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Research Article

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CYTOTOXIC ACTIVITY OF CRUDE EXTRACTS FROM *QUISQUALIS INDICA LINN.* (COMBRETECEAE)

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ABSTRACT

In this study, cytotoxic activity of Quisqualis indica Linn. (Combreteceae) crude extracts was investigated Peteoleum ether., ethylacetate,80 % ethanol and water extracts prepared from leaves and flower of Quisqualis indica Linn were tested for cytotoxic activity on L269cells using the MTT assay. MTT assay was used to evaluate the reduction of viability of cell cultures in the presence and absence of the extracts. Cell viability was inhibited to different extents by the extracts. The ethanol -leaves and water flower extracts of Quisqualis indica Linn were not cytotoxic at 500 µg mL-1. Both the ethanol-stem and water-root extracts exhibited weak cytotoxic activity. The Peteoleum ether -flower, ethylacetate leaves and flower or ethanol-flower extracts showed stronger cytotoxic activity than the others. However, the ethylacetate -flower extract exhibited the most effective cytotoxic activity at 500 µg mL-1 (70.3 %).

Keywords: Quisqualis indica Linn. cytotoxic activitiy; crude extracts; MTT assay

INTRODUCTION

Natural products have long been a fertile source of cure for cancer, which is projected to become the major cause of death in this century. There are at least 2, 50,000 species of plants out of which more than one thousand plants have been found to possess significant anticancer properties. While many molecules obtained from nature have shown wonders, there are huge number of molecules that still either remains untapped or studied in detail by the medicinal chemists[9]. Out of 121 prescription drugs in use today for cancer treatment, 90 are derived from plant species .The plant kingdom consists of about 400.000 plant species and is a huge reservoir of bioactive molecules, many of which have yet to be explored for various pharmaceutical applications. As a part of our on-going search for potent and selective anticancer compounds from natural products, we have been screening plant extracts for cytotoxic activities against L269 cell line. *Quisqualis indica* Linn. (Combreteceae) is a strong climber, ligneous vine that can reach from 2.5 meters to up to 8 meters. It is commonly known as Rangoon creeper. It is indigenous in Africa, Indo Malaysian region and cultivated all over

India. The plant is also used as a cough cure. In Amboina the leaves are given in a compound decoction for flatulent distension of abdomen. In India the leaves are given in a compound decoction for flatulent distension of abdomen, seeds are given with honey as an electuary for the expulsion of entozoa in children .Leaves contains rutin, trigonelline, L-proline, laspargine and quisqualic acid whereas flower gum contains pelargonidin-3-glucoside. Seed Oil contains linoleic, oleic, palmitic, stearic and arachidic acids. ellagitannins, quisqualin A and quisqualin B is present in fruits of this plant and flower contains linalool oxides (furanoid and pyranoid), 2,2,6-trimethyl- 6- vinyl-3-oxo tetrahydropyran, (E,E)-alphafarnesene,(Z)-3-hexenyl benzoate and benzyl benzoate8. Four Diphhenyl propanoids were isolated from stem bark of *Quisqualis indica.*[1,2,3,4] Many herbs have been evaluated in clinical studies and are currently being investigated phytochemically to understand their tumoricidal actions against various cancers. Major classes of anticancer compounds include alkaloids, terpenoids, flavonoids and lignans. We have chosen *Quisqualis indica* Linn whole plant, because terpenoids like taxol are currently being widely used in cancer chemotherapy[8].

Material and Methods

Collection and identifi cation of *Quisqualis indica* Linn

Selection and Collection of plant on the basis of ethno botanical survey, traditional use and literature survey. The mature flower and leaves of *Quisqualis indica* Linn were collected in the morning locally from Pathanamtitta District, Kerala,India, in the month of November 2009. The powdered drug packed in a paper bags & stored in air tight container until use.Identification and Authentication of here by Dr.Elizabeth.T.Mangatt, Professor and Head Dept. of Botany, Marthoma College Thiruvalla, Kerala, India (Voucher. No 138/17/OCT/2012)

Preparation of extracts

Crude extracts of leaves and flower were prepared by decoction of 10 g of each pulverized material in 100 ml for leaves and 200 mL for flowers of Peteoleum ether, Ethylacetate, 80 % Ethanol and Water for 2 days. Then samples were extracted at room temperature using a waring blender. Plant residues were removed by centrifugation (12000 rpm, 30 min, 10 ^{o}C) and the supernatant was filtered and evaporated to dryness under reduced pressure and/or lyophilized. In this way, ten different crude extracts were obtained: Peteoleum ether extract-leaves (PEE-L), Peteoleum ether -flower (PEE-F), ethylacetate extract- leaves (EAE-L), ethylacetate extract- flower (EAE-F), ethanol extract- leaves (EE-L), ethanol extract- flower (EE-F) and aqueous extract-leaves (AE-L), aqueous extract- flower (AE-F). AE-L and AE-F were dissolved in the medium (Eagle's mimimum essential medium, EMEM) and EE-L and EE-F in dimethyl sulfoxide (DMSO) respectively. The other extracts were dissolved in the solvents used for extraction and were then added to EMEM medium in appropriate concentrations

PHYTOCHEMICAL ANALYSIS

Preliminary Phytochemical studies of various *extract of Quisqualis indica* was performed for major classes of constituents like alkaloids, carbohydrates, protein , amino acid ,Terpinoids, Saponins, glycosides, steroids, tannins, flavonoid and phenolic compounds according to published standard methods[11]

Table 1. The yield of extracts Quisqualis indica

	Petroleum ether extract		Ethyl acetate Extract		Ethanol extract		Aqueous extract	
Yield* (w/w)	Leaves	Flower	Leaves	Flower	Leaves	Flower	Leaves	Flower
	50	67	32	50	92	125	73	116

****Weight (mg) of crude extract per 10 g of fresh plant material***

IN VITRO CYTOTOXICITY ASSAY

The cytotoxicity assays were performed according to the microculture MTT method. The cells were harvested (4,5–5,0 x 104 cells/well) and inoculated in 24 well microtiter plates. The cells were washed with phosphate buffered saline (PBS) and the cultured cells were then inoculated with and without the extract. After 72 h incubation, the medium is aspirated. 150 µL of MTT solution (5 mg mL-1 in PBS, pH 7,2) is added to each well and the plates are incubated for 4 h at 37 °C. After incubation, 800 µL of DMSO was added to the wells followed by gentle shaking to solubilize the formazan dye for 15 min. Absorbance was read at 540 nm and surviving cell fraction was calculated. The inhibition of cell viability was calculated by means of the formula:

% inhibition = (1-absorbance of treated cells/absorbance of untreated cells)x100

Table 2. Percent of inhibition of cell viability of extracts Quisqualis indica

Inhibition of cell viability (%)				
	Extract concentration (µg/mL)			
Crude extract	10	100	250	500

PEE-L	1.6 ±5.85b	-4 ±6.55	3.6 ±4.93	-5 ±2.64
PEE-F	5.3 ±2.08	11.3 ±2.30	32.6 ±4.16	56.6 ±2.08
EAE-L	9 ±5.29	45.3 ±9.50	47±7.54	76.3 ±4.16
EAE-F	19.6 ±10.50	52.5 ±6.80	56.5 ±14.22	86 ±2.64
EE-F	6.66 ±7.07	12 ±0.0	24 ±1.73	59.3 ±1.15
EE-L	15 ±2.64	7 ±2.0	11.6 ±2.08	26 ±2.64
AE-F	2.3 ±4.50	3 ±3.46	2 ±3.00	9.6 ±4.50
AE-L	1.13±2.44	7.3±4.16	0.4 ±0.92	1.8 ±2.88

Values are averages and standard deviations for 3 independent experiments .Mean values within the column followed by the same letter are not signifi cantly different by the Tukey's test at 0.05% probability level

RESULT AND DISCUSSION

Cytotoxic effect of plant extracts

Peteoleum ether, Ethylacetate, 80 % Ethanol and Water of *Quisqualis indica* Linn were tested for cytotoxic activities on L269 fibroblast cells. Extracts were prepared from leaves and

Flower of the plant and the yield of extracts were given inTable 1. The results of the cytotoxic activity of crude extracts from roots and stems of are summarized in Table 2. Cytotoxicity of extracts *Quisqualis indica* Linn was determined by MTT assay on the L269 fibroblast cell culture. The most toxic extract was found to be the EAE-F and EAE –L whereas PEE-L ,AE-F,AE-L and EE-F were nontoxic(Table 2). Cell viability was also inhibited to different extents by the extracts. In general, the flower extracts were much more cytotoxic than the leaves extracts except for the hexane extracts. Natural products have been regarded as important sources that could produce potential chemotherapeutic agents. Plant derived compounds; in particular have gained importance in anticancer therapy and some of the new chemotherapeutic agents currently available for use includes paclitaxel, vincristine, podophyllotoxin and camptothecin, a natural product precursor from water soluble derivatives. Obviously natural products are extremely an important source of medicinal agents. Although there are some new approaches to drug discovery, such as combinatorial chemistry and computer based molecular modeling design, none of them can replace the importance of natural products in drug discovery and development . New scientifi c strategies for the evaluation of natural products with biological activity require the implementation of large-scale screening programs. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of biological active agents (4, 21). Furthermore, the selection of crude

plant extracts for screening programs has the potential of being more successful in its initial steps than the screening of pure compound isolated from natural products (5, 13) *Quisqualis indica* Linn has not been screened yet for its fi ne chemicals which are potentially responsible of its biological activities. Therefore, the investigation of cytotoxic potential of *Quisqualis indica* Linn crude extracts is an initial step [9]. In this study, we evaluated the cytotoxic activity of flower and leaves parts of *Quisqualis indica* Linn and concluded that the PEE-L ,AE-F,AE-L and EE-F extracts of *Quisqualis indica* Linn were not cytotoxic at 500 µg mL-1. Both the ethanol-stem and water root extracts exhibited weak cytotoxic activity. The EAE-F and EAE –L stronger cytotoxic activity than the others. However, the chloroform-root extract exhibited the most effective cytotoxic activity at 500 µg mL-1 (72.3 %). The *L269 fibroblast* cells cultivated in the presence of 0.2 % of AE, PE, EE and EAE (the highest concentration used) used as a control showed a cell death ratio of 2 %. Reports have shown that crude plant extracts are more active pharmacologically than their isolated active principles . This may be due to the synergistic effects of the various components present in the extracts.

CONCLUSION

In summary, some of the *Quisqualis indica* Linn extracts investigated in this study appear to have a potential towards the cytotoxic activity. The future studies will investigate in greater
detail the action of *Quisqualis indica* Linn substances, and synthesize new and possibly more active derivatives for their pharmaceutical application

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