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Biochemical Investigations and Green Synthesis Characterization using Aqueous Extract of *Ageratum Conyzoides* L. Leaf

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ABSTRACT

Introduction: *Ageratum conyzoides* L., commonly known as Goat Weed, has long been utilized in traditional medicine for its therapeutic properties. This study aims to scientifically validate these claims and explore the potential applications of the plant in nanotechnology.

Methods: In this experimental study, the biochemical profile of *A. conyzoides* was assessed using standard analytical techniques. Phytochemical analyses, including the alkaline reagent test, Hager's test, foam test, and Liebermann's test, were performed on the extract following established methods, all indicating its medicinal potential and strong antioxidant activity. Antioxidant properties were evaluated through DPPH and NO radical scavenging assays, as well as the Ferric Reducing Antioxidant Power (FRAP) assay. Nanoparticles of *A. conyzoides* were characterized using UV-Vis spectroscopy and Fourier-transform infrared spectroscopy (FT-IR).

Results: Phytochemical analysis revealed the presence of flavonoids, alkaloids, tannins, saponins, and phenolic compounds, all contributing to its medicinal potential and strong antioxidant activity. Toxicological evaluations, including acute and sub-acute toxicity tests, as well as hematological and white blood cell analyses of treated Wistar rats, confirmed a positive and favorable safety profile for the extract. The results also demonstrated that the aqueous extract of *A. conyzoides* exhibited significant antimicrobial activity against a variety of pathogenic bacteria and fungi. Additionally, the extract facilitated the green synthesis of silver, copper, and zinc nanoparticles.

Conclusion: The results support the traditional medicinal use of *A. conyzoides* for treating various ailments, such as wounds and inflammation. Furthermore, the plant shows promising potential in natural antimicrobial applications, nanomedicine, and drug development.

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Introduction

A. conyzoides, commonly known as goat weed, is a tropical plant species belonging to the Asteraceae family (Santos et al., 2016). Originating from Central and South America, it has spread to various tropical and subtropical regions particularly in Africa, Asia, and South America (Singh et al., 2013), thriving in diverse habitats ranging from grasslands to disturbed areas, roadsides, and agricultural fields. Despite its classification as a weed in some regions, *A. conyzoides* has drawn significant attention for its diverse medicinal properties and traditional uses. This plant, distinguished by its hairy leaves and small, fluffy flowers that are typically blue, white, or purple, has shown notable antifungal potential due to its secondary metabolites such as chromenes, terpenoids, flavonoids, and coumarins (Tsivileva et al., 2022).

Historically, *A. conyzoides* has been valued for its medicinal properties and integrated into traditional healing practices across various cultures. Indigenous communities in areas where the plant is native have long used its leaves, stems, and roots either in fresh form or as dried powder to treat a variety of health conditions. In folk medicine, preparations of *A. conyzoides* have been utilized to address ailments such as wounds, fever, inflammation, respiratory infections, gastrointestinal disorders, and reproductive issues (Anand et al.,2022). Traditional healers and herbalists have recognized its analgesic, anti-inflammatory, antimicrobial, antidiarrheal, and antipyretic properties, often using it in the form of decoctions, infusions, poultices, or topical applications for therapeutic purposes (Chabi-Sika et al., 2023).

A. conyzoides contains a wide array of phytochemical constituents that contribute to its medicinal effectiveness (Mary and Giri, 2016). Studies have identified bioactive compounds such as alkaloids, flavonoids, terpenoids, phenolic acids, coumarins, and essential oils in various parts of the plant. These phytoconstituents exhibit a range of pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, analgesic, antidiabetic, antitumor, and wound healing properties (Omole et al., 2019). Due to its wide range of medicinal uses, further investigation into the plant's biochemical makeup and pharmacological properties is crucial to substantiate traditional claims and explore its potential in contemporary medicine. Nanotechnology provides a cutting-edge method to boost the medicinal effects of plant-derived compounds. Studies on the synthesis and characterization of metallic nanoparticles, such as silver, copper, and zinc, using plant extracts have yielded promising outcomes by improving bioavailability, enhancing therapeutic effectiveness, and reducing toxicity. This research focuses on utilizing the aqueous extract of *A. conyzoides* for synthesizing silver, copper, and zinc nanoparticles, with the aim of exploring both the plant's phytochemical components and their application in modern biomedicine. The study's objectives include identifying the phytochemical compounds in the aqueous extract of *A. conyzoides* leaves, assessing its antioxidant activity, and synthesizing three metallic nanoparticles (silver, copper, and zinc). Ultimately, this study seeks to integrate traditional knowledge with nanotechnology, enhancing the understanding of how plant-based nanoparticles can improve therapeutic outcomes.

Researchers have investigated the potential of *A. conyzoides* extracts and isolated compounds in preclinical studies, elucidating mechanisms of action and therapeutic applications that warrant further exploration. While traditional knowledge underscores the medicinal value of *A. conyzoides*, contemporary scientific research aims to validate and expand upon its therapeutic potential. Preclinical studies have provided insights into the pharmacological mechanisms underlying its traditional uses, shedding light on its safety profile, dosage regimens, and potential drug interactions (Atawodi et al.,2014).

Additionally, research into the phytochemical composition and bioactivity of *A. conyzoides* reveals great potential for developing novel pharmaceuticals, nutraceuticals, and herbal remedies aimed at various health conditions (Omole et al.,2019; Paul et al.,2022).

Recent advancements have highlighted the synergistic effects of its phytoconstituents, which can enhance the efficacy of traditional treatments and reduce the risk of side effects. For instance, the combination of its flavonoids and terpenoids has been shown to exert potent anti-inflammatory and analgesic effects, making it a promising candidate for developing new pain management therapies (Uritu et al., 2018). Furthermore, the antimicrobial properties of *A. conyzoides* have shown promise in combating antibiotic-resistant strains of bacteria (Odeleye et al., 2014), positioning it as a potential alternative or adjunct to conventional antibiotic treatments. *A. conyzoides* essential oils have also been extensively studied for their therapeutic potential. These oils have demonstrated significant antioxidant activity, which could be harnessed for preventing and managing oxidative stress-related diseases such as cardiovascular disorders and neurodegenerative diseases (Dangana et al., 2024). The integration of nanotechnology in phytotherapy, helps to utilize herbalmedicines in treating various diseases, including improving their delivery mechanisms. For clinical trials and therapeutic outcomes to be reliable, herbal products must be standardized, ensuring consistent quality and well-defined ingredients. The therapeutic effectiveness of herbal formulations is largely influenced by their phytochemical content. A major challenge is developing accurate analytical methods to profile these compositions, particularly for quantitative analysis of bioactive compounds. Nanotechnology-based herbal drugs have shown potential in enhancing solubility and bioavailability. Ayurveda, the ancient Indian medical science centered on herbal and herbo-mineral preparations, is particularly relevant in this field. Researchers have applied nanotechnology to insert and activate genes within the cell walls of *different plant*, which presents significant biotechnological opportunities. Nanotechnology is also being investigated for its potential to detect plant diseases at early stages. Researchers at Texas AgriLife Research are exploring this to protect crops from

potential outbreaks. Meanwhile, the U.S. Department of Agriculture is focusing on creating portable, user-friendly detection systems to combat bacterial, fungal, and viral threats to food and agriculture (Garg, 2010). Although isolating and extracting natural compounds from *Ageratum may* presents technical difficulties, plant-derived products continue to hold an important place in modern pharmacopoeias. Nanotechnology-based formulations address various challenges, such as poor solubility, low permeability, unstable bioavailability in biological environments, and rapid metabolism. These nanomaterials significantly enhance the pharmacokinetics and therapeutic effectiveness of derived drugs. Targeted delivery systems and combination therapies can further improve their performance. This research will examine the toxicological profile and antimicrobial properties of *A. conyzoides* nanoparticles to validate their pharmaceutical potential

Materials and Methods

Sample Collection and Identification

The collection and preparation of *A. conyzoides* (Goat Weed; Figure 1) involve a series of steps to ensure the integrity and efficacy of the aqueous extract as proper collection and preparation are crucial for the consistency and reproducibility of results in biochemical, antioxidant, toxicological, antimicrobial, and nanoparticle synthesis studies.

Figure 1. Goat Weed [Ageratum conyzoides (L.)] photographed from sight

The plant material (leaves) of goat weed was collected from Akure, Ondo State, Nigeria in January, 2024. This was done in the early morning to avoid moisture loss and maximize phytochemical content. The plant sample specimen was authenticated and deposited at the herbarium of the Department of Plant Science, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria. It was identified and confirmed by Mr. Omotayo, the Chief Technologist as *A. conyzoides*, Goat Weed, Ewe Imi Esu.

The leaves of *A. conyzoides* were carefully removed from the plant and rinsed under running tap water to remove any surface dust or contaminants. The cleaned leaves were then air-dried for 14 to 20 days, after which the leaves were ground into a fine powder.

Aqueous Extraction

The aqueous extraction of Ageratum conyzoides leaves adhered to WHO guidelines, using a 20:100 (w/v) ratio of powdered leaves to distilled water, the mixture was shacking continuously overnight on mechanical shaker till properly extracted, the mixture was filtered and the filtrate was stored at 4°C for stability and further use (Bouterfas et al.,2014; Sayompark et al., (2019).

Phytochemical Analysis of Fresh Sample

Test for Flavonoids (Alkaline Reagent Test)

To detect flavonoids, 2.5 mL of ammonia and 1 mL of concentrated sulfuric acid were added to 5 mL of the plant sample. The appearance of a yellow color indicated the presence of flavonoids in the sample (Saleem et al., 2014).

Test for Alkaloids (Hager Test)

For phytochemical analysis, 0.2 g of the plant sample was mixed with 3 mL hexane, shaken, and filtered (Astuti et al., 2011). After adding 5 mL of 2% HCl, the mixture was heated, filtered, and a few drops of picric acid were added. A yellow precipitate indicated alkaloids.

Test for Saponins (Foam Test)

For saponins, 10 drops of distilled water were added to 20 drops of the plant sample. The mixture was shaken vigorously. The persistence of foam indicated the presence of saponins (Sarfo-Antwi, 2017).

Detection of Phenolic Compounds (Ferric Chloride Test)

Ageratum conyzoides aqueous extract was subjected to a 5% ferric chloride solution, with a deep blue coloration indicating the presence of phenolic compounds, while treatment with a 10% lead acetate solution resulted in a white precipitate, confirming the presence of phenolic compounds (Tyagi and Agarwal, 2017).

Liebermann's test for Steroidal Nucleus

To test for steroids, 2.0 mL of acetic anhydride was added to 0.5 g of each solvent extract of the sample, followed by 2.0 mL of sulfuric acid (H2SO4) (Shodehinde and Oboh, 2013). A color change from violet to blue or green indicated the presence of steroids.

Some In-vitro Antioxidant Analyses of the Leaf Sample

Determination of DPPH Free Radical Scavenging Ability

The ability of the extract to neutralize 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was evaluated using a method adapted from Chaves et al., 2020. Briefly, 1.0 mL of the extract at various concentrations (20, 40, and 80 mg/mL) was added to separate test tubes. To each tube, 1.0 mL of a 0.1 mM methanolic solution of DPPH was added. The samples were then mixed and left to incubate in the dark at room temperature for 30 minutes. Following incubation, the absorbance of the solutions was measured at 516 nm. A decrease in absorbance indicated the extract's scavenging activity against DPPH free radicals.

Determination of Ferric Reducing Antioxidant Power

The ferric reducing antioxidant power (FRAP) of the extract was evaluated using a modified Pulido et al., 2017 method. A lower absorbance indicated higher ferric reducing power, reflecting the sample's antioxidant capacity.

Estimation of Vitamin C

Estimation of vitamin C content was determined using (Grace et al., 2014) method. Absorbance was measured at 520 nm, and ascorbic acid concentration was determined using a standard calibration curve and expressed as mg/g of the sample.

Green Synthesis and Characterization of the Synthesized Silver, Copper and Zinc Nanoparticles

Synthesis of Metal Nanoparticle

200 ml of the plant extract was weighed and poured into a 250 ml flat bottom quick fit flask and heated in a magnetic stirrer for few minutes. 50 ml of the prepared metal ion solution was then poured little by little. A color change was observed from the original colour of the extract to black brown showing a desirable formation of the nanoparticle. The mixture was fitted into a condenser and allowed to boil at an increased temperature of between 29 ^{o}C -70 ^{o}C. At the end of the desired heating, the mixture was poured into a clean 500 ml beaker and left to settle. Two distinct layers were observed. The upper layer (with no useful content) was poured out while the lower layer (containing the nanoparticle mixture) was poured into a 14 ml sample holder and centrifuged for 10 minutes. At the end of the centrifuging, two side layers were seen; the nanomaterial was at the lower side whereas the upper side (with no useful content) was poured out. The remaining mixture from beaker was repeatedly poured into a 14 ml sample holder and constantly centrifuged. At the end, distilled water was used to rinse the nanomaterial to remove unreacted substance. The nano material under the tube (sample holder) was oven dried and sent for characterization.

Characterization of the synthesized silver, zinc and copper nanoparticles

The biosynthesis of the AgNPs, CuNPs and ZnNPs in the solutions were monitored, identification of the synthesized nanoparticles and their size through (TEM) and characterized by measuring the UV–visible spectra of the solutions of the reaction mixture (Patil er al. 2023). UV–vis spectra were recorded on double beam spectrophotometer (Shimazdu, model UV-1800, Kyoto, Japan) from 300 to 800 nm at a resolution of 1 nm. The distilled water was used as a blank. Organic functional groups present in the leaf extract and AgNPs, CuNPs and ZnNPs were detected using FTIR. These measurements were carried out on a Shimazdu, Kyoto, Japan instrument in the diffuse reflectance mode at a resolution of 4 cm−1 in KBr pellets.

Toxicological Effect Analysis

The acute toxicity assay was carried out using the aqueous extract of *A. conyzoides* on a Wistar rat using 400mg/kg dose of the extracts for 24 hours, while the subacute toxicity was carried out using 200 mg/kg and 400 mg/kg doses of the extract administered to two rats respectively according to doses in all (4) four rats, to evaluate its effects and the rats behaviour for 21 days, the liver marker enzymes [Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)] were monitored and analyzed from the blood as described by the Ojewale et al methods.

Hematology Analysis

Automated hematology analyzer (Beckman Coulter) was used. Which involves using a blood sample diluted in an electrolyte solution to form a cell suspension, which is then introduced into the analyzer. The suspension flows through a narrow aperture surrounded by an electric field. As each blood cell passes through this sensing zone, it displaces electrolyte, temporarily altering the electrical resistance. This change generates a voltage pulse, with the height of each pulse reflecting the cell's volume. The analyzer captures these pulses, allowing precise determination of cell size and concentration, providing essential data for blood analysis.

Anti-microbial Analysis

In accordance to (Gumgumjee and Hajar 2015) agar well diffusion method, the procedures in the determination of antimicrobial activity using *A. conyzoides* aqueous extract was measured and recorded.

Results

The proximate composition analysis of *Ageratum conyzoides* demonstrates its richness in key nutritional components, including moisture, ash, crude fat, crude fiber, crude protein, and carbohydrates (Table 1).

Table 1: Proximate composition of *Ageratum conyzoides*

Parameters	Values (%)
Moisture content	6.89±0.01
Ash	13.34±0.01
Crude fat	19.04±0.003
Crude fibre	14.29±0.005
Crude protein	20.78±0.00
CHO (by difference)	25.66±0.01

The anti-nutrient composition of *A. conyzoides* was analyzed, showing the presence of oxalates, tannins, phytates, alkaloids, and trypsin inhibitors. These components were quantified to provide insights into the plant's biochemical properties (Table 2).

Table 2. Anti-nutrients composition of *Ageratum conyzoides*

Parameters	Values (%)
Oxalate (mg/g)	1.37±0.02
Tannin mg/L	28.45±0.02
Phytate (%)	0.25±0.02
Alkaloids	4.00±0.00
Trypsin inhibitor (%)	31.44±0.77

The mineral composition analysis of *A. conyzoides* revealed the presence of key elements, including potassium, sodium, phosphorus, calcium, magnesium, iron, zinc, copper, manganese, and chromium, with magnesium being the most abundant. Pb was not detected in the sample (Table 3).

Table 3. Mineral composition of *Ageratum conyzoides*

Parameters	Values (%)
K	5.50±0.01
Na	4.34±0.01
P	4.34±0.01
Ca	5.25±0.01
Cu	0.22±0.001
Cr	0.112±0.002
Mg	50.45±0.25
Fe	0.94±0.001
Mn	0.11±0.001
Pb	ND
Zn	1.73±0.002

ND: No detect

The phytochemical screening of the aqueous extract of *A. conyzoides* indicated the presence of various bioactive compounds, including saponins, phenols, tannins, flavonoids, alkaloids, terpenoids, steroids, glycosides, and phlobatannins. These findings confirm a diverse range of phytochemicals in the extract.

The results of the antioxidant composition analysis on the aqueous extract of *A. conyzoides* indicate a flavonoid content of 8.00% ± 0.00, a vitamin C concentration of 240.00 mg/100g ± 0.00, and a phenolic compound content of 3.18 mg GAE/g ± 0.04.

The extract exhibited a DPPH scavenging activity of 66.46% ± 0.08, a TBARS value of 0.02 mg MDA/g ± 0.00, a FRAP value of 16.86 mg (Vitamin C)/g ± 0.06, and a nitric oxide (NO) scavenging activity of 54.84% ± 0.3.

The antibacterial potential of *A. conyzoides* aqueous leaf extract and its nanoparticles indicate that the antibacterial activity varies significantly depending on the type of sample and the bacterial strain. Notably, the ZnNP exhibited the highest activity, particularly against Streptococcus faecalis, followed by moderate activity against other strains. Other samples, including the leaf extract and AgNP, demonstrated selective or minimal antibacterial effects.

The antifungal potential of *A. conyzoides* aqueous leaf extract and its nanoparticles against Fusarium oxysporium indicate that among the tested samples, ZnNP showed the highest mycelial growth inhibition (25.93%), followed by CuNP (14.82%). The leaf extract and AgNP did not exhibit measurable antifungal activity.

The sub-acute toxicity assessment of liver marker enzymes in rats treated with *A. conyzoides* aqueous extract is summarized in Table 4. At doses of 200 mg and 400 mg, the levels of AST, ALT, and ALP remained within normal ranges, indicating no significant hepatotoxicity. Slight variations in enzyme levels were observed between the two dosages.

Table 4. Sub-acute toxicity of liver maker enzymes of aqueous extract of *Argeratum conyzoides*

Parameters	Aqueous extract	
	200 mg	**400 mg**
AST (U/L)	37.20±0.15	38.13±1.11
ALT (U/L)	56.50±1.13	51.10±0.60
ALP (U/L)	66.63±0.89	57.66±0.40

The hematological parameters of rats treated with *A. conyzoides* aqueous extract at doses of 200 mg and 400 mg are presented in Table 5. Both dosages produced comparable effects on WBC, RBC, HB, and other blood indices, with variations within acceptable physiological ranges. Notably, statistical significance was observed for MCV and MCH between the two doses, suggesting potential dose-dependent effects. However, most parameters showed no significant differences, indicating overall hematological stability with the treatment.

Table 5. Haematology test on aqueous extract *Ageratum conyzoides*

Parameters	Aqueous extract	
	200 mg	**400 mg**
WBC (U/L)	6.10±0.70	6.10±0.70
RBC (U/L)	5.00±0.10	4.40±0.20
HB (U/L)	6.60±0.60	6.85±0.45
HCT (U/L)	36.50±0.50	38.00±1.00
MCV (U/L)	95.35±2.85	93.30±2.8
MCH (U/L)	25.15±0.55	27.50±0.80
MCHC (U/L)	26.35±0.25	27.30±1.60
PLT (U/L)	242.00±102.00	165.00±47.00

The white blood cell count analysis of rats treated with *A. conyzoides* aqueous extract at 200 mg and 400 mg doses is summarized in Table 6.

Table 6. White blood count on aqueous extract of *Ageratum conyzoides*

Parameters	Aqueous extract	
	200 mg	**400 mg**
N (U/L)	65.00±2.00	61.50±0.50
L (U/L)	27.50±2.50	34.00±2.00
M(U/L)	5.50±3.5	11.50±3.50
E(U/L)	1.50±0.50	3.00±1.00
B (U/L)	2.50±0.50	5.00±2.00

N: Neutrophils; L: Lymphocytes; M: Monocytes; E: Eosinophils; B: Basophils

The UV-Visible characterization of *A. conyzoides* leaf aqueous extract was performed using a UV-1800 Series spectrophotometer. The absorbance peaks were observed at 280.00 nm (1.700 absorbance) and 318.00 nm (1.333 absorbance). The spectrum indicates significant absorbance in the UV region, highlighting the potential presence of bioactive compounds.

The UV-Visible characterization of nanoparticles synthesized from the aqueous extract of *A. conyzoides* indicates the successful synthesis of AgNPs, CuNPs, and ZnNPs, with significant absorption observed in the UV-Vis region.

The FTIR spectrum of the aqueous extract of *A. conyzoides* reveals the presence of various functional groups, indicating a rich composition of bioactive compounds. The broad peak at 3280 cm^{-1} corresponds to O-H stretching, suggesting the presence of hydroxyl groups commonly found in phenols and alcohols. The peak at 1656 cm^{-1} is attributed to C=O stretching, indicative of carbonyl groups, which may originate from amides, carboxylic acids, or flavonoids. Peaks at 1438 cm^{-1} and 626 cm^{-1} suggest the presence of aromatic structures and alkanes, while the C-O stretching band around 1080-1015 cm^{-1} points to alcohols, ethers, or polysaccharides. These findings highlight the extract's composition of phenolic compounds, flavonoids, tannins, and glycosides, supporting its potential

therapeutic properties, such as antioxidant and antimicrobial activities (Figure 2).

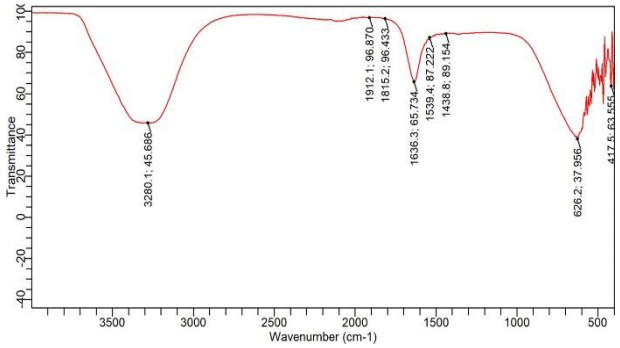

Figure 2. FTIR characterization of A. conyzoides leaf aqueous extract

The FTIR spectrum of silver nanoparticles (AgNPs) synthesized using Ageratum conyzoides reveals significant functional groups, indicating the role of biomolecules in nanoparticle stabilization and capping (Figure 3). The FTIR analysis confirms the presence of functional groups such as hydroxyl, carbonyl, and amide, as well as aromatic compounds, in the synthesized silver nanoparticles. These biomolecules, derived from *A. conyzoides*, play crucial roles in reducing silver ions and stabilizing the nanoparticles. The presence of the Ag-O band further confirms successful nanoparticle synthesis. This supports the bio-inspired method of AgNP production and highlights its potential applications in medicine and nanotechnology.

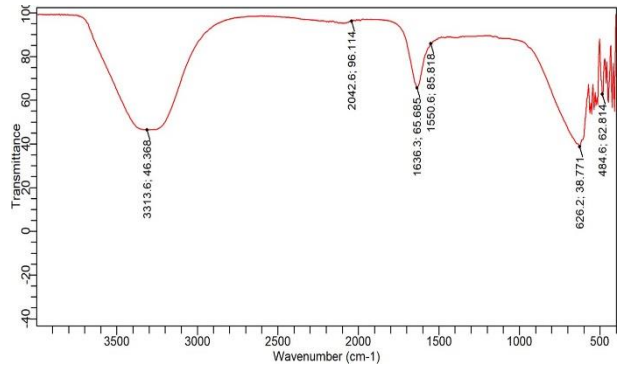

Figure 3. FTIR Characterization of A. conyzoides Silver Nanoparticles (AgNPs)

The FTIR spectrum of copper nanoparticles (CuNPs) synthesized using *A. conyzoides* shows the involvement of biomolecules in nanoparticle stabilization and capping. The broad peak at 3317 cm^{-1} indicates O-H stretching, representing hydroxyl groups likely from polyphenols, which play a role in the reduction and stabilization of CuNPs. The peak at 1632 cm^{-1} corresponds to C=O stretching, suggesting the presence of carbonyl groups from proteins, amides, or other plant metabolites acting as capping agents. The aromatic or phenolic compounds are indicated by a peak at 626 cm^{-1}, while the metal interaction band at 484 cm^{-1} confirms the successful synthesis of copper nanoparticles. These results highlight the role of plant-derived functional groups in reducing copper ions and stabilizing the CuNPs, emphasizing the eco-friendly synthesis process and the potential of A. conyzoides as a green source for nanoparticle production (Figure 4).

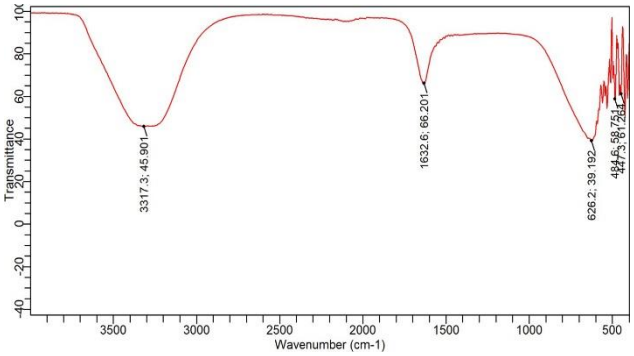

Figure 4. FTIR Characterization of A. conyzoides Copper Nanoparticles (CuNPs)

The FTIR spectrum of zinc nanoparticles (ZnNPs) synthesized using A. conyzoides reveals the presence of distinct functional groups, indicating their role in the stabilization and capping of nanoparticles. The broad absorption peak at 3261.4 cm^{-1} corresponds to O-H stretching vibrations, representing the presence of hydroxyl groups, likely from phenolic compounds or water molecules. The peaks at 2146.9 cm^{-1} and 1986.7 cm^{-1} could be attributed to C≡C or C≡N stretching vibrations, suggesting the presence of alkyne or nitrile groups. The peak at 1636.3 cm^{-1} corresponds to C=O stretching vibrations, indicating amides or carbonyl functional groups from proteins or flavonoids. Peaks at 1464.9 cm^{-1} and 1356.8 cm^{-1} represent C-H bending vibrations from alkanes. The strong absorption at 961.7 cm^{-1} and 626.2 cm^{-1} might relate to metal-oxygen vibrations, confirming the presence of Zn-O bonds, indicative of zinc oxide formation. These observations confirm the successful reduction and stabilization of zinc nanoparticles by bioactive compounds in A. conyzoides (Figure 5).

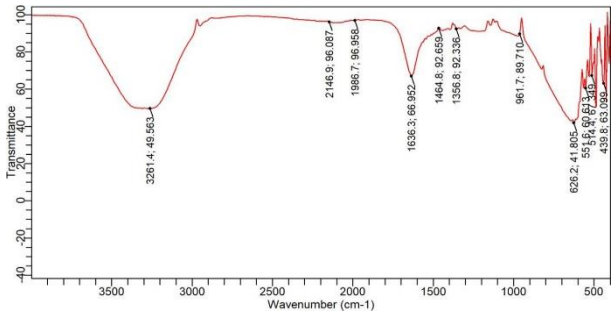

Figure 5. FTIR Characterization of A. conyzoides Zinc Nanoparticles (ZnNPs)

Discussion

In this study, Ageratum conyzoides possess a rich nutritional profile. The plant's low moisture content enhances shelf life and nutrient concentration (Bell, 2020). Its high ash content suggests significant mineral presence, vital for various biochemical functions (Alamgir, 2017). The notable crude fat content indicates it as a potential energy source, while the crude fiber is

beneficial for digestive health (He et al., 2022). The high crude protein content makes it valuable for tissue growth and repair, especially in regions with limited animal protein. The carbohydrate content underscores its role as an energy provider. Overall, these properties highlight its suitability for nutritional and therapeutic applications, supporting its traditional use in herbal medicine (Adelakun et al., 2022).

The plant contains antinutrients such as oxalates, tannins, phytates, alkaloids, and trypsin inhibitors, which may affect nutrient absorption. Oxalates can reduce calcium bioavailability and potentially lead to kidney stones, and tannins may inhibit protein and mineral absorption while offering antioxidant benefits. Low phytate levels suggest minimal impact on mineral absorption (Grases et al., 2017). Alkaloids have medicinal properties despite potential toxicity (Adamski, Z et al., 2020), and the high trypsin inhibitor content could hinder protein digestion if consumed in large quantities (Avilés-Gaxiola et al.,2018). Proper processing methods like cooking or fermentation are recommended to mitigate these effects and enhance safety and benefits.

Our study indicated that Ageratum conyzoides is rich in essential minerals, supporting various physiological functions. It contains significant levels of potassium, calcium, magnesium, phosphorus, sodium, copper, chromium, iron, manganese, and zinc. According to (Wang et al., 2023), minerals are crucial for cellular functions, bone health, enzymatic reactions, fluid balance, and immune function. The absence of lead indicates safety for consumption, enhancing its value in nutritional and therapeutic applications (Kumar and Prasad, 2018).

Ageratum conyzoides contains diverse bioactive compounds, including saponins, phenols, tannins, flavonoids, alkaloids, terpenoids, steroids, glycosides, and phlobatannins. These contribute to its anti-inflammatory, immune-boosting, antioxidant, antimicrobial, and anticancer properties (Anand et al., 2021) supporting its traditional medicinal use and potential for developing natural health products and pharmaceuticals (Singh and Sharma 2015; Panche et al., 2016; Yazarlu et al., 2021; Johnson et al., 2021)

The plant demonstrates strong antioxidant potential, effective in combating free radicals and preventing chronic diseases (Kaji et al.,2016). Its nanoparticles, particularly zinc nanoparticles, exhibit significant antibacterial and antifungal activities, suggesting potential applications in agriculture and medicine (Prabhu et al., 2017).

The sub-acute toxicity and hematological profile of Ageratum conyzoides (Goat Weed) are vital in determining the safety of its usage, particularly in therapeutic doses. Sub-acute toxicity studies focus on the biochemical and physiological changes that occur in the body after prolonged exposure to substances at varying concentrations. For Ageratum conyzoides, understanding the liver enzyme levels and hematological parameters helps in assessing its potential toxic effects and safe dosage range. Liver enzymes such as AST, ALT, and ALP are key indicators of liver health. Significant changes in these enzymes can suggest hepatotoxicity or liver stress, as AST is released when liver cells are damaged. ALT levels decreased from 56.50 U/L at 200 mg to 51.10 U/L at 400 mg, which may indicate less liver damage or potential protective effects at the higher dose. Similarly, ALP levels significantly dropped from 66.63 U/L to 57.66 U/L, hinting at reduced liver or bile duct stress at 400 mg. Overall, the changes in enzyme levels are mild, implying that Ageratum conyzoides causes minimal liver toxicity and may even offer some protective effects, particularly at higher doses indicating no significant hepato toxic effects. This suggests the extract's metabolic influence without pathological liver alterations (Adelakun et al., 2022). The hematological parameters of rats administered with 200 mg and 400 mg doses of aqueous extract of Ageratum conyzoides demonstrated notable effects on various blood components. The WBC count remained stable across both doses, with no significant impact on immune cell populations ($p = 0.226$), suggesting that the extract does not adversely affect overall white blood cell levels. However, there was a significant decrease in RBC count ($p = 1.33 \times 10^7$) at the higher dose, indicating a dose-dependent suppression of erythropoiesis, which could reflect a toxic or inhibitory effect on red blood cell production. Despite this reduction, the hemoglobin (HB) concentration showed a slight, non-significant increase ($p = 0.172$), potentially representing an adaptive response aimed at maintaining oxygen transport capacity even with fewer RBCs. This is further supported by a highly significant rise in hematocrit (HCT) ($p = 6.99 \times 10^6$), indicating a compensatory mechanism to preserve blood oxygen-carrying efficiency by increasing the concentration of red blood cells relative to plasma volume. There were also significant changes in MCV ($p = 0.0015$) and MCH ($p = 2.82 \times 10^7$), suggesting alterations in red blood cell size and hemoglobin content. Specifically, the higher dose led to a slight decrease in MCV, reflecting smaller RBCs, and a rise in MCH, indicating increased hemoglobin content per cell, possibly as part of the body's effort to maintain adequate oxygenation despite the decrease in RBC count. A notable finding was the marginally significant decrease in platelet (PLT) count ($p = 0.057$) at the higher dose, suggesting a potential impact on thrombopoiesis or platelet lifespan. This finding warrants further exploration to determine whether the extract affects platelet production or leads to increased platelet destruction. Additionally, the WBC differential counts revealed subtle but important changes in specific immune cell populations. Neutrophils showed a slight decrease, while lymphocytes, monocytes, eosinophils, and basophils increased with higher doses of the extract. The increase in lymphocytes suggests a potential enhancement of adaptive immune responses, while elevated monocytes point to improved capacity for phagocytosis and antigen presentation, supporting the immune system's role in combating infections and inflammatory processes. The increases in eosinophils and basophils are indicative of heightened responses to parasitic infections and allergic stimuli (Nakanishi, 2010; Eberle and Voehringer, 2016), suggesting that the extract may bolster the immune

system's ability to handle infections, inflammation, and allergic reactions(Harikrishnan and Balasundaram 2020) though, the potential for heightened allergic responses, especially at higher doses, necessitates further investigation, as increased eosinophil and basophil counts may reflect an increased susceptibility to allergic reactions.

The UV-Visible spectroscopy analysis of the aqueous extract from Ageratum conyzoides leaves revealed distinct absorbance peaks, particularly around 340 nm, indicative of phenolic compounds and flavonoids known for their antioxidant properties (Relhan et al., 2024). This suggests the presence of bioactive constituents that could contribute to antioxidant activity (Bessada et al., 2015), crucial for combating oxidative stress and promoting health benefits (Chahal et al., 2021). The characterization of silver nanoparticles synthesized from the plant showed a maximum peak absorbance indicative of surface plasmon resonance (SPR) also correlating with Chandraker et al., typically observed in the 400-500 nm range, signifying a high concentration of silver nanoparticles and demonstrating the effectiveness of using Ageratum conyzoides as a reducing and stabilizing agent (Aththanayaka et al., 2023; Shirsul et al., 2024). The UV-Visible spectroscopy of copper nanoparticles synthesized from the plant revealed a significant absorbance peak at approximately 600 nm, indicating the SPR phenomenon specific to copper nanoparticles, suggesting a spherical or quasi-spherical morphology (Chan, Y. B et al.,2022). This highlights the antimicrobial properties of copper nanoparticles, making them promising for applications in antimicrobial coatings, catalysis, (Usman et al., 2013) and biomedical uses (Vodyashkin et al., 2024). Similarly, the analysis of zinc nanoparticles synthesized from the plant displayed a distinct absorbance peak at approximately 400 nm, indicative of the SPR phenomenon specific to zinc nanoparticles. This suggests a predominantly spherical morphology (Al-Kordy et al., 2021) reflecting their stability and interactions in biological and environmental applications, with recognized antimicrobial properties and potential uses in drug delivery (Bessada et al., 2015), cancer therapy, and environmental remediation (Paul et al., 2022).

Fourier Transform Infrared (FTIR) spectroscopy analysis of A. conyzoides leaf aqueous extract and nanoparticles (AgNP, CuNP, ZnNP) reveals distinct peaks corresponding to functional groups such as aromatic compounds, alkyl groups, amides, phenolic compounds, carboxylic acids, esters, and hydroxyl groups. These analyses indicate the presence of bioactive compounds like phenolic antioxidants and highlight the nanoparticles' potential in biomedicine, environmental remediation, and antimicrobial applications. Silver nanoparticles (AgNP) show strong antimicrobial effects (Shankar and Rhim, 2015), copper nanoparticles (CuNP) exhibit antioxidant properties (Din et al., 2017) and zinc nanoparticles (ZnNP) demonstrate antimicrobial and catalytic capabilities (Benali et al.,2023), suggesting diverse applications in healthcare and environmental sustainability (Zahin et al., 2020).

Conclusion

The aqueous extract of Ageratum conyzoides (Goat Weed) from Akure, Ondo State, demonstrates significant potential across several scientific domains, including biochemical, antioxidant, toxicological, and antimicrobial studies. Notably, the extract facilitated the successful synthesis of silver, copper, and zinc nanoparticles, which were confirmed via UV-Visible spectroscopy through distinct surface plasmon resonance (SPR) peaks, particularly for silver nanoparticles. Biochemical assays revealed a rich presence of phytochemicals, such as flavonoids, tannins, and phenolics, which contribute to the plant's potent antioxidant properties. Antimicrobial testing showed that the nanoparticles effectively inhibited a variety of bacterial and fungal strains, indicating their potential as antimicrobial agents. While the findings affirm the plant’s bioactive properties and its potential in nanoparticle synthesis, further research is needed to fully assess the long-term toxicological effects and environmental impacts of these nanoparticles. Additionally, isolating and identifying the specific bioactive compounds of the extract using advanced techniques will provide deeper insights into its mechanisms of action and support the development of targeted therapeutic applications. Further clinical trials are essential to confirm the extract's safety and efficacy in humans. Additionally, exploring its integration into agriculture as a natural pesticide or growth enhancer could offer a sustainable alternative to chemical inputs.

Declarations

Conflict of interest

There is no conflict of interest among the authors.

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Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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