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In vitro antiproliferative effect of the root extracts from *Decalepis hamiltonii*, *Hemidesmus indicus* and *Utleria salicifolia*

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Abstract: This study was undertaken to examine the *in vitro* antiproliferative activity of the different solvents crude extracts of *Decalepis hamiltonii*, *Hemidesmus indicus* and *Utleria salicifolia* tubers roots. Extracts were screened for their possible antitumoral activity by Trypan blue and MTT assay against chronic myelogenous leukemia, K562. IC_{50} values were determined for all the extracts and were ranged from 0.085 to 0.25 mg/ml. The preliminary results obtained from these studies indicated that the extracts of decalepis hemiltonii, hemidismus indicus and utleria salacifolia tubers roots could be considered as natural resource of potential antitumor agents.

Introduction

It is well established that plants have been a useful source of clinically relevant antitumor compounds (Cragg *et al*., 1994). Indeed there have been worldwide efforts to discover new anticancer agents from plants. There are different approaches for the selection of plants that may contain new biologically active compounds (Cordell *et al*., 1991; Schwartsmann and Workman, 1993). One of the approaches used is the ethnomedical data approach, in which the selection of a plant is based on the prior information on the folk medicinal use of the plant. It is generally known that ethnomedical data provides substantially increased chance of finding active plants relative to random approach (Chapuis *et al*., 1988; Cordell *et al*., 1991). However, as for cancer, the disease is complicated and heterogeneous, which makes it difficult to be well diagnosed, especially by traditional healers. The ethnomedical information obtained for a plant extract that is used to treat cancer might therefore not be reliable (Cragg *et al*., 1994). Traditional Indian medicinal plants have been used in the treatment of different diseases in the country for centuries. There have been claims that some traditional healers in India can successfully treat cancer using herbal drugs. Indeed, some traditional healers who were interviewed recently in the country stressed that they have successfully treated patients presented with cancer or cancer related diseases.

Decalepis hamiltonii Wight & Arn., commonly called 'swallow root' is a monotypic genus of the family Periplocaceae. It is found in the Deccan peninsular region of Western Ghats. The root is used as a flavouring principle, appetizer, blood purifier (Anonymous, 1990) and preservative (Phadke *et al*., 1994). The plant finds use as a culinary spice due to its aromatic roots. The active principle in the root

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is a volatile compound called 2-hydroxy-4-methoxy benzaldehyde, which has bioinsecticidal and antimicrobial activity (George *et al*., 1999a, b). This highly important aromatic medicinal species have been subjected to overexploitation by destructive harvesting that has endangered the survival of this species in its wild habitat.

Hemidesmus indicus (L.) R. Br., commonly known as 'Indian Sarsaparilla' is a perennial, slender, laticiferous shrub. The plant has been used widely in the treatment of leucoderma, leprosy, skin diseases, asthma, bronchitis, hyperdipsia, ophthalmopathy, hemicrania, epileptic fits, dyspepsia, helminthiasis, diarrhea, dysentery, haemorrhoids, strangury, leucorrhoea, syphilis, general debility and as a blood purifier (Warrier *et al*. 1995). It is also used as a flavouring agent for the preparation of soft drinks (Sarasan *et al*., 1994). This plant has been found useful in several free radical-mediated disease conditions (Anoop and Jegadeesan, 2003). Also, three antioxidants lupeol, vanillin, and rutin occur within the plant (Gupta *et al*., 1992).

Utleria salicifolia Bedd. ex Hook.f., a laticiferous shrub is a critically endangered and endemic medicinal plant, which deserves special attention as it represents a monotypic genus with a very narrow restricted distribution. This plant is distributed only in Palakkad and Idukki districts of Kerala, Coimbatore and Dindigul districts of Tamilnadu. This species was originally recorded from Anamalai hills in Coimbatore, but it is apparently depleted in the type locality now. The plant is used to treat intestinal disorders, tuberculosis, asthma and skin diseases (Radhakrishnan *et al.,* 1998). Antiulcer activity of the plant has been reported by Rao *et al*. (2004).

On the basis of these findings we were interested to check whether the above mentioned species could be considered as a source of potential antitumor agents. To investigate this pharmacological property, preliminarily we screened the antiproliferative activity of different solvents extracts isolated from roots of decalepis hemiltonii, hemidismus indicus and utleria salacifolia tubers using chronic myelogenous leukemia (K562) cell line.

Material and Methods

Plant materials

Plants were collected from the field gene bank of Centre for Medicinal Plants Research (CMPR), Kottakkal, Malapuram district, Kerala, India. The species was identified from the Taxonomy Division of CMPR and voucher specimenbeen deposited in the 'CMPR' Herbarium.

Preparation of extracts

The plant (tuber roots) materials cut in to small picess were dried at room temperature and then powdered using a grinder. The tuber roots powder (200 gm) extracted by continuous extraction using soxhlet apparatus with solvents like petroleum ether, chloroform, and methanol. The resultant extracts were filtered and concentrated to dryness under reduced pressure below 40^0C in rotary evaporator and stored at 4^0C for further use. The yields of solvent extracts of tuber roots were 0.1%, 1.1% and 8.5% respectively.

Cytotoxicity assay

Human cell line, K562 was purchased from National Center for Cell Science, Pune, India. Cells were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml of penicillin, and 100 µg of streptomycin/ml and incubated at 37^0C in a humidified atmosphere containing 5% CO_2.

Trypan blue exclusion assay

Cell viability was monitored by the trypan blue exclusion assay. K562 Cells growing in exponential phase were seeded at a density of 0.75×10^5 cells/ml in a 6-well tissue culture plate for 24 h prior to the experiments and cells were exposed to 0.5 mg and 1 mg of different extracts (petroleum ether, chloroform and methanol). Since these dried extracts were dissolved in DMSO, DMSO was used as vehicle control. Cells were collected at intervals of 24 h and resuspended in 0.4% trypan blue and further incubated for 5 min, after which the number of viable cells was estimated in a haemocytometer chamber.

MTT assay

Cell proliferation was further assessed by MTT assay, which is based on the ability of viable cells to metabolize a yellow tetrazolium salt to violet formazan. Exponentially growing K562 cells were plated in duplicates at a density of 1×10^4 cells /well. After 24 h, plant extracts were added at a concentration of 0.5 mg and 1 mg. Cells were harvested after 48 and 72 h of treatment and incubated with MTT (0.5 mg/ml) for additional 4 h. The blue MTT formazan precipitate was then solubilized in detergent containing 50% N,N-dimethylformamide (Sigma–Aldrich, USA) and 10% of sodium dodecylsulphate (Amresco, USA) and incubated for 2 h. Absorbance was measured at 570 nm on a multiwell ELISA plate reader (Molecular Devices, USA) scanning spectrophotometer. Cells grown in culture media alone or with appropriate concentrations of DMSO were used as controls. The percentage cell proliferation was calculated as a ratio of OD value of sample to the OD value of control and IC_{50} values (concentration of compound causing 50% inhibition of cell growth) were estimated after 72 h of treatment (Table-1).

Results and Discussion

The search for anticancer agents from natural sources has been successful worldwide. Active constituents have been isolated and nowadays are used to treat human tumours. The hunt for anti-cancer agents from plant sources started in the 1950s with the discovery and development of the *Vinca* alkaloids, vinblastine and vincristine, and the isolation of the cytotoxic podophyllotoxins. Several promising new agents are in clinical development based on selective activity against cancer related molecular targets and some agents failed in earlier clinical studies. This work buttresses the need to continue to investigate traditional remedies with a view to isolating their active constituents since an estimated 60% of people living in developing countries depend on traditional medicine for their primary health care. This led to the screening of many new plants as a source of anticancer agents.

Table 1. ***In vitro*** **growth inhibitory activity (IC50 µg/ml) of different solvent extracts (crude) of *Decalepis hamiltonii*, *Hemidesmus indicus*, and *Utleria salicifolia***

Decalepis hamiltonii	**IC50 mg/ml**
Pet ether extract	0.1
Chloroform extract	0.085
Methanolic extract	0.25
Hemidesmus indicus	**IC50 mg/ml**
Pet ether extract	0.15
Chloroform extract	0.1
Methanolic extract	0.12
Utleria salicifolia	**IC50 mg/ml**
Pet ether extract	0.25
Chloroform extract	0.25
Methanolic extract	0.18

In our study we have used the extracts isolated from the tuberous roots of *Decalepis hamiltonii, Hemidesmus indicus* and *Utleria salicifolia*. To investigate the new property of growth inhibition of tumor cells by these species, we employed antiproliferative assays, trypan blue and MTT against chronic myelogenous leukemia (K562) cells. Results from these studies indicated that the crude extracts inhibit the cell proliferation significantly in a time dependent manner (Fig. 1 and 2). IC50 values were calculated after 72 h of exposure of these extracts and tabulated in Table 30. All the three species showed excellent inhibitory activity against the cell line tested at tested concentrations. Among *D. hamiltonii* root extract, chloroform and petroleum ether extracts exhibited more activity compared to methanolic extract. In case of *H. indicus* root extracts all the three extracts exhibit good antiproliferative activity. Taken together, the data obtained suggests that these plants extract could be considered as a natural resource of antitumor agents. In order to obtain some insight into the nature of active components responsible for the cytotoxic

activity, fractionation of the crude extracts and isolation of the compounds are underway.

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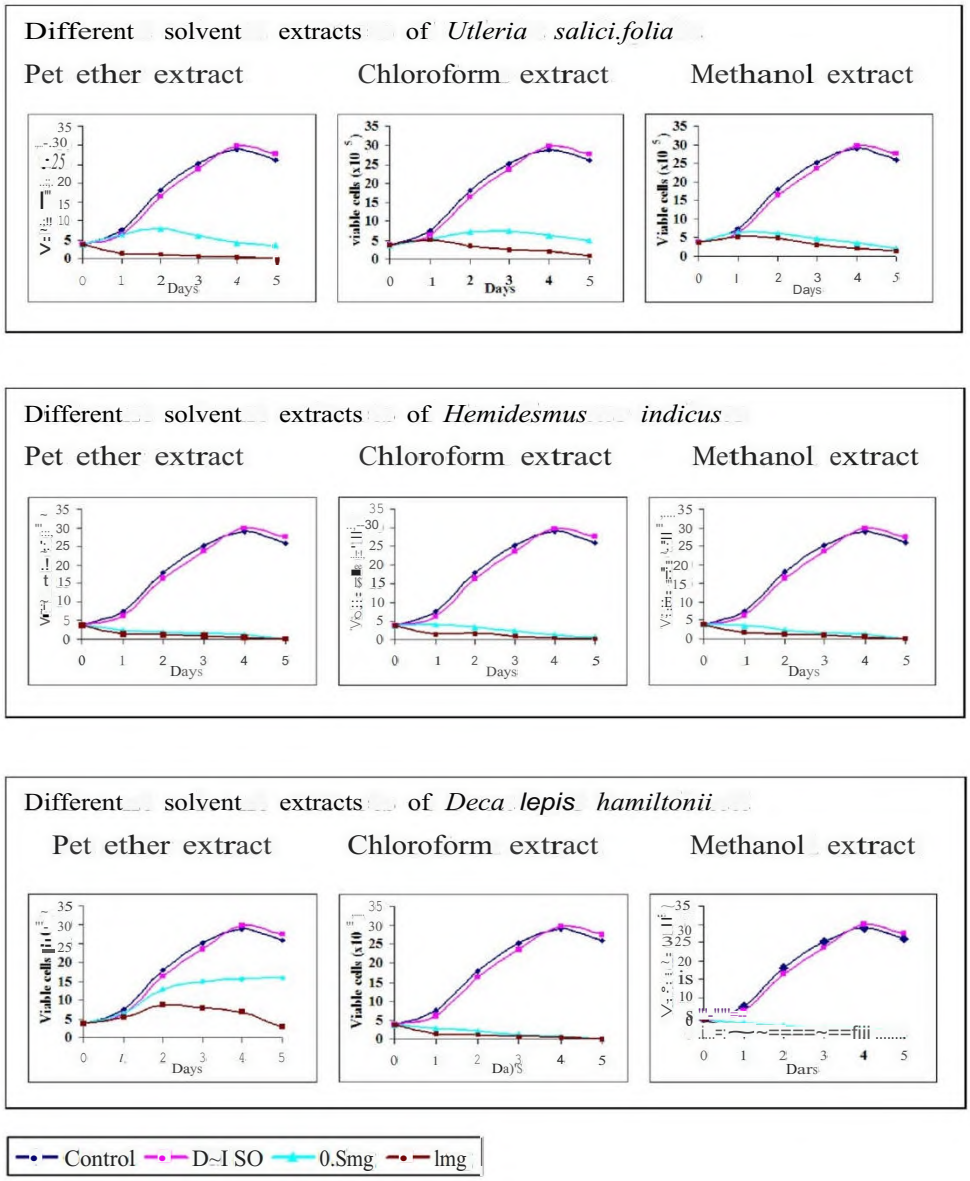
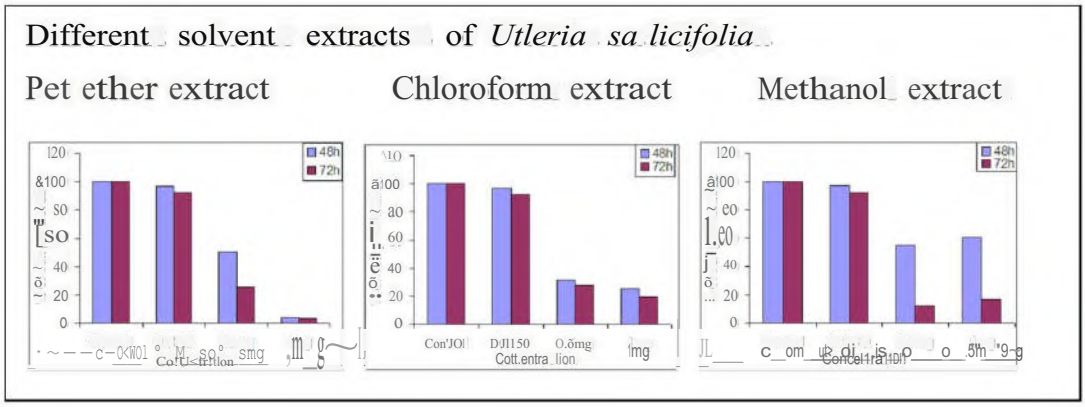
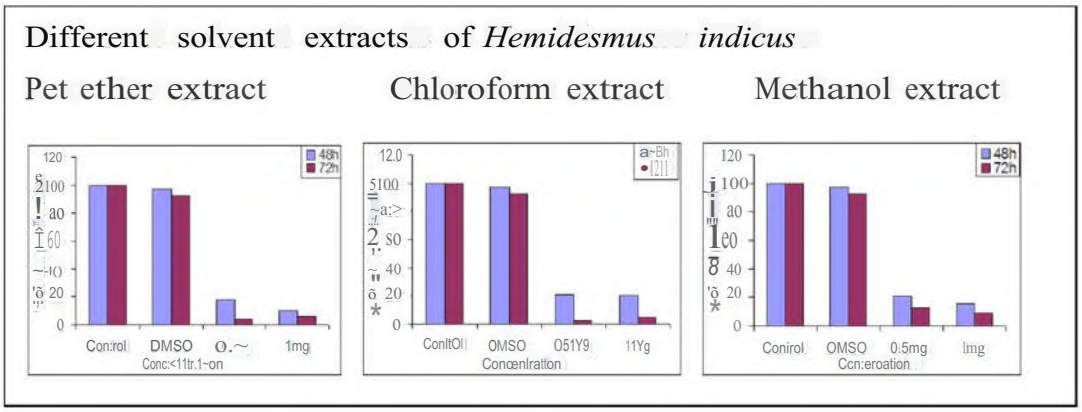


Fig 1. Dose and time-dependent effect of different solvent extracts of three plants on the viability of K562 cells measured by trypan blue assay. Approximately 0.75 x 10^5 cells /ml were cultured and extracts were added after 24 h of plating at a concentration of 0.5 mg/ml and 1mg/ml. In addition to the extracts, DMSO treated cells were used as vehicle control. From the time of addition of extracts, live cells were counted at an interval of 24 h, till the control cells reached stationary phase and the data was represented as a graph.





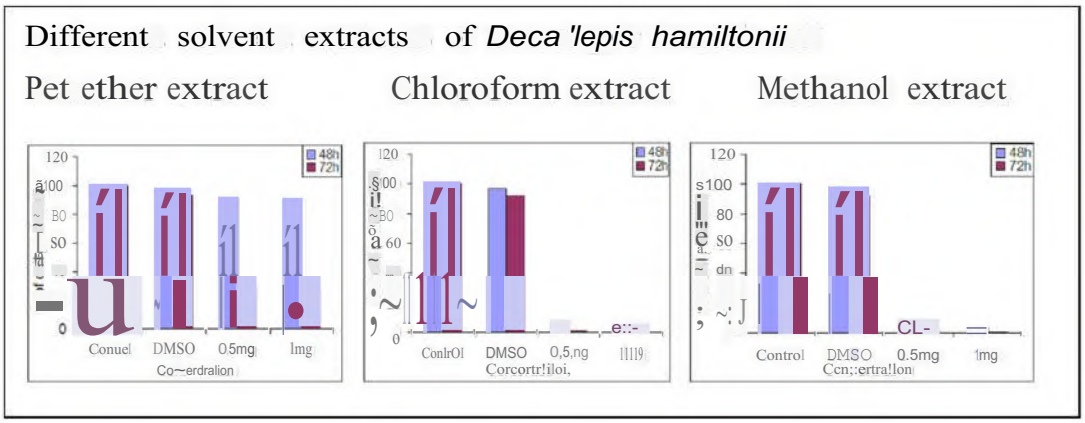


Fig. 2. Effect of different solvent extracts of three plants on proliferation of K562 cells, measured by MTT assay after 48 h and 72 h of exposure. Data are reported as mean value ±SEM of six wells for every dose level.

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