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A comparative – *invitro* study of anticancer effect of *mentha piperita, ocimum basilicum* and *coleus aromaticus* against human laryngeal epidermoid carcinoma (HEP-2) cell lines

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Aromatic plants of Lamiaceae family are known for its traditional medicine. The leaves of the plants are promoted in relieving sore throats, toothaches, colds, coughs, laryngitits, bronchitis, nasal congestion and inflammation of the mouth and throat. Ethanolic extracts of *Mentha piperita*, *Ocimum basilicum* and *Coleus aromaticus* were studied for the *invitro* cytotoxicity against Human Laryngeal epidermoid carcinoma (Hep-2 cell lines). The results showed that *Mentha piperita* treated Hep-2 cell lines with maximum cytotoxicity,*Ocimum basilicum* shown moderate cytotoxicity . When compared to the above two plants the *Coleus aromaticus* treated Hep-2 cells has very little or no cytotoxicity. The findings of the present investigation demonstrated that *Mentha piperita* significantly suppresses growth and induces apoptosis in Hep-2 cell lines.

Keyword: *Mentha piperita, Ocimum bascilium, Coleus aromaticus,* Cytotoxicity concentration, Hep-2 cells.

1. Introduction

Hep-2 cell line is derived from laryngeal carcinoma cells of human nasopharyngeal mucosa. Being slow-growing tumors, these cells develop in animal hosts as well as *invitro.* Cancer drug development is entering a remarkable new phase. The last two decades have brought remarkable progress in understanding the molecular basis of cancers.

Now it is doubtless that plants are the most vital source of several compounds which possess significant therapeutic values for cancer treatment. The antioxidant components of plant origin will protect, the body from free radicals, and thus prevent cancer. *Mentha piperita* L.(Lamiaceae family) is an ancient species known to Chinese, Greek and Arab Physicians. Peppermint tea is customarily used as a substitute for black tea refreshing drink. Apart from its use as condiment and as a flavouring agent, various medicinal properties are attributed to this tiny spicy herb, which range from dyspepsia, flatulence, indigestion, biliousness and to check morning sickness, nausea and summer diarrhoea[1]. Monoterpenes are non-nutritive dietary components. D-Carvone is an essential oil found in many medicinal and aromatic plants that are endowed with many biological activities. They have antioxidant, antimicrobial, fungicidal and insecticidal potentials [2].

D-limonene is a monocyclic terpene which is widely found in aromatic plants. Anti-tumour activities of the compound in several animal tumour models and *invitro* experiments have been reported. Limonene inhibits the development of gastric cancers possibly through increased apoptosis. It has been also used as antimicrobial, antiviral, expectorant, sedative, spasmolytic, and antibiotic agent[3]. The main volatile compounds identified by the gas chromatography-mass

spectrometric analysis of *Mentha piperita* were menthol, menthone, carvone and limonene [4] *Ocimum basilicum* known as the "royal herb" to the ancient Greeks, the botanical name is derived from the Greek "to be fragrant". It has anti-oxidant, anti-carcinogenic, radio-protective and free radical scavenging properties. It also has been recommended for the treatment of headaches,coughs,infections of upper respiratory tract,kidney malfunction and to eliminate toxins [7]. Eugenol is a major component of the essential oil of *Ocimum basilicum* and widely used as a flavouring agent in food products, pharmaceutical products and also as an analgesic in dentistry.

Coleus aromaticus commonly known as Karpuravalli is an aromatic erect, spreading plant with many branches, with fleshy leaves and stems. The juice of the leaves for dyspepsia, asthma, chronic coughs, bronchitis, flatulence and rheumatism. Eugenol, methyl eugenol and thymol were also found to occur in this plant.

2. Materials and Methods

2.1 Preparation of the Extract

5 gms of fresh leaves of *Mentha arvensis*, *Ocimum basilicum* and *Coleus aromaticus* were weighed using a weighing balance and washed with distilled water and grinded in mortar and pestle using 5 ml of 99% ethanol. Then they are poured in petri plates and kept in an incubator at 37ºC for 1 day. Air dried extracts were stored at 20®C until analysis.

2.2 Determination of Cell viability by Sulphorhodamine B (SRB) Assay

The sulphorhodamine-B (SRB) assay is used for cell density determination based on the measurement of cellular protein content.

SRB is a bright pink amino xanthene dye with two sulphonic groups. Under mild acidic conditions SRB binds to protein basic amino acid residues in TCA fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude.

The monolayer cell culture was trypsinized and cell count was adjusted to $1.0x10^5$ cell/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitre plate 0.1 ml of the diluted cell suspension (approximately 10,000 cell) were seeded. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed with the medium .100µl of the extract with different concentrations were added to the cells. The plants were then incubated at 37 ºC for 3 days with 5% CO_2 atmosphere. Microscopic examination were recorded for every 24 hours. After 72 hours, 25µl of 50% tri chloroacetic acid was added to the wells gently in such a way that it forms a thin layer over the extract to form an overall concentration of 10%.The plates were flicked of and incubated at 4 ºC for 1 hour.

The plates were washed five times with water to remove traces of medium, extract, serum and air-dried. They were stained with SRB for 30 min. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried. Tris base (10mM, 100 microlitre) was then added to the wells to solubilise the dye. The plates were shaken vigorously for 5 min. The absorbance was measured at 540 nm. The percentage growth inhibition was calculated using the formula given and CTC_{50} values were calculated.

$$\% \text{ Growth Inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of Control group}} \times 100$$

3. Result

From the SRB assay conducted with the vero cell lines and cancerous Hep-2 cell lines, the following results were obtained. The normal vero cell lines has the CTC_{50} value of 252.84, 251.96 & 250.12 against the *Mentha piperita*, *Ocimum basilicum* and *Coleus aromaticus* leaf extracts respectively. (Table 1)

Mentha piperita leaf treated Hep-2 Cell lines was found to have a minimum CTC_{50} value of 94, whereas in *Ocimum basilicum* it was found to be moderate of 104 and in case of *Coleus aromaticus* leaf treated Hep-2 cell lines CTC_{50} value was 186.

Table 1: Studies on Anticancerous Effects of *Mentha Piperita, Ocimum basilicum* and *Coleus aromaticus* on Hep -2 Cell Lines by SRB assay.

S.No	Plant name	SRB assay - CTC 50 Value µg/ml	
		Vero cell lines	Hep-2cell lines
1	*Mentha Piperita*	252.84	94
2	*Ocimum basilicum*	251.96	104
3	*Coleus aromaticus*	250.12	186

4. Discussion

From the above study, it was revealed that the maximum anticancerous effect was observed in *Mentha piperita* treated Hep-2 cell lines whereas in *Ocimum basilicum* treated samples the CTC_{50} value was moderate. When compared to the above two samples the *Coleus aromaticus* treated Hep-2 cells has a very little or no effect against cancer presented in fig 1.

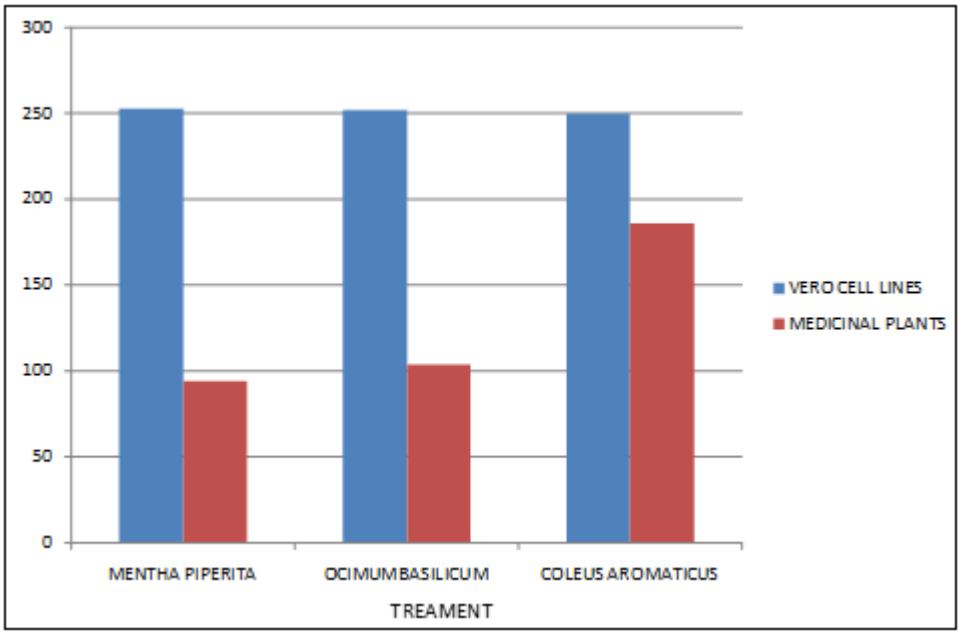

Fig 1: Anticancerous Effects of *Mentha Piperita, Ocimum basilicum* and *Coleus aromaticus* on Hep -2 Cell Lines by SRB assay.

The highest anticancerous property of the *Mentha piperita* leaves may be due to the presence of the monoterpenes called d-carvone and D-limonene in its leaf extracts. D-carvone has been found to reduce forestomach tumour formation and pulmonary adenoma formation induced by N-nitrosodimethylamine in mice [5].

D-limonene metabolites also cause G1 cell cycle arrest, inhibit post translational modification of signal transduction proteins and cause differential expression of cell cycle and apoptosis-related gene. Limonene inhibit the activity of HMG-COA reductase and reducing the possibility of cancer cell growth [6].

The mechanism of Caspase-8 activation and mitochondrial-mediated apoptotic pathways are involved in the action of d-limonene and d-carvone as an important role [5].

The anticancerous property of *Ocimum basilicum* may be due to the presence of Eugenol in its leaf extract. Eugenol inhibit the cancerous cells by inducing apoptosis and blocking the cells in G2/M phase [8].

Coleus aromaticus has very little effect on cancerous cell lines, which may be suggesting that the extract posses low concentration of secondary metabolites.

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