

Antioxidant dynamics in *Cajanus cajan* (L.) and *Melaleuca leucadendra* (L.): from plants to nanoparticles

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Abstract. This study marks a significant stride in nanotechnology, particularly in catalyst development, focusing on magnesium oxide nanoparticles and plant extracts from *Cajanus cajan* (L.) and *Melaleuca leucadendra* (L.), noted for their antioxidant properties. We aimed to unravel how these substances, both as traditional extracts and as nanoparticles, perform in various applications due to their antioxidant potential. Utilizing DPPH and FRAP assays, the research revealed that Ascorbic acid consistently exhibited strong antioxidant capabilities, serving as a reliable benchmark. Interestingly, *Cajanus cajan* (L.) and *M. leucadendra* (L.) extracts varied in their antioxidant effectiveness. A key finding was the pronounced increase in antioxidant efficacy when these extracts, particularly from *Cajanus cajan* (L.), were transformed into nanoparticles, as reflected in elevated FRAP values. This observation underscores the potential of nanoparticles to significantly enhance the effectiveness of plant extracts. The implications of this advancement are far-reaching, opening new avenues in the pharmaceutical and nutraceutical industries for developing therapeutic agents and antioxidant-rich foods. This research contributes notably to pharmaceutical sciences, emphasizing the vital role of nanoparticle technology in enhancing the antioxidant qualities of plant-based substances. It lays a solid foundation for further exploration into the mechanisms underlying nanoparticle-mediated improvements, offering valuable insights into the application of nanotechnology in health and nutrition.

1 Introduction

Nanotechnology's integration into advanced catalysts, especially at the nanoscale, marks a significant area of scientific and technological advancement. Magnesium oxide (MgO) nanoparticles stand out for their economic efficiency and effectiveness in facilitating organic transformations [1].

Characterized by their unique properties, such as high reactivity, crystalline structures, and large surface areas, these nanoparticles, including metal and metal oxides, are vital in various applications. Magnesium oxide's broad band gap lends itself to diverse uses from

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catalysis and hazardous waste detoxification to the production of refractory materials, heavy fuel oil additives, and superconducting and ferroelectric thin films. It's also used in developing reflective coatings and lithium-ion batteries [2]. Additionally, the eco-friendly synthesis of MgO nanoparticles using plant extracts is gaining industrial prominence [3].

Cajanus cajan (L.) Millsp., commonly known as pigeon pea, and *Melaleuca leucadendra* (L.), known as cajeput, are two plant species endowed with a plethora of medicinal properties and are frequently utilized in therapeutic practices. Pigeon pea, thriving over 4.3 million hectares in Asia and accounting for a yield of approximately 3.3 million tons annually [4], is distinguished as the sixth most extensively cultivated legume worldwide. The pigeon pea is particularly enriched with polyphenols, notably flavonoids, which are celebrated for their antioxidant capabilities, thus positioning it as a rich source of these advantageous compounds.

Research highlights *Cajanus cajan* (L.)'s broad range of health benefits, such as antioxidant [5], anti-inflammatory [6], glucose-modulating, and antimicrobial properties [7], making it suitable for medical and health-promoting food products. In traditional medicine, it's used to treat various conditions including respiratory and reproductive infections, dysentery, diabetes, hepatitis, and menstrual disorders [8].

Cajeput oil, extracted from *Melaleuca leucadendra* (L.) leaves, is widely used in health and personal care, including herbal medicine, cosmetics, and for its pharmacological benefits. Analytical studies reveal that monoterpenes are its main component [9], and it is known for antibacterial, antifungal, antioxidant, and soothing properties.

Plants with compounds like carotenoid derivatives, phenolics, flavonoids, and vitamins are crucial for their antioxidant properties, helping prevent and treat diseases caused by oxidative stress [10]. Research confirms the vital role of antioxidants in maintaining human health by reducing oxidative stress [11,12].

Antioxidants, categorized as enzymatic or nonenzymatic (Figure 1), play a crucial role in countering oxidative processes caused by free radicals, resulting in inert compounds [10]. They work through various methods, including catalyzing radical decomposition, acting as reducing agents, and chelating metals. Flavonoid antioxidants, known for their hydroxyl (-OH) groups, include catechins, carotenoids, β-carotene, lycopene, and diterpenes.

This study effectively uses DPPH and FRAP assays to measure antioxidant capacities, highlighting their reliability, ease of use, and quick results [10,13]. The aim is to investigate the antioxidant activity of *Cajanus cajan* (L.) and *Melaleuca leucadendra* (L.) extracts, both before and after nanoparticle treatment."

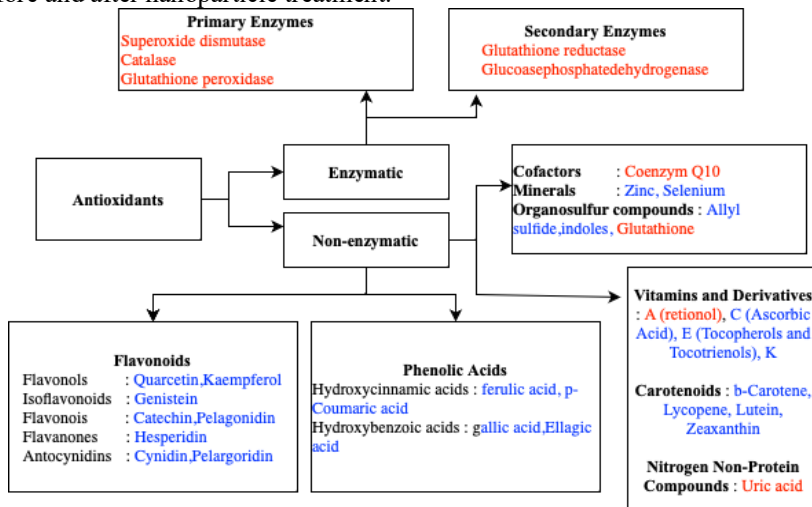


Fig. 1. Classification of antioxidants: Blue words represent exogenous antioxidants, while Red ones represent endogenous antioxidants [10,14].

2 Materials and method

2.1 Preparation of seeds of *Cajanus cajan* (L.) and Leaf *M. Leucadendra* (L.)

The seed samples of *Cajanus cajan* (L.) were sourced from Rote Island's forests in East Nusa Tenggara, and leaf specimens of *M. Leucadendra* (L.) were acquired from the Palangkaraya forests in Central Kalimantan (Figure 2a-b). These samples underwent a rigorous cleaning protocol to eliminate any contaminants. Post-cleaning, they were exposed to an ambient air-drying process under shade, crucial for preserving their natural biochemical constituents. The dried samples were then methodically ground into a fine powder using an advanced powder milling apparatus. For the extraction phase, 10 grams of this powdered material was immersed in 250 ml of a 75% ethanolic aqueous solution, maintaining a 1:1 volumetric ratio, for a period of five days. The resultant concoction was then filtered using a Buchner funnel equipped with Whatman No-1 filter paper and subsequently concentrated under reduced pressure in a BUCHI R210 vacuum concentrator. To finalize the process, the concentrated extract was lyophilized using a freeze-drying system, resulting in a fine powder that retains the plant's essential characteristics, crucial for further application in nanoparticle synthesis (Figure 2c-g).

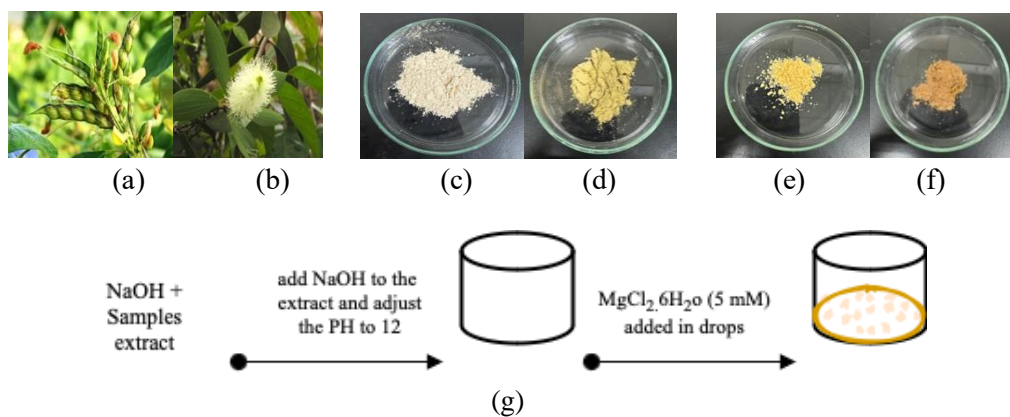


Fig. 2. (a) *Cajanus cajan* (L.) (b) *M. Leucadendra* (L.) (c) Powder seeds of *Cajanus cajan* (L.) (d) Powder leaf *M. Leucadendra* (L.) (e) freeze dried extract Seeds of *Cajanus cajan* (L.) (f) freeze dried extract leaf *M. Leucadendra* (L.) (g) flow chart of green synthesis.

2.2 Preparation of Mg nanoparticles using seeds of *Cajanus cajan* (L.) and leaf of *M. leucadendra* (L.)

In our research, we have refined the protocol for Mg nanoparticle green synthesis, originally proposed by John Sushma, (2016) [15]. Our method incorporates extracts from *Cajanus cajan* (L.) seeds and *M. leucadendra* (L.) leaves. The synthesis began with the dissolution of 100 mg of the plant's ethanolic extract in 30 ml of double-distilled water. We then adjusted the solution's pH to 12. Next, we slowly added 20 ml of a 5 mM MgCl₂ solution. This step resulted in a pale-yellow solution, signalling the formation of Mg nanoparticles. Nanoparticle isolation was achieved by centrifuging the solution at 10,000 rpm for a period of 10 minutes. Following this, the nanoparticles underwent two ethanol washes, ensuring their effective

purification. Finally, we resuspended the clean nanoparticles in double-distilled water, preparing them for in-depth analysis. This modified approach not only enables the efficient production of Mg nanoparticles but also effectively combines the therapeutic potentials of *Cajanus cajan* (L.) and *M. leucadendra* (L.) within the nanoparticles.

2.3 Characterization of Mg nanoparticles

We prepared Mg nanoparticles from *Cajanus cajan* (L.) seed extracts and *M. leucadendra* (L.) leaves and then applied various physical and chemical methods for their characterization. We captured the absorption spectra of these MgO nanoparticles using a UV-Visible Spectrophotometer (Perkin Elmer, USA) in the 200-800 nm range. To identify the functional groups in these particles, we employed FT-IR spectroscopy (Perkin Elmer, USA), analyzing the 400-4000 cm⁻¹ wave number range. These measurements, conducted at a resolution of 4 cm⁻¹ through 64 scans in transmission mode, were carried out under standard environmental conditions. This approach is pivotal for a comprehensive understanding of the nanoparticles' properties, a crucial aspect in pharmaceutical research.

2.4 1,1,-Diphenyl-2-picrylhydrazyl (DPPH) Assay

Our research rigorously explored the antioxidant effects of *Cajanus cajan* (L.) and *M. leucadendra* (L.) extracts, utilizing the DPPH assay for its proven efficacy in evaluating radical scavenging properties. For this purpose, we methodically prepared multiple samples of the extracts, each measuring 500 µl, at diverse concentrations for comprehensive analysis. These were then methodically combined with an equal volume of a 0.3 mM ethanolic DPPH solution for further analysis. This mixture was then incubated for 30 minutes in a dark environment. Post-incubation, absorbance readings were taken at 517 nm, using a water: ethanol (1:1) solution as the baseline and the DPPH solution as the control. The extracts' antioxidant activities were quantified by calculating the inhibition percentage, a crucial step in determining their potential efficacy in radical scavenging, a significant aspect in pharmaceutical and chemical research.

$$\text{Inhibition \%} = (1 - (A \text{ Sample})/A_0) \times 100 \% \quad (1)$$

In this formula, A(sample) represents the absorbance of the *Cajanus cajan* (L.) extract sample, while A₀ denotes the absorbance of the DPPH solution.

2.5 Ferric Ion Reducing Antioxidant Power (FRAP) assay

To accurately measure the antioxidant potential of *Cajanus cajan* (L.) and *M. leucadendra* (L.) extracts, our team employed the FRAP assay, aligning with the method established by Sivapalan, Dharmalingam, Ashokkumar, Venkatesan, and Angappan (2024) [16]. We rigorously tested the extracts, with concentrations varying from 1.25 to 20 µg/ml, against a precisely formulated FRAP solution. This solution consisted of a mixture of acetate buffer, TPTZ, and FeCl₃.6H₂O in a ratio of 10:1:1, ensuring a standardized approach for reliable results. For each evaluation, we mixed 60 µL of the extract with 1800 µL of the FRAP solution. This mixture was then incubated for 5 minutes at room temperature in a dark environment. We measured the absorbance at 593 nm, using water as a control for background readings. To quantify the antioxidant effectiveness of the extracts, we compared their absorbance levels with those of ascorbic acid, our chosen standard, thus assessing their ability to reduce ferric ions.

$$\text{FA} = ((A_{\text{std}} - A_{\text{blnk}})/A_{\text{std}}) \times 100 \% \quad (2)$$

2.6 Statistical test

The data analysis techniques used in this study comprise the independent samples t-test and the paired-samples t-test, with significance levels indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and a sample size of $N=3$ for each group. The independent samples t-test is a statistical method designed to compare the means of two unrelated groups. These groups are considered unrelated because the study involves distinct sample subjects. The fundamental principle of this test is to evaluate the differences in variations between the two data groups.

3 Results

3.1 Spectroscopic characterization of the Mg nanoparticles

3.1.1 UV-Visible spectrophotometry test of Mg nanoparticles (Left: C.C., Right: M.L.)

The absorption spectrum of MgO nanoparticles was measured using a UV-Visible spectrophotometer across the 200-800 nm range. As shown in Figure 3, distinct absorption peaks at 202 nm, 217 nm, and notably 275 nm confirm the successful synthesis of the nanoparticles. The peak at 275 nm is particularly significant, aligning with the findings of Sarfraz et al. (2023), who associate this wavelength with antioxidant activity, thereby supporting the antioxidative potential of the synthesized Mg nanoparticles [17].

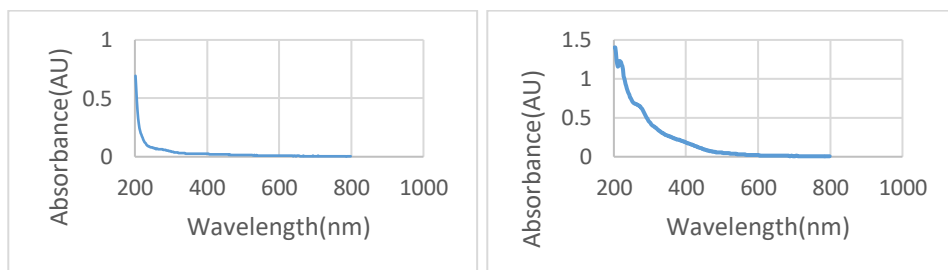


Fig. 3. Spectroscopic characterization of Mg nanoparticles.

3.1.2 FT-IR spectrophotometry Test of Mg Nanoparticles (Left: C.C., Right: M.L.)

FT-IR spectroscopy is a suitable technique to establish the adsorption affinity towards the surface of MgO Nanoparticles. The spectra of MgO, *Cajanus cajan* (L.) and *M. Leucadendra* (L.) are clearly illustrated in the Figure 4. The graph for *Cajanus cajan* (L.) and *M leucadendra* (L.) shows frequency ranges from 3000-3500, likely indicating the presence of amines and amides with O-H bonds. In the frequency range of 1000-1500 with strong intensity, the likely compound types are alcohols, ethers, carboxylic acids, and esters, characterized by C=O bonds [18].

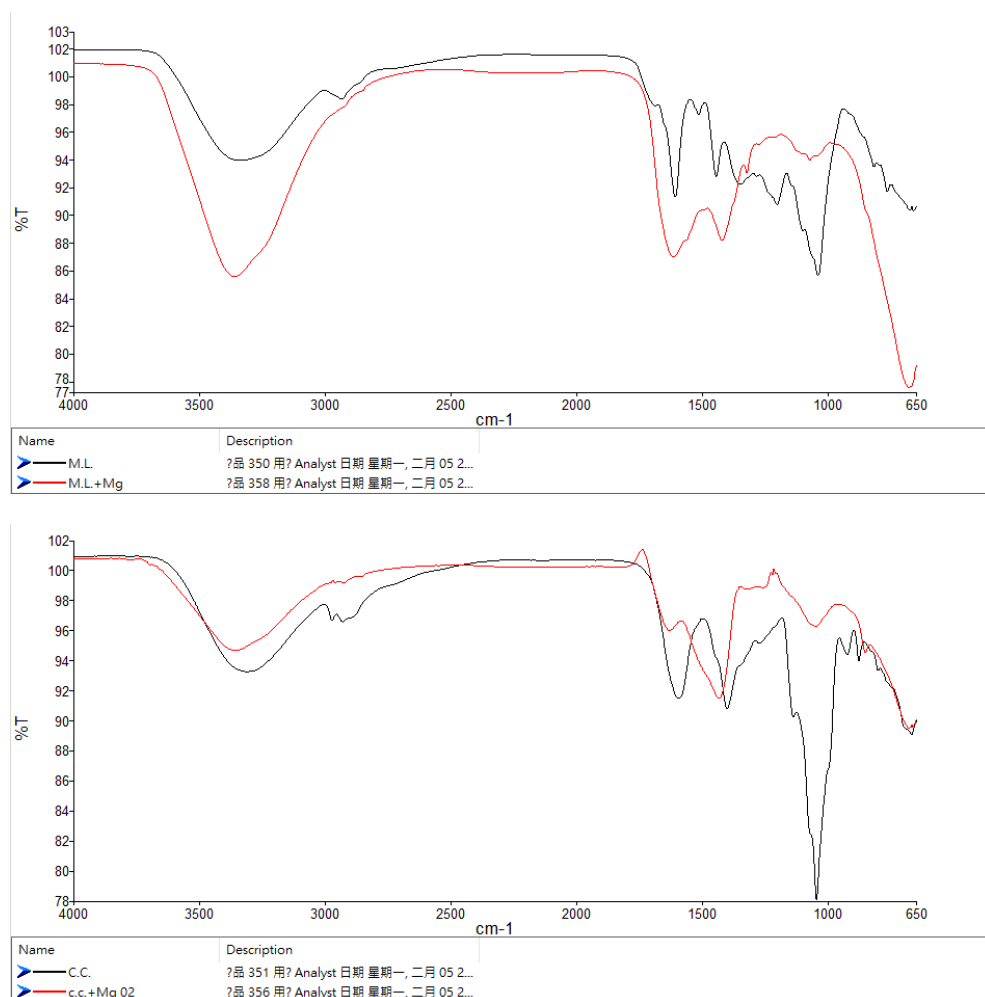


Fig. 4. Spectroscopic Characterization of Mg Nanoparticles (Top: black line *M. Leucadendra* (L.), red line *M. leucadendra* (L.) + Mg Nanoparticles, Bottom: black line *Cajanus cajan* (L.), red line *Cajanus cajan* (L.) + Mg Nanoparticles).

3.2 DPPH scavenging activity

3.2.1 Ascorbic acid, *Cajanus cajan* (L.) and *M. Leucadendra* (L.)

This study aimed to evaluate the effectiveness of different plant extracts, including Ascorbic acid, *M. leucadendra* (L.), and *Cajanus cajan* (L.), in combating free radicals, known for their damaging effects on cells. Notably, Ascorbic acid, renowned for its antioxidant capabilities, showed considerable effectiveness in neutralizing these harmful molecules. Concentrations of 10 $\mu\text{g}/\mu\text{l}$ and 20 $\mu\text{g}/\mu\text{l}$ were used in these assessments. It neutralized approximately 49% of free radicals at the lower concentration and exceeded 80% effectiveness at the higher concentration. This data underscores the robust antioxidant activity of Ascorbic acid in varying concentrations. The consistency of these results was quite precise, with very little variation. *Melaleuca leucadendra* (L.) also demonstrated good antioxidant activity, cutting down free radicals by about 27% at the lower dose and over 52%

at the higher one, although with slightly more variation at the higher concentration. *Cajanus Cajan*, however, was less effective, showing only about a 10% reduction at both concentrations (table 1), and its results varied more, especially at the higher dose. These observations give us a clearer understanding of how these different plant extracts perform in fighting oxidative stress.

Table 1. DPPH scavenging activity of ascorbic acid, *M. leucadendra* (L.) and *Cajanus cajan* (L.) in concentration 10-20 µg/µl.

Sample	DPPH Scavenging activity (%)			
	Concentration 10 (µg/µl)	STD	Concentration 20 (µg/µl)	STD
Ascorbic acid	48.79	0.001	80.61	0.004
<i>M. leucadendra</i> (L.)	26.85	0.001	52.52	0.088
<i>Cajanus Cajan</i> (L.)	9.86	0.001	10.56	0.148

Our research evaluated Magnesium chloride and Magnesium Nano Particles from *M. leucadendra* (L.) and *Cajanus Cajan* (L.) for neutralizing DPPH radicals at 10 µg/µl and 20 µg/µl concentrations (table 2). Magnesium chloride showed limited effectiveness, scavenging 2.14% and 4.67% of radicals at these concentrations, respectively, with consistent results. In contrast, Magnesium Nano Particles from *M. leucadendra* (L.) and *Cajanus Cajan* (L.) were more effective, with scavenging rates of 9.44% and 12.8%, and 5.07% and 5.93%, respectively, demonstrating greater antioxidant capacity.

Table 2. DPPH scavenging activity of magnesium chloride, Mg nano particles (*M. leucadendra* (L.)) and Mg nano particles (*Cajanus cajan* (L.)) in concentration 10-20 µg/µl.

Sample	DPPH Scavenging activity (%)			
	Concentration 10 (µg/µl)	STD	Concentration 20 (µg/µl)	STD
Magnesium chloride	2.14	0.001	4.67	0.201
Mg Nano Particles (<i>M. Leucadendra</i> (L.))	9.44	0.001	12.8	0.17
Mg Nano Particles (<i>Cajanus cajan</i> (L.))	5.07	0.001	5.93	0.145

This study employed statistical analysis, specifically an independent samples test, to compare the antioxidant effects before and after nanoparticle treatment in the DPPH assay. The test yielded a significance value of 0.004 and a mean difference of 31.523, indicating a substantial difference between the pre- and post-nanoparticle conditions.

After conducting Levene's test as shown in Table 3, we proceeded with a paired-samples T-test to assess the differences between individual groups, using the three optical density (OD) readings as data points. The significance levels were marked as *P < 0.05, **P < 0.01, ***P < 0.001, as detailed in Figure 5. This analysis focused on the antioxidant activities of *Melaleuca leucadendra* (L.) and *Cajanus Cajan* (L.), in both extract and nanoparticle forms, at concentrations of 10 and 20 µg/µl. While *Melaleuca leucadendra* (L.) exhibited an increase in antioxidant activity with higher concentrations, the extract from *Cajanus Cajan*

(L.) showed no significant changes. However, its antioxidant activity significantly increased when formulated as nanoparticles.

Table 3. Independent Samples Test for DPPH Assay of Extract and Nanoparticles.

		Levene's test for equality of variances		t-test for equality of means		
		F	Sig.	Sig.(2-tailed)	Mean Difference	Std. error difference
Concentration	Equal variances assumed	14.049	.004	.020	31.523	11.379
	Equal variances not assumed			.038	31.523	11.379

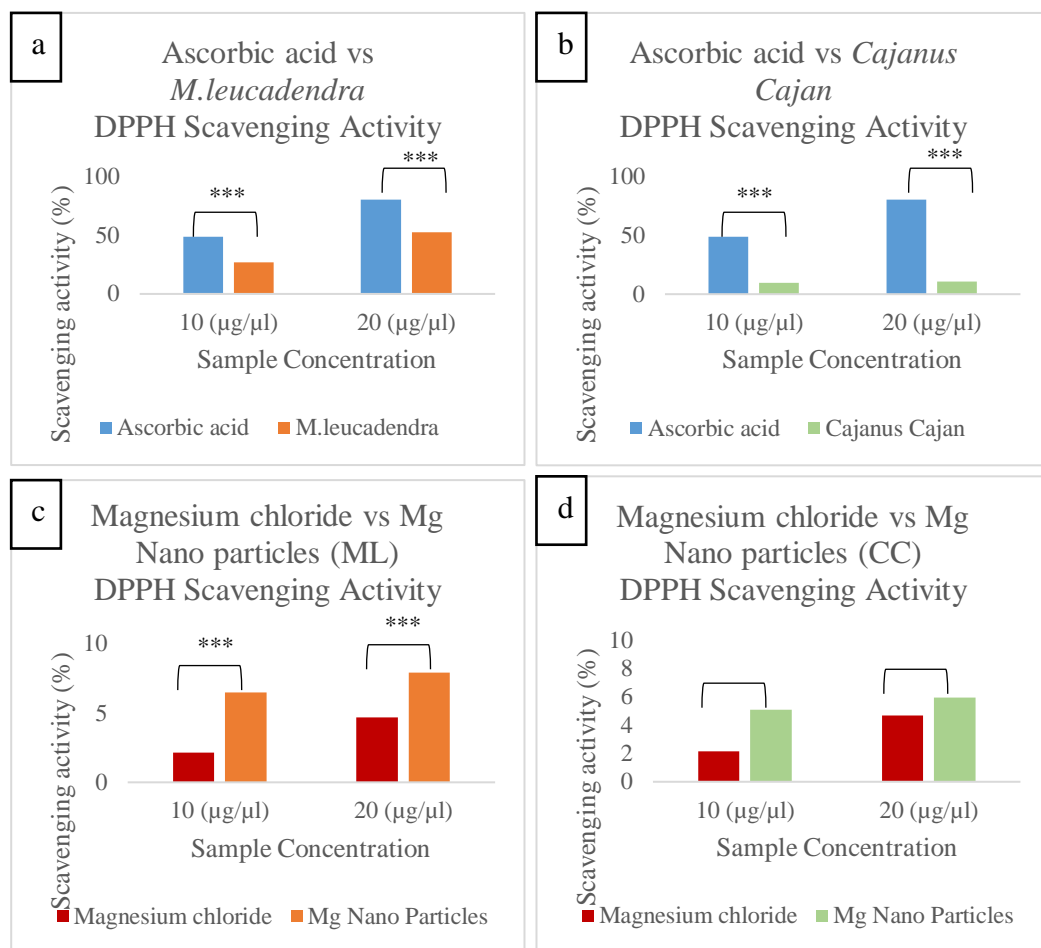


Fig. 5. DPPH scavenging activity of Ascorbic acid vs *M.leucadendra* (L.) extract (a), Ascorbic acid vs *Cajanus Cajan* (L.) extract (b), Magnesium Chloride vs Mg Nano Particles *M.leucadendra* (L.) (c) and Magnesium Chloride vs Mg Nano Particles *Cajanus Cajan* (L.) (d). Statistically significant different when *P < 0.05, **P < 0.01, ***P < 0.001.

3.3 FRAP Scavenging Activity

This analysis evaluates the FRAP values of substances at 10 and 20 µg/µl concentrations, highlighting Ascorbic acid as the most effective with 53.99% and 72.5% efficacy at these concentrations, respectively, and consistent standard deviations of 0.001. *Melaleuca leucadendra* (L.) showed moderate effectiveness (29.01% at 10 µg/µl and 43.35% at 20 µg/µl), while *Cajanus Cajan* (L.) had lower values (11.11% and 15.79% at the respective concentrations), all with precise standard deviations (table 4). This data positions Ascorbic acid as the most potent antioxidant among the substances tested.

Table 4. FRAP scavenging activity of ascorbic acid, *M. leucadendra* (L.) and *Cajanus cajan* (L.) in concentration 10-20 µg/µl

Sample	FRAP Value (%)			
	Concentration 10 (µg/µl)	STD	Concentration 20 (µg/µl)	STD
Ascorbic acid	53.99	0.001	72.5	0.001
<i>M. leucadendra</i> (L.)	29.01	0.001	43.35	0.001
<i>Cajanus Cajan</i> (L.)	11.11	0.001	15.79	0.001

Our research used the FRAP method to analyze the antioxidant capacities of various samples at 10 µg/µl and 20 µg/µl concentrations. Magnesium chloride demonstrated moderate antioxidant capacity, with FRAP values of 8.76% at the lower concentration and 9.62% at the higher. The results, presented with their standard deviations (0.001 for both concentrations), underscore each sample's precise antioxidant capability and the consistency of the findings.

Magnesium Nano Particles from *Melaleuca Leucadendra* (M. Leucadendra (L.)) exhibited moderate antioxidant activity with FRAP values of 6.5% at 10 µg/µl and 7.94% at 20 µg/µl, both with a consistent standard deviation of 0.001. In comparison, Magnesium Nano Particles from *Cajanus Cajan* (L.) showed significantly higher antioxidant strength, with FRAP values of 17.7% at 10 µg/µl and 30.08% at 20 µg/µl (table 5). These consistent results, demonstrated across the varying concentrations, indicate the superior antioxidant effectiveness of *Cajanus Cajan* (L.) Magnesium Nano Particles, followed by *M. Leucadendra* (L.) and magnesium chloride, with minimal standard deviations emphasizing the reliability of the data.

Table 5. FRAP scavenging activity of magnesium chloride, Mg nano particles (*M. leucadendra* (L.)) and Mg nano particles (*Cajanus cajan* (L.)) in concentration 10-20 µg/µl

Sample	FRAP Value (%)			
	Concentration 10 (µg/µl)	STD	Concentration 20 (µg/µl)	STD
Magnesium chloride	8.76	0.001	9.62	0
Mg Nano Particles (<i>M. Leucadendra</i> (L.))	6.5	0.001	7.94	0
Mg Nano Particles (<i>Cajanus Cajan</i> (L.))	17.7	0.001	30.08	0.001

We conducted an independent samples test to evaluate whether there was a significant difference in the FRAP assay before and after nanoparticle treatment. The analysis yielded an F-value of 5.968, a mean difference of 24.191, and a significance value of <0.035 (table 6). This indicates a significant difference between the pre- and post-nanoparticle conditions.

Table 6. Independent Samples Test for FRAP Assay of Extract and Nanoparticles

		Levene's test for equality of variances		t-test for equality of means		
		F	Sig.	Sig.(2-tailed)	Mean Difference	Std.error difference
Concentration	Equal variances assumed	5.968	.035	.041	24.191	10.296
	Equal variances not assumed			.054	24.191	10.296

Following Levene's test as presented in Table 6, we continued with a paired-samples T-test to assess the differences between individual groups using the three optical density (OD) readings as data points, with significance levels indicated as *P < 0.05, **P < 0.01, ***P < 0.001, as shown in Figure 6. This analysis measured the FRAP values for *Melaleuca leucadendra* (L.) and *Cajanus Cajan* (L.) in both extract and nanoparticle forms at concentrations of 10 and 20 µg/µl. *Melaleuca leucadendra*'s antioxidant activity increased with concentration but was less effective than Ascorbic acid, the positive control. However, in nanoparticle form, it outperformed magnesium chloride. In contrast, the extract of *Cajanus Cajan* (L.) showed no significant change in antioxidant activity at either concentration, but its performance improved significantly when in nanoparticle form.

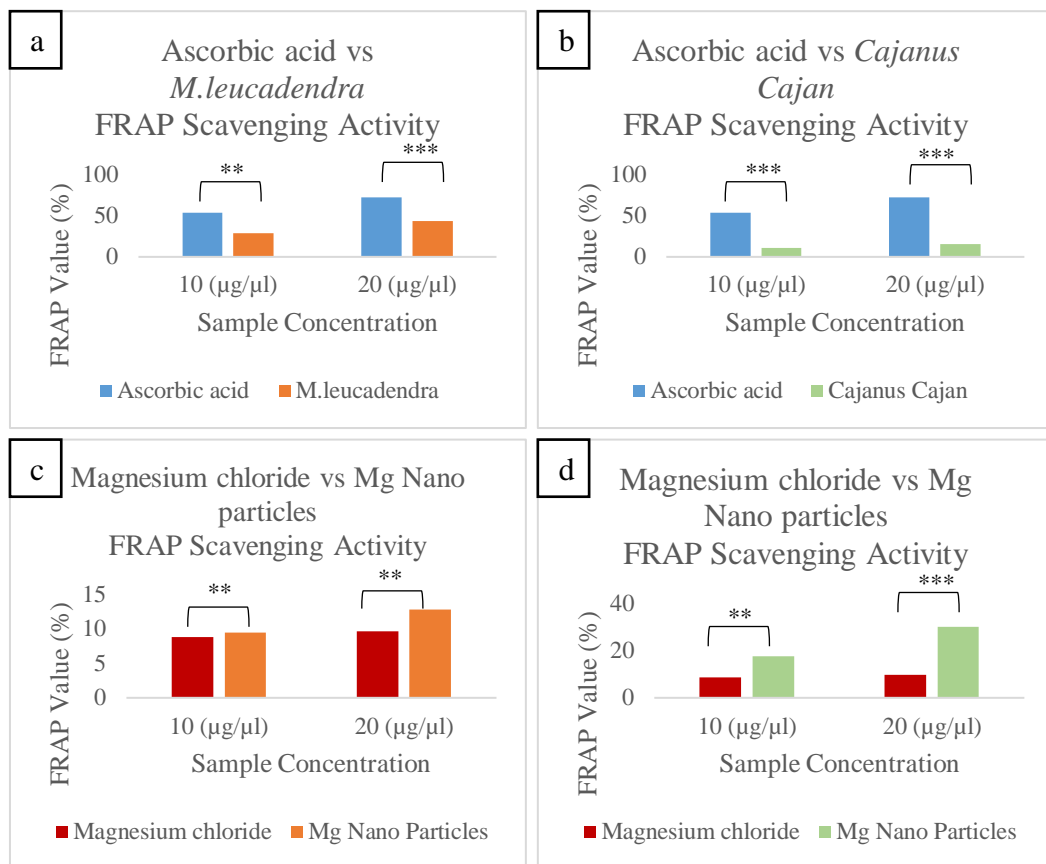


Fig. 6. FRAP scavenging activity of Ascorbic acid vs *M. leucadendra* (L.) extract (a), Ascorbic acid vs *Cajanus Cajan* (L.) extract (b), Magnesium Chloride vs Mg Nano Particles *M. leucadendra* (L.) (c) and Magnesium Chloride vs Mg Nano Particles *Cajanus Cajan* (L.) (d). Statistically significant different when *P < 0.05, **P < 0.01, ***P < 0.001

4 Discussion

Our extensive analysis of the DPPH scavenging activity and FRAP values in various samples, including extracts and nanoparticles from *M. leucadendra* (L.) and *Cajanus Cajan* (L.), provides insights into their antioxidant capabilities. The DPPH assay, a straightforward method to gauge the radical scavenging potential of compounds, is based on the reduction of the DPPH• radical, identifiable by a change in absorbance at 515 nm. In contrast, the FRAP assay evaluates the ability to reduce Fe³⁺ to Fe²⁺ in an acidic environment (pH 3.6), which is crucial for maintaining iron solubility and promoting redox reactions. In addition to these two methods, scavenging activity can also be measured using the Folin-Ciocalteu reducing capacity (FC) and ABTS assays. These four antioxidant tests are simple methods with high accuracy, requiring no special equipment, and can be implemented as "fixed time" assays with reaction times ranging from 12 to 30 minutes. However, their reactivity depends heavily on the sample type, which can lead to variable reaction times.

The DPPH and ABTS tests are antioxidant assays based on electron-deficient radicals. DPPH, a stable free radical, does not require fresh preparation unlike the ABTS cation

radical. ABTS testing is less susceptible to interference from colored samples due to its use of a higher wavelength. Generally, electron transfer occurs rapidly, whereas hydrogen transfer is relatively slower. This initial electron transfer is much quicker in ABTS tests due to the sterically hindered radical site in DPPH, which is less accessible to phenols. The FRAP and FC tests, both based on SET reactions. FC reduces Mo6^+ to Mo5^+ , resulting in a color change that can be monitored photometrically. The FC test is commonly used to determine phenolic content; however, it measures the reducing capacity, which directly correlates with phenolic content and antioxidant capacity, as it interacts with not only phenols but all oxidizable groups [19].

M. leucadendra (L.) and *Cajanus Cajan* (L.) extracts demonstrated different levels of antioxidant activity. *M.leucadendra* (L.) exhibited moderate effects, while *Cajanus Cajan* (L.) was less potent. These variations likely stem from their distinct phytochemical profiles. *Cajanus Cajan* contains compounds such as flavonoids, stilbenes, saponins, and alkaloids [20], whereas *M. leucadendra* (L.) is rich in Eugenol methyl ether, 1,8-cineole, terpinen-4-ol, and other compounds [21]. Flavonoid and Eugenol methyl ether are two compounds that potentially provide antioxidant properties to *Cajanus cajan* (L.) and *Melaleuca leucadendra* (L.) plants [22].

Furthermore, the conversion of magnesium chloride into nanoparticles, particularly those derived from *M. leucadendra* (L.) and *Cajanus Cajan* (L.), markedly influences their antioxidant activity. This is evidenced by an increase in the antioxidant activity of *M. leucadendra* (L.) and *Cajanus Cajan* (L.) nanoparticles compared to magnesium, as demonstrated in the DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Ability of Plasma) assays. The water-dispersible, monodispersed magnesium oxide nanoparticles had a uniform size of 20 nm.

The enhancement in antioxidant activity through the transformation into MgO nanoparticles may be explained by the intrinsic properties of these nanoparticles. Literature indicates that MgO, a functional inorganic material with chemodynamic therapy (CDT) capability, excels in generating reactive oxygen species (ROS) autonomously and in a hydrogen peroxide milieu. Notably, MgO facilitates the catalysis of oxygen dissolved in water into superoxide anion radicals ($\text{O}_2^{\bullet-}$) and hydroxyl radicals ($\bullet\text{OH}$) [23]. Singlet oxygen ($^1\text{O}_2$), dissolution of cations, internalization of nanostructures resulting in disintegration of the cell membrane, inhibition of enzyme activity and DNA synthesis, interruption of energy transduction, etc. Generally, ROS damage biomolecules, e.g. proteins, vitamins, and lipids (lipid peroxidation), due to the strong oxidation potential of ROS [24]. This augmented antioxidant capacity of MgO nanoparticles, when contrasted with plant extracts or magnesium chloride, could be due to their unique molecular composition and surface characteristics. Investigating whether the advantageous compounds found in *Cajanus Cajan* (L.) and *M. leucadendra* (L.), especially those not present in magnesium chloride, are preserved or amplified in the nanoparticle format is crucial.

Overall, our results highlight the diverse antioxidant activities among different substances and their nanoparticle versions. This study emphasizes the potential of nanoparticles, particularly those derived from *Cajanus Cajan* (L.) and *M. leucadendra* (L.), in enhancing antioxidant activities. This research contributes to the understanding of antioxidant properties and sets the stage for future investigations into using nanoparticle technology to boost antioxidant properties, with potential applications in therapeutic and functional food sectors.

5 Implication

5.1 Conclusion, limitation and future research avenues

This investigation has systematically assessed the antioxidant capacities of various substances, emphasizing the pronounced antioxidant strength of Ascorbic acid. The study also unveils the varied antioxidant activities of *M. leucadendra* (L.) and *Cajanus Cajan* (L.) extracts, with the former showing moderate effectiveness and the latter less so. The conversion of *Cajanus Cajan* (L.) into nanoparticle form notably enhances its antioxidant activity, illuminating the significant role nanoparticles may play in improving the qualities of traditional plant extracts.

The findings from this study are particularly compelling, indicating that nanoparticles have the potential to significantly boost the antioxidant efficacy of plant extracts. This is evidenced by the increased FRAP values observed in nanoparticle formulations derived from *Cajanus Cajan* (L.). The research contributes to the pharmaceutical sciences by reinforcing the importance of exploring both conventional extracts and their nanoparticle counterparts. By detailing the characteristics of antioxidant assays in Table 7 [19], the study offers a roadmap for assessing antioxidant strength in a diverse array of substances.

While the study's findings are substantial, they come with the caveat that only DPPH and FRAP assays were employed, as characterized in Table 7. This may have limited the detection of other compounds with potential antioxidant properties. Future research should, therefore, expand the range of methodologies used for antioxidant assessment, including but not limited to the Folin-Ciocalteu reducing capacity (FC) and ABTS assays. Such comprehensive future studies will likely offer broader insights into the antioxidant capabilities of both plant extracts and nanoparticle technology, thus paving the way for novel applications in health and nutrition sectors.

Table 7. Characteristic of Antioxidant assay [19]

	2,2- diphenyl-1-picrylhydrazyl (DPPH)	ferric reducing antioxidant power (FRAP)	Folin–Ciocalteu reducing capacity (FC)	2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)] free radical scavenging activity assay (ABTS)
Principle of method	Reaction with organic radical	Reaction with Fe(III) complex	Reaction with Mo (VI) complex	Reaction with organic radical cation
Reaction mechanism	Mixed mode (HAT & SET)	SET	SET	Mixed mode (HAT & SET)
End-product determination	Colorimetry (discoloration) at 518 nm	Colorimetry (color formation) at 593 nm	Colorimetry (color formation) at 750 nm	Colorimetry (discoloration) at 734 nm
Working pH	5-9	3.6	≈ 10	3-9
Polarity of antioxidants	Hydrophobic (only organic solvents)	Hydrophilic (only in aqueous solution)	Hydrophilic	Hydrophilic & Lipophilic

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