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Research Article

Studies on Antimicrobial, Biochemical and Three Metallic Nanoparticles on Aqueous Extracts of Justicia Carnea Leaf

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Abstract

Flamingo Flower *(Justicia carnea)*, historically valued for various applications, and has been researched for its medicinal properties. This study aims to investigate the biochemical composition, antioxidant activity, toxicological safety, antimicrobial efficacy, and green synthesis of the aqueous extract of flamingo flower. Standard analytical methods were used in determining the various parameters. The results of the analyses revealed the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds in the extract. It also exhibited strong antioxidant activities against DPPH and NO radicals with increased ferric reducing antioxidant power (FRAP) as well as significant levels of total phenols and flavonoids. The Acute and sub-acute toxicity analyses indicated no adverse effects from the aqueous extract on the Wistar rats. Hematological and white blood cell analyses in animals orally administered with the extract showed a positive safety profile. Furthermore, the green synthesis of metallic nanoparticles (silver, copper, and zinc) which were characterized using UV-Vis spectroscopy and FT-IR showed enhanced antimicrobial activity against various pathogenic bacteria and fungi, revealed some chemical properties based on the amount of light absorbed as well as identified some organic compounds based on their functional groups. Therefore, the study supports the traditional medicinal use of flamingo flower and suggests their potentials for developing natural antimicrobial agents, nanomedicine and drug development.

Key words: biochemical composition; antioxidant activity; toxicological safety; antimicrobial efficacy; green synthesis

Introduction

Justicia carnea, a flowering plant in the Acanthaceae family native to South America, particularly Brazil, is celebrated for its vibrant inflorescences and is widely cultivated worldwide as an ornamental shrub. Typically growing 1-1.5 meters tall with lanceolate to ovate dark green leaves, it produces dense spikes of tubular flowers in shades of pink, red, or white, arranged in verticillasters. These flowers attract pollinators such as bees and butterflies with their nectar-rich blooms. Justicia carnea thrives in well-drained soils with regular watering and partial shade, though it can tolerate full sun in cooler climates [1]. Beyond its aesthetic appeal, recent research has highlighted its potential medicinal properties, including antioxidant and anti-inflammatory activities, which may have implications for pharmaceutical and cosmetic applications. Conservation efforts are crucial due to habitat loss and fragmentation, underscoring the importance of habitat restoration and sustainable harvesting practices. *Justicia carnea* exemplifies tropical plant diversity, enhancing landscapes and supporting biodiversity through its role as a pollinator attractant [2].

Justicia carnea, a plant renowned for its vibrant flowers, plays a pivotal role in landscaping and horticulture worldwide due to its adaptability to diverse climates and soil conditions [3]. Its ecological significance lies in its ability to attract and support diverse pollinator populations, including bees, butterflies, and hummingbirds, thereby contributing significantly to local biodiversity and ecosystem stability.

Medicinally, *Justicia carnea* has been studied for its bioactive compounds, such as antioxidants and anti-inflammatory agents, which show promise in combating oxidative stress and inflammation. This potential has sparked interest in pharmacological research aimed at exploring new therapeutic treatments [4]. In terms of cultural importance, *Justicia carnea* is valued for its visual appeal in gardens and its symbolic representation of beauty and resilience. Its flowers are commonly used in floral arrangements, enhancing landscapes with color and texture. Conservation efforts are increasingly vital to manage *Justicia carnea* sustainably, preserving its natural habitats and minimizing negative impact on local biodiversity. Continued research and conservation practices are essential to fully understand and harness the benefits of this versatile plant species in various fields [5].

Materials And Methods

The process of collecting and preparing *Justicia carnea* (flamingo flower) for aqueous extract involves several critical steps to ensure the extract's integrity and efficacy across various studies. The plant material, specifically the leaves, was collected from Akure, Ondo State, Nigeria, on January 3, 2024, during the early morning to preserve moisture and maximize phytochemical content. The collected specimen underwent authentication and was deposited at the herbarium of the Department of Plant Science, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria. Authentication was confirmed by Mr. Omotayo, the Chief Technologist, and assigned the code number UHAE 2020068. This meticulous process ensures consistency and reproducibility in biochemical, antioxidant, toxicological, antimicrobial, and nanoparticle synthesis studies involving Justicia carnea [6].

Preparation Of Plant Leaf Extract

The leaves of *Justicia Carnea* 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 several days. Once adequately dried, the leaves were ground into a fine powder. A measured amount of 20 grams of this powdered leaf material was mixed with 100 mL of distilled water. This mixture was then agitated continuously overnight to facilitate thorough extraction of the leaf compounds [7].

Aqueous Extraction

Powdered leaves were mixed with distilled water in a 1:10 ratio (w/v) and heated at 70-80°C for 3-4 hours. After cooling to room temperature, the mixture was filtered to separate solid particles from liquid. Concentration was achieved using a rotary evaporator under reduced pressure if needed. The resulting extract was stored at 4°C for stability until future use [8].

Phytochemical Analysis (Fresh Samples)

The phytochemical analysis of fresh plant samples involved tests for flavonoids, alkaloids (Mayer's, Dragendorff's, and Wagner's tests), saponins (foam test), phenolic compounds (Ferric chloride and Lead acetate tests), and steroids (Liebermann's test). Each test utilized specific reagents to detect the presence of these compounds based on characteristic color changes or precipitate formations. Additionally, in vitro antioxidant analysis was conducted using the DPPH free radical scavenging assay [9].

Estimation Of Vitamin C

To estimate the vitamin C content, samples were oxidized with bromine water, neutralized with thiourea, and derivatized with DNPH. After dilution, absorbance at 520 nm was measured against standard ascorbic acid solutions (0.2-1.0 mL, 2 mg/mL). Concentration was calculated using a calibration curve [10].

Green Synthesis and Anti-Microbial Analyses

The experiment involves the biosynthesis of nanoparticles using *Justicia carnea* leaf extract. Initially, a leaf extract is prepared by mixing sliced leaves with distilled water, filtering, and refrigerating overnight. Three metal ion solutions are then prepared: 0.1 M silver nitrate, 1 mM copper sulfate, and 1 mM zinc sulfate, by dissolving respective salts in deionized water. These solutions serve as precursors for nanoparticle synthesis [11].

Synthesis Of Silver Nanoparticle

Justicia Carnea extract (200 ml) was heated with silver ion solution in a 250 ml flask, leading to a color change indicating nanoparticle formation. The mixture was boiled at 290-700°C for 12 hours, yielding a biphasic solution. The lower nanoparticle-containing layer was centrifuged and rinsed with distilled water to remove unreacted substances. Finally, the nanoparticles were dried in an oven for several days and sent for characterization.

Synthesis Of Copper Nanoparticles

Justicia carnea extract (200 ml) was mixed with a Copper ion solution, causing a color change to dark brown, indicating nanoparticle formation. The mixture was heated for 12 hours, then separated into upper and lower layers in a beaker. The lower layer, containing nanoparticles, was centrifuged and cleaned with distilled water before being oven dried for characterization [12].

Synthesis Of Zinc Nanoparticle

Justicia carnea extract (200 ml) was reacted with zinc ion solution in a 250 ml flask, resulting in a color change to dark brown, indicating reduction of nickel nitrate. The mixture was refluxed for 12 hours at 350°C, then separated into upper and lower layers in a beaker. The lower layer containing nanoparticles was isolated via centrifugation, washed with distilled water, dried, and prepared for characterization [13].

Characterization Of The Synthesized Silver, Copper And Zinc Nanoparticles

Nanoparticle biosynthesis of AgNPs, CuNPs, and ZnNPs was monitored using UV-visible spectroscopy (Shimadzu UV-1800) from 300 to 800 nm. Distilled water served as a blank for baseline correction. FTIR spectroscopy was employed to identify organic functional groups on leaf extract and nanoparticles, revealing their chemical composition and surface characteristics. This dual spectroscopic approach provided comprehensive insights into the synthesis and properties of nanoparticles [14].

Leaves were processed in two sets: one with 5 grams ground and extracted in 50ml ethanol and water, labeled "Aqueous" and "Ethanolic." Another set used 20 grams dry leaves with 300ml ethanol and water for extraction. Extracts were stored refrigerated post-filtration [15].

Liver Marker Enzyme Test

The study evaluated the effects of *Justicia carnea* extract at doses of 200 mg/kg and 400 mg/kg on liver function in Wistar rats over 21 days. Enzyme levels of ALT, AST, and ALP were measured to assess hepatoprotective or hepatotoxic effects. No acute toxicity was observed, and biochemical analysis showed significant alterations in enzyme levels, indicating potential impacts on liver function at higher doses of the extract. These findings suggest a need for further investigation into the safety and dosage of *Justicia carnea* extract in therapeutic [16].

Anti- Microbial Analysis

The study prepared concentrations of 100 mg/ml and 50 mg/ml of plant extracts using a double dilution method. Four bacterial isolates—*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*—were tested for antimicrobial activity. Glassware and media were sterilized rigorously, and bacterial inocula were standardized to 0.5 McFarland standard (10^5 cfu/ml). Antimicrobial activity was assessed using Mueller Hinton agar, with the zone of inhibition measured after 24 hours of incubation at 37°C [17].

Results

Parameters	Values (%)
Moisture Content	10.91±0.01
Ash content	16.20±0.27
Crude Fat	16.67±0.003
Crude Fibre	15.38±0.002
Crude Protein	27.71±0.01
CHO	13.13±0.1

Table 1: Proximate composition of Flamingo flower (*Justicia carnea*)

Parameters	Values
Oxalate (mg/g)	0.57±0.03
Tannin mg/L	24.68±0.03
Phytate (%)	0.27±0
Alkaloids	5±0
Trypsin inhibitor (%)	21.05±0.52

Table 2: Antinutrient composition of Flamingo flower *(Justicia carnea)*

Minerals	Values
K	7.42±0.02
Na	6.30±0.01
P	22.32±4.35E-15
Ca	5.37±0.01
Cu	0.18±0.001
Cr	0.12±0.001
Mg	46.65±0.15
Fe	1.42±0.003
Mn	0.08±0.001
Pb	0.02±0.002
Zn	1.1±0.0

Table 3: Mineral composition of Flamingo flower *(Justicia carnea)*

Phytochemical	Values
Saponin	+
Phenol	+
Tannin	++
Flavonoid	+
Alkaloid	++
Terpenoids	+
Steroids	+
Glycoside	+
Phlobatanine	++

Table 4: Phytochemical screening of Flamingo flower *(Justicia carnea)*

Parameters	Values
Flavonoid (%)	10.0±0.00
Vitamin C (mg/ 100g)	440.00±0.00
Phenolic Compounds (mg GAE/g)	1.98±0.02

Table 5: % Antioxidant composition of Flamingo flower *(Justicia carnea)*

SAMPLES	DPPH %	NO %	TBARS mgMDA/g	FRAP mg (Vit.C)/g
Flamingo Flower *(Justicia carne)*	50.43±0.16	41.08±0.25	0.05±0.001	17.39±0.06

Table 6: Free radical scavenging abilities of Flamingo flower *(Justicia carnea)*

Samples *Alcaligen*	*Pseudomonas Odorance*	*Streptococcus Syringiae*	*Salmonella faecalis*	*Enterobacter typhi*	*aerogens*
Justicia carnea Leaf extract	2.0	9.0	3.0	2.0	–
Justicia carnea AgNP	5.0	–	6.0	–	–
Justicia carnea CuNP	–	–	2.0	1.0	–
Justicia carnea ZnNP	8.0	3.0	8.0	3.0	3.0

Table 7b: Antifungal potential of blood leaf (*Justicia carnea*) aqueous extract and its metal nanoparticles on *Fusarium oxysporium*

Parameters	**Mycellial growth inhibition of *Fusarium oxysporium* (%)**
Justicia carnea Leaf extract	–
Justicia carnea AgNP	14.82
Justicia carnea CuNP	–
Justicia carnea ZnNP	14.82

Measurement Properties]
Wavelength Range (nm.): 200.00 to 900.00
Sampling Interval: 0.5
Scan Mode: Single
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm

No.	Wavelength	Absorbance
1	281.50	0.379

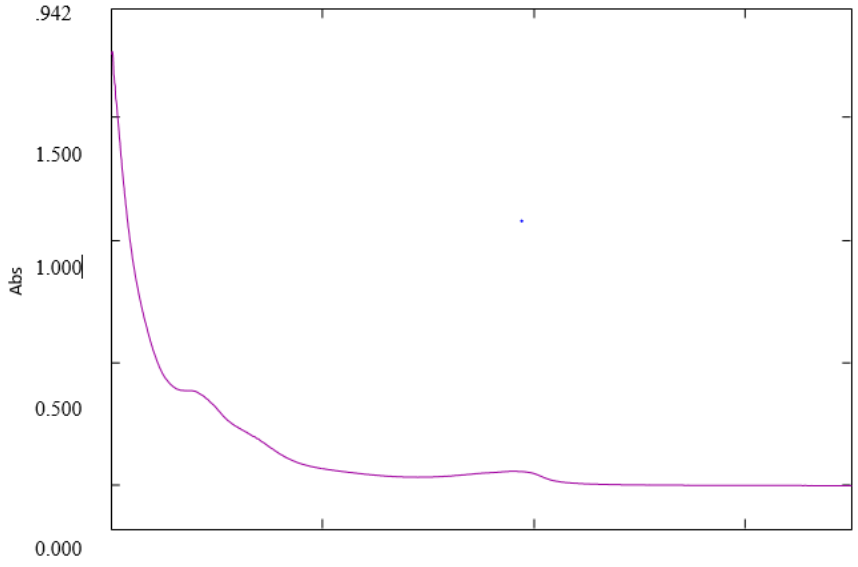

Figure 1.0a: The UV-Visible Characterization of Blood leaf (*Justicia carnea*) leaf aqueous extract

[Measurement Properties]
Wavelength Range (nm.): 200.00 to 900.00
Sampling Interval: 0.5
Scan Mode: Single
Instrument Type:
UV-1800
Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm

No.	Wavelength	Absorbance
1	276.50	1.243
2	333.50	0.721
3	587.50	0.152

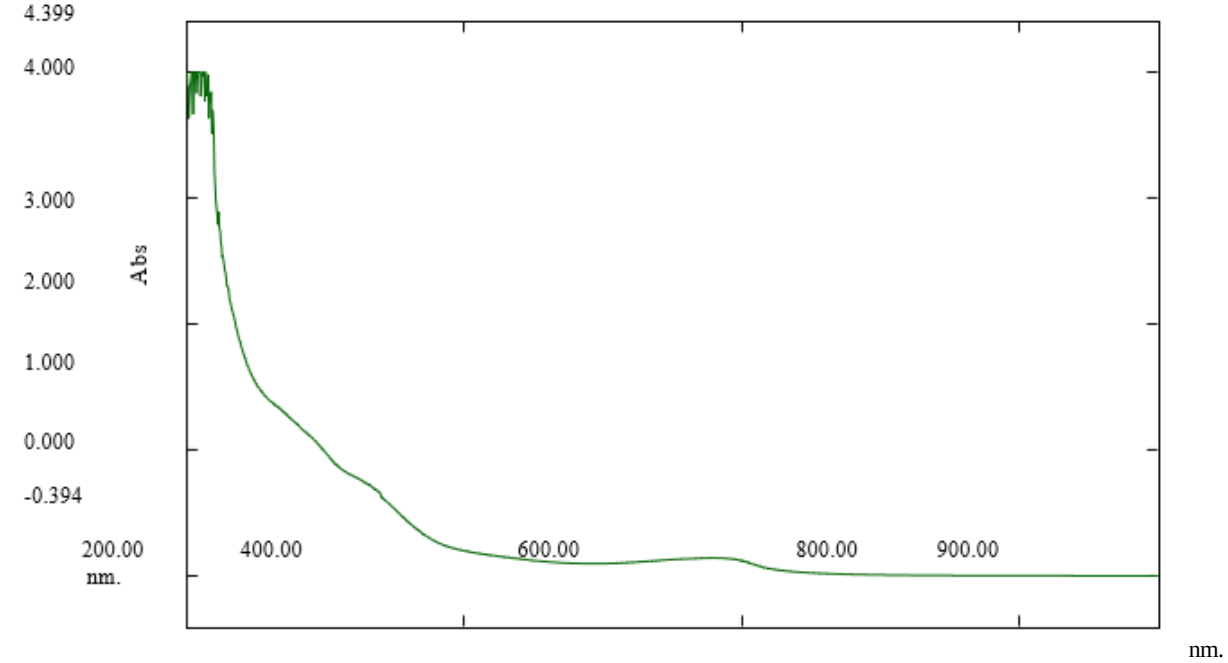

Figure 1.0d: The UV-Visible Characterization of Blood leaf (*Justicia carnea*) of zinc nanoparticle

Sample ID: Blood leaf (*Justicia carnea*) leaf aqueous extract
Sample Scans:32 Background Scans:32
Resolution:8 Range:4000 - 400

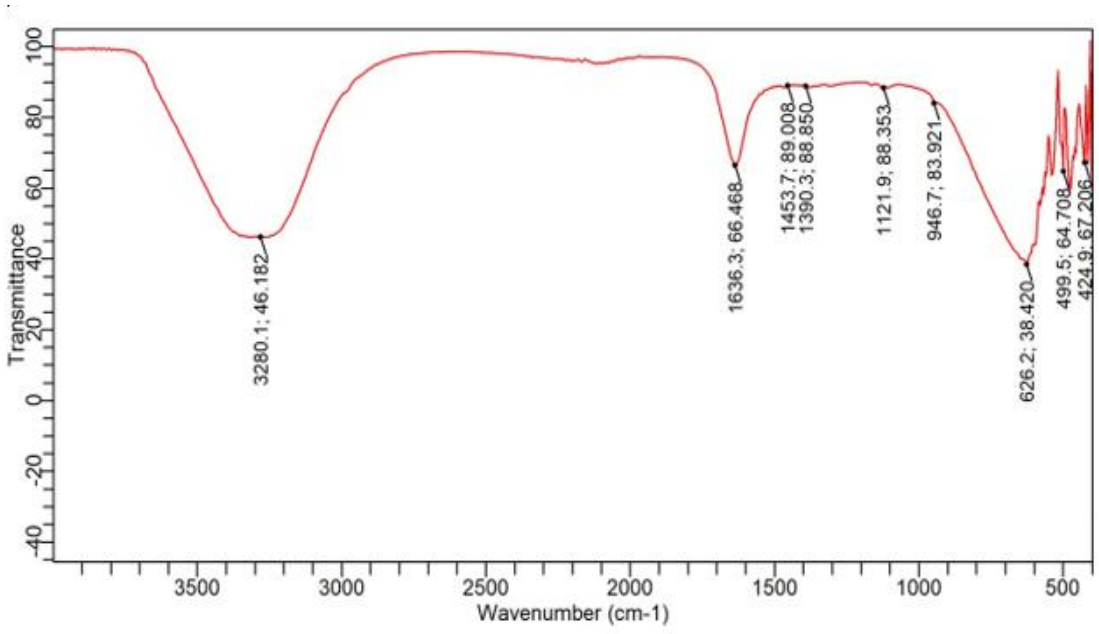

Figure 2:0a: FTIR characterization of Blood leaf *(Justicia carnea)* leaf aqueous extract

Peak Number	Wavenumber (cm^{-1})	Intensity
1	424.91651	67.20601
2	499.46327	64.70771
3	626.19276	38.41981
4	946.74381	83.92119
5	1121.92869	88.35253
6	1390.29701	88.85030
7	1453.66176	89.00789
8	1636.30131	66.46832
9	3280.05730	46.18249

Sample ID: Blood leaf *(Justicia carnea)* AgNP
Sample Scans:32 Background Scans:32
Resolution:8 Range:4000 - 400

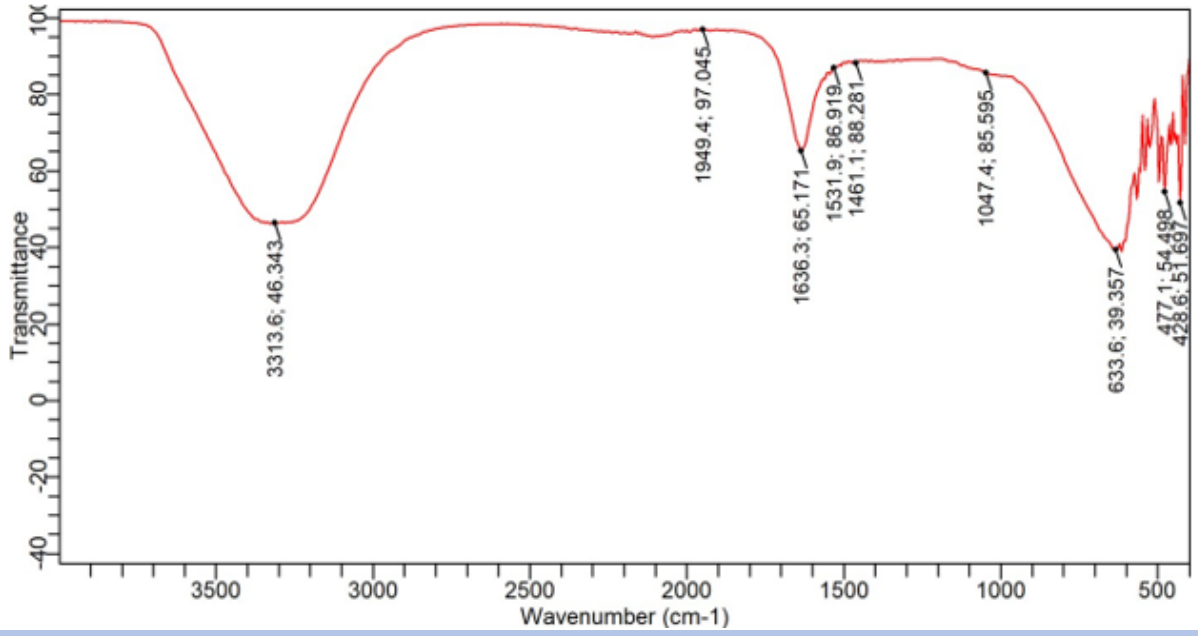

Figure 2:0b: FTIR characterization of Blood leaf *(Justicia carnea)* Silver nanoparticle (AgNP)

Peak Number	Wavenumber (cm^{-1})	Intensity
1	428.64385	51.69715
2	477.09924	54.49781
3	633.64743	39.35686
4	1047.38193	85.59515
5	1461.11643	88.28055
6	1531.93585	86.91934
7	1636.30131	65.17129
8	1949.39769	97.04453
9	3313.60334	46.34328

Sample ID: Blood leaf *(Justicia carnea)* CuNP
Sample Scans:32
Background Scans:32

Resolution:8 Range:4000 - 400

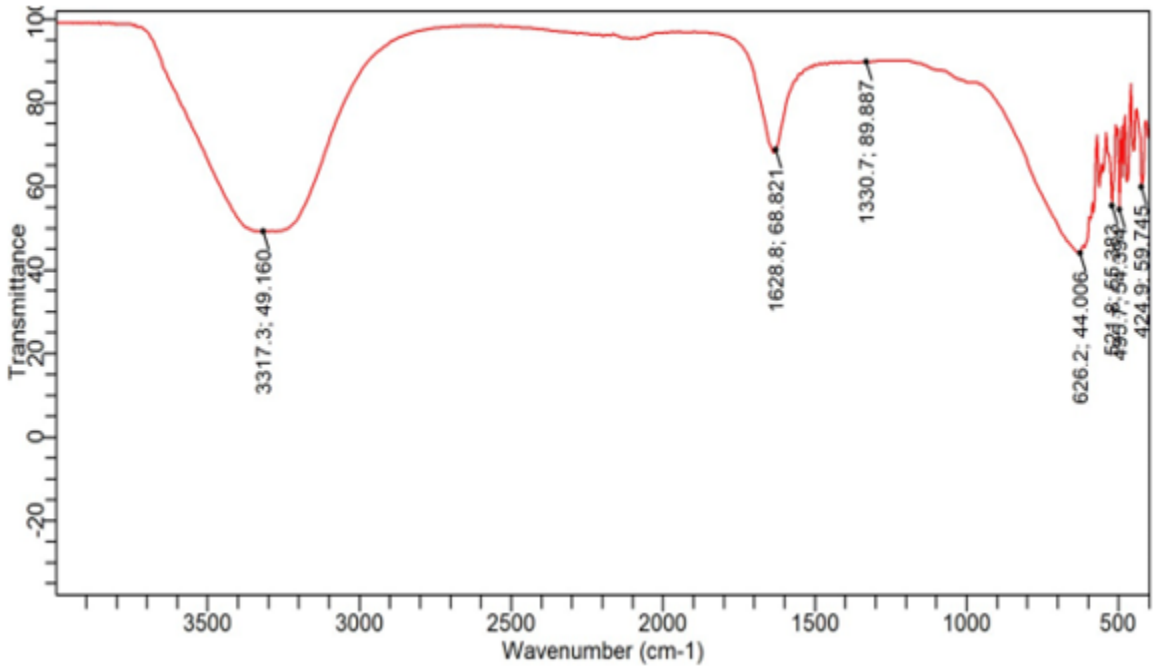

Figure 2.0c: FTIR characterization of Blood leaf *(Justicia carnea)* Copper nanoparticle (CuNP)

Peak Number	Wavenumber (cm^{-1})	Intensity
1	424.91651	59.74491
2	495.73593	54.39371
3	521.82730	55.38278
4	626.19276	44.00579
5	1330.65961	89.88691
6	1628.84663	49.159

Sample ID: Blood leaf *(Justicia carnea)* ZnNP
Sample Scans:32 Background Scans:32
Resolution:8 Range:4000 - 400

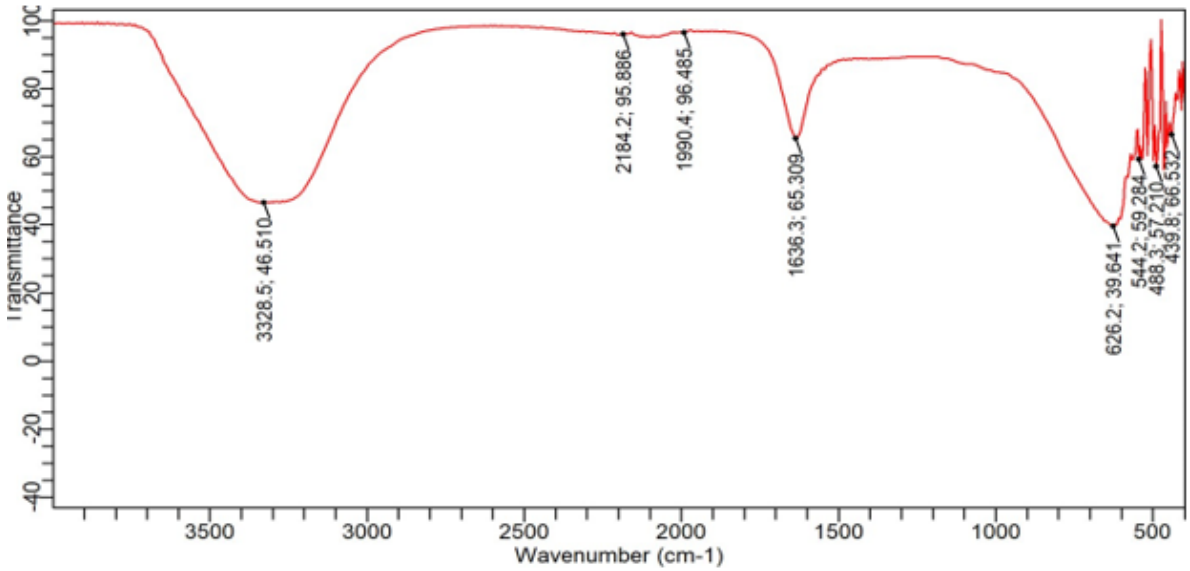

Figure 2.0d: FTIR characterization of Blood leaf *(Justicia carnea)* Zinc nanoparticle (ZnNP)

Peak Number	Wavenumber (cm^{-1})	Intensity
1	439.82586	66.53195
2	488.28126	57.20999
3	544.19132	59.28393
4	626.19276	39.64128
5	1636.30131	65.30913
6	1990.39841	96.48513
7	2184.21997	95.88619
8	3328.51269	46.50954

Discussion

Justicia carnea (Flamingo flower or Blood leaf) leaves exhibit a diverse nutritional and phytochemical profile [17]. Proximate analysis revealed a moisture content of 10.91 g/100g, ash content of 16.20%, and substantial levels of crude fat (16.67%), crude fibre (15.38%), crude protein (27.71%), and carbohydrates (13.13%). Antinutrient analysis indicated moderate levels of oxalate (0.57 mg/g) and tannins (24.68 mg/L), with low phytate content (0.27%) and significant trypsin inhibitor activity (21.05%).

Mineral composition highlighted significant levels of magnesium, phosphorus, and potassium, with moderate amounts of calcium, iron, and zinc, and trace elements like sodium, copper, chromium, manganese, and lead present. Phytochemical analysis revealed the presence of saponins, phenols, flavonoids (10.0%), terpenoids, steroids, glycosides, alkaloids (5 mg/g), and moderate levels of tannins and phlobatannins [18,19].

Flamingo flower leaves are rich in antioxidants, containing Vitamin C (440.00 mg/100g) and phenolic compounds (1.98 mg GAE/g). Antioxidant assays demonstrated significant free radical scavenging capabilities [20], (e.g., DPPH: 50.43%, NO scavenging: 41.08%) and lipid peroxidation inhibition (TBARS: 0.05 mg MDA/g), along with ferric-reducing antioxidant power (FRAP: 17.39 mg Vitamin C/g) [21].

Further characterization via FTIR spectroscopy of aqueous extracts and metal nanoparticles (AgNP, CuNP, ZnNP) derived from *Justicia carnea* confirmed the presence of characteristic functional groups such as hydroxyl (-OH), carbonyl (C=O), and methyl/methylene (C-H) groups, indicative of bioactive molecules and stabilizing agents [22]. These findings underscore the nutritional, antioxidant, and potential therapeutic properties of *Justicia carnea*, supporting its traditional medicinal uses and potential applications in pharmaceutical and dietary contexts [23].

Conclusion

The study revealed that "Ewe Eje," scientifically known as *Justicia carnea*, is a flowering plant indigenous to parts of tropical Africa, including Nigeria. In traditional medicine, it is valued for its purported therapeutic properties, which include treating skin infections, headaches, and rheumatism. The plant's leaves and roots are typically used, either brewed into teas or applied externally as poultices. The study revealed that *Justicia carnea* leaf is a good source of phytochemicals which have been reported to have various biochemical and physiological effects. The leaf is apparently a good source of antioxidants and their various nanonoparticles: Zinc nanoparticles, copper nanoparticles and silver nanoparticles have been tested against bacteria and the result showed that they possess antibacterial activities. Vitamin c is present.

Recommendation

The outcome of this research will be useful to all the communities, Pharmacological industries and traditionalist using this plant since they will be the first beneficiaries. It will create awareness to local communities to enhance the traditional knowledge with scientific finding. It will be useful to pharmaceutical companies in order for them to appreciate the benefits, the local users and most communities will be exposed to and the need to gives adequate orientation and advice on planting of the plant.

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