

Invitro anticancer Activity of Whole Plant Ethanolic Extract of *Evolvulus alsinoides* against Different Cell Lines

S.T. Mini & T.Abarna & V. Ramamurthy

P.G & Research Department of Biochemistry, Marudupandiyar College, Vallam Post, Thanjavur, 613 403, Tamil Nadu, India.

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ABSTRACT: Cancer is a debilitating disease resulting from uncontrolled proliferation. One major treatment strategy for cancer is the application of chemotherapeutic drugs which kill cancer cells. In this study the anticancer potentials of plants was investigated against Kato-III cell line (Human gastric carcinoma cell) and SHP-77 (human lung adenocarcinoma epithelial cell line). Cytotoxicity of plant extracts was determined by MTT assay. The results showed that the ethanolic extract of *Evolvulus alsinoides* possessed a moderate amount of anticancer activity and the IC_{50} value was recorded. The most potent anticancer activity was observed with the ethanolic entire plant extract of *Evolvulus alsinoides* with IC_{50} values of 55.9 μ g/ml and 57.9 μ g/ml on Kato-III and SHP-77 cells respectively. Phytochemical analyses revealed the presence of large amount of steroids and flavonoids in the potent plant extracts which may be suggested to play an important role in their anticancer activities. The ethanolic extract would be studied further for isolation and characterization of active components for lead optimization studies.

Key Words: Anticancer, cytotoxicity, *Evolvulus alsinoides*, SHP-77 and Kato-III cells, MTT assay

Introduction

Cancer refers to a group of disease that result from anomalous uncharacteristic growth in the cells. These cells divide and produce new cells in an unrestrained way that can spread throughout the body and injure vital organs. When cancer spreads to other parts of the body, this is called metastasis. This can occur when cancer cells enter the bloodstream or lymph system. These systems circulate all over the body and allow the cells to travel. Cancer is a universal problem. Even though many therapeutic options are available for some cancers including childhood leukemia and testicular cancer, but many metastatic solid tumours are not sensitive to current therapies thus emphasizing the significance of search on unconventional drug sources that can be more effective therapeutically to treat these deleterious cancer cells.

Cancer is one of the major human diseases and causes large suffering and economic loss worldwide. Chemotherapy is one of the methods of treating cancer. However the chemotherapeutic drugs are highly toxic and have devastating side effects. Various new strategies are being developed to control and treat several human cancers (Modha and Modha, 2007). Over 60% of anticancer drugs available in the market are of natural origin. Natural products are also the lead molecules for many of the drugs that are in use (Cragg et al., 1997). Therefore, the phytochemicals present in several herbal products and plants may have the potential to act as preventive or therapeutic agents against various human cancers (Modha and Modha, 2007). The increased popularity of herbal remedies for cancer therapy perhaps can be attributed to the belief that herbal drugs provide benefit over that of allopathy medicines while being less toxic. Since the conventional therapies have devastating side effects, there is a continuous need for search of new herbal cures of cancer (Aquil et al., 2006).

Apoptosis, or programmed cell death, is one of the most finely coordinated regulatory functions for maintenance of the homeostasis in the living organism. It involves the continuous checking of the cellular integrity and cascade-like events of self destruction when the integrity of the organism is endangered. Morphological hallmarks of apoptosis are nuclear condensation, cell shrinkage, membrane blebbing and the formation of apoptotic bodies. These changes are accompanied by biochemical features, including DNA fragmentation and the proteolytic cleavage of a variety of intracellular substrates.

Evolvulus alsinoides is an important popular Ayurvedic drug used to improve intelligence, memory and other higher mental functions. It is also used to treat bowel problems and to promote conception (Austin, 2008 and Sethiya et al., 2009). The entire plant was considered astringent, useful for treating hemorrhages and there are a variety of other medical applications including as an adaptogenic, antiphlogistic, antipyretic, antiseptic, aphrodisiac, febrifuge, stomachic, tonic, vermifuge, against asthma, bronchitis, scrofula, syphilis and to promote wound healing (Daniel, 2008 and Auddy et al., 2003). It is also used as an ingredient in formulations used in the management of diseases like psychosis, epilepsy and other

conditions where brain activities are affected (Sharma, 1983). It is used as best tonic for brain and nerves. It is included as a Medhya drug in the treatises of Ayurveda like Carakasamhita, Susrutasamhita and Ashtanga Hridaya. The popular Ayurvedic formulations where *Evolvulus alsinoides* is included as one of the ingredients are Brahma rasayana, Jeevanyadi ghrita, Brahmi ghrita, Vachadi ghrita, Naladi ghrita and Agastya rasayana (Madhavan *et al.*, 2008). The present investigation was taken up for evaluating the antiproliferative potential possessed by the whole plant ethanolic extract of *Evolvulus alsinoides* against different human cancer cell lines.

Materials and Methods

Collection of plant samples

The present study, the whole plants of *Evolvulus alsinoides* was collected from Nagercoil of Kanyakumari District, Tamil Nadu, India. The plant parts were identified taxonomically and authenticated according to various literatures, Flora of Madras Presidency and Wealth of India including other pertinent taxonomic literature.

Preparation of Extract

The dried and powdered of *Evolvulus alsinoides* (500 g) was extracted using soxhlet extractor by evaporating with 85% ethanol. The soxhlet extraction was carried out for 3 days and the extract was collected. The excess ethanol was evaporated by using vacuum evaporator. The sample is evaporated to dryness under boiling water bath at 60°C.

Phytochemical Analysis

The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The previously dried powdered (50 gm) were extracted in a Soxhlet apparatus with ethanol successively. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc. Chemical tests were identifying the phytochemicals as described (Sofowora, 1993; Trease and Evans, 1983; Harborne, 1973). Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Tumour cell lines

Cell lines of different tissue origin such as SHP-77 (human lung adenocarcinoma epithelial cell line) and Kato-III cell line (Human gastric carcinoma cell) were used. Cells were cultured in MEM (Minimum Essential Media) supplemented with Sodium Bicarbonate, EDTA, FCS (Foetal Calf Serum) and incubated in humidified atmosphere of 5% CO₂ and 37°C. The culture medium was changed every two days. All cell lines used were of human origin in order to more closely mimic how plant extracts would affect human cancer cells. Cells were generally cultured in 10 mL of appropriate medium in 75 cm² tissue culture (T-75) flasks at 37°C in a humidified atmosphere of 5% CO₂/ 95% air. Cells were passages weekly and medium replaced fortnightly.

MTT assay (Mossman, 1983)

Antiproliferative effects were measured in vitro by using MTT ([3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) assays. After treatment, the living cells were assayed by the addition of 20 µl of 5 mg/ml MTT solution. Finally, the reduced MTT was assayed at 545 nm wells with untreated cells were utilized as controls. Antiproliferative and cytotoxic effects were distinguished by cell number and the duration of treatment (72 h, 5000 cells/w, and 24 h, 25000 cells/w, respectively). Stock solutions of the tested materials were prepared with dimethyl sulfoxide (DMSO). The highest DMSO concentration (0.3%) of the medium did not have any significant effect on the cell proliferation. Extracts which demonstrated potent activity (growth inhibition > 50%) were selected for further in vitro testing (dose-response curve and cytotoxicity). To study the interactions between acridones and doxorubicin, a checkerboard method was applied. A series of 2-fold dilutions of the acridones was tested in combination with 2-fold dilutions of doxorubicin. The cell growth rate was determined with MTT staining drug interactions were evaluated according to the following system (fractional inhibitory index = FIX):

FIX < 0.5	Synergism	1 < FIX < 2	Indifferent effect
FIX = 0.51-1	Additive effect	FIX > 2	Antagonism

Result and Discussion

The result of phytochemical screening of the whole plants extract of *Evolvulus alsinoides* revealed that the presence of alkaloids, flavanoids, phytosterols, tannins and phenols (Table 1). The whole plants

extract of *Evolvulus alsinoides* used for the present work was choosing on the basis of their medicinal values. Previous study in the phytochemical screening of the crude extract revealed that the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids and steroids but reducing sugars, carbonyl (aldehyde) and Phlobatanin show negative results (Makinde *et al.*, 2007).

This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized by several workers (Sofowora, 1993). In the present study, it was clearly understood that the alcohol extracted maximum amount of the different type of metabolites present in the *Evolvulus alsinoides*. Boominathan and Ramamurthy (2009) reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

Anticancer activity of *Evolvulus alsinoides* was studied in different mammalian cell line. Anticancer activity of ethanolic extract of *E. alsinoides* as well as standard was determined through MTT cytotoxicity assay. In the preliminary study, the ethanolic extract showed the good yielding capacity of phytocompounds activity. In this regards, the present investigation the ethanolic extract of *E. alsinoides* was studied in Kato-III and SHP-77 cell lines and its result labelled in the table 2 and also made with standard drug tamoxifen.

The minimum cell viability (20.5%) and maximum cell inhibition (80.5%) were noted in 200 µg/ml concentration of *E. alsinoides*. The IC₅₀ value (55.9µg/ml and 57.9µg/ml) was calculated for anticancer activity of ethanolic entire plant extract of *E. alsinoides* against Kato-III and SHP-77 cell lines. The tamoxifen used as a standard for this study. In the standard, the minimum cell viability (16.6%) and maximum cell inhibition (83.4%) were observed in higher concentration. The percentage of cell inhibition was noted in the different concentrations of ethanolic extract of *E. alsinoides* ranges from 20 to 200 µg/ml. The lowest cell inhibition (20.5%) was recorded in the lowest concentration and highest cell inhibition (84.5%) was noted in the higher concentration of ethanolic extract of *E. alsinoides*.

Anticancer properties of many natural compounds isolated from different plant extracts have been reported. Research is being carried out throughout the world to find a lead compound which can block the development of cancer in humans. Nature has always been a great contributor towards this goal. Plant-derived natural products such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties, which include cytotoxic and chemopreventive effects (Abdullaev, 2001). They were the first agents to advance into clinical use for the treatment of cancer (Cragg and Newman, 2005).

Withania somnifera as a potential source of new molecules that can curtail cancer growth were studied by Dredge *et al.* (2003). *E. alsinoides* have also been shown to inhibit the growth of human cancer cell lines comparable to that produced by tamoxifen. The entire plant extract produced antiproliferative activity on SHP-77 and Kato-III tumor cell lines. Jayaprakasam *et al.* (2003) reported that the inhibitory concentrations obtained was 25.1±0.91 against colon cell line HCT-116, but in this study entire plant extracts from different cancer cell treatments of *E. alsinoides* cultivated in fly ash containing soil had shown more than 85% inhibition against human cell lines. Furthermore this study has reported growth inhibitory importance in *E. alsinoides* against various cancer cell lines i.e. SHP-77 and Kato-III tumor cell lines. Hence, this study has revealed remarkable anticancer potential in the whole plant extract of *E. alsinoides*.

Table 1: Qualitative Phytochemical screening on extracts of *Evolvulus alsinoides*

S. No	Name of Test	Test applied / Reagent used	Results
1	Alkaloids	A] Mayer's B] Wagner's C] Hagner's D]) Dragendorff's test	+ + + +
2	Flavanoids	HCl and magnesium turnings	+
3	Carbohydrate	Molisch's test	+
4	Tannins & Phenols	A] 10% Lead acetate B] FeCl ₃	+ +
5	Test for Steroids	A] Salkowski's Test B] Libermann-Burchard's Test	+ +
6	Gums & Mucilages	Alcoholic Precipitation	-
7	Fixed oil & Fats	Spot test	+

8	Saponins	Foam test	+
9	Phytosterols	LB test	+
10	Volatile oils	Hydro distillation method	+
11	Protein & free amino acids.	A] Biuret test B] Ninhydrin test C] Xanthoprotein test	+ + +

-, absents; +, present;

Table 2. Survival analysis of cancer cells treated with extracts of *E. alsinoides*

Concentrations ($\mu\text{g ml}^{-1}$)	Kato-III		SHP-77	
	Cell viability (%)	Cell inhibition (%)	Cell viability (%)	Cell inhibition (%)
20	79.5	20.5	74.7	25.3
40	67.3	32.7	62.4	37.6
60	56.6	43.4	53.3	46.7
80	48.8	51.2	45.7	54.3
100	37.6	62.4	40.3	59.7
125	29.5	70.5	28.2	71.8
150	21.2	78.8	22.3	77.7
200	16.3	83.7	15.5	84.5
Vehicle control (DMSO)	100	0	100	0

Conclusion

Nowadays herbs are extensively used for the research purpose and it possesses more than one chemical entity so it has been widely used for the research investigations. Anticancer properties of many natural compounds isolated from different plant extracts have been reported. Research is being carried out throughout the world to find a lead compound which can block the development of cancer in humans. Nature has always been a great contributor towards this goal. Furthermore this study has to prove the cytotoxic effects of ethanolic whole plant extract of *E. alsinoides* may be conducted in clinical trials on patients suffering from cancer disease. To the best of our knowledge, the present study concluded that the *E. alsinoides* have an anticancer activity against SHP-77 and Kato-III cell line. From this study, it is clear that *E. alsinoides* whole plant extract have significant anti-cancer activity in cell line. The anti-cancer activity is probably due to the presence of phytochemicals.

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