

Isolating flavonoid derivatives from *Asparagus aphyllus* L. extract and their Cytotoxic against tumor cells

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*Aislamiento de derivados de flavonoides del extracto de *Asparagus aphyllus* L. y su citotoxicidad frente a células tumorales*

*Aïllament de derivats de flavonoides de l'extracte d'*Asparagus aphyllus* L. i la seva citotoxicitat contra cèl·lules tumorals*

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ABSTRACT

This study used HPLC and real samples to quantify and identify the twenty-two phenolic acids that make up the phenolic pattern of *Asparagus aphyllus*. Using various chromatographic techniques, five flavonoid compounds—apigenin, quercetin, apigenin-4'-*o*-rhamnoside, quercetin-3'-*o*-rutinoside, and quercetin-7-methoxide—were extracted from the plant's aerial portions. The chromatographic methods of thin layer chromatography (TLC), paper chromatography (PC), and column chromatography (CC) are used to separate the flavonoid components. The structure elucidation of the pure compounds was done by different chemical and physical methods such as UV-spectrophotometer, ¹H & ¹³C NMR. Using the MCF7 (breast carcinoma cell line) and HCT (colon carcinoma cell line), the cytotoxic activity of the plant's successive extracts (petroleum ether, chloroform, and 70% ethanol) was determined; the IC₅₀ values varied. The highest activity was achieved by chloroform extract against breast carcinoma cell line (12.1 µg/ml), while ethanol extract (70%) had the most promising result at colon carcinoma cell line (16.2 µg/ml).

Keywords: *Asparagus aphyllus* L.; Flavonoids; phenolic; Cytotoxic Activity, plant extract.

RESUMEN

Este estudio utilizó HPLC y muestras reales para cuantificar e identificar los veintidós ácidos fenólicos que conforman el patrón fenólico de *Asparagus aphyllus*. Mediante diversas técnicas cromatográficas, se aislaron cinco compuestos flavonoides de las partes aéreas de la planta: apigenina, quercetina, apigenina-4'-*o*-rhamnósido, quercetina-3'-*o*-rutinosido y quercetina-7-metóxido. Para separar los componentes flavonoides se emplearon las técnicas cromatográficas de cromatografía en capa fina (TLC), cromatografía en papel (PC) y cromatografía en columna (CC).

La elucidación de la estructura de los compuestos puros se realizó mediante diferentes métodos químicos y físicos, como espectrofotometría UV y RMN ¹H y ¹³C. Utilizando las líneas celulares MCF7 (carcinoma de mama) y HCT (carcinoma de colon), se determinó la actividad citotóxica de los extractos sucesivos de la planta (éter de petróleo, cloroformo y etanol al 70%), obteniéndose distintos valores de IC₅₀. La actividad más alta se logró con el extracto de cloroformo sobre la línea de carcinoma de mama (12,1 µg/ml), mientras que el extracto de etanol (70%) obtuvo el resultado más prometedor sobre la línea de carcinoma de colon (16,2 µg/ml).

Palabra clave: *Asparagus aphyllus* L.; Flavonoides; Fenoles; Actividad Citotóxica; Extracto Vegetal



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RESUM

Aquest estudi va utilitzar HPLC i mostres reals per quantificar i identificar les vint-i-dues àcids fenòlics que conformen el patró fenòlic d'*Asparagus aphyllus*. Mitjançant diverses tècniques cromatogràfiques, es van aïllar cinc compostos flavonoides de les parts aèries de la planta: apigenina, quercetina, apigenina-4'-o-rhamnosida, quercetina-3'-o-rutinosida i quercetina-7-metòxid. Per separar els components flavonoides es van utilitzar les tècniques cromatogràfiques de cromatografia en capa fina (TLC), cromatografia en paper (PC) i cromatografia en columna (CC).

La elucidació de l'estructura dels compostos purs es va realitzar mitjançant diferents mètodes químics i físics, com espectrofotometria UV i RMN ¹H i ¹³C. Utilitzant les línies cel·lulars MCF7 (carcinoma de mama) i HCT (carcinoma de còlon), es va determinar l'activitat citotòxica dels extractes successius de la planta (èter de petroli, cloroform i etanol al 70%), obtenint-se diferents valors d'IC₅₀. L'activitat més alta es va aconseguir amb l'extracte de cloroform sobre la línia de carcinoma de mama (12,1 µg/ml), mentre que l'extracte d'etanol (70%) va obtenir el resultat més prometedor sobre la línia de carcinoma de còlon (16,2 µg/ml).

Paraules clau: *Asparagus aphyllus* L.; Flavonoides; Fenòlics; Activitat Citotòxica; Extracte Vegetal

1. INTRODUCTION

The World Health Organization (WHO) states that medicinal plants would be the greatest source of novel pharmaceuticals (Nascimento et al., 2000). Many ethnic groups use a variety of plant species to cure a wide range of illnesses, including skin conditions, asthma, malaria, diarrhea, and a number of other indications (Ansari et al., 2025; Dahanukar et al., 2000). The presence of compounds in medicinal plants known as phytochemicals that have a specific physiological effect on living cells is what gives these plants their therapeutic value. Herbal and homeopathic remedies, including phytochemicals, were employed to treat the illness (C. Chitravadivu, 2009; Sagar et al., 2024). According to Ahmed and Urooj (2009), these phytochemicals are non-nutritive and possess protective or disease-preventive properties (Ahmed & Urooj, 2009). Tannins, flavonoids, alkaloids, and phenolic compounds are the most valuable of these bioactive substances (Abd El-Halim et al., 2025; Dahiya & Purkayastha, 2012; El-Beltagi et al., 2025). Family Asparagaceae is placed in the order Asparagales of the monocots (Bremer et al., 2009). In earlier classifications, these species were involved in the family Liliaceae. Asparagaceae, with 2900 species includes 114 genera (Christenhusz & Byng, 2016). According to Chase et al. (2009), the genus *Asparagus* is a member of the Asparagaceae family (Chase et al., 2009). There are 300 species involved. Because the *Asparagus* genus contains steroidal saponins and sapogenins in different portions of the plant, it is thought to have medicinal value (Oketch Rabah HA, 1998). *Asparagus officinalis*,

or just asparagus, is the most well-known edible species. Numerous antioxidant elements are provided by asparagus, such as manganese, zinc, selenium, beta-carotene, vitamin C, and vitamin E (Pellegrini et al., 2003). *Asparagus* is used as an anticancer because it contains the enzyme "asparaginase," which is used in the medication of acute lymphoblastic leukemia (Gullett et al., 2010; Siddiqui et al., 2025; Xiao et al., 2022); also, as a digestive support food (Chrubasik et al., 2008).

Aphyllus L. is a perennial plant with woody, branching stems that can grow up to 100 cm in length. Smooth, green escabros, branches, and stems. The fruits are in the form of berries (5) 7-8 mm, black, with 1-3 seeds. It flowers from July to November. *Asparagus aphyllus* is used as a diuretic (Ali-Shtayeh M S, 2008) and antibacterial (Ali-Shtayeh et al., 2013). The existing literature and research have revealed a lack of reports on the phytochemical content of this plant. Therefore, the present investigation focuses on the qualitative and quantitative phytochemical estimation and biological screening of the aerial parts of *Asparagus aphyllus* L. growing in Egypt.

2. MATERIALS AND METHODS

2.1. Plant Materials

In the spring of 2014, fresh *Asparagus aphyllus* pieces were collected from Wadi Maged in the province of Mersa Matrouh, Egypt. Through comparison with herbarium specimens at the Desert Research Centre (Egypt), as well as the Botany Department of the Faculty of Science at Zagazig University, the plant was recognized. After two days of air drying in the shade, the aerial parts were dried for a full day at 40°C in an oven. Lastly, grind into a fine powder.

2.2. Phytochemical studies

2.2.1. HPLC analysis of phenolic compounds:

A sample of *Asparagus aphyllus* plants was used to extract its phenolic components (Ben-Hammouda et al., 1995). Identification of phenolic acid content of the plant sample was done by a Dionex Ultimate HPLC (Model 3000) using a Hypersil C18 RP column (250 x 4.6 mm) with 5 µm particle size. The mobile phase is acetonitrile and H₂O, with a flow rate of 0.7 ml/min using a diode array detector. The phenolic constituents of the plant were defined by their relative retention times. Peak area measurements were used to calculate each compound's concentration, which was then translated to µg of phenolic gm⁻¹ dry weight.

2.2.2. Determination of active compounds:

The powder of *Asparagus aphyllus* aerial parts (1.5 kg) was percolated in 70% ethanol for 24 h, and filtered off; the marc was extracted again by the same way (this process was repeated three times). The collective ethanol extracts were concentrated by rotavapor under reduced pressure at a temperature not exceeding 45°C until dryness. The obtained brownish sticky residue

was soluble in the least amount of water, treated with an excess of absolute ethanol, and filtered to remove inorganic salts and non-phenolic compounds. The ethanolic extract was concentrated again to a very small volume as described by Liu et al (1989). Then it was mixed with a small amount of silica gel and introduced on top of a column of silica gel. Elution started with pet. ether, CHCl₃, EA, and ethanol in case of increasing polarity. The separated bands formed from the column during elution were observed under visible and UV light. About 40 fractions (150 ml each) were collected; collective fractions were formed depending on the paper chromatography method using the system: n-Butanol: acetic acid: water (BAW) (4:1:5 v/v/v) upper phase. Four collective fractions [F1 (4.3 g), F2 (3.2 g), F3 (1.6 g), and F4 (9 g)] were obtained, containing six major flavonoid compounds and three phenolic acids.

The collective fraction F1 (4.3 g) was examined by preparative thin layer chromatography (PTLC) utilizing the system water/methanol/ethyl acetate (4:5:30) showing three bands under UV light, then each band was purified on column Sephadex LH-20 using methanol as an eluting system, giving three pure compounds (M1 = isoferulic acid, M2 = gallic acid, and M3 = elagic acid). Compounds (M5 and M7) were purely isolated from F2 (3.2 g) by application of silica gel column chromatography, and the eluting system was (petroleum ether, chloroform, and ethyl acetate). Two sub-fractions were received and then purified using the ethanol/water eluting method on column Sephadex LH-20. The resulting fractions showed one spot in each fraction when they were exposed to paper chromatography with the system. n-Butanol: Acetic Acid: Water (BAW) (4:1:5 v/v/v). The collective fraction F3 (1.6 g) was subjected to preparative paper chromatography using the system: n-Butanol: acetic acid: water (BAW) (4:1:5 v/v/v) upper phase showing two bands under UV light, then eluate of each band was purified on column Sephadex LH-20 using ethanol, giving two pure flavonoid compounds (M4 and M6). Compound (M8) was purely isolated from (0.9 g) of fraction F4 by application of silica gel column chromatography, and the eluting system was (pet. ether gradient to ethanol), followed by a Sephadex column using the eluting system ethanol/water.

2.2. Determination of Cytotoxic Activity

Potential cytotoxic activity of the different successive extracts of *Asparagus aphyllus* was tested (Skehan et al., 1990). MCF7 (Breast carcinoma cell line) and HCT (Colon carcinoma cell line) were employed in the test. The National Cancer Institute, Kasr El-Einy, Cairo, Egypt, provided the cell lines.

2.2.1. Procedures for Cytotoxic Activity:

Cells were grown in 96-well plates (104 cells per well) and allowed to sit for a full day before being exposed to extracts that caused the cells to adhere to the plate wall.

The cell monolayer was exposed to varying extract concentrations under test (0, 5, 12.5, 25, and 50 µg/ml). Wells were created in triplicate for every dosage.

The extract was incubated with the monolayer cells for 48 hours at 37 °C in a 5% CO₂ environment.

Cells were cleaned and stained with sulforhodamine B stain (SRB) after 48 hours.

The surplus stain was eliminated with acetic acid, and the associated stain was extracted using Tris-EDTA buffer.

The color intensity was measured using an ELISA reader.

The association between the extract concentration and survival fraction was plotted to obtain the tumor cell line's survival curve following the addition of the designated extract.

3. RESULTS AND DISCUSSION

3.1. Phytochemical studies

3.1.1. Phenolic acid identification:

Using a high-performance liquid chromatography apparatus (HPLC), the phenolic compounds found in the aerial parts of *Asparagus aphyllus* were identified. The compounds included pyrogallol, gallic, protocatechuic, catechin, catechol, epicatachin, vanillic, ferulic, iso-ferulic, e-vanillic, ellagic, benzoic, coumarin, and salicylic. (Table 1).

Table 1: HPLC of phenolic compounds of the aerial parts of *Asparagus aphyllus*.

P. No.	RT	Phenolic Compound	Results (ppm)
1	6.980	Pyrogallol	233.21
2	7.063	Gallic acid	7.94
3	7.404	4-Aminobenzoic	3.95
4	8.338	Protocatechuic	33.84
5	8.650	Catechein	823.30
6	9.015	Catechol	19.42
7	9.176	Chlorogenic a.	73.46
8	9.554	Epicatachin	107.12
9	9.698	P-OH-benzoic	46.78
10	9.773	Caffeine	37.92
11	10.047	Caffeic a.	7.82
12	10.104	Vanillic a.	106.71
13	11.453	P-coumaric	15.16
14	11.714	Ferulic a.	72.59
15	12.149	Iso- Ferulic a.	160.01
16	12.812	e-vanillic a.	1036.78
17	13.060	Ellagic a.	548.98
18	13.107	Benzoic a.	308.98
19	13.811	3,4,5-methoxycinnamic	6.38
20	13.945	Coumarin	21.01
21	14.227	Salicylic a.	112.93
22	15.082	Cinnamic a.	7.04

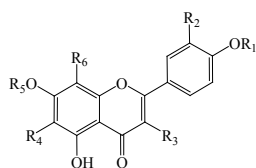
3.1.2. Isolation and identification of active compounds.

Asparagus aphyllus shares some similarities with other *Asparagaceae* species in its phenolic style. Physical investigation, including UV, ¹H, and ¹³C NMR, was used to clarify the structures of the isolated compounds. The isolated compounds (M1 = gallic acid, M2 = isoferulic acid, and M3 = elagic acid) were compared with an

authentic sample and previous published data (Barakat et al., 1987; Chen et al., 2010; Nawwar et al., 1994).

3.1.3. Identification of isolated flavonoid compounds

Asparagus aphyllus shares some similarities with other Asparagaceae species in its flavonoid pattern. The structures of the separated compounds were ascertained by ¹H and ¹³C NMR and UV spectroscopy. The separated compounds (M4-M8) were contrasted with previously released information. they are apigenin, apigenin-4'-rhamnoside, quercetin, quercetin-3'-rutinoside, and quercetin-7-methoxide (Czeczot et al., 1990; Harborne & Mabry, 1982; Malikov & Yuldashev, 2002; Pieroni et al., 1996) Figure (1), (Table 2).



- M4 = Apigenin
M5 = Quercetin
M6 = Apigenin-4'-O-rhamnoside
M7 = 7-methoxyquercetin
M8 = Quercetin-3-O-rutinoside
- R₁ = R₂ = R₃ = R₄ = R₅ = R₆ = H
R₁ = R₄ = R₅ = R₆ = H, R₂ = R₃ = OH
R₂ = R₃ = R₄ = R₅ = R₆ = H, R₁ = rhamnoside
R₁ = R₄ = R₆ = H, R₂ = R₃ = OH, R₅ = CH₃
R₁ = R₄ = R₅ = R₆ = H, R₂ = OH, R₃ = O-rutinoside

Figure 1: Isolated flavonoid compounds (M4-M9) of *Asparagus aphyllus*

3.2. Cytotoxic Activity (Anti-tumor).

Potential cytotoxic activity of the successive extracts of *Asparagus aphyllus* with different concentrations was tested against MCF7 (Breast carcinoma cell line) and HCT (Colon carcinoma cell line). Data obtained from Table 3 showed IC₅₀ (inhibition concentration) of the extracts of *Asparagus aphyllus* aerial parts for both MCF7 and HCT.

Results pointed out that the successive extracts of the plant showed different cytotoxic activity against MCF7 and HCT; however, all the tested extracts were capable of inhibiting the growth of MCF7 (Breast carcinoma cell line) and HCT (Colon carcinoma); the chloroform extract has the highest activity against the breast carcinoma cell line (12.1 µg/ml), and the ethanol 70% extract is the best result with the colon carcinoma cell line (16.2 µg/ml). Due to the presence of flavonoid components, including their glycosides and quercetin and apigenin, which influence signal transmission in angiogenesis and cell proliferation and have a curative effect on cancer (Fatemeh & Khosro, 2013). Naringin and naringinine have cytotoxic activity against lung carcinoma (A549) and white breast adenocarcinoma (MCF7) (two human cancer cell lines) by using cell viability assays, which gives a good result compared to Adriamycin (Atta et al., 2019).

Table 3. IC₅₀ of the successive extracts of *Asparagus aphyllus* aerial parts on MCF7 and HCT.

Successive extracts	IC ₅₀ (µg/ml) for MCF7	IC ₅₀ (µg/ml) for HCT
Pet. ether	13.2 µg/ml	16.7 µg/ml
Chloroform	12.1 µg/ml	19.6 µg/ml
Ethanol 70 %	16.1 µg/ml	16.2 µg/ml

4. CONCLUSION

Asparagus aphyllus L. contains flavonoids and phenolic compounds, which may be why the antitumor activity of the plant is attributed to them. The chloroform extract had the best IC₅₀ result with MCF7 (Breast carcinoma cell line), and the ethanol 70% extract

Table 2. UV, ¹H and ¹³C NMR data of flavonoid compounds.

Cpd	UVλ _{max} nm (MeOH)	¹ H-NMR δ(ppm)	¹³ C-NMR δ(ppm)
M4	266, 335	6.18 (d, J=2.5Hz, H-6), 6.47(d, J=2.5Hz, H-8), 6.77(s, H-3), 6.92(d, J=8Hz, H-3' and H-5'), 7.93(d, J=8Hz, H-2' and H-6')	163.8 (C-2), 102.8 (C-3), 181.5 (C-4), 161.3 (C-5), 98.7 (C-6), 163.6 (C-7), 93.9 (C-8), 157.2 (C-9), 103.6 (C-10), 121.1 (C-1'), 128.3 (C-2' and C-6'), 115.8 (C-3' and C-5') and 161.4(C-4').
M5	260, 370	7.79 (1H, d, J= 2.5 Hz, H-2'), 7.7 (1H, dd, J= 2.5 and 8.5 Hz, H-6'), 6.91 (1H, d, J= 8.5 Hz, H-5') 6.78 (1H, d, J= 2.5 Hz, H-8) and 6.49 (1H, d, J= 2.5 Hz, H-6).	147.5 (C-2), 136.5 (C-3), 176.5 (C-4), 161 (C-5), 99.5 (C-6), 166 (C-7), 94.5 (C-8), 156.7 (C-9), 104 (C-10), 123 (C-1'), 116 (C-2'), 145.4 (C-3'), 148.1 (C-4'), 116.5 (C-5'), 121 (C-6')
M6	267, 340	6.18 (d, J=2.5Hz, H-6), 6.47(d, J=2.5Hz, H-8), 6.77(s, H-3), 6.92(d, J=8Hz, H-3' and H-5'), 7.93(d, J=8Hz, H-2' and H-6'). Rhamnose moiety: 5.20 (1H, d, J= 4 Hz, H-1"), 3.1 – 3.9 (m, overlapped with water proton resonances, H-2" - H-5"), 0.8 (3H,d, J= 6 Hz, CH ₃ rhamnose)	163.8 (C-2), 102.8 (C-3), 181.5 (C-4), 161.3 (C-5), 98.7 (C-6), 163.6 (C-7), 93.9 (C-8), 157.2 (C-9), 103.6 (C-10), 121.1 (C-1'), 128.3 (C-2' and C-6'), 115.8 (C-3' and C-5') and 161.4(C-4'). Rhamnose moiety: 102.2 (C-1"), 70.8 (C-2"), 71.1 (C-3"), 71.6 (C-4"), 70.5 (C-5"), 18.01(CH ₃).
M7	260, 370	7.79 (1H, d, J= 2.5 Hz, H-2'), 7.7 (1H, dd, J= 2.5 and 8.5 Hz, H-6'), 6.91 (1H, d, J= 8.5 Hz, H-5') 6.78 (1H, d, J= 2.5 Hz, H-8) and 6.49 (1H, d, J= 2.5 Hz, H-6), 3.81 (3H, s, OCH ₃).	147.2 (C-2), 135.9 (C-3), 175.8 (C-4), 160.3 (C-5), 97.3 (C-6), 164.8(C-7), 91.8 (C-8), 156.0 (C-9), 103.9 (C-10), 121.8 (C-1'), 115.2 (C-2'), 145.0 (C-3'), 147.7 (C-4'), 115.5 (C-5'), 119.9 (C-6'), 55.9 (OCH ₃)
M8	350, 299, 266, 259	6.18 (d, J=2.5Hz, H-6), 6.37 (d, J=2.5 Hz, H-8), 7.55 (d, J=2.5 Hz, H-2'), 6.85 (d, J=8Hz, H-5'), 7.56 (dd, J=2.5 and 8 Hz, H-6'). Sugar moiety: 5.32 (1H, d, J=8 Hz, H-1" glucose), 4.50 (1H, d, J=2.5 Hz, H-1" rhamnose), 3.40(m, remaining sugar protons), 0.8 (1H, d, J=6Hz, CH ₃ -rhamnose).	146.9 (C-2), 135.5 (C-3), 175.8 (C-4), 160.7 (C-5), 98.8 (C-6), 164.1(C-7), 93.6 (C-8), 156.7 (C-9), 103.1 (C-10), 121.6 (C-1'), 115.3 (C-2'), 144.8 (C-3'), 148.5 (C-4'), 116.3 (C-5'), 120.2 (C-6') Glucose moiety: 101.2 (C-1"), 74.1 (C-2"), 76.5 (C-3"), 70.0 (C-4"), 75.9 (C-5"), 67.5 (C-6"). Rhamnose moiety: 100.8 (C-1"), 70.4 (C-2"), 70.6 (C-3"), 71.9 (C-4"), 69.1 (C-5"), 18.1 (CH ₃ -rhamnose).

had the best result with HCT (Colon carcinoma cell line). To understand how it functions, more biological research is needed to determine the cytotoxicity of the plant aerial parts' aqueous alcoholic extract.

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