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Zinc Oxide Nanoparticles Biosynthesis using *Leptadenia hastata* Leaf Extracts and their Potential as Antimicrobial Agents

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Abstract:- The study is to focus at the evaluation of the potential of the antibacterial activity of the synthesized Zinc oxide nanoparticle using *Leptadenia hastata* leaf extracts on selected bacterial; *E.coli, S. aureus, K. pneumonia*. Zinc oxide (ZnO) nanoparticles synthesised, UV, SEM and FTIR were used to characterise the nanoparticles. The particles were further subjected to evaluation of their antimicrobial activity. The FTIR studies was observed to have shown the several chemical functional groups, while the SEM results showed the synthesized nanoparticles were in the range below 100 nm and the size of the particles was slightly ellipsoidal as well as spherical. From the result it was observed that the synthesised nanoparticles have showed significant antimicrobial potentials against the gram negative and gram positive bacteria's (*E. Coli, S. aureus* and *K. Pneumonia*. This study being the first using *Leptadenia hastata* leaf extract should provide a new agent toward the fight of resistance bacterial and effective therapies in the field of medicine.

Keywords:- *Biosynthesis, Zinc oxide, nanoparticles, Leptadenia hastata, leaf, antimicrobial, characterization.*

I. INTRODUCTION

Green synthesis has been considered as another remedy in the field of medicine. Aside toxic chemical and physical method, biological method is considered using medicinal plants extract were used for the synthesis of nanoparticles. The surface and fraction of the atoms are responsible for the activity of the nanoparticles. This invention of green nanotechnology is considered eco-friendly and cost effective when compared to the others. The technology utilizes proteins as natural capping agents and its synthesis from plants utilize various secondary metabolites, enzymes, proteins and or other reducing agents which makes it suitable to use in various biomedical and clinical applications [1].

The advantage of green nanoparticles technology from ZnO is that they have a strong potential against the pathogenic microbes when applied in small concentration [2]. It was also reported that ZnO showed remarkable growth inhibition of bacteria [3]. Thus, impaired wound healing in ulcer patients is a major complication. The elevated infections by bacterial such as *Helicobacter pylori* and many more resistant bacterial may interfere in the proper healing process. If left untreated will lead to delay in the healing process, as such introducing nanoparticle is an answer to such disturbing ailment. Many antibacterial agents have limitations in the clinical applications, because of complications and resistance towards pathogenic microorganisms. [4, 5]

It was reported in some cases where few metal nanoparticles are known to have potential antimicrobial and consequential wound rejuvenating activity, but they also have some disadvantages related to the toxicity level [6]. This prompted the synthesize of nanoparticles by bio-assisted pathway to reduce their toxicity effect and provide better duodenal or stomach ulcer healing therapy to avoid and treat the resistance related pathogens.

Thus, this study may provide a remedy with the green synthesis nanoparticles and its high potential towards antibacterial activity to control the resistance effect of *Helicobacter phylori* as well as other ulcer causing bacteria. Since this area is less explored, there is a need to consider the potential of this nanoparticles as to cutile the menace and the pain attached to this wound ulcer.

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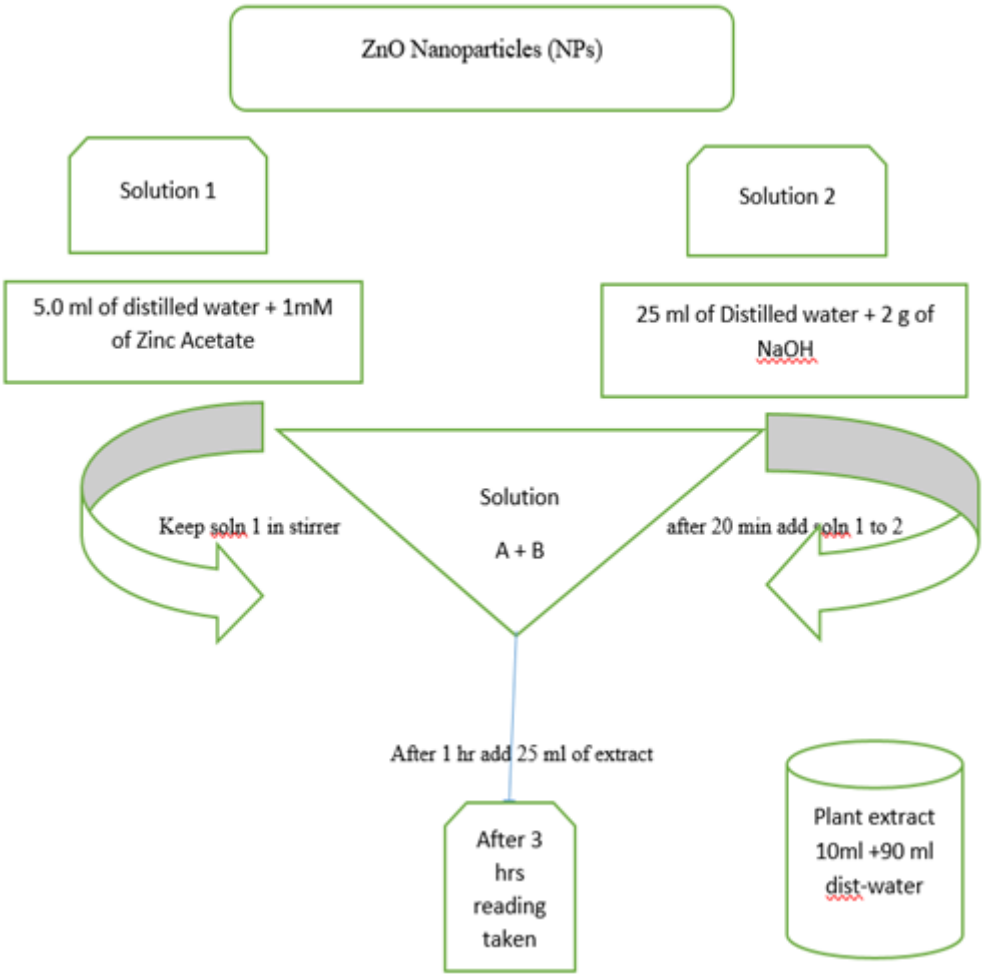

Fig 1:- Schematic representation of ZnO Nanoparticles (NPs) synthesis.

ISSN No:-2456-2165

Fig 2:- Leaves of *Leptadenia hastata*.

II. MATERIAL AND METHOD

➢ *Plant collection*

Leaves of *Leptadenia hastata* (Pers) Decne was collected in Michika local government in Adamawa state, Nigeria. It is one among the economical plant. Fresh green leaves were harvested in the months of August and September.

➢ *Preparation of the plant extract*

Fresh leaves (5g) were washed with distilled water, the samples were cut in to smaller pieces and soaked in a 250 mL cylinder flask containing 100 mL of distilled water. The sample was allowed to boil at 70 °C for 7 to 8 min. The sample extract was allowed to stand at room temperature and filtered through Whatman number-1 filter paper, and the filtrate was stored for further use.

➢ *Synthesis of ZnO Nanoparticles (NPs)*

1 mM Zinc acetate [Zn $(O_2CCH_3)_2$ $(H_2O)_2$] was dissolved in 50 ml distilled water and kept in stirrer for 1 hr, respectively as reported by Jamdagni *et al*. [7]. NaOH (20 mL) solution was slowly introduced into the Zinc acetate solution and 25 mL of plant extract was added to the same. The colour of the preparation was observed to change after 1 hrs. of incubation time. The solution was left in stirrer for 3 hrs. A light yellow colour was observed after the incubation time. This indicate the synthesis of ZnO Nanoparticles (NPs). The precipitate from the preparation was separated by centrifugation at 8000 rpm at 60 °C for 15 min and pellet collected from the filtered and dried at 80 °C using t hot air oven for 2 hrs and stored in vial for further studies.

➢ *ZnO Nanoparticles (NPs) Characterization*

The *Leptadenia hastata* ZnO nanoparticle was characterized based on Ultra Violet (UV) absorption spectra at 300 to 400 nm. The FTIR characterization was obtained using 2 mg of the Nanoparticles (ZnO), mixed with 200 mg of potassium bromide, the bromide used was FTIR grade to maintain standard, then pressed into a pellet for the characterization. The prepared sample was transferred into the sample holder and the spectra of the nanoparticles were recorded at a resolution of 4 cm^{-1} from the Fourier Transform Infra-Ray (FTIR) spectroscopy [8]. The Nanoparticles shape and size were analysed by using SEM.

❖ *Antibacterial activity of synthesized ZnO Nanoparticles (NPs)*

➢ *Preparation of Test Samples*

The *Leptadenia hastata* zinc oxide (ZnO) nanoparticle from *Leptadenia hastata* leaf water extract was evaluated as described by Isaac *et al*. [9]. 5mg of the sample was dissolved equally in 5mL of methanol to form a stock solution 1000 µg/mL. Lower concentration was also prepared of 10, 50, 100, 250, and 500 ppm from the stock solution. 1000 ppm concentration was again prepared to make the sixth concentration.

ISSN No:-2456-2165

➢ *Preparation of Bacteria Broth*

The selected bacteria were used to test the activity of the obtained green nanoparticles of Leptadenia hastata zinc oxide, *Staphylococcus aureus*, (S. aureus +ve), *Escherichia coli* (E. coli –ve) *and Klebsielia Pneumonia (K. pneumonia +ve)* were collected from Universiti Malaysia Sarawak Virology Laboratory. The nutrient broth was prepared as instructed by the manufacturers, 2.6 g of the broth was dissolved in distilled water (200 mL) and sterilization at a temperature of 121 ºC in an autoclave. The selected bacteria's were sub-cultured in a 10 mL of broth for about 16 hours in an incubator at 37ºC [10]. The optical density of the bacterial broth at 575 nm, the broth is ready for experiment when the turbity was observed within 0.6 to 0.9 OD [11]

➢ *Plate Inoculation*

The experiment was carried out in a biohazard cabinet as described by Isaac et al., [12]. The agar plate was allowed to stand for 5 to 10 minutes and sample applied. A volume of 10 µL each of 10, 50, 100, 250, 500 and 1000 µL of the sample and the negative control (disc of Methanol) and 30 µg of tetracycline (Positive) was introduced into the demarcated six millimetre disc. The plates were left for ten minutes at room temperature to allow the test sample to diffuse into the prepared test. The experiment was observed in triplicate for each of the bacteria used and incubated at 37oc for 18 to 24 hrs. The zone of inhibition were recorded in diameter (mm) to evaluate the antibacterial activity of the nanoparticles compared to the test control.

III. RESULT AND DISCUSSION

➢ *Scanning Electron Microscope (SEM)*

The scanning electron microscope was used to visualize the shape and size of the green nanoparticles. The sample images from SEM were seen in different magnification ranges which clearly demonstrated the presence of spherical shaped nanoparticle with mean average diameter of 70 nm Fig 3 [13].

➢ *Fourier Transform Infra-Red Spectrometry (FTIR)*

The result obtained from the FT-IR spectra and functional group involved in *Leptadenia hastata* ZnO Nanoparticle synthesis illustrated peak within the value of 1000-4000 cm^{-1} (Fig 4). Absorption band of ZnO Nanoparticle IR spectrum showed the presence of the functional group (O-H) which appeared at 3363.66 cm^{-1} bond. Figure 4 shows an IR absorption band at C-H bond of 2970.49 cm^{-1} this suggested the presence of methyl carbon in the chemical structure. With a double bond of C=C stretching at 1772.35 cm^{-1} and 1566.86 cm^{-1} was also observed in the spectrum of ZnO Nanoparticle. The peak observed was in the range of 1502.35 and 1409.81 which corresponded to C=C stretch in aromatic ring and C=O stretch in polyphenols and C-N stretch of Amide-I in protein.

From the result weak peaks was observe at 1089.98 cm-1, 1010.10 cm-1 and 886.75 cm-1, 830.25 cm^{-1}, 764.32 cm^{-1}, demonstrated the presence of C-O stretching in amino acid, C-N stretching, C-F, C-l, C-Br strong stretching, and C-H bending respectively. A very ignorable peak obtained at 686.62 cm^{-1} demonstrated the probable presence of C-Alkyl chloride and Hexagonal phase ZnO thus supported by the report of Yadurka et al. [14].

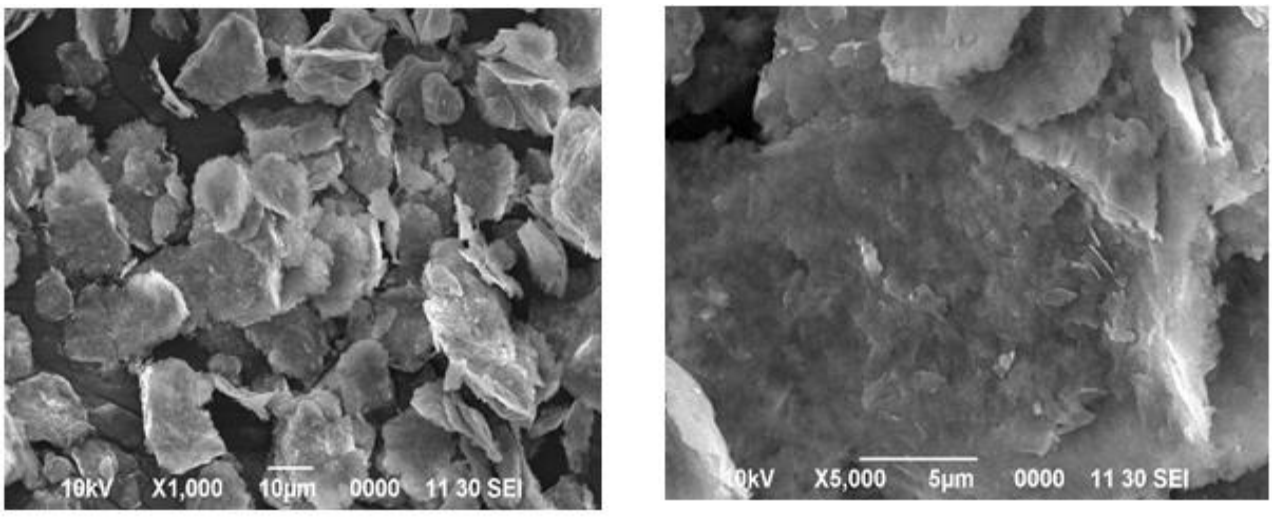

Fig 3:- SEM images of ZnO Nanoparticles (NPs) of *Leptadenia hastata* leaf in different magnification ranges

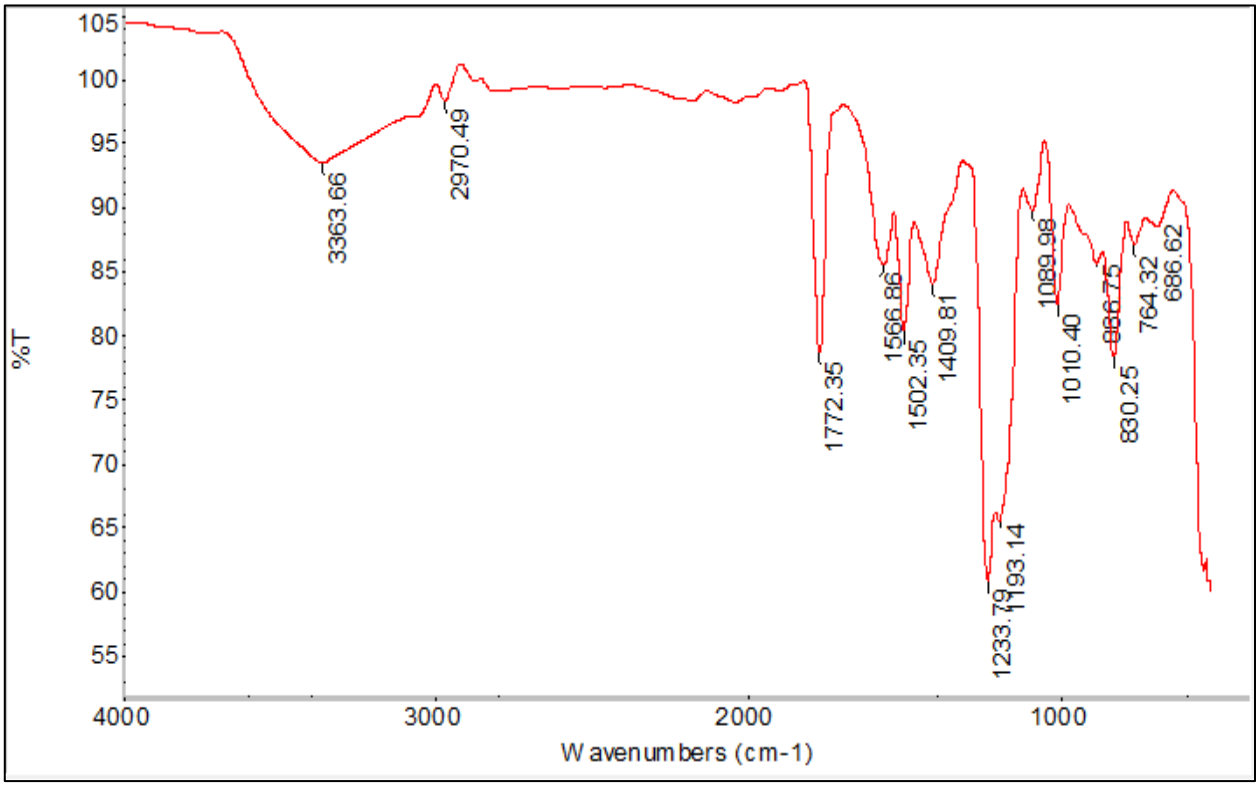

Fig 4:- FT-IR Spectrum of ZnO Nanoparticles (NPs) of *Leptadenia hastata* fresh leaf

➢ *UV-visible Analysis*

The UV-visible spectroscopy is usually conducted to confirm the synthesis of ZnO Nanoparticles. Conducting electrons start oscillating at a certain wavelength range due to surface Plasmon resonance (SPR) effect. Figure 5 represents the UV-visible spectra of freshly prepared Leptadenia hastata ZnO Nanoparticles. Peak observed at 380 nm clearly demonstrates the presence of ZnO Nanoparticles in the reaction mixture. Initial peak obtained at range of 420 nm got further raised due to oscillation of more electrons after 5 hrs which reports the continuous synthesis of Leptadenia hastata ZnO Nanoparticles.

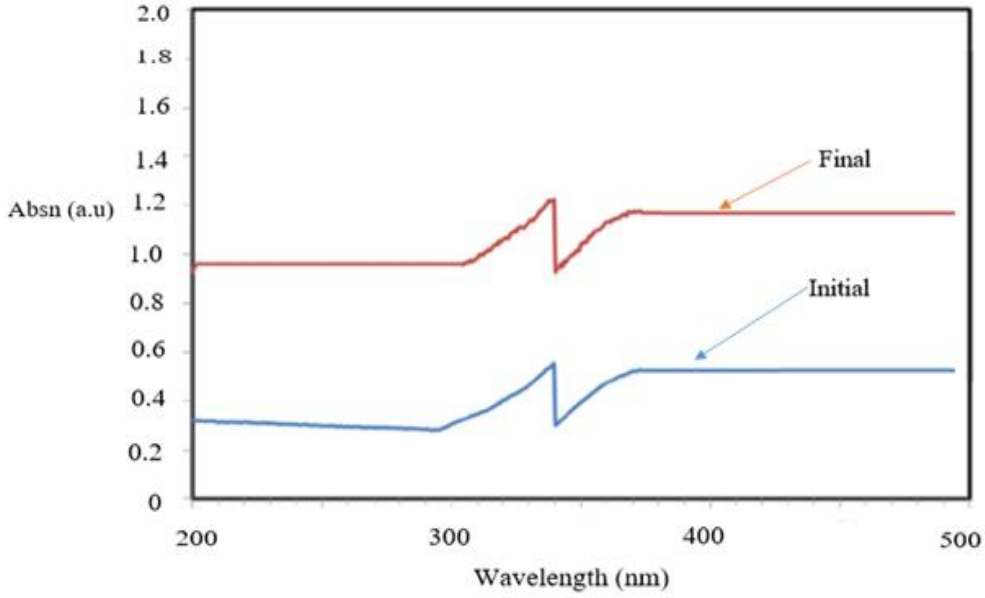

Fig 5:- UV-vs spectrum of *Leptadenia hastata* leaf ZnO Nanoparticles.

➢ *Leptadenia hastata ZnO Nanoparticle activity.*

Anti-bacterial effect of *Leptadenia hastata* fresh leaf ZnO Nanoparticles was tested against *S. aureus, E. coli, K. Pneumonia.* Tetracycline disc was used as a standard, from the table 1 the result clearly demonstrated that the nanoparticles showed antibacterial activity in a concentration dependant manner as shown in Figure 6 and Table 1.

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A significant activity was observed at 25 ppm to 1000 ppm, high zone of inhibition was obtained against all the bacteria at 1000 ppm with increase in concentration of the ZnO nanoparticles in all the gram-ve and gram +ve bacterium (*Staphylococcus aureus*, *Escherichia coli*, *Klebsielia Pneumonia*) of12.53 ± 0.12 mm, 12.34 ± 0.10 mm and 12.13 ± 0.12 mm, respectively. Weaker value was observed against all the bacterium at 25 ppm. However, the zone of growth Inhibition obtained using the nanoparticle was much significant when compared to the standard disc (Tetracycline) used which depicts the need of considering nanoparticle as an agent to compliment in the fight of resistance bacteria in the field of medical science. The entire tests were done in triplicate. From the result it was observed nanoparticles as an *in-vitro* antimicrobial activities.

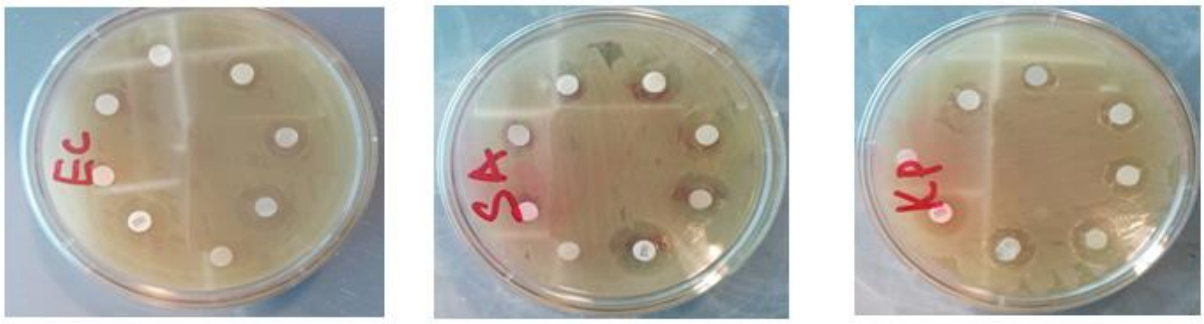

Fig 6:- Showing the growth inhibition of *Leptadenia hastata* ZnO Nanoparticles

Concentration (ppm)	*Escherichia coli* (Gram–ve),	*Staphylococcus aureus*, (Gram +ve)	*Klebsielia Pneumonia* (Gram +ve)
Control(tetracycline)	13.15 ± 0.10	13.12 ± 0.81	13.10 ± 1.10
25	9.97 ± 0.21[b]	7.75 ± 0.07	10.77 ± 0.23
50	11.20 ± 0.20	11.60 ± 0.10[b]	11.55 ± 0.07
100	11.85 ± 0.07	11.00 ± 0.10	11.65 ± 0.07
250	11.90 ± 0.14	11.67 ± 0.14	11.95 ± 0.07[b]
500	11.99 ± 0.10[b]	12.17 ± 0.25	11.97 ± 0.35
1000	12.53 ± 0.12[a]	12.34 ± 0.10[a]	12.13 ± 0.12[a]

Table 1:- Effect of *Leptadenia hastata* leaf zinc oxide (ZnO) nanoparticle on *Staphylococcus aureus*, (+ve), *Escherichia coli* (–ve) *and Klebsielia Pneumonia* (+ve)

Values are Mean ± SD
[a] Significantly ($p < 0.05$) higher at different concentration in each column
[b] Significantly ($p < 0.05$) higher at the same concentration in each row

IV. CONCLUSION

The study of *Leptadenia hastata* ZnO synthesis was carried out. This green synthesis was found to be eco-friendly, non-toxic and less usage of chemicals compared to the physical and chemical method. The presence of phytochemical constituents in the leaf extract helps in the synthesis of the ZnO nanoparticles by inducing redox reaction. The functional groups of phytochemicals such as amine, alkane and hydroxyl groups induced the formation of nanoparticles which are widely seen in the secondary metabolites, such as terpenoids, flavonoids, and alkaloids. The preliminary confirmation of the ZnO nanoparticles was measured using the Uv-visible spectroscopy at 380 nm, the SEM analysis demonstrate the size of the nanoparticles and the antibacterial activity of the *Leptadenia hastata* ZnO nanoparticles has confirmed the potential of the nanoparticles as an agent against bacterial.

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➢ *Conflict of Interest*
The authors declare no competing of interest.

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