Research Article

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Synthesis of ZnO and ZnO/PVA nanocomposite using aqueous *Moringa Oleifera* leaf extract template: antibacterial and electrochemical activities

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Abstract: The application of flexible polymer nanocomposites for food packaging to inactivate microorganisms associated with foods is the demand of the present-day food industry to assure quality throughout the packaging operation. The utilization of polyvinyl alcohol (PVA) assisted zinc oxide nanocomposite for food stuff packaging has been very attractive in the recent past. Nanostructured ZnO was synthesized at optimized pH (10.5) from different ratios of zinc acetate and *Moringa oleifera* leaf extract (1:7, 1:3, 1:1 and 3:1). ZnO coated polyvinyl alcohol (ZnO/PVA) nanocomposites were prepared from 5, 9, 13 and 16% by wt of ZnO and PVA using solution casting method. The thermal stability of ZnO synthesized with 1:1 ratio at pH 10.5 was investigated with TGA/DTA. The analytical techniques such as X-ray diffraction (XRD), ultra-violet visible analysis (UV-Vis), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscope (SEM) were used for the characterization of the synthesized ZnO and ZnO/PVA nanocomposites (NCs). The antibacterial activity of the synthesized ZnO and ZnO/PVA NCs were evaluated against gram negative *E. coli* and gram positive *S. aureus* bacteria. The electrochemical stability of ZnO/PVA NCs was also investigated by cyclic voltammetric (CV) method.

The thermogram of ZnO indicated that the oxide was found to be stable even beyond 500°C. The SEM analysis revealed rod shaped morphology for synthesized ZnO from 1:1 ratio at pH 10.5. But the nanocomposite prepared with 5% of ZnO of (1:1) at the same pH exhibited uniformly dispersed rod-shaped particle on the surface as well as in matrix of polyvinyl alcohol film. According to XRD result, ZnO synthesized with more percentage of plant extract resulted in the small size crystallites while that with low percentage of plant extract resulted in the larger crystallite size. The antibacterial inhibition efficiency of ZnO/PVA NCs was better and found to increase with increase in the amount of ZnO.

Keywords: Nanocomposite, Green synthesis, *Moringa oleifera*, antibacterial activity, morphology

1 Introduction

The spoilage and deterioration of food are mainly caused by food borne pathogens and other microorganisms, such as *Escherichia coli*, *Listeria monocytogenes*, etc., which mainly grows on food surfaces [1, 2]. Active packaging technologies are being developed as a result of increased consumer demand for processed foods. Active packaging provides interaction between food and packaging materials to maintain a microenvironment and extends the shelf life of food products [3, 4]. Active packaging has been mostly used to absorb oxygen, carbon dioxide, moisture, ethylene and release some substances such as antimicrobial agent and antioxidants [5]. Some of the most promising active packaging materials are antimicrobial films, which can be prepared by incorporating synthetic or natural antimicrobial agents into films [6]. Polymeric food packaging films are the most commonly used films with excellent performance [7]. Polymeric food package can protect food from microbial attack and has properties such as flexibility, strength, stiffness and a barrier to oxygen and moisture.

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The antimicrobial agent is mixed with the polymer and the active compound is distributed in the polymer matrix, homogeneously when polymer and agent are chemically compatible, or heterogeneously, forming a multiphase matrix in which sites enriched with the agent can be distinguished [8]. Recently, nanoparticles have been used to enhance mechanical performance of food packaging, such as flexibility, stability against humidity and temperature, reducing gaseous permeation, and ultraviolet light resistance [9]. In addition, they have antimicrobial activity as growth inhibitors, killing agents and antimicrobial carriers [10].

Metal oxide nanoparticles (MONPs) are more stable and have potent antimicrobial effect against microorganisms. MONPs such as titanium dioxide (TiO_2), zinc oxide (ZnO), copper oxides ($\text{CuO}/\text{Cu}_2\text{O}$), silica (SiO_2), aluminum oxide (Al_2O_3), magnesium oxide (MgO), and iron oxides (Fe_3O_4 and Fe_2O_3) have been commonly used for various applications. Among them, TiO_2 , ZnO and MgO have received special attention in the food packaging industry due to their strong antimicrobial effects. ZnO NP-loaded starch/polythene films tested for their ability to control pathogenic *E. coli* [11]. It was found that the film could prevent the bacterial contamination in food stuff by inhibition of *E. coli*. This possible action could be achieved by the production of reactive oxygen species, such as hydroxyl and superoxide radicals. ZnO -deposited polypropylene packaging film was prepared by using dielectric barrier discharge plasma treatment and exhibited better antimicrobial effect against pathogens *S. aureus* and *E. coli* [12].

Polyvinyl alcohol (PVA), a degradable polymer, easily dissolved in water, and a combination of ZnO nanoparticles and PVA results in nanocomposite with improved electrical, mechanical and optical properties. This non poisoning biodegradable nanocomposite can be used as a more effective and environmentally friendly material for food-stuff packaging [13]. In the present work, we report the synthesis of ZnO and polyvinyl alcohol (ZnO/PVA) nanocomposites (NCs) using *Moringa-oleifera* leaf extract and evaluation of their electrochemical and antimicrobial activities.

2 Materials and methods

2.1 Chemicals and Reagents

Polyvinyl alcohol (PVA) (Sigma Aldrich), with an average molecular weight of 17,000 GRG and 97% hy-

drolyzed was used without further purification. Zinc acetate ($\text{C}_4\text{H}_6\text{O}_4\text{Zn}\cdot 2\text{H}_2\text{O}$), NaOH , Mueller-Hinton agar and DMSO were purchased from Sigma Aldrich. Graphite powder and silicon oil were purchased from the local market. Reactant solutions were prepared by using doubly distilled water. Analytical grade chemicals reagents and solvents were used without further purification.

2.2 Collection of *Moringa Oleifera* Leaf

Healthy and Fresh *Moringa oleifera* leaves were collected from Dinsho Wereda, Bale Zone, Oromia Regional State, Ethiopia, after conducting the field survey. Dinsho (also called Gurie) is a village in south-central Ethiopia, located in the heart of the Bale Mountains with a latitude and longitude of $705^\circ\text{N}3945^\circ\text{E}$ and an elevation of 3207 meters.

2.3 Methods

2.3.1 Preparation of *Moringa-oleifera* leaf extracts (Broth solution)

The collected *Moringa-oleifera* leaves were washed thoroughly with distilled water to remove dust and other foreign particles and dried under shadow at room temperature. The dried leaves were ground to get fine powder. The extraction was carried out by taking 50 g of this powder in 300 mL deionized water ($\text{DI-H}_2\text{O}$). The obtained colloidal suspension was boiled at 50°C for about 1 hour with constant stirring using magnetic stirrer. The solution was allowed to cool at room temperature and filtered through Whatman number 1 filter paper. The filtered solution was stored in the refrigerator at 4°C for the synthesis of ZnO/PVA NCs. Broth solutions prepared from *moringa oleifera* aqueous leaves extract are as shown in Figure 1.

Figure 1: *Moringa oleifera* leaves broth solution prepared for this work.

2.3.2 Biosynthesis of ZnO NPs using *Moringa Oleifera* leaf extract

To synthesize ZnO/PVA NCs, four different volume ratios of 0.122 M $C_4H_6O_4Zn \cdot 2H_2O$ and broth solution of *Moringa oleifera* leaf extract were taken in the 250 mL volumetric flask as per the following procedure; 12.5 mL of 0.122 M $C_4H_6O_4Zn \cdot 2H_2O$ with 87.5 mL leaf extract (1:7), 25 mL of 0.122 M $C_4H_6O_4Zn \cdot 2H_2O$ with 75 mL of leaf extract (1:3), 50 mL of 0.122 M $C_4H_6O_4Zn \cdot 2H_2O$ with 50 mL of leaf extract (1:1) and 75 mL of 0.122 M $C_4H_6O_4Zn \cdot 2H_2O$ with 25 mL of leaf extract (3:1) were mixed in different 250 mL conical flasks.

In all of these cases, the solutions were stirred using a magnetic stirrer for about two and a half hours, by controlling pH with dropwise addition of 1M NaOH solution to pH = 9, 10.5 and 12 for all the mentioned ratios. The solution was centrifuged at 12000 rpm for 15 min three times fol-

lowed by repeated washing with ethanol and distilled water to remove the impurities. The obtained precipitate was collected using a ceramic crucible dish and allowed to dry in an oven at 100°C for about one hour. Finally, the dried precipitate was ground into a fine powder. The thermal stability of the synthesized sample was checked using a simultaneous DTA/TGA instrument. Based on the thermal analysis result, the obtained ZnO powder was subjected to an additional heat treatment in a furnace at 500°C for 2 h. The overall schematic of the procedure for the synthesis of ZnO nanoparticles is shown in Figure 2.

2.3.3 Preparation of ZnO/PVA nanocomposite films

The ZnO/PVA NC films were prepared by solution casting technique. ZnO synthesized with (1:1) ratio at pH = 10.5 was added in different weight percent ratios by (wt.%; 0 (free

Figure 2: Schematic diagram of green synthesis of ZnO nanoparticles.

Figure 3: Schematic of synthesis of ZnO/PVA NC.

Table 1: Ratio of precursor to plant extract used to prepare ZnO NPs

No	Volume of 0.122 M $C_4H_6O_4Zn \cdot 2H_2O$	Volume of leaf extract	Precursor to extract ratio	pH	Color of ZnO	crystallite size
a	12.5 mL	87.5 mL	1:7	10.5	Brownish color	7.57 nm
b	25 mL	75 mL	1:3	10.5	Light Yellow color	9.89 nm
c	50 mL	50 mL	1:1	10.5	Yellow color	10.13 nm
d	75 mL	25 mL	3:1	10.5	Bright yellow color	15.27 nm

of ZnO NP), 5, 9, 13 and 16) to PVA according to the formula given below (1):

$$x(\text{wt}\%) = 100 \times \frac{wf}{(wf + wp)} \quad (1)$$

where wf and wp represent the weights of ZnO and PVA polymer, respectively.

The NC films were prepared by dispersing different amounts of ZnO nanoparticles in 2.0 g of PVA dissolved in 50 mL distilled water at 80°C under vigorous stirring for 1 h to prevent any agglomeration. Lastly, the aqueous solution of ZnO/PVA nanocomposite was cast into Petri dishes and allowed to dry in a dust free chamber at room temperature for 5 days to obtain the films. The obtained pure PVA and ZnO/PVA NC films were characterized. The overall schematic of the experiment for making ZnO/PVA NC film is shown in Figure 3.

2.4 Characterization

The XRD diffraction patterns of the pure PVA film, ZnO powder and the composite films (PVA/ZnO) were recorded at room temperature using an X-ray powder diffractometer (XRD-700, *shimadzu co.* South Korea) equipped with Cu $K\alpha$ as radiation source ($\lambda = 1.54 \text{ \AA}$) in the 2θ (Bragg angles) range ($10^\circ \leq 2\theta \leq 80^\circ$) to report the information about their structure. The Fourier transform infrared spectra of the nano powder and the composites were analyzed in the range $400\text{--}4000 \text{ cm}^{-1}$ using the instrument (Perkin Elmer Spectrum 65 Spectrum BX FTIR-Model 6100, Japan) in the absorbance mode at a resolution of 4.0 cm^{-1} . Thermal stability of the ZnO was conducted with TGA/DTA (DTG60H *shimadzu co.* South Korea). The absorbance spectra (A) and the transmittance spectra (T) of the films were recorded at 200–1100 nm wavelength using a dual beam UV-Visible spectrophotometer (Perkin Elmer, D-7770 Ueberlingen, and Germany) and the morphological and compositional analysis of all the sample were done using SEM-EDX machine (JEOL-JSM-8040, 81F, Tokyo, Japan). The CV measurements were carried out using an electrochemical analyzer CHI608E Potentiostat in a three electrode system,

consisting of carbon paste electrode, platinum wire and Ag/AgCl as working, counter and reference electrodes, respectively and the electrolyte being a solution of 1.0 M KOH.

3 Result and Discussion

The colors of the biogenic ZnO nanoparticles obtained with different ratios of zinc acetate and *Moringa oleifera* leaf extract are presented in Table 1. The different colors obtained for various ZnO NPs are believed to be due to the variation in the particle sizes. In this regard the ratios of leaf extract played crucial role in capping the ZnO NPs.

3.1 Thermal analysis of synthesized Zinc oxide sample

Figure 4 depicts the TGA-DTA curves for the thermal transformation of ZnO NPs. TGA showed a weight loss in two steps at 100 and 500°C, while DTA showed one exothermic peak and one endothermic peak at 72.3°C and 394°C respectively. The endothermic peak at 72.3°C was due to

Figure 4: Thermal Analysis result of as synthesized ZnONPs.

removal of water and the peak at 394°C was due to decomposition of zinc hydroxide to form ZnO and (or) organic binders from leaf extracts. No considerable loss is observed after 500°C, indicating that the oxide is thermally stable above 500°C.

3.2 XRD Analysis

The XRD diffraction patterns presented in Figure 5 and Figure 6 are recorded within 10° to 80° range. The diffraction peaks observed at 2θ values of 32.01, 34.54, 36.33, 47.67, 56.73, 62.96, 67.04, 68.07, 69.34 and 72.91° corresponds to (100), (002), (101), (102), (110), (103), (200), (112), and (201) planes respectively for ZnO NPs synthesized from different ratios of salt and leaf extract [14–17].

It is found to be in good agreement with the reported values of the Joint Committee Powder Diffraction Standards data (JCPDS # 36-1451). No additional peaks were found indicating single phase of ZnO NPs was synthesized.

Figure 5: XRD diffraction patterns of synthesized ZnO NPs.

The average particle size of the prepared ZnO nanoparticles was evaluated by using Debye-Scherrer formula [18]. The measured values varied between 7.57 nm and 15.27 nm for samples from a to d as given in Table 1. ZnO NPs synthesised with 3:1 ratio exhibited the most intense peaks with crystallite size of 15.27 nm whereas that with 1:7 ratio resulted in smaller particle size of 7.57 nm. This is possibly due to higher concentration of capping agent in 1:7 ratio of precursor to plant extract.

The X-ray diffraction pattern (XRD) for pure PVA film exhibits characteristic peak ~ 20° which is related to the semi-crystalline nature of PVA membrane [19].

Figure 6: XRD pattern of pure PVA nano film.

The XRD diffraction patterns for ZnO/PVA composite films are shown in Figure 7(b-e). XRD patterns of the composite films showed a broad peak at $2\theta = 20.11^\circ$, which is attributed to the semi-crystalline nature of polyvinyl alcohol (PVA) as well as ZnO doped PVA composite films. Diffraction peaks observed at $2\theta = 32.01, 34.54, 36.33, 47.67, 56.73, 62.96$ and 67.04° , indexed with (100), (002), (101), (102), (110), (103), and (112) confirmed the presence of ZnO NPs.

Figure 7: XRD diffraction patterns of ZnO/PVA NCs.

As it described in the Figure 7, crystalline nature increases, as the dispersions of ZnO particles increase in the polymer. The XRD results indicated that the metal oxide polymer composite film consists of two phases; PVA and ZnO and no other additional phase was detected.

Table 2: XRD data of synthesized ZnO NPs with different ratios of precursors.

No	1:7 ratio		1:3		1:1		3:1	
	2-Theta (deg)	FWHM (deg)	2-Theta (deg)	FWHM (deg)	2-Theta (deg)	FWHM (deg)	2-Theta (deg)	FWHM (deg)
1	36.0487	1.05000	36.2443	0.77500	36.2316	0.79430	36.2839	0.54180
2	31.6889	1.00870	31.7752	0.79950	31.7637	0.75160	31.8129	0.53580
3	34.3985	1.04000	34.5404	0.79570	34.4814	0.77050	34.4747	0.46550
4	20.2670	0.59000	47.6161	1.04000	37.3359	0.47000	37.2759	0.38660
5	23.6370	1.12670	56.5385	0.87400	47.5769	1.10500	47.5933	0.75650
6	26.2454	1.19330	61.9437	0.68000	56.5705	0.94340	56.6308	0.60860
7	28.1159	0.42000	62.9774	1.05200	57.6252	0.58000	62.8946	0.73750
8	29.2843	0.76000	66.3223	0.82000	61.8237	0.76000	63.6232	0.41000
9	37.6756	0.00000	68.2468	1.61000	62.9092	1.15830	66.4223	0.79000

Table 3: XRD data for PVA and ZnO/PVA NCs

PVA		5%ZnO/PVA		9%ZnO/PVA		13%ZnO/PVA		16%ZnO/PVA	
2 θ (deg)	FWHM (deg)	2 θ (deg)	FWHM (deg)	2 θ (deg)	FWHM (deg)	2 θ (deg)	FWHM (deg)	2 θ (deg)	FWHM (deg)
43.9615	0.1967	20.009	2.53330	19.186	2.5433	20.35	2.4700	36.16	0.88490
64.3065	0.2218	21.738	0.00000	17.668	0.0000	36.88	0.7527	31.69	0.91370
77.4059	0.2655	36.461	0.78380	21.299	0.0000	22.76	0.0000	34.39	0.84060
77.7591	0.1200	23.094	0.00000	22.336	0.0000	23.77	2.0134	47.52	1.09330
37.7388	0.1984	23.693	2.32000	23.354	1.6000	32.40	0.7350	56.52	0.96280
21.4388	0.5866	25.729	1.04000	24.212	0.9200	35.08	0.5967	61.78	0.52000
64.6028	0.0960	31.967	0.82800	25.250	0.6000	41.34	0.5400	62.85	1.03070

3.3 UV-Vis analysis of PVA and ZnO/ PVA composite films

Figure 8 shows the UV-visible absorption spectra of PVA and ZnO/ PVA composite films. The absorption spectrum of pure PVA has one broad absorbance band at 290 nm and one weak band or shoulder at 330 nm. The weak band or shoulder is assigned to the electronic transitions $n \rightarrow \pi^*$ [20]. As it is shown in Figure 8, free PVA has poor absorptive in the visible region (longer wavelength) and also has small absorbance in the UV region as compared with its composite with ZnO NPs. Absorbance of the composite films increases with increasing ZnO concentration from 5% to 16%. The emission peak of ZnO/PVA composite film has been shifted to the blue region (shorter wavelength). The absorbance spectra of the ZnO/PVA NC films consist of the fundamental peak of the PVA and further absorbance band at 375 nm, corresponding to ZnO NP; which is 5 nm blue shift in comparison with the ZnO bulk material (380 nm) at room temperature [21].

Figure 8: UV-Vis spectra of PVA and ZnO/PVA NCs.

3.3.1 Optical band gap

The optical transitions in composite films can be easily understood by determining the optical band gap by Tauc's plot [22]. The frequency-dependent absorption coefficient is given by $(\alpha h\nu)^n = h\nu - E_g$ where α is the absorption coefficient, which calculated using the Beer-Lambert's relation, $\alpha = 2.303/A$ and t are the absorbance and the thickness

of the film, $h\nu$ is the incident photon energy, E_g is the optical band gap energy and (n) is an index characterizing the nature of the electronic transitions causing the optical absorption and can take values $n = 0.5$ and 2.0 for direct and indirect transitions respectively. Figure 9 shows the relationship between absorption edges $(\alpha h\nu)^2$ for pure PVA and ZnO/PVA composites as a function of photon energy ($h\nu$). The energy gap decreases with increase in the concentration ZnO NPs. This is probably due to the creation of new levels in the band gap, that facilitate the crossing of electrons from the valence band to the conduction band, consequently the conductivity increases and the band gap decreases.

Figure 9: Band gap energy of casted PVA film and ZnO/PVA composite film

3.4 FTIR analysis

The FT-IR spectra of uncalcined and calcined ZnO powder, PVA film and ZnO/PVA composite are depicted in Figure 10. As it can be seen from the figures the wave number of the spectra adjusted from 4000 cm^{-1} up to 500 cm^{-1} . The absorption band at 3444 cm^{-1} attributed to the O-H bond stretching, while absorption band in the range, 2929 cm^{-1} to 2858 cm^{-1} is related to C-H stretching for uncalcined and calcined ZnO nanoparticle [23], and the absorption band in the range of 1631 cm^{-1} to 1406 cm^{-1} is due to the stretching C-O bond. The analysis showed that, ZnO NPs before calcinations exhibited a strong sharp band than calcined ZnO. Calcined ZnO NPs had lost some organic functional groups. The intense absorption peaks in the region $430\text{--}550\text{ cm}^{-1}$ correspond to the standard peak of ZnO due to the stretching frequency of Zn-O bonds which confirmed the presence of metal-oxide [24].

As it is shown in Figure 10b, the intense absorptions peaks in the region $1300\text{--}1800\text{ cm}^{-1}$ correspond to the standard peak of free PVA film. The composite film wave num-

ber 3000 cm^{-1} is due to the O-H group. The band at 2182 cm^{-1} is due to the C-C bond. Moreover, the band at about 1700 cm^{-1} and 500 cm^{-1} for PVA and ZnO composites is indicative of the Zn-O bond which verifies the presence of ZnO nanoparticle in the PVA matrix. The change of the band from 837 cm^{-1} to 500 cm^{-1} in composite might be related to the interactions between ZnO nano particles and PVA.

3.5 Scanning Electron Microscopy

The surface morphologies of synthesized ZnO at different pH using 1:1 ratio of salt and leaf extract, free casted PVA film, and ZnO/PVA composite were studied by scanning electron microscopy (SEM) and the results are shown in Figures 11-13. Figure 11 shows that, the morphology of ZnO NPs synthesized at pH 9 with 1:1 mixing ratio of zinc acetate and leaf extract has flower shapes.

Figure 12, shows the morphology of ZnO NPs that are synthesized at pH 10.5 using 1:1 mixing ratio of zinc acetate and leaf extract. The SEM micrograph revealed the formation of nano rods of zinc oxide and the micrographs also revealed that the nano rods bond to each other. This morphological change might be due to the change of template structures with pH change of the solutions during the synthesis.

Figure 13, shows, the morphology of ZnO nanomaterials synthesized at pH 12 using 1:1 mixing ratio of precursors. The micrographs show, the existence of nano sheet and agglomerated nano rod morphology of ZnO. The size and shape of ZnO synthesized using biological active templates and capping agents in the leaf extract of *Moringa Oleifera* was influenced with the variation of pH during the synthesis of the nano structured materials [25, 26].

The Figure 14(a) below shows the surface morphology of the pure casted PVA polymer film. No observed particles dispersed in the matrix of PVA. But, Figure 14(b) shows, SEM morphology of ZnO/PVA NC with 5% ZnO dopant prepared using 1:1 ratio at pH of 10.5. It confirmed the uniform distribution of rod shaped ZnO in the matrix of PVA. Figure 14(c), the SEM micrograph of 16% by wt ZnO, distributed in the PVA matrix. In the case of higher amount of ZnO dopant (16% loading) the zinc oxide distribution was not uniform as revealed by SEM image.

Figure 10: a) FTIR spectra of uncalcined and calcined ZnO NP, b) FTIR spectra of free PVA and 5% ZnO/PVA composite films.

Figure 11: SEM micrographs of ZnO NPs synthesized at pH 9.

Figure 12: SEM micrographs of ZnO NPs synthesized at pH 10.5.

Figure 13: SEM micrographs of ZnO NPs synthesized at pH 12.

Figure 14: (a-c): SEM images of a) pure casted PVA, b) 5% ZnO/PVA c) 16% ZnO/PVA NC films synthesized at pH 10.5

Figure 15: Cyclic voltammogram of 1:1 ZnO NPs.

3.6 Electro chemical analysis

Electrochemical analysis is one of the important features which determine potential of materials. Figures 15 and 16 showed CV plots of ZnO NPs and ZnO/PVA NCs.

The presence of redox peaks in their cathodic and anodic scan is an indication of significant share of pseudo capacitance in the electrochemical process. It was found

Figure 16: Cyclic voltammogram of 5%ZnO/PVA NC.

that the difference in the peak potentials $\Delta E_{a,c}$ is comparatively lower for 13% ZnO/PVA composite (Table 4).

Table 4: Electrochemical parameters of CV analysis of ZnO NPs and ZnO/PVA NCs.

sample	mV/s	I_a	I_c	E_{pa}	E_{pc}	$\Delta E_{a,c}$
1:1ZnO NP	50	0.0000163	0.0000276	0.363	-0.398	0.035mV
5%ZnO/PVA	20	0.00006913	0.00000886	0.54	-0.4	0.14
	50	0.0000525	0.0000188	0.465	-0.404	0.061
9%ZnO/PVA	50	0.0000534	0.0000313	0.3923	-0.25	0.1423
13%ZnO/PVA	40	0.0000962	0.0001098	0.034	-0.3007	0.2667

Where: I_a is the anodic current, I_c is the cathodic current, E_{pa} is anodic peak potential, E_{pc} is cathodic peak potential and $\Delta E_{a,c}$ is the difference in the anodic peak potential and cathodic peak potential. The difference in the anodic (E_{pa}) peak potential and cathodic (E_{pc}) peak potential, i.e. $\Delta E_{a,c}$, is a direct measure of the redox reaction reversibility.

Figure 17: Measured Inhibition zones of *S.aureus* and *E.coli* with free PVA, ZnO NPs and ZnO/PVA NCs.

3.7 Anti-bacterial applications of ZnO NPs and ZnO/PVA nanocomposite

The antimicrobial activities of free casted film of polyvinyl alcohol, calcined and uncalcined ZnO NPs, and ZnO/PVA NCs were evaluated, against *S. aureus* and *E.coli* by disc diffusion method to investigate the inhibition ability of the developed materials. The inhibition zones on Figure 17 represented with the letters: A, B, C and D represents ZnO synthesized with ratios (1:7, 1:3, 1:1 & 3:1) before calcinations, while, E, F, G, and H represents synthesized ZnO with the

ratios of (1:7, 1:3, 1:1 & 3:1) heated at 500°C and I represents free PVA film, J, K, L and M represents ZnO/PVA NCs prepared with 5, 9, 13 and 16% of ZnO diffused in the matrix of PVA.

Based on the obtained results the antibacterial activity of PVA is not much significant as compared with ZnO nanoparticle. The antibacterial activity of synthesized zinc oxide before and after heat treatment is almost the same. This might be due to the removal of capping materials through washing from the surface of the synthesized particles, the synergetic effect of leaf extract with the metal

Table 5: Anti-bacterial inhibition zones of the synthesized nano materials: ZnO NPs (5 mg/ml), ZnO/PVA NCs (5 mm dispersed with 5 mg/ml ZnO).

Type of nano material	Bacterial type									
	<i>E.Coli</i> (gram negative) Zone of inhibition (mm)					<i>S.aureus</i> (gram positive) Zone of inhibition(mm)				
	I	II	III	$\bar{x}$	$\Delta\bar{x}$	I	II	III	$\bar{x}$	$\Delta\bar{x}$
Un 1:7 ZnO NP (A)	13	12.5	13	12.8	0.1	14	13.3	13.9	13.7	0.03
Un 1:3 ZnO NP (B)	11	11	11	11	0	12.5	13	13.5	13	0.17
Un 1:1 ZnO NP(C)	11	10.5	10.5	10.7	0.03	12.5	13	13	12.8	0.03
Un 3:1 ZnO NP (D)	11	10	10	10.3	0.03	12	13.5	12.5	12.6	0.07
Cal 1:7 ZnO NP (E)	11	12	11	11.3	0.03	12	14	12	12.6	0.07
Cal 1:3ZnO NP (F)	10	10.5	10	10.2	0.03	12	13	12	12.3	0.03
Cal 1:1 ZnO NP (G)	12.5	11	12	11.8	0.03	13	15	12	13.3	0.03
Cal 3:1 ZnO NP (H)	13	12	11	12	0	13	14	12.5	13.2	0.03
PVA(5mm)(I)	6	5.5	5	5.5	0	6	5.5	5	5.5	0
5%ZnO/PVA (J)	13	14	13.5	13.5	0	14	15	14.5	14.5	0
9% ZnO/PVA (K)	14	15	16.5	15.2	0.03	15	17	17.5	16.5	0
13% ZnO/PVA (L)	15	17	18.5	16.8	0.03	19.5	21	19	19.8	0.03
16% ZnO/PVA (M)	16.5	17.5	17	17	0	19	22	22.5	21.2	0.03

From the table, “un” stands for uncalcined and “cal” stands for calcined nano particle.

oxide is weak in antimicrobial activities. The results in the Table 5, revealed the fact that antibacterial activity of the tested composite sample was dependent on the concentration of ZnO NPs in the polymer matrix. It can be concluded that the finding of this work indicated ZnO NPs and ZnO/PVA NCs are better antibacterial agents against *S. aureus* and *E.coli* [27–32].

4 Conclusion

ZnO NPs and ZnO/PVA NCs were successfully synthesized using aqueous *Moringa oleifera* leaf extract template in various ratios of precursor salt and extract concentrations. The ZnO NPs synthesized from 1:1 ratio of the Zinc acetate salt and *Moringa oleifera* leaf extract at pH 10.5 exhibited rod shaped morphology. The particle sizes of ZnO NPs obtained from 1:7 ratio and 3:1 ratio were found to be 7.57 nm and 15.27 nm, respectively. Microstructure analysis by scanning electron microscopy confirmed the successful loading of ZnO NPs on the surface and matrix of polymer polyvinyl alcohol at low percentage of the oxide. The band gap of polyvinyl alcohol found to decrease with the increase in ZnO loading, while the electrochemical potential or stability of the nanocomposites found to increase with increasing in ZnO content. The anti-bacterial inhibition efficiency of ZnO/PVA NCs against gram negative *E.*

coli and gram positive *S.aureus* bacteria was found to be better and exhibited dependency on ZnO loading.

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