Cytotoxic Activity of Ethanolic Extract of *Myristica Fragrans* (Houtt) Against Seven Human Cancer Cell Lines

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Abstract The objective of the present study was to evaluate the *in-vitro* anti-cancer effects of ethanolic extract of seed of *Myristica fragrans* (Houtt) against seven human cancer cell lines ascribed to central nervous system, Colon cell (Colon502713, Colo205), Liver (Hep-2), Lung (A-549), Ovary OVCAR-5 and Prostrate (PC-5). The SRB assay was used to test cytotoxic activity for all the cell lines. The activity was evaluated at 100µg/ml concentration of test material. OVCAR-5 (Ovary) cell line showed highest cytotoxic activity (97%) Growth inhibition of colon 502713 and PC-5 (Prostrate) cell lines was 86%. SF-295(CNS) and Colo205 showed 73% growth inhibition, while Hep-2 (Liver) showed 72%. A-549 (Lung) showed no significant activity (50%). Our results indicate that the plant has a promising anti-cancer activity. The ethanolic extract of plant showed highest anti-cancer activity against OVCAR-5. Further investigation for active compound present in *Myristica fragrans* is required for cancer management and for the development of new anti-cancer drugs.

Keywords *Myristica Fragrans* (Houtt), Ethanolic Extract, OVCAR-5

obtained from the seeds is a popular condiment used as a spicing agent. The seeds are embedded in a white sweet-smelling pulp and are most economically important part of the tree. They are aromatic and are used after grinding to a powder as a condiment in food providing flavor resembling that of nutmeg (*Myristica fragrans*). They are also used as an aromatic stimulating additive to medicine and snuff. The nutmeg seed is one of four components of the fruit obtained from the nutmeg tree, *Myristica fragrans* Houtt (*Myristicaceae*). About 30-55% of the seed consists of oils and 45-60% consists of solid matter including cellulose materials. There are two types of oils: The "essential oil of nutmeg" also called the "volatile oil" accounts for 5-15% of the nutmeg seed and the "fixed oil of nutmeg" sometimes called "nutmeg butter" or expressed oil of nutmeg accounts for 24-40% of the nutmeg seed. The relative percentages of the different components will vary depending on the geographical origin of the nutmeg. In the present study we analyzed the cytotoxic activity of ethanolic extract of nutmeg for possible anti-cancer properties against several cells by SRB method.

1. Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain bioactive components of therapeutic value. *Myristica fragrans* is getting attention as a new avenue in treating various diseases. Nutmeg has been shown to possess a spectrum of pharmacological activities, including anti-bacterial, anti-inflammatory, anti-cancer, anti-diabetes and hepatoprotective activities. Historically many plant oils and extracts, such as ginger, garlic, curcuma, tea tree and clove have been reported to have antimicrobial properties. *Myristica fragrans*, a perennial edible plant belonging to Annonaceae family, is a berry that grows wild in the evergreen forests of West Africa and India. The seeds are economically and medicinally important. The kernel

2. Materials and Methods

2.1. Preparation of Ethanolic Extract from *Myristica Fragrans*

The seed was ground (500g) to a fine powder and was placed in a glass percolator of appropriate size. Sufficient quantity of solvent was added to submerge the plant material. After standing for about 16 h, percolate was collected and filtered, if necessary. The process was repeated four times for exhaustive extraction of the plant material. The ethanolic extract was evaporated to dryness under reduced pressure at 50°C using rotavapor and round bottom flask. The final drying was done in a vacuum desiccator. The extract was transferred to glass container of appropriate size. This formed the stock Ethanolic extract.

2.2. Source of Human Cancer Cell Line

Human cancer cell lines were obtained from National Centre for Cell Science, Pune-411007(India) and National Cancer Institute, DTCD, Fredrick Cancer Research and Development Centre, Fairview centre, Suite 205, 1003, West -7th Street Frederick MD-21701- 8527 (USA)

2.2.1. Selection of Human Cancer Cell Line

The cell lines were selected in such a way that all the cell line could grow on a single growth medium (RPMI-1640) in tissue culture flask (TCP) and the mass doubling time was such that enough cell were obtained for screening. Cell which were used were free from bacteria, yeast, mould, mycoplasma and in special cases from viruses at all the stages. If contamination appeared at any stage, the stock in which it occurred was discarded immediately. Cancer of central nervous system CNS, Lung cancer cell line A-549, Colon cancer cell lines, Colo-205, Colon 502713, Liver cancer cell line, Hep-2, Ovarian cancer cell line, OVCAR-5, Prostrate cancer cell line PC-5 were taken for the study.

2.3. Procedure for *In Vitro* Cytotoxicity of Extract

Cytotoxicity of test sample was performed against seven human cancer cell lines. 96 well flat bottom tissue culture plates were taken. There were four types of well in TCP, control blank (CB, without cells, complete growth medium only) and control growth (GC, with cell in absence of test material) to determine 100% growth. The growth in the presence of test material was determined from the difference of test growth (GT, cell with test material) and test control (CT, test material without cells).

The desired human cancer cell lines were grown in tissue culture flask at 37°C in an atmosphere of 5% CO₂ and 90% relative humidity in complete growth medium to obtain enough number of cells. The cells were harvested by the treatment of trypsin-EDTA and complete growth medium added. Viable cells were counted in haemocytometer and mortality checked by using trypan blue. Viable cell density was adjusted to 5000-40,000 cells/100µl depending upon the cell line (Monks *et al* 1991). Next, cell suspension 100 µl was added to each. Complete growth medium was added and incubated at 37°C for 24 h in an atmosphere of 5 % (v/v)CO₂

and 90 % (v/v) relative humidity in a CO₂ incubator. After 24 h, test material was added. Plates were incubated at 37°C for 48 h. The growth was determined after 48 h by SRB assay described below.

2.3.1. SRB Assay

SRB assay was carried out as described by Skehan *et al.*, 1990, using SRB dye. After 48 h incubation of cells with test material, the plates were taken out and 50µl of chilled 50% (w/v). TCA was gently layered on top of the medium in all the wells to produce a final concentration of 10%. Subsequently, tissue culture plate was incubated at 4°C in a refrigerator to fix the cells attached to the bottom of the wells. After 1 h, the plates were taken out from refrigerator and all the contents of all the wells were pipetted out and supernatant was discarded. The plates were washed five times with distilled water to remove traces of TCA, growth medium, low molecular metabolites, serum protein etc. For washing, the wells of tissue culture plates were filled with distilled water and the liquid in the wells was discarded by sharply flicking plate over sink. Plates were air dried and can be stored until use. SRB solution (100µl) was added to each well of the plates and the plates were incubated for 30 minutes at room temperature. The unbound SRB was removed quickly (to avoid desorption of protein bound dye) by washing the wells of the plates five times with 1% (v/v) acetic acid. Plates were then air dried and Tris buffer (100µl /well) was added to the plates. The plates were gently stirred for 5 min on a mechanical shaker and optical density was recorded on ELISA reader at 540 nm.

3. Results and Discussion

The ethanolic extract of *Myristica fragrance* showed more than 70% growth inhibition against all human cancer cell lines namely SF-295, Colon502713, Colo-205, Hep-2, OVCAR-5 and PC-5, respectively except A-549(Table1). The maximum cytotoxicity of the extract was shown by OVCAR-5 (97%). Two human cancer cell lines viz. Colon 502713 and PC-5 showed 86 % activity.

Table1. In vitro cytotoxic activity of ethanolic extract (100 µg/ml) of *Myristica fragrans* Hout against seven human cancer cell lines

Cell Line type		Myristica fragrans Hout			% Cytotoxic activity
		Plant part	Extract	Conc.µg/ml	
CNS	SF-295	Seed	Ethanolic	100	73%
Colon	Colon502713	Seed	Ethanolic	100	86%
Colon	Colon 205	Seed	Ethanolic	100	73%
Liver	Hep-2	Seed	Ethanolic	100	72%
Lungs	A-549	Seed	Ethanolic	100	50%
Ovary	OVCAR-5	Seed	Ethanolic	100	97%
Prostrate	PC-5	Seed	Ethanolic	100	86%

Figure 1. Growth-inhibitory effects of ethanolic extracts of *Myristica fragrans Houtt* against seven human cancer cell line. Cytotoxic activity was determined using SRB assay.

3.1. Discussion

In recent years, considerable interest has been generated on identifying naturally occurring chemopreventive substances capable of inhibiting or reversing the multistage carcinogenesis. A wide array of phenolic substances particularly those present in dietary and medicinal plants have been reported to possess substantial anti-carcinogenic and anti-mutagenic activities. Many of the natural products from a large number of aromatic and medicinal plants have found use in household treatment of a variety of elements. These products hold a great promise as new sources of drugs that have been used effectively for centuries for traditional medicine. Medicinal properties of these plants are due to presence of some active principal that produce definite biologic activity causing interference in some essential biologic pathway.

For testing the anticancer property, extracts of *Myristica fragrans Houtt.* plant was screened by using seven human cancer cell lines by established *in vitro* cytotoxicity assays. One of the ancient herbs known as nutmeg (*Myristica fragrans Houtt*) used as a condiment and spice in Indian foods obtained from the nutmeg tree (*Myristica fragrans*), a native of the Moluccas or Spice Islands (In India, it is grown in the Nilgiris, Kerala, Karnataka and West Bengal) shows great promise for cancer therapy as it inhibited the growth of all the seven human cancer cell lines.

The maximum activity of the extract was shown against OVCAR-5 (97%). Remarkably, the extract was active against all seven cell lines. Activity was above 70% against cell lines SF-295, Colon502713, Colo-205, Hep-2, OVCAR-5 and PC-5 respectively except A-549. 86% activity was observed against two human cancer cell line viz. Colon 502713 and PC-5. *Myristica fragrans Houtt* can serve as a candidate drug for cancer therapy and may very well be exploited for cancer treatment to provide a great promise and service to cancer patients.

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