Research Article

Cytotoxic Effects of Luteolin Isolated from *Feronia limonia* Linn.

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ABSTRACT

Aim: To evaluate cytotoxic properties of *Feronia limonia* Linn. in different test systems. **Methods:** In the present study, we have evaluated germination of green grams, viability of yeasts and potato disc assay for rapid and inexpensive screening of drugs exhibiting cytotoxic properties of the isolated compound in comparison with the standard drug. Seed germination parameters- rootlet growth and inhibition were assayed. Yeast viability by spectrophotometric and methylene blue staining method. **Results:** Zone of inhibition study against *Agrobacterium tumefaciens* and potato disc assay has shown a distinct interference of plant extracts against tumor formation and bacterial growth, there by its effect is attributing to antitumor and growth inhibitor properties. **Conclusion:** This screening test helps in identifying the potential plant types that can be used for medicinal purposes against specific microbe and also as a nutritional supplement for fighting against the free oxide radicals.

Key words: *Agrobacterium tumefaciens,* Herbal extracts, Potato disc, Seed germination, Yeast.

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INTRODUCTION

At present, cancer is the serious health problem in most of the developed and developing countries. Natural and synthetic drugs are being used to treat the cancer. However, due to the resistance of different allopathic medicine natural source is still preferred as they contain effective different chemical moiety such as flavonoids, triterpenoids and steroids[1-3] having pharmacological activities like Anti-ulcer,[4] Antihyperlipidemic,[5,6] anti-oxidant[7,8] and cytotoxicity.[9]

A number of plant extract exhibiting cytotoxic properties are used for the treatment of various cancers which interferes with the cell-cycle kinetics by inhibiting the proliferation of active mitotic cells affecting DNA damage during S-phase, formation of mitotic spindles in the M phase of the cell cycle,[10] microtubule dynamics and signaling pathways that finally leads to the mitotic arrest or apoptotic cell death.[11] Although there are plenty of anticancer agents are available, yet a continuous search for new compounds is being made for more efficient and safe drugs. Different experimental models are commonly used for the evaluation of anticancer and cytotoxic compounds involving *in vivo* studies that include xenografts in nude mice, carcinogen induced tumors in rodents, transgenic and knockout mice, *in vitro* studies on tumor cell lines,[12] plant and other test systems. Yet, there is a great emphasis on *in vitro* studies for the initial screening followed by validation in the animal model. A common model that has been widely used is the root tip meristem of onion which is a tissue of actively dividing cells.[13] However, due to chemical treatment of the bulbs for long term storage, this model poses problems in growing root tip meristems. The present study was therefore carried out using *Feronia limonia* Linn. with the aim of developing a rapid and inexpensive cytotoxicity test for the preliminary screening of new drugs.

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MATERIALS AND METHODS

Bioactive compound was isolated from fruits of *Feronia limonia*. The compound was identified as Luteolin using characterization methods was used in this study. The test sample Luteolin was dissolved in 30 % of Dimethyl sulfoxide.[14]

Yeast growth and viability assay

Saccharomyces cerevisiae was cultured in medium containing 1 % yeast extract, 2 % glucose, 2% peptone and test sample at 35°C. After incubation time, cell growth was studied using the spectrophotometer. Optical density of treated yeast cultures with different concentrations of Luteolin was recorded at wave length of 600 nm.[15]

Equal volume of methylene blue solution and yeast sample was mixed on a microscopic slide. Dead and living cells were counted by using the optical microscope. The experiment was carried out in triplicate. Viability was calculated using the following formula.[16]

$$\text{Total Cells} = \frac{(\text{Total Cells}) - (\text{Dead Cells})}{\text{Total Cells}} \times 100$$

Potato disc inhibition assay

The antitumor activity of the test compounds was evaluated by potato disc inhibition assay. Potato discs of 10 mm diameter and 0.5 mm thickness were used. *Agrobacterium tumefaciens* was cultured in Yeast extract media for 2 days at 28°C and then inoculated onto the potato discs aseptically. Each disc was placed on the surface of agar media and incubated at 28°C. The test samples were prepared using 1 mL of Luteolin; 1 mL of bacterial suspension and 0.25 mL of water. The control solution was prepared by adding 1.25 mL of water and 1 mL of bacterial suspension. An aliquot of 50 µL of solution was placed on the disc and incubated at 25°C for 10 days. On day 11, the discs were stained with Lugol's reagent (I_2 KI; 5 % I_2 and 10 % KI in distilled water).[17] The stained potato discs were viewed under10x dissecting microscope and the number of tumors were counted. The experiment was carried out in triplicate.

Phytotoxicity assay

Root length and seed germination parameters were used to assess the phytotoxicity. Whatman No. 1 filter paper was placed in a petri dish, 5 mL Luteolin, 5 mL of water was added. 10 seeds were placed on filter paper. The petri dish was tightly sealed and incubated at 23±2°C. Root length and germination was measured each day for 5 days. Petri dish containing only water was used as control. Each experiment was carried out in triplicate. A graph of root length against number of days and percentage of seed germination against number of days was plotted in comparison to the root length of the control sample used.

$$\%\ \text{inhibition of the root length} = \frac{\text{Root length in test sample}}{\text{Root length in control}} \times 100$$

RESULTS

According to the obtained results there was no significant difference in growth of yeast cells than the control sample in concentration of 0.5 mg/mL of the test compound but this difference is significant in concentration of 1.0 mg/mL ($p < 0.05$). Results obtained from staining with methylene blue showed that 0.5 and 1.0 mg/mL concentrations of *F. limonia* compound decreased viability of *Saccharomyces cerevisiae* cells up to 24 % and 46 %, respectively in comparison to the standard drug which decreased to 52 %. Effect of *F. limonia* plant isolated Luteolin[18] on wild-type growth (OD 660 nm) is shown in the Figure 1.

The tested compound had inhibited tumor formation in the potato disc. None of the samples were able to inhibit the growth of *A. tumefaciens*. The tested fractions did not affect the viability of the *A. tumefaciens* growth, but have shown the most inhibitory tumors effects with IC_{50} = 1.0 mg/mL. The Luteolin sample was able to inhibit tumor formation of 34 % in the concentration of 0.5 mg/mL and 54 % in the concentration of 1.0 mg/mL.

In seed germination assay, the suppression is 52 % for compound at concentration of 1.0 mg/mL when compared to the control and most significant with standard drug being observed. The gradient of root length suppression however varies over time over a range of 5 days and most significant results are observed for fourth and fifth day of the study. However, at concen-

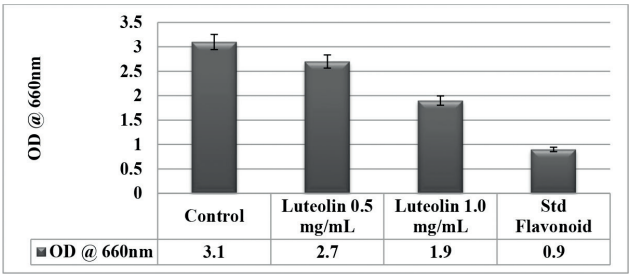

Figure 1: Growth of wild-type at different concentrations. The growth rate of yeast cells was measured in the presence of test compounds at 660 nm.

tration of 0.5 mg/mL a relatively lesser effects on root elongation is observed.

The isolated compound of *F. limonia* had shown a distinct inhibition of root elongation in terms of branch and root hair formation and main tube elongation represented in Figure 2. The test compounds on 4th day at concentrations of 0.5 and 1.0 mg/mL has shown drastic inhibition of 20 % and 32 % on seed germination in contrast to the control (Figure 3). The effects are significant for earlier days of germination until third day and at the day 4 and 5, the effect is almost nullified and the germination rate is same as that of control.

DISCUSSION

Saccharomyces cerevisiae is a brilliant model system in order to identify the compounds possessing anti-proliferative property and the mechanism of these compounds. Compounds having the anti-proliferative activity are the basic for anti-cancer and antifungal property.[19] Synthetic drugs can be replaced by plant drugs since they have significant effect with or without side effects. With this view we aimed to study for the therapeutic property of *F. limonia* particularly through *in vitro* assays. Based on the results of this study the isolated compound inhibits growth significantly and

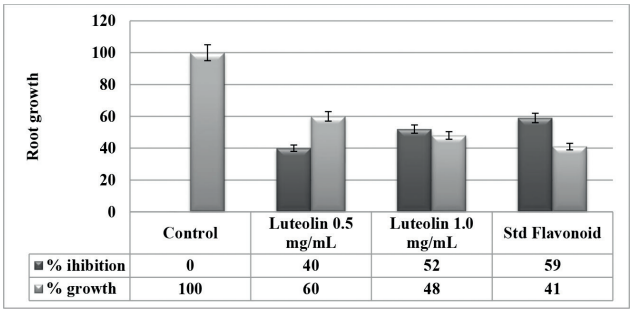

Figure 2: The growth rate of root in the presence of test compounds on 5th day.

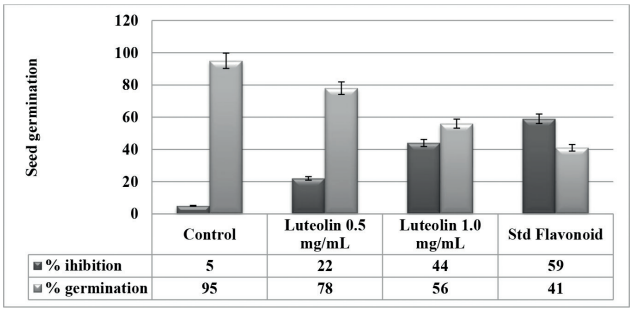

Figure 3: The rate of seed germination in the presence of test compounds on 5th day.

decreases survival of *Saccharomyces cerevisiae* cells thus confirms the anti-proliferative effect of *F. limonia*. Essential oil of the *Matricaria* plant showed that the compounds like flavonoids and α-bisabolol have antifungal effect on the *Candida albicans* yeast.[20] *Matricaria* hydroalcholic extract has antifungal property on *Candida albicans*. This effective concentration on *Saccharomyces cerevisiae* and *Candida albicans* may indicate that the anti-proliferative property of extract is more for the *Saccharomyces cerevisiae* yeast than the *Candida albicans* yeast. In present study *F. limonia* has decreased cell survival rate. Other studies conducted on the *Candida albicans* yeast did not yield the anti-proliferative property.[21] This difference in results can be caused by difference in extraction methods or difference in studied parts of the plant.

The activities of examined samples are based on the tumor inhibitory formation rather than the viability of the micro-organism. It is known that the capacity of inhibiting the formation of crown gall produced by *A. tumefaciens* in the potato disc and also consequent growth was in good correlation with the compounds present in the extract. The tumor produced which is similar histologically to animal or human ones. Thus the process of tumor induction by Ti-plasmid is the result of cell proliferation and blocking of apoptosis like in animal or human cancer cells.[22] Thus the crown gall assay or potato disc assay acts as the pre-screen for detecting novel plant based compounds for anti-tumor activity with minimal technical requirements.

The elongation of root is a significant marker of developmental stage of a plant and continues throughout the lifetime with differential rates depending on the system requirements. The isolated compound of *F. limonia* had shown a distinct inhibition of root elongation in terms of branch and root hair formation and main tube elongation. This action of test compound indicates the cross talk between the metabolites of plant and metabolic byproducts of seed germination. However, a relatively lesser effects on root elongation are observed. Phytotoxicity of plant extracts on root elongation thereby marks an inhibitory action on growth potential. Seed germination bioassay is one of the widely employed assays for phytotoxic activity.[23,24]

The present study explores the potent anti-proliferative activity which may be either because of a direct cytotoxic effect of the extract on normal cells or restriction of cell division in normal cell cycle. The test compounds used in the experiment has interfered with normal seed germination metabolism and later gets diluted and degraded by the byproducts of germination process.

CONCLUSION

The compound Luteolin isolated from *Feronia limonia* bears a high cytotoxic potential in terms of the phenotypic and chemical mode. The outcome of the present experiment therefore indicates that the Luteolin at two different concentrations regarded as a source for the development of anti-tumor agents. Further use of this plant, in designing several drugs targeting for bacteria and other microbes as bio-fertilizer or bio-pesticide for agricultural purposes is well supported.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

mg: Milligram; **mL:** milliliter; **µL:** Microlitre; **nm:** Nanometer; **°C:** Celsius; **OD:** Optical density.

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