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Evaluation of Anti -Cancer Potential of luteolin 7 -o- glucoside extracted from *Cuminum cyminum* and *Ocimum basilicum* in colon Cancer Cell Line

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Abstract

This study involved two plant species of different plant families, cumin (*Cuminum cyminum)* family Apiaceae and basil (*Ocimum basilicum)* family Lamiaceae of medical and economic importance. Aim: study luteolin o -7- glucoside in both species and evaluate its potential effect on colon cancer.

Method: extraction by (70% ethanol) using (Soxhlet and reflux), high-performance liquid chromatography (HPLC) for detection of compound , and *in vitro* evaluation of the potential biological activity of crude plant extracts and luteolin O-7 glycoside with anticancer activity of Colon HCT Cell lines were tested using (MTT) assay.

Results: of *in vitro* anticancer activity study conducted against colon cancer cell line showed moderate cytotoxicity of these plant extracts and luteolin 0 7 glucoside compounds against colon cancer. In which inhibition in viability of colon cancer cell by *Cuminum cyminum* (17.48% at concentration 250µg , 18.39 at concentration 500µg and 34 at 1000µg) , *Ocimum basilicum* showed inhibition in the viability of cancer cell (17.66 at 250 µg , 26.8 500µg and 40.6 at 1000µg) While luteolin 0 - 7-glucoside stander showed decrease in the viability of cancer cell by (29.14 at 250 µg 11.11 500µg and 39 at 1000µg

keywords: luteolin, colon cancer, cumin **,** basil

Introduction

Colorectal cancer is the third and second most common cancer in men and women. It is closely related to environmental and genetic risk.

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Approximately 5% of colon cancers are caused by two genetic disorders: Familial adenomatous polyposis and Lynch syndrome [1].
The transformation of normal colon cells into precancerous and ultimately cancerous cells requires a combination of somatic and/or genetic changes over approximately 10 to 15 years[2].

Cumin

Cumin (*Cuminum cyminum* L.) is a plant belonging to the Apiaceae family, native to the Mediterranean region, Turkestan and Egypt, and spread to many regions of the world. It is one of the oldest and most important commercial plants, and its cultivation usually requires 3-4 months of summer. It is drought resistant and grows in the Mediterranean climate.[3].

Different parts of the fennel plant (leaves, buds, roots, and flowers) have similarities and differences[4].

Cumin aldehyde ,α-Sabinin , α-Flandren, α-Kadinin, α-Pinene, p-Cymene , Linalool, α-Phellandrene, Safranal, Limonene, Linalyl acetate Luteolin , 1,8-Cineole, Methyl eugenol , Geraniol, α-Terpineol acetate

Basil

Basil (*Ocimum basilicum* L.) is a plant belonging to the Lamiaceae family, an important oilseed, culinary and medicinal plant. The essential oil of the plant, which grows in tropical and subtropical regions, is used in oral health and dental products as well as in the spice and food industry. O. basilicum, Ocimum, Lamiaceae, Lamiaceae, Class Magnolia, Phylum Magnolia ve Kingdom Plantae cinsinin bir slizidir [5]

Materials and methods

Materials

Trypsin/EDTA\Capricorn, DMF\Santa Cruz, RPMI 1640\Gibco, MTT Stain\Sigma, Fetal Bovine Serum\Gibco, Ethanol\BDH.

Methods of Extraction

Two hydroalcoholic extracts of basil and cumin were prepared by the reflux and Soxhlet extraction method.

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Reflux Extraction

Reflux extraction Crude extract samples were prepared by mixing 25 mg of leaf powder with 150 ml of 70% ethanol and boiling in a reflux at 70°C for 45 min.

Filter the ethanol extract, place it in a vial, and store at -4°C until used for analysis[6]

Soxhlet Extraction

Ethanol extract from Soxhlet extraction equipment, ethanol extract is prepared from dry cumin seeds using continuous Soxhlet extraction equipment.

The crude extract sample was prepared by boiling 15 g of dry cumin seeds (150 ml) with ethanol (70%) in a Soxhlet extractor. Extractions were performed for 10 h and the ethanolic extract of each extract was filtered; both crude extracts were stored at −4°C[7]

High-performance liquid chromatography (HPLC) analysis: HPLC conditions [8]

• Mobile phase: a mixture of 80% water and 20% trifluoroacetic acid in an acetonitrile solution
• C-18 chromatography column
• 1.0 milliliters per minute (ml/min)
• Column temperature: 22 degrees Celsius
• 20 µL of injection volume
• A UV detector with a wavelength of 290 nm is used for detection.

Cytotoxicity study

Methods

Cell culture

A mixture of cancer cells and normal cell lines were procured from the IRAQ Biotechnology Cell Bank Unit in Basra and cultured in RPMI-1640 with the addition of 10% fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. After each passage in Trypsin-EDTA, the cells were reseeded at 70% confluence and incubated at 37 °C with 5% CO2 [9].

Cytotoxicity Assays

To test the cytotoxic effect, a 96-well plate was used for the MTT cell viability assay.

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Each well was seeded with 1 x 104 cells for each cell line. The cells were exposed to c&u test compounds at a concentration of µg/ml for every compound after 24 hours or after they had grown into a confluent monolayer.

The 72-hour mark was used to determine the cell viability. Take the medium out and mix it with 28 µL of MTT solution (2 mg/mL). Then, put the cells in an incubator at 37 °C for 2 hours.

Add 100 µL of DMSO (dimethyl sulfoxide) after removing the MTT solution and incubate at 37 °C with shaking for 15 minutes[10]. The assay was carried out in triplicate and the absorbance at 620 nm (the test wavelength) was measured using a microplate reader.

Following this formula, one may determine the rate of cell growth inhibition (% of cytotoxicity): In this equation, PR is the growth rate, A is the average eye velocity of the untreated well, B is the treated well's eye velocity, and IR is the difference between 100 and PR.

The Results

High Performance Liquid Chromatography HPLC

HPLC analysis of two basil and cumin extracts detected luteolin o -7-glucoside as the major ingredient along with several minor ingredients.

Luteolin o-7-glucoside was determined by comparing the retention time (Rt) with standard luteolin o-7-glucoside (7.25 min) as shown in the figure (3.1).

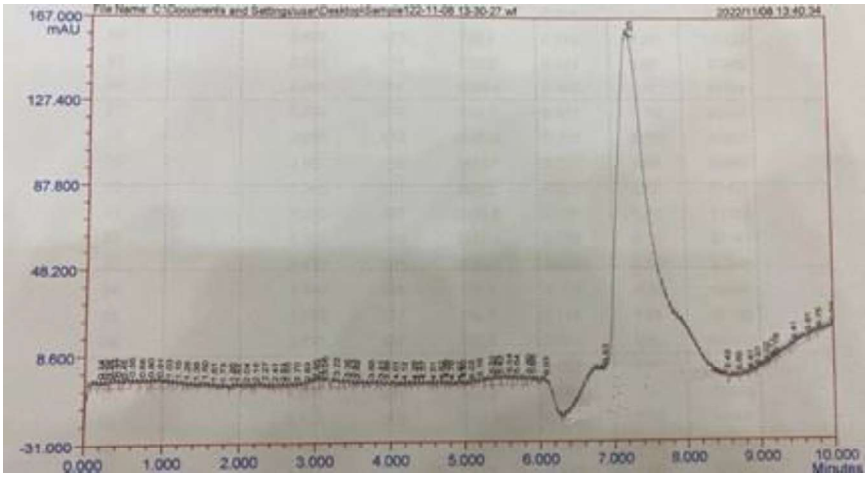

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Figure (1) HPLC Chromatogram of standard luteolin o -7- glucoside

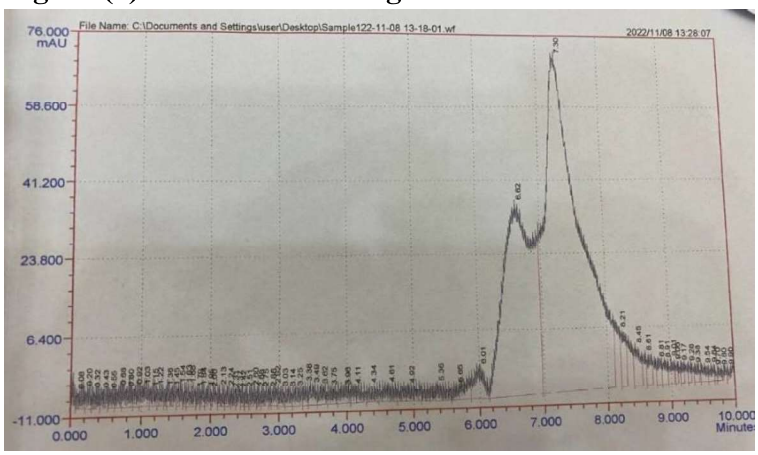

Figure (2) HPLC chromatogram of 70% ethanol reflux extraction of basil

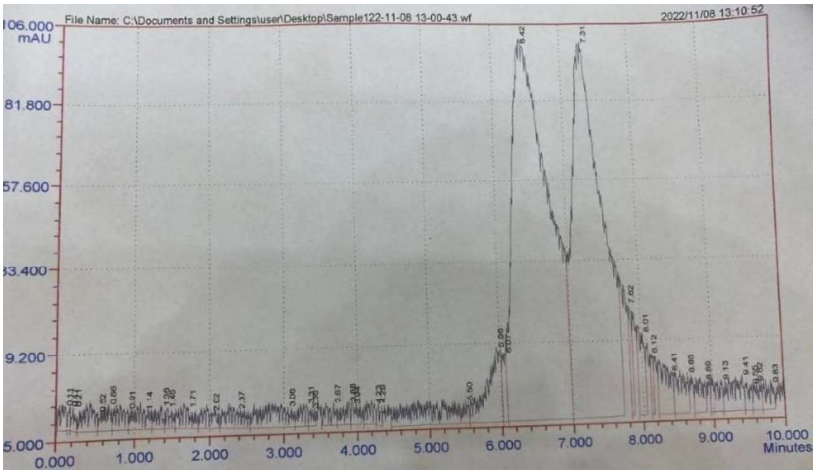

Figure (3) HPLC chromatogram of cumin extracted with Soxhlet with 70% ethanol

Cytotoxicity study

Cell line cytotoxicity study shows the following results for colon cancer cell line

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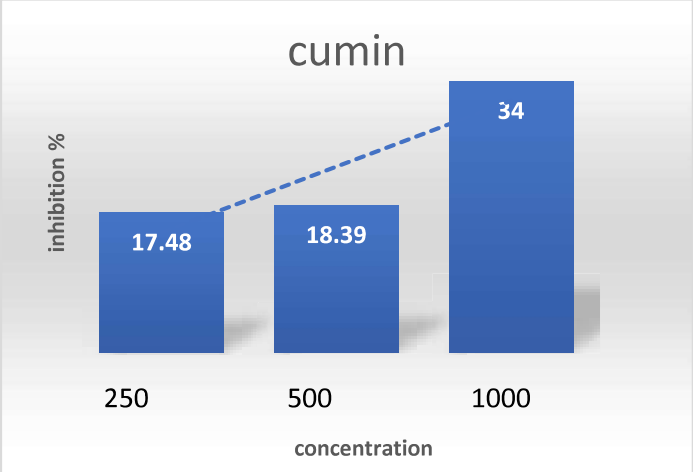

Figure (4) Anti proliferative activity of *Cuminum cyminum* crude extract against colon carcinoma cells HCT cell line
Cell viability was evaluated according to MTT assay, and the results showed that the percentage of cell growth inhibition in the treatment was
(250, 500 and 1000 µg/ml).

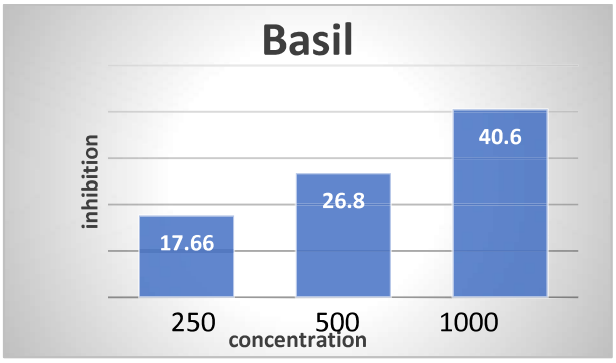

Figure (5) Anti proliferative activity of *Ocimum basilicum* crude extract against colon carcinoma cells HCT cell line
Cell viability was evaluated according to MTT assay, and the results showed that the percentage of cell growth inhibition in the treatment was
(250, 500 and 1000 µg/ml).

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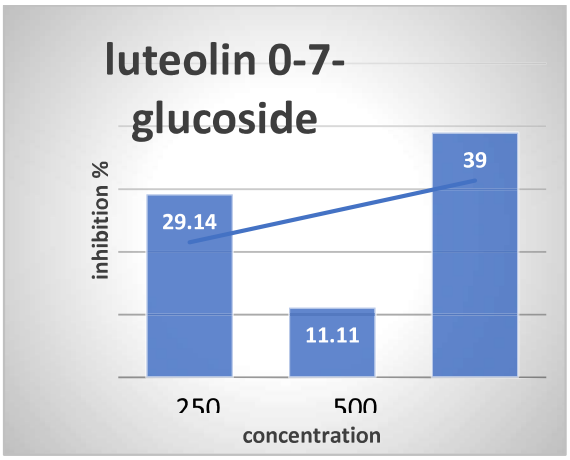

Figure (6) Anti proliferative activity of luteolin o -7 glucoside standard against colon carcinoma cells HCT cell line
Cell viability was evaluated according to MTT assay, and the results showed that the percentage of cell growth inhibition in the treatment was
(250, 500 and 1000 µg/ml).

Discussion

In this study, results showed inhibition in viability of colon cancer cell by *Cuminum cyminum* (17.48% at concentration 250µg , 18.39 at concentration 500µg and 34 at 1000µg) as in figures (4)
Ocimum basilicum showed inhibition in the viability of cancer cell (17.66 at 250 µg , 26.8 500µg and 40.6 at 1000µg) as in figures (5)
While luteolin 0 – glucoside stander showed decrease in the viability of cancer cell by (29.14 at 250 µg 11.11 500µg 39 and at 1000µg) as in figures (6)

Luteolin has demonstrated the ability to hinder the proliferation of HCT116 breast cancer cells by regulating apoptosis and cell cycle arrest in a p53-dependent manner, without inducing autophagy[11] .

Chen et al. conducted a study to examine the impact of luteolin on the interruption of the cell cycle in human cancer cells. In 2018, researchers revealed that luteolin induces cell cycle arrest in LoVo human cancer cells at the G2/M phase, leading to death[12] .

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Subsequent molecular investigations revealed that luteolin exerted an impact on the proliferation of LoVo cells by impeding the halt of the cell cycle at the transition from G2 to M phase and deactivating cyclin B1/cell division cycle 2 (CDC2), thereby prompting cell death. Apoptotic protease-activating factor 1 is activated through the involvement of cytochrome c and deoxyadenosine triphosphate [13]

According to a study conducted in 2022 by Song et al., luteolin had the following effects on colorectal cancer cells: it slowed their proliferation by modulating the MAPK pathway, which in turn caused cell cycle arrest, DNA damage, and apoptosis. Additionally, luteolin had the opposite effect on tumor growth and CRC xenografts. real-time model [14] .

Furthermore, Song et al. 2022 demonstrated that cisplatin is an effective chemotherapeutic on colorectal cancer cells (CRCs), and that when combined with luteolin, cisplatin enhances its effects by lowering cell survival and increasing the Apoptosis rate of HCT-116 and HT-29 cell counts, in comparison to cisplatin treatment alone.

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