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RESEARCH ARTICLE

IN VITRO CYTOTOXICITY POTENTIAL OF ETHANOL EXTRACT OF *SYZYGIUM SAMARANGENSE* (Wt.)

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ABSTRACT

The present investigation is carried out to study the *invitro* cytotoxicity of ethanol extract of *Syzygium samarangense* leaves on HeLa cell line by using MTT assay. Ethanol extract of *S. samarangense* showed concentration dependent activity on HeLa cell line with IC50 value of 40.5 µg/ml which shows that ethanol extract of *S. samarangense* posses significant cytoxicity.Moreover the preliminary phytochemical screening showed the presence of fatty acids, alkaloids, flavonoids, terphenoids, saponins, tannins and steroids which are responsible for its cytotoxicity. There are only a few reports are available for cytotoxicity of ethanol extract of *S. samarangense*.

Keywords: *S. samarangense*, *In vitro* cytotoxicity, HeLa cell and MTT assay.

1. INTRODUCTION

Plant based products are widely used for the various treatments from the early stages of human civilization (1). The medicinal plants are herbs that contain phyto active components which are used for disease remedies could be in any usual forms such as infusions, decoctions, tinctures, syrups, infused oils, essential oils, ointments and creams (2-4). Now a days due to various forms of pollutions and life styles of human much number of diseases are out breaking, especially cancer, is widely identified and cured by many new drugs, but these are having enormous side effects and high cost. So our aim of this present investigation is to evaluate *in vitro* cytotoxic effect of ethanol extract of *Syzygium samarangense* (Wt.) Walp. that belongs to the family *Myrtaceae.* Its native is Fiji, India, Indonesia, and Malaysia. The common names of *S. samarangense* are wax apple, love apple, Java apple. The genus comprises about 1200 to 1800 species (5) and has a native range that extends from Africa and Madagascar through Southern Asia east through the Pacific. Its highest level of diversity occurs from Malaysia to North Eastern Australia. The *Syzygium* species were reported to possess various pharmacological properties viz. anti diabetic, antifungal, anti-inflammatory, antibacterial, antioxidant, anti hyperlipidemic and anti proleferative activities. (6-10). From the literature survey, there are few reports are available for the *in vitro* cytotoxicity of the ethanol extract of *S. samarangenese* leaves. Hence the present study is designed to evaluate the cytotoxicity study and preliminary phytochemical screening of *S. samarangense* leaves.

2. MATERIALS AND METHODS

2.1. Collection of plant materials

Syzygium samarangense of plant family *Myrtaceae* was collected from area near Palani, Tamil Nadu, South India (Geographic coordinates of Palani, India Latitude: 10°27′01″N, Longitude: 77°31′15″ E Elevation above sea level: 328 m =1076ft). The plant sample was authenticated from Dr.Logamadevi Assitant Professor, Department of Botany and the voucher specimens have been kept for further reference.

2.2. Extraction Process

The leaves of *S. samarangense* were dried in shade for 10 days and tightly packed for further process. Air dried leaves of *S. samarangense* was chopped into small pieces. The coarse material was subjected to maceration and Soxhlet extraction by using different solvents. Solvents are used based on their increasing order of polarity i.e. Petroleum ether, Acetone and Ethanol. Solvent are used based on their increasing of polarity. The extract was subjected to vacuum distillation and was concentrated to yield brownish residues of 80g.

2.3. Extraction process

The 300 g of shade dried leaves of *S.samarangense* was extracted with Petroleum ether using Soxhlet apparatus. The extract was subjected to vacuum distillation and was concentrated to yield a green residue of (1.3) g. The defatted plant leaves of *S. samarangense* was again extracted with acetone and ethanol.

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2.4. Preliminary phytochemical screening

Petroleum ether, ethanol and acetone extracts of *S. samarangense* was subjected to qualitative chemical analysis to identify the nature of phytochemical constituents present in it. The following tests were evaluated to identify the phytochemicals present.

2.4.1. Test for steroids and terpenoids

Libermann – Burchard's regent test

The extract (50 mg) was dissolved in 2 ml acetic anhydride. To this one or two drops of concentrated H_2SO_4 were added slowly along the sides of the test tube. An array of colour change showed the presence of steroids and triterpenoids.

2.4.2. Test for flavonoids

Shinoda Test

The extract mixed with few ml of alcohol was heated with magnesium and then con. HCl was added under cooling. Appearance of pink colour indicates the presence offlavonoids.

2.4.3. Alkaline Reagent Test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

2.4.4. Test for phenols and tannins

Small quantity of 50% alcoholic extract was dissolved in water and 5% ferric chloride solution. Appearance of blue colour with ferric chloride indicates the presence of tannins and phenols.

2.4.5. Detection of Alkaloids

The extracts were dissolved in dil. H2SO4 and filtered. The filtrate was treated with Dragendroff' reagent, the appearance of orange brown precipitate indicated the presence of alkaloids.

2.5. In vitro cytotoxicity by MTT Assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinatedehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 hour of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37˚C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula. % cell Inhibition = 100- Abs (sample)/Abs (control) x100. Nonlinear regression graph was plotted between % Cell inhibition and Log10 concentration and IC50 was determined using Graph Pad Prism software.

3. RESULT AND DISCUSSION

3.1. Preliminary phytochemical screening

Petroleum ether, ethanol & acetone extracts of *S. samarangense* were subjected to qualitative chemical analysis to identify the nature of phytochemical constituents present in it. Which are shown in table.1

Table 1. Phytochemical constituents of *S. samarangense.*

S. No	COMPOUNDS	PET ETHER	ACETONE	ETHANOL
1	Fatty acids	+ + +	_	
2	Alkaloids	+	+ + +	+ +
3	Terpenpoids	+ + +	+	+ +
4	Flavonoids	_	+ + +	+ +
5	Steroids	+ + +	_	_
6	Saponins	+ +	+ + +	+ +
7	Carbohydrates	+ +	+ +	_
8	Phenolics	_	+ + +	_

-Indicates absence of compounds, + indicates smaller amount of compounds

+ + indicates high amount of compounds, + + +indicates very high amount of compounds

3.2. In vitro cytotoxicity study

MTT assay is widely used assay for plants and plant based products which measures cell viability of cancer cell lines.

Table 2. IC_{50} values for Ethanol extract of *S. samarangense* leaves.

Name of the extract	IC_{50} µg/ml	Name of the cell line
Ethanol	40.5 µg/ml	HeLa

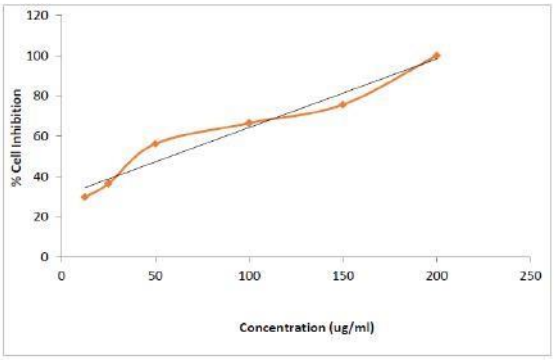

Fig. 1. % of cell inhibition at various concentration of Ethanol extract of *S. samarangense* leaves.

In this investigation ethanol extract of *S. samarangense* leaves was evaluated for *in-vitro* cytotoxicity studies using human breast cancer (HeLa). The viability of cancer cells after incubation with different concentrations of *S. samarangense* leaves (12.5, 25, 50, 100, 150 and 200 µg/ml) affected the viability of human breast cancer cell line HeLa in a dose dependent pattern and the IC 50 values was determined as 40.5 µg/ml. The results are shown in Table 2.

4. CONCLUSION

In the present study, the ethanol extract of *S. samarangense* leaves was evaluated for its phytochemical screening and *in vitro* cytotoxicity effects. It was found that the cell viability of cancer cell line was dose dependent pattern with IC50 µg/ml. This may be due to the presence of flavonoids, steroids and alkaloids. Further studies are in progress.

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