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A NOVEL APPROACH TOWARDS GREEN SYNTHESIS OF ZINC NANOPARTICLES FROM *BANIUM PERSICUM* AND ITS PHARMACOLOGICAL EFFICACY

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Keywords:

Zinc nanoparticles, *Banium persicum*, SEM, XRD, Antimicrobial Activity, Antiplatelet Activity

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ABSTRACT: Integration of nano-science in medicine leads to the development of biomedical products that help society in a faster and safer manner. In the present studies, Zinc nanoparticles were synthesized by green synthesis route using aqueous extracts of *Banium persicum*, and characterization was done through UV, SEM, TEM, FT-IR, and XRD. The synthesized nanoparticle was spherical in shape with an average size of 20 nm. Various bioactive compounds present in aqueous extract of this plant were responsible for bioreduction of nanoparticles. Further, these nanoparticles were tested for various biological activities. In antimicrobial activity maximum, anti-bacterial activity was observed against *S. aureus* at 120 µg/ml (IZ-18 mm),while in the case of fungus, maximum potential was observed at against *C. albigans* at 120 µg/ml (IZ- 30 mm). Further nanoparticles were screened for their antioxidant activity by LPO, Peroxidase, and Catalase assay and found they possessed potent antioxidant activity. The platelet aggregation of nanoparticles was assayed by Prothrombin (PT) and Activated Partial Thromboplastin time (APTT). In PT assay, maximum activity was observed at 6 µg/ml (128 sec), and in APTT it was found at 1 µg/ml (478 sec). Results showed the biosynthesis of Zinc nanoparticles using aqueous extract of *Banium persicum* is a clean, inexpensive and safe method that is free from toxic substance and consequently does not have any side effects.

INTRODUCTION: Nanotechnology is an emerging area of science, and the synthesis of nanoparticles (NPs) has been the most important step in the field of nanotechnology [1]. In the field of biology, nanoparticles have a variety of applications as vaccine/drug delivery systems, minerals, anti-bacterial, *etc*.

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A wide range of chemical and physical, and biological methods are being used for the synthesis of nanoparticles [2]. The major cause of the boom in the field of nanoscience in the last decade is its multiple applications in industry, agriculture, medicine, public health and business.

However, owing to various concerns such as being expensive, labour intensive, and toxic to the environment, there is an obvious requirement for cost-effective and environmentally benign methods for synthesis. Multiple studies have already demonstrated that NPs interactions with biological systems depend on size, shape, charge, and the constituent material of the nanomaterial. Thus, the

focus has now shifted to the biosynthesis of NPs [3]. With the growing need of environmentally friendly nanoparticles, researchers are using the green method for the synthesis of various metal nanoparticles for pharmaceutical applications [4]. This method has several advantages, namely low cost, simple, use of less toxic materials, most important is eco-friendly [5]. ZnO is an inorganic, nontoxic molecule having high antimicrobial and ultraviolet resistant features and thus used in industrialized, food wrapping, biomedical applications [6]. Nanomaterials have the potentiality of execution to conflict pathogenic bacteria, biofilm outline, and resistant infectious diseases [7]. Several reports revealed that ZnO NPs in the biomedical field like nano-diagnostics, as targeted drug delivery, gene delivery, and a vast number of pharmacological efficacy like anticancer, antidiabetic and antioxidant [8].

Bunium persicum is a plant of the Apiaceae family called wild caraway. It is used as an anticonvulsant, anti-diabetic, anti-asthma, antispasmodic, anti-epileptic, anti-obstruction, diuretic, and flow increaser of breast milk. Phytochemical profile of *B. persicum* (Boiss.) The plant bears flavonoids, phenolic acids, and aldehydes and a high content of mono-terpenes and sesquiterpenes contained in the essential oil present in plant [9-10]. It also exhibits good anti-inflammatory and anti-oxidative potential [11]. The primary phytochemicals investigation revealed the presence of triterpenes, glycosides, flavonoids, alkaloids, sterols, and tannins [12].

Recently, the existence and banquet of antimicrobial resistance is a serious issue in both developing and developed nations, leading to global crisis [13]. A stratagem for the repression of resistance needs to be innovated, executed, and evaluated, which should be focused on improving the rational use of antimicrobials and reducing prospects for the spread of resistant organisms [14]. It has been reported [15] that the metallic nanoparticles are meticulously being explored and broadly explored as potential antimicrobials. The antimicrobial potency of the nanoparticles is known to be a function of the surface area in communication with the microorganisms. Nanomaterials have shown broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, mycobacteria, and fungi [16]. Therefore, the search for new antimicrobial drugs from nanoparticles derived from natural sources has increased as a substitute for commercial drugs [17]. Antioxidants have the capacity which can carefully assemble with free radicals and inhibit the chain reaction prior to important compounds are destroyed. They act as scavengers by resisting and mending the damages initiated by free radicals, and so elevates the immune defense and reduces the prone of cancer and degenerative diseases. They are produced by the environment in situ or outwardly supplied by the mode of foods and supplements. They are released as a response to the oxidative process, and nanoparticles may interact with metal-sequestering proteins and antioxidants (from body fluids and intracellularly), which will probably induce variation in the surface features of the nanoparticle thus making them with reduced toxicity [18].

Arterial thrombosis persuaded by the accumulation of platelet are accountable for life-threatening disorders like uneven angina and reocclusion after angioplasty. So deferment of platelet accumulation is vital physiologically for anticipation and cure of cardiovascular diseases. During the initial stage of thrombosis, damage in blood vessels causes the production of adhesive proteins (such as collagen and von Willebrand factor) and soluble agonists (such as ADP and thrombin) at the injury site; which further leads to platelet adhesion, activation, and aggregation, therefore leads to the formation of a platelet-rich thrombus [19].

It has been reported that the synthesis of nanoparticles from biological sources is of keen interest due to synergistic properties valuated by such nanoparticles. Heparin (HP), when accumulated with nanomaterials, has been getting a lot of attention for its chemical and biological features. It has a number of therapeutic roles which can be increased when composited with nanoparticles and has been suggested in various biological applications.

MATERIALS AND METHODS: The chemicals used for the synthesis of Zinc nanoparticles were purchased from Hi-media, Jaipur, India. Doubly distilled water was used for the preparation of aqueous extracts. *In-vitro* assay reagents for antimicrobial, antioxidant and antiplatelet activity were purchased from Sigma Aldrich, India.

Preparation of Zinc Nanoparticles: The synthesis of nanoparticles *via* green route particles was synthesized biologically by an aqueous extract of *Banium persicum*. Initially, leaves were washed properly with distilled water. 0.44 M of Zinc sulphate was taken in 175 ml autoclaved distilled water in a conical flask and kept on a magnetic stirrer. 50 gm leaves were weighed and grinded properly in the mortar and pestle for 15 to 20 minutes in distilled water. These grinded leaves were centrifuged at 4 ºC, 10000 rpm for 5 min.

After the centrifugation, the supernatant was collected and added to the flask containing the solution of Zinc sulphate. It was kept overnight and the change in the color of the solution from light brown to dark brown was observed which indicates the reduction and synthesis of Zinc nanoparticles. These synthesized nanoparticles were carried out for characterization by UV, SEM, TEM, FT-IR and XRD and further tested for their biological assays.

Pharmacological Studies:

Anti-microbial Activity: Anti-bacterial activity of the synthesized nanoparticles was investigated by agar well diffusion method [20]

Activity index = Zone of inhibition of sample / Zone of inhibition of standard

Antiplatelet Activity: Blood samples were collected from KCJ Diagnostic center, near SMS Medical College, Jaipur, and subjected to centri-fugation. Centrifugation at 10000 rpm for 5.5 min, 0.2 ml platelet-rich plasma was separated from the sample, dissolved in isotonic $CaCl_2$.

Different hemostatic constraints *viz.* Prothrombin time (PT) and Activated partial thromboplastin time (APTT) were measured by using established protocol [21].

Antioxidant Activity: Various antioxidant assays like LPO, [22] Catalase [23], and Peroxidase [24] were carried out using established protocols.

RESULTS:

Synthesis and Characterization of ZnNPs: ZNPs are versatile semiconductors that display significant optical transparency and luminescent properties in UV-Visible (UV-Vis) regions [25]. Preliminary confirmation of nanoparticles synthesis was indicated by the visual change in the colour of the colloidal solution from brownish green to white. This change is caused due to Leaf extract served as a reducing agent [26].

FIG. 1: CHANGE IN COLOR INDICATES FORMATION OF NANOPARTICLES

UV–Visible Spectroscopy: UV–Visible absorption spectrum of synthesized nanoparticles is shown in **Fig. 1**. The distinct peak centered around 250 nm is specific for ZNPs which is due to their large excitation binding energy at room temperature. It is well known from absorption spectroscopy that the bandgap increases on decreasing particle size. There is also an opposite ratio between the band gap and the wavelength of absorption. As we know, the absorption for bulk Zn occurs around 385 nm. The high blue shift absorption for the synthesized ZNPs in comparison with the bulk ZnO can be due to a high decrease in particle size **Fig. 2**.

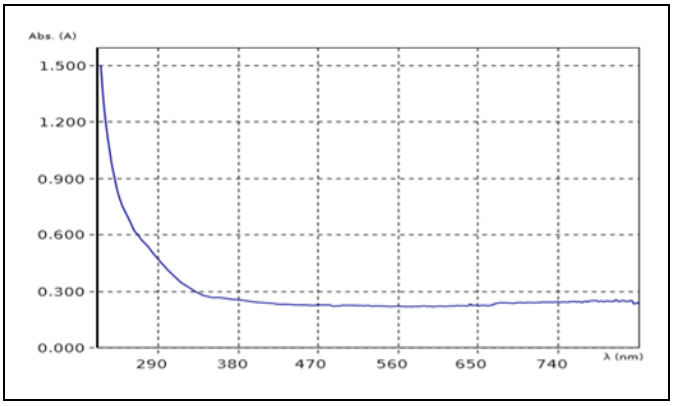

FIG. 2: SHOWING UV-SPECTROSCOPY OF $ZnNP_S$

FT-IR: FTIR spectroscopy was employed to determine the possible biomolecules in *Banium persicum* extract involved in reduction, capping, and efficient stabilization of synthesized ZnNPs. The absorption bands at 3600, 3500, 3400, 2900, 2800, 2100, 1700, 1600, 1500, 1300, 1100, 1000, 700, 600 and 500 cm^{-1} were observed. The strong peaks at 3600 cm^{-1} correspond to – O-H stretching confirms the presence of the OH group.

The band at 3500 cm^{-1} was attributed to primary amine N–H stretching vibration. The peak at 3400 cm^{-1} corresponds to N-H stretching vibration of primary amine 2900 cm^{-1} due to N-H stretching.

Furthermore, peaks assigned at 2800 C-H stretching Alcohol and 2100 cm^{-1} were due to C ≡C stretch having an Alkyne bond. Other intense bands at 1600 C=C stretching Alkyne, 1500 N-C stretching Nitro compound, and 1300 cm^{-1} were characteristic of phenyl C-O. Peaks at 1100 and 1000 cm^{-1} Alkoxy C-O were observed. The peak at 700 cm^{-1} reveals the alkyne C=C bond. The peak at 600 cm^{-1} C-I stretching Halo compound. Finally, the peak at 500 cm^{-1} due to C-I stretching Halo compound. These functional groups are found to be synergistically important in the reduction of NPs.

On the basis of the FTIR analysis, it is clear that the functional groups such as –OH (hydroxyl) –C=O (carbonyl) and C–N (amine) present in the leaves of *Banium persicum* were involved in the synthesis of ZnNPs, confirming the bio-reduction of Zn^{2+} ions to Zn.

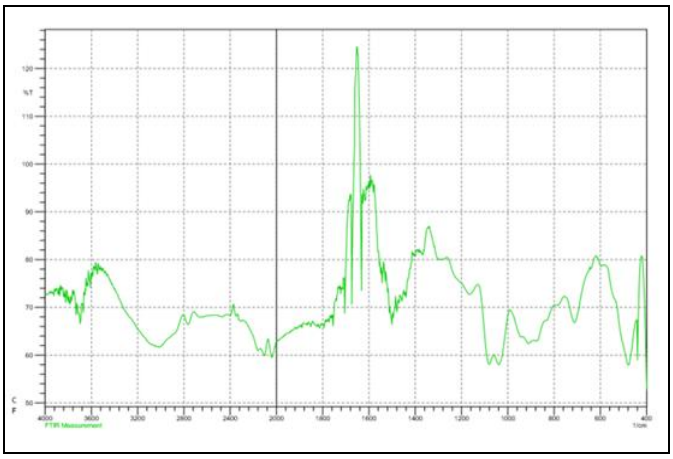

FIG. 3: SHOWING FT-IR OF ZnNPs

SEM and TEM: SEM analysis was carried out to determine the surface morphology and the topography of the prepared ZnNPs. SEM images revealed that the biosynthesized ZnNPs were mostly spherical in shape, as shown.

Direct imaging of ZnNPs was done to obtain quantitative measures of their size and morphology by TEM as shown, which significantly confirmed spherical shape and well-dispersed condition of ZnNPs. The size of ZnNPs varied between 80 and 390 nm. Smaller sized (80–90 nm) ZnNPs were obtained in **Fig. 4** and **5**.

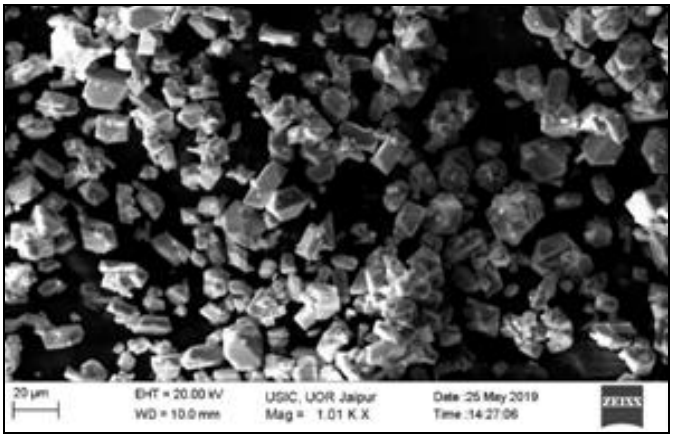

FIG. 4: SEM OF ZnNPs

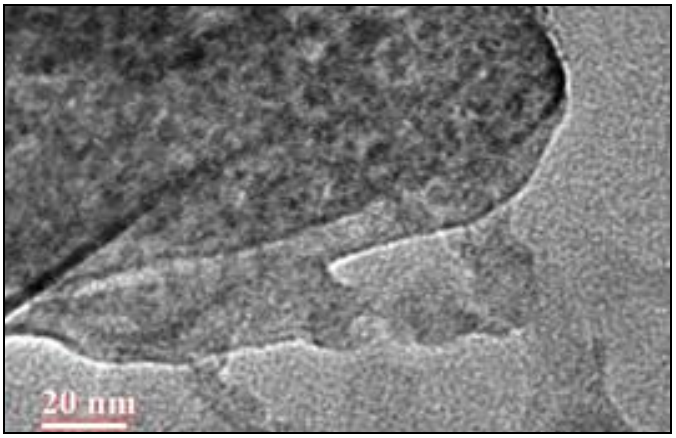

FIG. 5: TEM OF ZnNPs

DLS: DLS was used for measurement of average particle size, polydispersity index (PDI), and zeta potential of nanoparticles on high-performance particle zetasizer (HPPS-5001, Malvern, UK). The results are given as the average particle size obtained from the analysis of each of them measured three times **Fig. 6**.

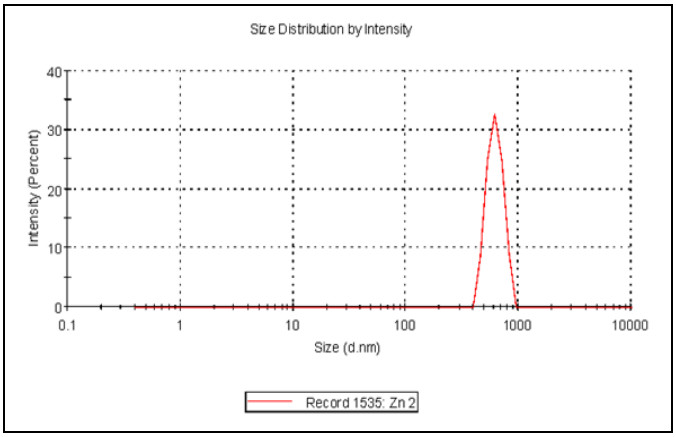

FIG. 6: DLS OF ZnNPs

XRD: X-ray diffraction (XRD) studies were carried out to confirm the synthesis of ZnNPs and characterize crystallinity and the phase pattern of

the ZnNPs. It was observed that 2Θ (in degrees) were in free of impurities as no other characteristics XRD peaks were observed. The mean grain crystalline size of green synthesized ZnNPs was calculated using the Debye–Scherrer formula D= Kλ βcosΘ where D is the average crystalline diameter size (Å), K is a constant (0.9), ƛ is the wavelength of the X-ray used (k = 1.54 Å), 'β' is the angular line width at the half maximum of diffraction (radians) and 'Θ' is the Bragg's angle (degrees).

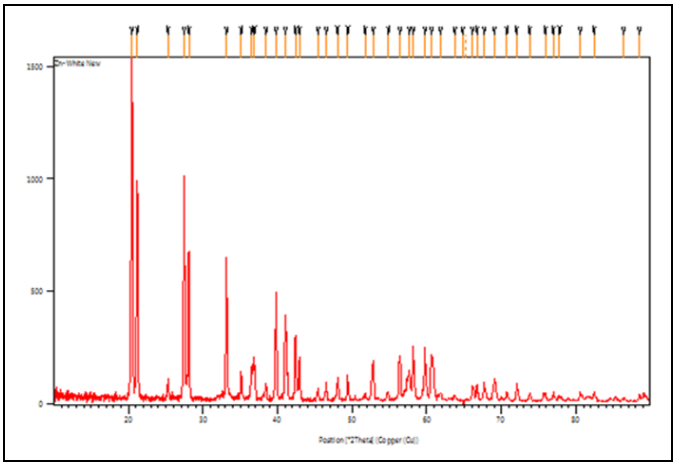

FIG. 7: XRD PATTERN OF ZnNPs

Pharmacological Activities:

Anti-microbial Activity: The anti-microbial activity was done using agar well diffusion method against clinically important microbes. Coating of NPs on disc might cause ambiguity, hence to assure uniform distribution of NPs, well diffusion method was preferred over. In the present investigation, it was observed that the ZnNPs exhibit potent anti-bacterial activity against *E. coli* at a concentration of 80 µg/ml the zone of inhibition was found to be 16 mm, further increasing dose level at 100 µg/ml the one was found to be 12 mm and minimum at 60 µg/ml. It was observed that against *S. aureus* maximum activity was observed at 120 µg/ml bearing zone of IZ-18 mm and minimum zone was observed (IZ-10 mm) at concentration 80 µg/ml. Against *B. subtilis* dose level of ZnNPs at 120 µg/ml had zone of IZ-14 mm which reduced with decrease in concentration and minimum at a concentration of 60 µg/ml (IZ-10 mm). In the case of fungal strains, maximum activity was observed against *C. albigans* at 120 µg/ml (IZ-30 mm) and minimum at 60 µg/ml (IZ-20 mm).

When it was studied against *T. reesei* maximum zone was observed at 80 µg/ml (20 mm) while minimum against at concentration 60 µg/ml (IZ-10 mm).

The rest of the strains were found to be partially resistant. High inhibitory activities of ZnNPs at high concentrations may be due to the interaction with the cytoplasmic components. Thus from the above research, it can be recommended that ZnNPs are potent anti-microbial agents **Table 1** and **2**.

TABLE 1: ANTI-BACTERIAL ACTIVITY OF ZnNPs

Concentration (in µg/ml)	*E. coli*	*S. aureus*	*B. subtilis*
60	IZ-10AI-0.55	IZ-12 AI=0.54	IZ-10 AI-0.5
80	IZ-16AI-0.88	IZ-10 AI-0.45	IZ-10 AI-0.5
100	IZ-12AI-0.66	IZ-14 AI-0.63	IZ-12 AI-0.6
120	IZ -10AI-0.55	IZ-18 AI-0.81	IZ-14 AI-0.7

Zone size is measured in mm, IZ – inhibition zone, and AI = zone of sample/zone of standard. Standard solution – ciprofloxacin. Standard solution -18 for *E. coli*, 22 for *S. aureus* and 20 for *Bacilus subtilis*.

TABLE 2: ANTIFUNGAL ACTIVITY OF ZnNPs

Concentration (in µg/ml)	*C. albicans*	*T. reesei*	*A. niger*	*F. oxysporum*
60	IZ-20 AI-0.5	Iz-10 AI-0.27	NIL	NIL
80	IZ-26 AI-0.65	IZ-20 AI-0.55	NIL	NIL
100	IZ-22 AI-0.55	IZ-18 AI-0.45	NIL	NIL
120	IZ-30 AI-0.75	IZ-16 AI-0.44	NIL	NIL

Zone size is measured in mm, IZ-Inhibition zone and AI-Zone of sample /Zone of standard. Standard solution-Ketokenazole Zone of inhibition of standard -40 mm for *C. albicans*, 36 mm for *T. reesei*, 32 for *A. niger* and 28 for *F. oxysporum*

Antiplatelet Activity: In thrombin time, all the concentrations of nanoparticles prolonged the clotting time as compared to control. Significant activity was observed at 6µgmL^{-1} (8.53 times of control and 3.31 times as compared to standard), which was maximum and increased in linear fashion **Table 3**.

TABLE 3: CLOTTING TIME OF ZnNPs AT DIFFERENT CONCENTRATIONS ASSAYED BY PT

Concentration, µg/ml	Clotting time (Sec.)	Standard	Control
1	55	3.66	1.48
2	85	5.66	2.29
3	39	2.6	1.05
4	120	8	3.24
5	25	1.66	0.67
6	128	8.53	3.31
7	54	3.6	1.45
8	50	3.33	1.35
9	104	6.93	2.81
10	56	3.73	1.51

Control – 37 s. Standard PT (plasma + PT reagent) – 15 s. * Denotes potency of the sample at different concentrations when compared with standard and control

TABLE 4: CLOTTING TIME OF ZnNPs AT DIFFERENT CONCENTRATIONS ASSAYED BY APTT

Concentration (in µg/ml)	Clotting time (Sec.)	Standard	Control
1	478	11.95	10.17
2	263	6.57	5.59
3	322	8.05	6.85
4	102	2.55	2.17
5	162	4.05	3.44
6	367	9.17	7.80
7	106	2.65	2.25
8	247	6.17	5.25
9	175	4.37	3.72
10	234	5.85	4.97

Control – 47 s. Standard PT (plasma + PT reagent) – 40 s. * Denotes Potency of the sample at different concentrations when compared with standard and control.

In this assay significant activity was observed at 1 μgmL^{-1} (10.17 times of control and 11.95 times as compared to standard, which increased slowly and was maximum **Table 4** with reduced in dose level.

TABLE 5: ANTIOXIDANT ACTIVITY OF ZN NANOPARTICLES (IN µm/l/g)

Catalase	Peroxidase	Lipid peroxidation assay
0.24	0.0392	4.65

In the present investigation, it was observed that synthesized nanoparticles possessed potent antioxidant activities when assayed by various antioxidant assays. In catalase and peroxidase, it inhibited the formation of oxygen free radicals, while in LPO they inhibited the further reduction of malondialdehyde to form reactive oxygen species **Table 5**.

DISCUSSION: Nanosized particles, of either simple or composite nature, possess unique physical and chemical features and represent a demanding material in the innovations of novel nano-devices that can be used in numerous physical, biological, biomedical, and pharmaceutical applications [27]. Physical methods for the synthesis of nanoparticles require high energy consumption. The chemical method usually leads to remaining toxic reactions and non-use of generated particles in biological applications. So attention has been focused by many researchers on the synthesis of nanoparticles as therapeutic drugs.

The metal-based nanoparticles possess potent antimicrobial activity [28], and keen researchers are engaged in innovating various nanoparticles as anti-bacterial agents. These nanoparticles have unique benefit over traditional chemical antibiotics. Generally, the antimicrobial efficiency of drugs depends on the particular binding with the surface and the metabolism of agents into the micro-organism. One of the most important challenge in innovation of such drugs is that microorganisms have evolved drug resistance for many generations. So till date, these antimicrobial drugs have been effective for therapy; but having various side effects. Therefore, an alternative way to overcome the drug resistance of various micro-organisms is needed now desperately. Thus in the present research, potent, antimicrobial activity of synthesized tungsten nanoparticles was observed.

Adenosine diphosphate is the main cause of platelet aggregation. The platelets are unexposed to ADP escape from such kind of mechanism. The ADP-activated platelets without nanoparticle treatment reduce the clotting time in terms of platelet aggregation. The reduction in the aggregation of found to be higher in nanoparticles treated samples than the non-treated platelets. NPs possess the potential to cross epithelial obstacles, reach the universal circulation and restrict with physiological platelet function elevating the jeopardy of cardiovascular disorders and vascular thrombosis [29]. Although some nanoparticles have been designed for therapeutic purposes, which has focused on the injured vascular site imitating platelet role or to increase blood clotting the potential not desired, pro-and/or anti-aggregating, properties of NPs are of the prime issue in the field of nanomedicine and may hinder the development of auspicious engineered NPs to the clinical trials.

In the present investigation, significant activity was observed at 1 μgmL^{-1} (10.17 times of control and 11.95 times as compared to standard, which increased slowly and was maximum with reduced in dose level when assayed by APTT while Significant activity was observed at 6 μgmL^{-1} (8.53 times of control and 3.31 times as compared to standard), which was maximum and increased in a linear fashion.

Nanomaterials denotes one of the most auspicious leading edge in the key area for efficient anti-oxidant agents. Few nanomaterials, including melanin, lignin, *i.e.* gold, platinum based nanoparticles, denotes intrinsic redox potential that is often connected with radical deceiving with superoxide dismutase-like and catalase-like activities. Redox sedentary nanomaterials can be distorted into antioxidants by splicing low molecular weight antioxidants on them [30]. In the present investigation in all antioxidant assays, nanoparticles inhibited the formation of free radicals, thus avoiding metabolic disorders.

CONCLUSION: Nanotechnology is very rapidly emerging in biological sciences as novel techniques are being developed to probe and manipulate the effect of single atoms and molecules against a wide range of microbes. The present research works a systematic and scientific approach to develop and investigate the nanoparticles and their biological activities against a range of routes.

There is a great scope of the study of nanoparticles and antifungal agents though having limited effectiveness. Our tactic is to assess the anti-microbial activity of Zinc nanoparticles to improve the effectiveness of the drug at low cost and its anticancer effect followed by its antiplatelet activity. Hence we can also predict that Zinc nanoparticles have potent antimicrobial, antiplatelet, and antioxidant activity.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest.

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