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Research Paper

Biochemical Investigations, Anti-microbial Activities and Green Synthesis of Three Metallic Nanoparticles of Aqueous Extract of *Polyalthia LongifoliaSonn*. Leaves

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Abstract
Introduction: *Polyalthia longifolia is a plant found in the tropical areas of the world and has been used as an ornamental tree. The leaves of the plant were investigated with the aim of ascertaining some biochemical and antimicrobial activities as well as green synthesis of some metallic nanoparticles of its aqueous extract. The leaves of the Polyalthia longifolia were obtained from within Ekiti State University compound in Ado-Ekiti, Nigeria. The leaves were removed from its plant, washed under running tap water to remove dust. They were then air dried for some days after which the leaves were crushed into powder. 20 g of powdered leaves was mixed with 100 mL of distilled water and shaken overnight for effective extraction. The resulting mixture was filtered with the help of muslin cloth and the filtrate was used for further analyses.*
Results: *The aqueous extract of the leaves was found to contain alkaloids, flavonoids, saponins, phenolics, and steroids. Results of the analyses showed that the extract possessed strong antimicrobial and antioxidant activities.The metallic nanoparticles were characterized with FTIR peaks around 3997, 3705, 2777, 1698, 1488 $cm^{-1}$for AgNP, CuNP and ZnNP. All the Nanoparticles showed maximum inhibitory activity against the tested pathogens.These plant extracts and their nanoparticles also show very high antibacterial and antifungal activities.*
Conclusions: *From the result obtained in this study, the plant obtained can be used for medicinal purpose and drug development.*
Keywords: *phytochemicals, antibacterial, antifungal, antioxidants, nanoparticles*

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I. Introduction

Medicinal plants constitute an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80% of the rural population depends on them as primary health care (Akinyemi, 2000). Plants have been used as sources of remedies for the treatment of many diseases since ancient times and people of all continents especially Africa have this old tradition. Despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Newman et al., 2000). However, plants used in traditional medicine are still understudied (Kirby, 1996). In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80% of the population uses medicinal plants as remedies (Kirby, 1996; Hostellmann and Marston, 2002).

According to the World Health Organization (WHO) the definition of traditional medicine may be summarized as the sum total of all the knowledge and practical, whether explicable or not, used in the diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing. Traditional medicine might also be considered as a solid amalgamation of dynamic medical known-how and ancestral experience. In Africa, traditional healers and remedies made from plants play an important role in the

health of millions of people. Traditional medicine has been described by the WHO as one of the surest means to achieve total health care coverage of the world's population. Numerous medicines have been derived from the knowledge of tropical forest people and clearly there will be more in the future.

One such medicinal plant is *Polyalthia longifolia* (Sonn.) Thwaites (PL) Belongs to Annonaceae family. Polyalthia is the Greek word poly means much (or) many and althea from lathes means cure, which shows multiple health benefits. It is commonly used as ornamental street tree due to its effectiveness in reducing noise pollution. The plant is used in traditional system of medicine for the treatment of fever, skin diseases, diabetes, hypertension and helminthiasis. Since this plant has an ability to be used as medicine for treatment of some medical complications, therefore there is a need to investigate the plant for its phytochemical composition, antioxidant properties, antimicrobial potentials and to assess the green synthesis of three metallic nanoparticles and characterization of the plant extract.

II. Materials and Methods

Plant identification and authentication

The leaves of *Polyalthia longifolia* was collected from Ekiti State University campus, Ado Ekiti Nigeria in November 2020. The plant sample specimen was authenticated and deposited at the herbarium of the Department of Plant Science and Biotechnology, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria. It was identified and confirmed by Mr. Omotayo, the Chief Technology with voucher number: UHAE 2020054 *Polyalthia longifolia* (Sonn), Wind breaker/Masquerade leaf.

Preparation of plant leaf extract

The leaves of the *Polyalthia longifolia* were removed from its plant, washed under running tap water to remove dust. The leaves were then air dried for some days after which the leaves were ground into powder. 20 grams of powdered leaves was mixed with 100 mL of distilled water and shaken overnight for effective extraction. The resulting mixture was filtered, and the filtrate of the plant leaves was taken and used for phytochemical analyses, in-vitro antioxidant and green synthesis analyses.

Phytochemical analysis of the fresh sample of the plant

Test for flavonoids

Procedure: 2.5 ml of ammonia and 1ml of concentrated sulphuric acid was added to 5ml of the plant sample. Indication of yellow color indicates the presence of flavonoid in the plant sample.

Test for alkaloids

a) **Mayer's test:** A fraction of the extract was treated with Mayer's reagent (1.36g of mercuric chlorate and 5g of potassium iodide in 100ml distilled water) and noted for a cream-colored precipitate.

b) **Dragendorff's test:** A fraction of the extract was treated with Dragendroff's reagent and observed for the formation of reddish orange precipitate.

(Bismuth nitrate 1.7g, glacial acetic acid 20mL, water 80mL and 100ml of 50% solution of KI in water, mix together and keep as stock solution. 10ml of stock, 20mL of glacial acetic acid make up for 100ml in water for working solution)

c) **Wagner's test:** A fraction of the extract was treated with Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of distilled water) and observed for the formation of reddish-brown precipitate.

Further test for alkaloids

For the purpose of phytochemical analysis of the selected plant, 0.2 g of the selected plant samples were added in each test tube and 3 ml of hexane were mixed in it, shaken well and filtered. Then 5 ml of 2% HCl was taken and poured in a test tube having the mixture of plant extract and hexane. The test tube with the mixture was heated, filtered few drops of picric acid was poured into the mixture. Formation of yellow color precipitate indicates the presence of alkaloids.

Test for Saponin (Foam Test)

Procedure: 10 drops of distilled water was added to 20 drops of plant sample. After shaking vigorously, persistence in the foam indicates the presence of Saponin.

Detection of phenolic compounds

a) **Ferric chloride test:** A fraction of the extract was treated with 5% FeCl3 solution and observed for the formation of deep blue color.

b) **Lead acetate test:** A fraction of the extract was treated with 10% lead acetate solution and observed for the formation of white precipitate.

Lieberman's test for steroidal nucleus

2.0mL of acetic anhydride was added to 0.5 g of each solvent extract of sample with 2.0 mL H_2SO_4. The color changed from violet to blue or green in some samples indicating the presence of steroids.

Determination of DPPH free radical scavenging ability

The 1,1- diphenyl-2-picryhydrazyl (DPPH) free radical scavenging ability of the extract was determined using the modified method of Gyamfi *et al*. (1999).

Briefly, 1.0 mL of different concentrations (20, 40 and 80 mg/mL) of the extracts was placed in respective test tubes. 1.0 mL of 0.1 mM methanolic DPPH solution was added tothe samples. These samples were vortexed and incubated in dark at room temperature for 30mins. The respective solutions were thoroughly mixed and incubated in the dark for 30mins before absorbance measured at 516nm. Decreased absorbance of the sample indicates DPPH free radical scavenging capability. Distilled water was replaced for the extract in the control. Percentage radical scavenging ability was calculated using the following expression:

$$\% \text{ DPPH radical scavenging ability} = 1 - \frac{\text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100$$

Determination of Nitric oxide (NO) radical scavenging ability

The modified methods of Jagetia and Baliga, 2004 was used to determine the Nitric oxide radical scavenging ability. Sodium Nitroprusside in aqueous solution at physiological pH 7.0 spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent [1.0 mL sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid at room temperature for 5 minutes with 1 mL of naphthylethylenediamine dichloride (0.1% w/v)]

Nitric oxide radical scavenging activity = $(Abs_{Control} - Abs_{Sample} / Abs_{Control}) \times 100$

Determination of ferric reducing antioxidant power

The reducing property of the extract was determined by the modified method of (Pulido et al., 2002). This method is based on the reduction of (Fe^{3+})ferricyanide in stoichiometric excess relative to the antioxidants. Different concentrations of the methanolic extract of the sample and its various fractions (10 – 50µg/mL) was added to 1.0 mL of 200mM of sodium phosphate buffer pH 6.6 and 1.0 mL of 1% potassium ferricyanide[$K_3Fe(CN)_6$]. The mixture was incubated at 50^oC for 20min, thereafter 1.0 mL of freshly prepared 10% TCA was quickly added and centrifuged at 2000rpm for 10min, 1.0 mL of the supernatant was mixed with 1.0 mL of distilled water and 0.25 mL of 0.1% of $FeCl_3$solution was added. Distilled water was used for blank without the test sample while control solution contained all other reagents except the 0.1% potassium ferricyanide. Absorbances of these mixtures were measured at 700 nm using a spectrophotometer. Decreased absorbance indicates ferric reducing power capability of sample.

The percentage Ferric reducing antioxidant power (%) was subsequently calculated as

$[(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$

Estimation of Total Phenolic Content

The extractable phenol content was determined on the extracts using the method reported by Singleton *et al*. (1999).0.2mL of the extract was mix with 1.5mL of 10% Folinciocalteau's reagent and 2ml of 7.5% Sodium carbonate. The reaction mixture will be subsequently incubated at 45^oC for 40mins, and the absorbance was measure at 700nm in the spectrophotometer, garlic acid would be used as standard phenol. The concentration of total phenols was expressed in mg/gm of dry extract as gallic acid equivalent, GAE from standard curve equation:

Total phenolic contents were expressed in terms of Y =0.005x +0.464 (R^2=0.961) mg of GA/gm of dry extract.

Determination of total flavonoid

The total flavonoid content of the extract was determined using a colorimeter assay developed by (Bao, 2005). 0.2ml of the extract was added to 0.3mL of 5% NaNO3 at zero time. After 5min, 0.6ml of 10% $AlCl_3$ was added and after 6mins, 2mL of 1M NaOH solution was added to the mixture followed by the addition of 2.1mL of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as mg gallic acid equivalent.

The absorption of standard garlic acid solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in duplicates. Total flavonoid contents were expressed in terms of Galic acid equivalent, GAE (standard curve equation:

Y =0.005x +0.464 (R^2=0.961) mg of GAE/mg of dry extract

Green synthesis and anti-microbial analyses

52.92 g of the crushed sample was weighed into a beaker and transferred to a 1000 ml quick fit round bottom flask where 500 ml of distilled water was added and heated in a mantle for 1 hour for proper extraction. At the end of the process, the substance was removed and poured into a Buckner funnel (lined with filter paper) for the first round of filtration. A second time filtration was done to obtain a clean filtrate (cotton wool was used at this point). The filtrate obtained was kept in the fridge at 4^0C for the nanoparticle synthesis.

Preparation of Metal Ions Solutions

Silver Solution (0.1 M): 17 g of silver nitrate salt was weighed into a clean beaker and then dissolved with deionized water and made up to the mark in a 1000 ml standard volumetric flask. The solution was labeled and kept.

Zinc Solution (0.1 M): 0.140g of Zinc sulfate salt was weighed into a beaker and then dissolved with deionized water and made up to the mark of a 500 ml standard volumetric flask. The solution was labeled and kept.
Copper Solution (0.1 M): 12.484 g of copper (II) sulfate penta hydrate salt was weighed into a beaker and then dissolved with distilled water and made up to the mark of a 500 ml standard volumetric flask. The solution was labeled and kept.

Characterization of the synthesized silver, zinc and copper nanoparticles

The biosynthesis of the AgNPs, ZNNPs and CuNPs in the solutions were monitored by measuring the UV–visible spectra of the solutions of the reaction mixture. UV–vis spectra were recorded on double beam spectrophotometer (Shimazdu, model UV-1800, Kyoto, Japan) from300 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, ZNNPs and CuNPs were detected using FTIR.

Anti-microbial analysis

Antibacterial and antifungal analyses were carried out on the synthesized nanoparticle and plant extract.50 mg of the synthesized samples were weighed and dissolved in 2 ml of distilled water separately and heated for few minutes to allow for proper dissolution. The extract was poured into a sterile petri-dish and antimicrobial disk was inserted respectively for proper pre-diffusion into the synthesized sample.

Disk Diffusion Method

The Disk Diffusion plate method was used for the antifungal and antibacterial analysis. A sterile nutrient agar (1.4 g/l) and sterile potato dextrose agar was prepared and poured into the sterile plate and allowed to gel. Culture of the organisms (bacteria for nutrient agar and fungi for potato dextrose agar) was taken from stock and inoculated into each agar. Various cells were made in each sterile plate. In each cell, the pre-diffused disk of the synthesized sample was introduced respectively, and the plate was left on the bench to allow proper diffusion of extract into the agar. The plates were incubated in an incubator at 37 ^{0}C for bacteria and 25 ^{0}C for fungi. The bacteria and fungi plates were observed after 24 hours of introduction. It was clear that some extract had clear zones of inhibition whereas some had none.

III. Results

Table 1.0: Phytochemical Screening of *Polyalthia longifolia* aqueous and ethanolic extracts

Plant Extract	**Saponin**		**Alkaloids**		**Flavonoids**		**Phenolic compound**		**Steroid**	
	H_2O	Ethanol	H_2O	Ethanol	H_2O	Ethanol	H_2O	Ethanol	H_2O	Ethanol
Polyalthia longifolia	+	-	+	-	+	+	++	-	-	++

- = Not present, + = Present, ++ = High, +++ = Higher

Table 2.0: Total Flavonoids contents of aqueous extract of *Polyalthia longifolia* leaf

Std. gallic acid concentration	20mg/ml	40mg/ml	80mg/ml	100mg/ml
Polyalthia longifolia	0.343 ± 0.006	0.553 ± 0.003	0.572 ± 0.006	0.587 ± 0.002

Values are mean deviation ± standard deviations of two numbers

Table 3.0: Phenolics content of aqueous extract of *Polyalthia longifolia* leaf

Std. gallic acid concentration	20mg/ml	40mg/ml	80mg/ml	100mg/ml
Polyalthia longifolia	0.429 ± 0.002	0.562 ± 0.006	0.667 ± 0.002	0.740 ± 0.006

Values are mean deviation ± standard deviations of two numbers

Table 4.0: Ferric reducing antioxidant power (FRAP) of aqueous extract of *Polyalthia longifolia*

Concentration	20mg/ml	40mg/ml	80mg/ml	100mg/ml
VitaminC	0.025 ± 0.003	0.174 ± 0.002	0.210 ± 0.006	0.246 ± 0.006
Polyalthia longifolia	0.269 ± 0.00	0.349 ± 0.006	0.372 ± 0.004	0.392 ± 0.003

Values are mean deviation ± standard deviations of two numbers

Table 5.0: Nitric oxide radical scavenging ability of aqueous extract of *Polyalthia longifolia*

Concentration	20mg/ml	40mg/ml	80mg/ml	100mg/ml
BHA	0.452 ± 0.004	0.562 ± 0.004	0.578 ± 0.006	0.608 ± 0.003
Polyalthia longifolia	0.428 ± 0.002	0.485 ± 0.006	0.513 ± 0.005	0.649 ± 0.006

Values are mean deviation ± standard deviations of two numbers

Table 6.0: 2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability of aqueous extract of *Polyalthia longifolia* leaf

Concentration	20mg/ml	40mg/ml	80mg/ml	100mg/ml
BHA	0.604 ± 0.006	0.622 ± 0.003	0.768 ± 0.004	0.901 ± 0.006
Polyalthia longifolia	0.790 ± 0.002	0.887 ± 0.003	0.924 ± 0.006	1.052 ± 0.004

Values are mean deviation ± standard deviations of two numbers

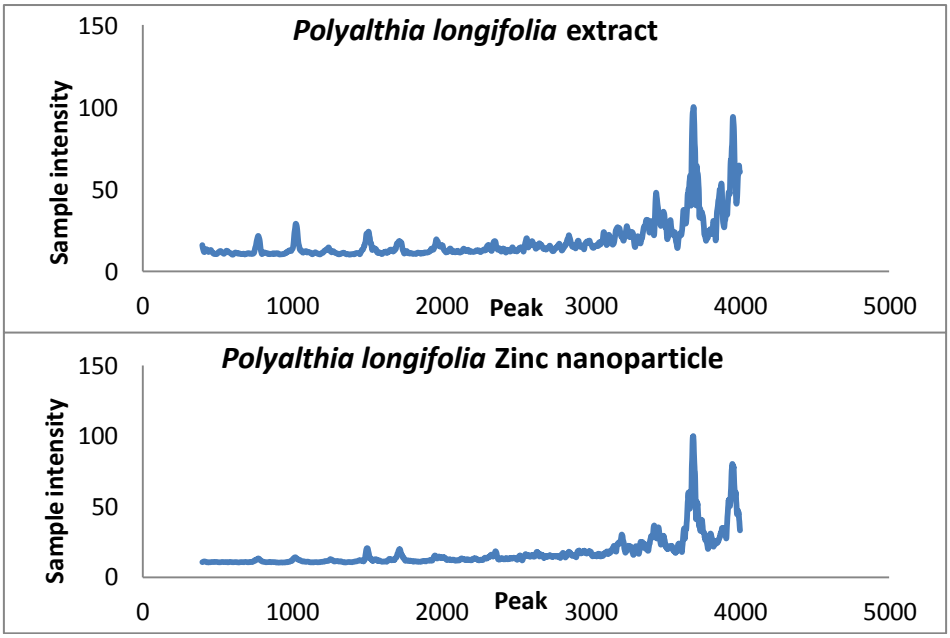
Table 7.0: Antibacterial activities of *Polyalthia longifolia* Extract and silver, zinc and copper nanoparticles.

	A	B	C	D	E	F	G	H
Polyalthia longifolia	3.0	-	-	-	-	-	-	-
*Polyalthia longifolia*ZnNP	2.0	-	-	7.0	2.0	-	-	-
*Polyalthia longifolia*AgNP	-	-	-	-	1.0	1.0	-	1.0
*polyalthia longifolia*CuNP	2.0	-	-	6.0	-	-	-	3.0
CIPRO	23	18	28	30	32	28	26	20

CIPRO = Ciproflacin (500mg)/20ml of distil water
A = *Ralstoniasolanecearum* B = *E.coli* C = *Pseudomonas glycinea* D = *Staphylococcus aureus*E = *Streptococcus faecalis* F = *Xanthromonasphasaoli* G = *Enterobacteraerogenes*
H = *Salmonella typhi*

Table 8 .0:Antifungal activities of *Polyalthia longifolia* Extract and silver, zinc and copper nanoparticles

	Zone of inhibition(mm)	
	Trichophyton verrycosumn(%)	*Epidermophyton floccosum*(%)
Polyalthia longifolia (extract)	63.46	29.59
*Polyalthia longifolia*AgNP	59.72	13.27
*Polyalthia longifolia*ZnNP	57.69	44.90
*Polyalthia longifolia*CuNP	48.08	15.31
Ketokonazole (standard)	80.00	70.00
Trymethroprime (standard)	80.77	89.80



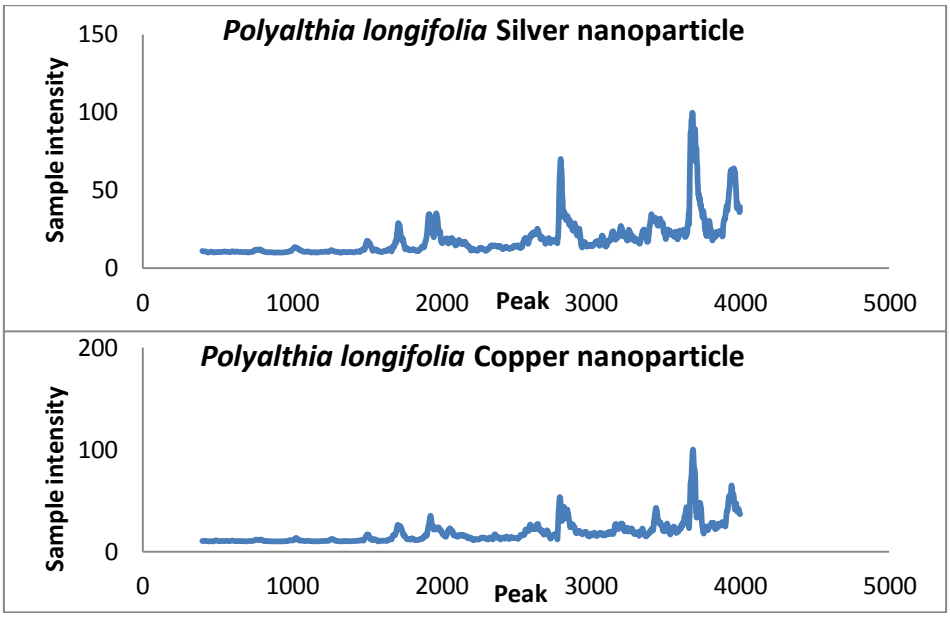


Figure 1.0 FTIR Spectra of *Polyalthia longifolia* extract and three metal nanoparticles

The obtained spectra from extract and the synthesized nanoparticles have the following peaks

Samples	**Peaks**
Polyalthia longifolia extract	3951, 3681, 3437, 2577, 2302, 1858, 1720, 1015,
Polyalthia longifolia ZnNP	3944, 3681, 3420, 3151, 2304, 1942
Polyalthia longifolia AgNP	3934, 3670, 3395, 2792, 1911, 1701
Polyalthia longifolia CuNP	3940, 3680, 3431, 3162, 2791, 2575, 2040, 1921

IV. Discussion

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents such as alkaloids, flavonoids, saponin, phenolics compounds in aqueous (H_2O) extract which are known to exhibit medicinal purpose as well as physiological activities and the presence of constituents such as flavonoids, steroids was observed in the ethanolic extract which also exhibit medicinal and physiological activities but, in a percentage, lower than aqueous extract. The phytochemicals tested are known to exhibit medicinal activity and physiological activity. Alkaloids are an important drug source and have been reported to possess antimicrobial, antioxidant, and cytotoxic activity (Rahman *et al*. 2009). The presence of biologically important phytochemicals in the *Polyalthia longifolia* extracts may contribute to their reported medicinal values and indicates that it is a potential source of drugs development (Edeogaet al., 2005). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant, such as phenols (Kazmiet al.,1994), essential oils (Cosentinoet al.,1999). terpenoids (Habtemariamet al.,1993; Taylor et al.,1996), alkaloids (Omulokoliet al.,1997) and flavonoids (Batista et al.,1994). Preliminary phytochemical analysis during the present study also ascertains the presence of some potential group of bioactive substances in line with what others have reported (Dafereraet al.,2003). The alkaloids, flavonoids, Saponins, phenolic compounds earlier reported by Bankole, (2016), Borokini and Omotayo, (2012) were equally found in the present study, suggesting the plant to be a rich source of these antioxidant phytochemicals (Livingstone et al., 1999).

From Table 2.0 it shows that as the amount of the sample increases, the concentration of total flavonoids also increases and vice versa, therefore, the higher the sample, the higher the total flavonoids content and the higher the antioxidant power whereas, the lower the sample, the lower the total flavonoids content and the lower the antioxidant power of the sample. Similar trends were obtained by (Adaramolaet al., 2017) in their study on methanolic and ethyl acetate extracts of the plant.

From Table 3.0 it shows that as the amountof the sample increases, the concentration of total phenolic content also increases, therefore, the higher the sample, the higher the phenolic content and the higher the antioxidant power while the lower the sample, the lower the phenolic content and the lower the antioxidant power of the sample. The observations reported in this study are also in consonance with was obtained by (Adaramolaet al., 2017) in their works on phenolic contents of fractions of methanol and ethyl acetate extracts of *Polyalthia longifolia* leaves

From the table 4.0 it shows that as the amount of the sample and standard ascorbic acid increases, the ferric reducing antioxidant power of the sample and the standard used also increase, therefore, the higher the value, the higher the ferric reducing antioxidant power and the lower the value, the lower the ferric reducing antioxidant power. The ferric reducing antioxidant power obtained in this study however agrees with what was reported for the methanolic and ethyl acetate fractions of *Polyalthia longifolia* extract by Adaramolaet al., (2017).

From the Table 5.0 above, it shows that as the concentration of sample increases, the nitric oxide radical scavenging ability of the sample and the standard used also increase, therefore, the higher the amount of the sample, the higher the nitric oxide radical scavenging ability and hence the higher the antioxidant power while the lower the value, the lower the antioxidant scavenging power. It had also been reported previously by Adaramolaet al., (2017) and Njoku al., (2011) in line with what was obtained in this study that the scavenging ability of the plant extract increased as concentration increased in the various solvents used.

From the table 6.0 it shows that as the concentration increases, the sample and the standard used also increases, therefore, the higher the value, the higher the antioxidant scavenging power and the lower the value, the lower the antioxidant scavenging power. The table also showed that the *Polyalthia longifolia* aqueous extract has high DPPH radical scavenging power than the standard used at various concentrations. On a general note, the results showed that the DPPH scavenging capacity of the extract and other antioxidant measuring capabilities displayed concentration dependent fashion for the aqueous extract in this study and methanolic, chloroform and ethyl acetate reported by Adaramola al., (2017) in their own work.

Table 7.0 shows that the extract *Polyalthia longifolia* only has antibacterial activity on a single microorganism *Ralstoniasolanecearum*while all the nanoparticles according to the result show

antibacterial activities on three different microorganisms.*Polyalthia longifolia*ZnNPhave antimicrobial effect on *Ralstoniasolanecearum, Staphylococcus aureus, Streptococcus faecalis*. *Polyalthia longifolia*AgNP have antibacterial effect on *Staphylococcus aureus*, *Streptococcus faecalis*, *Salmonella typhi*. *Polyalthia longifolia* CuNP have antibacterial effect on Staphylococcus aureus, *Ralstoniasolanecearum*, *Salmonellatyphi*. Although when comparing the three nanoparticles used, AgNP shows little percentage of antimicrobial activity when compared with ZnNP and CuNP which almost have equal percentage of antibacterial activities. Therefore, nanoparticles are preferred than the plant extract**.** Similarly, the biologically synthesized silver nanoparticles exhibited excellent antibacterial activity against the bacterial pathogens *Staphylococcus aureus* (Grampositive), *Escherichiacoli*, and *Pseudomonas aeruginosa* (Gram negative) (Singh *et al.*, 2007). It has been reported that antibacterial effect was size and dose dependent and was more pronounced against Gram-negative bacteria than Gram-positive bacteria. But the present study clearly shows that the synthesized nanoparticles (AgNP, CuNP, and ZnNP) have good antibacterial action against Gram-positive organism than Gram-negative organisms. The antimicrobial activities of colloidal silver particles are influenced by the dimensions of the particles. The smaller particles lead to the greater antimicrobial effects. The effect of antibacterial activity is higher in the case of silver nanoparticles synthesized at 60◦C compared to 25◦C because of being smaller in size (Singh *et al.*, 2007).

The Table 8.0 shows that the extract (*Polyalthia longifolia*) has highest percentage (63.46) of antifungal activities over *Trichophyton verrycosum* which will inhibit their growth perfectly, follow by ZnNP with (59.72), CuNP with (57.69) which also have the tendency to inhibit *Trichophyton verrycosum* growth but not as perfect has that of extract. AgNP with (48.08) has the least tendency of inhibiting *Trichophyton verrycosum* growth, though AgNP will still inhibit but may not be as perfect as others. CuNP has the highest percentage (44.90) which is not even a perfect percentage of antifungal activity over *Epidermophyton floccosum*, therefore has low tendency of inhibiting their growth. The extract (*Polyalthia longifolia*) has a very low percentage (29.59)of antifungal activity over *Epidermophyton floccosum* which cannot inhibit their growth perfectly and other nanoparticles ZnNP (13.27), AgNP (15.31) have poor tendency of inhibiting *Epidermophyton floccosum* growth. According to this result CuNP has highest percentage of antifungal activity compared to extract (*Polyalthia longifolia*), therefore nanoparticles are more preferred than extract.

Figure 1.0 showed the FTIR Spectra of the *Polyalthia longifolia* extract and the three metal nanoparticles; the obtained spectrum for the extract reveals numerous absorption peaks at 3951cm^{-1} and 3681$cm^{-1}$is due to C=O and/or O-H group of carboxylic acids and it is an indication of alcohol and phenol groups. And at 3432 cm^{-1} is due to the C≡C-H:C-H group indicating Alkynes. The medium absorption peak at 2302 cm^{-1} and 2577 cm^{-1} is due to O-H signifying carboxylic group but the band depends on whether it is saturated or unsaturated, dimerized or has internal hydrogen bonding. And at 1800 cm^{-1} and at 1720 cm^{-1} is due to C-C and/or C-H group signifying overtones or aromatic compounds. While for ZnNP, the band at 3944 cm^{-1}, and 3681 cm^{-1} is due to C-O and/or O-H indicating phenol and alcohol group. And at 3420 cm^{-1} and at 3151 is due to the C≡C-H:C-H group indicating Alkynes. At 2304 cm^{-1} is due to O-H signifying carboxylic group but the band depends on whether it is saturated or unsaturated, dimerized or has internal hydrogen bonding and 1942 cm^{-1} is due to C-C and/or C-H group signifying overtones or aromatic compounds. This nanoparticle (ZnNP) shifted the functional groups at 2577 $cm^{-1,}$,1858 $cm^{-1,}$,1720 cm^{-1} of plant extract. For AgNP, the band at

3934 cm^{-1}, and 3670 cm^{-1} is due to C-O and/or O-H indicating phenol and alcohol group. And at 3395 cm^{-1} is due to the C≡C-H:C-H group indicating Alkynes. At 2792 cm^{-1} is due to O-H signifying carboxylic group but the band depends on whether it is saturated or unsaturated, dimerized or has internal hydrogen bonding. At 1911 cm^{-1} and 1701 cm^{-1} is due to C-C and/or C-H group signifying overtones or aromatic compounds. This nanoparticle(AgNP) shifted the functional groups at 2577 $cm^{-1,}$,1858 $cm^{-1,}$,1720 cm^{-1} of plant extract.For CuNP, the band at 3940 cm^{-1}, and 3680 cm^{-1} is due to C-O and/or O-H indicating phenol and alcohol group. And at 3431 cm^{-1} and 3162 is due to the C≡C-H:C-H group indicating Alkynes. At 2791 $cm^{-1}$and 2575 cm^{-1} is due to O-H signifying carboxylic group but the band depends on whether it is saturated or unsaturated, dimerized or has internal hydrogen bonding. And between 2040 cm^{-1} and 1921 cm^{-1} is due to C-C and/or C-H group signifying overtones or aromatic compounds. This nanoparticle (CuNP) shifted the functional groups at 2577 $cm^{-1,}$ 1858 $cm^{-1,}$,1720 cm^{-1} of plant extract. Therefore, it can be concluded that the nanoparticles do not affect the carboxylic (C-O and/or O-H) and also C≡C-H: C-H since it is present in both the extract and the nanoparticles but the nanoparticles reduce the other functional groups, thus shifting it.The observations in this study however agrees with earlier study by Adeyemi et al., (2020) who reported the FTIR spectrometry of ZnNP, CuNP, and SAME (Spondiasmombin) to give wave number ranging from 895.71-3320.67, 747.02-3225.45 and 658.25-3674.49 cm-1 respectively. FTIR analysis showed that

SMAE acted as reducingand stabilizing agent while the NPs exhibited lower energy absorption band when compared to the plant extract.

V. Conclusions

The present study revealed that the *Polyalthia longifolia* plant act as a potential source of useful drug. The phytochemical screening has shown the presence of constituents such as alkaloids, flavonoids, saponin, phenolics compounds in aqueous (H_2O) extract and the presence of constituents such as flavonoids, Steroids, in ethanolic extract which are known to exhibit medicinal purpose as well as physiological activities. Demonstration of the antibacterial and antifungal activities of the aqueous extract and nanoparticles (ZnNP, CuNP, AgNP) extract of *Polyalthia longifolia* may help to discover new chemical classes of antibiotics substance that could serve as selective agents for infectious diseases chemotherapy and control. The present study clearly indicates that the synthesized nanoparticles have good antibacterial action against Gram-positive organism than Gram-negative organisms. Also, the antioxidant activities of the plant extract were assayed for Nitric oxide and DDPH radical scavenging ability while Flavonoids and phenolic compound quantification of the plant extracts carried out showed that there was inhibition in the formation of free radicals; indicating a possible antioxidant property, Synthesis of nanoparticles using Polyalthia longifolia leaf extract as reducing and capping agent used to increase the stability of the nanoparticles. This investigation has opened up the ability for using this plant in antimicrobial drug development for human application and also for medicinal purpose and general drug development. Nanoparticles were synthesized by *Polyalthia longifolia* leaves extract. The spectroscopic characterization from UV-visible and FTIR supports the stability of the biosynthesized nanoparticles. The nanoparticles were found to have wider antimicrobial activity in Grampositive than Gram negative organisms. We believe that the (AgNPs, CuNPs, and ZnNPs nanoparticle have great potential for applications in catalysis, biomedical, and pharmaceutical industries.

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