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In vitro studies of the anticancer action of *Tectaria cicutaria* in human cancer cell lines: G_0/G_1 p53-associated cell cycle arrest-Part I

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ABSTRACT

Objective: The rhizome of Tectaria cicutaria has been used in Indian traditional medicine for the treatment of various disorders. The objective of present investigation is to screen various extracts of the rhizomes of *Tectaria cicutaria* for anti-cancer activity and to investigate the mechanism involved.
Materials and methods: The rhizomes of *Tectaria cicutaria* were extracted with different solvents. In vitro anti-cancer activity of different rhizome extracts were studied in Human cancer Cell Lines using Sulphorodamine B (SRB) colorimetric cytotoxicity assay. The effect of ethanolic extract (TCe) on cell growth inhibition, modulation in gene expression, and induction of apoptosis using the K562 human leukemia cell line were studied. The extract was analyzed by GC-MS to identify their major chemical compounds.
Results: TCe shows antioxidant potential in both DPPH scavenging assay and reducing capacity. Flow cytometric analysis showed that 11 μg/ml of TCe arrested cell cycle progression at the G_0/G_1 phase. In the TCe treated K562 cells, the mRNA and protein expression level of p53 was strongly up-regulated in reverse transcription polymerase chain reaction. Furthermore, its downstream target p21 level was also increased. The GC-MS study has depicted results with the presence of twelve different compounds which will require significant further efforts for structure and putative identification.
Conclusion: The present work has for the first time, tried to elucidate the anti leukemic potential of *Tectaria cicutaria*. TCe was more potent in K562 cells, altering the cell cycle progression and inducing apoptosis.

1. Introduction

For prevention and treatment of several diseases plant and plant extracts have been widely used all over the world. According to recent studies conducted by the World Health Organization (WHO), about 80% of the world's population relies on traditional medicine.[1] Traditional medicine involves the use of plant extracts as well as their active principles. Indeed, several anti-cancer agents derived from plants have been discovered in the last decades.[2,3] Besides, many anticancer agents obtained from plant source have achieved pre-clinical or clinical development.[4,5]

Tectaria cicutaria (L.) Copel is a species of fern in the Tectariaceae family. Its rhizomes are short (Fig. 1), erect and dark brown or black in color with distinctive hard thick leaf bases. This fern is found all over in India as well as in the tropics throughout the world. It is known as *Kombadnakhi* in the local language. In Ayurveda rhizomes of *Tectaria cicutaria* has been used for the treatment of wide variety of disorders and conditions such as chest complaints, sprain, rheumatic pain, burns, poisonous bites,[6] toothache, gum complaints, and diarrhea.[7] In folklore medicine, it is also used in clinical conditions like tonsillitis, mental disorders, and obesity. Decoction as well as an infusion is used for the treatment of different types of gynecological disorders and inflammatory conditions in Ayurvedic medicine. So far there is no studies available pertaining to anti-cancer activity of *Tectaria cicutaria*.

In view of this fact, the present study was aimed to investigate the phytochemicals present in the *Tectaria cicutaria,* in vitro anti cancer activity of rhizome extracts of *Tectaria cicutaria* on different

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List of abbreviations

RT-PCR	Reverse transcription polymerase chain reaction
TCe	Ethanolic extract of *Tectaria cicutaria* rhizome
TGI	Total growth inhibition
GI_{50}	Growth inhibition of 50%
PBS	Phosphate-buffered saline
PS	Phosphatidylserine
FACS	Fluorescence activated cell sorting
TE buffer	Tris/EDTA buffer
Annexin FITC	fluorescein isothiocyanate

human cancer cell lines and further to understand the underlying molecular mechanism.

2. Materials and methods

2.1. Collection of plant material and preparation of extracts

Tectaria cicutaria was collected from Koyana Wildlife Sanctuary, Western Ghats of Sahyadri hills, Satara, India. The species of fern were identified with the help of experts using the standard floras.[8–10] Identification of the fern was confirmed by Dr. S. S. Sathe, Head/Dept of Botany, Padmabhushan Dr. Vasantraodada Patil Mahavidyalaya, Tasgoan, Maharashtra, India. Washed rhizomes were dried under shade, ground into powder and used for successive extraction. Coarse powder was subjected to hot successive continuous extraction in Soxhlet apparatus. 50 gm of powder was extracted with petroleum ether (400 mL). It was sequentially extracted with solvents of increasing polarity (ethyl acetate, ethanol and water). Each time before extracting with the next solvent, the material was dried at room temperature. The extracts were filtered and evaporated to dryness. Yield of the extract was recorded and extracts were stored at 4°, until analysis. These extracts were tested for the presence of active phytochemicals viz: tannins, alkaloids, phytosterols, triterpenoids, flavonoids, glycosides, saponins, carbohydrates, proteins, amino acids and fixed oils & fats using standard tests.[11]

Fig. 1. Rhizome of *Tectaria cicutaria.*

2.2. DPPH radicals scavenging and reducing power assay

DPPH radical is a widely used method to evaluate the free radical scavenging ability of natural compounds. This assay is based on the measurement of the scavenging ability of antioxidant substances toward the stable radical. 1.0 mL of various concentrations of extracts (10–50μg/mL) was mixed with 1.0 mL of 0.8 mmol/L DPPH solution. Each mixture was kept in the dark for 30 min and the absorbance was measured at 517 nm against a blank.[12] Ascorbic acid was used as standard. The inhibition percentage for scavenging DPPH radical was calculated according to the equation:

$$\%\text{decolorization} = \left[1 - \left(\frac{\text{AB sample}}{\text{AB control}}\right) * 100\right] \quad (1)$$

The reducing power ability of the extracts was evaluated by the method described by Oyaizu. The reaction mixture contained 1.0 mL of TCe (20–100 μg/mL), 2.5 mL of 1% potassium ferricyanide and 2.5 mL of 0.2 mol/L sodium phosphate buffer. The mixture was incubated at 50 °C for 30 min and the reaction was terminated by the addition of 2.5 mL of 10% trichloroacetic acid, followed by centrifugation at 3000 rpm for 10 min. 2.5 mL of the upper layer was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride and absorbance was measured at 700 nm against blank that contained distilled water and phosphate buffer. Increase in absorbance indicates increased reducing power of the sample. BHT was used as standard.[13]

2.3. Determination of cell proliferation in vitro (sulphorodamine B assay)

K562 (Human Leukemia Cell Line), KB (Human Nasopharyngeal Cancer Cell Line), HT29 (Human Colon Cancer Cell Line) and Colo205 (Human Colon Cancer Cell Line) were used for screening the anticancer activity of the different extracts of *Tectaria cicutaria* using the Sulphorodamine B (SRB) colorimetric cytotoxicity assay. The extracts were dissolved in DMSO and tested at concentrations of 10, 20, 40, 80 μg/ml. Adriamycin (Doxorubicin) was used as a positive control at the same concentration range. Cytotoxicity was expressed as the percent viable cells relative to cells incubated in the presence of 0.1% DMSO vehicle control. Growth inhibition of 50% (GI_{50}) was calculated from drug concentration resulting in a 50% reduction in the net protein increase. Each measurement was performed in triplicate.[14]

2.4. Apoptosis detection

The cells (1×10^6) were harvested and washed twice with ice-cold PBS. Subsequently, the cells were labeled with Annexin V and Dead Cell assay kit according to the manufacturer's instructions. This assay is based on the phosphatidylserine (PS) detection on the apoptotic cells surface, using fluorescently labeled Annexin V. The samples were determined by the Muse Cell Analyzer (Millipore, USA) and analyzed by software provided by Merck Millipore.

2.5. Cell cycle analysis

Analysis of DNA content and cell cycle distribution was done using muse cell cycle kit by flow cytometry. For fluorescence activated cell sorting (FACS) analysis, the treated K562 cells were incubated for 24 h. After overnight incubation cells were detached and pipetted out and spun at 300 × g for 5 min and washed once with 1 × Phosphate-buffered saline (PBS). The cells were fixed with 1 ml of ice cold ethanol and incubated at -20 °C for overnight. The

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