Biochemical Characterization, Antimicrobial Potency, and Metallic Nanoparticle Generation from *Tridax procumbens* Extracts

Abstract

Several studies have mainly focused primarily on seeking promising ways to solve major human diseases such as cancer, cardiovascular disease, and neuro-degenerative disease. In the quest for new antimicrobial and modifications of existing ones, interestingly, medicinal plants have emerged as the preferred and safest source of various medicines, as recommended by the World Health Organization (WHO). Plants and their bio-active compounds hold potential as antimicrobial agents to combat these diseases and are pioneers in the discovery of new medicines. *Tridaxprocumbens* is a highly promising species that produces secondary metabolites that have been reported to possess a variety of medicinal uses, including anti-anemic, anti-inflammatory, anti-diabetic, and anesthetic properties. This species has a long history of traditional use by different communities. This study aimed to investigate the medicinal properties of phytochemicals constituents and other biochemical components. The phytochemical screening, Vitamin C content antioxidants and antimicrobial properties as well as green synthesis of three metallic nanoparticles of the *Tridaxprocumbens* leaf extracts were determined using standard analytical procedures. The extracts of the leaves was found to contain medicinal important phytochemical constitutes; strong antioxidant and antimicrobial activities. The metallic nanoparticles were characterized with FTIR peaks around 4000, 3705, 2777, 1800, 1500 $cm^{-1}$for AgNP, CuNP and ZnNP. Both the plant extracts and all the nanoparticles exhibited maximum inhibitory activity against the tested pathogens. Based on the results obtained in this study, this species could potentially serve as an effective, safe, and affordable source of treatment of certain ailments and drug development against major human diseases that pose threats worldwide.

Keywords: cancer, cardiovascular disease, Biochemical Characterization, Antimicrobial Potency

Introduction

"Medicinal plants constitute an effective sources of both traditional and modern medicines. These plants have been shown to have genuine utility and about 80% of the rural population depends on them as primary health care" [1].

“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” [2].

“However, plants used in traditional medicine are still under studied” [3]. There is limited knowledge regarding their active constituents of many plants with only one or two being discovered at times. Moreover, these active constituents can be influenced by factors such as mineral composition, soil quality, plant age at harvest, and climatic conditions of the growth area.

“A comprehensive understanding of these factors would provide insights into the development of effective medicines. Herbal medicines typically act gently to support or enhance deficient body systems and processes that have become deficient or attempt to help remove excesses that have become preeminent. They usually tend to have several broad complementary or synergistic actions on physiological systems, which are usually in the same general therapeutic direction” [4].

“Therefore instead of using synthetic drugs for the cure of any disease, natural drugs which can reduce the side effect, toxicities of synthetic counterparts and also maximize therapeutic potential with effective and dynamic healing effects should be used. According to the reports about 8% of hospital admissions in the United States of America are due to adverse side effects of synthetic drugs and about 100,000 people each year die due to these toxicities” [5].

Synthetic drugs may have stronger potency, resulting in a more pronounced therapeutic effect, but they also carry an increased risk of side effects. This is not the case with natural drugs, as they exhibit fewer side effects, if any, and their multiple effects are attributed to the wide range of active constituents present in a single plant, which work synergistically.

“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 of such medicinal plants is *Tridax procumbens*, also known as coat buttons is a perennial plant from the Asteraceae family, native to Central and South America” [6,7].

"Since ancient times, this species has been used in Ayurveda in India" [8]. "Different substances such as oils, teas and skin poultices, among others, have been manufactured using this species" [9]. "T. procumbens has diverse pharmacological properties including but not limited to immune modulatory, anti-oxidant, anti-hepatotoxic, analgesic, antidiabetic, anti-inflammatory, antifungal, and antimicrobial activities" [7,10,11,12,13].

The versatility of a particular species of plant is most likely due to the plant's defense mechanisms, secondary metabolites such as flavonoids, alkaloids, tannins, carotenoids and saponins present in them. Therefore, it is necessary to investigate these plants' phytochemical compositions, antioxidant properties, antimicrobial potentials, as well as to generate and characterize the green synthesis of three metallic nanoparticles of the plant'saqueous extract.

Materials and Method

Plant identification and authentication

The leaves of *Tridax procumbens* was collected from Kabba, Kogi State, Nigeria in November,2020. The plant sample specimen was authenticated and deposited at the herbarium of the Department of Plant Science and Environmental Science, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria. It was identified and confirmed by Mr. Omotayo, the Chief Technologist with the herbarium number: UHAE 2020067

Preparation of plant leaf extract

The leaves of the *Tridax procumbens* were removed from its plant, washed under running tap water to remove dust. The leaves were then air dried for 14 days on laboratory benchafter which the leaves were ground into powder with average particle size of between 0.149 mm and 0.125 mm using appropriate mesh. 20 grams of powdered leaves was mixed with 100 mL of distilled water and ethanol respectively, shaken overnight for effective extractions. The resulting mixtures were filtered, and the filtrates of the plant leaves taken and used for phytochemical analyses, in-vitro antioxidant properties and green synthesis analyses.

Phytochemical analysis of the fresh sample of the plant

Test for flavonoids

Procedure: 2.5 ml of concentrated ammonia and 1ml of concentrated sulphuric acid was added to 5ml of the plant extract carefully. Indication of yellow color shows 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% $FeCl_3$ 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 Gyamfiand Aniya, (2002) method[14].

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 to the 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 using a UV-Visible spectrophotometer. 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:

% DPPH radical scavenging ability =1 - (Abs_{Sample}/$Abs_{Control}$) x 100

The results obtained in concentration dependent basis was plotted against varying concentration of butylated hydroxyanisole (BHA) standard solution using Microsoft excel computer program spreadsheet.

Determination of Nitric oxide (NO) radical scavenging ability

The modified method of Jagetia GC et al. (2004)[15] 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)]. The absorbance of reaction solution was measured using a UV-Visible spectrophotometer and calculated with this expression

Nitric oxide radical scavenging activity = ($Abs_{Control}$ – Abs_{Sample} /$Abs_{Control}$) x 100.

The results obtained in concentration dependent basis was plotted against varying concentration of butylated hydroxyanisole (BHA) standard solution using Microsoft excel computer program spreadsheet.

Determination of ferric reducing antioxidant power

The reducing property of the extract was determined by the modified method [16]. This method is based on the reduction of (Fe^{3+}) in 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 ferric cyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50^{o}C for 20 min, 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. Absorbance 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: $[(Abs_{control} – Abs_{sample}) /Abs_{control}] \times 100$

The results obtained in concentration dependent basis was plotted against varying concentration of ascorbic acid standard solution using Microsoft excel computer program spreadsheet.

Estimation of Total Phenolic Content

The extractable phenol content was determined on the extracts using the method reported by [17] 0.2mL of the extract was mix with 1.5mL of 10% Folin Ciocalteau's reagent and 2ml of 7.5% Sodium carbonate. The reaction mixture will be subsequently incubated at 45^{o}C for 40 mins, and the absorbance was measure at 700 nm 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.

The results obtained in concentration dependent basis was plotted against varying concentration of galic acid standard solution using Microsoft excel computer program spreadsheet.

Determination of total flavonoid

The total flavonoid content of the extract was determined using a colorimeter assay developed by [18] 0.2 mL of the extract was added to 0.3 mL of 5% NaNO3 at zero time. After 5min, 0.6 mL of 10% $AlCl_3$ was added and after 6 mins, 2 mL of 1.0M NaOH solution was added to the mixture followed by the addition of 2.1 mL of distilled water. Absorbance was read at 510 nm 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

The results obtained in concentration dependent basis was plotted against varying concentration of galic acid standard solution using Microsoft excel computer program spreadsheet.

Estimation of vitamin C

To the 0.5 and 1.0 mL of the filtered sample solution in separate standard volumetric flasks, few drops of bromine water were added until the solution became colored (to confirm the completion of the oxidation of ascorbic acid to dehydro-ascorbic acid). Then few drops of 10% thiourea solution was added to it to remove the excess bromine and thus the clear solution was obtained. Then 0.5 mL of 2,4-dinitrophenyl hydrazine (DNPH) reagent (2% in 9N H_2SO_4) was added and mixed thoroughly with the oxidized ascorbic acid and solution made up to 2 mL mark with the 10% acetic acid solution. Volumes of standard ascorbic acid (2mg/mL) solution ranging between 0.2-1.0 mL were treated similarly as the samples. The absorbance was read at 520 nm in a spectrophotometer. The concentration of ascorbic acid in the sample was calculated from the standard calibration curve in linear regression mode and expressed in terms of mg/g of sampleusing Microsoft excel computer program spreadsheet.

Green synthesis and anti-microbial analyses

Extract preparation

52.92 g of the crushed leaf 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): 8.50 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 500 ml standard volumetric flask. The solution was labeled and kept.

Zinc Solution (0.1M): 8.072 g 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 pentahydrate 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.

Synthesis of silver, zinc and copper nanoparticles

Equal volumes (20 mL) of 0.1 M $AgNO_3$, ZnCl and $CuSO_4$ solutions were added separately to the extract in a separate conical flask and the mixture was incubated on a hot plate at 70^oC under agitation for 10-45 min. The change in colour as a function of the bioreduction of respective metallic ion to metallic nanoparticles was monitored.

Characterization of the synthesized silver, zinc and copper nanoparticles

The biosyntheses 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.

Results

Table 1: Phytochemical Screening of *Tridax procumbens* 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
Tridax procumbens	+	-	+	-	+	+	++	-	-	+

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

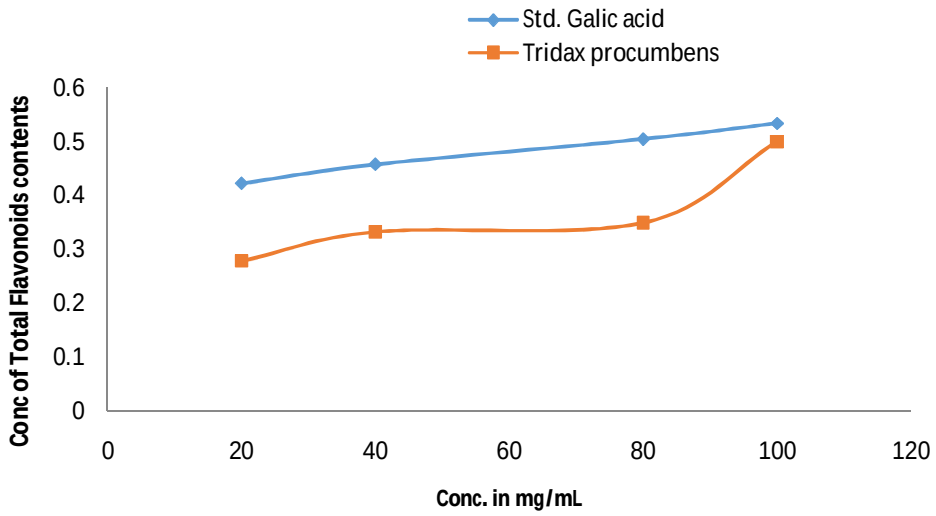


Figure 1: Total Flavonoids contents of aqueous extract of *Tridaxprocumbens* leaf

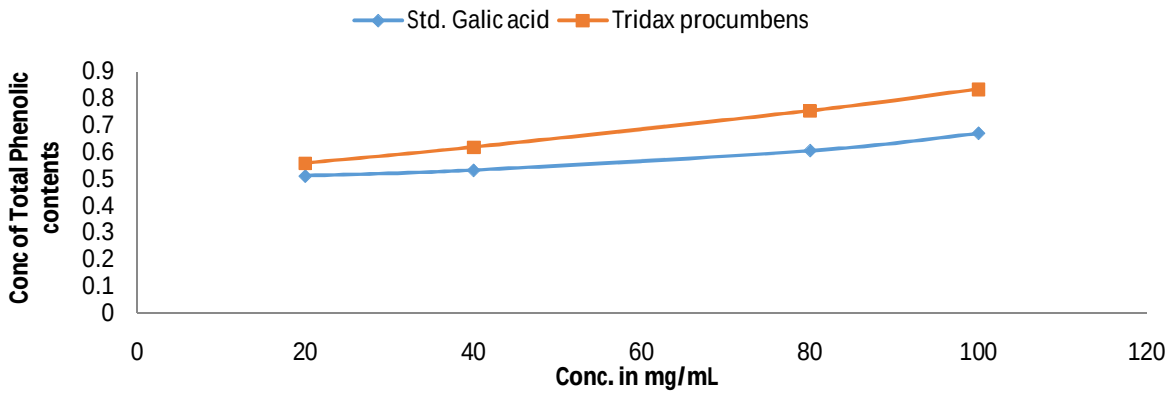


Figure 2: Total Phenolics content of aqueous extract of *Tridaxprocumbens* leaf

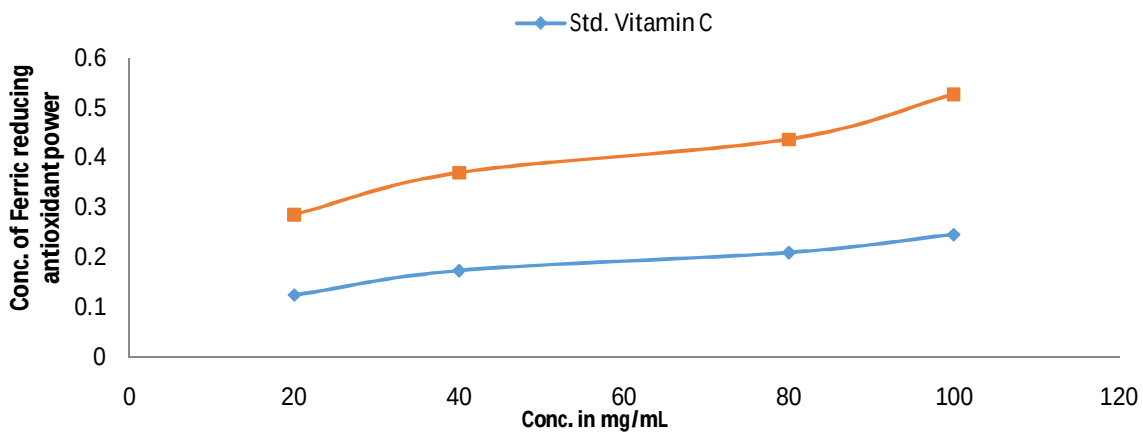


Figure3: Ferric reducing antioxidant power (FRAP) of aqueous extract of *Tridaxprocumbens* leaf

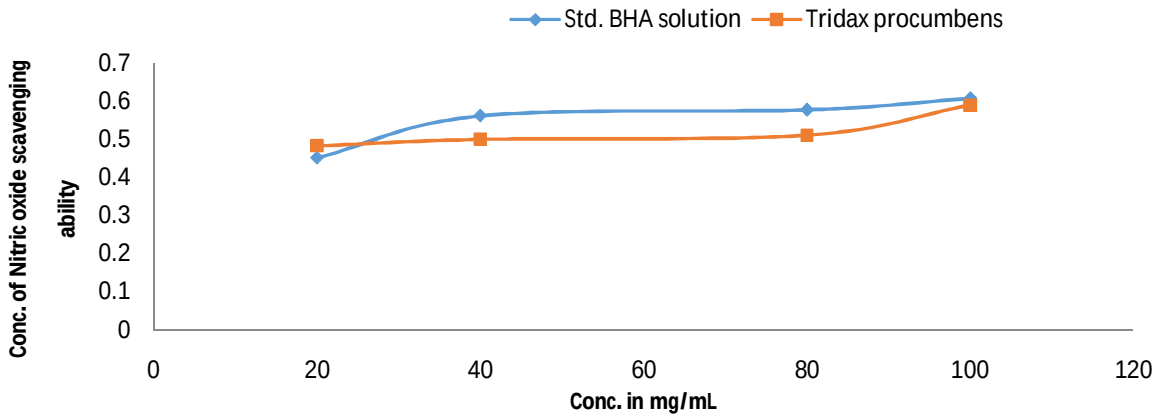


Figure 4: Concentration of Nitric oxide radical scavenging ability of aqueous extract of *Tridaxprocumbens* leaf

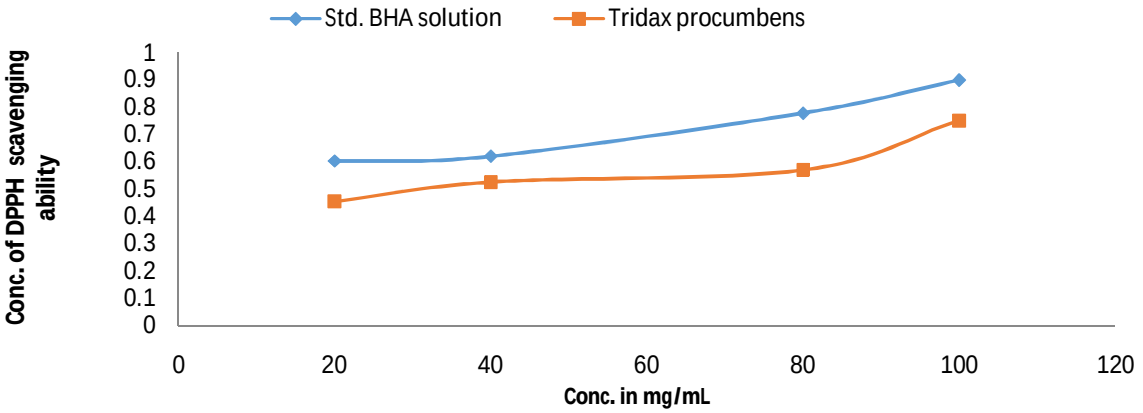


Figure 5. Concentration of 2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability of aqueous extract of *Tridaxprocumbens* leaf

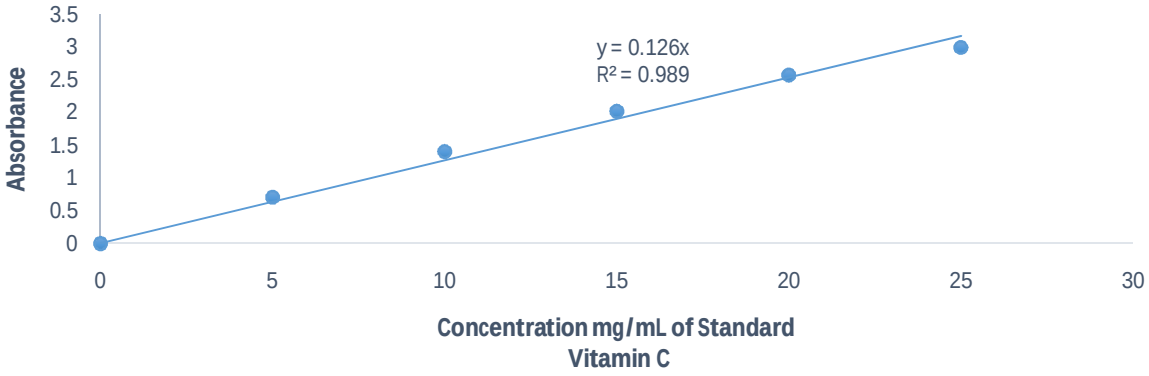


Figure 6: Calibration Curve for determination of Vitamin C content of *Tridaxprocumbens* leaf

The concentration of Vitamin C content in *Tridax procumbens* leaf was calculated from the graph with absorbance of 0.651 and slope of 0.1269 to be 51.3±0.01

mg/100g using y = 0.1269x

Table 2:Anti-bacteria activities of *Tridaxprocumbens*extract and silver, zinc and copper nanoparticle

	Zone of Inhibition (mm)							
	A	B	C	D	E	F	G	H
Tridax procumbens	12.0	17.0	6.50	12	-	-	7.0	7.0
*Tridax procumbens*ZnNP	6.0	-	-	9.0	2.0	1.0	5.0	9.0
*Tridax procumbens*AgNP	2.0	4.0	-	2.5	3.0	2.0	4.0	3.0
*Tridax procumbens*CuNP	5.0	3.0	4.0	5.0	6.0	2.0	6.0	3.0
CIPRO	23	18	28	30	32	28	26	20

CIPRO = Ciproflacin (500 mg/20 mL of distilled water

A = *Ralstonia solanecearum*B = *E. coli*

C = *Pseudomonas glycinea*D = *Staphylococcus aureus*

E = *Streptococcus faecalis*F = *Xanthromonasphasaoli*

G = *Enterobacteraerogenes* H = *Salmonella typhi*

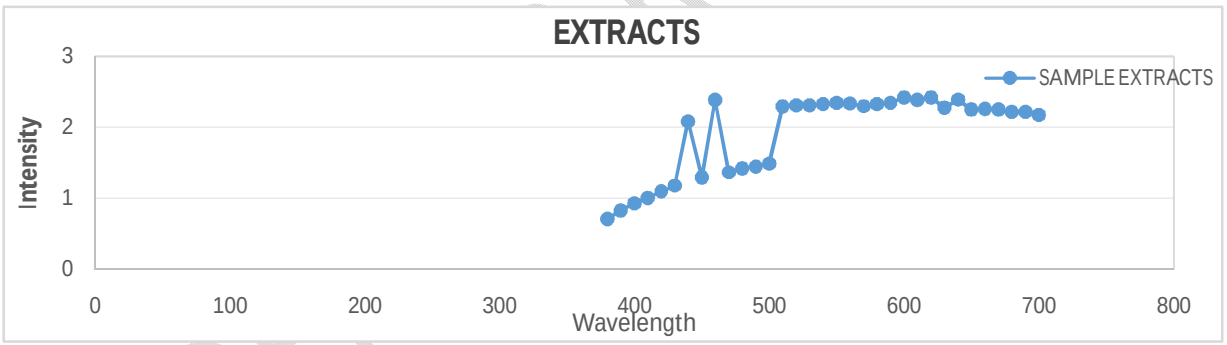
Table 3:Antifungal activities of *Tridaxprocumbens*extract and silver, zinc and copper nanoparticles

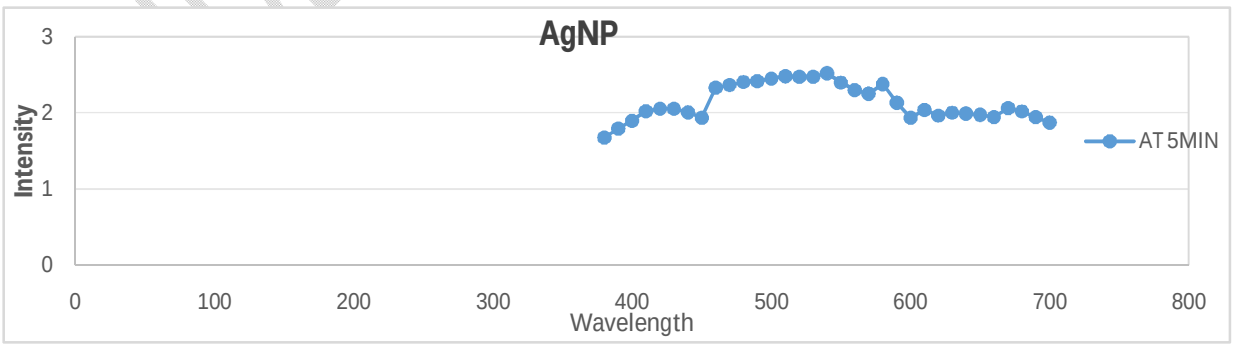
	% Zone of inhibition(mm)	
	Trichophytonverrycosumn	*Epidermophytonfloccosum*

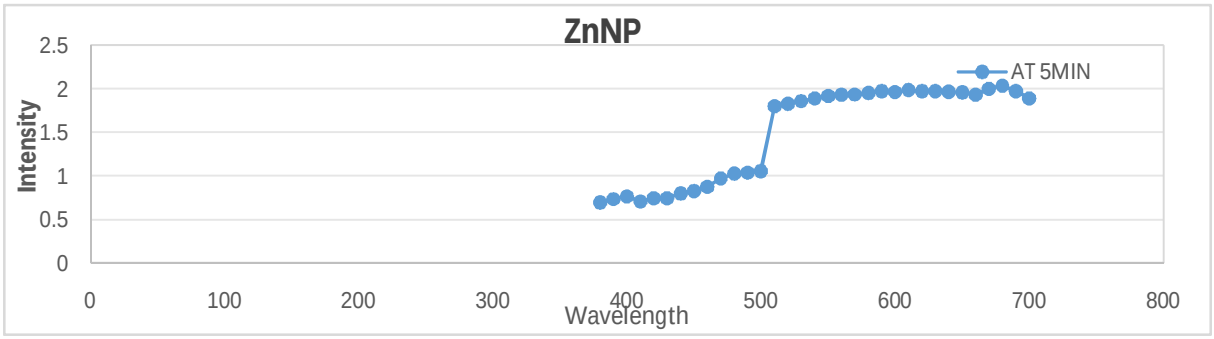
Tridax procumbens (extract)	69.23	43.88
*Tridax procumbens*ZnNP	50.00	42.86
*Tridax procumbens*AgNP	57.69	47.96
*Tridax procumbens*CUNP	55.77	21.43
Ketokonazole (standard)	80.00	70.00
Trymethroprime (standard)	80.77	89.80

Table 4: The obtained peaks of spectra from extract and the synthesized nanoparticles

Samples	Peaks
Extract	3964, 3677, 2894, 2642, 2428, 2240, 1986, 1723, 1507,1262, 1024 and 827
AgNP	3971, 3680, 3484, 2800, 2726, 1971, 1728 and 1568
ZnNP	3961, 3686, 3453, 2410, 2039, 1780 and 1566
CuNP	3888, 3684, 3503, 3256, 2853, 2671, 2005, 1732 and 1560







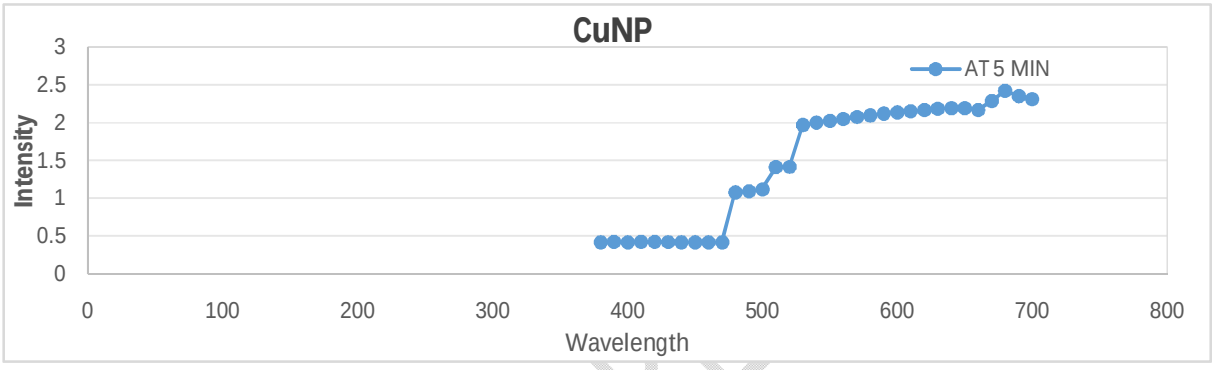
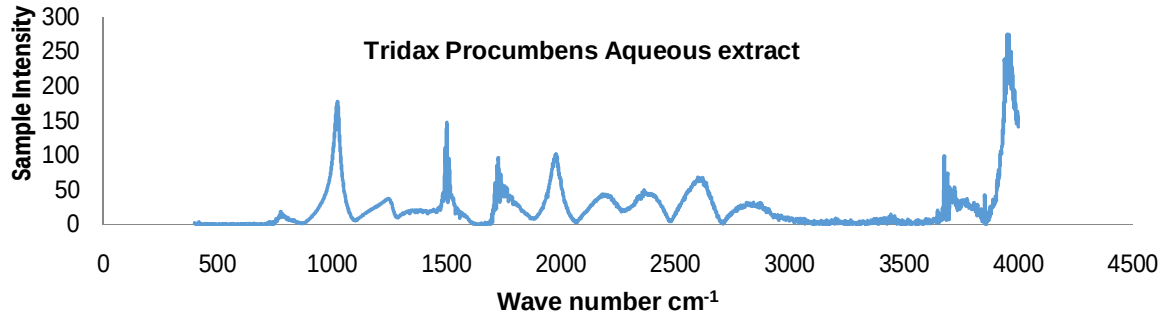
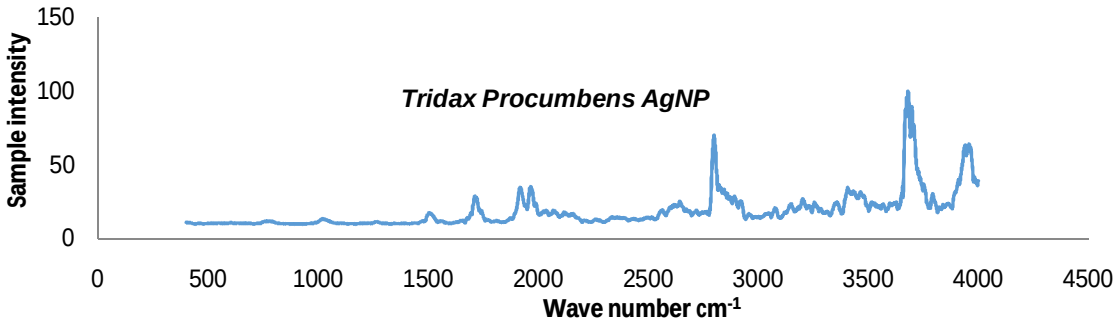


Figure 7: Wave scanning effects on for *Tridax Procumbens* extract, silver, Zinc and copper Nanoparticles





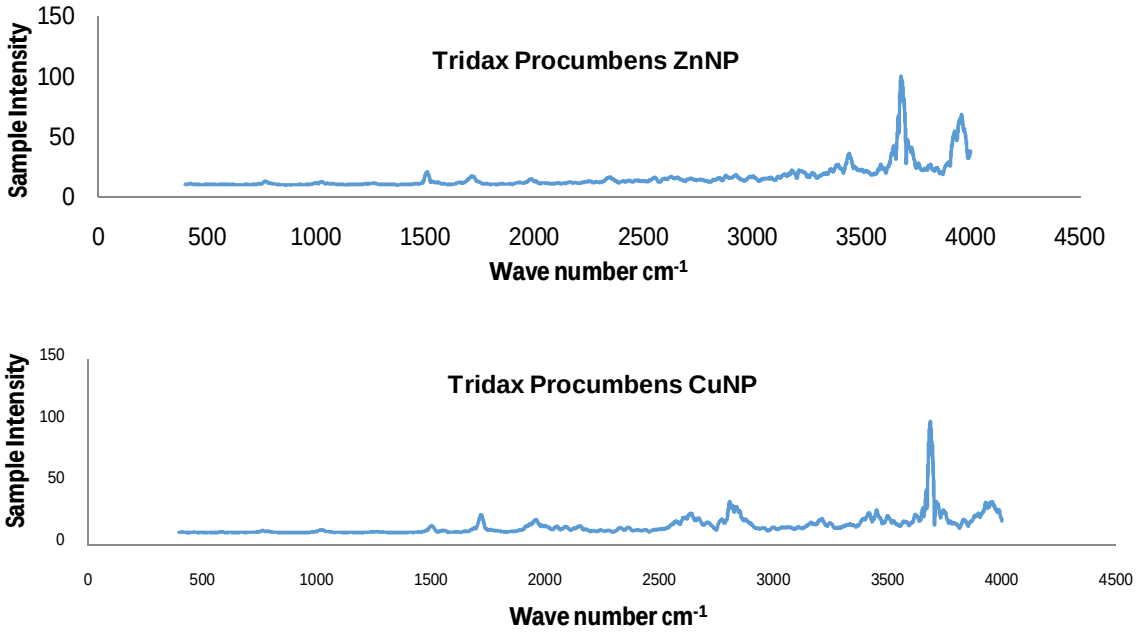


Figure 8: FTIR Spectra of *Tridax procumbens* extract, silver, zinc and copper Nanoparticles

Discussion

Table 1 displayed the phytochemical analysis of the ethanol and aqueous extracts of *Tridax procumbens* revealed the presence of constituents such as alkaloids, flavonoids, saponin, phenolics compounds and steroids. The results also showed that the aqueous extract contains more phytochemical constituents compared to the ethanolic extract, this may be due to the solubility of these compounds more in aqueous solution. These tested phytochemicals are known to exhibit medicinal and physiological activities by strengthening the immune system, reducing inflammation, preventing DNA damage, and inhibiting cancer cell growth [19]

Plant phytochemicals provide significant protection against free radicals as they neutralize their harmful effects, preventing them from attacking nucleic acids, lipids, and proteins. This plays a role in the etiology of major human diseases [20,21]. Additionally, the generation of reactive oxygen species (ROS) is associated with cancer and cardiovascular disorders. For example, fruits such as Prunus domestica, Syzygium cumini, Rubus ellipticus, and Prunus armeniaca were found to be rich in antioxidants [22]. Medicinal plants are often considered a

valuable gift from nature to humans [23]. Alkaloids are an important source of drugs and have been reported to possess antimicrobial, antioxidant, and cytotoxic activities [24]. Many plants have been used for their antimicrobial properties, which are attributed to compounds synthesized in the plant's secondary metabolism, such as phenols [25], essential oils [26], terpenoids [27,28], alkaloids [29] and flavonoids [30]. The preliminary phytochemical analysis conducted in this study also confirms the presence of some potential groups of bioactive substances consistent with previous reports [31]. The alkaloids, flavonoids, saponins, and phenolic compounds previously reported by [32,33] were also found in this study, suggesting that the plant is a rich source of these antioxidant phytochemicals [34]

In Figure1, it shows that as the sample amount increases, the concentration of total flavonoids also increases, and vice versa. Therefore, higher sample amounts correspond to higher total flavonoid content and antioxidant power, while lower sample amounts result in lower total flavonoid content and antioxidant power. The sample's aqueous extract also demonstrated higher antioxidant power than the standard (garlic) used at various concentrations. Similar trends were observed by [35] in their work. The flavonoids content of the leaf was slightly lower than the equal amount of the standard used.

Figure 2 shows that as the sample concentration increases, the concentration of total phenolic content also increases. Thus, higher sample amounts lead to higher phenolic content and antioxidant power, while lower sample amounts result in lower phenolic content and antioxidant power. The sample's aqueous extract also exhibited higher antioxidant power than the standard (garlic) used at various concentrations. Similar trends were reported by [36,37]] in their work. The phenolic content of the leaf was slightly higher than the equal amount of the standard used.

Figure 3 reveals that as the sample concentrations increase, the ferric-reducing antioxidant power of both the sample and that of standard also increases. Therefore, higher values indicate higher ferric-reducing antioxidant power, while lower values indicate lower ferric-reducing antioxidant power. The sample's aqueous extract also showed higher antioxidant power than the standard (ascorbic acid) used at various concentrations. The ferric-reducing antioxidant power obtained in this study agrees with what was reported by [37].

The ferric-reducing antioxidant power of the leaf was slightly lower than the equal amount of the standard used.

From Figure 4 above, it shows that as the sample concentration increases, the nitric oxide radical scavenging ability of both the sample and the standard used also increases. Hence, higher sample concentrations correspond to higher nitric oxide radical scavenging ability and overall higher antioxidant power, while lower values indicate lower antioxidant scavenging power. A similar result was previously reported by [38], which aligns with the findings of this study, showing increased scavenging ability as the concentration of the plant extract increased in various solvents used. The nitric oxide radical scavenging ability of the leaf was slightly lower than the equal amount of the standard used.

Figure 5 demonstrates that as the concentration increases for both the sample and the standard used, the antioxidant scavenging power also increases. Thus, higher values indicate higher antioxidant scavenging power, while lower values indicate lower antioxidant scavenging power. Overall, the results indicate that the DPPH scavenging capacity of the aqueous extract in this study and the methanolic, chloroform, and ethyl acetate extracts reported by [39] displayed a concentration-dependent fashion. The DPPH scavenging capacity of the leaf was slightly lower than the equal amount of the standard used.

It is evident that the aqueous extract from the leaves of *T. procumbens* possesses strong antioxidant activity. This indicates its ability to effectively quench or scavenge free radicals such as reactive nitrogen species (RNS) and reactive oxygen species (ROS), which are key factors in neurodegenerative diseases and oxidative stress. These free radicals can cause damage to biomolecules like lipids, proteins, and DNA, thereby influencing cell survival regulation, inflammation, and stress responses [40,41,42] *T. procumbens* has demonstrated protective activity against oxidative stress induced by ROS by increasing the levels of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, and catalase. These effects can be attributed to the diverse range of bioactive constituents present in the plant.

Figure 6 depicts that *Tridaxprocumbens* exhibits a high ascorbic acid content of 51.3±0.006 mg/100g. This finding further supports the strong antioxidant activity of the aqueous extract

from the leaves of this plant. Ascorbic acid, also known as vitamin C, is known for its significant antioxidant activity. It acts as a scavenger of ROS and helps prevent oxidative damage to vital biological molecules [43,44]. Therefore, plants with a higher content of ascorbic acid can serve as potent sources for protecting the human body against oxidative stress, inflammation, cancer, and infections.

Table 3 reveals that the *Tridax procumbens* extract possesses strong antibacterial activity against the tested pathogens, except for *Streptococcus faecalis* (E) and *Xanthomonas phaseoli* (F). On the other hand, the synthesized *Tridax procumbens*ZnNP exhibit strong antibacterial activity against the tested pathogens, except for *E. coli* (B) and *Pseudomonas glycinea* (C). Additionally, the *Tridax procumbens*AgNP demonstrate mild antibacterial activity against the tested pathogens, except for *Pseudomonas glycinea* (C). However, Tri*dax procumbens*CuNP shows mild antibacterial activity against all the tested pathogens. The results also indicate that the extract and all three nanoparticles exhibit complete antimicrobial activity against *Ralstonia solanacearum* (A), *Staphylococcus aureus* (D), *Enterobacter aerogenes* (G), and *Salmonella typhi* (H). *Tridax procumbens* extract was found effective against both Gram-positive and Gram-negative bacteria, consistent with the earlier report by [45]. Despite the structural dissimilarities and composition of the membrane of gram-negative bacteria [46,47]. Although the synthesized ZnNP, AgNP, and CuNP show more obvious impacts on gram-positive pathogens compared to gram-negative ones, this may be as a result the level of reduction of the nanoparticle as well as possible components of gram-negative bacterial membrane [48].

Table 4 shows that the *Tridax procumbens* extract has a higher percentage (69.23) of antifungal activity against *Trichophyton verrucosum,* effectively inhibiting its growth. The synthesized ZnNP, AgNP, and CuNP exhibit percentages of (50.00), (57.69), and (55.77) respectively, indicating their good potential to inhibit the growth of *Trichophyton verrucosum* as well. For *Epidermophyton floccosum*, the Synthesized AgNP shows a higher percentage (47.96), suggesting a tendency to inhibit its growth, while the *Tridax procumbens* extract has a percentage (43.88) indicating its antifungal activity against *Epidermophyton floccosum*. Previous studies have shown that nanoparticles offer faster solutions to human problems than

any other means [48]. But for the extract from the leaves of *Tridax procumbens* to show a percentage (69.23) greater than the synthesized nanoparticles and closer to the standards Ketoconazole (80.00) and Trimethoprim (80.77) against *Trichophyton verrucosum* demonstrates its potential as a very good medicinal plant against antifungals. This study aligns with the report that the antifungal activity of *T. procumbens* may be due to the presence of many bioactive compounds such as phenols, flavonoids, saponins, sterols, and fatty acids. The essential oils obtained from the flowers of *Tridax procumbens* L. were found to be active against the tested fungi [49].

Figure 7 showed the effects of wave-scanning pattern of the extracts, AgNP, ZnNP and CuNP . It showed varied intensities at various wavelengths between 400 and 700 nm in all.

Change in colour was observed in both extract and the different synthesized nanoparticles that is CuNP, ZnNP and AgNP. The absorption spectra of all the nanoparticles as recorded followed a similar but slightly different pattern as well as slightly different from that of *Tridax procumbens* aqueous extract.

In Figure 8, the FTIR Spectra of *Tridax procumbens aqueous* extract, silver, zinc and copper nanoparticles by green synthesis actually revealed observed reduction and change in the functional group positions of the nanoparticles when compared with the plant extract. The green synthesis approach has received significant attention for the synthesis of metallic nanoparticles in drug development as a result improved quality and actions. Although various chemical and physical synthesis methods are known, green synthesis is considered safer, more sustainable, and biologically acceptable. Plants and microbes are the main biological materials used for green synthesis. Since plants are generally considered freely available, easy to handle, harmless, and inexpensive materials for synthesizing various types of nanoparticles [50], recent studies show extensive research being conducted on the synthesis of iron oxide nanoparticles (IONP) using plants. One primary reason for this trend could be that bacteria and fungi require lengthy incubation periods, whereas phytochemicals present in plants are capable of reducing metal ions in a comparatively shorter time [51]. The results in Table 2 support these findings; the FTIR spectra of *Tridaxprocumbens* extract displayed observable multiple peaks from 834 cm^{-1}to3964cm^{-1} far more than the nanoparticles while the three metal nanoparticles show significant peaks from 1560 cm^{-1}at varied positions to 3971

cm^{-1}forAgNP, 3961 for ZnNP and 3988 cm^{-1} for CuNP. The broad peak at 3454 cm^{-1} is due to the OH stretching vibrations of phenol and carboxylic groups present in *Tridaxprocumbens* extract. In the extract, the band at 1636 cm^{-1} corresponds to amide CO stretching, and a peak at 2083 cm^{-1} can be attributed to the alkyne group present in the phytoconstituents of the extract. The observed peaks at 1024cm^{-1} to 1113 cm^{-1} indicate -C-OC- linkages or -C-O- bonds, as also defined in a previous study [52]. Bio-reduction of copper, silver, and zinc oxide in aqueous solutions was monitored by periodically sampling aliquot of the mixture and subsequently measuring the UV–Visible spectra. The synthesis of nanoparticles was confirmed using a UV-Visible spectrophotometer as a characterizing tool. The maximum absorbance peaks of copper and zinc oxide nanoparticles were observed at 300 nm and 275-375 nm, respectively. The lambda maxima of the synthesized nanoparticles were quite similar to those reported by [53].

Conclusion

The present study revealed that the *Tridax procumbens* plant acts as a potential source of useful phytochemicals such as alkaloids, flavonoids, saponins, and phenolic compounds in the aqueous extract and the quantification of flavonoids and phenolic compounds in the plant extracts carried out indicate enormous antioxidant potentials of the plant which are known to exhibit medicinal purposes as well as physiological activities. The antioxidant power of the aqueous extract of the *Tridax procumbens*to scavenge nitric oxide and DPPH radicals also depicts its medicinal potency against various types of diseases. The antibacterial and antifungal activities of the aqueous extract and nanoparticles (ZnNP, CuNP, AgNP) of *Tridaxprocumbens* may also help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease and chemotherapy as well as disease control. The present study also clearly indicates that the synthesized nanoparticles have better antibacterial action against Gram-positive organisms than Gram-negative organisms. The study also showed the possibility of using the aqueous leaf extract of*Tridaxprocumbens*as a reducing and capping agent for the synthesizing of the metallic nanoparticles. This investigation has opened up the possibility of using this plant in antimicrobial drug development for human application, as well as for medicinal and general drug development.Spectroscopic characterizations from UV-visible and FTIR supports the

study of the bio-synthesized nanoparticles. It is believed that the AgNPs, CuNPs, and ZnNPs nanoparticles have great potential for applications in drug designs and pharmaceutical industries as they are found to improve quality and actions of drug, safer, enhanced therapeutic effects and biologically acceptability.

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