Antiproliferative activity of four *in vitro* Zingiberaceae oils using human breast cancer cell line

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Abstract

India is one of the leading producers of aromatic essential oils in the world. Cancer is one of the most lives threatening disease leading to death than any other diseases worldwide. Members of Zingiberaceae family have been used from centuries as spice ingredient and as traditional medicine in the Asian regions. Nowadays medicinal plants are used because of their vast medicinal properties and aromatic rhizome essential oils which are beneficial for anticancer activity. In the present study, four plant species like Curcuma longa, Curcuma aromatica, Kaempferia galanga and Hedychium coronarium were assessed against human breast cancer cell line MCF7 by MTT assay. To validate the cytotoxicity activity, MCF7 cell line was treated against different concentrations of rhizome essential oils ranging from 6.25-100 uL respectively.

This study revealed that Kaempferia galanga essential oil was most significantly active in comparison to other oil samples tested against MCF7 cell line. Hence the cytotoxic study of essential oils could be used by pharmaceuticals and other industry for therapeutics.

Keywords: *Curcuma longa, Curcuma aromatica, Kaempferia galanga, Hedychium coronarium*, Cytotoxicity.

Introduction

Zingiberaceae family have been reported to possess compounds with anti-inflammatory, antioxidant and anticancer properties[8,17,26]. In Asian countries, historical background of medication shows that medicinal plants and their products have been utilized in cancer treatment[1]. The ginger species are widely used traditionally for treatment of stomach problems, sore throat, cough, liver complaints, rheumatism, fever, bruises, swelling, muscular pains and other various disorders. Cancer cells have uncontrolled growth characteristics for which effective inhibition of cancer proliferation is a method for cancer therapy[13].

Previous studies have been done on natural antioxidants with respect to their protective effect against free radical damage that may be the reason of cancer. So far, the anticancer activities of essential oils from plants have attracted much interest for which the secondary metabolites of medicinal plant are well known for their bioactivity such as chemopreventive agents. *Curcuma longa* is known worldwide for use in medicine, cosmetics, food flavouring and textile industries. Several pharmacological activities of turmeric like anti-inflammatory, hepato-protective, antimicrobial, wound healing, anticancer, anti-tumor and antiviral properties are due to the presence of curcumin present in its rhizome and essential oil found both in leaves and rhizomes[28].

Curcuma aromatica is another important plant in which rhizomes are used for flavouring and as colouring agent for food preparations in many parts of the world. Its essential oil has been reported to have antimicrobial and antifungal activity and is used in the treatment of early stages of cervical cancer[4]. Due to the presence of curcumin in its rhizome, it possesses anti carcinogenic properties and is used in the formulation of anticancer drug having inhibitory effect on the growth of tumors[14].

Similarly, *Kaempferia galanga* is used in the preparation of ayurvedic drugs, perfumery and cosmetic industries and also used as spice ingredient. It possesses antioxidant, antinociceptive and anti-inflammatory activities which would help in treatment of mouth ulcers, migranes, headache, rheumatism and swollen eye infections[5,31]. *Hedychium coronarium* is a very important medicinal plant used for the isolation of diterpenes possessing cytotoxic and inflammatory activities, leishmanicidal and antimalarial activities as well as antihypertensive activities.[7,21,32,34] Due to the presence of coronarin D in its rhizome, this plant inhibits NF-kB activation induced by different inflammatory stimuli and carcinogenesis. The price of the essential oil varies in international market due to high exploitation by local people and pharmaceutical industries.

In the present study we have extracted the rhizome essential oil from micropropagated plantlets of four plant species namely *C. longa*, *C. aromatica*, *K. galanga* and *H. coronarium* (CL, CA, KG and HC) for gas chromatography and mass spectrometry (GC-MS) analysis followed by its cytotoxicity study against human breast cancer (MCF7) cell line. There are few reports on rhizome oil analysis of these important medicinal species but no report on its *in vitro* rhizome oil analysis[30].

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity based on reduction of the yellow colored water soluble tetrazolium dye MTT to formazan crystals[2,22]. Thus we report the effectiveness of four *in vitro* rhizome essential oils as an anticancer agent and its mode of cell death on MCF7 cells as targeted cell by MTT assay.

Material and Methods

Plant material collection and GC-MS analysis: The plants were collected from different areas of Eastern Ghats and were maintained in the medicinal plant garden of Centre for Biotechnology, Siksha 'O' Anusandhan University, Bhubaneswar.

The bud explants from the rhizome were thoroughly washed in running tap water with a neutral liquid detergent (Extran, Merck) for 5 minutes. Then these explants were sterilized with 0.1 % mercuric chloride for 3-5 minutes for different plant samples in a laminar flow cabinet and 3 times rinsed in sterile distilled water to remove the traces of sterilants prior to inoculation. Explant were inoculated and cultures were established on basal Murashige and Skoog (MS) medium supplemented with various combination of kinetin (Kin), benzyl adenine (BA), indole- 3-acetic acid (IAA), naphthalene acetic acid (NAA) and adenine sulphate (Ads) with 30 gm/l of sucrose and 0.8 % of agar following our earlier reports[19,20,24].

In vitro regenerated plants with well developed shoots and roots were studied for rhizome oil extraction. This essential oil was extracted by hydro-distillation using a Clevenger's apparatus following the method[12]. A flask containing 100gm of sliced rhizomes in 500ml of distilled water was heated for 6 hours and the condensed vapour was separated in oil-water surface.

The transparent oil present at the upper most layers was collected in the microcentrifuge tube through the collecting chambers. The component identification was achieved by the GC–MS analysis using HP 6890 series GC (Hewlett-Packard, USA) coupled with a mass selective detector (MSD), HP 5973 series (Hewlett-Packard). Helium was used as a carrier gas and the sample was injected in split less mode in a column HP5 phenyl methyl siloxane [25µl film thickness × 320µm internal diameter × 30 m length of the column]. Mass spectra were acquired over a 40–400 atomic mass unit range. Compounds were identified by comparing the mass spectral data with those in the NIST library provided with software and with commercially available data. Temperature programming was: initial temperature 60^0C, ramping rate 3^0C, final temperature 243^0C, run time 61 min.

Culturing of cell lines, treatment groups and MTT cell viability assays: Human breast adenocarcinoma (MCF7) cell line was procured from National Centre for Cell Science, Pune, India. The assay controls used were medium control (medium without cells), negative control (medium with cells but without the experimental compound) and positive control (medium with cells and 10uM of Camptothecin). The cells were first subcultured in dulbecco modified eagle medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, 1% glutamine in tissue culture flasks incubated in a CO_2 incubator in a 5% CO and 95% humidity atmosphere.

After trypsinization, the cell count was done and the cell viability was tested by trypan blue dye exclusion method using haemocytometer. 200µl cell suspension was seeded into 96-well plate at required cell density without the test agent and the cells were allowed to grow for about 24 hours. Then appropriate 5 concentrations of the test agent were added i.e. 6.25, 12.5, 25, 50 and 100uL/mL and plates were incubated for 24 hrs at 37°C in a 5% CO_2 atmosphere. After the incubation period, the plates were taken out from the incubator, spent media were removed and MTT reagent was added to a final concentration of 0.5mg/mL of total volume. Then the plates were wrapped with aluminium foil to avoid exposure to light and incubated for 3 hours.

After incubation, the MTT reagent was removed and 100 µl of solubilisation solution (DMSO) was added. Finally, the absorbance of the 4 test samples as well as the control was taken on an ELISA reader at 630nm used as reference wavelength. The IC50 value was calculated by using linear regression equation i.e. Y =Mx+C where Y = 50, M and C values were derived from the viability graph[10,23].

Results and Discussion

The essential oil yield found in all four samples CL, CA, KG and HC was 0.49%, 0.52%, 0.73% and 0.41% respectively which was transparent in colour. In *C. longa* ar-tumerone of about 47.3%, in *C. aromatica* ar-tumerone of about 37.45%, in *K. galanga* ethyl p-methoxycinnamate of about 58.16% and in *H. coronarium* eucalyptol of about 46.19% was found to be the major compound. There are reports on *C. longa* rhizome oil analysis who also reported ar-tumerone as the major compound having antimicrobial activities against eye infections[29].

Earlier many scientists have reported on the rhizome oil composition of *C. longa* where mostly ar-tumerone was the major compound[9]. *C. longa* is also being used for their high commercial value as reported earlier. Almost all species of *Curcuma* are having properties of enhancing immune system due to the presence of curcumin in its rhizomes. In several studies, they have reported *C. aromatica* rhizome oil having camphor as the major compound which is contrary to our reports[3].

There are reports in which different *Curcuma* species alongwith *C. aromatica* have been discussed to have many active compounds which showed potential anticancer activity both in *in vitro* and *in vivo* models of cancer[16]. Other studies on *C. aromatica* rhizome oil to be effective against against melanoma cells (B16) and prostate cancer cells (LNCaP) have been performed[33]. Very few reports on GC-MS analysis of *Kaempferia* species and *Hedychium* species are available where we have reported earlier on *K. galanga* and *H. coronarium* micropropagated rhizome oil analysis[20,25].

Other reports from our laboratory on *H. coronarium* extract arrests cell cycle progression in HeLa cervical cancer[27]. The

quantitative composition and the relative proportions of the oil components are widely influenced by climatic, geographic and genotypic differences. In the present study, GC-MS analysis shows the presence of bioactive compounds which might be responsible for the medicinal activity of the four samples. The percentage inhibition at different drug concentrations (6, 12.5, 25, 50 and 100 mg/mL) of all samples against cancer cell lines along with the percentage inhibition of positive control (camphothecin) at 10 mM concentration is shown.

The MTT assay expressed the effective concentration required for 50% of MCF7 reduction against different concentrations of rhizome oil. The study shows that the best activity was found in KG followed by HC, CA and CL respectively (Table 1, Fig. 1 and Fig. 2). Other studies have been done on ginger essential oil to evaluate their cytotoxicty activity on Dalton's Lymphoma Ascites and Ehrlich Ascites Carcinoma cell lines[15].

The anticancer activity of 11 Zingiberaceae species was done using two different cell lines, HT-29 and MCF-7 cancer cells by MTT assay including *C. longa* which showed strong inhibitory effects on the growth of both cell lines but no effect was seen in *K. galanga*[6]. Other studies of the cytotoxic potential of rhizome essential oils from four different regions of the Western Himalaya (India) on *H. spicatum* have been done against lung, colon, breast, head, neck and cervix cancer cell lines[18]. Similarly, essential oil revealed potential cytotoxic activities in other species like *Populus alba* and *Rosmarinus officinalis* for MCF7 cancer cell lines by MTT assay[11]. Also other reports of high anticancer potential of essential oils on human lung cancer and prostate cancer cell lines are available[35].

But in the present study among all four samples, KG was significantly active against the cancer cell lines. The selectivity of the plant proved to be a promising anticancer potential by inhibiting MCF7 human breast cancer cell line. However, the search for new lead compounds from natural sources with more effective and less toxic compounds constitutes interesting alternatives for the development of anticancer drugs in breast cancer treatment.

Table 1
Percentage (%) of viability of MCF7 cells against *Curcuma longa* (CL), *Curcuma aromatica* (CA), *Kaempferia galanga* (KG) and *Hedychium coronarium* (HC) rhizome oil in different concentrations at an incubation period of 24hours

S. N.	Species name	Viability %						
		Untreated	Standard	Samples (in uL) treated in MCF7 cell				
				6.125	12.5	25	50	100
1	*C. longa*	100	50.91	77.65	63.98	57.34	41.90	27.97
2	*C. aromatica*	100	50.91	96.62	75.40	69.98	49.30	23.57
3	*K. galanga*	100	50.91	85.36	76.68	65.00	55.03	45.49
4	*H. coronarium*	100	50.91	96.24	88.53	61.89	53.10	47.48

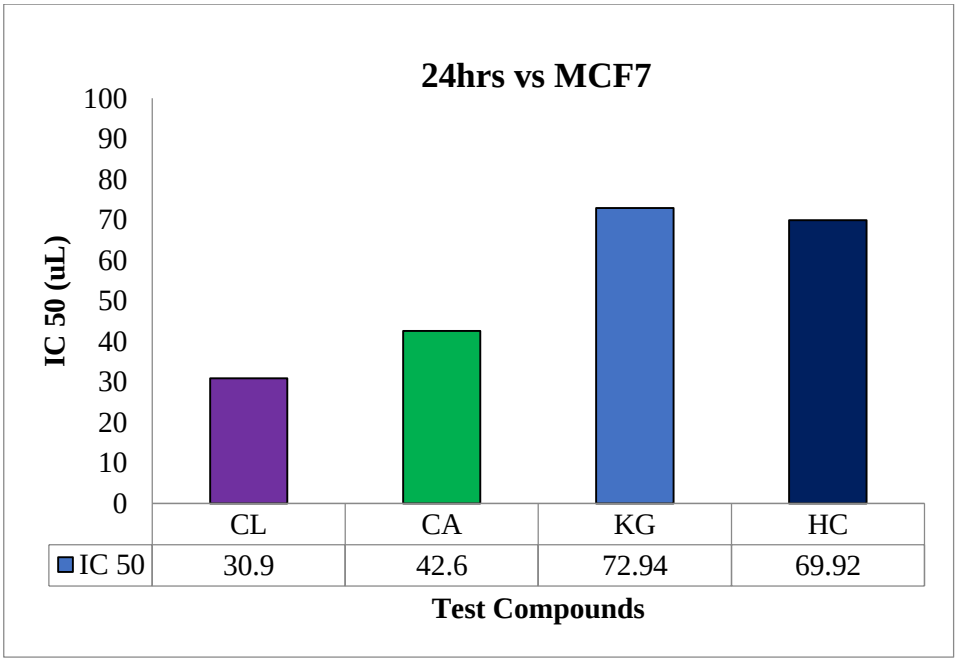

Fig. 1: Graph of IC50 concentrations of *Curcuma longa* (CL), *Curcuma aromatica* (CA), *Kaempferia galanga* (KG) and *Hedychium coronarium* (HC) against MCF7 cell line observed in the MTT assay after the incubation period of 24hours

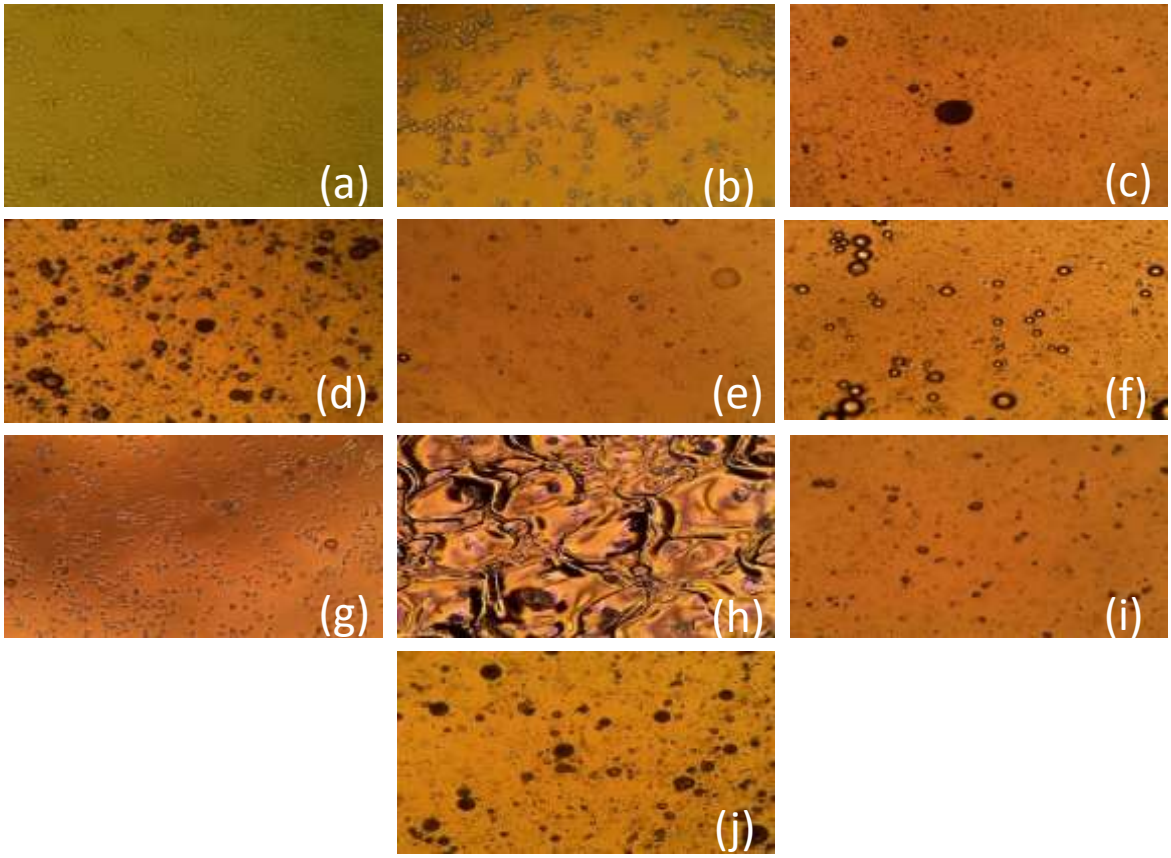

Fig. 2: Effects of rhizome oil and morphological changes observed in MCF7 cancer cell line (a) MCF7 cell control (b) 10uM of Camptothecin as standard or positive control (c, d) MCF7 activity of *C. aromatica* oil in 12.5 uL/mL and 100 uL/mL (e, f) *C. longa* oil in 25 uL/mL and 100 uL/mL (g, h) *K. galanga* oil in 6.25 uL/mL and 12.5 uL/mL (i, j) *H. coronarium* oil in 12.5 uL/mL and 100 uL/mL

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