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Synthesis, Characterization and Antibacterial Analysis of Zerovalent Iron Nanoparticles from *Cymbopogon Citratus and Vernonia Amygdalina* Plant Extracts as a Means of Sustainable Development Goal

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Abstract

Cymbopogon citratus and *Vernonia amygdalina* are tropical plants that are rich in phytochemicals which makes them to have antibiotic capability. Aqueous extracts of *Cymbopogon citratus* and *Vernonia amygdalina* leaves were used to produce Zerovalent Iron nanoparticles (ZVINPs) by reduction of ferric chloride solution. The ZVINPs were characterized by Fourier Transform Infrared Spectrophotometry (FTIR) for the identification of functional groups. The antibiotic effect of the ZVINPs was tested on four isolates, each at five different concentrations of 100%, 10%, 7.5%, 5.0% and 2.5%. The results showed that the efficacy of the *Cymbopogon citratus* and *Vernonia amygdalina* ZVINPs against some biotic isolates were 36% and 34.3% respectively. Also, FTIR spectroscopic measurements identified 4 functional groups and showed that the ZVINPs had no peak at 430-860 cm^{-1}, indicating that the iron has been reduced to its zerovalent form by the extracts. Thus, the phytochemicals present in the extracts served as effective reducing agent and the isolates were inhibited moderately at peak concentrations. The study concluded that the prepared ZVINPs had moderate antibacterial potential.

Keywords: Synthesis, Characterization, Antibiotic Activity, Zerovalency, Plant Extract, Nanoparticles.

1. Introduction

Nanoparticles are extremely small-sized materials exhibiting properties and characteristics that are utilized in different fields such as medicine, agriculture, engineering, environmental science (Dikshit *et al*., 2021). Previously, Zero Valent Iron Nanoparticles (ZVINPs) have been used due to their ease of contaminant removal from aqueous solutions (Fazlzadeh *et al*., 2017). The ZVINPs are synthesized by physical (Kharissova *et al*., 2015), laser ablation (Wang *et al*., 2012), electric arc discharge (Hosseynizadeh Khezri *et al*., 2012), microbial (Dikshit *et al*., 2021) and chemical methods (Bukka *et al*., 2019). The expensive instruments, high-energy (Kalyan Kamal *et al*., 2014), release of inimical chemicals (Ravindran *et al*, 2013), culture of cell (Baker *et al*., 2013) and wasteful purifications (Awwad *et al*., 2013) are some of the drawbacks to the effective synthesis these methods require. Previously, a variety of organic reducing agents like such as sodium borohydride ($NaBH_4$) (Pattanayak & Nayak, 2013), hydrazine, sodiumdodecyl sulphate (Kiruba *et al*., 2013) were utilized. These reducing agents cause environmental hazards (Sampath *et al*., 2014).

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Developing nanoparticles using plants as base material is a common occurrence. Green synthesis of ZVINPs using extracts of natural product is being resourced in the last few years. The plant-mediated ZVINPs produced is a cost-effective, simple and environmentally friendly alternative method (Wei *et al*., 2016). Various work has been done that support this assay.

Among the plants in existence, *Vernonia amygdalina* commonly called bitter leaf and *Cymbopogon citratus* often referred to as lemon grass. Bitter leaf contains phytochemical and pharmacological compounds which makes it an antibiotic agent and the active ingredients in lemon grass enable it to serve as an antimicrobial and insecticidal agent. These constituents portend the plants as useful materials in the green synthesis of zerovalent iron nanoparticles. These plants are sustainable because they are cheap and available all the year round (Mirghani *et al*., 2012).

The leaves of Lemon grass and bitter leaves are good sources of antioxidants and phenolic compounds (Simic *et al*., 2008). In this work, a simple green method was developed to synthesize ZVINPs using bitter leaf and lemon grass aqueous extracts as reductants and stabilizing reagents. This work also derived the antibacterial efficacy of the synthesized nanoparticles.

- ***Vernonia amygdalina***, a member of the daisy family, is a small shrub that grows in tropical Africa. *V. amygdalina* typically grows to a height of 2–5 m (6.6–16.4 ft). The leaves are elliptical and are up to 20 cm (7.9 in) long. Its bark is rough. *V. amygdalina* is commonly called bitter leaf in English because of its bitter taste.
- ***Cymbopogon citratus*** belongs to the grass family, Poaceae (syn. Gramineae). It is also called lemon grass because of its characteristic scent and it is widely distributed in all regions of the world. *Cymbopogon* is a genus comprising about 180 species, subspecies, varieties, and is a perennial aromatic grass having dense fascicles arising from short annulate, sparingly branched rhizome, leaf blade is linear and long, approximately 90cm long and 5cm wide (Purnima *et al*., 1999)**.**

2.0 Materials and Methods

2.1 Materials Used

The materials used for this study include *Vernonia amygdalina* and *Cymbopogon citratus* leaves; $FeCl_3.6H_2O$ was the metal salt used. The fresh leaves of *Vernonia amygdalina* and *Cymbopogon citratus* were gotten from a vegetable garden at Ganmo, Kwara State, Nigeria. Integrated Sunaf Scientific Supplies supplied the metal salt.

2.2. Method of Synthesis

2.2.1 Synthesis of ZVINPs

Bitter leaf and lemon grass aqueous extracts were used as a reducing agent for the synthesis of ZVINPs. The fresh leaves of bitter leaf (*Vernonia amygdalina*) were washed repeatedly with distilled water. Approximately 25 g of leaves were taken in a glass of 250 ml containing 75 ml of distilled water and then heated at 60° C for 20 minutes and filtered. The resulting filtrate was stored and used as reducing and stabilizing agents (Machado *et al*., 2013). The precursor and the reducing agent were mixed into clean sterilized flask in a 1:1 proportion. For the reduction of Fe ions, 5 ml filtered *V. amygdalina* extract was mixed with 5 ml of freshly prepared 0.1 M aqueous solution of $FeCl_3$ with constant stirring at room

temperature. The resulting solution was centrifuged for 30 minutes at 5000 rpm. The precipitate generated was freeze dried (for 24 hours), stored in sealed vial and dessicated before analysis.

The same procedure was repeated for lemon grass (*Cymbopogon citratus*) aqueous extract.

2.2.2 Antibacterial evaluation of ZVIPs

S. aureus was obtained in frozen form from the American Type Culture Collection (ATCC; ATCC 25923). The bacteria were thawed on ice for 20 minutes before being plated on an agar plate. The plate was dried before incubation for 16 hours in a standard cell culture environment (37°C, 5% CO_2, and 95% air). A single colony of *S. aureus* was selected using a 10 µL loop (Sigma, St. Louis, MO) and inoculated into centrifuge tubes containing 5 mL of tryptic soy broth. Bacteria in centrifuge tubes were then incubated at 37°C under agitation at 200 rpm for another 16 hours. At that point, the bacteria solution was diluted in tryptic soy broth to an optical density of 0.52 at 562 nm using a microplate reader (Spectra-Max300; Molecular Devices, Sunnyvale, CA). According to the standard curve correlating bacteria number with optical density, this value was equivalent to 5×10^6 cells/mL. The cells were further diluted in tryptic soy broth to 5×10^4 cells/mL before being added to a new centrifuge tube at 3 mL/tubes. Concentrated Iron Nanoparticles (IN) in solution were added to bacteria tubes at different doses [30 µg/mL (low dose), 300 µg/mL (medium dose), and 3 mg/mL (high dose)]. A tube of bacteria without nanoparticles served as a control. The IN solution was also added to tubes containing only tryptic soy at the same concentration as above and this served as a particle control. Bacteria were then incubated under agitation for four hours, 12 hours, and 24 hours before a 200 µL bacteria solution was transferred to a 96-well plate for optical density readings at 562 nm using a microplate reader (Tran *et al*., 2010).

2.3 Characterization

The prepared samples were characterized by Fourier Transform Infrared Spectroscopy (FTIR, Perkin–Elmer RX1).

FT-IR spectrum and characterization frequency denote bands of ZVINPs in the range of 400-4000 cm^{-1}.

3. Results and Discussion

3.1 FTIR Spectroscopy Analysis Result

The FT-IR spectrum of synthesized *Cymbopogon citratus* ZVINP (Fig. 1) displayed four strong bands around 3381.29, 1605.83,1391.42 and 1062.96 cm^{-1}. The spectrum shows stretching vibrations at 1605.83 cm^{-1} which corresponds to C=C, 3381.29 cm^{-1} corresponds to O-H stretching vibration, 1062.96 and 1391.42cm^{-1} corresponds to C-O-C, and C=N bands respectively.

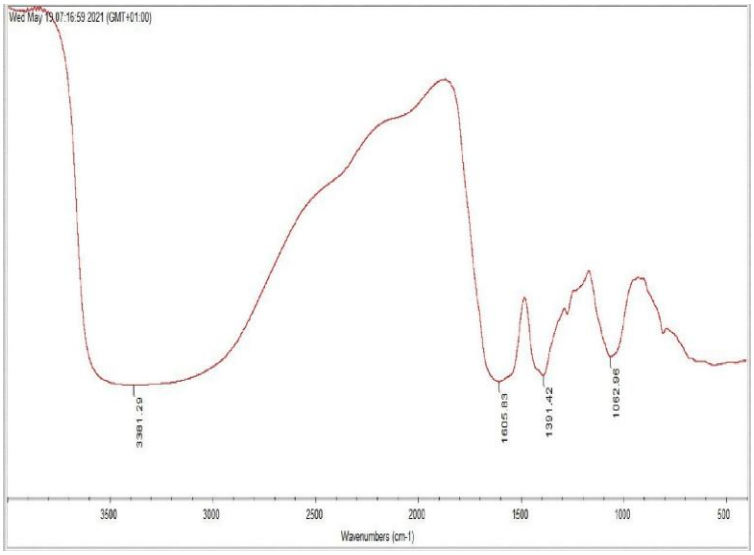

Fig. 1: FTIR Spectrum of *Cymbopogon citratus*

NanoparticlesGottimukkala *et al*. (2017**)** have reported the presence of similar bands at 3452 cm^{-1} for O-H, 1632 cm^{-1} for C=C, 2926 and 1383 cm^{-1} for C-H and C-N respectively when iron nanoparticles were synthesized from green tea extract. Sravanthi *et al*. (2018) also reported presence of similar bands: 3359 cm^{-1} for O-H group, 1594 cm^{-1} for C=C, 1039 and 1396 cm^{-1} for C-O-C and C-N groups respectively from synthesized *Calotropis gigantea* iron nanoparticles.

From the spectrum above, the broad and intense absorption peak at 3,381.29 cm^{-1} corresponds to the O-H stretching vibrations of phenols and carboxylic acids (Njagi *et al*., 2011; Kokila *et al*., 2015). This indicates the prominence of phenolic functional groups for the reduction of Fe^{3+} to Fe^{0}. Furthermore, the more available phenolic groups provide the favorable molecular arrangement for the delocalization of unpaired electrons. So, the *Cymbopogon citratus* extract enhances the property of effective scavenging of free radicals. On the other hand, anti-oxidant capacity and anti-radical property increases with the number of phenolic hydroxyl groups. The appearance of the peak at 3359 cm^{-1} suggests that the phenolic group present in *Cymbopogon citratus* extract may be involved in zerovalent iron nanoparticle (ZVINP) formation.

Mystrioti *et al*. (2015) reported that polyphenols were responsible for the reduction of Fe. From this spectrum, the absence of peaks at 430-860 cm^{-1} in FT-IR spectra of ZVINP *Cymbopogon citratus* shows that polyphenols present in the *Cymbopogon citratus* extract is responsible for reduction and stabilization of ZVINP, thus, no peak at 430-860 cm^{-1}.

The broad and intense absorption peaks at 3381.29 cm^{-1} corresponds to the O-H stretching vibrations of phenols and carboxylic acids (Njagi *et al*., 2011; Kokila *et al*., 2015). This indicates the involvement of O-H functional groups of *C. citratus* extract in the synthesis of ZVINP.

The bending vibration of C=N at 1391.42 cm^{-1} in the amide group indicates the involvement of the amino groups of the *C. citratus* extract in nanoparticles synthesis (Kokila *et al*., 2015).

The peak at 1605.83 cm^{-1} is in accordance with the C=C stretching of aromatic compounds.

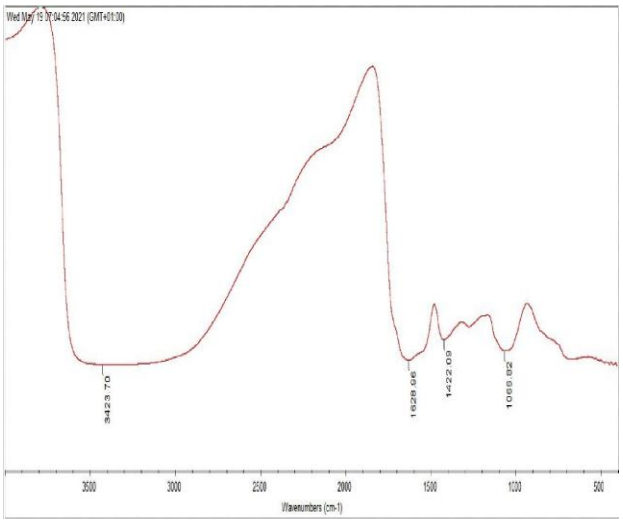

Fig. 2: FTIR Spectrum for *Vernonia amygdalina* Nanoparticles

FT-IR spectrum of Vernonia amagdalina ZVINP (Fig. 2), is almost similar to that of *C.citratus* nanoparticles (since zerovalent iron nanoparticles is synthesized also); but differs in that, the O-H stretching vibration peak is 3423.70 cm^{-1}, the C=C band is at 1628.96 cm^{-1}, C-O-C and C-N at 1066.82 and 1422.09 cm^{-1} respectively. The peak, 3423.70 cm^{-1} corresponds to polyphenols, which indicates the prominence of phenolic functional groups for the reduction of Fe^{3+} to Fe^{0}. Moreover, the phenolic groups provide the favorable molecular arrangement for the delocalization of unpaired electrons. So, the *V. amagdalina* extract has the property of effective scavenging of free radicals.

On the other hand, anti-oxidant capacity and anti-radical property increases with the number of phenolic hydroxyl groups. The appearance of a peak at 3423.70 cm^{-1}suggests that the phenolic group present in *V.amagdalina* extract is be involved in ZVINP formation. The earlier report by Mystrioti *et al*. (2015) indicated that polyphenols were responsible for the reduction of Fe. As observed from the spectrum above, the absence of Fe peak at 430-860 cm^{-1} shows that polyphenols present in the *V.amagdalina* extract is responsible for the reduction and stabilization of ZVINP; thus, no peak was observed for Fe at 430-860 cm^{-1}.

The broad and intense absorption peaks at 3423.70 cm^{-1} corresponds to the O-H stretching vibrations of phenols and carboxylic acids (Njagi *et al*., 2011; Kokila *et al*., 2015). This indicates the involvement of O-H functional groups of *V.amagdalina* extract in the synthesis of ZVINP.

The bending vibration of C=N at 1422.09 cm^{-1} in the amide group indicates the involvement of amino groups of the *V.amagdalina* extract in nanoparticles synthesis (Kokila *et al*., 2015).

The peak at 1628.96 cm^{-1} is in accordance with the C=C stretching of aromatic compounds.

Table 1: Wave numbers of Functional Groups in *Vernonia amygdalina* and *Cymbopogon citratus* Nanoparticles

Groups	***Cymbopogon citratus***	***Vernonia amygdalina* NP**
O-H	3381.29	**3423.70**
C=C	1605.83	**1628.96**
C=N	1391.42	**1422.09**

C-O-C	1062.96	**1066.82**

3.2 Nanoparticle reduction

The formation of ZVINPs of the filtrate was investigated by the observation of the change in the color of the solution. When extract and aqueous ferric chloride solutions were mixed, color of the solution immediately turns black from brown and intense fine black precipitates appeared in tubes. This alludes that, ZVINPs was formed as previously reported (Wei *et al*., 2016; Mystrioti *et al*., 2016; Ravikumar *et al*., 2016). *C. citratus* and *V.amygdalina* are mainly composed of polyphenol (Sathya, 2014), α-citral, β-citral limonene, citronellal, ß-myrcene and geraniol may be involved in reducing the Fe^{2+} ions to Fe^0 (Schaneberg and Khan, 2002). Biological components, particularly polyphenols are known to interact with metal salts through these functional groups and iniate their reduction to nanoparticles (Nadagouda *et al*., 2010).

The formation of nZVI with polyphenols took place through the following steps: (1) complexation with Fe salts, (2) simultaneous reduction of Fe ions, (3) capping oxidized polyphenols (Nadagouda *et al*., 2010). Polyphenols can directly complex and then reduce iron (Fe^{2+}) to zero valent (Fe^0) particles. The reduction and oxidation processes in the reaction depend on the reduction potentials (E^0) of reagents. The reduction potential of polyphenol is 0,57 V which sufficient to reduce Fe^{2+} ions to Fe^0 (reduction potential -0,44 V) (Wang *et al*., 2017). The general reaction mechanism of synthesis of Fe^{2+} with polyphenols and other polyol compounds can be written as:

$$nFe^{2+} + 2Ar\text{-}(OH)_n \rightarrow nFe^0 + 2nAr{=}O + 2nH^+$$

where, Ar is the phenyl group and, n is the number of hydroxyl groups oxidized by Fe^{2+} (Mystrioti *et al*., 2016).

3.3.1 Antibacterial Analysis

3.3.2 Antibacterial activity/sensitivity of *Cymbopogon citratus* and V*ernonia amygdalina* nanoparticles on bacteria

Table 2: Zones of Inhibition (mm) of Isolates at Varying Nanoparticles Concentrations:

S/N	Isolates	Peak	10%	7.5%	5.0%	2.5%	Control
1	B_1	0.0	0.0	0.0	0.0	0.0	0.0
2	E_1	4.0	0.0	0.0	0.0	0.0	0.0
3	G_1	5.0	0.0	0.0	0.0	0.0	0.0
4	H_1	6.0	0.0	0.0	0.0	0.0	0.0
5	B_2	4.5	0.0	0.0	0.0	0.0	0.0
6	E_2	0.0	0.0	0.0	0.0	0.0	0.0
*7	G_2	4.5	0.0	0.0	0.0	0.0	0.0
8	H_2	4.0	0.0	0.0	0.0	0.0	0.0

$B_{1,2}$ = *Bacillus subtilis* $E_{1,2}$ = *Bacillus cereus* 0.0= No zone of inhibition $G_{1,2}$ = *Providencia retteri* $H_{1,2}$ = *Providencia rettgeri*

>11 mm= sensitive

$_1$ = isolates for *Cymbopogon citratus*

7mm-10mm = moderately sensitive

$_2$ = isolates for *Vernonia amagdalina*

2mm-6mm= resistant

The antibiotic activity of *Cymbopogon citratus* and V*ernonia amygdalina* nanoparticles against some selected isolates showed that the peak concentration of the samples had the most significant results with the highest value of 6.0mm obtained from Isolate H_1 of the *Cymbopogon c.* sample. Although a zone of inhibition of 4.5mm was found in Isolate B_2 and G_2 of the V*ernonia amygdalina* sample at peak concentrations. However, all other concentrations (i.e. 7.5%, 5.0% and 2.5%) as peak concentration of B_1 (*Cymbopogon citratus*) as well as Isolate E_2 (V*ernonia amygdalina*) had no zone of inhibition as shown above.

3.2.2 Inhibition effect of antibiotics on soil bacteria

Table 3: Inhibition Zones of Antibiotics on Gram Negative Bacteria

Isolates	Inhibition Zones of Antibiotics in mm								
	CAZ	CPR	CRX	GEN	OFL	AUG	NIT	CXM	Average
B	20	20	20	20	20	20	20	0	17.5
E	0	20	0	19	20	20	20	0	12.38
G	0	20	15	20	20	7	5	0	10.88
H	0	20	0	20	20	20	20	0	12.5

Key:
CAZ: ceftazidimeGEN: gentamycin
NIT: nitrofurantan
CPR: ciprofloxacin OFL: ofloxacin
CXM: cefuroxime
CRX: cefuroxeme AUG: augmentin
>11 mm= sensitive
7mm-10mm = moderately sensitive
2mm-6mm= resistant

The inhibition zone of standard antibiotics on Gram negative bacteria isolated from soil showed that all isolates were sensitive to Ciprofloxacin (CPR) and Ofloxacin (OFL) while all the isolates were resistant to Cefuroxime (CXM). The least resistance(5mm) was found in Nitrofurantan (NIT) in Isolate G which was also found to be moderately sensitive to Augmentin (AUG). Also, Isolate A and B showed sensitivity to all the antibiotics excluding Cefuroxime (CXM).

3.3 Results Comparison

The results of the analysis for *Cymbopogon citratus* and *Vernonia amygdalina* nanoparticles on basis of antibiotic efficacy are as presented below:

Table 4: Antibiotic Efficacy of *Cymbopogon citratus* and *Vernonia amygdalina* Nanoparticles

Plant	Nanoparticle Peak Inhibition	Conventional Inhibition	Efficacy (%)
Cymbopogon citratus	6.0 mm	17.5 mm	34.3
Vernonia amygdalina	4.5 mm	12.5 mm	36.0

4. Conclusion

Stable zerovalent iron nanoparticles synthesis using bitter leaf and lemon grass as base materials were successfully carried out in the laboratory using iron chloride hexahydrate as the metal precursor. Solid nanoparticle samples were characterized with FT-IR for identification of the functional groups in the nanoparticles. The characterization clearly shows that the plant metabolites possess the potential to reduce the ferric cations. The antibiotic effect of the nanoparticles was experimented on, with a view to determine the efficacy of the nanoparticles.

The yields of the nanoparticles synthesized were 10% and 11% for bitter leaf and lemon grass respectively while the efficacy against isolates were 36% and 34.3% for bitter leaf and lemon grass respectively.

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