Phytochemicals and Bioactivities of Vinca (*Catharanthus roseus*) Leaf Extracts: Potential Roles in Lipid Oxidation Inhibition and Atherosclerosis Prevention *InVitro*

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ABSTRACT

This treatise strive to investigate the bioactive compounds and biological activities of Vinca (*Catharanthus roseus*) leaves grown in Egypt, focusing on their potential to inhibit lipid oxidation and protect against atherosclerosis *in vitro*. Nutritional analysis of the dried leaf powder revealed a well-balanced composition rich in protein, fat, fiber, and carbohydrates, supporting its use as a functional food. Extraction yields varied significantly by solvent, with 80% ethanol providing the highest yield, underscoring the role of solvent polarity in phytochemical extraction. Ethanol (VLEE) and methanol (VLME) extracts contained higher levels of phenolics, flavonoids, lycopene, tannins, terpenoids, and kaempferol, while the aqueous extract (VLAqE) showed higher levels of anthocyanins, alkaloids, triterpenoids, and saponins. VLEE exhibited the highest antioxidant activity (90.04%), followed by VLME (87.12%) and VLAqE (71.44%), with the lowest oxidation rate ratio (ORR = 0.099). In hydroxyl radical scavenging assays, VLEE again led with the lowest IC_{50} (39.21 µg/mL), compared to VLME (52.64 µg/mL), VLAqE (85.40 µg/mL), and caffeic acid (61.88 µg/mL). Antimicrobial testing confirmed VLEE's superior inhibition zones against *E. coli*, *S. aureus*, *Streptococcus* spp., *Salmonella* spp., and *Candida albicans*. In the β-carotene-linoleate model system, both VLEE and VLME at 50 and 100 ppm significantly delayed β-carotene degradation, with results comparable to BHT, while VLAqE showed weaker effects. These outcomes suggest ethanol and methanol are more efficient in extracting bioactive, low-polarity compounds such as flavonoids and polyphenols. In conclusion, Vinca leaf extracts, particularly those obtained with ethanol, exhibit strong antioxidant and antimicrobial properties, making them promising candidates for use in food preservation and nutraceutical development aimed at combating oxidative stress and atherosclerosis.

Keywords: chemical composition, extractive value, bioactive compounds, antioxidant activity, hydroxyl radical scavenging activity, antimicrobial activity.

INTRODUCTION

Cardiovascular diseases (CVDs), particularly atherosclerosis, remain the leading cause of mortality worldwide, with the World Health Organization (WHO) estimating they account for 32% of global deaths. In Egypt, coronary heart disease alone contributes to 32.4% of all deaths, placing the country among those with the highest CVD-related mortality rates globally (WHO, 2023). While conventional pharmacological interventions-such as statins, fibrates, and calcium channel blockers—have proven effective in managing CVDs, their use is often associated with undesirable side effects, including muscle pain, liver dysfunction, gastrointestinal issues, and a growing peril of rhabdomyolysis (Shah *et al.*, 2022). As a result, there has been a growing shift toward alternative and complementary medicine in regions such as Egypt. In these areas, medicinal herbs-such as garlic, ginger, hawthorn, chamomile, and cinnamon-are increasingly used due to their perceived lower risk of side effects and potential cardiovascular benefits (Yeh *et al.*, 2006). Nevertheless, patients need to consult healthcare professionals before incorporating such remedies, as scientific validation of their safety and efficacy is still ongoing. Ultimately, integrating evidence-based natural therapies with conventional medicine may enhance cardiovascular care while reducing adverse outcomes. In addition to improving cardiovascular health, strengthening pharmaceutical security and reducing dependence on imported medications is also vital. Among medicinal plants, Vinca (*Catharanthus roseus*) stands out as a high-value option. It is a fast-growing, resilient plant that can thrive in a wide range of climates and requires only moderate cultivation inputs, making it particularly suitable for regions facing medicine shortages.

Vinca (*Catharanthus roseus*) is a perennial herbaceous plant that belongs to the *Apocynaceae*

DOI: 10.21608/asejaiqjsae.2025.434600
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Received, May 20, 2025, Accepted, June 22, 2025.

family. Originally native to Madagascar, it is now widely cultivated in tropical and subtropical regions worldwide, including Egypt. This plant is recognized for its glossy green, opposite leaves and funnel-shaped flowers, which can be white, pink, or purple in color. Vinca thrives in well-drained soils and is not only used ornamentally but also holds significant medicinal value (Chaturvedi *et al.,* 2022). The leaves of Vinca are particularly rich in various chemical constituents, including alkaloids, phenolics, flavonoids, and fatty acids. A recent study highlighted the presence of potent alkaloids, such as vincristine and vinblastine, which are well-known for their anticancer properties. In addition to these alkaloids, compounds like vindoline, vindolicine, and vindolinine are found in the leaves and have shown potential in treating diabetes and conditions related to oxidative stress (Malhotra *et al.,* 2024). The leaves also contain phenolic compounds such as flavonol glycosides and caffeoylquinic acids, which are well-documented for their antioxidant properties. These compounds together contribute to the diverse pharmacological activities of the plant (Mendonce *et al.,* 2025).

The bioactive compounds present in Vinca exhibit a wide array of biological activities, particularly in the context of cancer treatment. Vincristine and vinblastine, two key alkaloids found in the plant, are renowned for their ability to inhibit cancer cell proliferation and are integral components of chemotherapy regimens for treating cancers such as leukemia and lymphoma (Mendonce *et al.,* 2025). Additionally, alkaloids like vindoline and vindolicine enhance glucose uptake in cells and inhibit key enzymes involved in insulin signaling, offering potential therapeutic benefits for the management of diabetes (Tiong *et al.,* 2013). Moreover, phenolic compounds in Vinca leaves significantly contribute to free radical scavenging, thereby reducing oxidative stress and potentially lowering the risk of chronic diseases like cardiovascular and neurodegenerative disorders. Additionally, Vinca extracts have shown antibacterial and antifungal properties, indicating their potential in treating various infections (Raza *et al.,* 2009). Furthermore, the plant's compounds exhibit anti-inflammatory effects by modulating inflammatory pathways, providing potential therapeutic relief in conditions characterized by chronic inflammation (Mendonce *et al.,* 2025).

In traditional medicine, Vinca has been widely used for its therapeutic properties. The leaves of the plant are consumed in various forms to manage conditions such as diabetes, hypertension, and infections (Sharma *et al.,* 2024). The bioactive alkaloids, particularly vincristine and vinblastine, have been integral in chemotherapy regimens, playing a crucial role in cancer treatment (Goswami *et al.,* 2024). Furthermore, the plant's antioxidant and anti-inflammatory properties participate to overall health and well-being (Hashim *et al.,* 2024). Despite these known benefits, there remains a significant need for further research to fully understand its therapeutic potential. Future studies should focus on standardization of plant extracts, clinical trials to confirm therapeutic efficacy, bioavailability studies, and formulation development (Khan *et al.,* 2024). Addressing these areas could position *Catharanthus roseus* as a valuable component in therapeutic nutrition, offering natural alternatives for managing various health conditions. Consequently, this aim at pursuits to analyze the bioactive compounds and biological sports of Vinca leaves cultivated in Egypt, with a focal point on their ability outcomes on lipid oxidation and in *vitro* protection in opposition to atherosclerosis.

MATERIALS AND METHODS

1. Materials

1.1. Vinca (*Catharanthus roseus* L.)

The Vinca (*Catharanthus roseus*) plants were obtained as a donation from Faculty of Science, Menoufia University, Shebin El-Kom, Egypt. The authenticity of the plant samples was confirmed by the plant taxonomy department at the Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt.

1.2. Chemicals

The bioactive compound standards, including gallic acid (GA), catechin (CA), α-tocopherol, linalool, ursolic acid, butylated hydroxytoluene (BHT), $CuSO_4$, dimethyl sulfoxide (DMSO), trichloroacetic acid (TCA), EDTA and 2-deoxy-d-ribose were sourced from Sigma Chemical Co., St. Louis, MO. Folin-Ciocalteus's phenol reagent was obtained from Merck (Dam-stadt, Germany). All other chemicals, reagents, and solvents, unless specified otherwise, were of analytical grade and were obtained from El-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Instruments, Cairo, Egypt.

1.3. Machines

In this treatise, absorbance for various assays was recorded using a UV-160A spectrophotometer from Shimadzu Corporation, Kyoto, Japan. The entirety nitrogen content was assessed utilizing a Micro-Kjeldahl semi-automatic apparatus from Velp, Italy. Additionally, the crude fat determination was carried out using a Soxhlet semi-automatic apparatus, also from Velp, Italy.

1.4. Biological model for atherosclerosis assay

Adult male albino rats, each weighing approximately 180±15g, were obtained from the Laboratory Animal Colony at the Vaccine and Immunity Organization in Cairo, Egypt. The rats were housed and maintained

under standard, healthy conditions, adhering to the guidelines set by the National Research Council's Institute of Laboratory Animal Resources, Commission on Life Sciences (NRC, 1996). The basal diet (BD) for the rats was formulated based on the recipe provided by Reeves *et al.* (1993).

2. Methods

2.1. Elaboration of Vinca (*Catharanthus roseus* L.) leaves powder

After the Vinca (*Catharanthus roseus* L.) plants were brought to the experimenter, the leaves were manually removed and sorted to discard the damaged and crushed ones. They were then washed with water and dried using paper towels. Vinca leaves samples were dehydrated in a warm air furnace (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 70°C for three hours. After drying, the dried leaves were ground into a fine powder using a high-speed mixer (Moulinex Egypt, ElAraby Co., Benha, Egypt). The powder that passed through a 40-mesh sieve was collected, placed in polyethylene bags, and stocked, in a refrigerator at 4°C for use in subsequent experiments.

2.2. Elaboration of Vinca (*Catharanthus roseus* L.) extracts

A 20 g of dried Vinca (*Catharanthus roseus* L.) leaf powder were mixed with 180 mL of water and homogenized. The mixture was then placed in a beaker and agitated at 200 rpm for 1 hour at room temperature using an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany). Afterward, the extract was filtered through Whatman No. 1 filter paper to separate the liquid from the solid residue. The remaining residue was subjected to two additional extractions, and the resulting extracts were combined. The solvent was then removed using a rotary evaporator at 50°C under reduced pressure (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). This extraction process was repeated using 80% hydro-methanol or ethanol as solvents (20% water + 80% organic solvent), petroleum ether, hexane and chloroform and the residual solvent was removed under reduced pressure at 40°C.

2.3. Proximate composition analysis

Vinca (*Catharanthus roseus* L.) leaf samples were analyzed for their chemical composition, including moisture, protein (calculated as total nitrogen × 6.25, using the micro-Kjeldahl method with a semiautomatic apparatus from Velp, Italy), fat (determined using a Soxhlet apparatus from Velp, Italy, with petroleum ether as the solvent), ash and fiber contents, following the methods outlined by A.O.A.C. (1995). The carbohydrate content was calculated by subtracting the percentage of moisture, protein, fat, ash, and fiber from 100%, as shown in the formula: Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % ash). The total energy (Kcal/100 g) of GCS samples was determined following the method outlined by Insel *et al.* (2002), using the equation: Total energy (Kcal/100 g) = 4 × (Protein % + Carbohydrates %) + 9 × (Fat %).

2.4. Bioactive compounds determination

The total phenolic content in Vinca (*Catharanthus roseus*) leaf extracts was quantified using the Folin-Ciocalteu reagent, as described by Singleton & Rossi (1965) and Wolfe *et al.* (2003), with results expressed as gallic acid equivalents (GAE). The total carotenoid content in the 80% acetone extract was measured using the method outlined by Litchenthaler (1987) and expressed as micrograms of carotenoid per gram of dry extract. Total flavonoids were estimated using the colorimetric method of Zhishen *et al.* (1999), with results expressed as catechin equivalents (CAE), calculated from the standard curve ($y = 0.0003x - 0.0117$, $r^2 = 0.9827$), in milligrams of catechin per gram of dry extract. The total anthocyanin content was determined following the procedure of Sharif *et al.* (2010) and expressed as milligrams of cyanidin-3,5-diglucoside per 100 grams. Lycopene was quantified using colorimetric methods that measure its absorbance at specific wavelengths according to the method described by Anthon and Barrett (2007). Kaempherol was determined using the colorimetric method of Marín *et al.* (2019). Polysaccharides were extracted and quantified following the procedure of Vazirian *et al.* (2014), using starch as the standard and the results were expressed as milligrams of starch equivalents per gram of dry weight (dw). Total terpenoids were extracted and measured as per the method of Ghorai *et al.* (2012), with linalool used as a standard and results presented as milligrams of linalool equivalents per gram of dry weight. Total triterpenoids were assessed according to Schneider *et al.* (2009), using ursolic acid as the standard, with results expressed in milligrams of ursolic acid per 100 grams. Tannin content was determined using the method of Van-Burden and Robinson (1981), with gallic acid (GA) as the standard for constructing the calibration curve to estimate tannin levels. Total alkaloids were determined using the method of Zhao and Wang (2010), with atropine serving as the standard for constructing the calibration curve, from which the alkaloid content was estimated. Finally, total saponin content was determined colorimetric using oleanolic acid as a standard according to the method of Le Bot *et al.* (2022).

2.5. Antioxidant activities

2.5.1. Antioxidant activity (AA)

The antioxidant activity (AA) of Vinca leaf extracts along with standards such as α-tocopherol and BHT, was evaluated using the BCB assay, with modifications based on the procedure outlined by Marco (1968). In a typical assay, 1 mL of a β-carotene solution (0.2 mg/mL in chloroform) was added to 50 mL round-bottom flasks, along with 0.02 mL of linoleic acid and 0.2 mL of Tween 20. The mixtures were then treated with 0.2 mL of 80% methanol (control) or the corresponding plant extract or standard. After evaporating the solvent under vacuum at room temperature, 50 mL of oxygenated distilled water was added, and the solution was shaken to form a liposome suspension. The samples were subjected to thermal auto-oxidation at 50°C for 2 hours. The absorbance of the solution at 470 nm was recorded using a Beckman DU-50 spectrophotometer at 10-minute intervals, and the rate of *β*-carotene bleaching was determined by performing linear regression analysis on the data over time. Each sample was tested in triplicate. BHT and α-tocopherol in 80% methanol served as controls. Antioxidant activity was calculated using four different methods: 1) plotting the absorbance against time to create a curve, with the absolute value of the slope representing the antioxidant value (AOX) as per Al-Saikhan *et al.* (1995); 2) calculating the antioxidant activity (AA) as the percentage inhibition relative to the control using the equation $AA = [(R_{control} - R_{sample}) / R_{control}] \times 100$, where R control and R sample are the bleaching rates of β-carotene in the reaction mixtures without and with the plant extract, respectively; 3) calculating the oxidation rate ratio (ORR) as per Marinova *et al.* (1994) using the equation $ORR = R_{sample} / R_{control}$, where R_{sample} and $R_{control}$ are as described previously; and 4) determining the antioxidant activity coefficient (AAC) based on Mallet *et al.* (1994), calculated using the formula $AAC = [(AbsS_{120} - AbsC_{120}) / (AbsC_0 - AbsC_{120})] \times 100$, where $AbsS_{120}$ is the absorbance of the antioxidant mixture at 120 minutes, $AbsC_{120}$ is the absorbance of the control at 120 minutes, and $AbsC_0$ is the absorbance of the control at time zero.

2.5.2. Hydroxyl radical scavenging activity (HRSA) assay

HRSA of the Vinca leaf extracts were determined according to the method of Halliwell and Gutteridge (1989). The reaction mixture contained 0.8 mL of phosphate buffer solution (50 mmol L^{-1}, pH 7.4), 0.2 mL of extracts/standard at different concentrations (12.5–150 $\mu g.mL^{-1}$), 0.2 mL of $FeCl_3$ (1 mmol L^{-1}), 0.2 mL of EDTA (1.04 mmol L^{-1}) and 0.2 mL of 2-deoxy-d-ribose (28 mmol L^{-1}). The reaction mixtures were kept in a water bath at 37 ^{0}C and added 0.2 mL of H_2O_2 (10 mmol L^{-1}) plus 0.2 mL of ascorbic acid, AA (2 mmol L^{-1}). After one hour of incubation at 37 ^{0}C, 1.5 mL of TBA (10 g L^{-1}) was added to the reaction mixture followed by 1.5 mL of HCl (25 %). The mixture was heated at 100 ^{0}C for 15 min and then cooled down with water. The absorbance (Abs) at 532 nm (UV-160A; Shimadzu Corporation, Kyoto, Japan) was measured and the HRSA (%) was calculated according to the following formula:

$$HRSC\ (\%) = [Abs\ A_0 - (Abs\ A_1 - Abs\ A_2)] \times 100 / Abs\ A_0$$

Where: Abs A_0, the absorbance of the control without a sample, Abs A_1, the absorbance after adding the sample and 2-deoxy-D-ribose, Abs A_2, the absorbance of the sample without 2-deoxy-d-ribose. Then, the % of inhibition was plotted against concentration, and from the graph, IC_{50} was calculated.

2.6. Antibacterial and antifungal tests

The microbial strains used for evaluating antimicrobial activity included *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* spp., *Salmonella* spp., and *Candida albicans*, all sourced from the Microbiology Department's culture collection at the Faculty of Agriculture, Damietta University, Damietta, Egypt. The antibacterial and antifungal properties of *Catharanthus roseus* (Vinca) leaf extracts were assessed using the agar well diffusion method, following the protocol established by Spooner and Sykes (1979).

2.7. Anti-atherosclerotic effect of Vinca leaf extracts *in vitro* [Inhibition of low-density lipoprotein (LDL) oxidation]

Inhibition of LDL oxidation (Anti-atherosclerotic effect) *in vitro* for the Vinca leaf extracts was determined according to the method of Princen *et al.* (1992). Adult male white albino rat, Sprague Dawley strain, serum was collected and diluted by phosphate buffer (50 mM, pH 7.4) to the concentration of 0.6%. Quantities of 5.0 ml diluted serum were mixed with 10 µl DMSO or 10 µl DMSO containing various concentrations of the Vinca leaf extracts. A 20 µl of $CuSO_4$ solution (2.5 mM) was added to initiate the reaction and the absorbance at 234 nm was recorded then was taken every 20 min thereafter for 140 min at room temperature. The final result was expressed by calculating the net area under the curve.

2.8. Lipid Oxidation (β-Carotene-linoleate model system)

The antioxidant activity of Vinca leaf extracts in the β-carotene-linoleate model system was determined according to the method of Chandrasekara and Shahidi (2010). In brief, β-carotene oil-in-water emulsion was prepared by dissolving 1 mg of β-carotene in 10 mL of chloroform. The β-carotene solution (0.2 mL) was mixed with linoleic acid (20 mg) and Tween 40 (200

mg) and chloroform was removed under a nitrogen stream. Oxygenated distilled water (50 mL) was added to the mixture which was then homogenized (Velp Company Homogenizer, Italy) at a speed of 13,000 *g* for 1 min. The resulting emulsion was kept at room temperature (24-26 ^{0}C) in a dark place. Vinca leaf powder extracts dissolved in 50% (v/v) ethanol (500 µL) with different concentrations (500 and 1000 ppm) were mixed with prepared emulsion (4.5 mL) to obtain a final concentration of 50 and 100 ppm and the mixtures were incubated at 50^{0}C in a dark place. After the designated time (0, 20, 40, 80, 120, 160, 200, 240, 280, 320, 360 min), the mixture was randomly taken to measure the absorbance (Abs) at 470 nm. The control and reference/standard were prepared in the same manner, except that distilled water and BHT (50 and 100 ppm) were used instead of the samples. The lower decrease in the Abs represented the ability to prevent oxidation of the system.

3. Statistical Analysis

All experiments were carried out in triplicate. Data were subjected to the analysis of variance (ANOVA) and mean comparisons were performed using Duncan's multiple range test (Steel and Torrie, 1980). Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS for Windows: SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

1. Proximate composition of Vinca leaves powder

The proximate composition of *Vinca (Catharanthus roseus*) leaves powder, as shown in Table (1), presents a nutrient-rich profile, indicating its promising potential as a dietary supplement or functional food ingredient. Data obtained from triplicate analyses revealed balanced levels of moisture, macronutrients, and energy content, with consistency observed across related studies, although variability may arise due to genetic, environmental, and methodological factors. The moisture content, ranging from 6.98 to 8.41 g/100g FW (mean 7.75 ± 0.73), is relatively low, contributing to longer shelf life and product stability, which is comparable to moisture levels observed in other leafy medicinal plants like *Moringa oleifera* and *Ocimum gratissimum* (Oduro *et al.*, 2008 and Oboh *et al.*, 2009), confirming effective drying techniques. The protein content (7.92 ± 0.55 g/100g FW) is also noteworthy and aligns closely with values reported by Kannan *et al.* (2020) for *C. roseus* varieties from Poland and India, further supporting its traditional role in enhancing metabolic functions and immunity. In comparison with other leafy vegetables like *Amaranthus hybridus* (Akaneme and Ani, 2013), Vinca exhibits comparable protein quality. The fat content (4.98 ± 0.31 g/100g FW), and this may enhance both palatability and caloric density, consistent with findings by Pandey *et al.* (2006) on the lipid content of medicinal plants. Ash content (6.28 ± 0.47 g/100g FW), representing total mineral content, is also in line with findings by Prakash and Gupta (2009), suggesting a rich profile of essential macro- and micronutrients vital for enzymatic and physiological functions. The predominant component, carbohydrate (73.07 ± 2.11 g/100g FW), contributes substantially to the total energy value of 328 Kcal/100g, echoing findings by Chaturvedi *et al.* (2022), and highlights its suitability as an energy-dense, plant-based dietary source, particularly for populations requiring affordable and nutritious plant materials. Overall, the nutritional profile of *Vinca (Catharanthus roseus)* leaves powder is comparable to other well-known medicinal plants such as *Moringa oleifera*, *Amaranthus hybridus*, and *Ocimum gratissimum*.

Table 1. Proximate composition (g/100g ww) of Vinca (*Catharanthus roseus*) leaves powder

Component	Mean ±SD
Moisture	7.75 ± 0.73
Total protein	7.92 ± 0.55
Crude fat	4.98 ± 0.31
Ash	6.28 ± 0.47
Carbohydrate	73.07 ± 2.11
Energy (Kcal/100g)	368.78 ±6.00

Each value represents the mean of three replicates ±SD. WW, wet weight.

Variations in the proximate composition between studies may stem from differences in environmental conditions, plant age, soil fertility, seasonality, drying methods, and analytical techniques (Pandey *et al.,* 2006; Kannan *et al.,* 2020; Arafa, 2021; Elhassaneen *et al.,* 2021a and Chaturvedi *et al.,* 2022).

2. Extractive value of Vinca leaf powder

The extractive value of plant material is a key parameter for determining the presence of phytochemicals soluble in specific solvents, indicating both the quantity and type of bioactive compounds extractable. In Table (2), the extractive values of Vinca leaves powder varied significantly depending on the solvent system used, reflecting differences in solvent polarity and extraction efficiency. Among all the solvents, hydro-ethanol (80% ethanol: 20% water) recorded the highest extractive value (6.48 ± 0.10%), closely followed by hydro-methanol (6.19 ± 0.07%), while chloroform resulted in the lowest yield (3.64 ± 0.09%). These findings are in line with reports by Kannan *et al.* (2020) who observed higher extraction yields for polar and semi-polar solvent mixtures, particularly ethanol and methanol aqueous solutions, due to their efficiency in solubilizing phenolics, alkaloids, and flavonoids. Water (3.93 ± 0.21%) and petroleum ether (4.05 ± 0.11%) also showed moderate extractive values, consistent with the nature of the compounds they are likely to primarily be glycosides and some non-polar volatile constituents. This supports the findings of Pandey *et al.* (2006) who noted that aqueous extractions typically yield polar phytochemicals, such as tannins and polysaccharides, while petroleum ether targets fatty acids and sterols. The relatively low extractive values observed with hexane (3.79 ± 0.05%) and chloroform are typical for non-polar solvents, which are less efficient at extracting hydrophilic phytochemicals prevalent in Vinca leaves. A similar trend was reported by Chaturvedi *et al.* (2022), who emphasized that non-polar solvents are limited in extracting the major active components such as vindoline and catharanthine—both of which are more soluble in alcohol-based solvents.

The extensively higher extractive values received with hydro-ethanol and hydro-methanol solvents suggest that combining water with alcohol enhances solvent polarity, main to extra green extraction of numerous phytochemicals. These results emphasize the significance of solvent preference in pharmacognostic and phytopharmaceutical studies. Supporting studies (El-Wazeer, 2011; Abd El-Khader, 2018; Elhassaneen *et al.,* 2018, 2022 and El-Soukoty, 2021) have shown that extractive values are dependable signs of natural product exceptional, purity, and authenticity, and also can discover adulteration. Furthermore, the presence of statistically tremendous variations ($p \leq 0.05$), indicated by using exceptional superscript letters in the desk, confirms that extraction efficiency depends at the solvent used. This insight is particularly important in phytochemical studies and formulation development, where it is crucial to achieve maximum extract yield while preserving the integrity and activity of bioactive compounds.

3. Bioactive compounds in Vinca leaf powder extracts

Table (3) illustrates how the type of solvent used significantly influences the extraction efficiency of bioactive and antinutritional compounds from *Vinca (Catharanthus roseus*) leaves, demonstrating that solvent polarity plays a critical role in determining phytochemical yield. Methanolic (VLME) and ethanolic (VLEE) extracts generally exhibited higher concentrations of most compounds compared to aqueous extracts (VLAqE), with notable exceptions being anthocyanins, triterpenoids, alkaloids, and saponins, which were more prominent in the aqueous extract. Ethanol produced the highest total phenolics (69.78 ± 1.56 mg GAE/g), closely followed by methanol (64.97 ± 3.23 mg GAE/g),

Table 2. Extractive value of Vinca (*Catharanthus roseus*) leaves powder using different organic solvents and water

Extraction medium	Mean extract (%) ±SD
Water (Distilled)	3.93 ± 0.21^{bc}
Hydro-ethanol (20% H_2O :80% EtOH)	6.48 ± 0.10^{a}
Hydro-methanol ((20% H_2O :80% MtOH))	6.19 ± 0.07^{a}
Petroleum ether	4.05 ± 0.11^{b}
Hexane	3.79 ± 0.05^{c}
Chloroform	3.64 ± 0.09^{c}

Each value represents the mean of four replicates ±SD. Values with different superscript letters in the raw are significantly different at $p \leq 0.05$.

while the aqueous extract had a substantially lower value (29.45 ± 2.17 mg GAE/g), consistent with Kannan *et al.* (2020), who reported the effectiveness of hydro-alcoholic solvents in extracting polyphenols due to their intermediate polarity. Flavonoid content was also significantly elevated in both alcoholic extracts. Conversely, anthocyanins were highest in the water extract (14.56 ± 2.22 mg/g), aligning with Chaturvedi *et al.* (2022), who noted water's efficacy in extracting these polar pigments. Carotenoids and lycopene, being less polar, were more efficiently extracted with methanol and ethanol, mirroring findings by Alam *et al.* (2020) that non-polar solvents enhance carotenoid recovery. Similarly, the yields of terpenoids and polysaccharides were greater in alcoholic extracts, attributed to their solubility in solvents of intermediate polarity, as reported by Pandey *et al.* (2006). Interestingly, triterpenoids, alkaloids, and saponins were predominantly found in the aqueous extract, possibly due to their hydrophilic or glycosidic properties, as highlighted by El-Wazeer (2011). Tannins followed the trend of being more abundant in ethanol and methanol extracts, a pattern consistent with Elhassaneen *et al.* (2022), who noted that phenolic structures like tannins respond well to polar organic solvents. Kaempferol, a flavonol compound, also showed higher levels in alcoholic extracts, supporting its preferential solubility in such solvents. The significant differences ($p \leq 0.05$) among extraction methods, as indicated by superscript annotations, underline the necessity of careful solvent selection in phytochemical investigations. In the context of phytopharmaceuticals and functional food development, the findings underscore the importance of selecting appropriate solvent systems to enhance the extraction of health-promoting bioactive compounds while limiting the presence of undesirable antinutritional elements. This observation aligns with prior conclusions by Abd El-Khader (2018) and El-Soukoty (2021), who highlighted that solvent type significantly affects the phytochemical profile of leafy medicinal plants. Numerous studies have shown that *Catharanthus roseus* (Vinca) extracts, rich in phenolic and flavonoid compounds, exhibit therapeutic potential in managing various health conditions, including diabetes, cancer, cardiovascular diseases, obesity, and age-related disorders (Elhassaneen *et al.*, 2016b, c; 2019 and 2024). Moreover, the presence of polysaccharides in Vinca is associated with immunomodulatory and antitumor effects (Wasser, 2005), whereas triterpenoids are recognized for their role as building blocks in the development of antineoplastic drugs (Ma *et al.*, 2005). Despite their benefits, tannins may exert adverse effects in animal nutrition by reducing feed intake and impairing protein utilization (Chung *et al.*, 1998). Kaempferol, a dietary flavonoid with phytoestrogenic activity, has been linked to reduced risk of chronic illnesses, particularly cancer, through its antioxidant properties and its regulatory influence on cellular mechanisms such as inflammation, programmed cell death, new blood vessel formation, and metastasis (Chen and Chen, 2013).

Table 3. Bioactive and antinutritional compounds content of Vinca (*Catharanthus roseus*) leaf powder extracts

Compound	VLME	VLEE	VLAqE
Total phenolics (mg gallic acid equivalent. g^{-1})	64.97 ± 3.23 ^{a}	69.78 ± 1.56^{a}	29.45 ± 2.17 ^{b}
Total carotenoids (mg catechin equivalent. g^{-1})	48.18 ± 2.91 ^{a}	39.56 ± 3.17 ^{b}	19.45 ± 1.09 ^{c}
Total flavonoids (mg rutin equivalent. g^{-1})	41.56 ± 4.17 ^{a}	43.90 ± 2.21 ^{a}	18.56 ± 2.10 ^{b}
Total anthocyanins (mg cyanidin-3,5-diglucoside. g^{-1})	6.76 ± 0.97 ^{b}	5.13 ± 1.65 ^{b}	14.56 ± 2.22 ^{a}
Lycopene (µg.g^{-1})	3.98 ± 0.55 ^{a}	4.11 ± 0.72 ^{a}	1.56 ± 0.06 ^{b}
Polysaccharides (mg starch. g^{-1})	29.91 ± 1.06 ^{a}	27.45 ± 0.54 ^{a}	11.32 ± 0.65 ^{b}
Terpenoids (mg linalool equivalent. g^{-1})	6.55 ± 1.45 ^{a}	7.73 ± 0.33 ^{a}	2.64 ± 0.21 ^{b}
Triterpenoids (mg ursolic acid equivalent. g^{-1})	2.17 ± 0.27 ^{b}	1.99 ± 0.24 ^{b}	4.17 ± 0.39 ^{a}
Tannins (mg catechine equivalent. g^{-1})	3.61 ± 0.41 ^{a}	4.16 ± 0.27 ^{a}	2.73 ± 0.24 ^{b}
Kaempherol (mg.g^{-1})	2.01 ± 0.12 ^{a}	2.07 ± 0.09 ^{a}	1.05 ± 0.06 ^{b}
Total alkaloids (mg quercetin equivalent.g^{-1})	1.81 ± 0.09 ^{ab}	1.67 ± 0.23 ^{b}	2.43 ± 0.17 ^{a}
Saponine (mg oleanolic acid equivalent.g^{-1})	2.16 ± 0.11 ^{b}	1.64 ± 0.17 ^{b}	3.17 ± 0.09 ^{a}

Each value represents the mean of four replicates ±SD. Values with different superscript letters in the same raw are significantly different at p≤0.05. VLME, Vinca leaves methanol extract, VLEE, Vinca leaves ethanol extract, VLAqE, Vinca leaves aqueous extract.

4. Biological activities of Vinca (*Catharanthus roseus*) leaf powder extracts

4.1. Antioxidant activity

Table (4) presents the antioxidant activity of Vinca (*Catharanthus roseus*) leaf powder extracts, highlighting the influence of solvent polarity on the extraction efficiency of bioactive compounds. The ethanol extract (VLEE) exhibited the highest antioxidant activity, with an Antioxidant Activity (AA) of 90.04%, followed by the methanol extract (VLME) at 87.12%, and the aqueous extract (VLAqE) at 71.44%. The oxidation rate ratio (ORR) was inversely related to antioxidant activity, with VLEE showing the lowest ORR (0.099), indicating superior antioxidant efficacy. The antioxidant activity coefficient (AAC), which reflects the overall antioxidant potential, was highest in VLEE (737.62), suggesting its dominance in scavenging free radicals. These findings align with previous studies indicating that ethanol and methanol are effective solvents for extracting antioxidants from plant materials. For instance, a study by Tiong *et al.* (2013) reported that methanol extracts of *C. roseus* exhibited significant antioxidant activity. Similarly, research by Bhutkar and Bhise (2011) demonstrated that ethanol extracts of *C. roseus* leaves possess substantial antioxidant properties. The higher antioxidant activity in ethanol and methanol extracts can be attributed to their ability to solvate a wide range of polar and non-polar compounds, enhancing the extraction of phenolics, flavonoids, and other antioxidants. Unlike the ethanol and methanol extracts, the aqueous extract demonstrated comparatively lower antioxidant activity. This reduced effectiveness may be due to the limited ability of water to dissolve certain key antioxidant constituents, which are better extracted using solvents of intermediate polarity. These results highlight the critical role of solvent selection in maximizing the yield of bioactive compounds, particularly when the goal is to develop therapeutic or nutraceutical products. Similar observations have been reported in earlier studies involving various plant sources containing a wide spectrum of phytochemicals akin to those found in Vinca (Elhassaneen *et al.,* 2016a, 2019, 2021a, 2023; Mashal, 2016; Sayed Ahmed, 2016; Aly *et al.,* 2017; Hallabo *et al.,* 2018; El-Nassag *et al.,* 2019; Abd Elalal *et al.,* 2021 and Gharib *et al.,* 2022). These works consistently emphasized the strong association between antioxidant capacity and the presence of functional compounds such as phenolics, flavonoids, carotenoids, polysaccharides, anthocyanins, terpenoids, triterpenoids, tannins, and alkaloids. Therefore, the differences observed in the antioxidant potential of Vinca extracts are likely reflective of variations in the types and concentrations of these active compounds.

Table 4. Antioxidant activity of Vinca (*Catharanthus roseus*) leaf powder extracts

Extract	Antioxidant value[a] AOX (A/h)	Antioxidant activity[b] AA (%)	Oxidation rate ratio[c] (ORR)	Antioxidant activity coefficient[d] (AAC)
VLEE	0.056± 0.006 [d]	90.04± 1.21 [b]	0.099± 0.003 [d]	737.62± 9.11 [b]
VLME	0.073± 0.004 [c]	87.12± 0.98 [c]	0.128± 0.002 [c]	686.86± 5.21 [c]
VLAqE	0.161± 0.009 [b]	71.44± 1.09 [d]	0.285± 0.012 [b]	414.26 6.09 [d]
Control	0.565± 0.004 [a]	0.00± 0.00	0.998± 0.005 [a]	0.00± 0.00
BHT, 50 mg/L	0.074± 0.002 [c]	86.92± 0.76 [c]	0.130± 0.005 [c]	683.38± 3.54 [c]
BHT, 100 mg/L	0.017± 0.001 [e]	97.03± 0.09 [a]	0.029± 0.003 [e]	859.14± 1.77 [a]
α-tocopherol, 50 mg/L	0.007± 0.002 [f]	98.76± 0.18 [a]	0.012± 0.001 [f]	889.21± 2.10 [a]

[a] Antioxidant value (AOX, A/h) = The absolute value of slope (Abs was plotted against time).

[b] Antioxidant activity (AA, %) = (R control - R sample) / R control x 100 where: R control and R sample were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively

[c] Oxidation rate ratio (ORR) = R sample / R control

[d] Antioxidant activity coefficient (AAC) = (Abs S120 - Abs C120) / Abs C 0 - Abs C 120) x 1000 where: .Abs S 120 was the absorbance of the antioxidant mixture at time 120 min, Abs C 120 was the absorbance of the control at time 120 min, and Abs C 0 was the absorbance of the control at zero time.

[e] Each value represents mean ±SD. Values with different superscript letters in the same column are significantly different at $p \leq 0.05$.

AqE, aquatic extract, MeE, methanol extract, EtE, ethanol extract

4.2. Hydroxyl radical scavenging activity

Figure (1) and Table (5) presents the hydroxyl radical scavenging potential of *Catharanthus roseus* leaf powder extracts using different solvents-aqueous (VLAqE), ethanol (VLEE), and methanol (VLME)-compared with caffeic acid as a standard. The IC_{50} values, expressed in µg/mL, represent the concentration at which 50% of hydroxyl radicals are neutralized. A lower IC50 value indicates stronger antioxidant activity. Among the tested extracts, VLEE (ethanol extract) exhibited the highest antioxidant activity, with an IC_{50} of 39.21 ± 1.08 µg/mL, which is significantly lower than that of both VLME (52.64 ± 0.98 µg/mL) and VLAqE (85.40 ± 2.54 µg/mL). The aqueous extract (VLAqE) demonstrated the least scavenging activity, showing the highest IC_{50} value among all samples. Caffeic acid, the reference antioxidant compound, showed an IC_{50} of 61.88 ± 0.47 µg/mL, which was better than VLAqE and VLME but inferior to VLEE. These results align with findings by Elhassaneen *et al.* (2016b) and Mashal (2016), who emphasized that ethanol-based solvents are more efficient in extracting phenolic compounds responsible for free radical scavenging. The superior activity of the ethanol extract is likely due to its efficiency in extracting a wider range of polyphenols and flavonoids, known for their strong hydrogen-donating and metal-chelating abilities (Sayed Ahmed, 2016 and Aly et al., 2017). The methanol extract also showed significant antioxidant potential, consistent with its known ability to solubilize a broad spectrum of phytochemicals (Hallabo *et al.*, 2018). However, its slightly lower activity compared to ethanol could be attributed to differences in compound polarity and solubility. Conversely, the aqueous extract's weaker antioxidant performance reflects the limited solubility of certain bioactive-especially non-polar phenolic compounds in water. Similar findings were observed by El-Nassag *et al.* (2019) and Gharib *et al.* (2022), where water-based extractions resulted in lower concentrations of potent antioxidants.

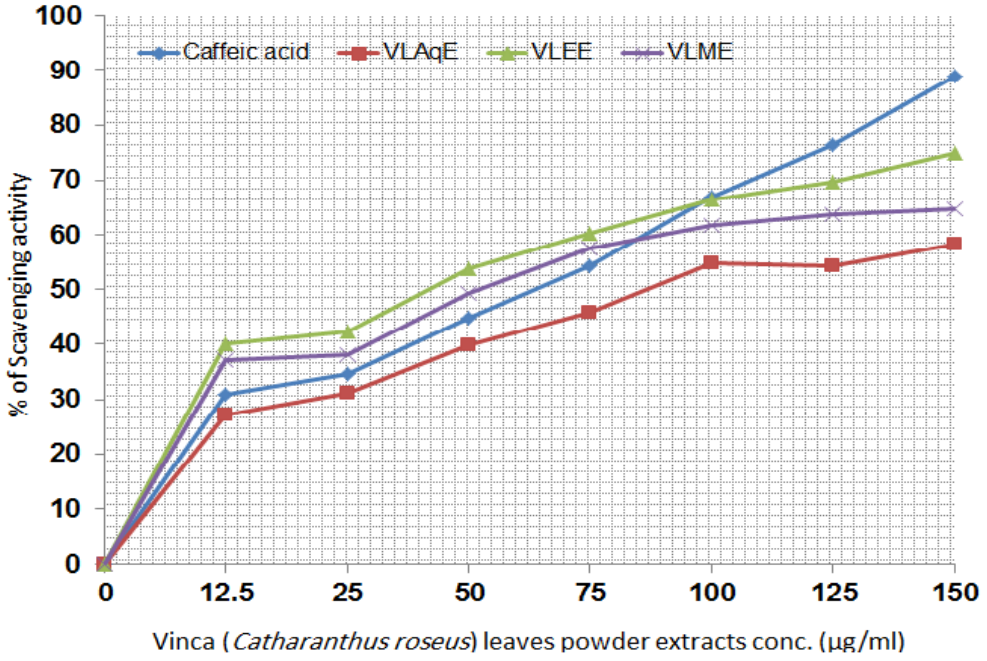

Figure 1. Hydroxyl radical scavenging activity of Vinca (*Catharanthus roseus*) leaves powder extracts. Each value represents the mean value of three replicates

Table 5. IC_{50} (Hydroxyl radical scavenging activity) of Vinca (*Catharanthus roseus*) leaf powder extracts

Name of sample	Caffeic acid	VLAqE	VLEE	VLME
IC_{50} (µg/mL)	61.88 ± 0.47 ^{b}	85.40 ± 2.54^{a}	39.21 ± 1.08 ^{d}	52.64 ± 0.98 ^{c}

Each value represents the mean value of three replicates ±SD. Different letters in the row mean significant differences ($p \leq 0.05$). VLAqE, aqueous extract, VLME, methanol extract, VLEE, ethanol extract.

The overall findings highlight the significant influence of solvent type on the efficiency of antioxidant compound extraction from *Catharanthus roseus* (Vinca) leaves. Among the solvents assessed, ethanol demonstrated the highest effectiveness in isolating hydroxyl radical scavengers, indicating its valuable application in the development of functional foods and plant-based therapeutics targeting oxidative stress. Notably, both ethanol (VLEE) and methanol (VLME) extracts showed greater hydroxyl radical scavenging activity than the standard antioxidant, caffeic acid (CA), pointing to their strong antioxidative potential. This enhanced activity suggests that these extracts may contribute to protective mechanisms against oxidative DNA damage, making them viable candidates for use in antimutagenic and anticarcinogenic interventions. Moreover, their capacity to neutralize hydroxyl radicals may help inhibit lipid peroxidation, a process closely linked with cellular damage and the onset of various degenerative diseases, thus reinforcing their potential health-promoting applications.

5. Antibacterial and antifungal activities of Vinca (*Catharanthus roseus*) leaf powder extracts

The data presented in Table (6) illustrate the antibacterial and antifungal potential of *Catharanthus roseus* (Vinca) leaf powder extracts prepared with different solvents—aqueous (VLAqE), methanol (VLME), and ethanol (VLEE). The inhibition zones (in mm) indicate that ethanol extract (VLEE) exhibited the strongest antimicrobial activity, followed by the methanol extract (VLME), while the aqueous extract (VLAqE) showed the weakest effect across all tested microbial strains. Among bacterial strains, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus spp.*, and *Salmonella spp.*, VLEE produced the highest inhibition zones (ranging from 27.86 to 43.65 mm), indicating strong antibacterial effects. Methanol extracts also exhibited considerable antimicrobial effects (21.62–36.54 mm), while aqueous extracts were significantly less effective, with inhibition against *E. coli* (8.53 mm) falling below the threshold for activity (10 mm), suggesting negligible action. Notably, for fungal **inhibition**, particularly *Candida albicans*, VLEE again showed the highest activity (45.07 mm), suggesting that ethanol is especially effective at extracting antifungal constituents.

This trend is consistent with previous findings that solvent polarity plays a critical role in extracting antimicrobial compounds. Ethanol and methanol, being moderately polar solvents, are more effective at solubilizing and extracting a wider range of bioactive molecules—especially alkaloids, flavonoids, and terpenoids—than water (Ramya *et al.,* 2008 and Naz *et al.,* 2015). These phytochemicals are known for their antimicrobial properties and may explain the enhanced activity of the VLEE and VLME extracts. In comparison to earlier studies, Saravanan *et al.* (2012) also reported strong antibacterial activity of *C. roseus* ethanol and chloroform extracts, particularly against *S. aureus* and *E. coli*, further supporting the current findings. Also, Jayraj *et al.* (2019) similarly confirmed that methanol and ethanol extracts of *Vinca rosea* significantly inhibited bacterial and fungal growth due to their high phenolic and alkaloid contents.

The relatively lower antimicrobial effect observed in aqueous extracts can be attributed to the limited solubility of hydrophobic antimicrobial compounds **in** water**,** thus leading to lower concentrations of active ingredients. Nevertheless, the aqueous extract still demonstrated moderate activity against *Streptococcus spp., Salmonella spp.,* and *C. albicans*, suggesting the presence of water-soluble antimicrobial compounds like certain tannins and saponins (Elhassaneen *et al.,* 2018). In conclusion, the previous data reinforce the potential of Vinca leaf extracts, particularly those prepared with ethanol, as effective antimicrobial agents. These extracts could serve as natural alternatives to synthetic antibiotics and antifungals, offering a promising avenue for the development of phytopharmaceuticals targeting a range of microbial infections.

Table 6. Antibacterial and antifungal activities of Vinca (*Catharanthus roseus*) leaf powder extracts

	Bacteria				Fungi
Extract	***Escherichia coli***	***Staphylococcus aureus***	***Streptococcus spp.***	***Salmonella spp.***	***Candida albicans***
VLAqE	8.53±0.56*	19.72±0.8 c	15.86±1.32 c	13.94±1.16 b	21.85±1.23 b
VLME	21.62±0.94b	36.54±1.1 b	33.93 ± 2.21 b	30.16±3.98 a	43.64±4.64 a
VLEE	27.86±1.78a	43.65±5.37 a	39.62 ± 0.89 a	30.48 ± 7.13 a	45.07±3.54 a

Each value represents the mean ±SD (n=3). Means with different superscript letters in the same row are significantly different at p≤0.05. Data in parenthesis means the percent of change from the control sample. *A Diameter of the inhibition zone less than 10 mm means the absence of activity. VLAqE, aqueous extract, VLME, methanol extract, VLEE, ethanol extract.

6. Implications for Lipid Oxidation and Atherosclerosis Protection *In Vitro*

6.1. Effect of Vinca (*Catharanthus roseus*) leaf powder extracts on lipid oxidation of β-carotene-linoleate model system

The results depicted in Figure (2) demonstrate the impact of *Catharanthus roseus* leaf powder extracts-namely ethanol (VLEE), methanol (VLME), and aqueous (VLAqE)-on lipid oxidation within a β-carotene-linoleate model system. For comparative purposes, the synthetic antioxidant butylated hydroxytoluene (BHT) was also included. All extracts, along with BHT, displayed notable antioxidant capacity, with VLEE showing the strongest inhibition of lipid oxidation, followed by VLME, VLAqE, and then BHT. The figure shows how the absorbance at 470 nm in all treated systems progressively declined over time ($p \leq 0.05$), indicating β-carotene degradation resulting from linoleic acid oxidation—a process initiated by free radical formation (Chandrasekara and Shahidi, 2010). Consistent with the findings of Kittiphattanabawon *et al.* (2012), this reduction was significantly delayed in samples treated with antioxidants due to their radical scavenging effects. Notably, extracts of Vinca leaves and BHT (both at 50 and 100 ppm) maintained significantly higher absorbance values than the control ($p \leq 0.05$), signifying effective protection against oxidation. There were no significant differences in antioxidant performance between VLME, VLEE, and BHT at equivalent concentrations, particularly at 100 ppm. However, the aqueous extract (VLAqE) consistently showed weaker antioxidant activity compared to ethanol and methanol extracts.

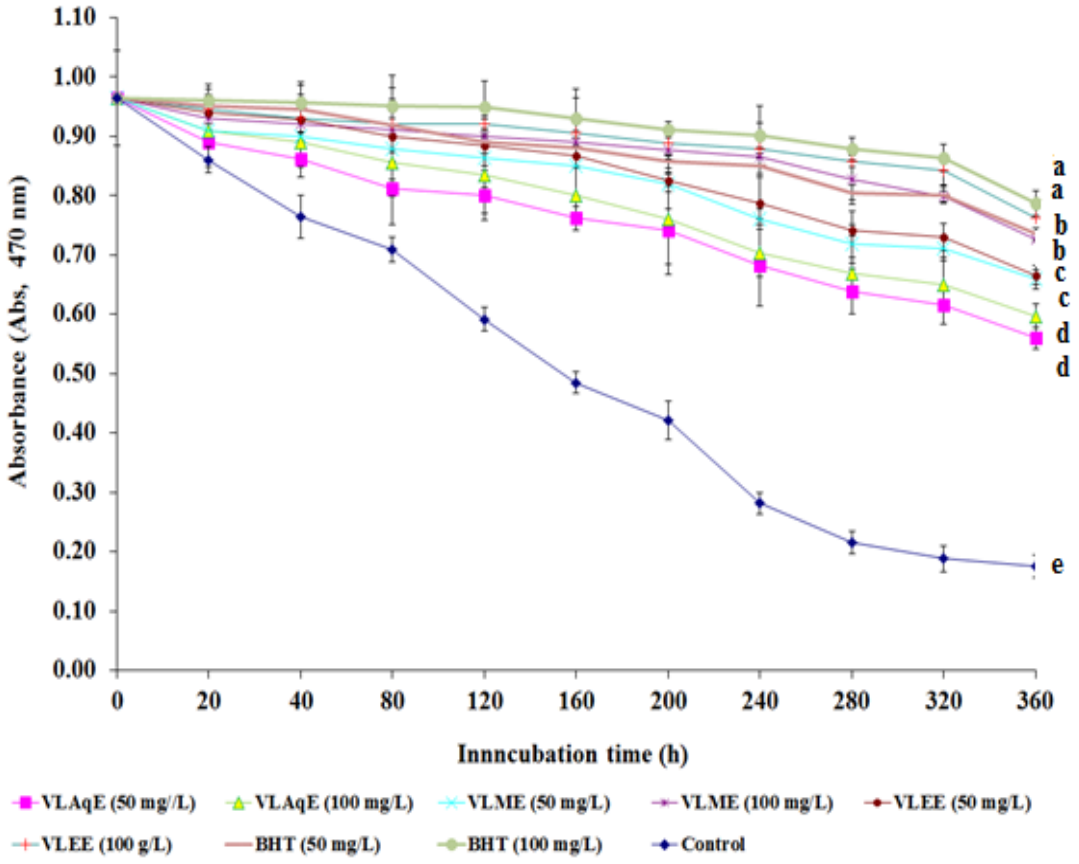

Figure 2. Changes in lipid oxidation of *β*-carotene-linoleate model system added with Vinca (*Catharanthus roseus*) leaf powder extracts at different concentrations.

Each value represents the mean ±SD (n=3). Means with different superscript letters in the same raw are significantly different at p≤0.05.VLEE, Vinca leaves ethanol extract, VLME, Vinca leaves methanol extract, VLAqE, Vinca leaves aquatic extract, BHT, butylated hydroxytoluene.

These outcomes suggest that ethanol and methanol extracts of Vinca leaves, especially at 100 ppm, substantially suppressed lipid oxidation in the model system, likely due to their content of electron or hydrogen-donating phytochemicals. The antioxidant performance of these extracts may be attributed to their richness in phenolic compounds, flavonoids, carotenoids, anthocyanins, lycopene, polysaccharides, terpenoids, triterpenoids, and kaempferol—bioactive constituents previously linked to radical-scavenging and antioxidant activities (Barros *et al.,* 2007; Elhassaneen & Sanad, 2009; Abd Elalal *et al.,* 2021; Elhassaneen *et al.,* 2019, 2021a,b and El-Nassag *et al.,* 2019). The observed differences in antioxidant efficiency among the extracts can be explained by the variation in solvent polarity, which affects the solubility and extraction efficiency of active compounds. Given that ethanol and methanol are less polar than water, they likely extracted more non-polar compounds from Vinca leaves-compounds known for stronger antioxidant effects in lipid systems. Supporting this, Zhong and Shahidi (2011) concluded that non-polar antioxidants perform more effectively than polar ones in emulsified systems. Consequently, VLEE and VLME proved to be more capable of delaying lipid oxidation in this model.

These findings align with previous investigations, such as those on *Ginkgo biloba* leaf extracts, where ethyl acetate fractions showed antioxidant effects comparable to synthetic agents like BHT and ethoxyquin (Li *et al.,* 2016). Similarly, BHT is widely recognized for its ability to prevent lipid oxidation in food systems (Huang and Weng, 2007). In conclusion, Vinca leaf extracts, particularly those obtained through ethanol extraction, show promise as natural antioxidants, making them suitable alternatives to synthetic preservatives in the food and nutraceutical industries.

6.2. Anti-atherosclerotic effect of Vinca (*Catharanthus roseus*) leaf powder extracts *in vitro* [Inhibition of low-density lipoprotein (LDL) oxidation]

Figure (3) presents the concentration-dependent inhibition of $CuSO_4$-induced oxidation of low-density lipoprotein (LDL) by different extracts of *Catharanthus roseus* leaf powder. All tested extracts demonstrated significant inhibitory effects on LDL oxidation, with the ethanol extract showing the highest potency, followed by the methanol and aqueous extracts.

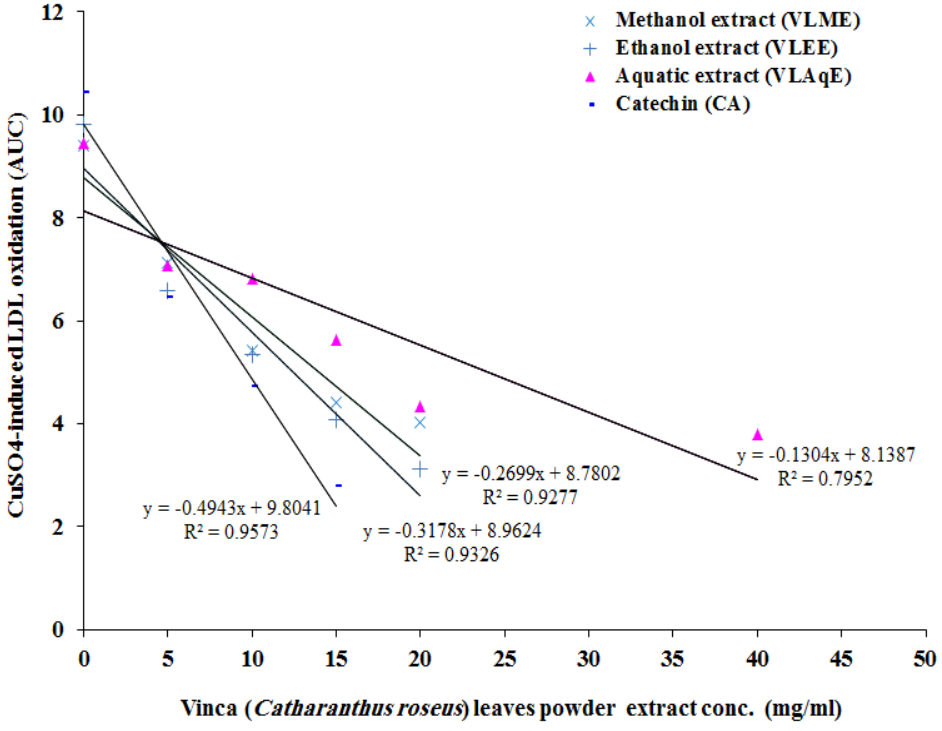

Figure 3. Dose-dependent inhibition of CuSO4-induced LDL oxidation *in vitro* by Vinca (*Catharanthus roseus*) leaf powder extracts

These results are consistent with earlier findings that confirm the antioxidant potential of *C. roseus* leaves. Ethanol extracts, in particular, are rich in flavonoids such as catechin hydrate, rutin hydrate, and kaempferol, which are known for their strong antioxidant actions (Pereira *et al.,* 2009). Similar patterns of LDL oxidation inhibition have been observed in other plant species, such as *Plectranthus glandulosus*, where extract concentrations correlated with increased antioxidant activity (Zouheira *et al.,* 2020).

The differences in antioxidant efficiency among the extracts can largely be explained by their varying solvent polarities and phytochemical profiles. Less polar solvents like ethanol and methanol are more effective at extracting non-polar bioactive compounds, including specific flavonoids and alkaloids, which have been recognized for their ability to neutralize free radicals and protect lipids from oxidative damage (Mardani-Nejad, 2021). These compounds often act as hydrogen donors, stabilizing reactive oxygen species. Supporting this, several *in vivo* studies have demonstrated that phenolics—the predominant bioactives in *C. roseus*—can reduce LDL oxidation by enhancing antioxidant enzymes such as glutathione (GSH) and glutathione reductase (GSH-Rd), and by suppressing NADPH-driven lipid peroxidation in tissues like the liver and lungs (Majid *et al.,* 1991; Elbasouny *et al.,* 2019; El-Gamal, 2020; Elhassaneen *et al.,* 2020 and Gouda *et al.,* 2024). Moreover, similar bioactive-rich extracts from other medicinal plants have shown comparable LDL oxidation-inhibiting properties (Aly *et al.,* 2017; Abd Elalal *et al.,* 2021; Elhassaneen *et al.,* 2021a,b and 2023). Given that LDL oxidation is a critical initiating step in the development of atherosclerosis due to its role in vascular inflammation and endothelial dysfunction (Poznyak *et al.,* 2021), the strong antioxidant activity of Vinca leaf extracts-especially those obtained using ethanol-suggests a potential application in preventing cardiovascular diseases, where the inhibition of LDL cholesterol oxidation plays a critical role in atherosclerosis prevention. These extracts also hold promise for use in food preservation and nutraceutical products, offering natural and effective alternatives to synthetic antioxidants.

CONCLUSION

The present look at highlights the tremendous dietary and bioactive ability of *Catharanthus roseus* (Vinca) leaf powder extracts. Proximate analysis discovered a high content material of essential macronutrients—proteins, fats, fibers, and carbohydrates—emphasizing its value as a nutrient-wealthy factor. The bioactive profile severa notably with solvent kind: ethanol extracts contained higher levels of usual phenolics, flavonoids, lycopene, terpenoids, tannins, and kaempferol, at the same time as aqueous extracts have been richer in anthocyanins, triterpenoids, alkaloids, and saponins. This variation displays the effect of solvent polarity on extraction performance, underlining the importance of solvent selection based totally on intended recovery or dietary use. Ethanol and methanol extracts exhibited sturdy antimicrobial hobby towards both bacteria and fungi, and especially the ethanol extract confirmed strong antioxidant activity by using inhibiting lipid peroxidation. This antioxidant impact is applicable to mitigating oxidative stress and preventing LDL oxidation, a key element in atherosclerosis. Overall, the findings support the potential application of ethanol-extracted Vinca leaves as functional ingredients in food preservation, pharmaceuticals, and nutraceuticals, offering a natural alternative.

Ethical considerations

Ethical approval for this research was obtained from the Scientific Research Ethics Committee of the Faculty of Specific Education, Tanta University, Tanta, Egypt.

Conflict of interest

The authors declare that there are no competing interests or potential conflicts related to the publication of this manuscript.

Authors' Contribution

All authors contributed equally to every phase of the research process. This included the initial design and formulation of the study protocol, as well as its subsequent refinement and validation. They actively participated in executing the experimental procedures, supervising laboratory work, collecting and analyzing data, and interpreting the outcomes. Additionally, each author was involved in compiling relevant literature, drafting the manuscript, critically reviewing its content for accuracy and coherence, and approving the final version for publication.

ABBREVIATIONS

AA, antioxidant activity, Abs, absorbance, AAC, antioxidant activity coefficient, CA, catechin, DMSO, dimethyl sulfoxide, GA, gallic acid, HRSA, hydroxyl radical scavenging activity assay, LDL, low density lipoprotein, MDA, malonaldehyde, MeE, methanol extract, ORR, oxidation rate ratio, SD, standard deviation, TBA, thiobarbituric acid, VLAqE, Vinca leaf aqueous extract, VLEE, Vinca leaf ethanol extract, VLME, Vinca leaf methanol extract.

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الملخص العربي

المواد الكيميائية النباتية والأنشطة البيولوجية لمستخلصات أوراق نبات الفينكا (*Catharanthus roseus*): الأدوار المحتملة فى تثبيط أكسدة الدهون والحماية من تصلب الشرايين معمليا خارج الجسم

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تهدف هذه الدراسة إلى التحقيق في المركبات النشطة بيولوجيًا والخصائص الحيوية لأوراق نبات الفنكا (*Catharanthus roseus*) المزروعة في مصر، مع التركيز على قدرتها المحتملة في تثبيط أكسدة الدهون والحماية من تصلب الشرايين معمليا خارج الجسم. كشفت التحاليل الغذائية لمسحوق الأوراق المجففة عن تركيبة غذائية متوازنة وغنية بالبروتين والدهون والألياف والكربوهيدرات، مما يدعم استخدامها كمكون غذائي وظيفي. تفاوتت كميات المستخلصات الناتجة بشكل ملحوظ حسب نوع المذيب المستخدم، حيث أظهر الإيثانول بنسبة ٨٠% أعلى كفاءة استخلاص، مما يسلط الضوء على أهمية قطبية المذيب في استخلاص المركبات النباتية الفعالة. احتوت مستخلصات الإيثانول (VLEE) والميثانول (VLME) على نسب أعلى من الفينولات والفلافونويدات والليكوبين والتانينات والتربينويدات والكايمبفيرول، بينما أظهر المستخلص المائي (VLAqE) نسبًا أعلى من الأنثوسيانين، والقلويدات، والتريتربينويدات، والصابونينات. أظهر مستخلص الإيثانول (VLEE) أعلى نشاط مضاد للأكسدة بنسبة ٩٠,٠٤%، يليه الميثانول(٨٧,١٢%)، ثم المستخلص المائي (٧١,٤٤%)، وكان له أقل نسبة لمعدل الأكسدة (ORR = 0.099). في اختبارات التخلص من الجذور الحرة من نوع الهيدروكسيل، سجل VLEE أقل قيمة IC_{50} ٣٩,٢١ ميكروجرام/مل، مقارنة بـ VLME(٥٢,٦٤ ميكروجرام/مل)، VLAqE (٨٥,٤٠ ميكروجرام/مل) وحمض الكافيك (٦١,٨٨ ميكروجرام/مل). وأكدت اختبارات النشاط الميكروبي قدرة VLEE الفائقة في تثبيط *E. coli*، و*S. aureus*، و*Streptococcus* spp.، و*Salmonella* spp.، و *Candida albicans*. وفي نظام نموذج β-كاروتين–لينوليك، أظهر VLEE و VLME بتركيزات ٥٠ و١٠٠ جزء في المليون فعالية في تأخير تحلل البيتا كاروتين، وكانت النتائج مماثلة لمضاد الأكسدة الصناعيBHT، بينما أظهر VLAqE تأثيرًا أضعف. تشير هذه النتائج إلى أن الإيثانول والميثانول أكثر كفاءة في استخلاص المركبات النشطة ذات القطبية المنخفضة مثل الفلافونويدات والبوليفينولات. وعليه، تُظهر مستخلصات أوراق الفنكا، خاصة المستخلصة بالإيثانول، خصائص قوية كمضادات أكسدة وكمضادات ميكروبية، مما يجعلها واعدة للاستخدام في حفظ الأغذية وتطوير المنتجات الغذائية الصحية الطبيعية لمقاومة الإجهاد التأكسدي وتصلب الشرايين.

الكلمات المفتاحية: التركيب الكيميائي، قيمة الاستخلاص، المركبات النشطة بيولوجيًا، النشاط المضاد للأكسدة، نشاط التخلص من الجذور الحرة، النشاط الميكروبي.