Gas Chromatography-Mass Spectrometry-based Characterization and Multitarget Bioactivities of Argan (*Argania spinosa*) Oil

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Argan oil exhibits promising pharmacological activities supported by its rich phytochemical composition, as confirmed by gas chromatography-mass spectrometry analysis. Major constituents included trans-13-Octadecenoic acid (20.0%) and Isopropyl tetradecanoate (25.6%), along with antioxidant-rich terpenoids and phenolics. Antimicrobial assays demonstrated strong inhibition against *Bacillus subtilis* and *Candida albicans*, with low minimum inhibitory, minimum bactericidal, and minimum fungicidal concentration values (31.2 µg/mL), though limited activity was noted against *Salmonella typhi*. Argan oil showed dose-dependent inhibition of α-amylase and α-glucosidase, with IC_{50} values of 15.1 µg/mL and 26.2 µg/mL, respectively, suggesting antidiabetic potential. It also inhibited butyrylcholinesterase (IC_{50} = 15.9 µg/mL), indicating possible neuroprotective properties. Antioxidant activity assessed by 2,2-diphenyl-1-picrylhydrazyl assay showed a concentration-dependent increase, with IC_{50} = 4.97 µg/mL, approaching the effectiveness of ascorbic acid at high doses. Lipase inhibition (IC_{50} = 25.10 µg/mL) highlighted potential anti-obesity effects. Cytotoxicity on Caco-2 cells was significant (IC_{50} = 36.17 µg/mL), with morphological damage correlating with dose, suggesting antiproliferative effects. These activities are likely due to bioactive compounds, such as unsaturated fatty acids and tocopherols, which influence inflammation, apoptosis, and enzyme regulation. Overall, the findings support the therapeutic potential of argan oil as a natural agent in managing microbial infections, oxidative stress, diabetes, obesity, and cancer.

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INTRODUCTION

The argan tree (*Argania spinosa* (L.) Skeels) is a unique and endemic species of the Sapotaceae family, found exclusively in southwestern Morocco. It holds exceptional ecological, cultural, and socioeconomic value, serving as a vital component of local livelihoods and traditional medicine (Chakhchar *et al.* 2021). In the current decade, there has been a growing global interest in traditional medicinal plants, not only for their therapeutic potential but also for their ethnopharmacological significance (Abdelghany *et al.* 2019). Among its most valuable products, argan oil has gained widespread attention for its nutritional, cosmetic, and medicinal uses. Rich in bioactive compounds—including monounsaturated (up to 80%) and saturated (up to 20%) fatty acids, tocopherols, sterols, and polyphenols—argan oil exhibits strong lipophilic antioxidant properties (Khong and Chan 2022). These components appear to contribute to its effectiveness in preventing noncommunicable diseases, such as cardiovascular disorders, hypercholesterolemia, and obesity, as well as in treating dermatological conditions.

Argan oil, which is renowned for its high nutritional and bioactive compound content, is widely utilized in the food industry, cosmetic formulations, and traditional therapeutic practices (El Yamani *et al.* 2024). Beyond the oil, various parts of the argan tree—such as leaves, seeds, pulp, bark, and roots—are traditionally used to manage a broad spectrum of ailments, including diabetes, rheumatism, eczema, ulcers, gastritis, dysentery, and fevers (Bourhim *et al.* 2018). In addition, its applications in skin hydration, regeneration, and anti-aging treatments make it a cornerstone of natural skincare regimens. Mechqoq *et al.* (2021) reported notable antimicrobial activity from argan leaves oil.

To better understand the bioefficacy of argan oil, detailed chemical profiling using gas chromatography–mass spectrometry (GC-MS) has emerged as a vital tool. Although GC-MS has been used previously, it continues to be employed for characterizing the phytochemical composition of argan oil. GC–MS analysis was used to ensure the consistency and accuracy of the results and to update the data according to the different agricultural and climatic conditions of the oil source used in our current study. Recent studies indicate that the chemical composition of argan oil can vary depending on these factors (Słomczyńska *et al.* 2025); therefore, it was essential to perform the analysis again to provide up-to-date and reliable data supporting the biological results presented in this investigation. The GC-MS method facilitates the identification of various phytochemical constituents, including fatty acids, sterols, alcohols, and volatile compounds, many of which are linked to pharmacological effects (Haouame *et al*. 2025). The complex interplay of these compounds underlies argan oil's broad-spectrum biological activities, which extend beyond antioxidant effects to include anti-obesity, anticancer, antimicrobial, and neuroprotective (anti-Alzheimer) potentials.

Alzheimer's disease and obesity are two major global health concerns with increasing prevalence and profound public health implications. Alzheimer's disease, the most common form of dementia, is a progressive neurodegenerative disorder characterized by cognitive decline, memory loss, and behavioral disturbances. Despite decades of research, current therapeutic options remain limited, largely symptomatic, and unable to halt disease progression (Al-Rajhi *et al.* 2023). In contrast, obesity is a chronic metabolic disorder marked by excessive fat accumulation, often resulting in insulin resistance, cardiovascular complications, and increased risk of Type 2 diabetes. The growing burden

of these disorders underscores the urgent need for effective preventive and therapeutic strategies, particularly those derived from natural, safe, and multifunctional bioresources such as medicinal plants and their bioactive oils (Al-Rajhi *et al.* 2022). The antimicrobial activities of argan oil have been reported in other studies but on limited microorganisms, namely *L. acnes* and *Prevotella intermedia* (Lall *et al.* 2019), as well as *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Almuhayawi *et al*. 2024). According to Lall *et al.* (2019), several cancer cells including epidermoid, breast adenocarcinoma cells, pigmented melanoma skin, and cervical carcinoma cells were not inhibited up to a dose of 1000 µg/mL. However previous report indicated that *A. spinosa* extract can exhibit anticancer potential towards human breast adenocarcinoma cell line (Babili *et al.* 2010). In addition to the previously studied antimicrobial and anticancer activities, the present work also investigates the anti-obesity and anti-Alzheimer potential of argan oil, thereby expanding its possible medical applications. In this context, the current investigation aims to characterize the chemical composition of argan oil using GC-MS and to evaluate its *in vitro* antioxidant, antimicrobial, anti-obesity, anticancer, and anti-Alzheimer activities. This multidisciplinary approach provides insight into the therapeutic value of argan oil and supports its potential applications in functional food, pharmaceutical, and nutraceutical industries.

EXPERIMENTAL

GC-MS Analysis of Argan Oil

The chemical ingredients of argan oil (obtained from Morocco Pure Produce company, Rabat, Morocco; extracted from the Seeds of *Argania spinosa*) was analyzed using a Thermo Scientific ISQ 7000 GC-MS system. A 1.00 µL aliquot of the oil was inserted in splitless mode into the gas chromatograph, with the injector maintained at 250 °C. The oven temperature program started at 45 °C and was held for 1 min, followed by a ramp at 3 °C/min to 200 °C with a 3-minute hold, then raised at 3 °C/min to a final temperature of 280 °C, which was held for 10 min. The total runtime was governed by the oven method settings. Helium was employed as the carrier gas at a constant flow rate of 1.5 mL/min. The mass spectrometer operated in electron ionization (EI) mode with a transfer line temperature of 240 °C and an ion source temperature of 250 °C. Spectral data were collected in the positive ion mode over a mass range of 50 to 1000 amu with a scan interval of 0.2 s.

Antimicrobial Potential of Argan Oil

The antimicrobial potential of argan oil was estimated *versus* selected microbial strains *via* the well diffusion technique. Test bacteria included *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Klebsiella pneumoniae* (ATCC13883), and *Salmonella typhi* (ATCC 6539). Molds included *Candida albicans* (ATCC 10221) and *Aspergillus niger* (ATCC 16888). Bacteria were maintained on nutrient agar while fungi on Sabouraud dextrose agar (SDA) slants at 4 °C. Briefly, microbial suspensions were formulated in sterile saline to match a turbidity equivalent to 0.5 McFarland standard (1.5×10^8 CFU/mL). Sterile Mueller-Hinton agar (MHA) (for bacteria) and SDA (for molds) were decanted into Petri dishes and allowed to solidify. Each plate was uniformly

inoculated with the microbial suspension employing a sterile cotton swab. Wells (6-mm diameter) were punched aseptically into the agar employing a sterile cork borer (Al-Rajhi and Abdelghany 2023). Argan oil (100 µL) was injected in each well, diluted in DMSO (Dimethyl sulfoxide). Negative controls (solvent only) and positive controls (standard antibiotics such as ampicillin for bacteria or fluconazole for fungi) were also included. Cultures were incubated for 24 h at 37 °C for bacteria and at 28 °C for 48 to 72 h for molds. After the period of incubation, the width of the clear (inhibition) zone around each well was recorded. A broth microdilution method was employed in the detection of minimum inhibitory concentration (MIC) in 96-well plates. Serial two-fold dilutions of argan oil were prepared in Sabouraud Dextrose broth (for molds) and Mueller-Hinton broth (for bacteria), followed by inoculation with standardized microbial suspensions. Plates were incubated at 37 °C for bacteria and at 28 °C for 48 to 72 h for molds. MIC was labelled as the lowest concentration showing no observable microbial development. The minimum bactericidal concentration (MBC) was determined by subculturing 10 µL from wells without visible growth onto agar plates and incubating under the same conditions (Selim *et al.* 2024). The MBC was the lowest concentration viewing no bacterial colony growth. Similarly, the Minimum fungicidal concentration (MFC) was evaluated by transferring aliquots from MIC wells onto SDA. The MFC was recorded as the lowest concentration that resulted in complete growth inhibition.

Evaluation of Anti-Alzheimer Activity of Argan Oil *via* Butyrylcholinesterase (BuChE) Inhibition

The BuChE inhibitory potential of argan oil was assessed using a modified Ellman's colorimetric method. Briefly, butyrylthiocholine iodide was applied as the substrate. In addition, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) was applied as the chromogenic agent. Reaction mixtures contained various concentrations of argan oil (1.95 to 100 µg/mL), DTNB (0.3 mM), BuChE enzyme (from equine serum), and phosphate buffer (pH 8.0). The mixtures were preincubated at 37 °C for 10 min, followed by the addition of the substrate to initiate the reaction. Absorbance changes were recorded at 412 nm for 5 min using a microplate reader. The inhibitor standard was Rivastigmine. The % of enzyme inhibition was assessed relative to a control lacking the test sample. The IC_{50} quantity, demonstrating the concentration needed to inhibit 50% of BuChE activity, was documented through dose-response investigation.

Evaluation of Anti-oxidant Activity of Argan Oil *via* DPPH Assay

The antioxidant action of argan oil was determined by utilizing the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. A methanolic solution of DPPH (0.1 mM) was prepared and mixed with various concentrations of argan oil (1.95 to 1000 µg/mL). The reaction mixtures were incubated in the dark at 25 °C for 30 min. Absorbance was then measured at 517 nm using a UV-Vis spectrophotometer (Model UV-1800, Shimadzu, Kyoto, Japan). Ascorbic acid served as the reference antioxidant. The percentage of radical scavenging activity was calculated by comparing the absorbance of samples with that of the control (DPPH solution without oil). The IC_{50} value, representing the concentration required to scavenge 50% of DPPH radicals, was calculated from the dose-response curve (Alsalamah *et al.* 2023).

Evaluation of Anti-Obesity Potential of Argan Oil Through Inhibition of Pancreatic Lipase

To investigate the potential of argan oil in inhibiting pancreatic lipase, a colorimetric *in vitro* assay was conducted. The enzyme porcine pancreatic lipase was made in Tris-HCl buffer (pH 7.4), and p-nitrophenyl butyrate (p-NPB) was used as the substrate. Argan oil samples were made at various quantities (1.95 to 1000 µg/mL) in suitable solvents and preincubated with the enzyme solution at 37 °C for 15 min. Following this, p-NPB was amended to start the reaction. The mixture after 30 min of reaction, the absorbance of the liberated p-nitrophenol was quantified at 405 nm using a microplate reader. Orlistat served as a positive control. The inhibitory activity was designed by comparing the absorbance quantities of samples treated by oil against the control (enzyme without inhibitor). Percentage inhibition was plotted against concentration to determine the IC_{50} value using dose-response curve fitting. This method allowed for assessment of the lipase inhibitory effect of argan oil as a natural anti-obesity agent.

Activity of Argan Oil Against Caco-2 Cells

The cytotoxic effect of argan oil on human colorectal adenocarcinoma (Caco-2) cells was evaluated using the MTT assay. Caco-2 cells were propagated in Dulbecco's Modified Eagle's Medium (DMEM) complemented with fetal bovine serum (FBS) (10%), 1% penicillin-streptomycin, and maintained in a humidified incubator with 5% CO_2 at 37 °C. Cells were sowed in 96-well plates (1×10^4 cells/well) and allowed to adhere for one day. Argan oil was dissolved in DMSO and applied at varying concentrations (0 to 1000 µg/mL) for one day. After dealing, 20 µL of MTT reagent (5 mg/mL) was added to each well and incubated for 4 h. The resulting formazan crystals were dissolved in DMSO, and absorbance was judged at 570 nm utilizing a microplate reader. Viability of Caco-2 was expressed as a percentage relative to untreated controls. IC_{50} values were calculated using nonlinear regression analysis. Caco-2 cells were examined under an inverted microscope with phase-contrast to detect any morphological changes after treatment (Qanash *et al.* 2022).

Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD) using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA) from at least three independent replicates.

RESULTS and DISCUSSION

Based on Table 1, after GC-MS analysis (Fig. 1) of argan oil, the chemical profile reveals a diverse composition of bioactive compounds, including fatty acids, esters, alcohols, terpenoids, phenolic derivatives, and peptides. The major components in argan oil include fatty acids and esters: trans-13-octadecenoic acid (19.98%), isopropyl tetradecanoate (25.59%), ethyl octadecanoate (2.29%), hexadecanoic acid (2.88%), and octadecanoic acid (1.46%). Other components belong to terpenes and terpenoids, including Isopropyl-trimethyl-oxabicyclo (4.63%), nerolidol-epoxyacetate (3.43%), and eucalyptol (1.54%). In addition, there were also phenolic compounds, including soeugenol (0.88%),

4-allyl-2-methoxy-phenol (0.34%), and benzopyran derivatives. In contrast, minor constituents belonging to sterols and alcohols, such as cholestan-3-ol (0.22%), 1-heptatriacontanol (0.28%), and hexadecanol (0.31%), were detected in argan oil. The application of GC-MS analysis in this study allowed for the detection of a wide range of compounds present in argan oil, with a highest portion belonging to the fatty acid group. This analytical approach has been effectively employed to profile up to 18 distinct fatty acids, as demonstrated in recent work by Słomczyńska *et al.* (2025), highlighting its reliability for comprehensive lipidomic screening. In the current study's findings, major fatty acids, such as trans-13-octadecenoic acid and hexadecanoic acid, were among the dominant constituents, which are frequently associated with bioactivities such as antimicrobial and anti-inflammatory effects. Among the terpenoid constituents, nerolidol and its epoxy derivatives were identified in notable doses. These ingredients are of particular interest due to their previously recognized antimicrobial efficacy, particularly against airborne pathogens, as observed by Krist *et al.* (2015). Another important compound detected was eucalyptol, a monoterpenoid ether recognized for its broad pharmacological profile, which includes anti-inflammatory, antioxidant, and antimicrobial activities. Moreover, Hoch *et al.* (2023) emphasized its emerging role in addressing complex health conditions, such as neuropathic disorders, Alzheimer's disease, and certain cancers, further reinforcing the therapeutic potential of argan oil.

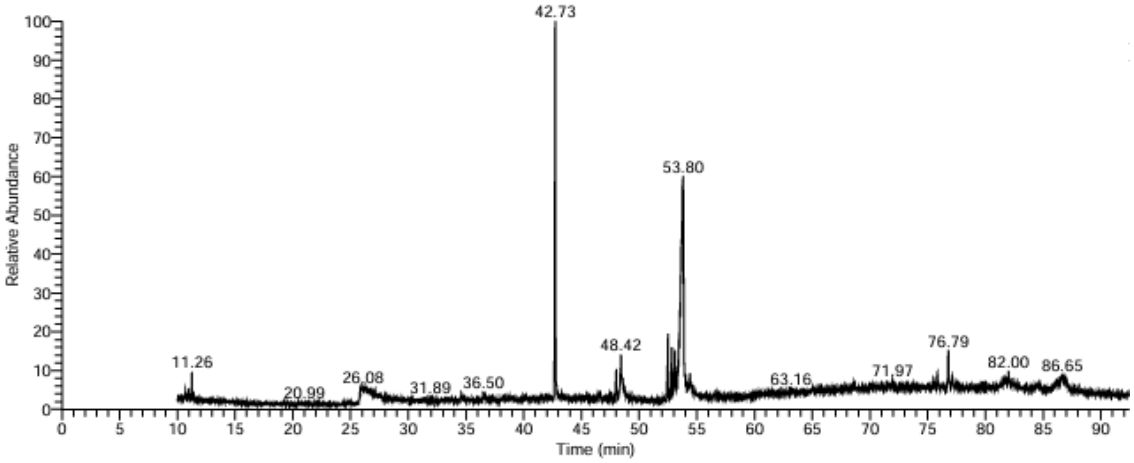

Fig. 1. GC-MS chromatogram of argan oil showing the retention times and relative abundance of identified compounds

Table 1. Identified Bioactive Constituents of Argan Oil Through GC-MS

Area %	Molecular Weight	Molecular Formula	Name of Compound	RT
0.22	2025	$C_{79}H_{131}N_{31}O_{24}S_4$	Apamin	10.09
0.09	1027	$C_{44}H_{61}N_{13}O_{12}S_2$	Vasopressin Desglycinamide	10 .16
0.53	154	$C_{10}H_{18}O$	p-Menthane, 1,4-epoxy	10.69
0.63	136	$C_{10}H_{16}$	4-Isopropenyl-1-methyl-1-cyclohexene	10 .98
1.54	154	$C_{10}H_{18}O$	Eucalyptol	11.26
0.88	164	$C_{10}H_{12}O_2$	Isoeugenol	25.86
0.34	164	$C_{10}H_{12}O_2$	4-Allyl-2-Methoxy-phenol	25.91

Area %	Molecular Weight	Molecular Formula	Name of Compound	RT
0.16	173	$C_5H_8ClN_5$	1,3, 5-Triazine-2,4-diamine, 6-chloro-n-ethyl	29.62
0.17	341	$C_{22}H_{31}NO_2$	3-Oxo-20-Methyl 11-à-ydroxycon	29.89
0.29	489	$C_{28}H_{43}NO_6$	(5á)Pregnane-3,20á-di ol, 14à,18à-[4-methyl-3-o xo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	31.89
0.62	220	$C_{15}H_{24}O$	1H-3A,7-Methano Azulen-5-OL, Octahydro-,8,8 Trimethyl-6-meth ylene	34.60
0.31	242	$C_{16}H_{34}O$	Hexadecanol	36.95
0.28	430	$C_{27}H_{42}O_4$	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)	38.18
0.22	400	$C_{28}H_{48}O$	Cholestan-3-ol, 2-methylene-, (3á,5à)	39.03
0.18	450	$C_{32}H_{66}$	Dotriacontane	40.63
0.28	270	$C_{18}H_{34}D_2O$	2,2-Dideutero octadecanal	41.86
25.59	270	$C_{17}H_{34}O_2$	Isopropyl tetradecanoate	42.73
0.29	274	$C_{18}H_{30}D_6O$	2,2,3,3,4,4 Hexadeutero Octadecanal	43.29
0.20	344	$C_{18}H_{16}O_7$	4H-1-Benzopyran-4-One, 2-(3,4-Dimethoxyphenyl)-3,5-dihydroxy-7-methoxy	43.66
0.20	256	$C_{17}H_{36}O$	1-Hexadecanol, 2-Methyl	45.29
0.22	258	$C_{16}H_{34}S$	Tert-Hexadecanethiol	45.65
0.20	282	$C_{18}H_{34}O_2$	9-Octadecenoic acid (Z)	46.07
0.78	280	$C_{19}H_{36}O$	12-Methyl-E,E-2,13-octadecadien-1-ol	46 .60
0.25	346	$C_{19}H_{22}O_6$	Isochiapin B	47.02
0.28	536	$C37H_{76}O$	1-Heptatriacotanol	47.43
2.29	312	$C_{20}H_{40}O_2$	Ethyl Octadecanoate	48.00
2.88	256	$C_{16}H_{32}O_2$	Hexadecanoic acid	48.42
0.08	330	$C_{19}H_{38}O_4$	2,3-Dihydroxypropyl palmitate	48.97
0.28	536	$C_{37}H_{76}O$	1-Heptatriacontanol	50.08
0.23	268	$C_{17}H_{32}O_2$	7-Methyl-Z-tetradecen-1-ol acetate	50.44
0.20	450	$C_{32}H_{66}$	Dotriacontane	51.54
4.63	306	$C_{20}H_{34}O_2$	Isopropyl-1,5,9-trimethyl-15-oxabicyclo[10.2.1]pentadeca-5,9-dien-2-ol	52.49
3.43	296	$C_{17}H_{28}O_4$	Nerolidol-epoxyacetate	53.11
19.98	282	$C_{18}H_{34}O_2$	Trans-13-Octadecenoic acid	53.82
1.46	284	$C_{18}H_{36}O_2$	Octadecanoic acid	54.41
3.33	394	$C_{29}H_{46}$	24-Norursa-3,12-diene	76.79
0.41	610	$C_{27}H_{30}O_{16}$	6,8-Di-C-á-Glucosylluteolin	77.50
0.23	268	$C_{17}H_{32}O_2$	7-Methyl-Z-tetradecen-1-ol acetate	50.44
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0.41	610	$C_{27}H_{30}O_{16}$	6,8-Di-C-á-Glucosylluteolin	77.50

The argan oil showed varying levels of antimicrobial activity against different microbes, with inhibition zones ranging from 15 mm to 23.5 mm (Table 2 and Fig. 2). The largest inhibition zones were observed against *B. subtilis* (23.0 mm) and *C. albicans* (22.2 mm), with the lowest MIC values (31.2 µg/mL), indicating high susceptibility. Their corresponding MBC/MFC values (62.5 µg/mL) confirm strong bactericidal/fungicidal potential. In contrast, the smallest zone was found against *S. aureus* (15.2 mm). *S. typhi* showed weak inhibition (15.8 mm) and required higher MIC (125 µg/mL) and MBC (500 µg/mL) values, suggesting reduced effectiveness of the oil against this strain. The standard drugs caused measurable zones of inhibition, mostly in the range of 15.6 to 21.2 mm. *A. niger* was not inhibited by the oil, while it was inhibited by the standard (17.8 mm). For a comparison with standard drugs, some microbes like *B. subtilis* and *C. albicans*, the oil's performance was close to the standard, indicating it could be a promising alternative or complementary agent. In contrast, with *K. pneumoniae*, *S. aureus*, and *S. typhi*, the oil showed moderate inhibition, which, while lower than the standard, still indicates potential for therapeutic use. The antimicrobial activity results reflect the action of trans-octadecenoic acid, eucalyptol, nerolidol epoxyacetate, fatty acid esters, and phenolic alcohols like isoeugenol. The antimicrobial results observed in the current study align with previously reported findings on the biological activity of *Argania spinosa* derivatives. While the current study's results showed notable inhibition zones and low MIC values—particularly against *B. subtilis* and *C. albicans*—the antimicrobial efficacy of oil is known to vary depending on the microbial strain. For example, Lall *et al.* (2019) reported a relatively high MIC value of 500 µg/mL against *C. acnes*, while no inhibitory effect was observed against *P. intermedia*, even at concentrations as high as 12.5 mg/mL, indicating limited activity against certain anaerobic bacteria. Moreover, the antimicrobial spectrum of argan oil has been further supported by the work of Nafis *et al.* (2021), who demonstrated its inhibitory action against both Gram-positive and Gram-negative bacteria, including *B. subtilis*, *S. aureus*, and *Escherichia coli*. These findings reinforce the results of the present study, in which argan oil showed selective but significant inhibition, particularly against Gram-positive bacteria and *Candida*, while showing no activity against *A. niger*. Such variability is likely due to differences in oil composition, extraction methods, and microbial susceptibility.

Table 2. Antimicrobial Activity of Argan Oil (AO) Compared with Standard Antibiotics or Antifungals (AB/AF), Against Tested Microorganisms

Tested Microbes	Zone of Inhibition (mm)		MIC (µg/mL)	MBC/MFC (µg/mL)
	Argan Oil	Standard	Argan Oil	
B. subtilis (ATCC 6633)	23.00 ± 0.5	21.17 ± 0.29	31.25	62.5
S. aureus (ATCC 6538)	15.17 ± 0.29	17.17 ± 1.04	62.5	62.5
K. pneumoniae (ATCC13883)	17.00 ± 2.0	15.87 ± 0.23	62.5	125
S. typhi (ATCC 6539)	15.83 ± 0.29	16.00 ± 1.00	125	500
C. albicans (ATCC 10221)	22.17 ± 0.29	20.50 ± 0.5	31.25	62.5
A. niger (ATCC 16888)	NA	17.83 ± 0.29	…..	…….

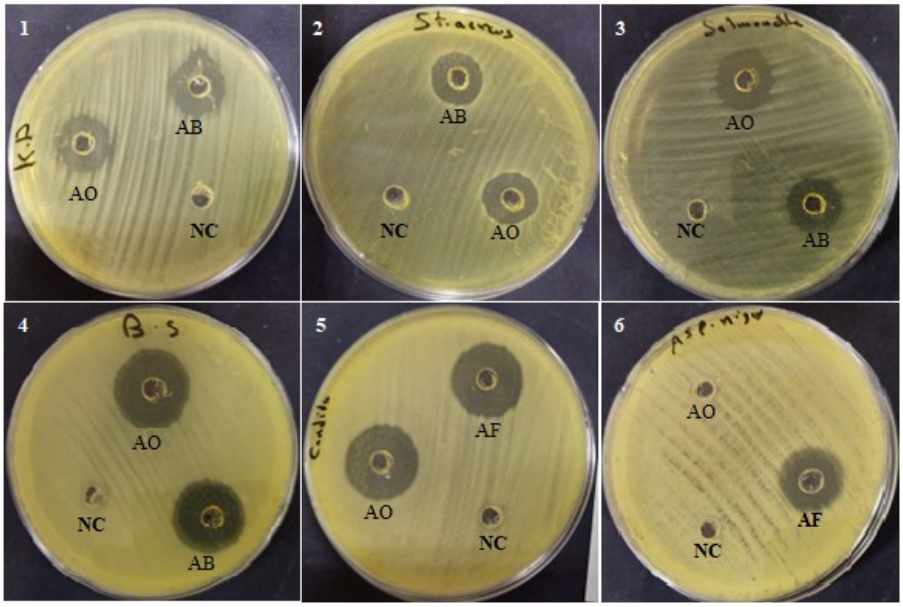

Fig. 2. Antimicrobial activity of argan oil (AO) compared with standard antibiotics or antifungals (AB/AF), and negative control (NC) against tested microorganisms: (1) *K. pneumoniae*, (2) *S. aureus*, (3) *S. typhi*, (4) *B. subtilis*, (5) *C. albicans*, and (6) *A. niger*

Inhibition of α-amylase and α-glucosidase by acarbose (a known antidiabetic drug) and argan oil across a range of concentrations (0 to 1000 µg/mL) is reported in Table 3. The values are reported as means ± standard deviation. At the bottom, IC_{50} values (the concentration required to inhibit 50% of enzyme activity) are also listed. Both acarbose and argan oil exhibited dose-dependent inhibition of α-amylase and α-glucosidase. Inhibition increased steadily with increasing concentration for both agents. Across all concentrations, acarbose consistently exhibited higher inhibitory activity than argan oil. For example, at 31.2 µg/mL, acarbose inhibited glucosidase by 76.4%, while argan oil showed 52.3% inhibition. The values of IC_{50} (α-amylase: 15.1 µg/mL, α-glucosidase: 26.2 µg/mL) confirm that argan oil is potent as an antidiabetic agent. Although IC_{50} of argan oil was found to be higher than acarbose (α-amylase: 3.07 µg/mL, α-glucosidase: 2.84 µg/mL), its inhibitory activity was notable and suggests it could be a natural alternative or adjunct in managing postprandial hyperglycemia. Argan oil's potent inhibition of these enzymes highlights its potential role in functional foods or nutraceuticals targeting type 2 diabetes. Its dual inhibition (both amylase and glucosidase) may help slow carbohydrate digestion and glucose absorption, reducing blood sugar spikes after meals. The antidiabetic potential of argan oil was assessed in a study by El Adib *et al.* (2015), which demonstrated that the methanolic fraction of argan oil effectively inhibited α-amylase activity. Likewise, Daoudi *et al.* (2020) confirmed the antidiabetic potential of argan oil through its inhibitory effects on both α-glucosidase and α-amylase enzymes. A recent study by Alaoui *et al.* (2024) demonstrated the hypoglycemic potential of argan fruit pulp, reporting effective α-amylase and α-glucosidase inhibitory activity, which are crucial targets for controlling postprandial hyperglycemia. These findings align with earlier research by Kamal *et al.* (2021), who highlighted the antidiabetic efficacy of bioactive fractions from *Argania spinosa*,

particularly saponin-rich extracts and argan oil. In their work, the saponin fraction exhibited more potent activity than conventional inhibitors, suggesting a synergistic or enhanced mechanism of action. Considering the current chemical profile, the presence of phenolic structures and fatty acid esters in our argan oil sample may also account for similar enzyme-inhibitory activities, supporting its potential role as a natural antidiabetic agent.

Table 3. Antidiabetic of Argan Oil *Via* Inhibition of α-Amylase and α-Glucosidase Compared to Acarbose

Concentration (µg/mL)	α-Amylase Inhibition		α-Glucosidase Inhibition	
	Acarbose	Argan oil	Acarbose	Argan oil
0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1.95	43.0 ± 1.8	24.2 ± 1.0	40.4 ± 0.2	17.9 ± 2.0
3.9	52.47 ± 0.40	32.40 ± 1.74	50.87 ± 1.0	25.30 ± 2.65
7.81	58.43 ± 0.12	42.30 ± 2.20	60.17 ± 0.12	35.27 ± 1.51
15.62	64.47 ± 0.32	50.80 ± 1.05	68.90 ± 1.73	43.70 ± 2.0
31.25	72.07 ± 0.29	58.33 ± 1.01	76.40 ± 2.65	52.30 ± 1.0
62.5	79.40 ± 0.36	68.60 ± 0.52	83.20 ± 2.00	61.20 ± 0.85
125	87.20 ± 0.35	78.50 ± 0.40	89.80 ± 0.17	70.20 ± 2.05
250	91.70 ± 1.06	85.00 ± 0.50	92.23 ± 0.23	79.00 ± 1.0
500	96.33 ± 1.08	92.00 ± 2.00	96.50 ± 0.30	87.90 ± 1.73
1000	98.20 ± 0.70	95.47 ± 1.31	98.20 ± 0.20	93.40 ± 2.03
IC_{50} µg/mL	3.07 ± 0.15	15.08 ± 0.02	2.84 ± 0.03	26.16 ± 0.02

Argan oil showed a dose-dependent increase in BuChE inhibition, though much weaker at low concentrations (Table 4), where the inhibition increased from 6.13% to 80.9%. Slight increases in the inhibition of BuChE were observed up to 1.56 µg/mL, while a large increase from 23.6% at 3.12 µg/mL to 80.9% inhibition at 100 µg/mL was recorded. Despite a weaker initial effect, argan oil exhibited notable BuChE inhibition at higher doses, suggesting potential anti-Alzheimer activity. Compared to Rivastigmine, inhibition increased steadily with concentration, starting at 37.7% (0.195 µg/mL) and reaching 94.1% at 100 µg/mL.

Table 4. Anti Alzheimer Potential of Argan Oil *via* Butylcholinesterase (BuChE) Inhibition Compared to Rivastigmine

Concentration (µg/mL)	BuChE Inhibition	
	Rivastigmine	Argan Oil
0.0	0.0 ± 0.0	0.0 ± 0.0
0.195	37.7 ± 0.35	6.13 ± 0.06
0.39	46.5 ± 0.36	9.70 ± 0.17
0.78	54.2 ± 1.15	11.20 ± 0.10
1.56	59.0 ± 0.20	14.60 ± 0.44
3.125	66.6 ± 0.30	23.60 ± 2.0
6.25	72.8 ± 0.10	38.37 ± 0.46
12.5	77.5 ± 0.40	44.43 ± 0.06
25	85.4 ± 1.30	51.57 ± 0.15
50	90.3 ± 0.20	65.80 ± 0.17
100	94.1 ± 0.17	80.90 ± 1.0
IC_{50} µg/mL	0.58 ± 0.10	15.93 ± 0.66

The consistent rise and IC_{50} (0.58 ± 0.10 µg/mL) confirm strong, dose-dependent BuChE inhibition by Rivastigmine. The IC_{50} (15.93 ± 0.66 µg/mL) of argan oil indicated its ability to inhibit BuChE activity. The inhibition of cholinesterase enzymes has been demonstrated to improve neurotransmission mechanisms in individuals with Alzheimer's disease. To support the current study's findings, El Mostafi *et al.* (2020) suggest that an argan oil–enriched diet may offer neuroprotective effects against the development of certain neurological and neuropsychiatric aspects of mental disorders.

The DPPH assay results showed that argan oil exhibited dose-dependent antioxidant activity, with increasing concentrations leading to progressively higher free radical scavenging compared to standard drug (ascorbic acid) (Table 5). At the lowest concentration (1.95 µg/mL), ascorbic acid exhibited a mean scavenging activity of 45.53% ± 1.00, while argan oil demonstrated 39.07% ± 1.10, indicating a lower initial antioxidant potential. As concentrations increased, the scavenging activities of both samples rose substantially. At intermediate doses (31.2 to 125 µg/mL), ascorbic acid maintained stronger activity than argan oil. For instance, at 62.5 µg/mL, ascorbic acid achieved 82.90% ± 0.52, while argan oil reached 77.00% ± 1.73. Despite this, the gap narrowed with increasing dose. It is worth mentioning that at higher concentrations (250 to 1000 µg/mL), argan oil showed comparable or even slightly higher scavenging than ascorbic acid. For instance, at 500 µg/mL, argan oil had a mean of 95.83% ± 0.12, compared to 93.57% ± 0.25 for ascorbic acid. At 1000 µg/mL, both approached complete scavenging activity, with argan oil at 97.70% ± 1.00 and ascorbic acid at 96.20% ± 1.00. The IC_{50} values of ascorbic acid was 2.31 ± 0.01 µg/mL, while argan oil was 4.97 ± 0.21 µg/mL. This shows that ascorbic acid is approximately twice as potent as argan oil, requiring a lower dose to achieve 50% inhibition of free radicals. However, argan oil's IC_{50} value still reflects strong antioxidant potential, particularly considering it is a complex natural extract rather than a pure compound.

The results clearly demonstrate that argan oil exhibited a dose-dependent inhibition of pancreatic lipase activity in parallel with orlistat (Table 5). However, the magnitude of inhibition differed notably between the two, with orlistat showing a markedly higher potency at all tested concentrations. At lower concentration 1.95 µg/mL, orlistat achieved around 40% inhibition, while argan oil induced only about 21%, indicating that orlistat is nearly twice as effective at this level. This pattern persisted across all concentrations, though the gap between their activities began to narrow at higher doses. For instance, at 1000 µg/mL, orlistat reached nearly 98% inhibition, while argan oil achieved 88%, suggesting that argan oil did approach full inhibition but required much higher concentrations to approach that level of inhibition. The IC_{50} values of orlistat and argan oil were 4.23 ± 0.03 and 25.10 ± 0.17 µg/mL, respectively. Despite the IC_{50} value of argan oil being higher than orlistat, argan oil exhibited promising inhibitory activity and maintained a consistent upward trend with increasing concentrations. Given its natural origin and likely lower toxicity compared to synthetic drugs such as orlistat, argan oil may hold value as a complementary or alternative agent in the management of obesity or lipid metabolism disorders. Its effectiveness, although inferior to orlistat, may still be clinically relevant, especially in long-term or preventive applications where synthetic drug use is limited due to side effects. Argan oil is naturally rich in powerful antioxidants, such as vitamin E, sterols, and phenolic compounds, which play a vital role in protecting the body against harmful agents (Bouchab *et al.* 2023). These antioxidant properties contribute to the

remarkable health benefits of argan oil. According to an earlier study, Drissi *et al.* (2004) reported that argan oil lowers LDL cholesterol and possesses antioxidant activities. These effects suggested that argan oil may serve as a natural dietary option for reducing cardiovascular risk. The antioxidant activity of argan oil may be attributed to its bioactive compounds. Abdalla *et al.* (2024) reported that argan oil contains numerous antioxidants, vitamin E, and essential fatty acids.

Table 5. Antioxidant Effect *via* DPPH Scavenging and Anti-obesity Effect *via* Lipase Inhibition of Argan Oil at Different Concentration Compared with Standard Compounds

Concentration (µg/mL)	Antioxidant *via* DPPH Scavenging (%)		Anti-obesity *via* Lipase Inhibition	
	Ascorbic Acid	Argan Oil	Orlistat	Argan oil
0.0	0.0 ±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1.95	45.53 ± 1.00	39.07 ± 1.10	40.0 ± 2.0	21.1 ± 1.25
3.9	52.00 ± 0.26	46.57 ± 1.15	47.73 ± 0.15	28.0 ± 2.0
7.81	59.03 ± 0.68	54.43 ± 0.06	55.80 ± 1.0	35.7 ± 1.0
15.62	67.50 ± 0.87	61.50 ± 0.90	63.97 ± 0.21	44.5 ± 1.11
31.25	76.13 ± 1.05	69.27 ± 0.21	70.57 ± 0.31	54.3 ± 0.44
62.5	82.90 ± 0.52	77.00 ± 1.73	77.70 ± 0.40	62.0 ± 1.0
125	88.77 ± 1.11	84.50 ± 1.95	85.30 ± 1.05	70.4 ± 1.0
250	93.40 ± 0.10	92.20 ± 0.00	91.90 ± 1.40	78.8 ± 0.95
500	93.57 ± 0.25	95.83 ± 0.12	94.90 ± 3.0	80.1 ± 2.00
1000	96.20 ± 1.00	97.70 ± 1.00	97.70 ± 0.53	87.6 ± 0.44
IC_{50} µg/mL	2.31 ± 0.01	4.97 ± 0.21	4.23 ± 0.03	25.10 ± 0.17

The data show a clear dose-response relationship, where as the concentration of argan oil increased, cell viability decreased, indicating increased toxicity. At the lowest tested concentration (31.2 µg/mL), toxicity already exceeded 40%, indicating moderate cytotoxic activity at low doses. Maximal toxicity (~97%) was reached between 250 and 1000 µg/mL, suggesting a plateau effect at high doses, where increasing concentration does not significantly increase toxicity further. Tukey's *post hoc* test assigned different significance letters (a through g) to each concentration group, confirming that each increase in dose caused a statistically significant increase in cytotoxicity. This relatively low IC_{50} 36.17 ± 1.04 µg/mL indicates strong cytotoxic potential of argan oil on Caco-2 cells. In the context of cancer research, a lower IC_{50} in the range of 10 to 100 µg/mL is often considered indicative of promising anti-cancer or anti-proliferative activity. Figure 3 indicates that control cells appear elongated, attached, and confluent. There is no visible damage or rounding, but clear cell boundaries and spreading, indicating normal viability. The micrographs confirm the dose-dependent cytotoxic effect of argan oil on Caco-2 cells: Low concentrations (≤ 31.25 µg/mL): Mild changes, partial loss of adherence; Moderate concentrations (62.5 to 125 µg/mL): Major morphological alterations, rounding, and detachment; High concentrations (≥ 250 µg/mL): Near-complete cell death, minimal viable cells observed. Argan oil is widely recognized for its health-promoting properties, largely attributed to its abundance of bioactive compounds, including unsaturated fatty acids and tocopherols. In particular, it contains high levels of linoleic and oleic acids. Researchers have proposed several pathways through which these unsaturated fatty acids may exert

their antiproliferative effects. One of the anticancer effects of argan oil is its ability to reduce the production of eicosanoids derived from arachidonic acid, which may help inhibit the growth of cancer cells. Moreover, unsaturated fatty acids are known to trigger specific alterations in gene expression. For instance, oleic acid can downregulate the overexpression of HER2 (erbB-2), a prominent oncogene involved in the initiation, aggressive progression, and metastasis of several human cancers. Carrillo *et al.* (2012) reported that unsaturated fatty acids are well recognized for their capacity to trigger apoptosis in a range of cell lines. A central mechanism in this process involves the disruption of mitochondrial membrane potential, which results in mitochondrial dysfunction and increased production of reactive oxygen species (ROS). The elevated levels of ROS are closely associated with the initiation of apoptotic cell death in various cancer cell types. Polyphenols found in argan oil have been shown to inhibit the proliferation of prostate cancer cell lines, including DU145, LNCaP, and PC3 (Bennani *et al.* 2007).

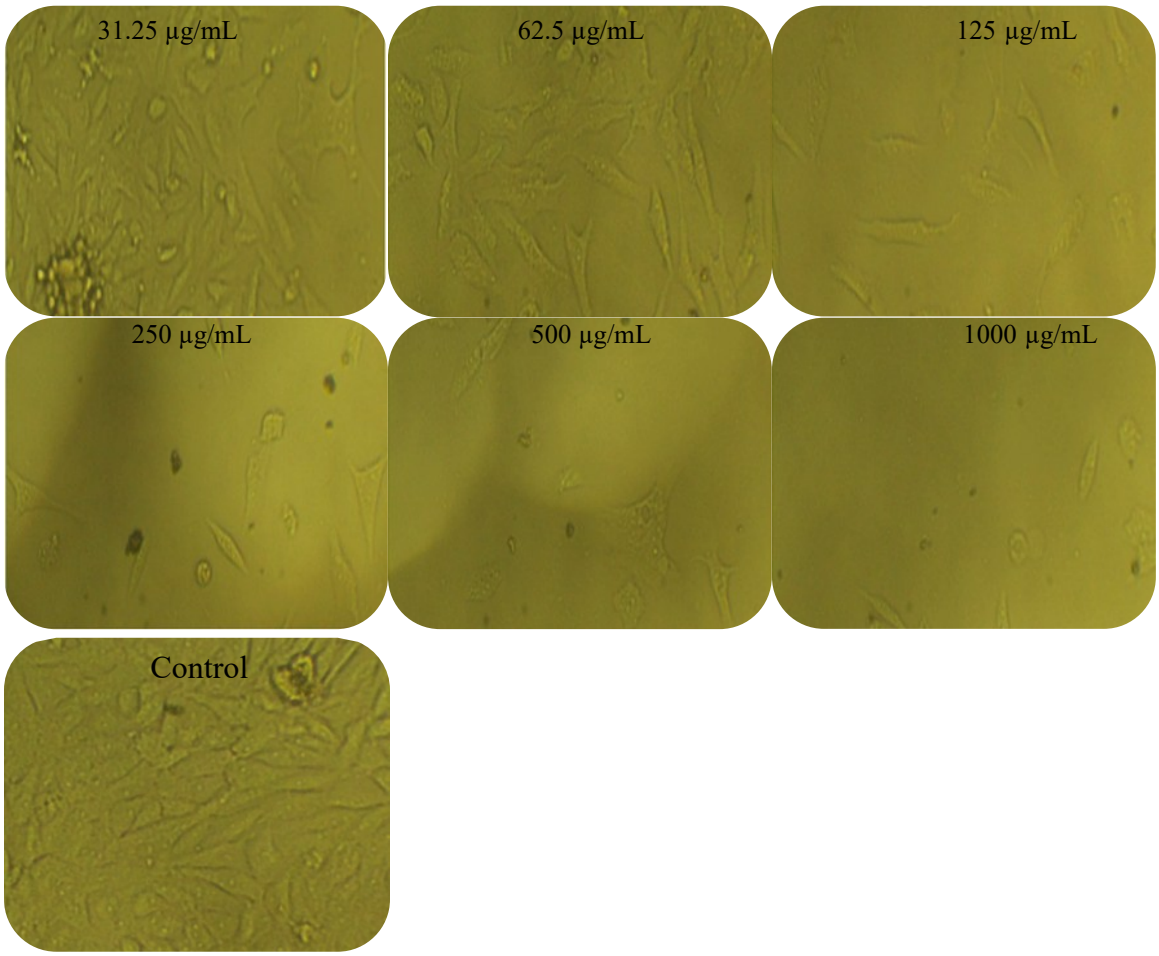

Fig. 3. Morphological changes in Caco-2 cells after treatment with argan oil

Table 6. Cytotoxic Effect of Argan Oil on Caco-2 Cells at Various Concentrations

Concentration (µg/mL)	Mean Toxicity (%) ± SD
0.00	0.00 ± 0.00
31.25	42.87 ± 1.14
62.50	77.59 ± 0.57
125	91.34 ± 1.02
250	96.73 ± 0.07
500	97.05 ± 0.04
1000	97.17 ± 0.06

CONCLUSIONS

1. Argan oil contains a diverse range of bioactive compounds—especially fatty acids, terpenoids, and phenolics—that contribute to its strong antimicrobial activity strains.
2. Argan oil showed notable antioxidant capacity through DPPH scavenging and anti-obesity *via* lipase inhibition, with performance improving at higher concentrations, making it a promising natural therapeutic and food additive agent.
3. Argan oil demonstrates dose-dependent cytotoxicity against Caco-2 cells, with a low IC_{50} value, highlighting its potential for further exploration in anticancer applications.
4. The bioactive properties reported here are indeed noteworthy, but further in vivo studies and pathway analyses are required to substantiate their therapeutic potential for clinical applications

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