

A Study on Extraction of Apigenin Flavonoid from Parsley Plants: A Natural Synergy for Cancer Prevention and Therapy

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Abstract - Apigenin is a low-toxicity flavonoid with several beneficial bioactivities. It is a secondary metabolite bioflavonoid that shows pharmacological activities such as antibacterial, anticancer, antidiarrheal, antiemetic, and hemostatic effects. Apigenin is extracted from different parts of parsley plants by using the Soxhlet Extraction method. Parsley (*Petroselinum crispum*), a flowering species of the Apiaceae family, is native to Greece, Morocco, and the former Yugoslavia. In traditional medicine, parsley has been used as a carminative, gastrotonic, diuretic, urinary tract antiseptic, anti-urolithiasis, antidote, and anti-inflammatory agent. It is also used for treating dermal diseases, amenorrhea, dysmenorrhea, gastrointestinal disorders, hypertension, cardiac and urinary diseases, otitis, cold, and diabetes. Apigenin is a phytochemical that occurs along with tannins, alkaloids, triterpenoids, steroids, flavonoids, and saponins. Rich in parsley, celery, celeriac, and chamomile tea, apigenin has health-promoting properties, including use as a natural sleep aid, antidiabetic, and anticancer compound. This flavonoid induces relaxation as it binds to brain receptors that promote sleep. The main aim of this paper is to extract apigenin flavonoid from parsley plant parts. Parsley leaves and flowers are abundant in phenolic compounds, present as aglycones (flavones and flavonols) and glycosides. In this study, apigenin was extracted from parsley leaves using Soxhlet extraction, followed by hydrolysis and recrystallization. A combination of apigenin and lecithin was also synthesized using a solvent method. Several extraction parameters were tested to evaluate yield, with Soxhlet extraction 5.5 h, 65 °C, solid-to-solvent ratio 8:400 as the reference and the purification done by column chromatography. UV-Visible analysis confirmed that the structure of apigenin remained stable after extraction and purification.

Keyword - Apigenin Flavonoid, Soxhlet Method, UV-Visible Spectrophotometer, Anticancer Agent, Parsley Plant.

INTRODUCTION

Apigenin is a low-toxicity flavonoid with several beneficial bioactivities. The parsley belong to the family of Apiaceae and is a aromatic herbs. Rich in parsley, celery, celeriac, and chamomile tea, apigenin has health-promoting properties, including use as a natural sleep aid, antidiabetic, and anticancer compound. This flavonoid induces relaxation as it binds to brain receptors that promote sleep[1]. The main aim of this paper is to extract apigenin flavonoid from parsley plant parts. Parsley leaves and flowers are abundant in phenolic compounds, present as aglycones (flavones and flavonols) and glycosides[4]. It is a secondary metabolite bioflavonoid that shows pharmacological activities such as antibacterial, anticancer, antidiarrheal, antiemetic, and hemostatic effects[6][7]. Apigenin is extracted from different parts of parsley plants by using the Soxhlet Extraction method. This is a reference method to determine the efficiency of MAE in

extraction of apigenin flavonoid from the combination of different parts of parsley plants[9][10].The separation of apigenin was also synthesized using a solvent method[12][15]. The separation of apigenin flavonoid from combination of different parasts of parsley plants by using Soxhlet extraction method 5.5 h, 65 °C, solid-to-solvent ratio 8:400 (8 gm & 400 ml of ethanol as the reference and the purification done by column chromatography[18][20]. UV-Visible spectrophotometer used as analysis confirmed that the structure of apigenin remained stable after extraction and purification process[25][27]. Then we are using the HPLC technique for the determination of the purity of the apigenin flavonoid[28].



Parsley plant

Processing of Plant Material and Plant Extraction Preparation: Fresh or dried parsley leaves (*Petroselinum crispum*) were collected. If fresh, leaves were dried and then ground into fine powder to increase surface area for extraction[32].

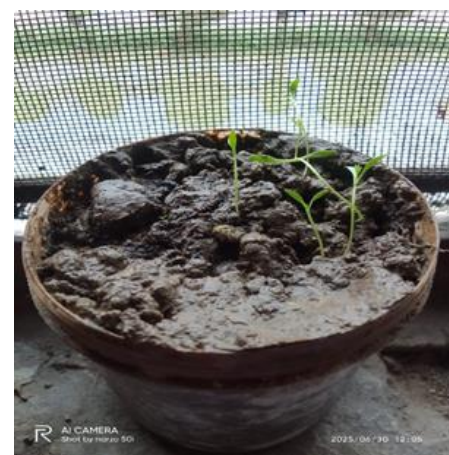


Fig 2. Plant Material Fresh or dried parsley leaves

II. METHODOLOGY

Soxhlet Extraction

A Soxhlet extraction apparatus was set up. Ethanol was used as the extraction solvent. About 8 g of powdered parsley leaves were loaded into a cellulose thimble. The solid-to-solvent ratio was 8 g leaves to 400 ml ethanol. Extraction was performed at 65 °C for approximately 5.5 to





Fig 3. Soxhlet Extraction of Parsley Plant

6 hours. Ethanol repeatedly washes through the parsley powder dissolving apigenin and other compounds. The solvent with dissolved compounds was collected. Optical Density of Soxhlet Extraction process is 0.27 nm.

Column Chromatography

The crude extract was concentrated by evaporating the ethanol solvent using a rotary evaporator at 40 °C. The concentrated extract was mixed with silica gel (60 mesh), dried, and then loaded on a glass chromatography column packed with silica gel. Elution was performed with solvents of increasing polarity: pure hexane, then mixtures of chloroform and hexane (ratios 25:75, 50:50, 75:25), and finally pure chloroform and methanol if required. Fractions were collected for analysis. Optical Density of Column Chromatography is 0.34 nm.

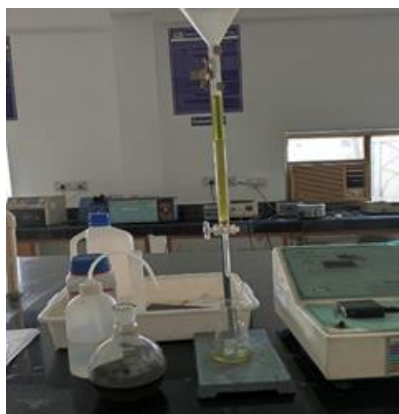


Fig 4. Column Chromatography

UV-Visible Spectrophotometer:

Used to analyse the extract fractions. The maximum absorbance wavelength of apigenin is 337 nm. Used to confirm the presence and stability of apigenin after extraction and purification.

Photo colorimeters provide a simple, cost-effective alternative to UV-Vis spectrophotometers for measuring absorbance in plant extracts, particularly for flavonoids like apigenin via colorimetric assays (e.g., aluminium chloride method). They use filters for specific wavelengths in the visible range, suitable for coloured complexes formed in flavonoid quantification.



Fig 5. Photo colorimeter

Prepare the plant extract (e.g., ethanol Soxhlet extract of leaves containing apigenin) and perform a colorimetric flavonoid assay first to generate a coloured complex measurable by the photo colorimeter.

- Turn on the photo colorimeter and allow it to stabilize (typically 10-15 minutes); select the appropriate filter for the assay wavelength (e.g., green filter ~420-450 nm for All₃-flavonoid complex, as apigenin derivatives absorb in visible after reaction).
- Fill a clean cuvette (usually 1 cm path length) with the blank solution (e.g., extraction solvent like 80% ethanol or assay reagents without extract); insert into the instrument.
- Calibrate to zero absorbance by adjusting the zero knob or auto-zero function until the display reads 0.000 absorbance.
- Remove the blank cuvette and rinse it thoroughly with distilled water, then with the test sample.
- Pipette the organized pattern (e.g., 1-five mL of diluted extract + reagents which include 5% Nano₂, 10% All₃, 1M NaOH for overall flavonoids; incubate 10-15 min for color development) into the cuvette; Wipe the exterior to avoid fingerprints.
- Insert the sample cuvette, look ahead to the readings to stabilize (10-30 seconds), and document the absorbance fee.
- Repeat for replicates (3x) and standards (apigenin calibration curve: 10-a hundred GO/mL); Calculate the

concentration the usage of Beer's regulation: $A = \epsilon \cdot c \cdot l$, wherein linearity is confirmed ($R^2 > 0.99$).

Integration with Extraction Process

Place after extract fractionation (post-Soxhlet: 8g leaves/400 mL ethanol, 65°C, 5.5-6h) and before HPLC purity check (86-90%). Use to screen fractions for total flavonoids at ~520 nm post-colorimetric reaction, confirming apigenin stability vs. UV-Vis at 337 nm.

Advantages in Workflow

Offers portability and low cost for initial quantification in resource-limited labs, correlating well with UV-Vis (ANOVA $p > 0.05$). Ideal for your biotech workflow before HPLC, aligning with green chemistry for flavonoid screening.

Result Discussion:

Apigenin was successfully extracted from parsley leaves with Soxhlet extraction. Purified by column chromatography with solvent systems to separate fractions rich in apigenin.



Fig 6. Apigenin Sample

UV-Visible analysis confirmed the structural stability of apigenin. HPLC analysis confirmed high purity of the extracted apigenin. The flavonoid shows pharmacological activities including anticancer, antibacterial, antidiarrheal, antiemetic, and hemostatic effects.

Parsley is a chemically active medicinal herb with various pharmacological effects. Soxhlet extraction coupled with column chromatography and UV-Vis/HPLC provides an effective method for isolating apigenin. Extracted apigenin retained chemical stability and purity. The methodology provides promising means for obtaining bioactive flavonoids from parsley for therapeutic use.

Sample Preparation

Fresh or dried parsley leaves are collected as raw material. The leaves are dried (if fresh) and ground into a fine powder to increase the surface area for extraction.

Extraction by Soxhlet Method

A Soxhlet extraction apparatus is set up. Ethanol is used as the solvent for extraction. The powdered parsley leaves are loaded into a cellulose thimble or extraction chamber. A solid-to-solvent ratio of approximately 8 g parsley leaves per 400 ml ethanol is maintained. The extraction is carried out at around 65 °C to 70 °C for about 5.5 to 6 hours, allowing ethanol to repeatedly wash through the parsley powder, dissolving apigenin and other compounds. After extraction, the solvent containing the dissolved compounds is collected.

Purification by Column Chromatography

The crude extract is concentrated by evaporating the solvent using a rotary evaporator at about 40 °C. The concentrated extract is then adsorbed onto silica gel (60 mesh) by mixing and drying. This solid mixture is loaded onto a glass chromatography column packed with silica gel. Elution is carried out using solvents of increasing polarity, starting from hexane, followed by mixed ratios of chloroform and hexane (e.g., 25:75, 50:50, 75:25), and finally chloroform and methanol if needed. Fractions are collected and analysed to identify those containing purified apigenin.

Verification of Apigenin

UV-Visible spectrophotometry is used to check the chemical stability of apigenin after extraction and purification. The characteristic absorbance peak confirms its presence. High-Performance Liquid Chromatography (HPLC) is performed to assess the purity and quantify apigenin content in the purified fractions. Purity levels of up to about 86% to 90% can be achieved with this method.

III. CONCLUSION

Microbial Activity Test:

Media Type: Nutrient Agar (as indicated by the "NA" label on the lid). This is a general-purpose medium that supports the growth of a wide range of non-fastidious organisms.

Growth Pattern: The plate shows confluent growth or a "lawn" of bacteria rather than isolated colonies. This usually happens when the sample concentration is very high or the inoculation (like a spread plate) was very dense.

Morphology: The growth appears creamy/off-white with a slightly granular texture towards the edges.

Key Considerations for Your Experiment

Since you are working with plant-associated bacteria (endophytes or rhizobacteria), here are a few things to keep in mind:

Colony Isolation: If you want to identify specific bacterial species, you should perform a streak plate method using a sample from this dish. This will allow you to see individual colonies and differentiate between different types of bacteria present in the plant.

Incubation: Plant bacteria often grow best at temperatures between 25°C and 30°C. If the plate was incubated at 37°C, you might be selecting for species that are more adapted to human/animal environments.

Contamination Check: Because the growth is so dense, it is difficult to tell if this is a pure culture or a mixture. Gram staining would be a great next step to see the cellular morphology (rods, cocci, etc.).

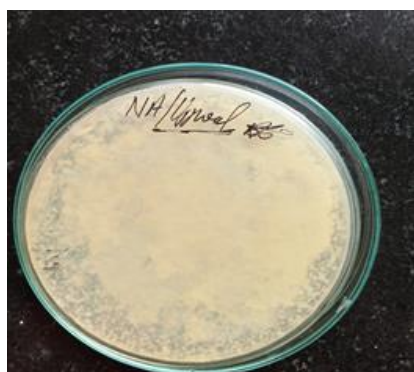


Fig 7. Nutrient Agar Medium

Parameter	Condition/ Result
Sample	Parsley leaves
Extraction Method	Soxhlet Extraction
Solvent	Ethanol
Extraction Time	5.5 hours
Extraction Temperature	65 °C
Solid-to-Solvent Ratio	8 g / 400 ml
Purification Method	Column Chromatography
Analytical Technique	UV-Visible Spectrophotometry
Stability of Apigenin	Stable after extraction
Bioactivities Highlighted	Anticancer, antibacterial, antidiarrheal, antiemetic, hemostatic
Additional Synthesis	Apigenin-Lecithin combination
Microbial Activity Test (NA Media)	rods, cocci

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