



Green Synthesis, Characterization and Application of Zinc Oxide Nano-particles as an Anticancer Agent Using *Arbutus pavarii* Pamp. Leaves Extract

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Abstract

In view of the advantages of green synthesis, a novel green route for the synthesis and stabilization of zinc oxide nanoparticles (ZnO-NPs) using extract of *Arbutus pavarii* Pamp. (*A. pavarii* Pamp.). ZnO-NPs were characterized through UV-Visible Spectroscopy (UV-Vis), scanning electron microscopy (SEM), X-ray diffraction analysis (XRD), Zeta potential analysis, Fourier-transform infrared spectroscopy (FT-IR) and Energy dispersive X-ray measurements (EDX). Their morphology and structure were determined through a combination of these techniques. The formation of ZnO-NPs is monitored by recording the UV-Vis absorption spectra for surface Plasmon resonance (SPR) peaks (~410 and 650 nm). SEM show uniform spherical particles obtained by this green method.. ZnO-NPs of variable particle size (16.67 - 31.01 nm) are highly uniform in size and have a fairly uniform distribution. *A. pavarii* Pamp. extract was shown to be an ideal candidate for obtaining much-improved uniformity in particle size and producing more efficient ZnO-NPs with similar particle sizes. The particles, though distinct, were primarily coated with the organic leaf extract, making them stable over long periods of time and ideal for biotechnology applications. In addition, cytotoxicity analysis of biosynthesized ZnO-NPs on two cell lines, lung cancer cells (A549) and lymphoma cancer cells (CCRF-CEM), revealed that ZnO-NPs in combination could function as an anticancer agent. Our findings demonstrated that the biosynthesized ZnO-NPs of *A. pavarii* Pamp. have promising medical uses as safe anticancer medications.

Keywords: Green synthesis, Zinc Oxide nanoparticles, *Arbutus pavarii* Pamp., anticancer.

التخليق الأخضر لجسيمات اكسيد الزنك النانوية وتوصيفها وتطبيقها كعامل مضاد للسرطان باستخدام

مستخلص أوراق نبات "*Arbutus pavarii* Pamp."

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المخلص

نظراً لمزايا التخليق الأخضر، تم تطوير مسار أخضر مبتكر لتخليق وتثبيت جسيمات اكسيد الزنك النانوية (ZnO-NPs) باستخدام مستخلص نبات "*Arbutus pavarii* Pamp.". جرى توصيف الجسيمات النانوية المخلقة باستخدام تقنيات متعددة شملت: مطيافية الأشعة فوق البنفسجية والمرئية (UV-Vis)، والمجهر الإلكتروني الماسح (SEM)، وحيود الأشعة السينية (XRD)، وتحليل جهد زيتا (Zeta potential)، ومطيافية الأشعة تحت الحمراء بتحويل فورييه (FT-IR)، وقياسات تشتت الطاقة للأشعة السينية (EDX). وقد تم تحديد مورفولوجيا (شكل) وبنية الجسيمات من خلال الجمع بين هذه التقنيات. تمت متابعة تكوين جسيمات اكسيد الزنك النانوية عن طريق تسجيل أطياف الامتصاص (UV-Vis) ورصد قمة رنين البلازمون السطحي (SPR) عند الأطوال الموجية (حوالي 410 و 650 نانومتر). وأظهر الفحص بالمجهر الإلكتروني الماسح (SEM) الحصول على جسيمات كروية متجانسة باستخدام هذه الطريقة الخضراء؛ حيث تراوحت أحجام الجسيمات بين 16.67 و 31.01 نانومتر، مع تميزها بتجانس عالٍ في الحجم وتوزيع



منتظم إلى حد كبير. كما تبين أن مستخلص " *A. pavarii Pamp* " يُعد خياراً مثالياً للحصول على تجانس أفضل بكثير في أحجام الجسيمات وإنتاج جسيمات أكسيد الزنك النانوية أكثر كفاءة ذات أحجام متقاربة. ورغم أن الجسيمات كانت متميزة (منفصلة)، إلا أنها كانت مغلقة في الغالب بمستخلص الأوراق العضوي مشكّلة تجمعات صغيرة، مما منحها استقراراً لفترات طويلة وجعلها مثالية للتطبيقات في مجال التكنولوجيا الحيوية. وبالإضافة إلى ذلك، كشف تحليل السمية الخلوية لجسيمات الزنك النانوية المُخلّقة حيويّاً - عند اختبارها على خطّين خلويين: خلايا سرطان الرئة (A549) وخلايا سرطان الليمفوما (CCRF-CEM) عن إمكانية عمل هذه الجسيمات كعامل مضاد للسرطان. وقد أظهرت نتائجنا أن جسيمات الزنك النانوية المُخلّقة حيويّاً باستخدام مستخلص " *A. pavarii Pamp* " تمتلك استخدامات طبية واعدة كأدوية آمنة مضادة للسرطان.

الكلمات المفتاحية: التخليق الأخضر، جسيمات أكسيد الزنك النانوية، اريباتس بفاري بامب (القطلب الليبي)، مضاد للسرطان.

1. Introduction

Nanotechnology is a fast-growing area of expertise that offers diverse applications in various fields such as medicine, manufacturing, biotechnology, and chemicals. Nanoparticles are capable of being produced with a surface to volume ratio of 1-100 nm, which produces at least one dimension between 1 and 100 nm [1], giving nanoparticles a massive surface area [2]. Nanoparticles exhibit distinct physical, biological, and chemical characteristics, in addition to their conventional material properties, not just particle size, but also their surface area and volume ratio[3].

The synthesis of nanoparticles can be achieved through a range of chemical and physical procedures [4,5] as these methods are costly and expose users to hazardous organic solvents and toxins that are detrimental to both the environment and human health [6,7]. Bio-based nanoparticle synthesis has been identified as a low-cost, sustainable, and safe alternative that does not require extensive medical training. The use of fungi, bacteria, whole plants, and natural extracts has been utilized as a non-toxic approach to generate and stabilize nanoparticles as a non-toxic technique[8].

Natural and plant extracts have many advantages over other eco-friendly synthesis methods, including being widely used, easily expandable, quickly accessible, safe to handle, and less expensive [9]. *A. pavarii Pamp* is an endemic species native to El-Jabel El-Akhdar region in Libya (specifically found in areas such as El-Jardard) and belongs to the Ericaceae family [10-12]. The *A. pavarii Pamp.* tree is 1.5 to 3 m in height as described in the Libyan flora, in the description of its species [13]. In folk medicine, it is used for relief and protection against various diseases, in addition to the plant leaves, as well as plant roots. A number of compounds used in the medicinal industry were collected from *A. pavarii Pamp.* leaves and stems [13].

Limited phytochemical studies on this plant revealed the presence of simple phenolics e.g., arbutin, gallic acid and polyphenolics, including flavonoids and tannins e.g., apigenin, epicatechin, hesperidin, kaempferol, naringin, quercetin and rutin, and some triterpenes and sterols [14-17]. Rutin was found to be the most abundant flavonoid, while kaempferol was the least in the aerial parts of *A. pavarii Pamp.* Arbutin appears to be the chemotaxonomic marker compound of the genus *Arbutus* L., as this compound is present in other species of this genus. Despite its uses in the Libyan traditional medicine, there are only a few published reports

depicting mainly its antioxidant, antimicrobial and cytotoxic activities available to date [14,16,18,19].

The ZnO-NPs have been attracted worldwide attention for their unique features, distinguishing them from their more significant components, such as flexible semi-conductor and piezoelectric capabilities [20]. ZnO-NPs are characterized by high conductivity, long-term stability, superior thermal and electrical conductivity, and excellent antibacterial potential. It is proposed that the size, crystallinity, band gap, and surface area of ZnO-NPs contribute to their antibacterial activity [21]. Consequently, drugs, cosmetics, and animal food supplements are just a few of the applications for ZnO-NPs [22].

ZnO-NPs have been shown to be a promising anticancer agent as it has the potential to destruct tumor cells with a minor effect on healthy cells; besides, several studies have proven the effectiveness of ZnO-NPs against several cell lines in a dose-dependent manner, such as MCF-7 and HepG-2 [23].

Research Gap:

The study addresses the lack of a novel, safe, eco-friendly, and cost-effective green synthesis route for producing and stabilizing zinc oxide nanoparticles (ZnO-NPs) using the endemic Libyan plant *A. pavarii Pamp.* leaf extract as a capping and reducing agent.

Despite the traditional medicinal uses of *A. pavarii Pamp.*, there is a scarcity of comprehensive scientific reports evaluating the *in vitro* cytotoxic, anti-proliferative, and anticancer efficacy of its biogenically synthesized ZnO-NPs, specifically against lung cancer (A549) and lymphoblastic leukemia (CCRF-CEM) cell lines.

Research Objective:

To develop a novel, eco-friendly method for synthesizing ZnO nanoparticles (ZnO-NPs) using *A. pavarii Pamp.* leaf extract and to thoroughly characterize their structural, morphological, and optical properties using advanced techniques such as UV-Vis, SEM, XRD, Zeta potential, FTIR, and EDX.

Additionally, to assess the antioxidant constituents and activities, hemolytic cytotoxicity on human red blood cells (RBCs), and the targeted *in vitro* anticancer and anti-proliferative efficacy of the biosynthesized ZnO-NPs against lung cancer (A549) and lymphoma (CCRF-CEM) cell lines.

2. Materials and Methods

2.1 Plant Collection

Samples of *A. pavarii Pamp.* (shmeri) leaves were collected from a local agricultural field during the winter season in October 2025 around the Al-Himadah area which is located 70 km east of the city of Benghazi and 30 Km west of Al-Marj city.

2.2 Plant Extraction

A total of 0.1g of fresh *A. pavarii Pamp.* leaves were gathered and cleansed with distilled water. The leaves were then allowed to air dried until their weight remained constant at room temperature (nearly four days), then ground into powder. Afterward, the plant extract was prepared by 0.1g the leaves powder in 50 mL solvents (acetone, diethyl ether, ethanol, and methanol) utilizing an Erlenmeyer flask and shaking it at a temperature of 80 C° while stirring magnetically at 1000 rpm for 30 s. ambient temperature on a rotary shaker, as previously

reported. The extract that was created was then centrifuged and stored at 4 C° in the refrigerator for phytochemical screening and active component evaluations[24].

2.3 Green Synthesis of ZnO-NPs from Extract of *A. pavarii* Pamp

50 mL of clear plant extract was added to 50 mL of ZnCl₂ solution (1mM, freshly prepared) and incubated under shaking in the dark conditions. The resultant dark brown solution indicated the ZnCl₂ reduction and formation of ZnO-NPs, which were separated by centrifuge processes for 10 min [25]. The precipitated ZnO-NPs were washed several times with distillate water and absolute ethanol to ensure purity, then dried at 60 C° for 24 hr. before any application Figure 1.

Gas Chromatography-Mass Spectrometry (GC–MS) Analysis.

The GC-MS analysis was performed using a Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, Waltham, MA, USA) to analyze the *A. pavarii* Pamp. extract for its active components. Chromatographic separation was achieved using a TG–5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness). Helium (99.999% purity) served as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was maintained at 250 C°, and samples (1.0 µL) were introduced in splitless mode. The GC oven temperature programming and sample preparation were completed as described previously [26]; the temperature was initially held at 60 C° for 2 min, gradually ramped at a rate of 5 C°/min to 280 C°, and finally maintained at this temperature for 5 min. Mass spectra were acquired using electron ionization (EI) mode at a standard ionization energy of 70 eV, with the transfer line and ion source temperatures set at 280 C° and 230 C°, respectively. Spectral data were recorded in full scan mode across a mass-to-charge ratio (m/z) range of 40–500 amu. These specific chromatographic and mass spectrometric parameters enabled a rigorous evaluation of the analytical results. The chemical components were recognized and validated by matching their retention times and fragmentation patterns against the Wiley Registry/NIST-MS library database, as well as matched mass spectra in literature data[27].

Antioxidant action

The antioxidant action of *A. pavarii* Pamp. extracts was examined by three different approaches:

DPPH radical scavenging activity

The methods employed differed just a little from the approach described by [28] and [29], where 3.9 mL of a DPPH (2, 2 Diphenyl -1-picrylhydrazyl) solution (concentration: 0.03 g L⁻¹ in pure methanol) was combined with 0.1 mL of an ethanolic extract aliquot. After a good shake, the mixture was left at room temperature in the dark for an hour. The resulting solution's absorbance at 517 nm was measured in comparison to the blank. The percentage of scavenged DPPH was calculated using this formula.

$$\text{DPPH scavenging \%} = (A_o - A_s / A_o) \times 100$$

where A_s and A_o stand for the absorbance of the sample and the blank at 517 nm, respectively.

Assay of ferric reducing power (FRP)

The ferric reducing influence of each extract was calculated with some alterations [30]. For the procedure, a mixture containing one milliliter of the ethanolic extraction samples was incubated at 50 C° for 20 min, 1 mL of potassium ferricyanide K₃Fe(CN)₆ (1% w/v), and 1 mL of sodium phosphate buffer (pH 6.6) with an amount of 0.2 M was combined. 1 mL of trichloroacetic acid (20 %, w/v) was added, and after that, the mixture was centrifuged for 15 min at 5000 rpm. The upper layer (1 mL) was combined with 0.2 mL of fresh FeCl₃ (0.1% w/v), and a blank without

potassium ferricyanide was prepared to measure absorbance at 700 nm. A higher absorbance denotes a higher reducing power.

Assaying of phosphomolybdate reagent (PMA)

The phosphomolybdate method, with some modifications, was employed to assess the extracts' overall antioxidant capability, using ascorbic acid as the standard [31]. Three milliliters of phosphomolybdate reagent (containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were combined with one milliliter of the ethanolic extract solution. The tubes were incubated for an hour in a bath of boiling water. The absorbance at 695 nm was measured in relation to the blank after the samples had cooled to room temperature.

Antioxidant compounds assay:

Calculating the phenolic content:

The total phenolic content of the various extracts under investigation was calculated by mixing the extract with 1 milliliter of Folin-Ciocalteu reagent and 1mL of 2% Na₂CO₃.

After 30 min, the mixture's absorbance was measured spectrophotometrically at 650 nm. The outcome is presented in (mg/g DM) and was calculated using a standard phenol (gallic acid) [32].

Estimation of flavonoid content:

A fraction of 0.5 mL of plant extract in ethanol was combined separately with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water to determine the flavonoidal content. The absorbance at 415 nm was measured after a 30-minute incubation period at room temperature. A standard flavonoid (quercetin) was used to establish a standard curve for flavonoid measurement (in mg/g DM). In the blank, the same volume of distilled water was used in place of the 10% aluminum chloride [33].

calculations of ascorbic acid content:

A minor correction was made to the ascorbic acid measurement. Two mL of 2% Na-molybdate, 1mL of 0.15N H₂SO₄, 1mL of 1.5 mM Na₂HPO₄, and 1mL of various ethanolic extracts made up the ascorbate reaction mixture in the presence of sulfosalicylic acid. After 40 min of incubation at 60 C° in a water bath, it was cooled and centrifuged for ten minutes at 3000 rpm. At 660 nm, the absorbance was determined. A constructed ascorbic acid calibration curve was used to determine the ascorbic acid content in mg g⁻¹ dry matter (DM) [34].

Estimation of saponin content

Saponin was quantitatively estimated after various adjustments. 100 µL of extract was added to 100 µL of vanillin reagent (8%, w/v in 99.9% ethanol). Test tubes were placed in an ice-cold water bath, and 2.5 mL of 72% (v/v) sulfuric acid was added slowly on the inner side of the wall. After mixing, the contents were warmed in a 60 C° water bath for 10 min and then cooled in an ice-cold water bath. Absorbance was calculated at 544 nm using a spectrophotometer against the reagent blank, and the content of total saponins was given as mg/g DM [35].

Determination of tannin content

The estimation of the tannin contents was done with a modification. Freshly made ammonium ferric citrate solution and 20% ammonia solution should be added to the extract in a clean test tube, allowed to sit at room temperature for ten minutes, and then measured spectrophotometrically at 525 nm [36].

Characterization of the Prepared Zinc Oxide Nanoparticles

Greenly synthesized ZnO-NPs derived from *A. pavarii pamp.* leaves extract were instrumentally characterized using different methods as follows:

UV-Visible Spectroscopy:

Utilizing ultraviolet-visible spectroscopy (Shimadzu, UV-1800, Japan), the absorption in the UV-visible region was estimated. It is also capable of evaluating the material's quality. The spectrum analysis verified that leaves extract-mediated ZnO-NPs were formed. A spectrometer was utilized to compute the UV-visible spectra of the produced ZnO-NPs by continuously scanning from 350 nm to 800 nm. Distilled water was employed as a reference for baseline correction [37].

Scanning electron microscopy (SEM) examination:

The samples of synthesized ZnO-NPs were examined for morphology using a JSM-6360 LA microscope (Tokyo, Japan) with the SEM technique at an acceleration voltage of 20 KV and a magnification of 40,000^x. This method involves directing a stream of electrons from the electron gun at the sample holder. At the top of the instrument, the electron gun is located, and a vacuum is created in the chamber to guide the electron beam toward the sample. The trajectory of the electron beam is regulated by lenses and electromagnetic fields within the instrument. An accelerated electron beam striking the sample results in some electrons being backscattered, some passing through the sample, and some producing secondary electrons. The detector monitors these secondary and backscattered electrons. Finally, the sample's morphology can be recorded via the computer.

X-ray diffraction (XRD) analysis:

XRD is a crucial non-destructive method for analyzing various materials such as crystals, fluids, and powders. It is highly significant for stress measurements and texture analysis, in addition to its significance in chemical characterization. We examined the phase, structural characteristics, and grain size of the synthesized nanoparticles in our X-ray diffraction investigation. A Shimadzu XRD-7000 (Kyoto, Japan) diffractometer with a CuK radiation beam ($\lambda = 0.154060$ nm), set up to 30 KV and 30 mA, and measuring data at $10^0 - 80^0$ in 2θ was used to acquire XRD patterns.

Zeta potential

The zeta potential of particles synthesized at the nanoscale was determined using a Zetasizer Nano series (Malvern Nano-Zs) model ZEN3600, UK, exclusively to evaluate surface charge and suspension stability. A sample of 0.01 g was added to 10 mL of distilled water, and it was then sonicated using (FLAC- ULTRASONIC BATHS 2020 MOD: LBS2 10LT) for 20 minutes before measurement. The particle size distributions were subsequently recorded. The zeta potential of nano-synthesized particles was assessed at a refractive index of 1.7 and an absorption value of 0.01 within the temperature range of 25-30 °C. The viscosity was measured at 0.8872, while the solvent (water) had a refractive index of 1.33 and a dielectric constant of 78.5[38].

Fourier-transform infrared (FTIR) spectroscopy

To classify the potential biomolecules and surface functional groups responsible for the reduction of nanoparticles and capping of the bio-reduced nanoparticles, FTIR analysis was performed at room temperature using a Nicolet 6700 FTIR spectrometer.

Certain chemical bonds react to particular frequencies when exposed to infrared light by vibrating more quickly in several modes, such as stretching, rocking, and scissoring. An infrared spectrum is a visual depiction of this response that can be identified. FTIR is a method

for obtaining an infrared spectrum that provides information on the sort of bonding present in the sample. FTIR is the most favored approach for evaluating the quality of the manufactured nanoparticles because it allows for the study of all phases of matter, which sets it apart from other infrared spectrum analytical techniques [39].

Since every material is a particular combination of atoms, no two compounds have the same infrared spectrum. As a result, infrared spectroscopy may produce a qualitative analysis that positively identifies each type of material used in the various nanoparticles. The magnitude of the spectrum's peaks can also be used to determine the quantity of material known as the fingerprint area; the vibrational modes that differ between molecules are helpful in differentiating one molecule from another that has comparable functional groups [40].

Energy Dispersive X-ray (EDX) measurements

The Energy Dispersive X-ray (EDX) microanalysis is an elemental analysis method linked to electron microscopy, relying on the production of characteristic X-rays that indicate the presence of elements in the specimens. In this study, EDX spectra were measured using a Si(Li) EDS detector (Thermo Scientific) coupled with Scanning Electron Microscopy (SEM).

For sample preparation, a few microliters of the sample solution were dropped onto standard carbon-coated copper grids (Plano) and air-dried. It is important to emphasize that these grids were employed solely as a low-background support substrate for SEM-EDX analysis, and no Transmission Electron Microscopy (TEM) imaging was performed. This method allows for a thorough examination of the elemental makeup, as well as the size, shape, and morphology of the nanoparticles via SEM. To ensure accuracy, the particle dispersion on the grid must be adequate; otherwise, EDX measurements were selectively performed on isolated particles to avoid cross-excitation and overlapping X-ray signals from adjacent particles.

In-vitro Biological Application of nanoparticles

Antioxidant constituents and activity

The antioxidant content (phenolic, flavonoids, ascorbic acid, saponin, and tannins) as well as antioxidant activity estimated by DPPH, FRP, and PMA contents in the three greenly synthesized nanoparticles, ZnO-NPs, were estimated following the methods mentioned earlier.

Cytotoxic activity

The cytotoxicity of the various greenly synthesized *A. pavarii* Pamp. leaves extract and ZnO-NPs was assessed using an MTT cell proliferation test kit, assessing cell viability on the WI-48 cell line using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide. For 48 hr., cells were placed in plates and subjected to different concentrations of ZnO-NPs. The cells were then incubated in a new medium containing MTT solution after being washed with phosphate buffer saline (PBS). An enzyme-linked immunosorbent assay (ELISA) plate reader was used to detect optical density at 550 nm following thorough shaking [41].

Anticancer activity

To study the effect of the three *A. pavarii* Pamp. greenly synthesized nanoparticles as an anticancer agent compared to the *A. pavarii* Pamp. extract, their anticancer activity against the two cell lines, A549 RRIDs: CVCL_0023 (lung cancer cells), Sigma-Aldrich catalogue no. 86012804 and CCRF-CEM RRID: CVCL_0207 (lymphoma cancer cells), ATCC company, Manassas, USA, was assessed. The carcinomatous cells of the lung and lymphoma were incubated at 37 C° in a humid environment made up of 95% air and 5% CO₂, using Dulbecco's Modified Eagle's Medium (DMEM). The carcinomatous cells were cultured at 37 C° for 24 h in 96-well plates containing 100 µL of DMEM to achieve the target of 5 * 10⁸ cells/well. Each

sample (50 μL) at concentrations of 1, 5, 10, 15, 30, 60, and 120 $\mu\text{g/mL}$ was added to a separate container. The plate was incubated for 24 h at a temperature of 37 °C. After centrifugation of the plate to remove the supernatant, it was washed with phosphate buffer saline. 15 μL of the MTT reagent (concentration: 0.5 mg/mL) was added to every well. For 4 h at a temperature of 37 °C, the plate was put in an incubator. In order to dissolve the formazan crystals (which are an MTT reduction product), 150 μL of DMSO was added to each well, and a shaker was used for mixing for 10 min. Optical density (OD) of the formazan products was measured at a wavelength of 570 nm. The percentage of viable cells was best determined using the following formula, with the aid of the following equation;

$$\% \text{ Cell viability} = (\text{OD sample} / \text{OD control}) * 100$$

Statistical Analysis

Using the Costat software on the Windows platform, the statistical analysis in this work was carried out according to recommendations. The data are shown as mean standard deviation (SD), and each experiment was measured in triplicate. The LSD test was used to perform a one-way ANOVA at a 0.05 significance level. Statistical analysis was performed using CoStat software (version 6.400, CoHort Software, Monterey, California, USA) on the Windows platform. All experiments were conducted in triplicate, and data are expressed as mean $\pm$ standard deviation (SD). The data were subjected to a one-way analysis of variance (ANOVA), and the Least Significant Difference (LSD) test was employed to determine significant differences between means at a significance level of $p \leq 0.05$ [42].

3. Result and Discussion

3.1 Antioxidant constituents of *A. pavarii* Pamp. leaves in different extracts.

To evaluate the potential antioxidant constituents of *A. pavarii* Pamp. leaves, various solvents (methanol, diethyl ether, ethanol and acetone) were used to screen a range of antioxidant compounds as well as activities Table1, which may be considered as indicators for its antioxidant impact. In estimating antioxidant activity, three different methods were utilized. The displayed results revealed a noteworthy antioxidant activity in methanolic extract followed by ethanol, acetone and then diethyl ether. Methanolic extract comprised the highest DPPH (83.4%) reducing capacity, the pronounced FRP content (31.7mg/g DM) and the significant PMA constituent (46.9 mg/g DM) compared to the other three solvents. Regarding antioxidant compounds, the presented results in Table 1 displayed a set of antioxidant compounds with a noteworthy impact of methanol as solvent compared to others. Additionally, phenolic, flavonoids and ascorbate in the methanolic extract exhibited the most pronounced concentrations (208.5, 11.6 and 12.3 mg/g DM), respectively, compared to the other three solvents. Moreover, the results showed that the methanolic extract contains considerable amounts of secondary metabolites, such as saponin and tannins with the maximum values (44.6 and 99.4 mg/g DM), respectively.

Recently, the use of plant extracts gained great attention as it is considered a safe, eco-friendly and cheap source [43]. The application of these natural substitutes in many environmental sectors: agriculture, industry and medical sectors spread quickly [44,45]. Notably, *A. pavarii* Pamp. which is endemic to Al-Jabal El-Akhdar on the eastern coast of Libya [46], revealed many significant compounds that have been documented to have medicinal importance, such as flavonoids (apigenin, epicatechin, hesperidin, quercetin, naringin, rutin) and simple phenolics (arbutin, gallic acid, pyrogallol) [16].

The findings of our investigations demonstrate a noticeable constituent of antioxidant compounds of *A. pavarii* Pamp. extract: phenolics and flavonoids, and this result is in accordance with [47,48] in extracts prepared from different parts of *A. pavarii* Pamp. .

Antioxidants have lately become a topic of increasing interest to health, science researchers, and medical experts. Furthermore, the rich constituents of the extract with saponins and tannins (antioxidant compounds) may serve as a good antimicrobial mediator. Several saponins are characterized by being antimicrobial agents and are considered protective mediators against prospective illness [49]. Further, tannins, as secondary metabolites, have a role as potential biological antioxidants used for the defense against oxidative damage, which has been implicated widely [50].

It is widely believed that antioxidants from natural sources increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart disease, stroke, and neurodegenerative diseases [51]. The main characteristic of an antioxidant is its ability to trap free radicals which in turn results in oxidative stress [52]. Our results regarding antioxidant activity revealed notable antioxidant activity shown by high DPPH scavenging activity, the decrease in its absorbance at 517 nm induced by antioxidant molecules, and its visually noticeable discoloration from purple to yellow [53]. Our finding is matching with, and this is because of the high antioxidant content of *A. pavarii* Pamp. extract which could reduce the DPPH molecule and decrease its absorbance [54].

3.2 Bioactive Compounds Identified in Extract by GC-MS

The GC-MS analysis of the *A. pavarii* Pamp leaves extract revealed the presence of several active compounds. Table 2 and Figure 2 provided details on the numerous extract components identified at different retention times (RT). Amongst the 13 active compounds identified, distinct relative abundance levels were observed for Melezitose at 18.85, 19.16 and 19.21, with areas reached (7.74, 10.01 and 11.21 %), Hexadecanoic acid (8.09 %), and Arabinitol, pentaacetate (3.12%). At an RT of 42.91 min, Vitamin E had a relative abundance of 16.57% while at 44.71 min, Betulin was the most abundant (7.15 %). Additionally, at an RT of 44.89 min, α -Amyrin was found to have a relative abundance of 19.95 %.

To confirm the constituents of *A. pavarii* Pamp. leaves extract with phytochemicals that may participate in its biological effects as cytotoxic and anticancer agents mentioned earlier, GC-MS was performed. It is well recognized that GC-MS is one of the most efficient, reproducible, and well-used analytical platforms for research. This is because of the reproducible, robust, and selective nature of the method, as well as the great number of well-established libraries of metabolite databases available. In Table 2 and Figure 2. Melezitose was checked as a potential therapeutic agent for the treatment of multiple disorders [55]. Melezitose is an oligosaccharide sugar and is classified as a non-reducing sugar because it does not possess a free aldehyde or ketone functional group [56]. It is commonly found in certain plants [65] and has anti-infectivity and anti-cancer therapeutic impacts [57]. A substantial decrease in A549 cell viability induced by Melezitose was observed, evident in cell blebbing. These findings collectively underscore the robust cytotoxicity of melezitose against lung cancer, emphasizing its potential as a new therapeutic avenue to treat lung cancer [58].

Hexadecanoic acid is proposed to have cytotoxic activity using the MTT assay and has an anticancer effect on human colorectal carcinoma (HCT-116) cells. This effect is due to its interaction with DNA topoisomerase-I [59].

17-Octadecanoic acids were the most predominant constituent present in *A. pavarii* Pamp extract, and similar results were obtained from *Dioscorea alata* leaf extract, which also demonstrated the presence of several varieties of organic acids [60]. It was found to have cytotoxicity against cancer cells [61].

Moreover, vitamin E, known as tocopherols, a potent peroxy radical scavenger, is a chain-breaking antioxidant that prevents the propagation of free radical damage in biological

membranes [62]. Vitamin E is the most abundant liposoluble antioxidant compound in the human body, and its modulatory effects regarding signal transduction, cellular pathways and many gene expressions have lately gained notoriety [63]. Besides, botulin, a very strong antioxidant with several pharmaceutical benefits was also present in our extract and has potent anticancer properties [64].

In the same regard, α -Amyrin, a pentacyclic triterpenoid compound widely distributed in plants and pharmacologically shown to have a wide range of activity including anti-inflammatory, anti-ulcer, anti-hyperlipidemic, anti-tumour, and hepatoprotective effects [65]. These findings indicate that the modulatory potential of α -Amyrin against oxidative stress possibly involves a mechanism related to its ability to block the selective inhibitors of ROS (reactive oxygen species) as antioxidants bringing about significant inhibition of the formation $O_2^{\bullet-}$, HO_2 , $HO_2^{\bullet}$, H_2O_2 and $\cdot OH$ [63].

3.3 Characterization of Synthesized ZnO-NPs

3.3.1 UV-Visible Spectroscopy of ZnO-NPs:

It is reported that phytochemicals are involved directly in the reduction of zinc ions and the formation of nanoparticles [66]. The important phytochemicals involved are flavones, aldehydes, ketones, carboxylic acids, and terpenoids, as prescribed by previous work [67]. The UV-Visible spectrum of the ZnO-NPs was recorded in the wavelength range of 350–800 nm; this technique is widely accepted and used to examine the optical properties of nano-sized particles. The displayed data show that the UV absorption spectra, resulting from the reduction of metal ions using *A. pavarii* Pamp. leaves extract has dispersed nanoparticles with a broadening peak in the absorbance band at different wavelengths according to the metal. ZnO-NPs demonstrates multiple absorption bands across the UV-visible region in Figure 3.

Regarding UV-visible spectroscopy, the formation of diverse peaks in Figure 3, which provides confirmation of the synthesis of nanoparticles from the plant extract; this finding is in accordance with previously reported results [68]. The ZnO-NPs sample shows a broad absorption profile, with major features centered near 410 nm and 650 nm. The ZnO-NPs lacks a sharp SPR peak, which is consistent with the fact that Zn does not exhibit strong SPR in the visible region. The observed bands may be associated with charge transfer transitions or interactions of Zn^{2+} ions within the host matrix. Moreover, these results prove the standard ZnO absorption manner, as all oxide materials have broad band gaps and have a tendency to shorter wavelengths; besides, the material tends to have shorter wavelengths when it is in nanoscale [69].

3.3.2. Scanning Electron Microscopy (SEM) Analysis

Figure 4 provides a detailed view into the morphological and particle size distribution characteristics of zinc oxide nanoparticles (ZnO-NPs), synthesized through green methods from *A. pavarii* Pamp. extract. The SEM images highlight distinct features for each type of nanoparticle. The ZnO-NPs image reveals a more heterogeneous structure, featuring smaller aggregates. Morphological analysis via SEM revealed that the average size of the amorphous ZnO-NPs ranged from 16.67 to 31.01 nm. The zinc oxide nanoparticles show less aggregation [70], pointing to a more stable synthesis process that minimizes clustering. The relatively narrow size distribution of ZnO-NPs suggests effective control over the synthesis parameters, resulting in well-dispersed nanoparticles [71].

3.3.3. X-ray Diffraction Studies

The phase purity and crystalline nature of the green-synthesized zinc oxide nanoparticles (ZnO-NPs) were investigated using X-ray diffraction (XRD) analysis. As illustrated in Figure 5, the XRD pattern of the synthesized ZnO-NPs exhibits a broad diffraction hump without any sharp or well-defined Bragg peaks. This broad feature, observed predominantly in the 2θ range of 15° to 45° , with maximum hump intensity centered around $2\theta = 35^\circ$, is a characteristic signature of an amorphous or semi-crystalline phase.

The absence of sharp crystalline peaks indicates that the synthesized nanoparticles do not possess a long-range crystalline order, which is highly typical for biogenically synthesized nanoparticles. This amorphous behavior can be attributed to the capping action of the bioactive molecules (such as phenolics, flavonoids, and other organic constituents) present in the *A. pavarrii* Pamp. leaf extract. These organic biomolecules successfully coat the surface of the nanoparticles during the reduction and stabilization process, acting as a biological shield that suppresses further atomic ordering and crystallization. Similar amorphous patterns for plant-mediated zinc oxide nanoparticles have been reported in previous literature, confirming that the biological matrix plays a vital role in stabilizing the nanoparticles in an amorphous state, which often enhances their surface reactivity and biological applications [72].

3.3.4. Zeta Potential

The displayed data in Figure 6 presents the zeta potential distribution of ZnO-NPs, synthesized using green methods. Zeta potential is a crucial indicator of the stability of nanoparticle suspensions, with higher magnitudes (either positive or negative) suggesting better stability due to increased electrostatic repulsion, which prevents aggregation. In Figure 6, the zeta potential for ZnO-NPs is recorded as -34.1 mV, indicating the greatest stability. The strong electrostatic repulsion among ZnO-NPs effectively prevents aggregation, ensuring a well-dispersed and stable suspension. This high stability is confirmed by the SEM image in Figure 4, where ZnO-NPs exhibit a fairly uniform distribution with particle sizes ranging from 16.67 nm to 31.01 nm, representing the greatest stability and indicating a more stable synthesis process that prevents significant clustering. Similar results were obtained where the leaf extracts of *Sageretia thea* were used to produce plant-mediated ZnO with cubic structures and an average diameter of 20 nm [73]. Numerous investigations corroborate our findings; according to Karam et al., the ZnO-nanoparticles were spherical and had an average size between 39.4 and 51.86 nm [74].

3.3.5. Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR analysis is crucial for identifying the functional groups on the nanoparticles' surfaces, providing insights into the stabilization and capping agents involved in the green synthesis process. Figure 7 presents the FTIR spectrum of ZnO-NPs. The broad peak at 3265.71 cm^{-1} is due to O-H stretching, signifying the presence of hydroxyl groups; peaks at 2928.39 cm^{-1} are related to C-H stretching. The absorption bands at 1617.90 cm^{-1} and 1384.27 cm^{-1} can be attributed to C=O stretching and C-H bending respectively. The peaks observed at 1103.77 cm^{-1} and 612.59 cm^{-1} may correspond to C-O and C-X (halogen) stretching vibrations. The FTIR spectrum of Zn-NPs indicated some broad peaks related to the hydroxyl group, carboxylate group and halogen. These functional groups suggest that the nanoparticles are capped and stabilized by organic molecules from the *A. pavarrii* Pamp. leaves extract used in the green synthesis, preventing aggregation and maintaining stability similar to a previously reported study [75]. The rich constituent of negatively charged groups may function for the reduction of Zn ions and efficient synthesis of NPs under natural environments [9].

3.3.6. Energy-dispersive X-ray spectroscopy (EDX)

The elemental composition analysis of the studied nanoparticles from the EDX plot of the SEM images is shown in Figure 4. The EDX spectra revealed that the required phase of ZnO is present in the samples and confirmed the formation of the synthesized ZnO-NPs. EDX spectrum, which displayed varying signal intensities of various binding energies (keV) on the X-axis, confirmed the existence of different elements in the sample. At 1 keV, the presence of a strong signal indicated the existence of metallic Zn in the biosynthesized sample, the EDX spectrum of the ZnO-NPs sample. Clearly, Zn and O are constituents of the sample, with a mass of 21.92 % for Zn at nearly 1 keV and 38.89% for O, elucidating the formation of ZnO-NPs. The EDX profile of ZnO-NPs in Figure 8. Furthermore, ZnO-NPs also represented elemental Zn and O, ensuring the formation of ZnO-NPs [76].

Overall, our results are in accordance with [77], and the existence of oxygen and carbon in the EDX profile may be a consequence of the synthesis of the sample and may be due to using a zinc grid coated with carbon for sample loading [78], as well as the occurrence of carbon as a result of capping molecules in the extract.

The horizontal axis 2θ represents the diffraction angle 10° to 90° , and the vertical axis (intensity) represents the intensity of the reflected radiation (in relative units). The presence of a broad peak at approximately $2\theta = 31^\circ$ indicates that the material is not completely crystalline or semi-crystalline (amorphous/semi-crystalline). The broad shape of the peak reflects the small size of the crystals (nanocrystalline nature). The absence of sharp, defined peaks indicates the lack of strong crystallinity as in pure crystalline materials and may be due to bio stabilization resulting from plant compounds in the extract, which may prevent the full growth of crystals. Also, the presence of the long tail on the right side (at larger angles) may indicate a wide distribution in particle size, as the *A.pavarii Pamp.* extract from which ZnO-NPs are made contains phenolic and terpenoid compounds that act as reducing and stabilizing agents, which may affect the crystallization pattern [76].

3.4. Biological Activities of Greenly synthesized ZnO-NPs

3.4.1. Antioxidant Constituent and Activity

In order to check the role of synthesized nanoparticles derived from *A. pavarii Pamp.* leaves extract as cytotoxic and anticancer agents, their antioxidant constituent was performed. The results displayed in table 1 differentiate the constituents of antioxidant activity as well as compounds in *A. pavarii Pamp.* synthesized ZnO -NPs.

Concerning the antioxidant capacity of the synthesized nanoparticles, ZnO-NPs contributed the most pronounced values of DPPH, FRP, and PMA (70.71 %, 10.12, and 14.62 mg/g DM, respectively). In addition to that, synthesized ZnO-NPs comprised high antioxidant compounds resembling phenolic, flavonoids and ascorbate (56.13, 9.93, and 4.11 mg/g DM, respectively) . Regarding saponin and tannin contents, ZnO-NPs Table 3 contained the highest constituents of both compounds, 11.22 and 16.92 mg/g DM respectively.

Greenly synthesized ZnO-NPs derived from *Eucalyptus globulus* proved their antioxidant activity through DPPH assay [73]; in the same regard, another work confirms our study results, where ZnO-NPs prepared through *Sesbania sesban* extract display similar DPPH radical scavenging impact as Ag and Cu-NPs [61]. ZnO-NPs generated from different plant extracts have apparently revealed substantial antioxidant activity [79].

A variety of antioxidant analyzes have been completed, along with free radical scavenging, antioxidant capacity, and reducing power estimations in biogenic ZnO-NPs generated from *Ziziphora clinopodioides* leaf extract and confirmed superior radical scavenging characteristics and overall antioxidant properties above typical values [80].

Besides, in our study, the capacity of *A. pavarii* Pamp. and its greenly synthesized nanoparticles ZnO-NPs as an antioxidant was shown to be induced by their reducing ability which may be considered a substantial indicator of their potential antioxidant characteristics, and this may have a role in changing free radicals to more steady products and hence ending free radical initiated chain reactions [81]. In this regard, the DPPH method is known to provide consistent information regarding the antioxidant capacity of definite extracts, products or compounds and all our studied greenly synthesized nanoparticles exhibited activity against DPPH. The total antioxidant potential of *A. pavarii* Pamp. and its greenly synthesized nanoparticles, demonstrated by its FRP and PMA content, is in accordance with [82], which gives the extract its benefit as well as its derived nanoparticles.

3.4.2. Cytotoxic and Anticancer Activity of ZnO-NPs

To study the effect of greenly synthesized nanoparticles ZnO-NPs derived from *A. pavarii* Pamp. extract on the viability of human red blood cells (RBCs), the cytotoxic effect was studied. The presented results in Figure 9 and Table 4, plotting hemolysis of RBCs against different nanoparticle concentrations revealed that all nanoparticles display low cytotoxic impact at lower concentrations and cytotoxic properties were more significant at higher nanoparticle concentrations. Remarkably, ZnO-NPs demonstrated high cytotoxicity. The half-maximal inhibitory concentration (IC_{50}) values, which represent the concentration at which 50 % of the cell population is killed or inhibited, were reported to be 14.8 $\mu\text{g/ml}$ for ZnO-NPs.

The anticancer activity of *A. pavarii* Pamp. and its greenly synthesized ZnO-NPs was tested against two cancer cell lines: lung cancer cells (A549) (Sigma-Aldrich catalogue no. 86012804), and lymphoblast's cancer cells (CCRF-CEM) (ATCC company, Manassas, USA). A549 is an epithelial cell that was isolated from the lung of a 58-year old, white male with carcinoma, while, CCRF-CEM are human lymphoblast's isolated from the peripheral blood of a Caucasian female, 4-year old with acute lymphoblastic leukaemia, and these cell lines are used for immuno-oncology and immunology research.

The depicted results in Figures 10, 11, revealed that increasing the concentration of *A. pavarii* Pamp. extract dose resulted in cell increasing inhibition in concentration-dependent manner till complete death at 250 $\mu\text{g/ml}$ of used materials and IC_{50} was 1.8 % in A549 cell line and 7.26% in the CCRF-CEM cell line.

Furthermore, it is evident that ZnO-NPs exhibit a marked anticancer effect across all tested concentrations. This suggests that the composition of the nanoparticles plays a significant role in their cytotoxicity towards cancer cells. Remarkably, at the highest concentration tested (250 $\mu\text{g/mL}$), ZnO-NPs showed cytotoxic impact against A549 and CCRF-CEM cells.

The most pronounced effect in the A549 cell line was recorded under ZnO-NPs application with IC_{50} reaching 12.2 $\mu\text{g/ml}$ application, while IC_{50} in the CCRF cell line was 26.6 $\mu\text{g/ml}$ in ZnO-NPs application. Cancer is one of the leading causes of mortality, resulting in one in six deaths (or 9.6 million people) in 2018 alone, with 70% of deaths from cancer occurring in middle- and low-income countries [83]. In addition to numerous therapies, cancer nanotechnology has unfolded a newer horizon of interdisciplinary research across chemistry, medicine, engineering and biology, focusing on the novel advancement in cancer detection, diagnosis and treatment [70]. Side effects and a high cost were reported with some of the approved chemotherapeutic agents, and as a result, developing alternative medicines to combat this deadly disease is critical.

Hence, synthesized nanoparticles show high yield, solubility and high stability. Among several synthetic methods for Nano preparation, biological methods seem to be simple, rapid, non-

toxic, dependable which can produce well-defined size and morphology under optimized conditions for traditional research [84].

All the greenly synthesized nanoparticles studied in this work (ZnO-NPs) in Table 3 and Figure 9 reveal a cytotoxic impact in concentration dependent manner, findings in a concentration accordance with [85], assumed that some of the nanoparticles possess a high cytotoxic effect on cancerous cells. In the same regard, the impact of synthesized nanoparticles (ZnO) derived from *A. pavarii Pamp.* extract as anticancer agents on the lung and lymphoblastic leukemia cell lines presented in Figure 10, 11 are in accord with the results obtained by [86,87] and supported the finding that natural extracts have moderate cytotoxic effects against MCF7, HepG2, and U2OS cells, and also has shown inhibitory effects on the proliferation of various cancer cell lines, eg, the Raji B-cell lymphoma stomach cancer line.

Additionally, crucial agents responsible for impeding tumour growth under *A. pavarii Pamp.* application are the rich contents of phenolic compounds. Among these compounds are arbutin, gallic acid, and pyrogallol, which play a significant role in inducing cell death and restraining invasion in the affected cells [16]. The *A. pavarii Pamp.* is famous for containing flavonoids, glycosides, sterols and triterpenes, which have roles in triggering apoptosis in cancer cells but have no opposing impacts on normal healthy cells [88].

Notably, the extract of *A. pavarii Pamp.* may possess the ability to impede the growth of cancerous cell lines through many routes; this an effect was observed without any accompanying cytotoxicity towards normal human cells [86]. The antiproliferative activity of *A. pavarii Pamp.* aerial parts on various cancerous cells such as breast (MCF7 and T47D), hepatocarcinoma HepG2, and lung (A549) cancer cells was previously evaluated by the MTT assay, and the results were less than 30 µg/ml [49]. Plant-based NPs have resulted in excellent biological activity with less toxicity to normal cells and are highly toxic to cancerous cells. This makes these NPs a promising candidate for future cancer treatment.

In other studies, regarding cancerous cell lines, it has been indicated that nanoparticles may interact with phosphorus and sulfur-containing compounds, and their interaction may cause damage to DNA and proteins, resulting in cancerous cell death [89].

There is ample proof that ZnO-NPs have anticancer effects. As a result, when ZnO-NPs anti-proliferative effects on several cells were investigated, the MTT assay demonstrated their efficacy [90]. ZnO-NPs have been prepared by Moghaddam et al. in another study, and their anticancer impact on breast cancer was estimated using a novel yeast strain. The cytotoxicity of ZnO-NPs is strong, and the mechanism causing this cytotoxicity is more likely to be apoptosis than cell cycle arrest [91].

According to [92]. Zinc may have this impact through mitochondria by causing a loss of membrane potential and the release of apoptotic proteins such as cytochrome C into the cytosol. Furthermore, ZnO-NPs may have this effect by suppressing antiapoptotic genes like *Bcl-2* and *JERK/2* and/or inducing apoptotic genes like *p21*, *p53*, *JNK*, and *Bax* [82]. ZnO-NPs can trigger the apoptotic signaling cascade and release dissolved zinc ions into cells [83]. Sharma et al. assessed the impact of ZnO-NPs and the possible therapeutic mechanisms using HepG2 human liver cancer cells. In HepG2 cells, ZnO-NPs were observed to enhance cytotoxicity and genotoxicity, which were mediated via reactive oxygen species (ROS). caused malfunction of the mitochondria [93].

Conclusion

Arbutus pavarii Pamp. extract was used in the environmentally friendly production of zinc oxide nanoparticles. The analysis of produced zinc nanoparticles using spectroscopic and

microscopic methods including UV-Visible, SEM, XRD, Zeta potential, FTIR, and EDX, confirms the formation of zinc oxide nanoparticles. ZnO-NPs exhibited excellent antioxidant activity and cytotoxic and anticancer activity and the impact of ZnO-NPs on molecular evaluations. Our results confirm the potential of *A. pavarii* Pamp. for the synthesis of ZnO-NPs in a simple, fast and ecofriendly way. ZnO-NPs were identified by a clear peak in the UV-visible spectrum at about ~410 and 650 nm. The XRD results confirmed the efficiency of the synthesis process of ZnO-NPs, indicating the presence of the characteristic peaks of ZnO-NPs, representing the amorphous phase of ZnO-NPs. While XRD confirmed the amorphous nature of the synthesized ZnO-NPs, SEM analysis demonstrated that the average particle size ranged between 16.67 - 31.01 nm, which was confirmed by XRD and SEM analysis. EDX spectrum results confirmed the presence of zinc and oxygen in the synthesized ZnO-NPs. FTIR studies clearly showed the formation of ZnO-NPs and indicated that the plant extract contains various phytochemicals, which work as capping and stabilizing agents for the synthesized ZnO-NPs. This was confirmed by the Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The findings made it abundantly evident that the precursors were essential to the structure and surface appearance of ZnO-NPs. ZnO-NPs have been shown in our study to have a positive effect as an anticancer agent, and this was proven through cytotoxicity and anticancer properties. Regarding ZnO-NPs, the SEM image shows uniformity in distribution with less aggregations, pointing to a more stable synthesis process that minimizes clustering. The relatively narrow size distribution of Zn-ONPs ranging from 16.67 nm to 31.01 nm suggests effective control over the synthesis parameters, resulting in well-dispersed nanoparticles.

Recommendations:

- 1- Expand the investigation of biogenically synthesized ZnO-NPs from *in vitro* cell line models to *in vivo* animal models to validate their therapeutic safety, pharmacokinetics, and actual antitumor efficacy in living systems.
- 2- Explore the potential formulation of these green-synthesized ZnO-NPs into safe, alternative, and cost-effective pharmaceutical medications for cancer treatment to minimize the severe side effects and high costs associated with conventional chemotherapeutic agents.

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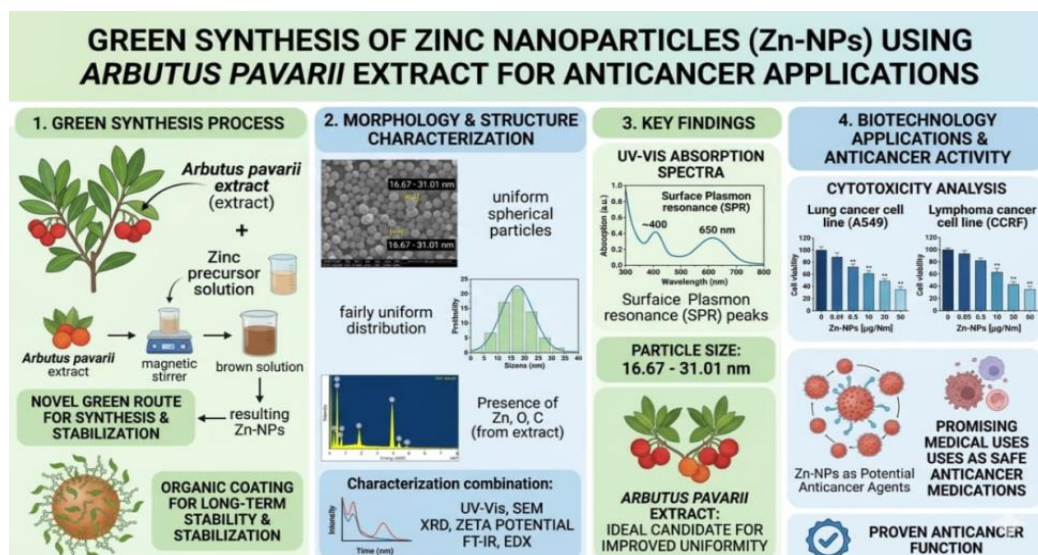


Figure .1 : Green synthesis of Zinc Oxide nanoparticles (ZnO-NPs) from *A.pavarii* Pamp. extract, characterization and application.

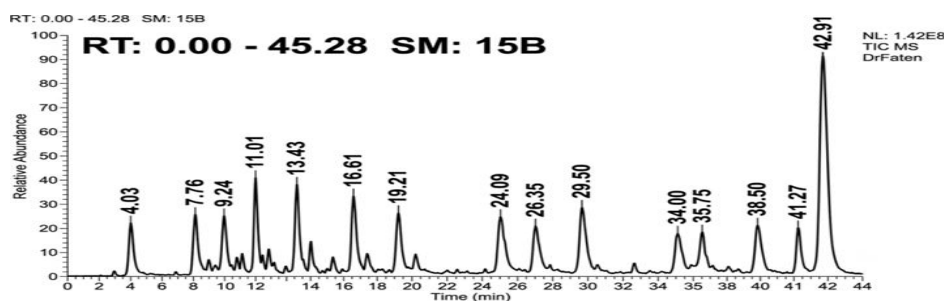


Figure.2 : The most abundant compounds of *A. pavarii* Pamp. extract using GC-MS.

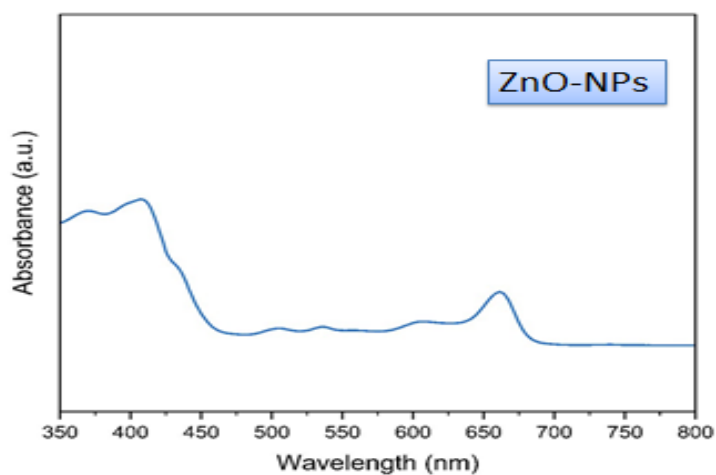


Figure.3: UV-Visible spectroscopic analysis of *A. pavarii* Pamp. greenly synthesized nanoparticles ZnO-NPs .



Figure .4:Morphological and particle characterization of the prepared ZnO-NPs illustrated by SEM analysis.

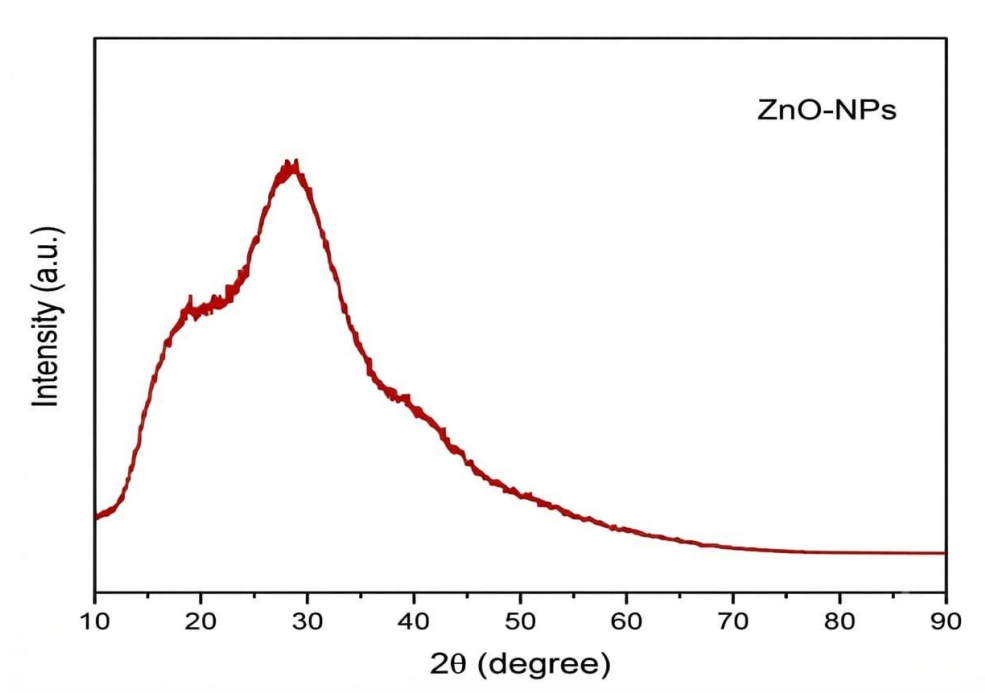


Figure .5 : XRD spectrum of *A. pavarii* Pamp. greenly synthesized nanoparticles ZnO-NPs.

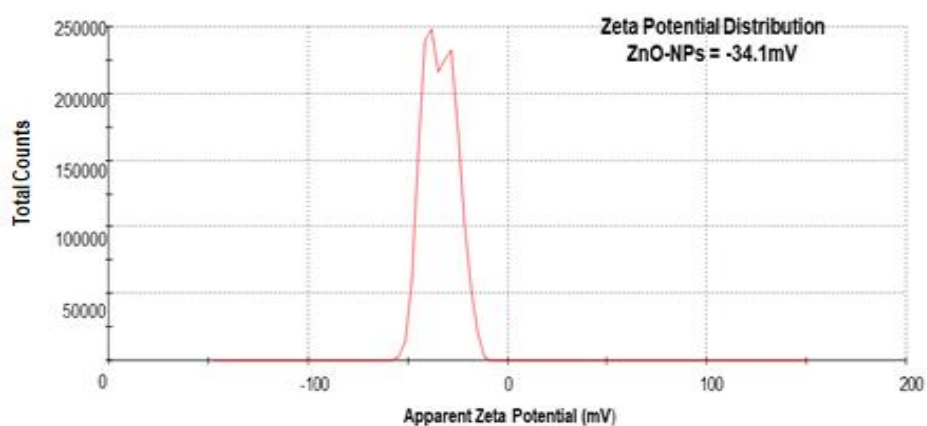


Figure.6 : The particles surface charge (Zeta potential) of *A. pavarii* Pamp. greenly synthesized nanoparticles ZnO-NPs.

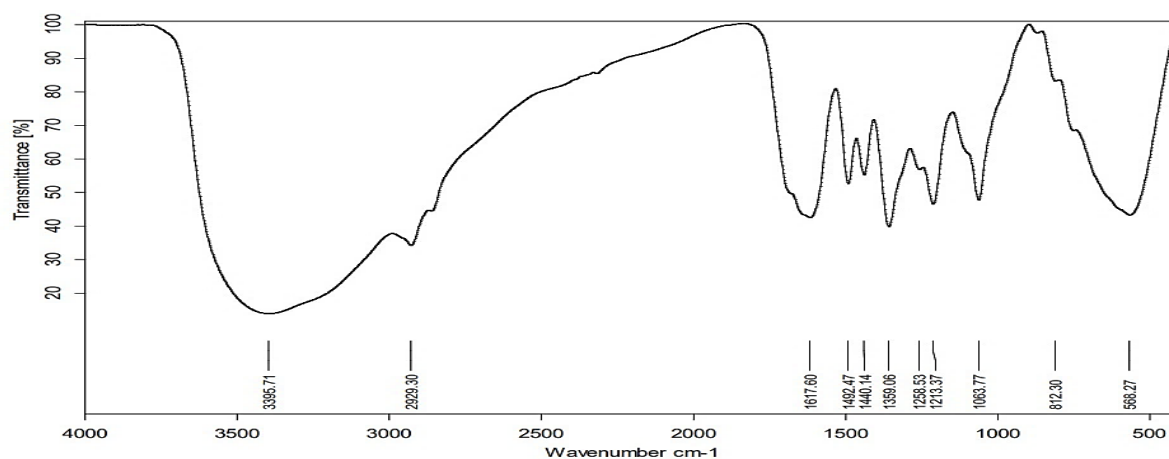


Figure. 7: Fourier transform infrared spectra (FT-IR) of *A. pavarrii* Pamp. green synthesized nanoparticles ZnO-NPs.

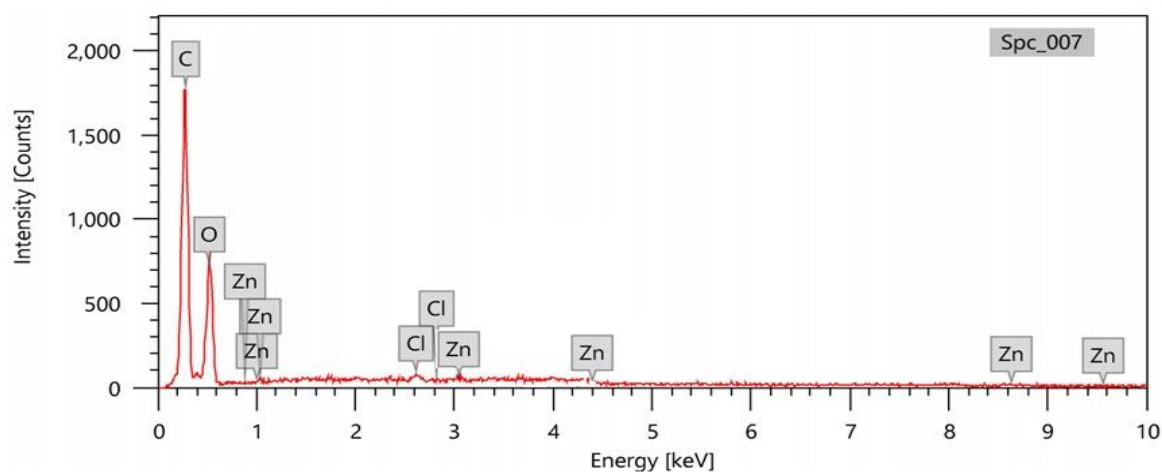


Figure. 8 : Energy-dispersive X-ray spectroscopy (EDX) data of green synthesized: ZnO-NPs derived from *A. pavarrii* Pamp extract.

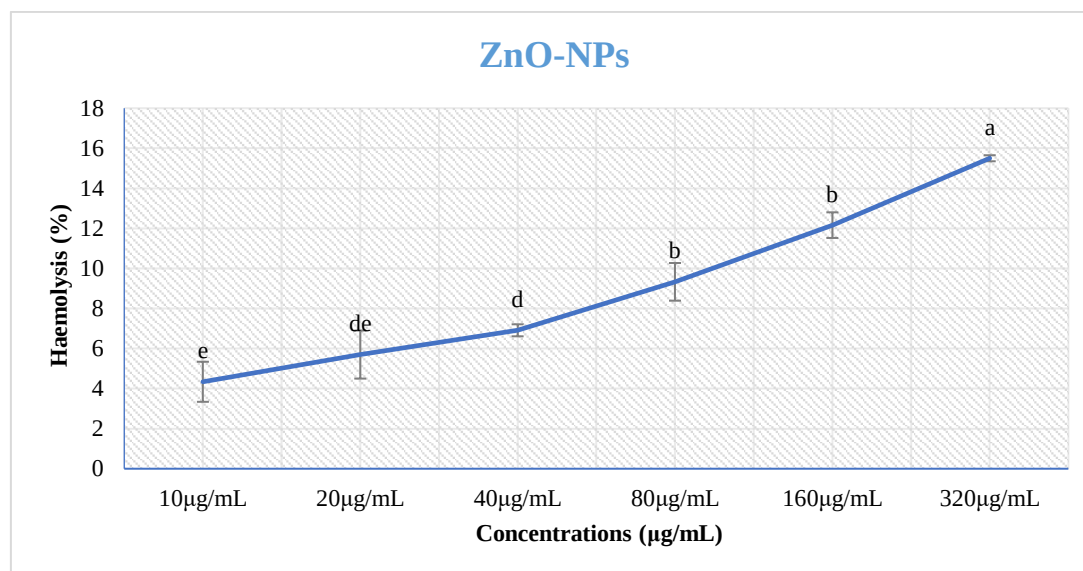


Figure. 9 : The effect of different concentrations (320, 160, 80, 40, 20 and 10 µg/ml) of *A. pavarrii* Pamp., synthesized ZnO-NPs on hemolysis of human RBCs. Data are means of three different replicates $\pm$ standard deviations SD. Different letters on row illustrate statistically significant variations at $P \leq 0.05$.

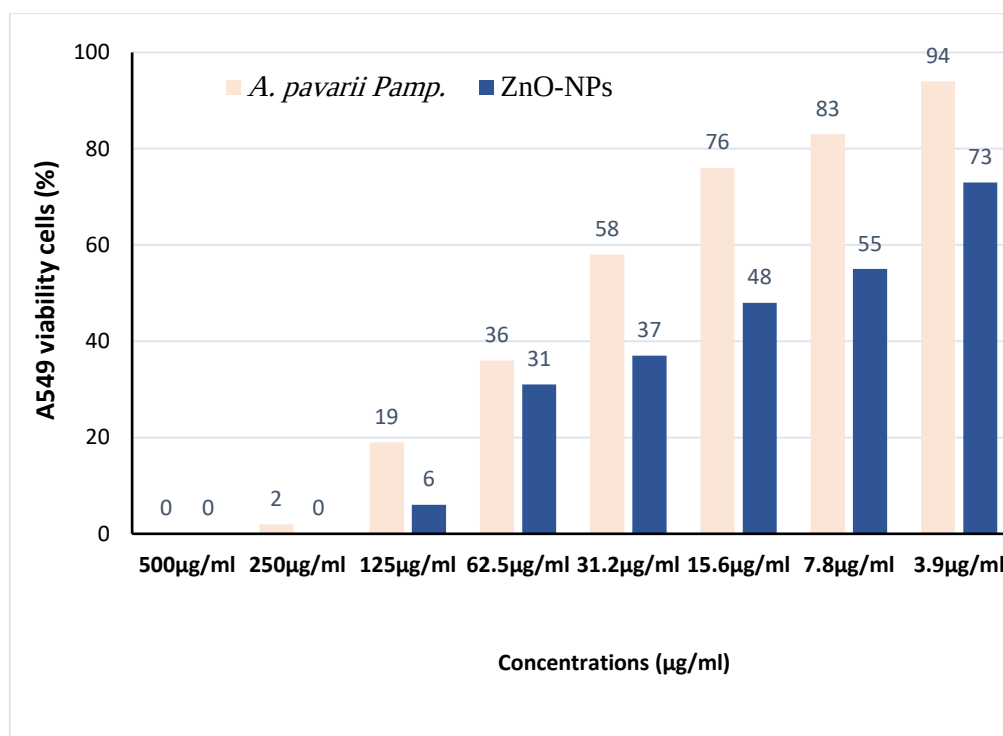


Figure.10: The effect of different concentrations (500, 250, 125, 62.5, 31.2, 15.6, 7.8 and 3.9 µg/ml) of *A. pavarrii pamp.*, synthesized ZnO-NPs on viability of lung (A549) cell line. Data are means of three different replicates $\pm$ standard deviations SD. Different letters on column illustrate statistically significant variations at $P \leq 0.05$

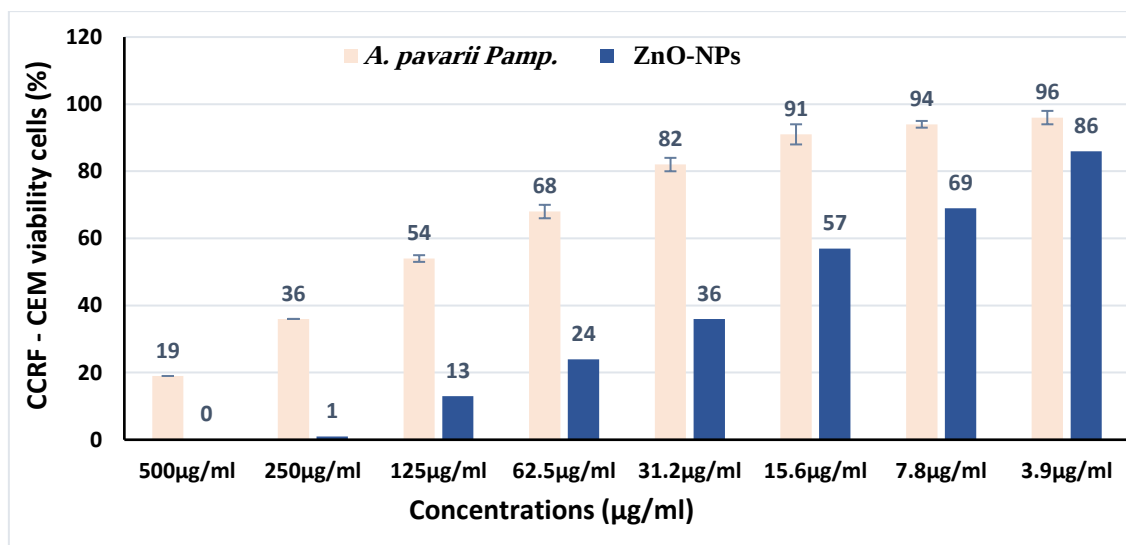


Figure.11: The effect of different concentrations (500, 250, 125, 62.5, 31.2, 15.6, 7.8 and 3.9 µg/ml) of *A. pavarrii Pamp.*, synthesized ZnO-NPs on viability of lymphoblastic leukemia (CCRF-CEM) cell line. Data are means of three different replicates $\pm$ standard deviations SD. Different letters on column illustrate statistically significant variations at $P \leq 0.05$.

Table 1: Phytochemical screening of different solvents of *A. pavarrii Pamp.* leaves extract (methanol, diethyl ether, ethanol, and acetone) active compounds

Parameters	Solvent				Unit
	Methanol	Diethyl ether	Ethanol	Acetone	
DPPH	83.41 $\pm$ 0.73 ^a	79.03 $\pm$ 1.51 ^c	82.30 $\pm$ 0.41 ^{ab}	80.70 $\pm$ 1.41 ^{bc}	%
FRP	31.72 $\pm$ 0.62 ^a	8.11 $\pm$ 0.51 ^c	30.40 $\pm$ 1.41 ^a	20.10 $\pm$ 0.51 ^b	mg/g DM
PMA	46.90 $\pm$ 1.11 ^a	12.42 $\pm$ 0.11 ^d	37.31 $\pm$ 1.13 ^b	32.61 $\pm$ 1.01 ^c	

Phenolic	208.51±7.61 ^a	142.81±2.20 ^c	159.21±10.0 ^b	153.12±8.51 ^{bc}	
Flavonoids	11.60±0.12 ^c	5.22±0.41 ^d	14.71±0.42 ^a	13.93±0.72 ^b	
Ascorbate	12.30±2.50 ^a	7.22±2.02 ^b	11.61±1.91 ^a	4.12±0.73 ^b	
Saponin	44.61±4.80 ^a	28.11±1.81 ^c	37.73±1.32 ^b	31.21±1.61 ^c	
Tannins	99.42±2.10 ^a	44.90±3.00 ^d	72.21±3.71 ^b	56.91±4.20 ^c	

Table 2: The most abundant compounds of *A. pavarii* Pamp. extract using GC-MS.

No	RT (min)	Area (%)	Compound	Molecular formula
1	18.85	7.74	Melezitose	C ₁₈ H ₃₂ O ₁₆
2	19.16	10.01	Melezitose	C ₁₈ H ₃₂ O ₁₆
3	19.21	11.21	Melezitose	C ₁₈ H ₃₂ O ₁₆
4	24.09	1.43	17-Octadecynoic acid	C ₁₈ H ₃₂ O ₂
5	26.35	8.09	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂
6	29.50	7.97	(Z,Z,Z)-9,12,15-octadecadienoic acid 2,3-dihydroxy propyl ester	C ₂₁ H ₃₆ O ₄
7	29.99	4.49	octadecadienoic acid	C ₁₈ H ₃₆ O ₂
8	40.10	1.29	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(isopropyl)	C ₂₀ H ₃₄ O ₂
9	41.27	3.12	Arabinitol, pentaacetate	C ₁₅ H ₂₂ O ₁₀
10	42.91	16.57	Vitamin E	C ₂₉ H ₅₀ O ₂
11	44.71	7.15	Betulin	C ₃₀ H ₅₀ O ₂
12	44.89	19.95	á-Amyrin	C ₃₀ H ₅₀ O
13	45.27	0.98	Lupeol	C ₃₀ H ₅₀ O

Table 3 : Phytochemical constituents and activity of synthesized nanoparticles. Data are means (n=3) ± SD.

Parameters	Zinc Oxide Nanoparticles (ZnO-NPs)	Unit
DPPH	70.71±1.03 ^b	%
FRP	10.12±0.22 ^c	mg/g DM
PMA	14.62±1.02 ^b	
Phenolic	56.13±1.53 ^b	
Flavonoids	9.93±0.32 ^a	
Ascorbate	4.11±0.71 ^c	
Saponin	11.22±0.01 ^b	
Tannins	16.92±2.21 ^b	

Note: Values are expressed as mean ± SD (n=3). Different superscript letters (b,c) within the same column indicate significant differences at P < 0.05 according to Duncan's multiple range test.

Table 4 : The effect of different concentrations (320, 160, 80, 40, 20 and 10 µg/mL) of *A. pavarii* Pamp., synthesized ZnO-NPs on hemolysis of human RBCs. Data are means of three different replicates ± standard deviations SD.

In vitro hemolytic activity and (IC₅₀) value (Cytotoxicity on RBCs)

Concentration (µg/mL)	Hemolysis %
10	4.34±1.85
20	5.70±2.25
40	6.91±2.30

80	9.33±1.94
160	12.16±1.64
320	15.50±0.15
IC₅₀ (µg/mL)	14.8 µg/mL

IC₅₀: sample concentration that causes 50% hemolysis of RBCs. The data are expressed as mean ± SEM (n=3).