol and the temperature of 60^0C was set and left for 6hours

Qualitative antibacterial activity of the plant extract

The antibacterial activity of the plant extracts was evaluated against the predominant isolates by well diffusion method. Sterile Nutrient Agar (Composition g/L: Peptone: 5g; Yeast extract: 5g, Beef extract: 3g, Sodium chloride: 5g, Agar 15 g; Final pH (7.0 ± 0.2) plates were prepared and allowed to solidify. About 0.1 *Staphylococcus aureus* and *Escherichia coli* were swabbed uniformly over the agar surface. Under sterile conditions, 6mm wells were cut on the agar surface of each NA plates. Each of plant extracts (25µl, 50µl, 100µl) in 5% dimethyl sulfoxide (DMSO) were loaded into the well and the plates were incubated at 37ºC for 24 - 48h. The activity was evaluated in terms of zone of inhibition around the wells of each extract in all the inoculated NA plates. The inhibition clear zones were measured and recorded in millimeter.

Anti-cancer activity of the plant extract

Cell apoptosis assay

MCF-7 breast cancer cell line is an established and well-characterized cell line that has demonstrated reproducible results. RPMI-1640 medium supplemented with fetal bovine serum used as culture medium.

The cells were grown in RPMI-1640 medium supplemented with 10% FBS. Cells ($\sim 10^5$) were seeded in the well and incubated at 37 °C for 24 h. Samples were added at different concentration in triplicate. Cells without sample served as control. The plate was incubated for 24 h. After incubation, the medium was completely removed and centrifuged. To the pellet EtBr stain (1µg/ml) was added in and observed under inverted phase contrast microscope.

MTT assay

Cell culture

MCF-7 (Breast cancer cell) 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% CO2 at 37oC.

Procedure

The sample A was tested for *in vitro* cytotoxicity, using MCF-7 cells by 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cultured MCF-7 cells were harvested by

trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×105 cells/ml cells/well (200µL) 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 A in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 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 an inverted microscope. Finally, the medium together with MTT (220µL) were aspirated off the wells and washed with 1X PBS (200µl). Furthermore, to dissolve formazan crystals, DMSO (100µL) 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 percentaege cytotoxicity and cell viability was calculated using the below formula.

Cytotoxicity = [(Control – Treated)/ Control] * 100
Cell viability = (Treated/Control) * 100

RESULTS AND DISCUSSION

Qualitative antibacterial activity of the plant extract

The *Cyanthillium cinereum* plant extract showed significant antibacterial activity against both the test pathogens. The zone of inhibition of plant extract against *Escherichia coli* was found to be 21mm and 19mm against *Staphylococcus aureus* (Table 1). The gram negative organism *E. coli* was found to be more sensitive than the gram positive organism *S. aureus.* The strong antibacterial activity of the *Cyanthillium cinereum* plant extract is due to the presence of bioactive compounds such as phenols, saponins and tannins.

Crude methanolic extracts of *C. cinereum* were tested for their antibacterial activities against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis (Tantengco et al., 2016)*. The leaf and stem extracts of *V. parviflora* and root extracts of *C. cinereum* exhibited high antibacterial activity against *Staphylococcus aureus*. Root extracts of *C. cinereum* exhibited inverse dose-response relationship. The anti-staphylococcal activity was the highest at the lowest concentration (25 mg/mL) and it decreased as the concentration increased. The phytochemical characteristics of petroleum ether, ethanol and aqueous extracts of *Cyanthellium cinereum* was screened by Varsha et al., 2015 and revealed the presence of certain phytocompounds. The results revealed the presence of certain bioactive compounds in leaf extracts of *C.cinereum*. Petroleum ether extract shown the presence of alkaloids, tannins, saponins and glycosides, other constituents like phenols, steroids flavonoids, carbohydrates, proteins, phlobtannins and terpenoids were found to be absent. Majority of the compounds like alkaloids, phenols, tannins, steroids, glycosides, flavonoids, carbohydrates and terpenoids were present in ethanolic extracts of *Cyanthellium cinereum*. Alkaloids, phenols, saponins and phlobtannins are the compounds that were screened in aqueous leaf extracts.

Cell apoptosis assay

Morphological changes of MCF-7 cells were noted on treatment with *Cyanthillium cinereum* plant extract at different concentrations (5, 25, 50, 75 and 100µl) using an inverted microscope. The untreated cells were used as negative control. In comparison to untreated cells, plant extract treated cells showed typical features of decrease in cell and cell death at the morphological level such as rounding off of cells, cell shrinkage, and detachment from the substrate which accumulated in a dose dependent manner, thus indicating that *Cyanthillium cinereum* plant extract induces cell death by apoptosis in these cancer cells (Figure 1).

MTT assay

Cyanthillium cinereum plant extract exhibited a significant reduction in cell viability as compared with control in all MCF-7 breast cancer cell lines. The cell viability of the 60µg/ml treated cancer cell was found to be 82.47% and 100µg/ml was 72.28%. The cytotoxic percentage for 60µg/ml plant extract was observed as 19.65 and 100µg/ml was 29.68%. Plant extracts exerted dose dependent cytotoxic and cell viability effect (Table 2). Thus, Cyanthillium cinereum plant extract possess a strong anti-cancer activity against MCF-7 breast cancer cells.

Conclusion

Soxhlet apparatus is used for the extraction of bioactive metabolites in Cyanthillium cinereum leaves. Ethanol is used as solvent for extraction process. The qualitative antibacterial activity of the plant extract was carried out using well diffusion method. Cell apoptosis and MTT assay were carried out in MCF-7 breast cancer cell lines. The zone of inhibition of plant extract against Escherichia coli was found to be 21mm and 19mm against Staphylococcus aureus. The gram negative organism E. coli was found to be more sensitive

than the gram positive organism S. aureus. In comparison to untreated cells, plant extract treated cells showed typical features of decrease in cell and cell death at the morphological level such as rounding off of cells, cell shrinkage, and detachment from the substrate which accumulated in a dose dependent manner, thus indicating that Cyanthillium cinereum plant extract induces cell death by apoptosis in these cancer cells. Cyanthillium cinereum plant extract exhibited a significant reduction in cell viability as compared with control cells. Plant extracts exerted dose dependent cytotoxic and cell viability effect. So far, only a relative handful of the plant kingdom has been evaluated for pharmacologically active plant substances with potential efficacy against cancer. Cyanthillium cinereum plant extract possess a strong antibacterial and anti-cancer activity which can be used as a new promising drug and has a wide range of applications in pharmaceutical industry.

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S. No	Test organism	Zone of inhibition (mm)
1	*Escherichia coli*	21
2	*Staphylococcus aureus*	19

Table 1: Qualitative antibacterial activity of the plant extract

S. No	Concentration (µg/ml)	% Cell viability	% cytotoxicity
1	20	93.56	7.39
2	40	86.13	16.22
3	60	82.47	19.65
4	80	78.34	23.01
5	100	72.28	29.68

Table2: Determining the cell cytotoxicity of MCF-7 cells for Cyanthillium cinereum plant extract

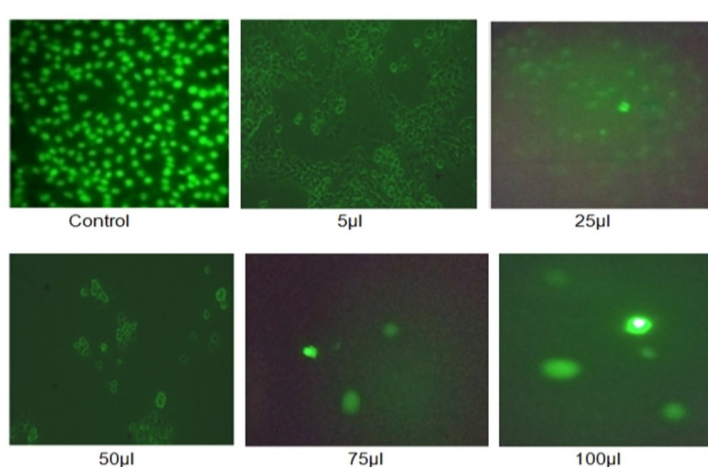

Figure 1: MCF-7 Apoptosis assay