Egypt. J. Chem. **Vol. 67**, No. 12 pp. 347 - 363 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

UPLC-QTOF/MS Profiling of *Portulaca Oleracea* Extract and Green Biosynthesis of Gold Nanoparticles with Anticancer Potential

Nada T. El-Bayioumy[1], Magdy K. Zahran[2], Hanaa M. El-Rafie[3], Maha I. Fatthalla[2], Mohamed S. Abdel-Aziz[4], Asmaa S. Mohamed[5]*

[1] Forensic laboratory, Ministry of Interior General Administration of Criminal Evidence Investigation, 417 Ramses, El-Abaseya El-Bahareya, El Weili, Cairo , Egypt
[2] Chemistry Department, Faculty of Science, Helwan University, Ain-Helwan, Cairo, 11795, Egypt
[3] Pharmacognosy Department, Pharmaceutical and Drug Industries Research Institute, National Research Centre, 33 El Bohouth St., P.O. 12622 (ID: 60014618), Dokki–Giza, Egypt
[4] Microbial Chemistry Department, Biotechnology Research Institute, National Research Centre, Cairo, Egypt.
[5] Medicinal Chemistry Department, Theodor Bilharz Research Institute, Kornaish El Nile, Warrak El-Hadar, Imbaba (P.O. 30), Giza 12411, Egypt

Abstract

Recently, the nanotechnology sector has opened new avenues for enhancing the therapeutic potential of plant-based compounds. Nanoparticles, especially those synthesized from plant extracts, have shown great promise in improving the delivery and efficacy of anticancer agents. These plant-derived nanoparticles can target cancer cells more precisely, minimizing side effects and maximizing therapeutic outcomes. A chemical profiling study using UPLC-QTOF-MS/MS was conducted to explore the *Portulaca oleracea* (*P. oleracea*) phytoconstituents that exert the biological activities. Fifty compounds, comprising phenolic acids, organic acids, flavonoids, coumarins, fatty acids, alkaloids and amino acids were recognized via chemical characterization. The Folin.-Ciocalteu technique was utilized to assess the overall phenolics content of the obtained fractions, while the total amount of flavonoid was obtained using $AlCl_3$. Moreover, the antioxidant ability was measured by ABTS, FRAP and DPPH techniques. Our findings revealed that the methanolic fraction has the greatest level of flavonoid (121.47 ±1.41 mg RE/g extract) and phenolic amounts (555.15 ± 1.14 mg GAE / g extract) among the examined extracts also the total alkaloid and the total saponin percent of the methanolic extract are 1.79% and 1.28% respectively. Moreover, methanol extract also show the highest antioxidant activity IC_{50} = 6.87, 21.31, 20.18µg/mL for ABTS, DPPH and FRAP respectively, this activity could be explained by the extract's high flavonoid and phenolics content. The water extract of *P. oleracea* was employed in producing gold nanoparticles AuNPs. UV-Vis spectrophotometric analysis and transmittance electron microscope (TEM) were used for analyzing the biosynthesized AuNPs. Additionally, the anti-cancer potential of the water extract and its biosynthesized AuNPs was explored by MTT assay against several cancer cell lines. The findings demonstrated that the the synthesized gold nanoparticles (AuNPs) was active against mammary gland breast cancer MCF-7 with an IC_{50} of (29.44±0.5) µg/mL compared to the reference medication, staurosporine STA with an IC_{50} of (12.24±0.68) µg/mL where in case of hepatocellular carcinoma HepG-2 cell lines the AuNPs has IC_{50} of (14.51±1.02) µg/mL, compared to staurosporine STA IC_{50} (5.20±0.87) µg/mL. The IC_{50}valuesofAu-NPs were 31.14±0.87µg/mL for cervical cancer HeLa, 18.11±2.1 µg/mL for colon cancer cell lines Caco-2, 21.0±0.99 µg/mL, for HCT116 and17.47±1.3 µg/mL, for kidney cancer cell line UO-31 cell line. Across all cancer cell lines tested, the synthesized gold nanoparticles (AuNPs) from *P. oleracea* extract consistently demonstrated lower IC_{50} values compared to the crude water extract, indicating that the synthesis of AuNPs enhances the anticancer activity of the plant extract, this can be attributed to several factors, including their small size, which allows for better penetration into cancer cells, and their ability to induce reactive oxygen species (ROS), leading to apoptosis. The combination of plant-based bioactive compounds and nanotechnology provides a promising strategy for developing effective anticancer therapies.

Keywords: *Portulaca oleracea*; Gold nanoparticle; Green synthesis; UPLC-QTOF-MS; Anticancer, Antioxidant.

*Corresponding author e-mail: asmaasalah25@yahoo.com.;(Asmaa S. Mohamed). https://orcid.org/0000- 0001- 9397- 0556
EJCHEM use only: Received date 11 September 2024; revised date 04 October 2024; accepted date 15 October 2024
DOI: 10.21608/ejchem.2024.320054.10399

1. Introduction

Cancer remains among the most significant global challenges, despite advances treatment modalities. The continuous quest for more effective, less toxic therapies underscores the need for novel anticancer drivers. Historically, plants have been a precious source of medicinal constituents, with many traditional remedies based on plant extracts demonstrating significant anticancer properties [1, 2]. These plant-derived compounds offer a diverse chemical arsenal capable of inhibiting cancer cell growth and proliferation [3]. Recently, nanotechnology has opened new avenues for enhancing the therapeutic potential of plant-based compounds. Nanoparticles, especially those synthesized from plant extracts, have shown great promise in improving the delivery and efficacy of anticancer agents [4]. These plant-derived nanoparticles can target cancer cells more precisely, minimizing side effects and maximizing therapeutic outcomes. Emerging studies have demonstrated the successful application of plant-derived nanoparticles in combating various types of cancer. These nanoparticles not only enhance the bioavailability of plant compounds but also improve their selective uptake by cancer cells, thereby increasing their therapeutic efficacy [5].

Numerous studies have been conducted regarding noble metal nanoparticles creation, owing to the great and diverse potential of these nanoparticles in biological, therapeutics, pharmaceutical, biomedical and environmental applications [6]. Out of the several existing methods, AuNPs can be synthesized using “green” methods utilizing plant materials such as flower, leaves, fruit, seed, bark and roots [7]. Utilizing plant biomasses may reduce metallic ions producing metallic nanoparticles. Naturally occurring chemicals in plant extracts may exert stabilizing, capping, and reducing properties on synthesized metal nanoparticles [7]. Gold nanoparticles AuNPs fabrication by using plants may be more favourable than the physical and chemical approaches as these processes are economical, simple to carry out, comprehensive, eco-friendly, and may not require high pressure, temperature, or any hazardous chemicals. Many different types of plants including herbs, vegetables, fruits, trees, shrubs, and fungi have been explored for gold nanoparticle synthesis [8].

Purslane, or *Portulaca oleracea* L., is a perennial herbaceous plant belonging to the Portulacaceae family, which grows easily in a variety of soil types and climates and is widely dispersed around the world [9]. It is frequently utilized as traditional meal and dietary supplement because of its high nutritional content and health advantages. Numerous studies have demonstrated the plant's abundance in numerous significant phytochemicals, including proteins, carbohydrates, alkaloids, terpenoids, and flavonoids. Purslane also contains carotenoids, minerals and vitamins. Furthermore, *P. oleracea* has been recognized for its antioxidant, anticancer, skeletal muscle relaxant, antidiabetic, anti-insomnia, hepatoprotective, anti-inflammatory, neuroprotective and cholesterol-lowering activities [10-12]. From conventional medicine, Purslane is employed to cure a number of illnesses, including dysentery, diuresis, wound healing, bronchitis, gonorrhoea, and antiseptic properties [13-14]. Additionally, *P. oleracea* is reported to have a potential role in gold uptake and could tolerate both soluble and nanosized gold. However, the potential of *P. oleracea* for producing gold nanoparticles through biosynthesis is still unknown, and hence this paper was undertaken to explore the biosynthesis of AuNPs utilizing *P. oleracea* and characterize it using different experimental techniques including UV-Visible and TEM. The synthesized AuNPs were examined for their biological applications including selective cytotoxicity against cancer cell line using human tumor cell lines. Additionally, this work aims to investigate the antioxidant potential and determine the main components of *P. oleracea* extract responsible for the biological activity.

2. Experimental

2.1. *Collection and extraction of P. oleracea*

The *P. oleracea* aerial parts were harvested from local fields in Beheira governorate, Egypt during February–March 2020. Once the plant was shade-dried, a mortar pestle was used to pulverize it, and then sieved to attain 0.20 -0.60 mm particle sizes. The fine powder was kept in storage at – 30°C. Subsequently 10 g of *P. oleracea* powder in 500 mL of 90% methanol was extracted using the ultrasound-assisted extraction (UAE) technique for 40 min at 50°C using Nade Scientific Instruments, SK-24TC, 25 kHz, China, an ultrasonic cleaner. After centrifuging the material for ten minutes at 10,000 rpm, the supernatant was removed and dried by evaporating it [15]. Fractionation of the crude extract of *P. oleracea* was done by using a variety of solvents such as petroleum ether (60-80), methylene chloride, ethyl acetate, *n*-butanol and water.

2.2. *Phytochemical analysis*

The reported methods were carried out in order to perform the phytochemical analysis of the *P. oleracea* derived fractions [16-21].

2.3. ***Determination of total phenolic contents (TPC) and total flavonoid contents (TFC):***

The overall amount of phenolic compounds in the extracts was assessed via phosphomolybdenum assay according to Osman *et al*. [22]. Mossalem *et al*.'s technique [23] was also used to calculate the total flavonoid amount.

2.4. ***Determination of the overall alkaloids content***

The estimation of alkaloids has been done according to the standard method [20].

2.5. ***Determination of total saponin content***

The explained approach by Obadoni *et al* [24] was followed with minor adjustments to evaluate the overall saponin content.

2.6. ***Ultra-performance liquid chromatography/ quadrupole-time-of-flight mass spectrometer analysis (UPLC-QTOF-MS)***

The phytochemical profiling of the methanolic extract of *P. oleracea* were analyzed via a an Axion AC system (Kyoto, Japan) in conjunction with a triple TOF 5600+ system fitted with a Duo-Spray source operating in (ESI) the electrospray ionization mode (AB SCIEX, Concord, Canada) and attached with an autosampler, a precolumn (Phenomenex, 0.5 µm × 3.0 mm, USA), and an Xbridge C18 column (Waters Corporation, Milford, MA, USA) all of which were maintained at 40°C and a flow rate of 300 µL/min as formerly explained [25].

2.7. ***Antioxidant activity***

2.7.1. Radical scavenger potential by ABTS assay

Radical scavenger potential by ABTS technique of the extract and its fractions was valued by a procedure reported by Bakchiche *et al*. [26]. The ABTS+ solution was made and left to stand at room temperature for sixteen hours in the dark. After that, 40 milliliters of deionized water were added to 1 milliliter of the solution to create a working ABTS+ solution, which had an absorbance of 0.70 at 734 nm. The test samples (15 µL) were added to ABTS+ working solution (1.485 mL). The absorbance was measured at 734 nm after 10 minutes. ABTS radical-scavenging activity (%) = $(A_c - A_s) / (A_c) \times 100$ was the formula used to compute ABTS free radical-scavenging activity. The absorbance of the ABTS radical + methanol is called A_c, and the absorbance of the ABTS radical + sample extract / standard is called A_s. The graph of the percentage of the ABTS scavenging action against extract concentration was used to determine the extract concentration that provided 50% radical scavenging activity (IC_{50}). An antioxidant standard called Trolox was utilized to compare the activity.

2.7.2. Ferric reducing antioxidant power (FRAP) method

The FRAP assay was used to assess the antioxidant power of extracts against ferric reduction. When antioxidants are present, the complex 2,4,6-tripyridyl-S-triazine (TPTZ)-Fe^{3+} is reduced to TPTZ-Fe^{2+} (colored form), and this change in absorbance is measured using the FRAP method [27]. The solution for this experiment was prepared by mixing 25 mL of 300 mmol/L acetate buffer, 2.5 mL of 10 mmol/L TPTZ in 40 mmol/L HCl, and 2.5 mL of 20 mmol/L $FeCl_3.6H_2O$. We made samples using different *P. oleracea* solvent extracts at varying concentrations (50–500 µg/mL). After combining 900 µl of FRAP reagent with 100 µl of extract, the reaction mixture was incubated for 4 minutes at 37°C. The absorbance of the mixture was measured at 593 nm, with quercetin serving as the standard. The mean of three readings was calculated and expressed as mM $FeSO_4$ equivalent/mg extract.

2.7.3. DPPH radical inhibition method

The DPPH scavenging ability was established using a methodology that has been previously published [28]. The following equation was utilized to calculate the inhibitory activity (%):$[(Ab_c-Ab_t)/Ab_c] \times 100$, where Ab_c represents the control absorbance and Ab_t represents the standard or extract absorbance. The IC_{50} was determined by regression analysis.

2.8. ***Biosynthesis of gold nanoparticles (AuNPs)***

Twenty gram *P. oleracea* leaves were thoroughly washed to remove particulate materials and additional water was eradicated by wrapping them between filter papers. In 2000 mL volumetric Erlenmeyer flask, 500 mL distilled water were added to 20g leaves and left for 30 minute at 80°C and then the obtained extract was filtered. The production of gold nanoparticles (AuNPs) in a safe environmental manner was done as previously investigated by Hassanisaadi *et al*. [29]. Au^{3+} ions were reduced to Au^0 using the water extracts. A 5mL *P. oleracea* extract was added to 45mL of 5mM $HAuCl_4$ to generate 5mM Au^{3+} ion, and the mixture was kept at 25°C overnight. It was possible to keep the bottles shaking continuously to prevent agglomeration and enhance biosynthesis. The gold solution (faint yellow) treated with distilled water was kept as control. A bio-fabrication of AuNPs was observed 24 h following the mixing the $HAuCl_4$ solution with plant extract.

2.9. *Characterization of biosynthesized gold nanoparticles*

2.9.1. UV- Visible spectroscopic analysis

Ecofriendly reduction of tetrachloroauric acid ($HAuCl_4$) to gold nanoparticles AuNPs was observed after 24 hrs. A UV–Vis spectrograph was recorded between 200 and 800 nm in scanning speed by UV–visible Shimazu 2401PC spectroscopy [30].

2.9.2. Transmittance electron microscopic analysis (TEM)

The suspension of AuNPs biosynthesized by plant extract was examined by electron microscope (JEOL 1200 EX). To create TEM sample, carbon-covered copper grids were coated with a drop of the AuNPs solution suspension, the samples have been allowed for drying on the grid for four minutes. TEM micrographs were used to determine the size and form of AuNPs derived from *P. oleracea* [31].

2.10. *Anticancer potential via (MTT) micro culture tetrazolium assay*

Using malignant cell lines from humans, specifically hepatocellular carcinoma (HePG-2), cervical malignant (HeLa), human colon malignant cell lines (Caco-2, HCT116), human kidney malignant cell line (UO-31) and mammary gland breast cancer (MCF-7), the cytotoxicity against tumor cell line was performed in accordance with the MTT assay as previously described [32]. VACSERA Company, Egypt, provided the cell lines. Staurosporine STA, an anti-cancer medication, served as the baseline for comparison [33].

2.11. *Analytical statistics*

Every outcome was examined statistically via the computerized SPSS program, edition "13". The findings were displayed as the mean ± standard deviation.

3. Results and Discussion

3.1. *Ultrasound-assisted extraction (UAE) and Phytochemical examination*

Conventional extraction techniques utilized extensively in order to extract the plant constituents. But these techniques have various shortcomings, including extended periods of extraction, poor extraction effectiveness and excessive use of energy [15]. In order to overcome these constraints, recent developments in extraction technology have prompted the creation of novel extraction techniques. Among these techniques is (UAE) ultrasonic assisted extraction that has proven incredibly effective at plants extraction. Ultrasonic assisted extraction is unique because practicality and adaptability in manufacturing environment [34-35]. The ultrasonic waves in UAE induce the breakdown of cell membrane and improve the flow of materials present inside the cells into the solvent used for extraction. Thus ultrasonic assisted extraction shortens extraction times and greatly increases extraction proficiency. The plant extracts were qualitatively examined and the results in Table 1 had showed the existence of phytoconstituents as phenols, flavonoid, alkaloid, saponins, glycoside, steroid and tannins.

Phenolic and flavonoids act an important role in overall physiological routes, so they consider important criteria for assessing the biological potency plant extract [36]. In the current research, the overall flavonoids and phenolics amount of methanol extract of *P. oleracea* and its derived fractions is evaluated, (Table 2). The findings revealed that the methanolic extract contained considerably higher total phenolics (555.15 ± 1.14 mg of GAE/g extract) after that butanol extract (439.91 ± 0.29 mg of GAE/g extract), this could maybe due to the superior polarity of methanol [36]. Also, the methanol extract of *P. oleracea* contained higher total amount of flavonoids (121.47 ± 1.41 mg of RE/g extract) afterward the butanol extract (99.10 ± 0.81 mg of RE/g extract), this investigation is consistent with the previous findings. The total amount of flavonoids and phenolics was more in methanol fraction relative to other *P. oleracea* extracts. Phenolics possess an extensive array of biological characteristics, such as the ability to modify gene expression and their anti-oxidant and anti-carcinogenic qualities. Flavonoids are biologically active substances that resi st bacteria, ulcers, arthritis, angiogenic disorders, and malignant diseases. They also prevent the creation of mitochondrial adhesion [37].

3.2. *Identification of secondary metabolites of P. oleracea via UPLC-QTOF-MS spectrometer*

Combining UPLC with high resolution mass spectrometry (MS/MS) is an effective method for quickly identifying a wide range of chemical compounds; it provides precise mass and formula information as well as the ability to distinguish between isomeric compounds [38]. To obtain the profile of *P. oleracea* metabolites, UPLC-QTOF-MS analysis operated in the negative ionization mode was performed. The molecular weights, fragmentation patterns and published information on the plant were used to identify the structures of the detected compounds. Fifty compounds in all were found in the methanolic extract of *P. oleracea* and identified (Table 3, Fig.1). These compounds were categorized as flavonoids, flavonols, phenolics, carboxylic acids, indole carboxylic acids, coumarin glucoside derivative, amino acids, fatty acid esters, hydroxy fatty acids, dipeptides, Imidazole and alkaloids.

Table 1: Phytochemicals screening of different *P. oleracea* extracts

Phytoconstituents	Methanol. extract	Petroleum ether extract	Methylene. Chloride extract	Ethyl acetate extract	*n*-Butanol extract
Flavonoids	+	+	-	+	+
Phenolics	+	-	+	-	+
Tannins	+	+	-	+	-
Alkaloids	+	-	-	-	-
Terpenes	+	+	-	+	+
Saponins	+	-	-	-	-
Steroids	-	-	-	+	+
Glycosides	+	-	-	+	+

(+) presence, (−) absence

Table 2: Total phenolics content (TPC), total flavonoids content (TFC), percentage of alkaloids and saponins contents of *P. oleracea* extracts

Sample	TFC (mg RE/g extract)	TPC (mg GAE / g extract)	Total alkaloids %	Total saponins %
Methanol	121.47 ±1.41	555.15 ± 1.14	1.79 ± 0.14	1.28±0.18
Petroleum ether	34..19 ± 1.01	33.24 ± 0.06	0.87 ± 0.10	0.24 ± 0.02
Methylene chloride	56.12± 0.69	21.24 ± 2.07	0.91± 0.09	0.79 ± 0.08
Ethyl acetate	87.98 ± 0.47	427.25 ± 1.51	1.23 ±0.26	0.26 ± 0.06
n-butanol	99.10 ± 0.81	439.91±0.29	2.21 ± 0.17	0.169±0.68

Results are the average of triplicate experiments (n=3) and values expressed as the mean ± standard deviation, the measurement unit for the total amount of phenolic compounds is milligram gallic acid equivalent per gram extract (mg GAE/g ext.), while the measurement unit for the concentration of flavonoid is milligram rutin per gram extract (mg RE/g ext).

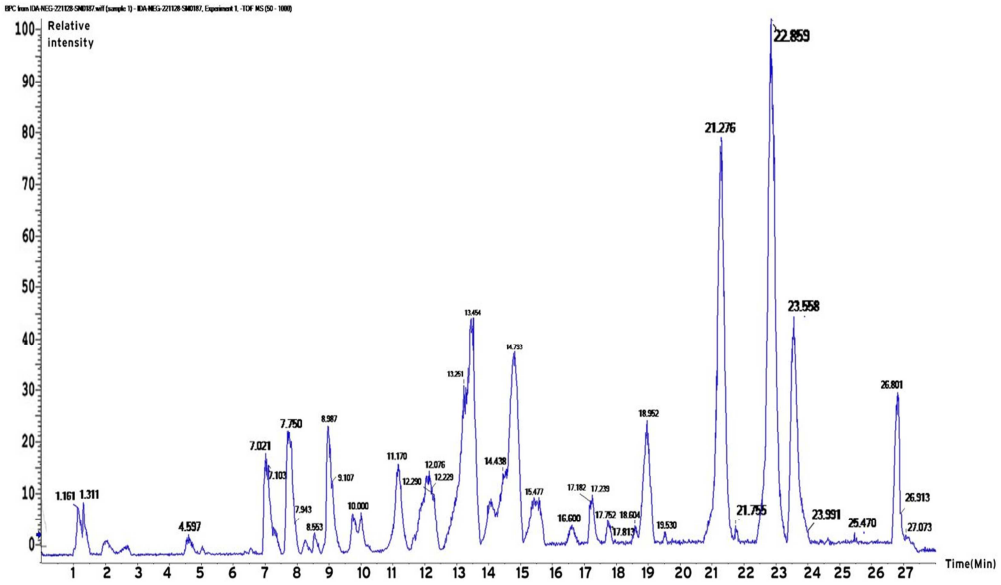


Figure 1: UPLC-QTOF-MS chromatogram of *P. oleracea* extract in negative mode

Table 3: List of the identified compounds of *P. oleracea* methanolic extract by UPLC-QTOF-MS in negative mode

Identified Compound	Retention time (min)	Exact mass	Mass fragmentation	Elemental formula	Class of compound	Ref.
Citric acid	1.0936	191.0556	163,130,128,111,103	$C_6H_8O_7$	Carboxylic acids	[53]
Gluconic acid	1.120933	195.0524	177,151,129,121	$C_6H_{12}O_7$	Carboxylic acid	[4]
D-(+)-Malic acid	1.1339	133.048	115,71,59	$C_4H_6O_5$	Fatty acids	[56]
L-5-Oxoproline	1.1460	128.0344	128	$C_5H_7NO_3$	Amino acids	[4]
1,2-Benzenediol	1.1466	109.028	108,	$C_6H_6O_2$	Hydroxybenzene	[51]
L-(-)-Phenylalanine	1.1599	164.073	147,120	$C_9H_{11}NO_2$	Amino acids	[4]
2-Isopropylmalic acid	1.1699	175.059	157,115,113	$C_7H_{12}O_5$	Hydroxy fatty acids	[4]
Protocatechuic acid	1.1729	153.020	153,109,91	$C_7H_6O_4$	Phenolic acids	[57]
Citramalic	1.1855	147.066	116,98	$C_5H_8O_5$	Fatty acids	[58]
4-Hydroxy benzoic acid	1.1923	137.02	93,65	$C_7H_6O_3$	Phenolic acids	
Maleic acid	1.2120	115.037	71	$C_4H_4O_4$	Carboxylic acids	[51]
Ferulic acid	1.2384	193.071	178,149,134,105	$C_{10}H_{10}O_4$	Phenolic acids	[4]
L-(-)-Mandelic acid	1.2779	151.061	107,79,77,51	$C_8H_8O_3$	Carboxylic acids	[52]
5-Methoxysalicylic acid	1.3055	167.033	108,91,65	$C_8H_8O_4$	Phenolic acids	[48]
Coumaric acid	1.3194	163.038	119	$C_9H_8O_3$	Phenolic acids	[4]
Citraconic acid (Mesaconic)	1.3204	129.054	129, 99, 85, 83, 68,55	$C_5H_6O_4$	Carboxylic acids	[49]
Gentisic acid	1.3330	153.018	153,109,108,91,84,81	$C_7H_6O_4$	Phenolic acid	[57]
2-Hydroxy-4-methylpentanoate	1.3600	149.070	130,102	$C_6H_{12}O_3$	Fatty acid esters	[56]
Isoferulic acid	1.3613	193.071	161, 133, 115, 105, 77		Phenolic acids	[4]
1H-indole-3-carboxylic acid	2.3068	160.039	116	$C_9H_7NO_2$	Indolecarboxylic acid	[13]
Glycyl-L-proline	2.996667	171.1042	153,127,111	$C_7H_{12}N_2O_3$	Dipeptide	[10]
Itaconate	5.31438	129.0928		$C_5H_6O_4$	Dicarboxylic acid	[4]
Xanthine	5.8155	151.040	136,108,92	$C_5H_4N_4O_2$	Alkaloids	[75]
Kaempferol-3-*O*-robinoside-7-*O*-rhamnoside	5.5912	739.195	592, 430,284	$C_{33}H_{40}O_{19}$	Flavonol glycoside	[59]
Kaempferol-3-*O*-(6-p-coumaroyl)-glucoside	6.0538	593.151	400,284	$C_{30}H_{26}O_{13}$	Acylated flavanol	[60]
Methyl Jasmonate	6.3108	223.135	205,189,183, 161, 131	$C_{13}H_{20}O_3$	Fatty acids	[4]
Carnosine	6.4418	225.109	209,197,181,156,110	$C_9H_{14}N_4O_3$	Dipeptides	[61]
Dicrotalic	6.5864	161.026	144,133,117,99,77,65	$C_6H_{10}O_5$	Carboxylic acid	
Scopoletin	6.612817	191.0357	176, 148, 85	C10H8O4	Coumarin derivative	[62]
Kaempferol-3-*O*-glucoside	6.8011	447.094	331, 284,255, 227	$C_{21}H_{20}O_{11}$	Flavonoids	[60]
Beta-Indoleacetic acid	6.8276	174.055	144,130,116,86,77	$C_{10}H_9NO_2$	Carboxylic acids	[63]
Alpha-methyl serine	6.9593	118.028	90,50	$C_4H_9NO_3$	Amino acids	[4]
Kaempferol-7-neohesperidoside	7.7157	593.146	463,233	$C_{27}H_{30}O_{15}$	Flavonoid glycoside	[39]
Daidzein	8.6673	253.049	224,208,184,133,116	$C_{15}H_{10}O_4$	Isoflavone	[64]
Allantoin	8.9065	157.124	157	$C_4H_6N_4O_3$	Imidazoles	[65]
Kaempferide	9.8421	299.058	284,237,199,137,80,65	$C_{16}H_{12}O_6$	Flavonols	[60]
Apigenin	10.149	269.046	117	$C_{15}H_{10}O_5$	Flavone	[66]
Luteolin	10.333	285.037	241,177,133,109,65	$C_{15}H_{10}O_6$	Flavone	[66]
Inosine	10.5726	267.0298	211	$C_{10}H_{12}N_4O_5$	Alkaloids	[54]
Acacetin	10.863	283.104	253	$C_{16}H_{12}O_5$	Flavonoids	[67]
Linolenic acid	11.1699	277.144	233,205, 103,59	$C_{18}H_{30}O_2$	Fatty acids	[10]
Chlorogenic acid	12.635	353.101	191	$C_{16}H_{18}O_9$	Esterified Phenolic acids	[68]
Neochlorogenic acid	12.655	353.104	191,179,135,85	$C_{16}H_{18}O_9$	Esterified Phenolic acids	[68]
Caffeic acid	12.714	179.107	161,135,107,91	$C_9H_8O_4$	Phenolic acids	[69]
Formononetin	14.298	267.154	252, 224, 199	$C_{16}H_{12}O_4$	Isoflavone	[70]
L-beta-homotyrosine	15.305	194.079	163,134	$C_{10}H_{13}NO_3$	Amino acids	[4]
Esculin	15.64152	339.1977	183	$C_{15}H_{16}O_9$	Coumarins glucoside derivative	[71]
5-Aminoimidazole-4-carboxamide-1-ribofuranosyl 5'-monophosphate	16.179	337.205	97	$C_9H_{15}N_4O_8P$	Amino imidazoles	[72]
Alpha-Linolenic acid	17.9255	277.144	261, 233,205, 103,59	$C_{18}H_{30}O_2$	Omega-3-fatty acids	[10]
Gamma-Linolenic acid	18.9600	277.144	261, 233,205, 103,59	$C_{18}H_{30}O_2$	Omega-6-fatty acids	[10]

3.2.1. Flavonoids

Flavonoids are one of the very prevalent and significant active phytoconstituents of *P. oleracea*, such as kaempferide, acacetin, apigenin, daidzein, formononetin, kaempferol-3-O-(6-p-coumaroyl)-glucoside, astragalin, robinin, kaempferol-7-neohesperidoside and luteolin. The previous studies found that during mass analysis, each flavonoid subgroup has a distinct fragmentation pattern [39].

The most common way that flavonoids fragment is through the retro-Diels-Alder of the C-ring. Small molecules like C_2H_2O (42 Da), CO. (28 Da), 2CO. (56 Da), 3CO (84 Da), COO (44 Da), and CO + COO (72 Da) may also be significantly lost. Flavonoids with methylation additionally include a definite ion [MH CH_3], identified by the loss of CH_3 (15 Da) [40-41].

3.2.2. Phenolic and carboxylic acids

Phenolic acids are frequently identified secondary metabolites in nearly all of the plant profiling investigations. Generally, phenolic acids produced an ion $[M–H]^–$ due to the loss of H and daughter ion $[M\ H\ 44]^–$ corresponding to loss of CO_2 from the carboxy group [25, 42]. In our research, we found ten phenolic acids comprising two esterified acids (chlorogenic and neochlorogenic) and eight free acids (protocatechuic acid, 5-methoxysalicylic, gentisic, ferulic, coumaric, 4-hydroxybenzoic, caffeic, and isoferulic).

2,5-Hydroxybenzoic acid (gentisic acid) was recognized by a molecular ion peak at m/z 153 and at 137$[M\text{-}H\text{-}OH]^-$, 109 $[M\text{-}H\text{-}CO]^-$, 108 $[M\text{-}2H\text{-}CO]^-$, 91 $[M\text{-}H\text{-}CO_2]^-$, 84and 81 [43]. Because there was a signal at m/z 193 of a molecular ion peak $[M\text{-}H]^-$, and a base peaks $[M\text{-}H\text{-}CH_3]^–$ at m/z 178, $[M\text{-}H\text{-}COO]^–$ 149 and $[M\text{-}H\text{-}CH_3\text{-}COO]^–$ 134, ferulic acid was recognized. 3-hydroxy-4-methoxycinnamic acid (isoferulic acid) was tentatively identified by $[M\text{-}H]^-$ peak at m/z 193 with daughter ions at 161, 133, 115, 105, and 77 [44]. Moreover, a compound had signal at m/z 163 demonstrated $[M\text{-}H]^-$ and daughter ions at 119 corresponding to loss of COO, and 59; it was tentatively recognized as p-coumaric acid (4-hydroxycinnamic acid) [4]. Protocatechuic acid was confirmed by its typical fragmentation pattern of m/z 153, 109, 91[45]. A signal demonstrated an $[M\text{-}H]^-$ m/z at 179 and daughter ions at 161$[M\text{-}H\text{-}H_2O]^-$, 135 $[MH\text{-}CO_2]^-$, and 107 $[M\text{-}H\text{-}COO\text{-}CO]^-$; it was tentatively recognized as caffeic acid [46]. Neochlorogenic acid was tentatively recognized from $[M\text{-}H]^-$ signal at m/z 353 and ions signals at 191 [M-H-caffeic acid portion]$^-$, 179 [M-.H- quinic acid portion]$^-$, 161, 135 and 85 [47]. 4-Hydroxybenzoic acid demonstrated by molecular ion peak at m/z 137 followed by a peak corresponding to the loss of carboxylic group $[M\text{-}H\text{-}CO_2]^-$ at 93 [43]. A signal was identified as 5-methoxysalicylic acid with molecular ion peak at 167 followed by fragmentation signals at m/z 108, 91, and 65 [48]. Citraconic acid was identified with peaks at m/z 129, 129, 99, 85, 83, 68, 55 [49]. A signal was identified as gluconic acid due to its molecular ion peak at m/z 195 followed by peaks at m/z 177,151,129,121 [4]. A signal corresponding to loss of H, at m/z 133 and fragment ions at 115 (because of loss of H_2O), 87, and 71; Malic acid was the tentative identification [50]. A signal showed molecular peak at m/z 115 and a fragment peak at 71 corresponding to the loss of carboxylic group; it was recognized to be maleic acid [51]. L-(-)-Mandelic acid also detected with molecular ion signal $[M\text{-}.H]^-$m/z at 151 and ions at107, 79, 77, 51. Additionally, dicrotalic exhibited molecular peak $[M\text{-}.H]^-$ m/z at 161 and ions 144, 133, 117, 99, 77, 65 [52].

3.2.3. Fatty acids

A signal was identified as 2-hydroxy-4-methylpentanoate due to its molecular ion peak at m/z 149, the carboxyl group loses CO_2, leading to a fragment with m/z 102. A common fragmentation involves the loss of acetic acid, resulting in a peak at m/z 130. A signal was identified as 2-isopropylmalic acid, its molecular ion peak $[M\text{-}H]^-$ at m/z 174, loss of CO_2 and CH_3COOH) often occurs, yielding fragments around m/z 148 and 130. The isopropyl group can also fragment, leading to peaks around m/z 116. A compound at m/z 147 was identified as citramalic acid, frequently loses CO_2, producing fragments around m/z 116. Further fragmentation can lead to peaks around m/z 116 and 98 (loss of H_2O). In addition malic acid appear at m/z 133$[M\text{-}H]^-$ with fragmentation at m/z 116 and 98 due to loss of H_2O and CO_2 respectively. A signal $[M\text{-}H]^-$ at 277 m/z and ions at 261 233corresponding to loss of OH, 233 corresponding to loss of CO_2, 205, 103, and 59 it was tentatively recognized as omega-3 fatty Acid (α-linolenic acid). A signal at m/z 233 $[M\text{-}H]^-$ and ions at 205 $[M\text{-}CH_3\text{-}OH]^-$, 189$[M\text{-}H\text{-}COO]^-$, 183 ,161 ,131, 99 it was tentatively recognized as methyl jasmonate. As a consequence of the extremely high content of fatty acids which are not often generated in earthly plants—fatty acids are particularly employed in ethno medicine and pharmacology [53].

3.2.4. Alkaloids

As shown in Table 3, two alkaloids component were identified in the tested extract as inosine and xanthine [54].

3.2.5. Coumarins derivatives

Scopoletin (coumarins derivative) was identified through a molecular ion peak at m/z 191 and fragment ions.at 176, 148, 85 [55]. Also Esculin (coumarins glucoside derivative) show a molecular ion peak at m/z 339 and daughter ions peak at 177 [M-H-glucose moiety]$^-$

3.2.6. Others

A peak corresponding to the molecular ion.at 109 m/z and peak at 108, 91 [M-H-H_2O]$^-$, 65, and 53; it was tentatively recognized as1, 2-dihydroxy benzene (pyrocatechol) [55]. In addition, *P. oleracea* extract contain three amino acids that detected as phenylalanine, L-beta-homotyrosine-HCl, alpha-methyl-DL-serine [4].

3.3. *Antioxidant potential of P. oleracea extracts*

The phytoconstituents of *P. oleracea were* responsible for its antioxidant potential [73]. To evaluate its antioxidant capacity, a number of assays have been utilized, such as DPPH, FRAP, and ABTS. The findings demonstrated that purslane's methanolic extract exhibited potent antioxidant activity (Table 4). ABTS IC_{50}=6.87µg/ml; DPPH IC_{50} = 21.31µg/ml; FRAP =20.18 mM $FeSO_4$ equivalent/mg extract, and it is associated with increased levels of total phenolics, omega-3 fatty acids [74]. Antioxidants are vital to maintaining health of people because they reduce the possibility of free radicals damaging cells [10]. *P. oleracea* has a wide variety of chemical constituents many of which have been discovered and found to have antioxidant properties. Such as alkaloids, inosine and xanthine exhibited antioxidant activities [75-76]. Another research found reduction in nitric oxide and in peroxidation of lipid in rat's liver. These results collectively imply that *P. oleracea* provides defense against free radicals.

3.4. *Characterization of gold nanoparticles from P. oleracea aqueous extract*

3.4.1. Colour change and UV/vis absorbance

The green production of AuNPs through *P. oleracea* aqueous extract was monitored using UV–visible spectroscopy where the first clue to the biosynthesis of AuNPs is the solution's color shift. The colour of tetrachloroauric acid ($HAuCl_4$) solution was gradually changed from yellow to purple or red (Fig. 2-a). Reaction mixture color changes result from collective electron oscillations on the AuNP surface, the UV/Visible of the produced AuNP was scanned by spectrophotometer (Shimadzu UV 2041PC). The produced gold nanoparticles exhibited characteristic surface Plasmon resonance at 537nm for *P. oleracea* (Fig. 2).

3.4.2. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) findings revealed that the biosynthesized gold nanoparticles (AuNPs) showed a typical particle size of 8.8 nm to 25 nm for *P. oleracea* (Fig. 3).

3.5. *A Anticancer activity of P. oleracea extract and its biosynthesized gold nanoparticles*

The anticancer potential of *P. oleracea* water extract and its synthesized gold nanoparticles (AuNPs) was evaluated across six different human tumor cell lines: hepatocellular carcinoma (HePG-2), cervical cancer (HeLa), human colon cancer (Caco-2 and HCT116), human kidney cancer (UO-31), and mammary gland breast cancer (MCF-7). Currently MTT dye is commonly known as a safe approach of examining the rate of cell proliferation.

Dehydrogenase enzymes of active cells reduce MTT (yellow colour) to give intracellular formazan (purple colour) which can be measured spectrophotometrically. Specifically, in our work we used the MTT assay to evaluate the anticancer activity of the *P. oleracea* extract and its biosynthesized AuNPs .The half-maximal inhibitory concentration (IC_{50}) values for each treatment were compared with staurosporine (STA), a potent anticancer agent known for its ability to induce apoptosis.

The findings in Table 5 demonstrated that the IC_{50} against HePG-2 cells value for the extract was 20.78±2.11 µg/mL, demonstrating moderate anticancer activity against liver cancer cells. The IC_{50} value of AuNPs synthesized from *P. oleracea* improved significantly to 14.51±1.02 µg/mL, suggesting that the AuNPs synthesized from *P. oleracea* extract have enhanced potency compared to the crude extract, indicating that nanoparticle synthesis enhances bioavailability and cellular uptake. Moreover, the IC_{50} value for the extract was 44.52±1.30 µg/mL, indicating lower anticancer activity against HeLa cells and the IC_{50} value decreased to 31.14±0.87 µg/mL, reflecting improved anticancer efficacy with the use of AuNPs. Additionally, the IC_{50} values in colon cancer cells Caco-2 were 26.19±1.54 µg/mL for the extract alone and 18.11±2.1 µg/mL for the synthesized AuNP formulation, suggesting better efficacy with the AuNP formulation. While for HCT116 (Human Colon Cancer) the IC_{50} value of *P. oleracea* extract was 33.41±1.09 µg/mL, showing relatively moderate anticancer activity, but the synthesized AuNPs: The IC_{50} value improved significantly to 21.0±0.99 µg/mL, demonstrating a marked increase in efficacy. As with other cell lines, the synthesized AuNPs from

P. oleracea extract exhibited superior anticancer activity compared to the extract alone, though STA remained the most potent. Also the synthesized AuNPs displayed greater anticancer activity against human kidney cancer (UO-31) and Mammary Gland Breast Cancer (MCF-7) than the extract.

Finally, across all cancer cell lines tested, the synthesized gold nanoparticles (AuNPs) from *P. oleracea* extract consistently demonstrated lower IC_{50} values compared to the crude water extract, indicating that the synthesis of AuNPs enhances the anticancer activity of the plant extract. This can be attributed to the improved bioavailability, cellular uptake, and stability of the bioactive compounds in the nanoparticle form.

The enhanced anticancer activity of AuNPs can be attributed to several factors, including their small size, which allows for better penetration into cancer cells, and their ability to induce reactive oxygen species (ROS), leading to apoptosis. The combination of plant-based bioactive compounds and nanotechnology provides a promising strategy for developing effective anticancer therapies. Treatments can now be specifically and directly targeted to malignant cells and tumors through the use of nanotechnology. Additionally, nanotechnology offers a special combination of tools for overcoming drug resistance, delivering medication via biological barriers, and coupling therapeutic agents with imaging techniques to provide synergistic effects. As a result, the biosynthesized AuNPs loaded with plant extract showed a significant potential for effectiveness. [77].

AuNPs can trigger apoptotic pathways in cancer cells, leading to programmed cell death. The phytochemicals from *P. oleracea* may synergistically enhance this effect**.** Gold nanoparticles can increase ROS levels in cancer cells, causing oxidative stress and subsequent cell death. The antioxidant properties of *P. oleracea* might possibly be involved in adjusting this effect. Functionalization of gold nanoparticles with specific ligands or antibodies can enable targeted delivery to cancer cells, improving the therapeutic index while minimizing side effects on healthy tissues. To increase the effectiveness of conventional chemotherapy drugs, gold nanoparticles can be added. The natural compounds from *P. oleracea* may also have synergistic effects used alongside these treatments. Our findings show a diverse array of compounds as phenolic acids, fatty acids, carboxylic acids, amino acids and flavonoids. These compounds are well-known because their roles in the metabolism of plant and potential health benefits in humans (e.g., anti-cancer properties). The existence of multiple phenolic acids such as gentisic, ferulic acid, caffeic acid etc. , are known for their antioxidant properties, which can help protect cells from oxidative stress and damage-factors associated with cancer development. Research indicates that phenolic acids can induce apoptosis in cancer cells, inhibit cell proliferation. These mechanisms are especially relevant in cancer cell lines such as breast, colon, and prostate cancer [78].

As mentioned in the results, IC_{50} values were harmonized with the current findings; the previous studies showed that phytoconstituents derived from *P. oleracea* stimulate apoptosis in cervical cancer [79]. Zheng *et al*. [80] showed that Portulac-erebroside A isolated from *P. oleracea*, significantly reduced human liver cancer cell. Fatty acids, particularly unsaturated fatty acids like alpha-linolenic acid and gamma-linolenic acid, have shown promise in preventing cancer cell growth. They can modulate inflammation and have been associated with reduced risk in various cancer types. Specifically, certain fatty acids can alter cell membrane fluidity, thereby affecting receptor signaling and gene expression involved in cell growth and apoptosis. Cell lines from colorectal cancer, breast cancer cell line and colon carcinoma have been observed to respond favorably to treatments with omega-3 fatty acids. Carboxylic acids, including short-chain fatty acids like citric acid and malic acid might suggest insights into metabolic pathways. These compounds have vital roles in energy production and metabolism. Furthermore, flavonoid is a broad class of polyphenolic substances with potent antioxidant properties. It has been established that they have anti-inflammatory and antiproliferative properties on several kinds of cancer cells. Flavonoids that have demonstrated the ability to block the progression of the cancer cell cycle and stimulate apoptosis include daidzein, acacetin, luteolin, and kaempferol. Studies utilizing cell lines as leukemia, lung and breast cells have exhibited the potential of flavonoids to impede the growth of tumors [81- 82]. In agreement to our characterizing the *P. oleracea* extract, include flavonoids and their polyphenolic derivatives [83-85]. Flavonoid-derived nanoparticles exhibit encouraging anticancer properties [86]. It has recently been demonstrated that apigenin induces apoptosis, which potency and dramaticallyinhibits the proliferation of human gliobl astoma cell lines [87–89]. Moreover, kaempferol has demonstrated anti-glioma activity by promoting apoptosis and the production of ROS [90]. Thus, polyphenolic substances including apigenin and kaempferol as well as polysaccharides coexist with *P. oleracea's* cytotoxic actions on human glioma cells. Also, *P. oleracea* blocked the HepG2 and HeLa cells proliferation [91]. Also *P. oleracea* leads to cytotoxicity in colon cancer, breast cancer and kidney cancer cell lines. In addition, amino acids can affect the metabolism and growth of cancer cells, especially those that are involved in the creation of proteins and signaling molecules. Proline or phenylalanine may

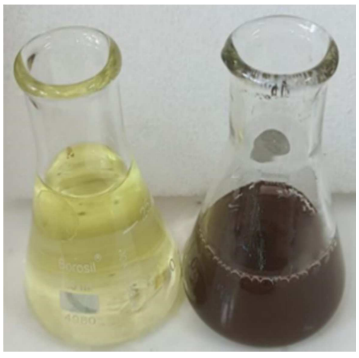
have the ability to boost cytotoxic action in addition to improving the affinity for binding the cancer cell membrane [92-93]. Thus, the findings of this research indicate that the *P. oleracea* extract 's constituents exhibit a complex interaction with cancer cell lines via a range of methods, such as triggering apoptosis, lowering inflammation, preventing cell division, and altering metabolic pathways. These substances' interactions may have synergistic effects that increase their potential as therapeutic agents for the prevention and treatment of cancer. Further investigation into these chemicals could pave the road for the creation of potent medicines or nutraceuticals targeted at the fight against cancer. However, because condensed tannins negatively regulate transcription factors, growth factors, receptor kinases, and other carcinogenic molecules, they have also been noted as interesting anticancer possibilities [94].

Table 4: Antioxidant activity of *P. oleracea* extracts

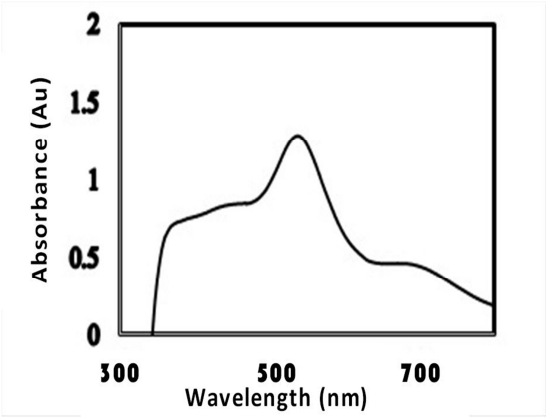
Sample	ABTS (IC_{50} µg/ml)	DPPH (IC_{50} µg/ml)	FRAP (mM $FeSO_4$ equivalent/mg extract)
Methanol	6.87±0.33	21.31±1.07	20.18±1.41
Petroleum ether	55.46±1.08	84.17±0.58	1.35±2.15
Methylene chloride	43.12±1.15	67.51±3.22	7.11±1.87
Ethyl acetate	8.10±1.04	33.91±2.01	17.14±0.86
n-Butanol	15.71±0.49	39.11±3.07	16.28±0.19
Quercetin	-	-	22.14±1.05
Ascorbic acid	-	3.21±0.55	-
Trolox	1.99±1.07	-	-

Table 5: Anticancer activities of *P. oleracea* extract and its biosynthesized AuNPs via MTT Assay

	IC_{50} (µg/mL)		
human tumor cell lines	*P. oleracea* extract	AuNPs from *P. oleracea*	Staurosporine STA
(HePG-2) Hepatocellular carcinoma	20.78±2.11	14.51±1.02	5.20±0.87
(HeLa) Cervical cancer	44.52±1.30	31.14±0.87	12.47±0.79
(Caco-2) Human colon cancer cell lines	26.19±1.54	18.11±2.1	9.25±1.8
(HCT116) Human colon cancer cell lines	33.41±1.09	21.0±0.99	7.31±1.1
(UO-31) Human kidney cancer cell line	29.11±0.46	17.47±1.3	9.11±1.3
(MCF-7) Mammary gland breast cancer	43.11±1.77	29.44±0.55	12.24±0.68



(a)



(b)

Figure 2: a) Colour change from faint yellow to purple after the reduction of $HAuCl_4$ by *P. oleracea* extract. b) UV/Vis of gold nanoparticles (AuNPs) biosynthesized by *P. oleracea* extract.

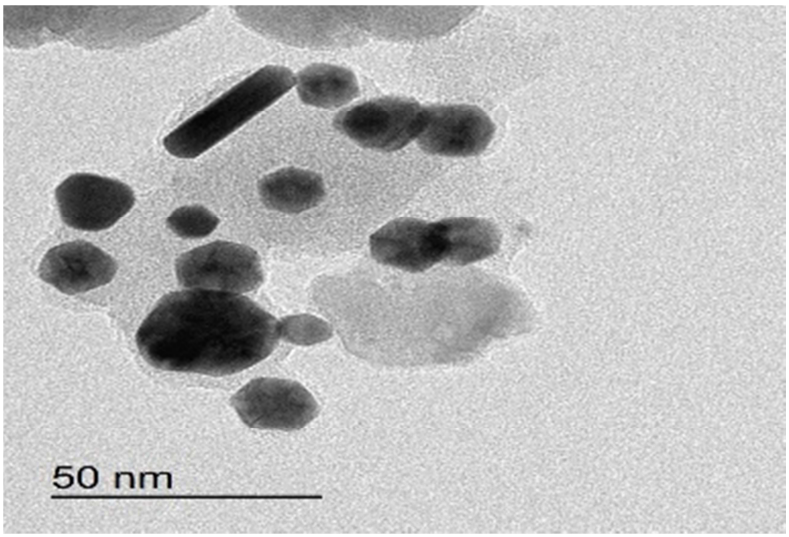


Fig. 3: TEM image of AuNPs biosynthesized by *P. oleracea* extract.

4. Conclusion

Our findings revealed that the methanolic fraction of *P. oleracea* has the greatest level of flavonoid (121.47 ±1.41 mg RE/g extract) and phenolic amounts (555.15 ± 1.14 mg GAE / g extract) among the examined extracts also the total alkaloid and the total saponin percent of the methanolic extract are 1.79% and 1.28% respectively. Moreover, methanol extract also show the highest antioxidant activity IC_{50} = 6.87, 21.31, 20.18µg/mL for ABTS, DPPH and FRAP respectively, this activity could be explained by the extract's high flavonoid and phenolics content. The UPLC-QTOF-MS utilized in the analysis of *P. oleracea* extract chemical profiling in this current study demonstrated the abundance of flavonoids , flavonols, phenolic acids, carboxylic acids, indole carboxylic acid, coumarins glucoside derivative, amino acid, fatty acid esters, hydroxy fatty acids, dipeptides, imidazole and alkaloids. Combining the water extract of *P. oleracea* in the green synthesis of metal nanoparticles led to the preparation of bioactive gold nanoparticle AuNPs that exhibited significant anticancer potential by MTT assay against tumor cell lines specifically, HePG-2 (IC_{50} 14.51±1.02µg/mL), HeLa(IC_{50} = 31.14±0.87µg/mL), Caco-2 (IC_{50}= 18.11±2.1 µg/mL), HCT116 (IC_{50} =21.0±0.99 µg/mL), UO-31(IC_{50} =17.47±1.3 µg/mL) and MCF-7(IC_{50} 29.44±0.5 µg/mL) compared to the reference medication, staurosporine STA , it exhibit promise as a novel approach in cancer, eliminating the requirement for artificial chemotherapeutic drugs. Across all cancer cell lines tested, the synthesized gold nanoparticles (AuNPs) from *P. oleracea* extract consistently demonstrated lower IC_{50} values compared to the crude water extract, indicating that the synthesis of AuNPs enhances the anticancer activity of the plant extract. This can be attributed to the improved bioavailability, cellular uptake, and stability of the bioactive compounds in the nanoparticle form. However, in all cases, STA showed the highest anticancer efficacy, underscoring its role as a powerful anticancer agent. The enhanced anticancer activity of AuNPs can be attributed to several factors, including their small size, which allows for better penetration into cancer cells, and their ability to induce reactive oxygen species (ROS), leading to apoptosis. The combination of plant-based bioactive compounds and nanotechnology provides a promising strategy for developing effective anticancer therapies.

Consequently, the synthesis of AuNPs in accordance with phytonanotech objectives will serve as a starting point for scientific advancements in the future.

5. List of abbreviations

°C: Degree in Celsius.
ABTS: Diammonium salt of (2,2-azinobis-(3ethylbenzothiazoline-6-sulfonic acid
AuNPs: Gold nanoparticles
BHT: butylated hydroxyl toluene

Caco-2: Human colon cancer cell line
DPPH: 1,1'- Diphenyl-2-PicrylHydrazyl.
FRAP: Ferric reducing antioxidant power
HCT116: Human colon cancer cell line
HeLa: cervical cancer cell line
HePG-2: Hepatocellular carcinoma cell line
IC_{50}: Median Inhibitory concentration.
MCF-7: Mammary gland breast cancer cell line
MTT: "3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide" assay
nm: Nanometer.
SD: Standard Deviation.
STA:Staurosporine
TEM: Transmission electron microscopy
Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
UO-31: Kidney cancer cell line
UPLC-QTOF-MS/MS: Ultra-Performance Liquid Chromatography/ Quadrupole-Time-of-Flight Mass Spectrometer analysis

6. Conflicts of interest

There are no conflicts of interest to declare.

7. References

[1] Balunas, M. J., & Kinghorn, A. D. (2005). Drug discovery from medicinal plants. Life Sciences, 78 (5), 431–441. https://doi.org/10.1016/j.lfs.2005.09.012

[2] Newman, D. J., & Cragg, G. M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of Natural Products, 75 (3), 311–335. https://doi.org/10.1021/np200906s

[3] Cragg, G. M., & Newman, D. J. (2005). Plants as a source of anti-cancer agents. Journal of Ethnopharmacology, 100 (1-2), 72–79. https://doi.org/10.1016/j.jep.2005.05.011

[4] Alshammari, F. H., Alam, M. B., Naznin, M., Kim, S., & Lee, S. (2022). Optimization of *Portulaca oleracea* L. extract using response surface methodology and artificial neural network and characterization of bioactive compounds by high-resolution mass spectroscopy. Arabian Journal of Chemistry, 16, Article104425. https:// doi.org/ 10.1016 /j.arabjc .2022.104425

[5] Singh, P., Pandit, S., Mokkapati, V. R. S. S., Garg, A., Ravikumar, V., & Mijakovic, I. (2018). Gold nanoparticles in diagnostics and therapeutics for human cancer. International Journal of Molecular Sciences, 19 (7), 1979. https://doi.org/10.3390/ijms1907197.

[6] Hutchinson, N., Wu, Y., Wang, Y., Kanungo, M., DeBruine, A., Kroll, E., Gilmore, D. J., Eckrose, Z., Gaston, S., Matel, P., Kaltchev, M., Nickel, A. M., Kumpaty, S., Hua, X., & Zhang, W. (2021). Green synthesis of gold nanoparticles using upland cress and their biochemical characterization and assessment. NCBI. https://www.ncbi.nlm.nih.gov

[7] Aljabali, A. A., Akkam, Y., Al Zoubi, M. S., Al-Batayneh, K. M., Al-Trad, B., Abo Alrob, O., Alkilany, A. M., Benamara, M., & Evans, D. J. (2018). Synthesis of gold nanoparticles using leaf extract of *Ziziphus zizyphus* and their antimicrobial activity. NCBI. https:// www. ncbi.nlm.nih.gov.

[8] Nabil A. Ibrahim, Basma M. Eid, Mohamed S. Abdel-Aziz, Green synthesis of AuNPs for eco-friendly functionalization of cellulosic substrates, Applied Surface Science, 2016, (389), 118-125, https:// doi.org/ 10.1016/ j.apsusc. 2016.07.077.

[9] Fernández-Poyatos, M. del P., Llorent-Martínez, E. J., & Ruiz-Medina, A. (2021). Phytochemical composition and antioxidant activity of Portulaca *oleracea*: Influence of the steaming cooking process. NCBI. https://www.ncbi. nlm. nih.gov.

[10] Uddin, M. K., Juraimi, A. S., Hossain, M. S., Nahar, M. A. U., Ali, M. E., & Rahman, M. M. (2014). Purslane weed (*Portulaca oleracea*): A prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. The Scientific World Journal, 2014, 951019. https://doi.org/10.1155/2014/951019.

[11] Zhou, Y. X., Xin, H. L., Rahman, K., Wang, S. J., Peng, C., & Zhang, H. (2015). *Portulaca oleracea* L.: A review of phytochemistry and pharmacological effects. NCBI. https://www. ncbi.nlm.nih.gov.https://doi.org/10.1155/2015/925631

[12] Petropoulos, S. A., Fernandes, A., Dias, M. I., Vasilakoglou, I. B., Petrotos, K., Barros, L., & Ferreira, I. C. F. R. (2019). Nutritional value, chemical composition and cytotoxic properties of common purslane (*Portulaca oleracea* L.) in relation to harvesting stage and plant part. Antioxidants, 8, 293. https://doi.org/10.3390/antiox8080293

[13] Durgawale, T. P., Khanwelkar, C. C., & Durgawale, P. P. (2018). Phytochemical analysis of *Portulaca oleracea* and *Portulaca quadrifida* extracts using gas chromatography-mass spectrometry. Asian Journal of Pharmaceutical and Clinical Research, 11(9), 204-207. https://doi.org/10.22159/ajpcr.2018.v11i9.26662

[14] Voynikov, Y., Gevrenova, R., Balabanova, V., Doytchinova, I., Nedialkov, P., & Zheleva-Dimitrova, D. (2019). LC-MS analysis of phenolic compounds and oleraceins in aerial

parts of *Portulaca oleracea* L. Journal of Applied Botany and Food Quality. 92. 298-312. https://doi.org/10.5073/JABFQ.2019.092.041

[15] Yang, R. F., Geng, L. L., Lu, H. Q., & Fan, X. D. (2017). Ultrasound-synergized electrostatic field extraction of total flavonoids from *Hemerocallis citrina Baroni*. Ultrasonics Sonochemistry, 34, 571-579. https://doi.org/10.1016/j.ultsonch.2016.06.037

[16] Scalbert, A. (1991). Antimicrobial properties of tannins. Phytochemistry, 30, 3875-3883. https://doi.org/10.1016/0031-9422(91)83426-L

[17] Mabry, T. J., Markham, K. R., & Thomas, M. B. (1970). The systematic identification of flavonoids. In The ultraviolet spectra of flavones and flavonols (Part II, Ch. V, pp. 41-164). Springer Verlag. https://sydney.alma.exlibrisgroup.com/permalink/61USYD_INST/1c0ug48/alma991032145213005106

[18] Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (2010). Textbook of pharmacognosy (31st ed.). Nirali Prakashan. https://pha.tanta.edu.eg/files/textbook%20of%20pharmacognosy%20and%20phytochemistry.pdf

[19] Harborne, J. B. (1993). Phytochemistry. Academic Press. https://link.springer.com/content/pdf/10.1007/978-94-009-5570-7.pdf

[20] Harborne, J. B. (2005). Phytochemical methods: A guide to modern techniques of plant analysis (3rd ed.). Springer Pvt. Ltd.

[21] Mondal, M., Hossain, M. S., & Das, N. (2019). Phytochemical screening and evaluation of pharmacological activity of leaf methanolic extract of *Colocasia affinis*. Clinical Phytoscience, 5, 8. https://doi.org/10.1186/s40816-019-0100-8

[22] Osman, E. E. A., Mohamed, A. S., Elkhateeb, A., Gobouri, A., Abdel-Aziz, M. M., & Abdel-Hameed, E. S. S. (2022). Phytochemical investigations, antioxidant, cytotoxic, antidiabetic and antibiofilm activities of *Kalanchoe laxiflora* flowers. European Journal of Integrative Medicine, 49, 102085. https://doi.org/10.1016/j.eujim.2021.102085

[23] Mossalem, H. S., Abuelenain, G. L., Mohamed, A. S., Mohamed, T. S., Zayed, K. M., & Mahmoud, S. S. (2021). Assessment of *Ocimum basilicum* extracts on *Schistosoma mansoni* infected *Biomphalaria alexandrina* snails and mice. Egyptian Journal of Aquatic Biology & Fisheries, 25(5), 253-270. https://dx.doi.org/10.21608/ejabf.2021.198598

[24] Obadoni, B. O., & Ochuko, P. O. (2001). Phytochemical studies and comparative efficacy of the extracts of some haemostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Sciences, 8, 203-208. https://doi.org/10.4314/gjpas.v8i2.16033

[25] Hegazy, M. M., Metwaly, A. M., Mostafa, A. E., Radwan, M. M., Mehany, A. B. M., Shymaa, E. A., Magdeldin S., Afifi W.M., ElSohly, M. A. (2021). Biological and chemical evaluation of some African plants belonging to *Kalanchoe* species: Antitrypanosomal, cytotoxic, antitopoisomerase I activities and chemical profiling using ultra-performance liquid chromatography/quadrupole-time-of-flight mass spectrometer. Pharmacognosy Magazine, 17(73), 179–192. http://dx.doi.org/10.4103/pm.pm_232_20

[26] Bakchiche, B., Guenane, H., Ramazan, E., Yilmaz, I., Mohamed, A. S., & El-Shazly, M. A. (2024). Mineral contents, antioxidant and antimicrobial activities of Algerian *Terfezia claveryi* extracts. FABAD Journal of Pharmaceutical Sciences, 49(1), 19-36. https://doi.org/ 10.55262/ fabade czacilik .1326710

[27] Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft & Technologie, 28, 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

[28] Guenane, H., Rezzoug, M., Bakchiche, B., Cheraif, K., Mohamed, A. S., & El-Shazly, M. A. (2024). Antioxidant properties and mineral contents of different solvent extracts of some medicinal plants cultivated in Algeria. Tropical Journal of Natural Product Research, 8(1), 5987-5991. https://doi.org/10.26538/tjnpr/ v8 i1.39

[29] Hassanisaadi, M., Bonjar, G. H. S., Rahdar, A., Pandey, S., Hosseinipour, A., & Abdolshahi, R. (2021). Environmentally safe biosynthesis of gold nanoparticles using plant water extracts. Nanomaterials, 11 (2033). https://doi.org/10.3390 /nano11082033.

[30] Raut, R. W., Kolekar, N. S., Lakkakula, J. R., Mendhulkar, V. D., & Kashid, S. B. (2009). Photosynthesis of silver nanoparticles using *Gliricidia sepium* (Jecq). Current Nanoscience, 5 (1), 117–122. http://dx.doi.org/10.2174/157341309787314674

[31] Elavazhagan, T., & Arunachalam, K. D. (2011). *Memecylon edule* leaf extract mediated green synthesis of silver and gold nanoparticles. International Journal of Nanomedicine, 6, 1265–1278. https://doi.org/10.2147/IJN.S18430.

[32] Mohamed, T. S., Refahy, L. A., Romeih, M. H., Hamed, M. M., Ahmed, H. O., & El-Hashash, M. A. (2022). Chromatographic isolation and characterization of certain bioactive chemical ingredients of *Phyllanthus emblica* extracts and assessment of their potentials as antiviral and

anticancer agents. Egyptian Journal of Chemistry, 65 (1), 179–192. https://doi.org/10.21608/EJCHEM.2021.59156.3498

[33] Amer, M., Shawer, E., Abdelhameed, M., Sabour, R., Elshazly, M., & Mohamed, A. (2024). The potential of *Brachionus plicatilis* extract against bacterial infection and cancer: In vitro and in silico studies. Egyptian Journal of Chemistry, 67 (9), 713–725. https://doi.org/10.21608/ejchem.2024.285871.9651.

[34] Chen, C. J., Wang, W. Y., Wang, X. L., Dong, L. W., Yue, Y. T., Xin, H. L., Ling, C. Q., & Li, M. (2009). Anti-hypoxic activity of the ethanol extract from *Portulaca oleracea* in mice. Journal of Ethnopharmacology, 124, 246-250. https://doi.org/10.1016/j.jep.2009.04.028.

[35] Liao, J., Guo, Z., & Yu, G. (2021). Process intensification and kinetic studies of ultrasound-assisted extraction of flavonoids from *peanut* shells. Ultrasonics Sonochemistry, 76, Article 105661. https://doi.org/10.1016/j.ultsonch .2021 .105661.

[36] Naz R., Roberts T.H., Bano A., Nosheen A., Yasmin H., Hassan M.N., Keyani R., Ullah S., Khan W., Anwar Z. (2020). GC-MS analysis, antimicrobial, antioxidant, antilipoxygenase and cytotoxic activities of Jacaranda mimosifolia methanol leaf extracts and fractions. *PLoS One* 15(7),e0236319. https://doi .org/10.1371/journal.pone.0236319.

[36] Ahmed, R., Tariq, M., Hussain, M., Andleeb, A., Masoud, M. S., Ali, I., Mraiche, F., & Hasan, A. (2019). Phenolic contents-based assessment of therapeutic potential of Syzygium cumini leaves extract. *PloS one*, *14*(8), e0221318. https://doi.org/10.1371/journal.pone.0221318.

[37] Ghareeb, M. A., Sobeh, M., Aboushousha, T., Esmat, M., Mohammed, H. S., & El-Wakil, E. S. (2023). Polyphenolic profile of *Herniaria hemistemon* aerial parts extract and assessment of its anticryptosporidiosis in a murine model: In silico supported in vivo study. Pharmaceutics, 15, 415. https://doi.org/10.3390/pharmaceutics15020415

[38] Zhu, H., Wang, Y., Liu, Y., Xia, Y., & Tang, T. (2010). Analysis of flavonoids in *Portulaca oleracea* L. by UV–vis spectrophotometry with comparative study on different extraction technologies. Food Analytical Methods, 3, 90–97. https://doi.org/10.1007/s12161-009-9091-2

[39] Choi, H. J., Naznin, M., Alam, M. B., Javed A., Alshammari F.H., Kim S., Lee S. (2022). Optimization of the extraction conditions of *Nypa fruticans Wurmb* using response surface methodology and artificial neural network. Food Chemistry, 381. 132086, https://doi.org/10.1016/j.foodchem.2022.132086

[40] Alam, M.B., Naznin, M., Islam, S., et al, 2021. High resolution mass spectroscopy-based secondary metabolite profiling of Nymphaea nouchali (Burm. f) stem attenuates oxidative stress via regulation of MAPK/Nrf2/HO-1/ROS pathway. Antioxidants (Basel Switzerland) 10. https://doi.org/10.3390/antiox10050719.

[41] Mostafa, A. E., Atef, A., Mohammad, A. E., Jacob, M., Cutler, S. J., & Ross, S. A. (2014). New secondary metabolites from *Dodonaea viscosa*. Phytochemistry Letters, 8, 10–15. https://doi.org/10.1016/j.phytol.2013.12.008

[42] Ammar, S., Abidi, J., Luca, S. V., Boumendjel, M., Skalicka-Woźniak, K., & Bouaziz, M. (2020). Untargeted metabolite profiling and phytochemical analysis based on RP-HPLC-DAD-QTOF-MS and MS/MS for discovering new bioactive compounds in *Rumex algeriensis* flowers and stems. Phytochemical Analysis, 31, 616–635. https://doi.org/10.1002/pca.2928

[43] Sinosaki, N. B. M., Tonin, A. P. P., Ribeiro, M. H. D. M., Poliseli, C. B., Roberto, S. B., Silveira, R. d., & Meurer, E. C. (2020). Structural study of phenolic acids by triple quadrupole mass spectrometry with electrospray ionization in negative mode and h/d isotopic exchange. Journal of the Brazilian Chemical Society.https://doi.org/10.21577/0103-5053.20190197.

[44] Slimen, I., Mabrouk, M., Chaabane, H., Najar, T., & Abderrabba, M. (2017). LC-MS analysis of phenolic acids, flavonoids and betanin from spineless *Opuntia ficus-indica* fruits. Cell Biology, 5(2), 17–28.

[45] Elessawy, F. M., Bazghaleh, N., Vandenberg, A., & RW. (2020). Polyphenol profile and comparisons of seed coats of five pulse crops using semi-quantitative liquid chromatography-mass spectrometric method. Phytochemical Analysis, 31, 458–471. https://doi.org/10.1002/pca.2909

[46] Cao, D., Wang, Q., Jin, J., Qiu, M., Zhou, L., Zhou, X., Li, H., & Zhao, Z. (2018). Simultaneous qualitative and quantitative analyses of triterpenoids in *Ilex pubescens* by ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Phytochemical Analysis, 29*, 168–179. https://doi.org/10.1002/pca.2725

[47] Müller-Schwarze, D., & Houlihan, P. W. (1991). Pheromonal activity of single castoreum constituents in beaver, *Castor canadensis*.

Journal of Chemical Ecology, 17 (4), 715–734. https://doi.org/10.1007/BF00994195

[48] Kaiquan, Y., Jian, L., Yuqi, C., Min, Z., Jiaxin, L., Shani, L., Qing, L., Ling, L., Lilan, Y., & Qingjiao, H. (2024). The chemical profiling and identification of *Abelia macrotera* (Graebn. et Buchw.) Rehd. based on UHPLC-Q-Exactive Orbitrap MS and study their antioxidant, anti-tyrosinase and anti-inflammatory activities. Arabian Journal of Chemistry, 17 (2), 105575. https://doi.org/10.1016/j.arabjc.2023.105575.

[49] Ghareeb, M., Sobeh, M., Rezq, S., El-Shazly, A., Mahmoud, M., & Wink, M. (2018). HPLC-ESI-MS/MS profiling of polyphenolics of a leaf extract from *Alpinia zerumbet* (Zingiberaceae) and its anti-inflammatory, anti-nociceptive, and antipyretic activities in vivo. Molecules, 23(12), 3238. https://doi.org/10.3390%2Fmolecules23123238

[50] Elshazly, M., Mahmoud, H., Saad, A., Refahy, L., Ghareeb, M., & Rizk, S. (2023). UPLC-QTOF/MS-assisted chemical profiling of *Daucus carota* leaf extract and evaluation of its antioxidant, antimicrobial and antibiofilm activities: Evidence from in vitro and in silico studies. Egyptian Journal of Chemistry. https://doi.org/10.21608/ejchem.2023.209102.7935.

[51] Liu, G., Liu, A., Yang, C., Zhou, C., Zhou, Q., Li, H., Yang, H., Mo, J., Zhang, Z., Li, G., Si, H., & Ou, C. (2023). *Portulaca oleracea* L. organic acid extract inhibits persistent methicillin-resistant *Staphylococcus aureus* in vitro and in vivo. Frontiers in Microbiology, 13, 1076154. https://doi.org/10.3389/fmicb.2022.1076154.

[52] Kumar, A., Sreedharan, S., Kashyap, A. K., Singh, P., & Ramchiary, N. (2021). A review on bioactive phytochemicals and ethnopharmacological potential of purslane (*Portulaca oleracea*L.). Heliyon, 8 (1), e08669. https://doi.org/10.1016/j.heliyon.2021.e08669.

[53] Kai-Jun, T., Yu, C., Jing, L., Xu, T., Lu-Ping, Y., Tian-Yun, J., Jia-Shun, Z., Yu, Z., Tao, S., Xiao-Ning, W., Dong-Mei, R., & Lan, X. (2023). Two new tetrahydroisoquinoline alkaloids from *Portulaca oleracea*. Phytochemistry Letters, 53, 155–160. https://doi.org/10.1016/j.phytol.2022.12.006.

[54] Tang, J., Dunshea, F. R., & Suleria, H. A. R. (2019). LC-ESI-QTOF/MS characterization of phenolic compounds from medicinal plants *hops* and *juniper berries* and activity. Foods, 9 (1), 7. https://doi.org/10.3390/foods9010007

[55] Al-Quwaie, D. A., Allohibi, A., Aljadani, M., Alghamdi, A. M., Alharbi, A. A., Baty, R. S., Qahl, S. H., Saleh, O., Shakak, A. O., Alqahtani, F. S., Khalil, O. S. F., El-Saadony, M. T., & Saad, A. M. (2023). Characterization of *Portulaca oleracea* whole plant: Evaluating antioxidant, anticancer, antibacterial, and antiviral activities and application as quality enhancer in yogurt. Molecules, 28(15), 5859. https://doi.org/10.3390/molecules28155859.

[56] Hossain, M., Brunton, N., Martin-Diana, A. B., & Barry-Ryan, C. (2010). Characterization of phenolics composition in Lamiaceae spices by LC-ESI-MS/MS. Journal of Agricultural and Food Chemistry, 58 (19), 10576–10581. https://doi.org/10.1021/jf102042g.

[57] Lou, Y.-R., & Pichersky, E. (2022). Deep roots and many branches: Origins of plant-specialized metabolic enzymes in general metabolism. Current Opinion in Plant Biology, 66, 102192. https://doi.org/10.1016/j.pbi.2022.102192.

[58] March, R. E., Miao, X. S., & Metcalfe, C. D. (2004). A fragmentation study of a flavone triglycoside, kaempferol 3-O-robinoside-7-O-rhamnoside. Rapid Communications in Mass Spectrometry, 18, 931–934. https://doi.org/10.1002/rcm.1428

[59] Miao, L., Zhou, C., Zhang, H., Cheong, M. S., Tan, Y., Wang, Y., Zhang, X., Yu, H., & Cheang, W. S. (2023). *Portulaca oleracea* L. (Purslane) extract protects endothelial function by reducing endoplasmic reticulum stress and oxidative stress through AMPK activation in diabetic obese mice. Antioxidants, 12(12), Article2132. https://doi.org/10.3390/antiox12122132.

[60] Wang, C. Y., Li, Y. R., Pan, C., Chen, J., Jiang, W., Li, W. N., & Yan, X. J. (2020). Quantitative analysis of carnosine, anserine, and homocarnosine in skeletal muscle of aquatic species from East China Sea. Biochemistry and Biophysics Reports, 25, Article 100880. https://doi.org/ 10.1016 /j.bbrep.2020.100880.

[61] Çakır, D. K., Zannou, O., & Koca, I. (2022). Scopoletin contents and antioxidant properties of some edible plants of Black Sea regions. Discovery Food, 2 (7). https:// doi.org/ 10.1007 /s44187-022-00010-y.

[62] Matsuda, F., Miyazawa, H., Wakasa, K., & Miyagawa, H. (2005). Quantification of indole-3-acetic acid and amino acid conjugates in rice by liquid chromatography-electrospray ionization-tandem mass spectrometry. Bioscience, Biotechnology, and Biochemistry, 69 (4), 778–783. https:// doi.org/ 10.1271/ bbb.69.778.

[63] Choi, B.-R., Hong, S. S., Han, X., Hwang, J. S., Lee, M. H., Hur, J. D., Hwang, B. Y., & Ro, J. S. (2005). Antioxidant constituents from *Portulaca oleracea*. Natural Product Sciences, 11, 229–232. https://doi.org/10.1016/j.foodchem.2006.09.025

[64] Wang, H., & Wang, C. (2018). Isolation, characterization and analgesic activity of natural allantoin from *Portulaca oleracea* seed. Modern Chemistry & Applications, 6. https://doi.org/ 10.4172/2329-6798 . 1000249.

[65] Liu, W., Kong, Y., Zu, Y., Fu, Y., Luo, M., Zhang, L., & Li, J. (2010). Determination and quantification of active phenolic compounds in pigeon pea leaves and its medicinal product using liquid chromatography-tandem mass spectrometry. Journal of Chromatography A, 1217 (27), 4723–4732. https://doi.org/10.1016/j.chroma.2010.05.027.

[66] He, Y., Long, H., Zou, C., *et al*. (2021). Anti-nociceptive effect of *Portulaca oleracea* L. ethanol extracts attenuated zymosan-induced mouse joint inflammation via inhibition of Nrf2 expression. Innate Immunity, 27 (3), 230–239. https://doi.org/10.1177/1753425921994190.

[67] Wang, Y., Wen, J., Zheng, W., Zhao, L., Fu, X., Wang, Z., & Xiao, W. (2015). Simultaneous determination of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid and geniposide in rat plasma by UPLC-MS/MS and its application to a pharmacokinetic study after administration of Reduning injection. Biomedical Chromatography, 29 (1), 68–74. https://doi.org/10.1002/bmc.3241.

[68] Chen, H. J., Inbaraj, B. S., & Chen, B. H. (2012). Determination of phenolic acids and flavonoids in *Taraxacum formosanum* Kitam by liquid chromatography-tandem mass spectrometry coupled with a postcolumn derivatization technique. International Journal of Molecular Sciences, 13(1), 260–285. https://doi.org/10.3390/ijms13010260.

[69] Tay, K. C., Tan, L. T., Chan, C. K., Hong, S. L., Chan, K. G., Yap, W. H., Pusparajah, P., Lee, L. H., & Goh, B. H. (2019). Formononetin: A review of its anticancer potentials and mechanisms. Frontiers in Pharmacology, 10, 820. https://doi.org/10.3389/fphar.2019.00820.

[70] Cai, T., & Cai, B. (2023). Pharmacological activities of esculin and esculetin: A review. Medicine (Baltimore), 102 (40), e35306. https://doi.org/10.1097/MD.0000000000035306

[71] Bhattacharjee, S., & Singh, A. (2024). Antioxidant and LC-MS screening of *banana pseudostem*. agriRxiv. https:// doi.org/ 10.5281/ zenodo.20240232351.

[72] Alam, M. A., Nadirah, T. A., Mohsin, G. M., Saleh, M., Moneruzzaman, K. M., Aslani, F., Juraimi, A. S., & Alam, M. Z. (2021). Antioxidant compounds, antioxidant activities, and mineral contents among underutilized vegetables. International Journal of Vegetable Science, 27, 157–166. https://doi.org/10.1080/19315260.2020.1748785

[73] Siriamornpun, S., & Suttajit, M. (2010). Microchemical Components and Antioxidant Activity of Different Morphological Parts of Thai Wild Purslane (Portulaca oleracea). *Weed Science*, *58*(3), 182–188. https://doi.org/10.1614/WS-D-09-00073.1

[74] Yang, Z., Liu, C., Xiang, L., & Zheng, Y. (2009). Phenolic alkaloids as a new class of antioxidants in Portulaca oleracea. *Phytotherapy research :PTR*, *23*(7), 1032–1035. https:// doi.org/ 10.1002/ptr.2742.

[75] Dkhil M.A., Moniem A.E.A. , Al-Quraishy, S. & Saleh R.A. (2011). Antioxidant effect of purslane (*Portulaca oleracea*) and its mechanism of action. Journal of Medicinal Plants Research, 5 (9), 1589–1593. https://api.semanticscholar.org/CorpusID:86088730

[76] Ion, D., Niculescu, A. G., Păduraru, D. N., Andronic, O., Mușat, F., Grumezescu, A. M., & Bolocan, A. (2021). An Up-to-Date Review of Natural Nanoparticles for Cancer Management. Pharmaceutics, 14(1),18. https://doi.org/10.3390/pharmaceutics14010018.

[77] Zhao, R., Gao, X., Cai, Y., Shao, X., Jia, G., Huang, Y., & et al. (2013). Antitumor activity of *Portulaca oleracea* L. polysaccharides against cervical carcinoma in vitro and in vivo. Carbohydrate Polymers, 96, 376–383. https://doi.org/10.1016/j.carbpol.2013.03.088

[78] Zhao, R., Zhang, T., Ma, B., & Li, X. (2017). Antitumor activity of *Portulaca oleracea* L. polysaccharide on HeLa cells through inducing TLR4/NF-kappaB signaling. Nutrition and Cancer, 69(1), 131–139. https://doi.org/10. 1080 / 01635581.2017.1249440.

[79] Zheng, G.-Y., Qu, L.-P., Yue, X.-Q., Gu, W., Zhang, H., & Xin, H.-L. (2014). Portulacerebroside A induces apoptosis via activation of the mitochondrial death pathway in human liver cancer HCCLM3 cells. Phytochemistry Letters, 7(1), 77–84. https://doi.org/10.1016/j.phytol. 2013.11.004.

[80] Davis, W. L., & Matthew, S. B. (2000). Antioxidants and cancer III: Quercetin. Alternative Medicine Review, 5(3), 196–208. https://www.researchgate.net/publication/12449946

[81] Kumar S., Pandey A.K. Chemistry and biological activities of flavonoids: an overview. Scientific World Journal. 2013 Dec 29;2013:162750. https://doi.org/10.1155/2013/162750.

[82] Hashemzehi, M., Khazdair, M., Kiyanmehr, M., Askari, V., & Boskabady, M. (2016). *Portulaca oleracea* affects muscarinic receptors of guinea

pig tracheal smooth muscle. Indian Journal of Pharmaceutical Sciences, 78, 388–394. https://api.semanticscholar.org/CorpusID:37429596

[83] Askari, V. R., Rezaee, S. A., Abnous, K., Iranshahi, M., & Boskabady, M. H. (2016). The influence of hydro-ethanolic extract of *Portulaca oleracea* L. on Th1/Th2 balance in isolated human lymphocytes. Journal of Ethnopharmacology, 194, 1112–1121. https://doi.org/10.1016/j.jep.2016.10.082

[84] Boskabady, M. H., Hashemzehi, M., Khazdair, M. R., & Askari, V. R. (2016). Hydro-ethanolic extract of *Portulaca oleracea* affects beta-adrenoceptors of Guinea pig tracheal smooth muscle. Iranian Journal of Pharmaceutical Research, 15, 867. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5316266/

[85] Dobrzynska, M., Napierala, M., & Florek, E. (2020). Flavonoid nanoparticles: A promising approach for cancer therapy. Biomolecules, 10, 1268. https://doi.org/10.3390/biom10091268

[86] Coelho, P. L., Oliveira, M. N., da Silva, A. B., Pitanga, B. P., Silva, V. D., Faria, G. P., et al. (2016). The flavonoid apigenin from Croton betulaster Mull inhibits proliferation, induces differentiation and regulates the inflammatory profile of glioma cells. Anticancer Drugs, 27, 960–969. https://doi.org/10.1097/cad.0000000000000413

[87] Kim, B., Jung, N., Lee, S., Sohng, J. K., & Jung, H. J. (2016). Apigenin inhibits cancer stem cell-like phenotypes in human glioblastoma cells via suppression of c-Met signaling. Phytotherapy Research, 30, 1833–1840. https://doi.org/10.1002/ptr.5689

[88] Stump, T. A., Santee, B. N., Williams, L. P., Kunze, R. A., Heinze, C. E., Huseman, E. D., et al. (2017). The antiproliferative and apoptotic effects of apigenin on glioblastoma cells. Journal of Pharmacy and Pharmacology, 69, 907–916. https://doi.org/10.1111/jphp.12718

[89] Sharma, V., Joseph, C., Ghosh, S., Agarwal, A., Mishra, M. K., & Sen, E. (2007). Kaempferol induces apoptosis in glioblastoma cells through oxidative stress. Molecular Cancer Therapeutics, 6, 2544–2553. https://doi.org/10.1158/1535-7163.mct-06-0788

[90] Chen, T., Wang, J., Li, Y., Shen, J., Zhao, T., & Zhang, H. (2010). Sulfated modification and cytotoxicity of *Portulaca oleracea* L.polysaccharides. Glycoconjugate journal, *27*(6), 635–642.https:// doi.org/ 10.1007 / s10719-010-9307-0.

[91] Dennison, S. R., Whittaker, M., Harris, F., & Phoenix, D. A. (2006). Anticancer alpha-helical peptides and structure/function relationships underpinning their interactions with tumor cell membranes. Current Protein & Peptide Science, 7, 487–499. https://doi.org/10.2174/138920306779025611.

[92] Ahmaditaba, M. A., Shahosseini, S., Daraei, B., Zarghi, A., & Houshdar Tehrani, M. H. (2017). Design, synthesis, and biological evaluation of new peptide analogues as selective COX-2 inhibitors. Archiv der Pharmazie, 350, e1700158.https://doi.org/10.1002/ardp.201700158.

[93] AlMalki, F. A., Hassan, A. M., Klaab, Z. M., Abdulla, S., & Pizzi, A. (2021). Tannin nanoparticles (NP99) enhance the anticancer effect of tamoxifen on ER+ breast cancer cells. Journal of Renewable Materials, 9, 2077–2088. https://doi.org/10.32604/jrm.2021.016173