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Research Article

Anti-Cancer Activity of Methanolic Extract of Root and Leaves of *Stereospermum Colais*

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ABSTRACT

Stereospermumcolais is a tree nearly glabrous except the flower. Leaves primarily compound and different parts of tree is used pharmacologically such as anti-diabetic, anti-cancer antioxidants etc. In present study anticancer effect of leaves and root of *Stereospermumcolais* was evaluated by using XTT assay. XTT Assay is an internationally accepted in vitro method for anticancer drug screening. Percentage of viable cells can be measured by intensity of orange colored formazan produced is directly proportional to the cell viability. In the present anti-cancer study, two types of human cell line such as MCF7, A Human breast carcinoma cell line and MOLT-4, a human T lymphoblastic leukemia cell line used and adjusted to density of 10[4]-10[5]. It was observed that extracts from root and leaves of *Stereospermumcolais* Buch showed dose dependent % cell viability in MOLT-4 and MCF-7 in vitro model

Keywords: *Stereospermumcolais*, Anticancer assay, XTT assay

INTRODUCTION

Cell differentiation of proliferation is maintained by growth factors that attach to cell surface receptors which connect to signaling molecules. Transcription factors are activated by these molecules which bind to DNA to facilitate the production of proteins, resulting in cell division. Improper function of any step in this regulatory cascade responsible abnormal cell proliferation, a fundamental cause of various human pathological conditions, most notably cancer and aging[1].

Determining mechanisms responsible for alterations in cell cycle proficiency is crucial to understanding various human disorders, most notably cancer. Cell proliferation assays have been mostly utilized to check cell cycle regulatory factors such as growth factors, mutagens, cytokines, anddrugs[2].

These assays have existed from classical [3H]-thymidine insertion, to 5'-bromo-2'-deoxy-uridine (BrdU) insertion, to WST-1, WST-8, MTT, or XTT methods. In comparison to the traditional radioactive assay or the time consuming BrdU assay, WST-1, WST-8, MTT, and XTT have the benefited of being easy to perform in a micro titer plate skipping washing steps. These assays can be performed within three to four hours.

Stereospermumcolais is a tree nearly glabrous except the flower. Leaves primarily compound. Corolla tubular, thinly villous and without tube rose, lobes yellow or pale rose, crisped. Fruits linear capsules, loculicidally 2- valved. Seeds compressed or subtigonous, with a membranous wing on each side. Flowers and fruits during March-July. Leaves imparipinnate, large, clustered towards the end of the branchlets, leaflets 3-6 pairs; flowers yellow, tinged with red, fragrant, in lax drooping terminal cymose panicles; fruits capsules, cylindric, slender, curved, somewhat rough with elevated white specks; seeds winged at each end, wing membranous. The roots are dull brown, cylindrical, hard and heavy with transversely extended lenticels[3]. It is commonly found in India, Myanmar, Sri Lanka; in the Western Ghats-South, Central andsouth Maharashtra Sahyadris[4].

Materials and Methods

Plant material

The root and leaves of *Stereospermumcolais* were collected from the Pathnurghat (TalukaArdhapur, Dist. Nanded, M.S.) in the month of August 2011. Roots of small diameter (1-3cm) are collected in a container containing 70% alcohol to

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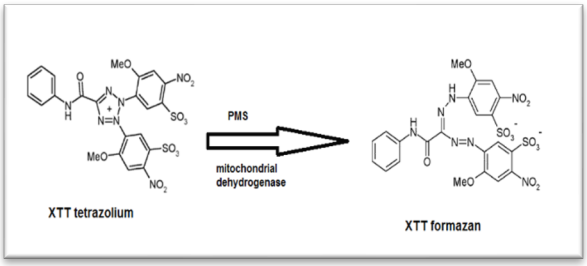
keep specimen in fresh condition for microscopical examination.



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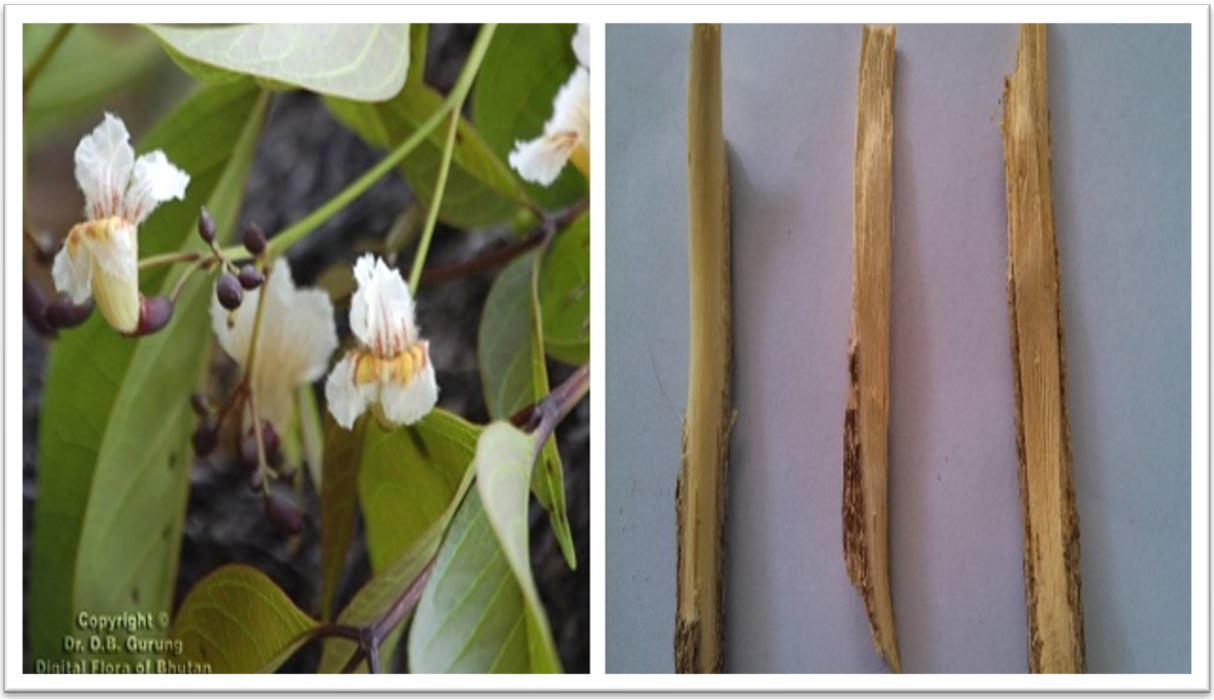

Figure 1: Leaves, flowers and Root of *stereospermumcolais*

Extraction

Extraction Procedure for root and leaves of *Stereospermumcolais*

The collected, cleaned and powdered leaves of plant *Stereospermumcolais* Buch. were used for the extraction purpose .500gm of powdered material was evenly packed in the Soxhlet apparatus. It was then extracted successively with various solvents of increasing polarity such as petroleum ether,chloroform and methanol. Each time plant material was air dried to remove the residual solvent.

Principle of the Assay

In any in vitro model XTT Cell Proliferation Assay Kit gives an easy-to-use tool for studying inference and inhibition of cell proliferation. The assay is mainly depending on the extracellular reduction of XTT by NADH produced in the mitochondria by trans-plasma membrane electron transport and an electronmediator[5]. XTT gives formazan (a water-soluble compound) which is to be dissolved directly into the culture medium, which eliminates the need for a further solubilization step.

Reagent preparation

Reagents

1. XTT reagent (vial 1)
2. Electron mediator solution (vial 2)

Procedure

The electron mediator solution was thawed before use then it was used to reconstitute the entire vial of XTT reagent. mix well. If the entire vial of reconstituted XTT reagent will not be utilized in a single experiment, it is recommended that you aliquot and store it at -20^{0}C. When it will be stored at -20^{0}C, the reconstituted XTT reagent can be stable for

several months. During entire process avoid repeated freeze/thaw cycles.

Cells

- MOLT-4, a (human T lymphoblastic leukemia cell line).
- MCF7, a (human breast carcinoma cell line) was obtained from the NCCS (National Centre for Cell science), PUNE. These cell lines were maintained in RPMI1640 supplemented with 10% (v/v) foetal bovine serum.

Plate Set Up

There is none specific pattern for utilizing the wells on the plate. A typical experimental plate will include wells without cells, wells with cells treated with experimental compounds and wells of untreated cells. We recommend that each treatment be performed in triplicate and that you record the contents of each well on the template sheet.

Assay procedure

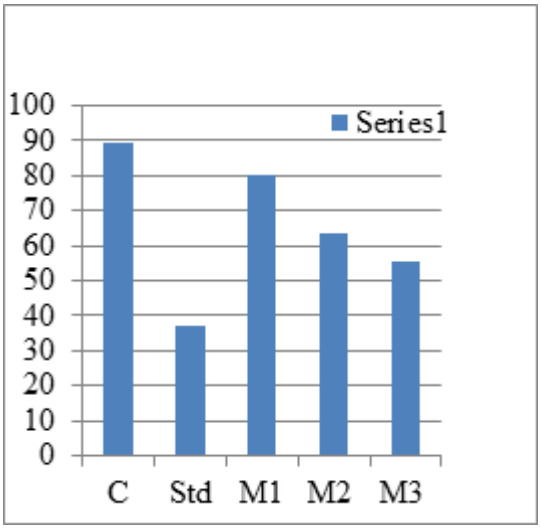

Figure 2: Effect of methanolic extract of root of SCB on MCF7cell line viability

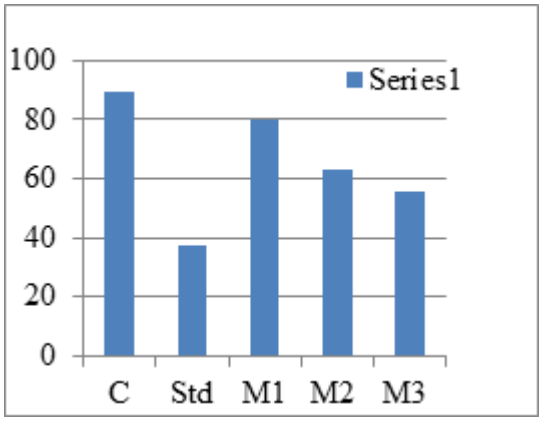

Figure 3: Effect of MERSC on MOLT-4 cell line viability

- Cells were seeded in a 96-well plate at a density of 10^4-10^5cell/well in 100µl of culture medium with or without compounds to be tested. Then cells were cultured in a CO_2 incubator at 37^0C for 24-48 hours.
- 10µl of the reconstituted XTT mixture was added to each well using a repeating pipetting.
- Mixed gently for one minute on an orbital shaker.
- Incubated the cells for two next hours (adherent culture) to four hours (suspension culture) at 37^0C in a CO_2 incubator.
- Before reading the plate, it was mixed gently on orbital shaker for one minute to ensure homogeneous distribution of color.
- Measured the absorbance of each sample using a microplate reader at a wavelength of 450 nm.
- Absorbance is directly proportional to the number of the live cells in culture. The relative

cell viability in percentage was calculated by using formulae given as follows[6,7].

Table 1: Composition of Assay Mixtures for XTT

Gr. no.	Name	Composition
1	MCF7 control	MCF 7+culture medium+ PBS+ XTT mixture
2	MCF7standard	MCF 7+ culture medium+ 5-Flurouracil+ XTT mitxure
3	MCF 7 M_1	MCF 7+ culture medium+ M_1 solution+ XTT mixture
4	MCF 7 M_2	MCF 7+ culture medium+ M_2 solution+ XTT mixture
5	MCF 7 M_3	MCF 7+ culture medium+ M_3 solution+ XTT mixture
6	MOLT-4 control	MOLT-4 + culture medium+ PBS+XTT mixture
7	MOLT-4standard	MOLT-4 + culture medium+ 5-Flurouracil+XTTmixture
8	MOLT-4 M_1	MOLT-4 + culture medium+ M_1 solution+ XTT mixture
9	MOLT-4 M_2	MOLT-4 + culture medium+ M_2 solution+ XTT mixture
10	MOLT-4 M_3	MOLT-4 + culture medium+ M_3 solution+ XTT mixture

All above mixtures were prepared and absorbances were recorded in triplicate.
Standard - 20µg/ml stock solution
M_1-Methanolic extract- 10µg/ml solution
M_2- Methanolic extract- 100µg/ml solution
M_3- Methanolic extract- 500µg/ml solution

Percentage cell viability =

$$\frac{\text{Absorbance of drug treated cells}}{\text{Absorbance of control cells}} \times 100$$

RESULTS AND DISCUSSION

SUMMARY AND CONCLUSION

XTT Assay is an internationally accepted in vitro method for anticancer drug screening. Percentage of viable cells can be measured by intensity of orange colored formazan produced is directly proportional to the cell viability. In the present anti-cancer study, two types of human cell line such as MCF7, A Human breast carcinoma cell line and MOLT-4, a human T lymphoblastic leukemia cell line used and adjusted to density of 10[4]-10[5]. It was observed that extracts from

Table 2: Effect of Methanolic extract of root of SCB on MCF7cell line by viability Assay

Group no	Design of Treatment	% viability
1	Control	100
2	Standard solution(20µg/ml)	26.2
3	M_1solution(10µg/ml)	87.7
4	M_2solution (100 µg/ml)	71.9
5	M_3solution (500 µg/ml)	59.9

Values are expressed as mean ± S.E.M., ** (P<0.01), Dunnett's test compared with MCF7 control.
Standard - 20µg/ml stock solution
M_1- MERSC 10µg/ml solution
M_2- MERSC 100µg/ml solution
M_3- MERSC 500µg/ml solution

Table 3: Effect of Methanolic extract of root of SCB on MOLT-4 cell line by viability Assay

Group No	Design of Treatment	% Viability
1	Control (MOLT-4)	100
2	Standard solution(20µg/ml)	27.2
3	M_1solution(10µg/ml)	86.7
4	M_2solution (100 µg/ml)	81.4
5	M_3solution (500 µg/ml)	64.9

Values are expressed as mean ± S. E. M., ** (P<0.01), Dunnett's test compared with MOLT-4 control.

root and leaves of*Stereospermumcolais*Buch showed dose dependent % cell viability in MOLT-4 and MCF-7 in vitro model. DEPO at doses10, 100 and 500 µg/ml elicited 87.7, 71.9, 59.9 % cell viability respectively. While CEPO at doses 10, 100 and 500µg/ml exerted 86.1, 74.6 and 63 % cell viability respectively. This indicated that with increased concentration of extract there was increase in cytotoxic activity.

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