



## Apoptotic Activity of *Acorus calamus* on Oral Cancer Cell Lines

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### ABSTRACT

*Acorus calamus* is a plant used in traditional medicine. The *Acorus calamus* most extensively investigated phytochemically and pharmacologically. Cancer is characterized by a rapid and uncontrolled formation of abnormal cells. Apoptosis or programmed cell death is a highly organised physiological process to eliminate damaged or abnormal cells. Extractives of different parts of *Acorus calamus* and calamus oil are widely used now in pharmaceuticals, traditional systems of medicines for a number of ailments *Acorus calamus* decreased cell viability in malignant cells in a concentration dependent manner.

**Keywords:** *Acorus calamus*, traditional medicine, apoptotic activity.

### INTRODUCTION

In the normal situations, the cells grow and divide as the body needs them. This process is disturbed when a new cell from the body does not require and old cells don't die when they should. These extra cell lumps together form a growth or tumour<sup>1</sup>. Cancer is characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumour, or proliferate throughout the body, initiating abnormal growth at other sites. If the process is not arrested, it may progress until it causes the death of the organism<sup>2</sup>. APOPTOSIS or programmed cell death is a highly organised physiological process to eliminate damaged or abnormal cells. It also plays a major role in embryogenesis where apparently normal cells undergo apoptosis. It is involved in maintaining homeostasis in multicellular organisms<sup>7</sup>.

Since long times plants have been provide essential nutritional values, medicinal properties and physiological effect to life and are a good source of food. Traditional medicine refers to the application, approach, knowledge and belief in incorporating plant and animal based properties in remedies for the purpose of treating or preventing disease as well as to maintain the well-being of an individual. Herbal remedies have been used to cure a variety of disorders like diabetes, sexual malfunction, and urinary tract infections in females, cardiovascular diseases, and weight control and used to cure many other ailments. Plants have a long history of use in the treatment of cancer. Plants have played an important role as a source of effective anti-cancer agents and it is reported that over 60% of currently used anti-cancer agents are derived from natural sources, including plants, marine organisms and micro-organisms<sup>1</sup>.

*Acorus calamus* (*vasambu*) Ayurvedic medicine is documented for treatment of insomnia, neurosis, and remittent fevers. Extractives of different parts of *Acorus*

*calamus* and calamus oil are widely used now in pharmaceuticals, traditional systems of medicines for a number of ailments *Acorus calamus* decreased cell viability in malignant cells in a concentration dependent manner.

$\beta$ -asarone an important chemical constituent of *A. calamus* has also been reported to possess antibacterial activity. The synergistic anthelmintic activity of *A. calamus* and *Vitex negundo* was studied by Merekar *et al.*<sup>4</sup> the induction of apoptosis leads to an activation of caspases. These enzymes are cysteine proteases that are produced by the cell as inactive pro-enzymes. After activation the pro-caspase is hydrolysed to its active form, caspase. Caspases belong to a family of proteases which are responsible for the cell death induction. The activation of the caspase induces the cell fragmentation and thus the development of "apoptoticbodies"<sup>10</sup>.

Many of the plants have been studied scientifically and proved to be beneficial anti-cancer agents. In medicine, particularly in the field of cancer, the use of herbs is increasingly enhanced especially with the excessive use of synthetic drugs and awareness of their toxicity, which contributed in oncology, leading to a favourable reconsideration of the medicinal practices made from natural herbal. Despite the divergent bioactivities of the plant medicines against various diseases, active components of most plant extracts have not been elucidated thoroughly, due their complex mixtures. The ability of agents to attach to carrier molecules directed to specific tumours, shows highly cytotoxic natural products to the tumours<sup>1</sup>.

### MATERIALS AND METHODS

#### Preparation of *Acorus calamus* extract

Powdered *Acorus calamus* was added to the solvent dichloromethane and allowed to settle for some time.



This was done to increase the efficiency of the extraction process.

### Maintenance of KB cell lines

Cell culture flasks were selected by the method of confluency. They were observed under inverted microscope. In order to maintain cell line, the technique of subculturing was performed. This is to facilitate cell growth by extraction of cells from the existing medium and placing them into a fresh new medium. For cell maintenance, enzymatic methods using TPVG were prevalently used. Growth medium is then extracted completely from the flasks and the cells were further subjected to incubation at 37°C after the addition of the enzyme. This, initially, detach the cells from the surface.

### Treatment of KB cells with *Acorus calamus* extract

Aliquot of the extract (125 micro grams and 75 micro grams) was added and incubated with KB cell lines.

### Isolation of DNA

1\*10 to the power of 6 cells were incubated with 100µl of cell lysis buffer at room temperature for one hour. This was centrifuged for 15 min at 3000rpm at 4°C to sediment the cell debris. To the supernatant equal volume of phenol: chloroform: isoamylalcohol mixture was added to the supernatant and mixed well. This was centrifuged at 5000 rpm for 15min. The supernatant was transferred to new tube. The 3rd step was repeated once. To the final aqueous phase 40µl of 3.5M ammonium acetate was added, to this ice cold isopropanol was added to precipitate the DNA. This was incubated at -20°C for 1hour, followed by the centrifugation at 10000 rpm for 15min. The pellet was retained and washed with 70% ethanol and stored in 20- 50µl of TE buffer. The samples were analysed in 2% agarose gel stained with Ethidium bromide.

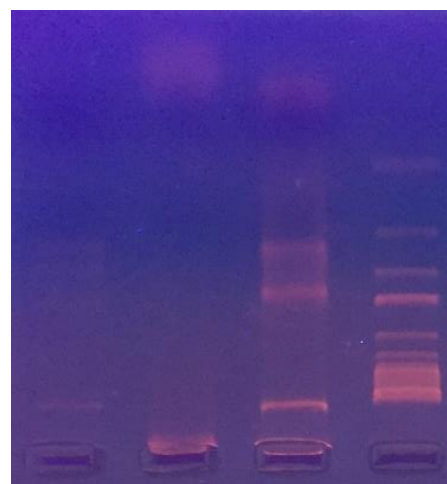
### DNA fragmentation by agarose gel electrophoresis

The extracted DNA is loaded to Agarose gel with the loading dye, DNA fragments was visualised under UV transilluminator. DNA fragmentation was observed with all the three concentrations of *Acorus calamus* extract on oral cancer cell lines by agarose gel electrophoresis method. Apoptosis has been characterised biochemically by the activation of a nuclear endonuclease that cleaves the DNA into multimers of 180-200 base pairs and can be visualised as an 'oligosomal ladder' by standard agarose gel electrophoresis. This proves that *Acorus calamus* extract shows apoptotic activity on the oral cancer cells by degrading its DNA. Hence *Acorus calamus* has the potential to be an anti-cancerous drug.

### RESULTS AND DISCUSSION

The conventional anticancer drugs which are being used, acts on both normal cells and tumour cells, causing brutal side effects and tumour resistance. Anticancer activity by apoptotic induction by herbs doesn't show any side effects.<sup>10</sup> Natural herbs can be used extensively to

prevent and treat cancer. Caspases are responsible for the programmed cell death or apoptosis. It begins by the activation of intrinsic and extrinsic pathways. Once activated these destructive proteases proceed to systematically deconstruct the cell to ensure its effective removal without damage to surrounding cells and tissues. Hence *Acorus calamus* helps by producing these caspases to induce apoptosis on the oral cancer cell line.<sup>9</sup>



Lane 1 – 1 kb ladder

Lane 2 – DNA from cells treated with 125 µg sample

Lane 3 – DNA from cells treated with 75 µg sample

Lane 4 – DNA from untreated cells

### CONCLUSION

The mechanism that can activate caspases may therefore represent a possibly feasible approach for effective tumour treatment which has several advantages over both conventional therapies and the more current "designer" approaches. The secondary metabolites from herbs are always promising with antioxidant and anticancer activity. The ability of apoptotic induction by *Acorus calamus* extract can be utilised in anticancer formulation.<sup>10</sup> Anticancer and antioxidant property of certain medicinal herbs can therefore be used to treat trauma over a longer period of time which is always very promising. Phytochemicals present in this herb may have antioxidant property. Therefore, *Acorus calamus* may be used in the treatment of cancer.<sup>9</sup>

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