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Green Synthesis of Zn Nanoparticles Using Aqueous Leaves Extract of Portulaca Quadrifida and Portulaca Oleracea and Determination of Its Antioxidant, Antibacterial and Antidiabetic Activities

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ABSTRACT
Green synthesized nanoparticles derived from plant sources are receiving a lot of interest because of their inherent qualities, which include affordability and environmental friendliness. Zinc nanoparticles (NPs) are potentially useful in various fields such as catalysis, electronics, biomedical, etc. In this study, zinc nanoparticles were synthesized using P. oleracea and P. quadrifida (L.) leaves aqueous extract green synthesized nanoparticles are characterized by UV –Vis spectroscopy, FTIR spectroscopy, Scanning electron microscopy. The antibacterial activity was found at 30mm at the Zone of inhibition in each leaf sample and the maximum DPPH radical scavenging activity was 93.1% at 100 µg/mL and the maximum Fe3+ reduction was 38.80 at 120 µg/mL concentration in P. quadrifida leaves extract and the maximum phosphomolybdenum reduction was 75 at 100 µg/mL concentration P. quadrifida leaves extract. The size observed in SEM characterization is specifically 140 nanoparticles, it suggests a relatively narrow size distribution within P. quadrifida sample. FTIR characterization shows the maximum peak value of 2098.55 cm−1 and it confirms the presence of aromatic compound with C-H bending with Zn nanoparticles. overall, this study, green Synthesized zinc nanoparticles can be a safe substitute for synthetic materials and a viable option for anti-inflammatory, antibacterial, and antioxidant drugs utilized in the pharmaceutical and biomedical sectors.

Keywords - Green synthesis, zinc nanoparticles, P. oleracea, P. quadrifida, leaf extract

I. INTRODUCTION

Nanoparticles (NPs) are materials with a size range of 1 to 100 nm.Based on their characteristics, forms, or sizes, they can be divided into several classes. Fullerenes, metal NPs, ceramic NPs, and polymeric NPs are among the various groups. Because of their large surface area and nanoscale size, NPs have special physical and chemical characteristics. It is stated that their size affects their optical characteristics, resulting in varying colors because of absorption in the visible spectrum. Their distinct size, shape, and structure also affect their toughness, reactivity, and other characteristics. Although nanoparticles have always been present in nature, mostly in the form of dust and smoke. NPs are formed of threelayers. The first layeris the surface, which can be functionalized with a range of small molecules, metal ions, surfactants, and polymers. The second layer is shell, which is entirely distinct chemically from the core and the third layer is core, which is the main part of the NP and is typically used to refer to the NP itself Shin et al., (2016). Inorganic nonmetallic solids, or ceramic nanoparticles, are created by heating and then cooling a material. They exist in forms that are porous, hollow, dense, polycrystalline, and amorphous Sigmund et al., (2006).Silvera Batista CA et al., (2015) reviewed that the smaller particles of the same substance frequently have characteristics that are noticeably different from bigger particles. As an atom's diameter typically ranges from 0.15 to 0.6 nm, a significant portion of the material in a nanoparticle is located within a few atomic diameters of the particle's surface. The techniques used to characterize nanoparticles fall into a number of broad groups. To describe the form, size, and location of individual nanoparticles, microscopy techniques provide photographs of them. The two most common techniques are scanning probe microscopy and electron microscopy. The physical and chemical properties of a nanoparticle can undergo significant changes as its size reduces.

International Journal of Pharmaceutical Research and Applications
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Applications in areas like as sensors, medical diagnostics, the understanding and manipulation of these optical properties Micheal Bolarinwa Fabiyi et al.,(2023).

P. quadrifida is an annual, succulent plant that forms mats. This species' natural range is unclear; many people refer to it as pantropical. Worldwide, P. quadrifida has been introduced into several new nations. P. quadrifida is an agricultural weed in its native region; there are no records of it acting as an invasive weed elsewhere. This plant does not compete much, but because it is a halophyte and can withstand dry spells and low soil fertility, it can easily take over in these environments.Due to its culinary and medicinal qualities, this species may be purposefully introduced Chris Parker (2014). P. quadrifida is also utilized in traditional medicine for purposes such as anti-abortifacient and as a veterinary lactation stimulant. In addition, it is used as a diuretic, pain reliever, vermifuge, and for parasite infections, kidney problems, lung issues, stomach problems, and venereal diseases. Additional uses listed by Prota et al., (2014) include the following: as a diuretic; as a sedative, analgesic, and cardiotonic; as a treatmentfor fever, urinary tract disorders, worm diseases; as a tonic and choleretic; as a treatment for dysentery; and as an external application for ulcers, eczema, and dermatitis. Anti-cancer activity against human colon cancer HT-29 cells was validated by Mulla and Paramjyothi Swamy's (2012) study. Antifungal effectiveness against Candida albicansand Aspergillus niger was confirmed by Naidu and Babu (2009).Studies conducted in vitro have shown that intestinal smooth muscle is directly affected by spasmolytic action.The ancient, worldwide species known as portulaca oleracea, or purslane, relies heavily on selffertilization. Because of this, there are local populations that exhibit a range of physical and physiological characteristics encoded in their DNA. Purslane hasn't, however, been divided into several microspecies Lagrand et al., (1992). Primarily an annual, P. oleracea has the potential to become perennial in tropical regions. Emerging from a taproot, the glabrous, fleshy stems can range in color from purplish-red to green and form mats when upright. They are also meaty. In rich soils, the leaves can measure anywhere from 40 mm by 15 mm to 60 mm by 25 mm. At the end of the stem, flowers are arranged in a bunch. A carina that resembles wings and is 3–4 mm long and can cover the fruit is formed when the two sepals unite at the base of the ovary. The capsule has a diameter of 4 to 9 mm and opens at or slightly below the middle. The style branches are 3-6. Mature seeds are black, although immature seeds can also be red or brown. The seeds typically have granulated to flat-stellate surfaces and are 0.6–1 mm long. Other patterns, on the other hand, may appear with elevated tuberculate and stellate surfaces Wallingford UK (2021).

II. MATERIALS AND METHODOLOGY

2.1. SAMPLE COLLECTION AND PROCESSING

The Fresh leaves of P. quadrifida and P. oleraceae were collected from the garden. The leaves were completely air dried in the shade before being ground into the fine powder. The powdered leaves were maintained in an air-tight container at room temperature (28 ± 2 °C) and kept away from light until use.

2.2. PREPARATION OF PLANT EXTRACT

The air-dried powder (20 g) of P. quadrifida and P. oleraceae leaves was taken and immersed in 400 mL of deionized water (dH2O). The extraction process was performed via the Aqueous Extraction method. The solvent (d.H2O) and powder layer were filtered using muslin cloth first and then Whatman filter paper. The filtrate solution of P. quadrifida and P. oleraceae leaf extract was kept in a refrigerator to be utilized for further use.

2.3. SYNTHESIS OF Zn NANOPARTICLES

The filtered leaves extract was heated at 50 °c for 10 minutes. And then after 20mL of 0.1 M of Zinc acetate dihydrate solution was added. The Zinc acetate dihydrate solution was dissolved in 50 mL of distilled water was added drop by drop.The solution was prepared and it was kept under the centrifugation process.

2.4. COLLECTION OF Zn NANOPARTICLES

The Zinc Acetate dihydrate solution with plant extract was centrifuged at 8000 rpm for 15 mins. After the pellet was collected from each sample. The unwanted particles were removed from the sample using ethanol. Then after the Zinc Nanoparticles were collected from each Micro centrifuge tube. The synthesized Zn nanoparticles can be stored for the various assay and the collect microfuge tube and it was used for the characterization process.

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2.5. SAMPLE PREPARATION FOR ASSAY

The leaves extracts were taken into the beaker and it was kept in the boiling process for few minutes. Then the methanol was added into the solutions to get the separations of the final solutions form.

2.6. PHYTOCHEMICAL ANALYSIS

Plant extracts are subjected to phytochemical analysis, which is the examination of the different chemical constituents that give plants their physiological, pharmacological, and nutritional characteristics. These substances may consist of glycosides, alkaloids, flavonoids, phenolic compounds, terpenoids, saponins, tannins, and many more. To comprehend the possible health advantages of plants and their useful uses in agriculture, medicine, and nutrition, phytochemical study is crucial.

2.7 ANTIBACTERIAL ACTIVITY

The antibacterial activity was tested against human pathogens by agar well diffusion methods. Bacteria was grown in NB medium and incubated for 24 hrs at the room temperature. The petriplates was sterilized by autoclave at 121 °C for 30 minutes. The Solidify agar was poured into the petriplates and after solidifying, the Staphylococcus aureus and Streptococcus pyogenes cultures was swabbed on agar medium using sterilizes buds. After 15 minutes the wells were punched.The wells were formed the various concentrations of 250µl, 500 µl,1000 µl and the antibiotic was used as positive control. The plates were incubated for 37 °C for 24hrs.The antibacterial activity was measured by zone of inhibition in mm.

2.8 ANTIOXIDANT ASSAY

2.8.1. DPPH (2,2-Diphenylpicrylhydrazyl) ASSAY

The leaf samples were added to the test tube in the range of 20 – 120 µl. Methanol was added to the test tube in 1mL. Then, 2,2-Diphenylpicrylhydrazyl (DPPH) was added in 1mL.It was kept in the dark for incubation for 30 minutes. Calculate the OD value at 517nm. The ability of methanol extract of P. quadrifida leaves to scavenge free radicals formed was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The maximum $DPPH^{\cdot}$ radical scavenging activity was 93.1% at 100 µg/mL and 120 µg/mL concentration in hexane extract

2.8.2. FERRIC REDUCING ASSAY

The leaf sample was added to the test tube in the range of 20 – 120 µl. Methanol was added to the test tube in 1 ml. Take 0.100 g of K3[Fe (CN)]6 in 100 mL distilled water and add Fe3+ reducing buffer in 1 ml. Incubate in a water bath for 20 mins at 50°C.After, TCA was added in 500 µl. Then, 100 µl of FeCl3 was added to each test tube. Observe the OD value at 700 and calculate the percentage of reduction.

2.8.3. PHOSPHOMOLYBDENUM ASSAY

The leaf sample was added to the test tube in the range of 20–120 µl. Methanol was added to the test tube in 1 mL of Phosphomolybdenum reagent was added. It was incubated for 90 minutes at 95°C. The OD value was observed at 695 nm, and the percentage of reduction was calculated.

III. RESULT AND DISCUSSION

3.1. PHYTOCHEMICAL ANALYSIS

The phytochemical analysis of P. quadrifida leaves extract showed the presence of Terpenoids, Phenol, Tannins, Glycosides, Carbohydrates, Quinones and proteins compounds and it showed the absence of Alkaloids, Flavonoids, Saponins, Steroids compound.

SI NO	PHYTO CONSTITUTES	TEST	RESULT
1	Alkaloids	Dragendroffs reagent	-
2	Terpenoids	CHCl3 + conc H2SO4	+
3	Flavonoids	NaOH solution	-
4	Phenols	FeCl3 solution	+
5	Glycosides	Pyridine + SNP + conc H2SO4	+
6	Saponins	Foam test	-
7	Steroids	Acetic anhydride solution + conc H2SO4	-
8	Tannins	H2SO4 + lead acetate solution	+
9	Carbohydrates	Alcoholic alpha nepthal solution + conc H2SO4	+
10	Proteins	Conc H2SO4	+
11	Quinones	MeOH + conc H2SO4	+

Table 1: Qualitative analysis of P.quadrifida leaf extract

International Journal of Pharmaceutical Research and Applications
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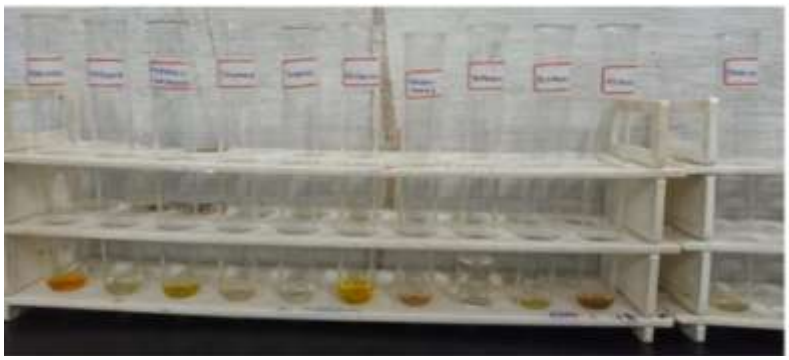

Fig 1: Qualitative analysis of P.quadrifida leaf extract

3.2. UV – VIS SPECTROSCOPY

UV-vis spectroscopy is a very useful and reliable technique for the primary characterization of synthesized Zinc nanoparticles.The absorption spectrum was recorded by using a UV – Vis (U-2900) double beam spectrophotometer in between a wavelength scan of 200- 800nm. The maximum absorbance was found to be 0.648 with the wavelength of 335nm. It confirms the presence of Zinc nanoparticles in the leaves extract.

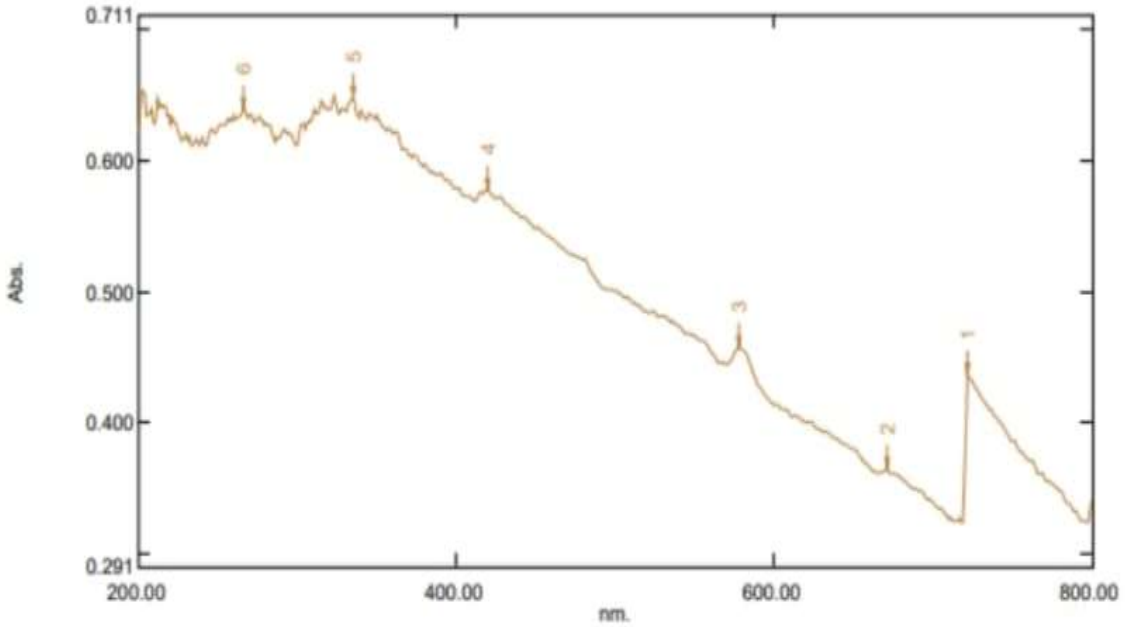

Fig 2: Uv – vis spectroscopy peak table analysis

3.3 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

FTIR analysis is used to identify the functional group involved in green synthesized Zn nanoparticles. At a wavelength of 500-4000 cm−1, the FTIR scanned with the resolution of 4.0 cm−1.The peak of 1000 cm−1 and 1616.36 cm−1 indicates the C=C bending and the alkene compound present in it and it was appeared like strong.The peak value of 2918.80 cm−1 and 3153.6 cm−1 have indicates the presence of Carboxylic acid with O-H stretching respectively and their appearance is strong and broad. The peak of 3880.78 cm−1And 3981.08 cm−1 have confirmed the presence of alcoholic compound with O-H stretching and it was appears like Medium and sharp. FTIR characterization shows the maximum peak value of 2098.55 cm−1 and it confirms the presence of aromatic compound with C-H bending.

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 1109-1118 www.ijprajournal.com ISSN: 2456-4494

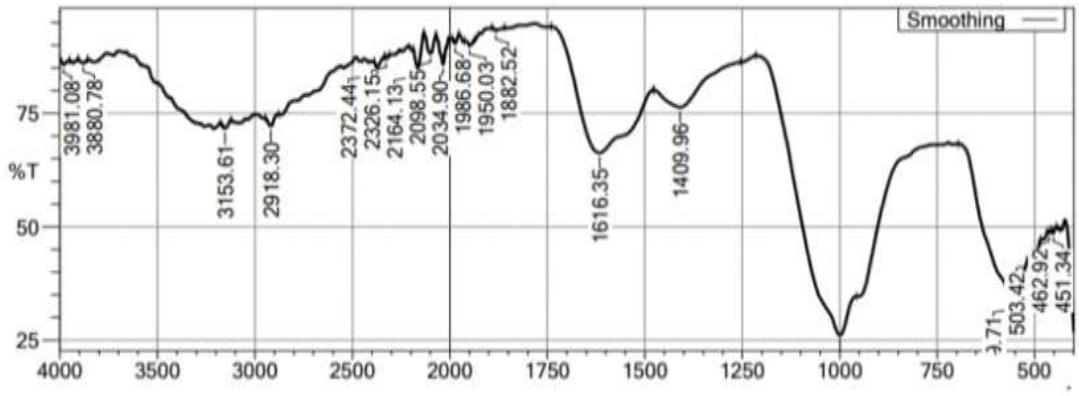

Fig 3: FTIR analysis peak table analysis

3.4 X-RAY DIFFRACTION

The XRD pattern of green synthesized Zn NPs using ALE of P.quadrifida is illustrated in below Figure 16. The XRD diffraction peaks existed at 2θ angles. The most intense peak corresponding to (33) plane located at 36. 35°.Furthermore, the XRD pattern revealed no additional peaks other than the characteristic Zn peaks, confirming the purity of the produced Zn NPs.

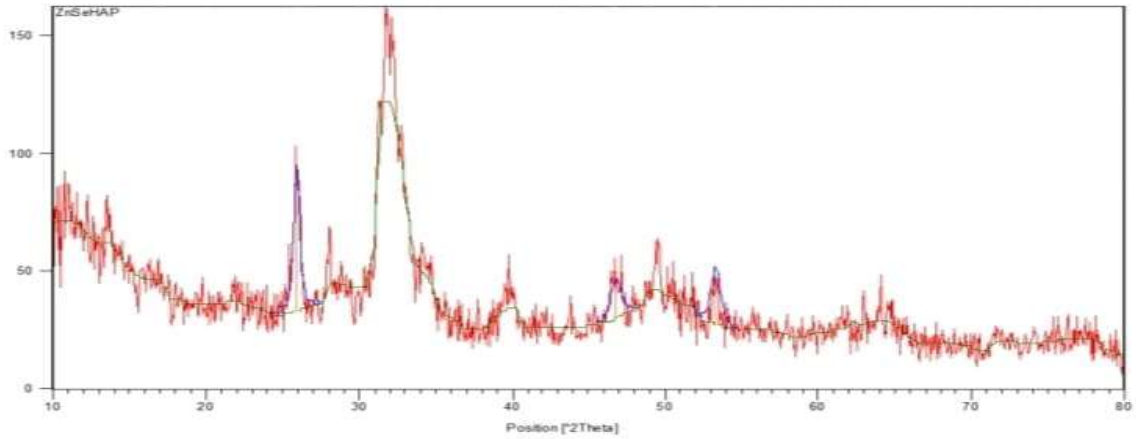

Fig 4: - ray diffraction peak table analysis

3.5. ANTIBACTERIAL ASSAY

The leaves extract of P. oleracea exhibited a zone of inhibition of 16mm against Gram positive bacteria of Staphylococcus aureus and Streptococcus pyrogens at the concentrations of 1000 µl/mg.

BACTERIAL STRAIN	ZONE OF INHIBITION (mm)			
	Leaf sample			Tetracycline
	250	500	1000	std
Staphylococcus aureus	14 mm	15mm	16mm	30mm
Streptococcus pyrogens	15mm	17mm	19mm	34mm

Table 2: Antibacterial assay of P. oleracea leaf extract

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 1109-1118 www.ijprajournal.com ISSN: 2456-4494

The leaves extract of P. quadrifida exhibited a zone of inhibition of 17mm against Gram positive bacteria of Staphylococcus aureus and Streptococcus pyrogens at the concentrations of 1000 µl/ml.

BACTERIAL STRAIN	ZONE OF INHIBITION (mm)			
	Leaf sample			Tetracycline
	250	500	1000	std
Staphylococcus aureus	17mm	19mm	22mm	33mm
Streptococcus pyrogens	14mm	16mm	19mm	30mm

Table 3: Antibacterial assay of P. quadrifida leaf extract

3.6 ANTIOXIDANT ACTIVITIES

3.6.1 DPPH RADICAL SCAVENGING ASSAY

DPPH radical scavenging assay is a decolorization assay that will measure the capacityof antioxidants to directly scavenge DPPH radicals by monitoring its absorbance using spectrophotometer at wavelength of 517 nm. The ability of methanol extract of P. quadrifida leaves to scavenge free radicals formed was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The maximum DPPH˙ radical scavenging activity was 93.1% at 100 µg/mL and 120 µg/mL concentration in hexane extract.

SAMPLE IN µl	OD VALUE IN nm	% OF INHIBITION
BLANK	0.190	-
10µl	0.131	31.052
20µl	0.080	57.89
30µl	0.034	82.1
40µl	0.024	87.3
50µl	0.013	93.1
60µl	0.013	93.1

Table 4: DPPH assay of P. quadrifida leaf extract

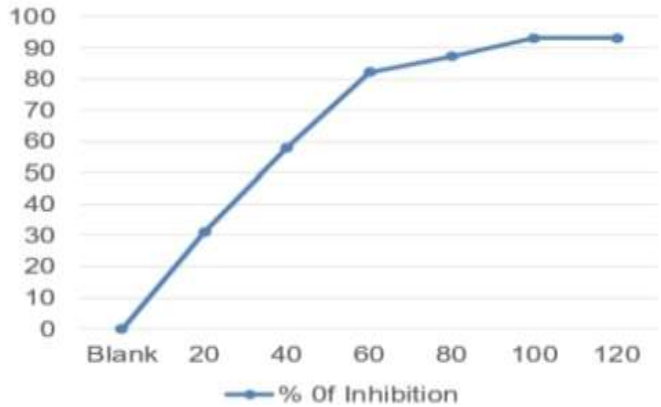

Fig 5: Graphical representation of DPPH assay

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 1109-1118 www.ijprajournal.com ISSN: 2456-4494

3.6.2 FERRIC (FE3+) REDUCING POWER ACTIVITY

The maximum Fe3+ reduction was 38.80 at 120 µg/mL concentration in P. quadrifida leaves extract. In this assay, higher absorbance of the reaction mixture indicates higher reduction potential. The reducing capacity of the extract was performed using Fe3+ to Fe2+ reduction assay as the yellow colour changes to green or blue colour depending on the concentration of antioxidants.

SAMPLE IN µl	OD VALUE IN nm	% OF REDUCTION
BLANK	0.735	-
20µl	0.837	12.18
40µl	0.913	19.49
60µl	0.961	3.51
80µl	1.052	30.13
100µl	1.076	31.69
120µl	1.202	38.80

Table 7: Ferric (fe3+) reducing assay of P. quadrifida leaf extract

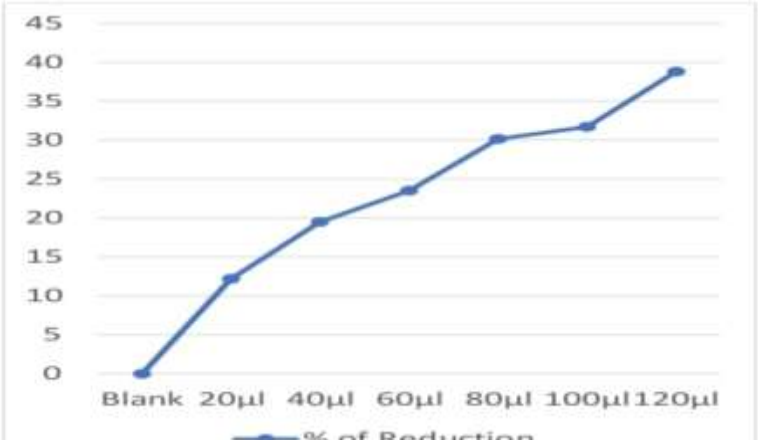

Fig 6: Graphical representation of Ferric reducing Power activity

3.6.3 PHOSPHOMOLYBDENUM REDUCTION ACTIVITY

The total antioxidant activity of methanol extract of P. quadrifida leaves was measured by phophomolybdenum reduction method which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm. The maximum phosphomolybdenum reduction was 75 at 100 µg/mL concentration.

SAMPLE IN µl	OD VALUE IN nm	% OF REDUCTION
BLANK	0.014	-
20µl	0.032	56.25
40µl	0.034	58.82
60µl	0.036	61.11
80µl	0.042	66.60
100µl	0.056	75.00
120µl	0.039	64.10

Table 8: Phosphomolybdenum reduction activity of p. quadrifida leaf extract

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 1109-1118 www.ijprajournal.com ISSN: 2456-4494

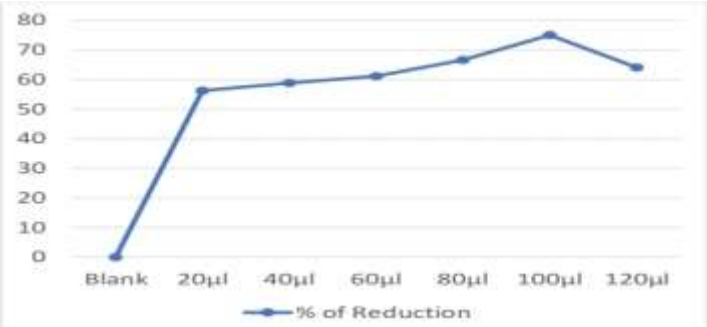

Fig 7: Graphical representation of Phosphomolybdenum assay

3.7 ANTIDIABETIC ACTIVITY

The Antidiabetic activity of test samples was determined by slightly modified method. Different concentration of test samples (20-120 µg/mL) was mixed with 1 mL of methanol, followed by 20 µL of alpha amylase solution (1 % w/v). The mixture was then incubated in room temperature at 37°C for 5 min. 500 µL of starch (1% w/v) was added to each mixture, mixed well and incubated in room temperature at 37°C for 60 min. 100 µL of freshly prepared 1N Hydrochloric acid was added to terminate the enzymatic reaction. The antidiabetic activity was found at 88.78 % of inhibition at the absorbance level of 595 nm with the concentration of 20 µg/ml. The examined results were used for the conducting the research purpose.

S.NO	Conc µg/ml	Absorbance at 595nm	% of inhibition
1	Control	0.107	-
2	20	0.012	88.78
3	40	0.017	84.11
4	60	0.026	75.70
5	80	0.038	64.48
6	100	0.048	55.14
7	120	0.054	49.54

Table 9: Antidiabetic activity of P. quadrifida leaf extract

3.8 SCANNING ELECTRON MICROSCOPY (SEM)

The Sequential range of micrometre range has been performed for the identification of nanoparticle of the sample P. quadrifida from the range of 2 µm to 40 µm. The size observed in SEM characterization is specifically 140 nanoparticles, it suggests a relatively narrow size distribution within P. quadrifida sample.

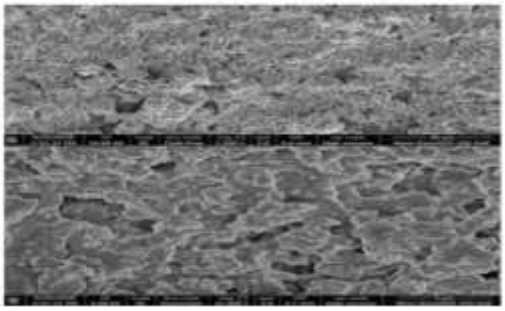

Fig 8: Scanning electron microscopy result of P. quadrifida leaf extract

IV. CONCLUSION

The result of this study indicates the P. quadrifida and P. oleracea leaves that can be environmentally sustainable process of synthesizing zinc nanoparticles using extract from the P. quadrifida and P. oleracea leaves with successful the antidiabetic, antioxidant and antibacterial activities. zinc nanoparticles were synthesized using P. odoratissimum and P. quadrifida (L.) leaves aqueous extract green synthesized nanoparticles are characterized by UV –Vis spectroscopy, FTIR spectroscopy, Scanning electron microscopy. The antibacterial activity was found at 30mm at the Zone of inhibition in each leaf sample and the maximum DPPH radical scavenging activity was 93.1% at 100 µg/mL and the maximum Fe3+ reduction was 38.80 at 120 µg/mL concentration in P. quadrifida leaves extract and the maximum phosphomolybdenum reduction was 75 at 100 µg/mL concentration P. quadrifida

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leaves extract. The size observed in SEM characterization is specifically 140 nanoparticles, it suggests a relatively narrow size distribution within P. quadrifida sample. FTIR characterization shows the maximum peak value of 2098.55 cm−1 and it confirms the presence of aromatic compound with C-H bending. These nanoparticles were encouraging the fact the natural materials are used to synthesize nanoparticles highlights the variety of biomedical uses for which they may be employed to fully realize the therapeutic potential of these activities more investigation is needed to optimize synthesis parameters and comprehend the underlying mechanisms.

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