Objective 1

Chapter 4

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Identification and efficacy assessment of Indigenous herbs as reducing agents for green synthesis of Zn-based nanomaterials.

4.1. Introduction

Metallic nanoparticles can be synthesised by bacteria, fungi, and plants, each with unique advantages and disadvantages[1–3]. Biological nanoparticle production is influenced by various aspects such as intracellular or extracellular synthesis, growth temperature, synthesis time, extraction ease, and proportion synthesised against the percentage removed from sample[4]. Selecting the appropriate biological approach requires consideration of various factors. Plants have an advantage over microorganisms since they are locally available, inexpensive, and environmentally friendly. This approach uses phytochemicals found in plant tissues as reducing, capping, chelating, and stabilizing agents[5–8]. Furthermore, the use of plant extracts enables the utilisation of locally available and renewable resources. Bioactive compounds include polyphenols, flavonoids, ascorbic acid, alkaloids, terpenes, and reductase[9–11]. Metallic nanomaterials are typically found as monometallic or bimetallic compositions. Monometallic nanoparticles (MNPs) are made up of a single type of metal with distinct physical and chemical properties. Bimetallic nanoparticles (BNPs) have received a lot of attention in the research and technical spheres over the last decade due to their unusual optical, electrical, magnetic, and catalytic capabilities, which are usually markedly different from their monometallic counterparts[12–15]. Bimetallic NPs are formed by combining two different metals and can have a extensive range of morphologies and structures[16]. They often exhibit more fascinating features than the corresponding monometallic NPs, which is attributable to the synergistic capabilities of the two metal sections.

Studies highlight plants like *Peltophorum innerme* Roxb. commonly known as Rukal. *Peltophorum pterocarpum* (formerly known as *Peltophorum ferrugineum*), commonly known as Copperpod or Yellow-flamboyant, is renowned for its antioxidant properties. *Polygonum microcephalum* Wall. ex D. Don, generally known as *Rongkhangmani*. *Polygonum microcephalum* Wall. ex D. Don, popularly known as Asiatic Knotweed, has been recognized for its antioxidant properties due to the presence of various phytochemicals, including phenolic compounds and flavonoids. Research has shown that leaf extracts of *Polygonum*

microcephalum possess significant antioxidant activity attributed to the presence of phenolic compounds and flavonoids. These compounds exhibit free radical scavenging activity, thus protecting cells from oxidative stress-induced damage[17]. Studies have demonstrated that extracts derived from the whole plant of *Chrysalidocarpus lutescens* H. Wendl commonly known as Golden Cane Palm for their rich array of active constituents, involving flavonoids as well as phenolic compounds, with medicinal and nanoparticle synthesis applications. Similarly, another study by Aziz et al. [18] investigated the antioxidant properties of *Aquilaria malaccensis* leaf extracts using DPPH and ferric reducing antioxidant power (FRAP) assays. The study demonstrated dose-dependent antioxidant activity, suggesting the presence of bioactive compounds with antioxidant properties. The plant species have the potential to reduce ions of metal to zerovalent colloidal nanoparticles.

4.2. Materials

The chemicals employed in the current investigation were of analytical grade and were bought from Merck, Alfa Aesar, Hi-Media, Sigma-Aldrich. All chemical reactions, including the fabrication of leaf extracts, stock solutions, and working solutions, were carried out using ultrapure distilled deionized water (Milli-Q, Millipore Co., Bedford, MA, USA). The pH was adjusted using solutions of 0.1 M HCl and 0.1 M NaOH.

4.2.1. Requisites

Beakers, flasks, test tubes, pipettes, Hot plates, water baths, reflux apparatus and measuring cylinders.

4.2.2. Statistical Analysis

The statistical analysis were done using IBM SPSS (Statistical Package for Social Sciences) v26.

4.3. Raw materials for green synthesis of nanoparticles

The Plant species were identified and collected following the standard protocol for taxonomic identification[19]. Fresh green withered leaves of *Peltophorum inerme* Roxb, *Polygonum microcephalum* Wall. ex D. Don., *Chrysalidocarpus lutescens* H. Wendl., *Crinum asiaticum* L., and *Aquilaria malaccensis* Lam. were collected from Tezpur, Assam. Plant leaves were air dried at room temperature, finely ground using mortar and pestle, sieved into fine powder, and stored in an airtight sterile glass jars for further studies[20].

4.3.1. Proximate Analysis of the raw material

Proximate examination of green leaves of *Peltophorum inerme* Roxb, *Polygonum microcephalum* Wall. ex D. Don., *Chrysalidocarpus lutescens* H. Wendl., *Crinum asiaticum*

L., and *Aquilaria malaccensis* Lam. was performed using the approach outlined by Gafar et al.[21]. Ash content, moisture content, volatile organic content (VOC), and fixed carbon percentage are all measured quantitatively during proximate analysis. The values for these parameters were determined as follows:

4.3.1.1. Moisture Content

An empty crucible was constructed, and 10 g of new leaf material were carefully inserted. The crucible containing the leaf material was weighed to ascertain its starting weight. The leaf material was then dried at 105 °C in a hot air furnace. The drying process lasted 24 hours to guarantee complete desiccation. After the drying phase, the crucible containing the dried leaf material was removed from the furnace. Finally, the crucible containing the dried leaf material was weighed again to calculate the final dry weight, thereby finishing the procedure.

$$= \frac{(W2-W3)}{(W2-W1)} \text{ X } 100 \quad (1)$$

4.3.1.2. Volatile Content

The dried leaf sample was carefully placed in a closed crucible for subsequent processing. The crucible containing the samples were heated at 750 °C for seven minutes. Following the heating operation, the sample containing crucible was allowed to cool to room temperature using a desiccator. When the sample reached room temperature, the final weight of the crucible and its contents was recorded.

$$\frac{(W3-W4)}{(W2-W1)} \text{ X } 100 \quad (2)$$

4.3.1.3. Ash Content

The residue material left in the crucible was carefully weighed to ascertain its mass. The material was then transported to a muffle furnace and incinerated at 900 °C for 30 minutes. After the cremation was finished, the crucible containing the sample were allowed to cool to room temperature. Following the cooling time, the ultimate weight of the crucible and leftover material after cremation were reported.

$$\frac{(W5-W1)}{(W2-W1)} \times 100 \quad (3)$$

Here, W1 = Weight of an empty crucible, W2 = Weight of crucible with leaf, W3 = Weight of crucible + leaf (after oven drying at 105 °C), W4 = Weighed crucible + leaf (after muffle treatment at 750 °C), W5 = Weighed crucible + leaf (after muffle treatment at 900 °C).

4.3.1.4. Fixed Carbon

Calculated it by the difference between moisture, volatile, and ash content. Fixed Carbon=100-(Moisture content%+Volatile content%+Ash content%).

4.4. Preparation of Aqueous Leaf Extracts (ALE)

The Aqueous Leaf Extract (ALE) was prepared using the method described by Gogoi et al. (2015)[22]. In brief, 20g of leaf powder were blended with 100 mL of distilled water and heated at 80°C for 1 hour, while continuously stirring at 250 rpm with a magnetic stirrer. The mixture was allowed to cool to room temperature before filtering. The resultant filtrate was collected and used as a leaf extract. It was then kept at 4°C for further use. All chemicals and reagents used in the experiment were of analytical quality and obtained from reliable sources such as Merck, Alfa Aesar, and Hi-Media.

4.4.1. Phytochemical analysis of prepared leaf extract

The phytochemical study identified biochemical components in the leaf extract, including flavonoids, saponins, tannins, cardiac glycosides, quinones, steroids, alkaloids, terpenoids, and reducing sugars[23]. The qualitative tests were conducted as follows:

4.4.1.1. Flavonoids

Flavonoids are a prominent class of polyphenols found in all plant species. They are structurally composed of several benzene rings, resulting in a varied array of C_{15} aromatic compounds. Extensive research demonstrates their effectiveness as antioxidants and free radical scavengers. In a laboratory setting, 1 mL of leaf extract was mixed with 5 mL of diluted ammonia (NH_3), followed by 1 mL of concentrated sulfuric acid. The appearance of a yellow tint during the reaction indicated the presence of flavonoids.

4.4.1.2. Tannins

Tannins, which are soluble in both water and alcohol, are found in many plant sections, including roots, barks, leaves, stems, and exterior tissues. Notably, its capacity to transform chemicals into leather results in a distinctive tan colour. This acidic character is accredited to the presence of phenolic or carboxylic groups. To test for tannins, 1 mL of leaf extract was added to a test tube, followed by two drops of 15% ferric chloride ($FeCl_3$). The appearance of a blue-black colouring during the reaction revealed the existence of tannins[24].

4.4.1.3. Cardiac Glycosides

Glycosides, water-soluble phytochemicals found in cell sap, are colorless and crystalline substances composed of carbon, hydrogen, oxygen, and occasionally nitrogen and sulfur. Structurally, glycosides consist of a carbohydrate moiety, typically glucose, and a non-carbohydrate component known as aglycone or genin. To detect cardiac glycosides, 1 mL of

leaf extract was mixed with 2 mL of glacial acetic acid in a test tube, followed by the addition of 1 drop of 15% ferric chloride ($FeCl_3$) and 1 mL of concentrated sulfuric acid (H_2SO_4). The appearance of a brown coloration at the interface indicated the presence of cardiac glycosides.

4.4.1.4. Saponins

Saponins are high molecular weight compounds characterized by the combination of a sugar molecule with a triterpene or steroid glycone. They can be classified into two main groups: steroid saponins and triterpene saponins. Soluble in water but insoluble in ether, saponins undergo hydrolysis to yield aglycones, like glycosides. Notably toxic, saponins can cause haemolysis of blood and are implicated in cattle poisoning incidents. To test for saponins, 1 mL of leaf extract was mixed with 5 mL of distilled water in a test tube. After vigorous shaking, the mixture was observed for frothing, indicative of the presence of saponins[25].

4.4.1.5. Steroids

Plant steroids, also known as steroid glycosides, are among the most prevalent phytoconstituents with therapeutic applications such as arrow poisons or cardiac drugs[26]. Anabolic steroids have demonstrated the ability to enhance nitrogen retention in osteoporosis and animals suffering from wasting illnesses. To determine the presence of steroids, 1 mL of leaf extract was mixed with 2 mL of concentrated sulfuric acid in a test tube. A change in color from violet to blue green served as an indication of the presence of steroids.

4.4.1.6. Terpenoids

Terpenoids represent the most abundant class of plant chemicals, characterized by cyclic hydrocarbons containing functional groups such as -OH and -COOH, among others. The Salkwoski test was employed to determine the presence of terpenoids. In this test, 1 mL of the extract was mixed with 2 mL of chloroform and 3 mL of concentrated sulfuric acid in a test tube. The appearance of a reddish-brown color at the interface confirmed the presence of terpenoids.

4.4.1.7. Anthroquinones

Anthraquinones are derivatives of phenolic and glycosidic compounds, primarily derived from anthracene, resulting in various oxidized derivatives like anthrones and anthranols. Common derivatives such as chrysophanol, aloe-emodin, rhein, salinosporamide, luteolin, and emodin exhibit a double hydroxylation at positions C-1 and C-8. To test for the presence of anthraquinones, 1 mL of leaf extract was mixed with 5 mL of benzene and 2.5 mL of dilute ammonia (NH_3) in a test tube. The mixture was vigorously shaken, and the appearance of a pink-red colour at the lower phase confirmed the presence of anthraquinones.

4.4.1.8. Alkaloids

Alkaloids comprise a diverse group of cyclic nitrogen-containing compounds, exceeding 12,000 known variants, found in over 20% of plant species. They represent the largest group of secondary chemical constituents, primarily consisting of ammonia compounds. Alkaloids are nitrogen bases synthesized from amino acid building blocks, featuring various radicals replacing one or more hydrogen atoms in the peptide ring, often containing oxygen. The degree of basicity varies significantly based on the molecule's structure and the presence and location of functional groups. To determine the presence of alkaloids, Wagner's test was employed. In this test, 1 mL of leaf extract was mixed with 3 drops of Wagner's reagent, comprising 2 grams of iodine and 6 grams of Potassium Iodide dissolved in 100 mL of distilled water. A reddish-brown coloration indicated the presence of alkaloids[24].

4.4.1.9. Quinines

It is characterized by aromatic rings with two ketone substitutions, which play a crucial role in binding to adhesins, forming complexes with the cell wall, and inactivating enzymes. To test for the presence of quinones, 1 mL of leaf extract was combined with 1 mL of concentrated H_2SO_4 in a test tube. The mixture was vigorously shaken for 5 minutes, resulting in the development of a red coloration.

4.4.1.10. Reducing Sugar

Sugars act as reducing agents due to the presence of free aldehydic and ketonic groups. All monosaccharides are considered reducing sugars. To test for the presence of reducing sugars, 1 mL of leaf extract was mixed with an equal volume of Benedict's reagent in a test tube. The mixture was then boiled in a water bath for 5 minutes. The solution turned green in colour, indicating the presence of reducing sugars.

4.5. Quantitative phytochemical analysis

Quantitative phytochemical analysis encompasses the precise quantification of distinct chemical constituents within plant extracts, employing a diverse array of analytical methodologies. These investigations afford crucial insights into the abundance of bioactive molecules, including phenolics, antioxidant capacity. The meticulous determination of these compounds facilitates a comprehensive comprehension of the therapeutic and nutritional attributes inherent to botanical specimens.

4.5.1. Total Antioxidant Capacity

The total antioxidant assay followed Umamaheswari et al., 2008[27] protocol using ascorbic acid as standard. Samples were dissolved in 95% methanol to 1 mg/ml and diluted for testing. A 0.1 ml aliquot of each sample was mixed with 1 ml of reagent solution containing 0.6 M

sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. After incubating at 95°C for 90 minutes, samples cooled to room temperature. Absorbance was measured at 765 nm against a blank containing only the reagent solution. Ascorbic acid served as the standard, ensuring rigorous scientific comparison.

$$\text{Antioxidant capacity (\%)} = \frac{(\text{absorbance of control} - \text{absorbance of sample})}{(\text{absorbance of control})} \text{ X } 100$$

4.5.2. DPPH radical scavenging activity assay

The fractions' free radical scavenging activity was evaluated in vitro using the 2,20-diphenyl-1-picrylhydrazyl (DPPH) test, as previously described[28]. DPPH (80 µg/ml) was produced using methanol. The extracts were serially diluted from their 1 mg/ml stock solutions. After mixing 2 ml of each solution with 2 ml of DPPH and waiting 30 minutes, the absorbance was measured at 517 nm. Ascorbic acid was utilised as the standard. An IC50 value was derived using a concentration-response curve. The % inhibition of DPPH free radicals was estimated using the following formula:

$$\text{Scavenging activity (\%)} = \frac{(\text{absorbance of control} - \text{absorbance of sample})}{(\text{absorbance of control})} \text{X } 100$$

4.5.3. Total Phenolic Content

The total phenolic content of the extracts was evaluated using a standard procedure[29]. A volumetric flask was filled with 1 ml of extract solution containing 2000 µg. 45 mL of distilled water and 1 mL of Folin-Ciocalteu reagents were then added and forcefully agitated. After 3 minutes, add 3 ml of a 2% Na_2CO_3 solution and shake intermittently for 2 hours. The absorbance was measured at 760 nm. Results were represented as micrograms of gallic acid equivalent (GAE) per mg of plant extract.

4.5.4. Reducing power assay

The reducing power assay was conducted using Oyaizu's method[30]. Mix 2.5 mL of plant extract (1 mg/mL) with 2.5 mL of 0.2 mol/L sodium phosphate buffer and 1% potassium ferricyanide were mixed. After incubating at 50°C for 20 minutes, add 2.5 mL of trichloroacetic acid solution. Centrifuge at 650 rpm and 25°C for 10 minutes. The supernatant (5 mL) was combined with 5 mL of distilled water and 1 mL of ferric chloride solution. The absorbance was measured at 700 nm. Ascorbic acid was utilised as the standard.

4.6. Green synthesis of Zn-based nanoparticles

The green synthetic pathways for Zn Monometallic, Zn-Cu Bimetallic, and Zn-Cu-Fe-Mn Quadrimetallic Nanoparticles (MNP, BNP and QMNP) are illustrated in Fig.4.4.

Cold-stored Aqueous Leaf Extract (ALE) from *Chrysalidocarpus lutescens*, known for its high phenolic content, served as the reducing agent for synthesis of MNP, BNP and QMNP following the methodology outlined by Gogoi et al. (2015)[22]. To prepare the leaf extract, air-dried *C. lutescens* leaves were milled into powder form using a disc milling tool. Subsequently, 200 g each leaf powder was mixed with 3000 mL of deionized water (20% w/v) in different glass beakers and heated on hot plates at 80°C for an hour while being stirred continuously at 300 RPM[31]. After cooling to room temperature, the extract was double filtered using Whatman filter paper and stored at 4ºC for later use in nanoparticle synthesis.

For the synthesis of Zn Monometallic Nanoparticles (MNP), 0.5 M zinc oxide solution was used. For Zn-Cu Bimetallic Nanoparticles (BNP), equimolar concentrations of 0.5 M Zinc Oxide and 0.5 M Copper sulphate precursor salts were utilized. Similarly, for Zn-Cu-Fe-Mn Quadrimetallic Nanoparticles (QMNP), 0.5M equimolar concentration of Zinc oxide, Copper Sulphate, Ferric Chloride Anhydrous, Manganous sulphate monohydrate. Additionally, 5% polyethylene glycol (PEG) (w/v) was added to enhance nanoparticle dispersibility. The reaction conditions were optimized by varying Aqueous Leaf Extract (ALE) concentrations (1–10%) (v/v) against individual beakers where MNP, BNP and QMNP is ought to be tailored. At temperatures ranging from 60–80 °C, with continuous stirring and the addition of PolyEthylene Glycol (PEG) solution to a final volume of 1 L in different beakers. pH values of 5, 7, and 9 were achieved by adjusting with 1 M NaOH and/or 1 N HCl. The reaction mixture, stirred at 400 rpm, exhibited colour changes indicative of nanoparticle formation, with brown precipitation observed at 80 °C. After multiple rounds of washing with deionized water, the nanoparticles were dried overnight in a hot air oven. Subsequently, centrifugation at 7500 rpm for 30 min was performed thrice to remove uncoordinated biomolecules, yielding a pellet for further characterizations.

4.7. Results

4.7.1. Proximate Analysis of green leaves

Here, in this study, Proximate analyses of the plant leaves showed that compared to *Peltophorum inerme Roxb., Polygonum microcephalum Wall. ex D. Don., Crinum asiaticum L., Aquilaria malaccensis Lam.* the leaves of *Chrysalidocarpus lutescens H.Wendl.,* has shown the highest amount of Ash content which means it has high mineral content (Table 4.1.)

4.7.2. Qualitative Analysis of Aqueous Leaf Extracts (ALE)

In the study, the plant leaf extracts of *Polygonum microcephalum Wall. ex D. Don., Chrysalidocarpus lutescens H.Wendl.* showed the presence of tannins, flavonoids, cardiac

glycosides, anthroquinones, terpenoids, steroids, saponins, alkaloids, quines and reducing sugar. However, some exceptions were noted in *Aquilaria malaccensis Lam., Crinum asiaticum L., Peltophorum inerme Roxb*. (Table 4.2).

4.7.3. Quantitative Analysis of Aqueous Leaf Extracts (ALE)

4.7.3.1. Total Antioxidant Capacity

In the study, *Chrysalidocarpus lutescens* H. Wendl. and *Peltophorum inerme Roxb.* Showed highest antioxidant capacity throughout the concentrations, more prominently in 40,60,80 and 100 µg $ml^{-1.}$ Contrastingly, *C. asiaticum* L. and *A. malaccensis* Lam. revealed lower antioxidant capacity throughout the concentration, more particularly in 10 and 20 µg ml^{-1} (Fig.4.1)

4.7.3.2. DPPH Free Radical Scavenging Activity

In this study, the DPPH scavenging activity assay, it was observed that *C. lutescens* H.Wendl. has the highest antioxidant potential. The 50% inhibition of free radicals has long been employed as a measure of antioxidant activity. In this investigation, both the plant extract and the standard significantly scavenged the DPPH radical as concentrations increased. The methanol extract of *C. lutescens* H. Wendl. (20 µg ml^{-1}) has the lowest IC50 value compared to other sample extracts. However, adding a larger amount of the plant's leaf extract to the DPPH assay combination reduced the degree of inhibition (Table 4.2.).

4.7.3.3. Total Phenolic Content (TPC)

In this study, it was documented that increasing concentration, increases the phenolic content also across all plant leaf extracts. However, a significant increase has been noted in *C. lutescens* H. Wendl. and in *P. microcephalum* Wall. ex D. Don. at 80 and 100 µg ml^{-1} (Fig 4.2.)

4.7.3.4. Reducing Power Assay

In this investigation, the reducing capacity of *P.inerme* Roxb. is phenomenon at 80 µg ml^{-1} compared to *Polygonum microcephalum* Wall. ex D. Don*., Crinum asiaticum* L*., Aquilaria malaccensis* Lam. on the other hand *Chrysalidocarpus lutescens* H.Wendl., records all time high in 100 µg ml^{-1} (Fig 4.3)

4.7.4. Green Synthesis of Monometallic Nanoparticles (MNP), Bimetallic Nanoparticles (BNP) and Quadrimetallic Nanoparticles (QMNP)

Synthesis of nanoparticles was confirmed by change in colour. Nanoparticles have unique optical properties due to their size, shape, and composition. As a result, they have different light absorption and scattering properties than bulk materials.

Table 4.1. Proximate analysis of green leaves of *Peltophorum inerme* Roxb*., Polygonum microcephalum Wall. ex D. Don., Chrysalidocarpus lutescens H.Wendl., Crinum asiaticum L., Aquilaria malaccensis* Lam*.*

Plant species	**Ash Content (%)**	**Fixed Carbon (%)**	**Moisture Content (%)**	**Volatile matter (%)**
Peltophorum inerme Roxb.	40.12	53.32	74.4	38.8
Polygonum microcephalum Wall. ex D. Don	1.895	33.54	89.1	3.7
Chrysalidocarpus lutescens H.Wendl.	45.23	8.23	57.7	5.3
Crinum asiaticum L.	42.67	38.57	87.3	8.6
Aquilaria malaccensis Lam.	42.23	29.23	62.9	24.1
LSD	**0.80**	**1.01**	**0.94**	**0.56**

Table 4.2. Qualitative and Quantitative (DPPH free radical reducing assay) phytochemical screening of Aqueous leaf extracts

Plant Species	DPPH free radical scavenging activity IC50 value		Phytochemicals										DPPH Free radical Scavenging activity						
	Concentration of plant extract (µg ml^{-1})	IC50 value	Tannin	Flavonoid	Cardiac glycosides	Saponins	Steroids	Terpenoids	Anthroquinones	Alkaloids	Quinines	Reducing sugar	10 µg/ml	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	LSD ($p \leq 0.05$)
***Aquilaria malaccensis* L.**	60	63.5 ±1.3	+	+	+	-	+	+	+	+	-	+	43.5±2.9	52.4 ±1.3	65.5 ±1.7	63.5 ±1.3	72.3 ±1.5	73.4 ±1.3	**1.438**
***Crinum asiaticum* L.**	40	48.3±1.3	+	+	+	+	+	-	+	+	-	+	50±1.6	42.9±1.2	48.3±1.3	41.5±1.4	40.1±1.3	50.0±1.5	**1.198**
***Chrysalidocarpus lutescens* H.Wendl.**	20	82.1±1.6	+	+	+	+	+	+	+	+	+	+	82.6±1.3	82.1±1.6	81.9±1.4	81.8±1.5	82.6±1.7	82.1±1.5	**1.438**
***Polygonum microcephalum* Wall. ex D. Don**	40	83.5±1.5	+	+	+	+	+	+	+	+	+	+	79.9±1.5	81.1±2.5	83.6±1.5	83.3±1.5	79.9±1.2	81.1±1.4	**1.233**
***Peltophorum inerme* Roxb.**	20	76.0±1.2	+	+	+	+	-	+	+	-	+	+	75.1±1.5	76.1±1.2	75.8±1.7	71.2±2.4	69.1±1.9	56.8±2.5	**1.349**

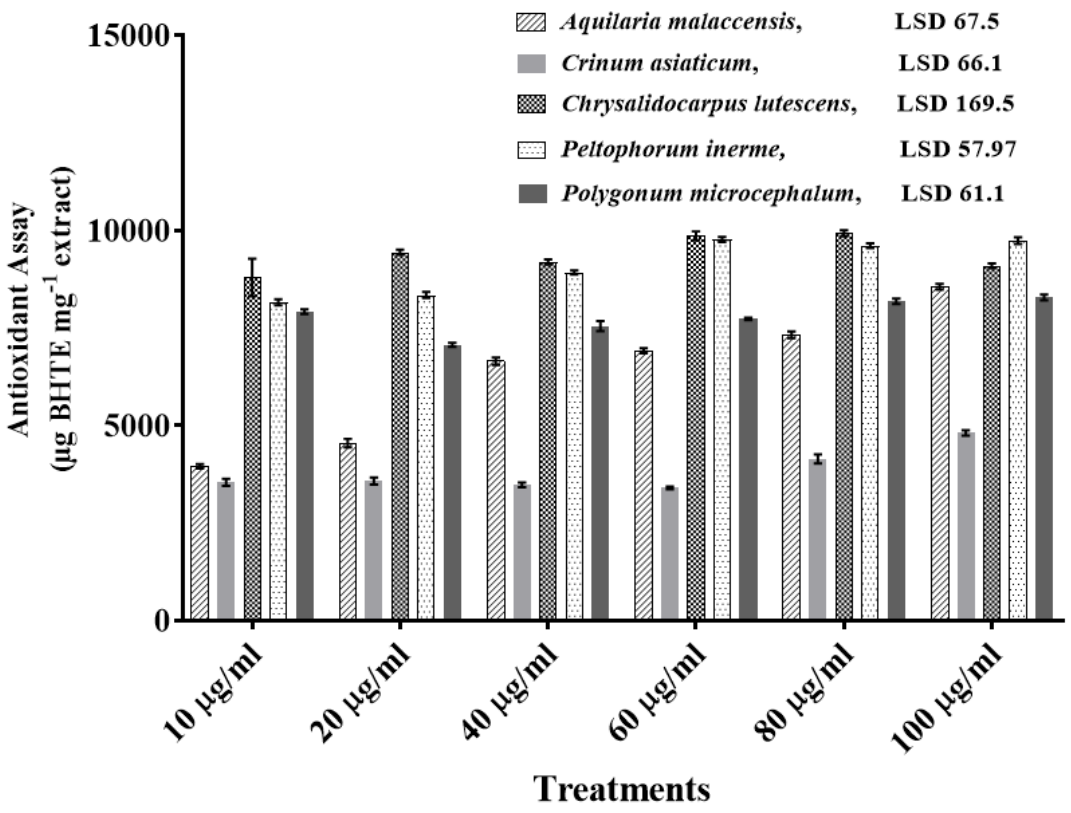

Fig 4.1. Total Antioxidant Capacity of Aqueous Leaf Extract (ALE) of *Peltophorum inerme* Roxb.*, Polygonum microcephalum* Wall. ex D. Don.*, Chrysalidocarpus lutescens* H.Wendl., *Crinum asiaticum* L.*, Aquilaria malaccensis* Lam.

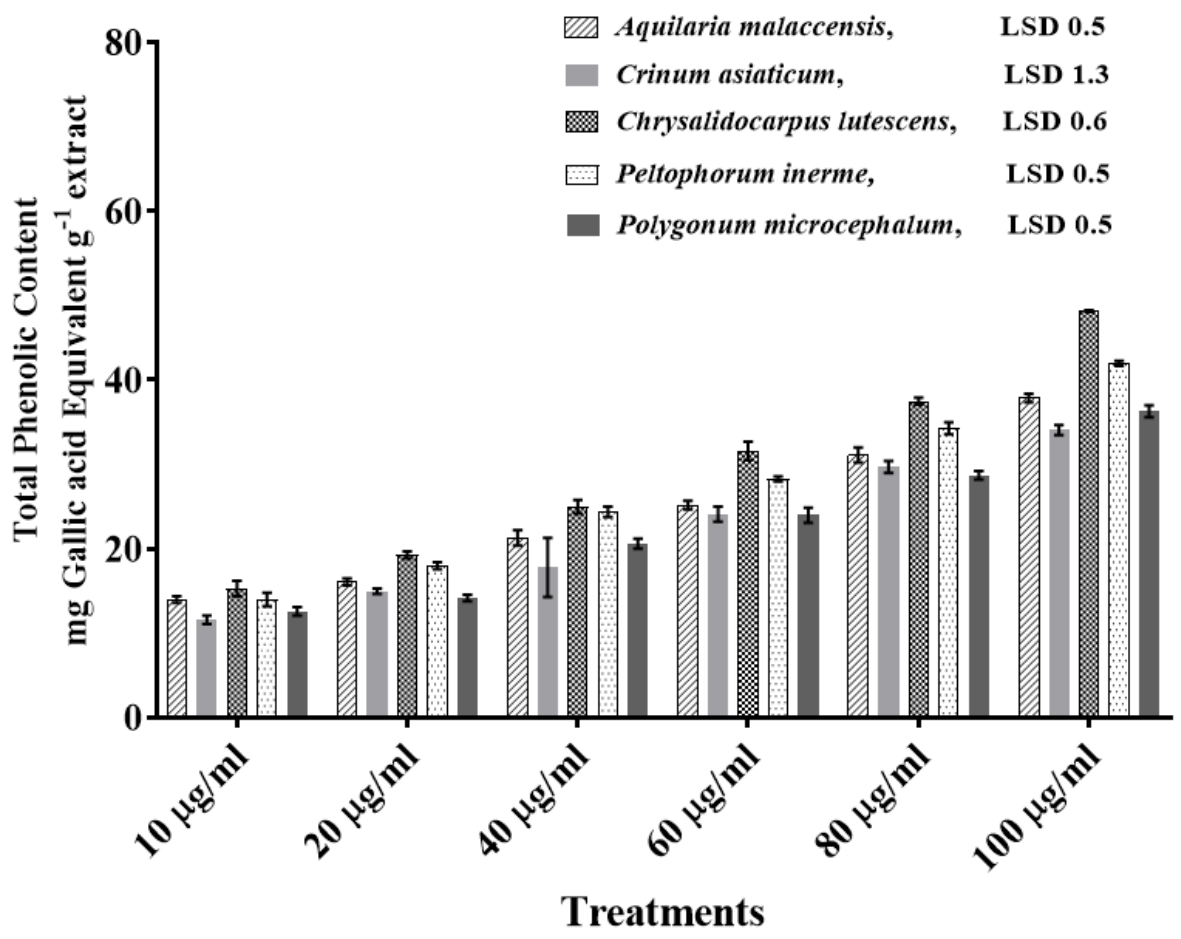

Fig 4.2. Total Phenolic Content of Aqueous Leaf Extract (ALE) of *Peltophorum inerme* Roxb.*, Polygonum microcephalum* Wall. ex D. Don.*, Chrysalidocarpus lutescens* H.Wendl., *Crinum asiaticum* L.*, Aquilaria malaccensis* Lam.

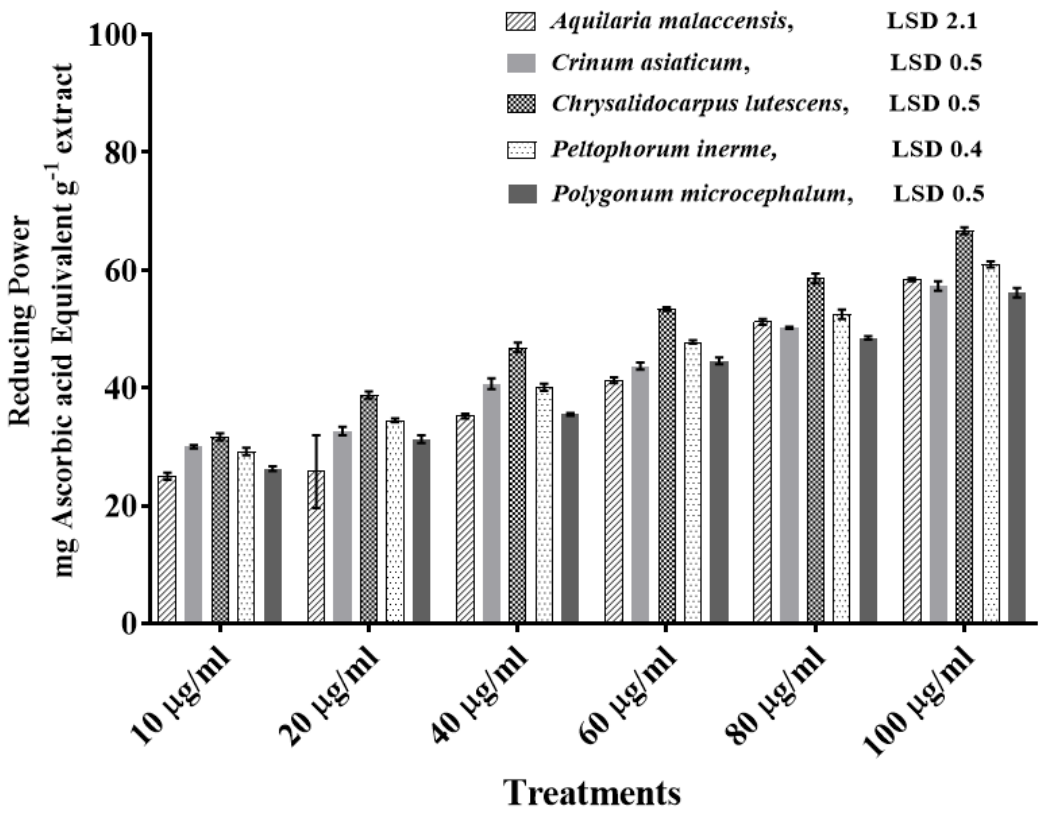

Fig 4.3. Reducing Power Assay of Aqueous Leaf Extract (ALE) of *Peltophorum inerme* Roxb., *Polygonum microcephalum* Wall. ex D. Don., *Chrysalidocarpus lutescens* H.Wendl., *Crinum asiaticum* L., *Aquilaria malaccensis* Lam.

Fig. 4.4. Plant aqueous leaf extract mediated green synthesis of mono, bi, and quadrimetallic nanoparticles

4.8. Discussion

The proximate analysis of green leaves of selected plant species revealed high moisture content (89.1%) in *Polygonum microcephalum* followed by *Crinum asiaticum*, while fixed carbon and ash content were comparatively low for *Polygonum microcephalum* (33.54% and 1.895%, respectively). This examination reveals important information about the leaves' nutritional composition and prospective applications in a variety of sectors. The moisture content is calculated by drying a sample of leaves to a fixed weight and determining the percentage of water loss. The ash content represents the leaves' inorganic mineral content, which is produced by burning the sample at high temperatures to eliminate organic stuff. Carbohydrate content is computed by subtracting the amount of moisture, ash, protein, fat, and fibre from 100%. Overall, proximate analysis gives useful information on the nutritional composition of green leaves and can be used to determine their suitability for a diversity of uses such as food, feed, and biofuel production[32–34]. *Chrysalidocarpus lutescens* showed high ash content and low moisture content which clearly signifies those leaves had high amount of minerals (Table 4.1). The list of phytochemicals confirmed in the leaf extracts of Aquilaria malaccensis, Crinum asiaticum, Chrysalidocarpus lutescens, Polygonum microcephalum, and Peltophorum inerme are summarized in (Table 4.2). Reducing sugars, flavonoids, alkaloids, tannins, terpenoids, quinones, and saponins served as stabilizing and reducing agents during NP synthesis. Flavonoids, alkaloids, tannins, and terpenoids donate electrons, which reduce metal ions to NPs, while quinones and saponins stabilize the NPs, preventing aggregation and maintaining homogeneity[35,36]. Earlier studies also reported the presence of flavonoids, tannins, and terpenoids in the plant extracts of *Peltophorum inerme, Chrysalidocarpus lutescens*, and *Polygonum microcephalum* that affect metal ion speciation in plant systems[37]. Flavonoids are efficient metal chelators and show high affinity towards metals, especially transition metals; the hydroxyl and carbonyl groups found in flavonoids can help in metal ions speciation and accelerate the reduction of metals to NPs[38,39]. According to Mazumder et al. [40], tannins significantly reduce and cap metal ions to their zerovalent form. The phenolic groups in tannins donate electrons to the -OH radicals that stabilize and prevent agglomeration of the NPs[41]. In this investigation, phytochemicals are the primary reducing agents that helped in the successful nucleation of NPs (Table 4.2). Additionally, phytochemicals form a bio-organic coating on the surface of the NPs that helps in stabilization and improves the NPs' biocompatibility[22]. FTIR studies have confirmed the presence of functional groups originating from phytochemicals and bound to the surface of the MNPs and BNPs. The reducing power of the leaf extracts of *Aquilaria malaccensis*, *Crinum asiaticum*, *Chrysalidocarpus lutescens*, *Polygonum*

microcephalum, and *Peltophorum inerme* was spectrophotometrically confirmed through DPPH antioxidant activities. Table 4.2 shows the values (%) of the inhibition of DPPH radical scavenging activity most significant in *Chrysalidocarpus lutescens* ($p \leq 0.05$). DPPH radical production was suppressed by 50% i.e., 20 mg L-1 of *Chryasalidocarpus lutescens* with an IC50 value of 82.12±1.6. In contrast, 40 mg L-1 of *Polygonum microcephalum* had an IC50 value of 83.5±71.5, proving that the antioxidants are the primary reducing agents in the synthesis of MNPs and BNPs[42]. The IC50 value for *Chrysalidocarpus lutescens* extract was high among the other four plants, which inferred *C. lutescens* phytoextract is ecologically safe for the synthesis of NPs for agricultural or hydroponic applications that will not affect the valuable microbes associated with plant growth. *Chrysalidocarpus lutescens* ALE may have good radical scavenging properties due to the ability of phytochemicals to donate hydrogen atoms[22]. Based on the phytochemical profile and IC50 values, *C. lutescens* was chosen to synthesize MNP and BNP. The outcomes of this study are consistent with the earlier reports on phytochemicals and the criteria for screening plant species for green synthesis of nanomaterials[22,43]. El-Shafey et al.[44] examined the effectiveness of *C. lutescens* phytoextract for synthesizing ZnO nanoparticles from zinc nitrate solution for the first time. Green synthesis of MNP, BNP and QMNP was confirmed by the color transformation of the ALE-salt solution from light brown to a prominent brown shade, mediated by the phytochemicals present in the *Chrysalidocarpus lutescens* ALE[45].

4.9. Conclusion

To summarize, the green synthesis of zinc monometallic, zinc-copper bimetallic and zinc-copper-iron-manganese quadrimetallic nanoparticles with diverse shapes and sizes by plant extracts is a promising route in nanotechnology. This environmentally friendly strategy uses the reducing and stabilizing characteristics of plant extracts to create nanoparticles with specific features. The synthesis process has multiple advantages, including low cost, scalability, and biocompatibility, making it appropriate for an extensive range of applications in domains such as catalysis, sensing, and biomedicine. Furthermore, the heterogeneous character of the synthesized nanoparticles provides chances to investigate new features and applications. As research in this area continues, better optimization of synthesis settings and characterization techniques will improve our understanding of nanoparticle formation and enable the production of new materials with tailored attributes and performance. Ultimately, the plant extract-supported green synthesis of multimetallic nanomaterials shows considerable promise for tackling food insecurity, sustainable agriculture and biomedical avenues which holds the key

concerns keeping in mind about dose response relationship, phytotoxicity, ecotoxicity and lethal doses while encouraging sustainability and environmental stewardship.

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