Biol. Stud. 2025, 19(4), 59–74 • doi: https://doi.org/10.30970/sbi.1904.850
www.https://publications.lnu.edu.ua/journals/index.php/biology

UDC: 577.1.084:[582.661.56:599.323.4:616.13-004.6]

EFFECT OF *OPUNTIA FICUS-INDICA* FRUIT NANO AND ALCOHOLIC EXTRACTS IN INDUCED-ATHEROSCLEROTIC RATS

Ahmed Ali Al-Alfayyadh ,
Nashwan Ibrahem Al-Lehebe , ***Nameer Saadallah Ezzat***

Department of Chemistry, College of Education for Pure Sciences, University of Mosul
Mosul 41002, Iraq

Al-Alfayyadh, A. A., Al-Lehebe, N. I., & Ezzat, N. S. (2025). Effect of *Opuntia ficus-indica* fruit nano and alcoholic extracts in induced-atherosclerotic rats. *Studia Biologica*, 19(4), 59–74. doi:10.30970/sbi.1904.850

Background. Atherosclerosis is a serious chronic disease that affects many people of all ages and requires continuous medication. To reduce the risk of side effects from these medications, the search for a less harmful natural treatment continues.

Materials and Methods. This study was designed to isolate an alcoholic extract from the fruit *Opuntia ficus-indica* (prickly pear), which was analyzed using high-performance liquid chromatography (HPLC). Silver nanoparticles were also prepared by loading silver nitrate onto the alcoholic extract, and their formation was verified by monitoring colour change, FESEM, and FT-IR, respectively. The effects of these extracts were studied in male atherosclerotic rats by assessing glucose, lipid profile, liver, and kidney function as well as troponin, myoglobin, and creatine kinase.

Results. HPLC analysis of the alcoholic extract showed polyphenol compounds: gallic acid, quercetin, ferulic acid, rutin, kaempferol, cinnamic acid, Tannic acid at concentrations of 88.9, 80.6, 45.2, 74.6, 50.6, 12.6, 20.7 μg/kg of prickly pear fruit, respectively. Green synthesis of silver nanoparticles produced a distinct colour change when the mixture was kept in a cold, dark place. FESEM indicated the presence of spherical nanoparticles with a size of 25 nm. The FT-IR also showed peak shifts when comparing the alcoholic extract with the nano-extract. The alcoholic extract produced a lower effect on lipid profiles, cardiac markers, creatinine levels, liver functions, and lipid peroxidation compared to the atherosclerotic control. Also, the nano-extract reduced the levels of kidney and liver function tests, as well as lipid peroxidation, lipid profiles, troponin, and creatine kinase-MB. The results also showed an increase in glutathione

levels in atherosclerotic rats treated with the extracts compared to the untreated atherosclerotic control group.

Conclusion. High levels of polyphenol compounds were found in the alcoholic extract of prickly pear fruit. The results demonstrated that silver nanoparticle extract was more effective than the alcoholic extract in most parameters. These findings suggest that such extracts may reduce symptoms or complications of this disease.

Keywords: atherosclerosis, nanoparticles, troponin I, creatine kinase, myoglobin

INRODUCTION

Atherosclerosis is the primary cause of heart disease and strokes, making it the main underlying factor in the leading causes of deaths worldwide. Although its risks can be reduced by lowering cholesterol levels, current treatments do not adequately address the role of inflammation, which is equally important in the development of heart and vascular diseases (Charo & Taub, 2011). The number of people affected by arteriosclerosis increases with age, meaning this disease is more common among the elderly. Therefore, doctors consider it a disease of aging (Tohirova & Shernazarov, 2022). When low-density lipoprotein cholesterol accumulates in the inner wall of the vessels in the form of plaques, the walls of the blood vessels harden, lose their elasticity, and eventually narrow due to the accumulation of fats and deposits. This makes it difficult for blood to flow to organs, and in severe cases, the blood vessels may become completely blocked. Cholesterol is primarily stored in the artery's inner lining as low-density lipoprotein (LDL). This deposit causes the arteries to constrict over time, severely reducing blood flow and, as a result, causing tissue oxygen deprivation (Wolf & Ley, 2019). The accumulation of fat inside cells is one of the early causes of atherosclerosis development. The formation of foam cells from macrophages is associated with the effect on LDL receptors, allowing these cells to absorb fats containing low-density lipoprotein. LDL is the main source of cholesterol accumulation in foam cells (Poggio *et al*., 2014).

Inflammation plays a role in atherosclerosis from the earliest stages to the development of complications, making it a leading and growing cause of death and disability globally (Soehnlein & Libby, 2021).

The prickly pear *Opuntia ficus-indica* (OFI), a local variety from Mexico, is now widely distributed in many geographic regions around the world. The *Opuntia ficus-indica* cactus is a plant that contains many beneficial properties, as it is a source of dietary fiber and vitamins, as well as many other bioactive compounds with anti-diabetic, anti-inflammatory, and antimicrobial properties. Total phenolic and total flavonoid content were found to be significantly present in the OFI fruit extract after qualitative and quantitative phytochemical screening in vitro (Soehnlein & Libby, 2021). Scientific studies have shown that different parts of this plant, including the seeds, pulp, peel, and leaves, have therapeutic potential and are safe for human use. Publications since 2020 have addressed many aspects of the *Opuntia ficus-indica* cactus in the field of pharmacy (Al-Naqeb *et al*., 2021; Kaur *et al*., 2012).

OFI shows several important biological activities for its high content of antioxidants, flavonoids, ascorbate, carotenoids, and phenolic acids. In addition to phytochemical components, bio-peptides and soluble fiber have been identified that contribute to the medicinal properties of this plant. OFI has been reported to be effective against diabetes, high blood pressure, tumours, dermatosis, prostatitis, rheumatism, stomach ache, wounds, colitis, and some viral diseases (Rasoulpour *et al*., 2017).

MATERIALS AND METHODS

Plant Collection and Preparation

Opuntia ficus-indica
Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots
Order: Caryophyllales
Family: Cactaceae
Genus: *Opuntia*
Species: *O. ficus-indica* (Aruwa *et al*., 2018)

Firstly, 1000 grams of fresh prickly pear fruit were collected from the Mosul local market in Iraq (imported from India). The fruit was crushed in a blender (Magic Bullet 600-Watt) with a 4:1 ratio of distilled water. The mixture frozen and thawed three times, followed by ultrasonic treatment for 60 min, and finally stored at -4 °C. It was then filtered and separated using a refrigerated centrifuge, and finally dried with a lyophilizer to obtain the crude extract.

Extraction of polyphenols. Polyphenol compounds were extracted from the residue of the previous step using a Soxhlet extraction system with ethanol as solvent at 70 °C for 12 h (Sridhar *et al*., 2021).

Preparation of $AgNO_3$ solution. A stock solution was prepared by dissolving 16.98 g of silver nitrate in 100 mL of deionized water (DW). From this, 1 mL was diluted with 1000 mL of DW to yield a 1 mM solution (Alnuaimi *et al*., 2019).

Green synthesis of silver nanoparticles. Five milliliters of prickly pear alcoholic extract were added to 95 mL of $AgNO_3$ solution (1 mM). The solution was subjected to ultrasonication, followed by mixing with a magnetic stirrer and storage in dark vials for 24 h. To obtain a clear supernatant, the mixture was centrifuged for 10 min at 10000 rpm and 4 °C, yielding a colloid preparation that was stored in dark vials. The color change of the solution was monitored for five days.

Characterization and examination of silver nanoparticles. The solution was centrifuged for 10 min at 5000 rpm to remove any precipitate. After obtaining a stable color, the supernatant was centrifuged for 30 min at 12,000 rpm. To remove any residual plant extract, the pellet was washed with deionized water. The final pellet was resuspended in DW before identification.

Identification of the polyphenol compounds in the extraction. Polyphenols were quantified individually through reversed-phase HPLC analysis using a SYKAM HPLC system with a UV detector.

Nano-extract. The field-emission scanning electron microscope (FE-SEM) was used to diagnose and confirm the green synthesis of silver nanoparticles from the alcoholic extract of prickly pear fruit. Also, the FT-IR technique was used. The color change of the extract from yellow to brown indicated the initial formation of nanoparticles.

Experimental animals. Twenty male albino rats, aged 2–3 months, were bred in a laboratory setting. The animals were housed in regular cages with a 12 h dark/12 h

light cycle at a controlled temperature of 25 °C. The Animal Ethics Committee at the University of Mosul approved the study protocol on 15/2/2023 (Ref: UM.VET.2023.065).

Atherosclerosis induction. Cholesterol (Sigma-Aldrich) was dissolved in coconut oil, and the doses were determined according to the body weight of the animals at 500 mg/kg. Lipid profiles were measured for 25 days after atherosclerosis was confirmed (Ram *et al.*, 2014).

Animal experimental protocol. The animals were divided into four groups (n = 5 each). Group 1 received oral doses of cholesterol (500 mg/kg; positive control). Group 2 consisted of healthy animals (negative control). The animals in groups 3 and 4 were administered polyphenol and nano-extracts, respectively, at a dose of 39.78 mg/kg body weight for 30 days (Taha, 2022).

Blood sample collection. All animals were anesthetized with ether, and blood was collected from the orbital sinus puncture into EDTA test tubes, then centrifuged at 4000 rpm (4 °C) for 10 min immediately after collection. The serum was stored in a deep freeze until analysis. The aortic and heart tissue samples were collected at the end of the experiment and preserved in a 0.9% physiological saline solution (Kassim, 2012). Atherosclerosis induction was confirmed by taking tissue samples from the heart and coronary arteries for each group.

Parameters analysis. All parameters were measured in the serum sample.

Lipid profile. Total cholesterol (T-Cho) and triglycerides (TG) were measured using an enzymatic method with a commercial kit (Biolabo, France). High-density lipoprotein (HDL-C) was also measured using an analysis kit (Kostner, 1976). VLDL-C and LDL-C were calculated according to the equation (Friedewald *et al.*, 1972).

Glutathione (GSH). Antioxidant glutathione was measured using the modified method of Ellman's reagent [5.5′-dithiobis (2-nitrobenzoic acid), DTNB]. DTNB, as a reagent, interacts with glutathione (GSH) and is reduced by the thiol group (-SH) to form a colored compound measured at 412 nm (Sedlak & Lindsay, 1968).

Malondialdehyde (MDA). The amount of MDA produced was estimated as an indicator of lipid peroxidation. The process is based on the interaction of lipid peroxides (MDA) with thiobarbituric acid (TBA) in an acidic medium. The product's absorbance was measured at 532 nm (Muslih *et al.*, 2002).

Creatinine and urea. The levels of creatinine and urea were estimated enzymatically using a Biosystems analysis kit (Spain) (Burtis *et al.*, 1999).

Transaminase enzymes. The activity of AST and ALT was measured by the enzymatic method using a ready-made assay kit (Bio Labo, France).

Troponin I (cTnI), creatine kinase (CK-MB), and myoglobin. Cardiovascular markers were measured using a kit from Life Sign MI (United States) (Adams *et al.*, 1993).

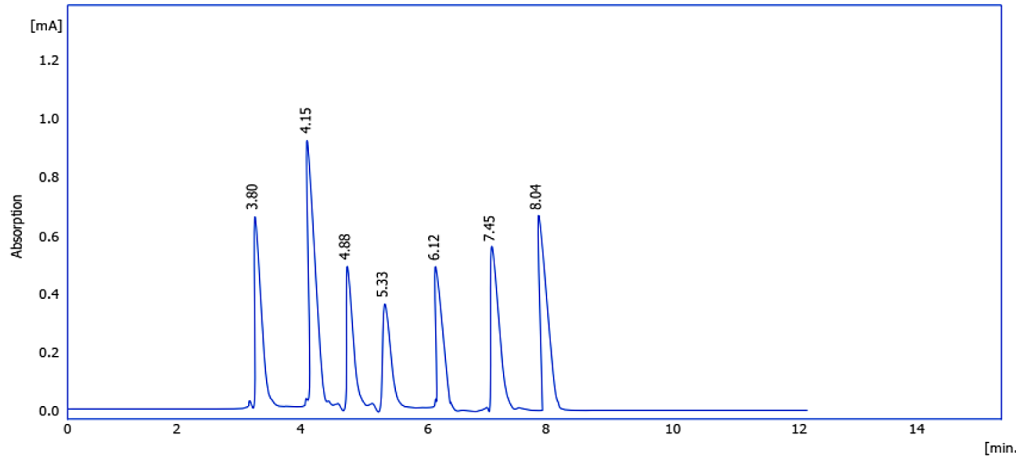
Statistical analysis. Statistical analysis was performed using IBM SPSS Statistics, version 27.0.1.0. A Duncan one-way analysis of variance (ANOVA) was applied. The results were presented as mean (X) ± standard deviation (SD), and comparisons were made between treatment and control groups. Statistical significance was set at $p \leq 0.05$ (George & Mallery, 2024).

RESULTS AND DISCUSSION

Phenolic and flavonoid compounds in the alcoholic extract were identified and quantified using HPLC analysis, based on comparison with standard compounds (**Table 1**, **Fig. 1**). The contents of polyphenol compounds were as follows: gallic acid (88.9 µg/kg), quercetin (80.6 µg/kg), ferulic acid (45.2 µg/kg), rutin (74.6 µg/kg), kaempferol (50.6 µg/kg), cinnamic acid (12.6 µg/kg), and tannic acid (20.7 µg/kg) in prickly pear fruit. These results were obtained by comparing the retention time of the standard polyphenol peaks, as shown in **Table 1** and **Fig. 2**.

Table 1. **Standard phenolic compounds and phenolic compounds identified in the raw alcoholic extract**

Peaks No.	The retention time for standard substances	The retention time for the polyphenol extract	Analysis of peaks	Concentration, µg/gm
1	3.80	3.80	Ferulic acid	45.2
2	4.15	4.15	Gallic acid	88.9
3	4.88	4.88	Cinnamic acid	12.6
4	5.33	5.33	Quercetin	80.6
5	6.09	6.12	Rutin	74.6
6	7.35	7.45	Kaempferol	50.6
7	8.00	8.04	Tannic acid	20.7



Result chromatography Table (Uncal - F:\ sample)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.80	254123.66	641.58	17.00	17.00	0.10	
2	4.15	365987.49	895.42	20.00	20.00	0.15	
3	4.88	135662.14	530.24	11.00	11.00	0.05	
4	5.33	114203.05	388.49	10.00	10.00	0.02	
5	6.12	121452.16	450.14	12.00	12.00	0.05	
6	7.45	144528.97	560.32	14.00	14.00	0.05	
7	8.04	152642.65	620.11	16.00	16.00	0.10	
	Total	1288600.12	4086.15	100.00	100.00		

Fig. 1. HPLC chromatogram: illustrates polyphenolic compounds in the alcoholic extract

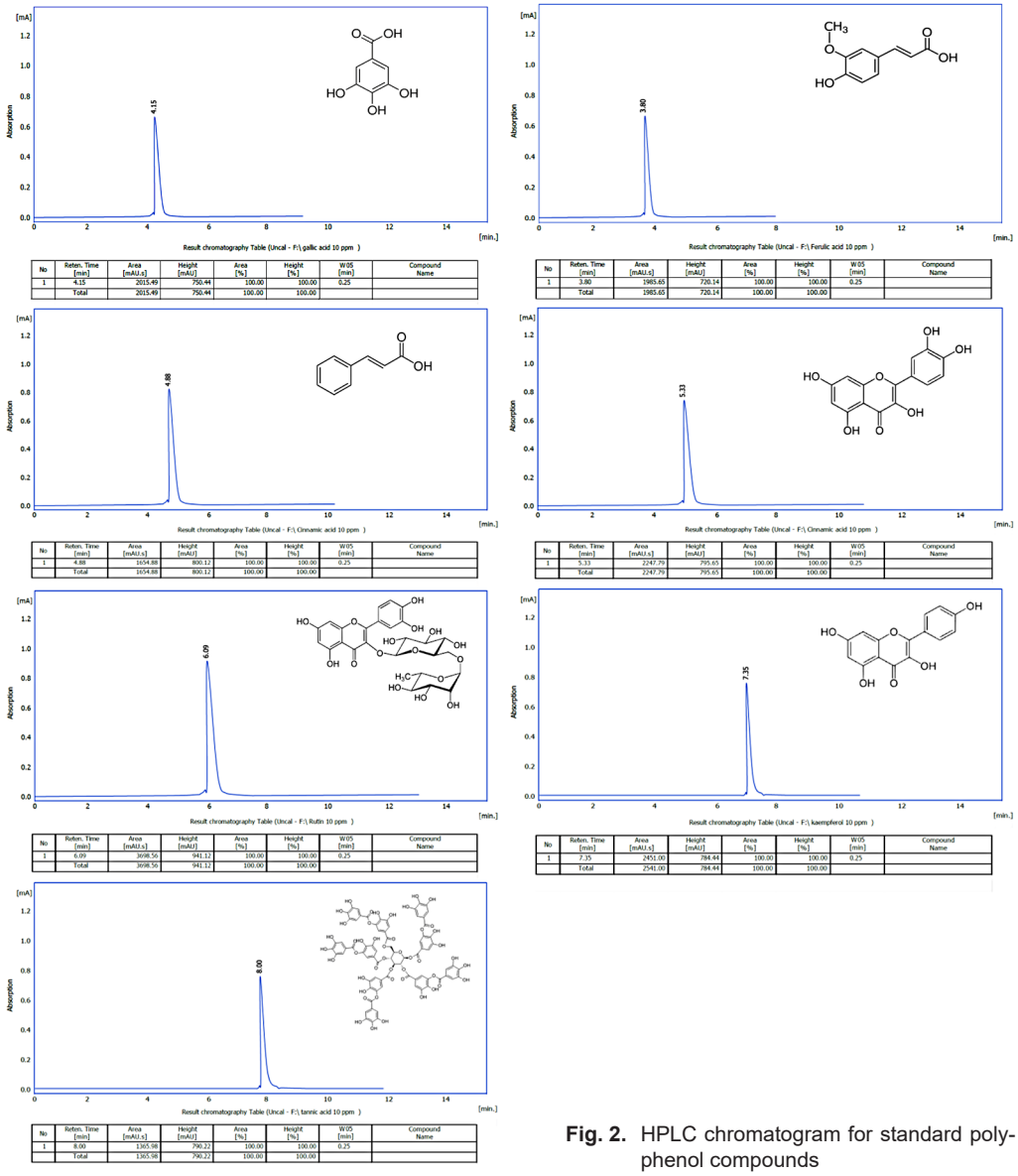


Fig. 2. HPLC chromatogram for standard polyphenol compounds

Green Synthesis of Silver nanoparticles using prickly pear alcoholic extract. OFI alcoholic extract was used to synthesize AgNPs. The color change of the reaction mixture was monitored visually. Following the addition of 1 mM $AgNO_3$ solution to the prickly pear fruit extract, the solution initially exhibited a yellowish color. Color intensity gradually deepened to dark brown as the incubation time increased (Kalimuthu *et al.*, 2008), as shown in **Fig. 3**.

Characterization of silver particles by FESEM. The image in **Fig. 4** shows that the silver nanoparticles were spherical (nano-spherical). This shape is considered one of the most effective chemically and biologically in nanotechnology, with applications as an

anti-tumor and antibacterial agent. The nanoparticles were uniform and narrowly distributed, indicating that the synthesis method used was highly successful in obtaining well-organized silver nanoparticles with a sharp distribution coefficient. In our study, nanoparticles with sizes less than 100 nm were obtained, with an average size of 25.52 nm (**Fig. 4**). Therefore, the silver particles prepared in this study are classified as nanoparticles, as they fall within the range of 1–100 nm, but outside the range of 1–15 nm, which corresponds to quantum particles that can cause complications in biological applications. Moreover, their spherical size was within the effective range for silver nanoparticles (Salih *et al*., 2019).

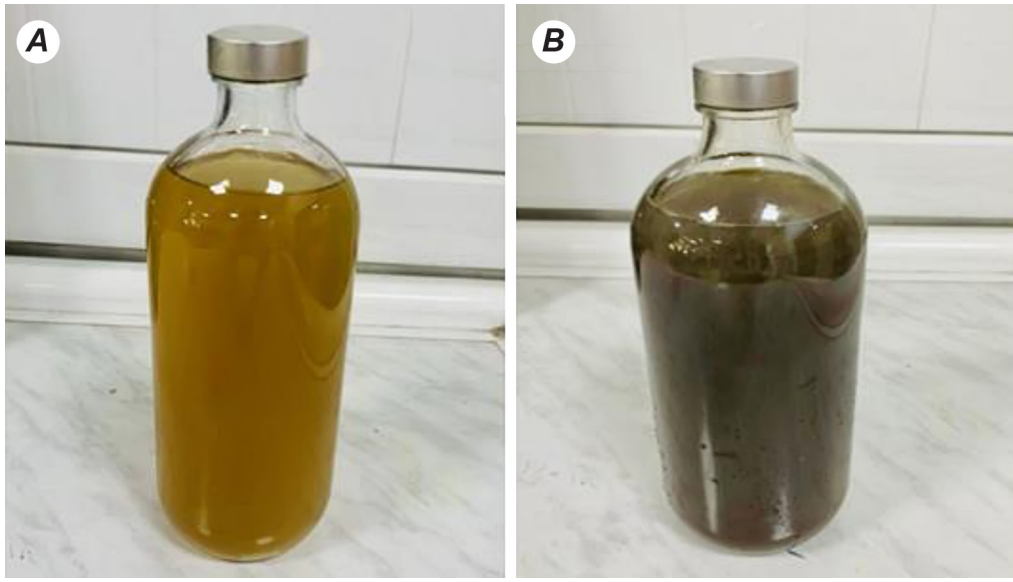


Fig. 3. The colors changed to silver particles after adding the extract (***A***) and after five days (***B***)

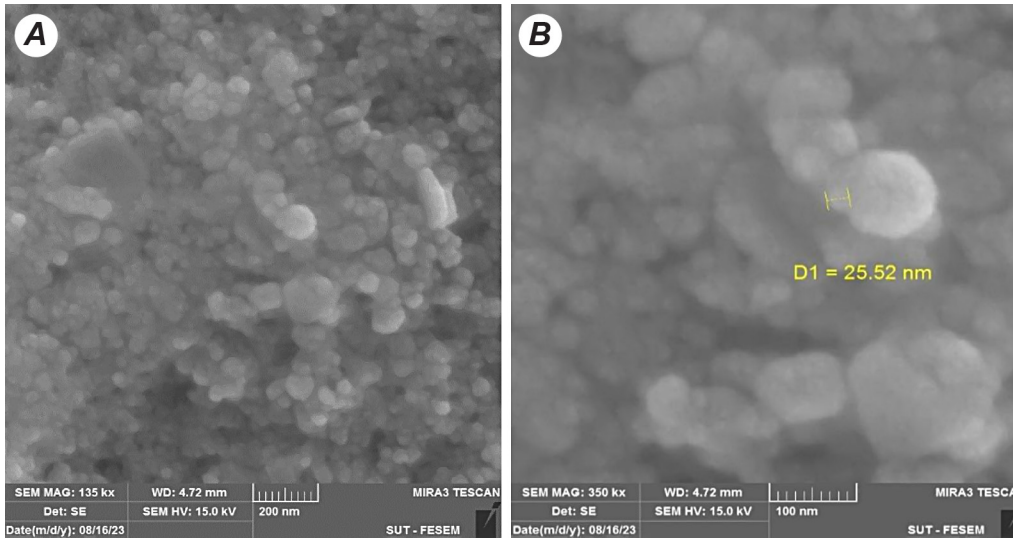


Fig. 4. FESEM images of silver nanoparticle extract: ***A*** – at 200 nm and ***B*** – at 100 nm

FT-IR spectral analysis of AgNPs. The FT-IR spectral analysis confirmed the formation of silver nanoparticles in the alcoholic extract of OFI fruit. The FT-IR spectrum of the alcoholic extract obtained from the prickly pear fruit appears in **Fig. 5*A***, while the spectrum of AgNPs is shown in **Fig. 5*B***. The FT-IR spectrum of AgNP clusters exhibited peaks at 3327, 2845, 2112, 1637, 1451, 1411, 1112, and 1016 cm^{-1}. It is worth noting that the peak at 3327 cm^{-1} represents the O–H vibrations of carboxyl and phenol functional groups. The peak at 2112 cm^{-1} suggests the binding of AgNPs to CH. The peak visible in the 1727 cm^{-1} region is attributed to the C=O ester peak in the extract. The peak shift of the extract in the O–H vibration is observed, and a peak appears at 1041 cm^{-1} due to the bending of C=O, which disappears, and a sharp alcohol peak appears at 1016 cm^{-1}. The peak at 1637 cm^{-1} is attributed to the C=C of the alkene (Bin Bakri *et al.*, 2021).

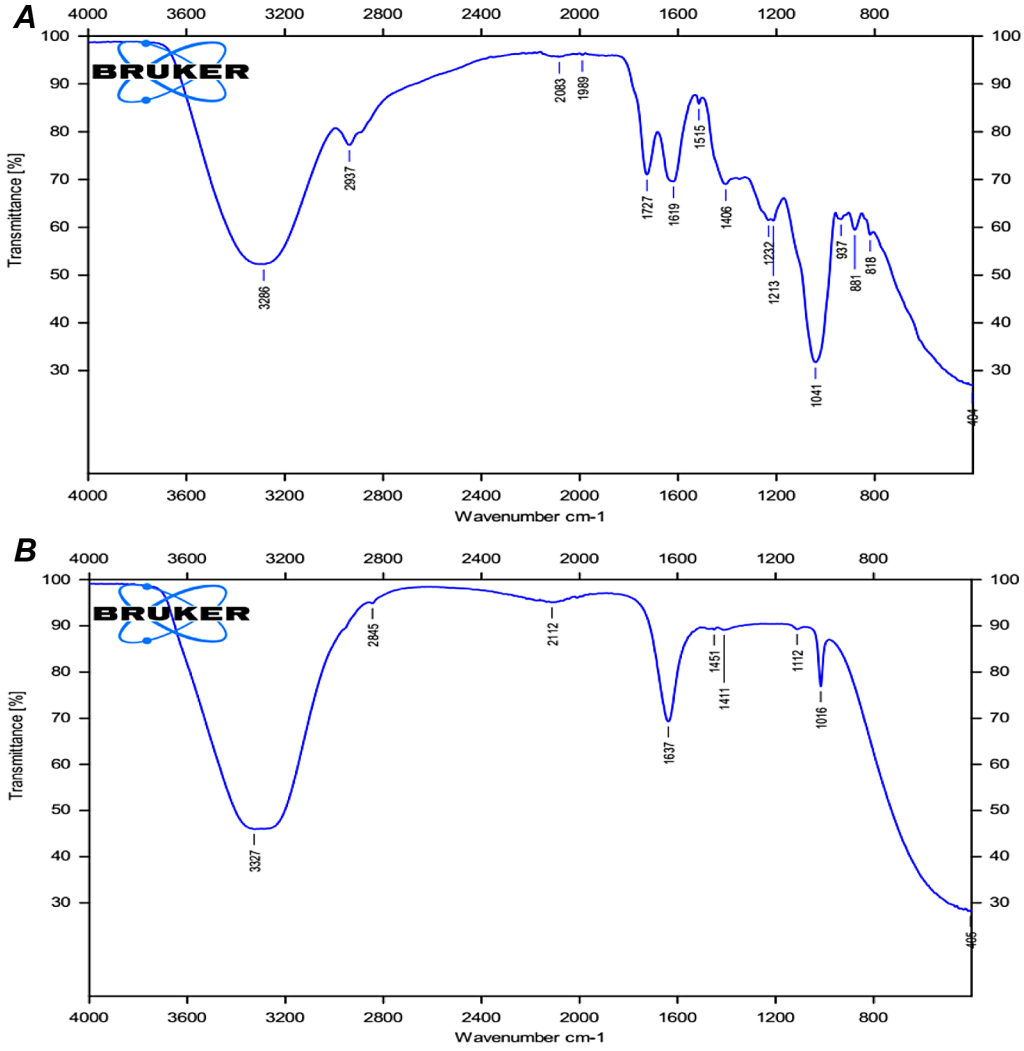


Fig. 5. ***A*** – values of the bands that appeared in FT-IR and belong to the nano-alcoholic extract; ***B*** – values of the bands that appeared in FT-IR and belong to the alcoholic extract

Biochemical parameters

Total cholesterol. As shown in **Table 2**, cholesterol levels were significantly increased ($p \leq 0.05$) in the atherosclerosis-induced group (+ve) compared with the healthy control (-ve). The development of atherosclerosis is closely linked to accumulation of fats in the arteries, especially cholesterol. Inflammatory cytokines of the IL-1 type, produced by the arteries, stimulate the generation of molecules that adhere to fatty substances in the blood vessel lining (Sprague & Khalil, 2009). Treatment of animals with alcoholic and nano-extracts of prickly pear fruit significantly reduced ($p \leq 0.05$) cholesterol levels compared with the atherosclerotic control group. Gallic acid is considered one of the most important polyphenolic compounds in the extract that reduce fat levels, and may influence lipase activity (*Jang et al.*, 2008).

Triglycerides (TG). The results showed a significant increase in the level of TG in the +ve group compared with the -ve group. This may result from triglycerides serving as precursors to inflammatory mediators such as prostaglandins and leukotrienes, with a decrease in the enzyme lipoprotein lipase responsible for the decrease in TG in LDL, its major carrier (Gabani *et al.*, 2023). Treating animals with the extract and nano-extract led to a significant reduction in triglyceride levels ($p \leq 0.05$) compared with the +ve group. This may be due to improved insulin receptor sensitivity, linked to quercetin – a flavonoid present in the extract (Adefegha *et al.*, 2025) a decreasing transfer of TG from fatty tissues, and stimulation of lipoprotein lipase activity in cellular tissues. In addition, lipoxygenase plays a significant role in the lipolysis dysfunction process.

High-density lipoprotein cholesterol (HDL-C). The results showed a significant decrease in HDL-C levels in the +ve group compared to the -ve group. The development of atherosclerosis leads to a disruption in lipid metabolism, increasing the transfer of cholesterol esters from HDL-C to VLDL-C, which enriches HDL-C with triglycerides and accelerates its degradation. Additionally, the fatty substance Apo-A is destroyed with important implications for the construction of HDL-C (Pikto-Pietkiewicz *et al.*, 2005). These effects may be attributed to a decrease in the activity of the LP-lipase enzyme and inhibition of the lecithin-cholesterol acyltransferase enzyme (LCAT), which is necessary for HDL-C (Arnold *et al.*, 2021). Treatment of animals with alcoholic extracts led to a significant increase in HDL-C levels ($p \leq 0.05$) compared with the +ve group. This may be attributed to flavonoids in the fruit extract that increase LCAT enzyme levels, thus raising HDL-C levels (Roghani & Baluchnejadmojarad, 2010).

Low-density lipoprotein cholesterol (LDL-C). The results in **Table 2** illustrate a significant increase in LDL levels in the +ve group compared to the -ve group. This is my be attributed to reduced clearance from the blood serum by macrophages and hepatocytes, or to a defect in the synthesis of particles on its surface or their receptors (Apo-B) (Haas *et al.*, 2013). When the animals were treated with OFI pear fruit alcoholic extract and nano-extract, the results showed a significant decrease ($p \leq 0.05$) in LDL-C levels, except for the nano-extract, which showed a non-significant decrease compared to the atherosclerosis control group. The reduction of LDL-C levels by the extracts may be related to their antioxidant activity, which protects LDL-C in the liver from oxidation. This plays an important role in removing excess cholesterol from cells and returning it to the liver until needed. The extracts also help prevent the deposition of fatty particles in the blood vessels, as cholesterol is transferred from LDL-C after esterification to HDL-C,

thus facilitating the removal of free cholesterol through metabolic pathways. This leads to a reduction in Apo-B particles and an increase in Apo-AI particles. Furthermore, unsaturated fatty acids enhance esterification (Shrestha *et al.*, 2018).

Very low-density lipoprotein cholesterol (VLDL-C). The results showed a significant increase in VLDL levels in the atherosclerosis control group compared to the healthy control, as its concentration depends on the level of TG. This increase may be attributed to a defect in some receptors, such as Apo-B. When animals were treated with OFI alcoholic extract and nano-extract, the results showed a significant decrease ($p \leq 0.05$) in VLDL levels. Some polyphenols are able to alter Apo-B secretion and hepatic enzyme activity, which can reduce the release of VLDL particles (Zern *et al.*, 2003).

Table 2. **Lipid profile in serum of the atherosclerotic, healthy, and treated animals**

Groups No = 4	Lipid profile, mg/dL				
	Mean±SD	Triglyceride mean±SD	HDL-C mean±SD	LDL-C mean±SD	VLDL-C mean±SD
Control +ve	106.88±8.62 a	161.96±14.20 a	41.02±14.88 a	34.53±15.98 a	30.46±5.00 a
Control -ve	58.57±0.75 b	68.44±8.61 b	67.02±7.77 b	20.23±12.36 b	12.16±3.73 b
Alcoholic extract	64.39±11.72 c,d,b	101.50±17.33 c	79.9±11.8 c,b	14.08±3.09 c,b	22.05±4.92 c
Nano-extract	67.28±10.13 d,c,b	69.52±13.44 d,b	23.22±1.48 d	25.79±2.81 d,a	13.90±2.68 d,c,b

Note: the difference are significant ($p \leq 0.05$): a – vs group "Control +ve"; b – vs group "Control -ve"; c – vs group "Alcoholic extract"; d – vs group "Nano-extract"

Creatinine and urea. The results in **Table 3** show a significant increase in the levels of both creatinine and urea levels in the +ve group compared to the -ve group. It has been hypothesized that dyslipidemia damages the kidneys and contributes significantly to the development of renal failure. Dyslipidemia may damage glomerular capillary endothelial and mesangial cells, as well as podocytes. Mesangial cells express receptors for LDL and oxidized LDL (Cases & Coll, 2005). The results in the table showed a significant decrease ($p \leq 0.05$) in urea levels when animals were treated with the nano-extract compared to the +ve group. The extended bioavailability effect of the nano-loaded extract, which ensures its delivery to the target damaged tissue, potentially plays a protective role in kidney tissue repair by removing free radicals, inhibiting inflammatory reactions, and improving kidney function (Huang *et al.*, 2025). The alcoholic extract showed a non-significant decrease in urea levels compared to the atherosclerotic control group. However, creatinine levels decreased significantly when animals were treated with the alcoholic and nano extracts. This may be attributed to the antioxidant and anti-inflammatory properties of these extracts, which may have a positive protective effect on kidney tissues by scavenging free radicals, thereby reducing or preventing kidney toxicity resulting from free radical accumulation that can cause functional and structural kidney damage (Yokoyama *et al.*, 2022).

Transaminases ALT and AST. Table 3 shows a significant increase in the concentration of both AST and ALT in the +ve group compared to the -ve group. Arterial stiffness causes an increase oxidative stress on blood vessel walls, leading to plaque rupture, and these enzymes are also linked to lipid metabolism risks (Deb *et al*., 2018). The results in the table show a significant decrease ($p \leq 0.05$) in AST and ALT levels compared to the atherosclerotic control group when animals were treated with prickly pear fruit alcoholic extract and nano-extract. This may be attributed to the antioxidants contained in the extracts that limit the formation of free radicals, and possess anti-inflammatory properties.

Malondialdehyde (MDA). The results in **Table 3** indicate a significant increase in MDA levels in the +ve group compared to the -ve group. This increase may be due to the enhanced lipid peroxidation resulting from oxidation of unsaturated fatty acids with carbon-carbon double bonds, which is associated with atherosclerosis, as well as decreased antioxidant levels in heart and artery diseases, especially glutathione (Iftikhar *et al*., 2023). Treatment with prickly pear fruit alcoholic and nano-extracts significantly decreased ($p \leq 0.05$) MDA levels compared to the atherosclerotic control group. This may be attributed to the compounds with antioxidant activity, present in these plants, that help in treating diabetes, cancer, arterial stiffness, oxidative stress, epilepsy and cardiovascular problems (Al-Bajari *et al*., 2019; Pizzino *et al*., 2017).

Glutathione (GSH). The results in the **Table 3** indicate a significant decrease in GSH levels in the +ve group compared to the -ve group. This decline may result from oxidative stress caused by reactive oxygen species, which are one of the causes of arteriosclerosis (Esquivel-Gutiérrez *et al*., 2021). Treatment with prickly pear fruit alcoholic and nano-extracts led to a significant increase ($p \leq 0.05$) in GSH levels compared to the control group with induced atherosclerosis. This may be due to their antioxidant properties that limit the production of free radicals and play a defensive role in protecting tissues, including cardiovascular tissue (Degroote *et al*., 2019; Thygesen *et al*., 2007).

Table 3. **Effect of alcoholic and nano-extract on liver, kidney functions, and lipid peroxidation in atherosclerotic rats**

Groups No = 4	Parameters					
	Creatinine mg/dL mean±SD	Urea mg/dL mean±SD	AST U/mL mean±SD	ALT U/mL mean±SD	GSH mmol/L mean±SD	MDA mmol/L mean±SD
Control +ve	2.09±0.39 a	56.90±8.21 a	182.00±8.68 a	352.5 ±47.16 a	1.72±0.46 a	1.39±0.26 a
Control -ve	0.76±0.57 b	42.00±5.24 b	145.33±14.01 b	220.25±44.28 b	3.38±0.68 b	0.48±0.28 b
Alcoholic extract	1.42±0.34 c,d,b	54.00±4.74 c,a	126.8±9.41 d,c,b	178.5±19.73 c,d,b	4.53±0.65 c	0.71±0.26 c,d,b
Nano-extract	1.12±0.1 d,c,b	28.00±4.35 d	121.6±16.13 d,c	132.0±62.55 d,c	2.82±0.88 d,b	0.38±0.11 d,c,b

Note: the difference are significant ($p \leq 0.05$): a – vs group “Control +ve”; b – vs group “Control -ve”; c – vs group “Alcoholic extract”; d – vs group “Nano-extract”

Cardiac markers. Table 4 shows a significant increase in the levels of cTul, CK-MB, and myoglobin in the blood serum of the +ve control group compared to the -ve group. This is due to necrosis of the heart muscle cells, which occurs when part of the heart is deprived of oxygen due to the blockage of the coronary arteries that supply the heart muscle with oxygenated blood. Without oxygen, the cells supplied by the artery begin to die (Webster, 2009). When animals were treated with prickly pear fruit alcoholic extract and nano-extract, a significant decrease ($p \leq 0.05$) in the levels of both cTnI and CK-MB was observed compared to the atherosclerotic control group. This decrease may be attributed to the properties of the plant compounds, which help counteract atherosclerosis, oxidative stress, and cardiovascular problems. In contrast, the decrease in myoglobin levels was not significant.

Table 4. **Effect of alcoholic and nano-extract on blood serum cardiac marker in atherosclerotic rats**

Groups No = 4	Cardiac marker, ng/mL		
	cTnI mean ± SD	CK-MB mean ± SD	Myoglobin mean ± SD
Control +ve	0.28±0.17 a	87.68±8.70 a	174.90±25.61 a
Control -ve	0.06±0.02 b	45.90±3.87 b	118.88±7.84 b
Alcohol extract	0.11±0.01 c,d,b	57.17±13.00 c,d,b	151.05±25.57 c,d,a
Nano-extract	0.12±0.04 d,c,b	69.32±10.89 d,c,b	178.92±22.25 d,c,a

Note: the difference are significant ($p \leq 0.05$): a – vs group "Control +ve"; b – vs group "Control -ve"; c – vs group "Alcoholic extract"; d – vs group "Nano-extract"

CONCLUSION

The alcoholic extract of prickly pear fruit was found to contain considerable levels of polyphenol compounds. The results demonstrated that the nano-silver extract was more effective than the alcoholic extract for most parameters studied. These findings suggest that such extracts may help reduce the symptoms or complications associated with atherosclerosis.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of the University of Mosul, College of Education for Pure Sciences, and the Department of Chemistry for this research topic.

CONFLICT OF INTEREST

The study was conducted without any financial or commercial relationships that could be interpreted as a potential conflict of interest.

ANIMAL RIGHTS

This article includes animal studies. Ethical approval for the study was obtained from the University of Mosul (Protocol No 50, 15 Feb 2023).

AUTHOR CONTRIBUTIONS

Conceptualization, [N.I.A]; methodology, [N.S.E.]; validation, [N.I.A]; formal analysis, [N.I.A.]; investigation, [N.I.A.]; resources, [A.A.A.]; writing – original draft preparation, [N.I.A]; writing – review and editing, [N.I.A]; visualization, [N.S.E]; supervision, [A.A.A]; project administration, [A.A.A]; funding acquisition, [-].

REFERENCES

Adams, J. E., Abendschein, D. R., & Jaffe, A. S. (1993). Biochemical markers of myocardial injury. Is MB creatine kinase the choice for the 1990s? *Circulation*, 88(2), 750–763. doi:10.1161/01.cir.88.2.750
Crossref ● PubMed ● Google Scholar

Adefegha, S. A., Ogundare, P. O., Oboh, G., Esatbeyoglu, T., & Papenbrock, J. (2025). An overview on the mechanisms of encapsulation of polyphenols used to help fight diabetes mellitus. *Discover Food*, 5(1), 185. doi:10.1007/s44187-025-00455-x
Crossref ● Google Scholar

Al-Bajari, S. A., AL-Lehebe, N. I., & AL-Alsadoon, L. H. (2019). Study of serum antioxidants, trace elements and leptin levels in epilepsy patients. *Indian Journal of Public Health Research & Development*, 10(2), 784. doi:10.5958/0976-5506.2019.00390.5
Crossref ● Google Scholar

Al-Naqeb, G., Fiori, L., Ciolli, M., & Aprea, E. (2021). Prickly pear seed oil extraction, chemical characterization and potential health benefits. *Molecules*, 26(16), 5018. doi:10.3390/molecules26165018
Crossref ● PubMed ● PMC ● Google Scholar

Alnuaimi, M. T., Hamdan, N. T., Abdalraheem, E., & Aljanabi, Z. Z. (2019). Biodegradation of malathion pesticide by silver bio-nanoparticles of *Bacillus licheniformis* extracts. *Research on Crops*, 20(spl), 79–84. doi:10.31830/2348-7542.2019.138
Crossref ● Google Scholar

Arnold, N., Lechner, K., Waldeyer, C., Shapiro, M. D., & Koenig, W. (2021). Inflammation and cardiovascular disease: the future. *European Cardiology Review*, 16, e20. doi:10.15420/ecr.2020.50
Crossref ● PubMed ● PMC ● Google Scholar

Aruwa, C. E., Amoo, S. O., & Kudanga, T. (2018). *Opuntia* (Cactaceae) plant compounds, biological activities and prospects – a comprehensive review. *Food Research International*, 112, 328–344. doi:10.1016/j.foodres.2018.06.047
Crossref ● PubMed ● Google Scholar

Bin Bakri, M. K., Rahman, Md. R., Khui, P. L. N., Jayamani, E., & Khan, A. (2021). Use of sustainable polymers to make green composites. In: M. R. Rahman (Ed.), *Advances in sustainable polymer composites* (pp. 109–129). Elsevier. doi:10.1016/b978-0-12-820338-5.00005-9
Crossref ● Google Scholar

Burtis, C. A., Ashwood, E. R., & Tietz, N. W. (1999). *Tietz textbook of clinical chemistry*. Philadelphia: W.B. Saunders.
Google Scholar

Cases, A., & Coll, E. (2005). Dyslipidemia and the progression of renal disease in chronic renal failure patients. *Kidney International*, 68, S87–S93. doi:10.1111/j.1523-1755.2005.09916.x
Crossref ● PubMed ● Google Scholar

Charo, I. F., & Taub, R. (2011). Anti-inflammatory therapeutics for the treatment of atherosclerosis. *Nature Reviews Drug Discovery*, 10(5), 365–376. doi:10.1038/nrd3444
Crossref ● PubMed ● PMC ● Google Scholar

Deb, S., Puthanveetil, P., & Sakharkar, P. (2018). A population-based cross-sectional study of the association between liver enzymes and lipid levels. *International Journal of Hepatology*, 2018(1), 1286170. doi:10.1155/2018/1286170
Crossref ● PubMed ● PMC ● Google Scholar

Degroote, J., Wang, W., Vergauwen, H., De Smet, S., Van Ginneken, C., & Michiels, J. (2019). Impact of a dietary challenge with peroxidized oil on the glutathione redox status and integrity of the small intestine in weaned piglets. *Animal*, 13(8), 1641–1650. doi:10.1017/s1751731118003166
Crossref ● PubMed ● Google Scholar

Esquivel-Gutiérrez, E. R., Manzo-Avalos, S., Peña-Montes, D. J., Saavedra-Molina, A., Morreeuw, Z. P., & Reyes, A. G. (2021). Hypolipidemic and antioxidant effects of *Guishe* extract from *Agave lechuguilla*, a Mexican plant with biotechnological potential, on streptozotocin-induced diabetic male rats. *Plants,* 10(11), 2492. doi:10.3390/plants10112492
Crossref ● PubMed ● PMC ● Google Scholar

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, 18(6), 499–502. doi:10.1093/clinchem/18.6.499
Crossref ● PubMed ● Google Scholar

Gabani, M., Shapiro, M. D., & Toth, P. P. (2023). The role of triglyceride-rich lipoproteins and their remnants in atherosclerotic cardiovascular disease. *European Cardiology Review*, 18, e56. doi:10.15420/ecr.2023.16
Crossref ● PubMed ● PMC ● Google Scholar

George, D., & Mallery, P. (2024). *IBM SPSS statistics 29 step by step: a simple guide and reference*. New York: Routledge. doi:10.4324/9781032622156
Crossref ● Google Scholar

Haas, M. E., Attie, A. D., & Biddinger, S. B. (2013). The regulation of ApoB metabolism by insulin. *Trends in Endocrinology & Metabolism*, 24(8), 391–397. doi:10.1016/j.tem.2013.04.001
Crossref ● PubMed ● PMC ● Google Scholar

Huang, S., Yang, X., Ma, J., Li, C., Wang, Y., & Wu, Z. (2025). Ethanol extract of propolis relieves exercise-induced fatigue via modulating the metabolites and gut microbiota in mice. *Frontiers in Nutrition*, 12*,* 1549913. doi:10.3389/fnut.2025.1549913
Crossref ● PubMed ● PMC ● Google Scholar

Iftikhar, K., Siddique, F., Ameer, K., Arshad, M., Kharal, S., Mohamed Ahmed, I. A., Yasmin, Z., & Aziz, N. (2023). Phytochemical profiling, antimicrobial, and antioxidant activities of hydroethanolic extracts of prickly pear (*Opuntia ficus indica*) fruit and pulp. *Food Science & Nutrition*, 11(4), 1916–1930. doi:10.1002/fsn3.3226
Crossref ● PubMed ● PMC ● Google Scholar

Kalimuthu, K., Babu, R. S., Venkataraman, D., Bilal, M., & Gurunathan, S. (2008). Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. *Colloids and surfaces B: Biointerfaces*, 65(1), 150-153. doi:10.1016/j.colsurfb.2008.02.018
Crossref ● PubMed ● Google Scholar

Kassim, H. (2012). Effect of fenugreek seeds extract on liver cells and enzymes of albino male. *Iraqi Journal of Science*, 53(1), 62–67.
Google Scholar

Kaur, M., Kaur, A., & Sharma, R. (2012). Pharmacological actions of *Opuntia ficus indica*: a review. *Journal of Applied Pharmaceutical Science*, 2(7), 15–18. doi:10.7324/japs.2012.2703
Crossref ● Google Scholar

Kostner, G. (1976). Letter: enzymatic determination of cholesterol in high-density lipoprotein fractions prepared by polyanion precipitation. *Clinical Chemistry*, 22(5), 695–695. doi:10.1093/clinchem/22.5.695a
Crossref ● PubMed ● Google Scholar

Muslih, R., Al-Nimer, O., & Al-Zamely, M. (2002). The level of malondialdehyde after activation with (H_2O_2 and $CuSO_4$) and inhibited by desferoxamine and molsidomine in the serum of patients with acute myocardial infection. *National Journal of Chemistry*, 5, 139–148.
Google Scholar

Pikto-Pietkiewicz, W., Wolkowska, K., & Pasierski, T. (2005). Treatment of dyslipidemia in patients with diabetes mellitus. *Pharmacological Reports*, 57, 10–19. Retrieved from http://if-pan.krakow.pl/pjp/pdf/2005/s_10.pdf
PubMed ● Google Scholar

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, 2017(1), 8416763. doi:10.1155/2017/8416763
Crossref ● PubMed ● PMC ● Google Scholar

Poggio, P., Branchetti, E., Grau, J. B., Lai, E. K., Gorman, R. C., Gorman, J. H., Sacks, M. S., Bavaria, J. E., & Ferrari, G. (2014). Osteopontin-CD44v6 interaction mediates calcium deposition via phospho-Akt in valve interstitial cells from patients with noncalcified aortic valve sclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 34(9), 2086–2094. doi:10.1161/atvbaha.113.303017
Crossref ● PubMed ● PMC ● Google Scholar

Ram, H., Jatwa, R., & Purohit, A. (2014). Antiatherosclerotic and cardioprotective potential of *Acacia senegal* seeds in diet-induced atherosclerosis in rabbits. *Biochemistry Research International*, 2014(1), 436848. doi:10.1155/2014/436848
Crossref ● PubMed ● PMC ● Google Scholar

Rasoulpour, R., Afsharifar, A., Izadpanah, K., & Aminlari, M. (2017). Purification and characterization of an antiviral protein from prickly pear (*Opuntia ficus-indica* (L.) Miller) cladode. *Crop Protection*, 93, 33–42. doi:10.1016/j.cropro.2016.11.005
Crossref ● Google Scholar

Roghani, M., & Baluchnejadmojarad, T. (2010). Hypoglycemic and hypolipidemic effect and antioxidant activity of chronic epigallocatechin-gallate in streptozotocin-diabetic rats. *Pathophysiology*, 17(1), 55–59. doi:10.1016/j.pathophys.2009.07.004
Crossref ● PubMed ● Google Scholar

Salih, B. D., Ali, A. H., Alheety, M. A., Mahmood, A. R., Karadağ, A., & Aydın, A. (2019). Biosynthesis of Ag nanospheres using waste phoenix dactylifera argonne: a prospective anticancer and antibacterial. *Materials Research Express*, 6(10), 105063. doi:10.1088/2053-1591/ab3bad
Crossref ● Google Scholar

Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry*, 25, 192–205. doi:10.1016/0003-2697(68)90092-4
Crossref ● PubMed ● Google Scholar

Shrestha, S., Wu, B. J., Guiney, L., Barter, P. J., & Rye, K.-A. (2018). Cholesteryl ester transfer protein and its inhibitors. *Journal of Lipid Research*, 59(5), 772–783. doi:10.1194/jlr.r082735
Crossref ● PubMed ● PMC ● Google Scholar

Soehnlein, O., & Libby, P. (2021). Targeting inflammation in atherosclerosis – from experimental insights to the clinic. *Nature Reviews Drug Discovery*, 20(8), 589–610. doi:10.1038/s41573-021-00198-1
Crossref ● PubMed ● PMC ● Google Scholar

Sprague, A. H., & Khalil, R. A. (2009). Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochemical Pharmacology*, 78(6), 539–552. doi:10.1016/j.bcp.2009.04.029
Crossref ● PubMed ● PMC ● Google Scholar

Sridhar, A., Ponnuchamy, M., Kumar, P. S., Kapoor, A., Vo, D.-V. N., & Prabhakar, S. (2021). Techniques and modeling of polyphenol extraction from food: a review. *Environmental Chemistry Letters*, 19(4), 3409–3443. doi:10.1007/s10311-021-01217-8
Crossref ● PubMed ● PMC ● Google Scholar

Taha, I. (2022). Effect of active compounds for *Quercus* fruit on some biochemical parameters and tissue aorta in induced atherosclerosis rats. *Scientific Journal for the Faculty of Science-Sirte University*, 2(2), 62–72. doi:10.37375/sjfssu.v2i2.84
Crossref ● Google Scholar

Thygesen, K., Alpert, J. S., & White, H. D. (2007). Universal definition of myocardial infarction. *Journal of the American College of Cardiology*, 50(22), 2173–2195. doi:10.1016/j.jacc.2007.09.011
Crossref ● PubMed ● Google Scholar

Tohirova, J., & Shernazarov, F. (2022). Atherosclerosis: causes, symptoms, diagnosis, treatment and prevention. *Science and Innovation,* 1(D5), 7–12.
Google Scholar

Webster, K. (2009). Mitochondrial death channels. *American Scientist*, 97(5), 384. doi:10.1511/2009.80.384
Crossref ● PubMed ● PMC ● Google Scholar

Wolf, D., & Ley, K. (2019). Immunity and inflammation in atherosclerosis. *Circulation Research*, 124(2), 315–327. doi:10.1161/circresaha.118.313591
Crossref ● PubMed ● PMC ● Google Scholar

Yokoyama, H., Masuyama, T., Tanaka, Y., Tsubakihara, I., Kondo, K., & Yoshinari, K. (2022). Acyl-CoA:diacylglycerol acyltransferase 1 inhibition in the small intestine increases plasma transaminase activity via the activation of protein kinase C pathway. *The Journal of Toxicological Sciences*, 47(1), 19–30. doi:10.2131/jts.47.19
Crossref ● PubMed ● Google Scholar

Zern, T. L., West, K. L., & Fernandez, M. L. (2003). Grape polyphenols decrease plasma triglycerides and cholesterol accumulation in the aorta of ovariectomized guinea pigs. *The Journal of Nutrition*, 133(7), 2268–2272. doi:10.1093/jn/133.7.2268
Crossref ● PubMed ● Google Scholar

Received / Одержано	Revision / Доопрацьовано	Accepted / Прийнято	Published / Опубліковано
24 April, 2025	15 July, 2025	17 December, 2025	30 December, 2025