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**Research Paper**

# Formulation and Characterization of bi-metallic nanoparticles of *Musa paradisiaca* pulp and peel for antibacterial potency .

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***ABSTRACT***
*Metal nanoparticles possess large surface energy; hence have the ability to absorb small molecules. Smaller nanoparticles containing $10^4$ or less atoms are referred as to nanoclusters. Metal nanoclusters are complicated in their bonding. Metals can be used for the preparation of nanoparticles can used as the delivery system like: Na, K, Mg, Zn, Cu, Fe, Mn, Au, Ag, Co etc. The biosynthesis of inorganic nanoparticles including metallic nanoparticles, sulfide nanoparticles, oxide nanoparticles and other typical nanoparticles. The application of biosynthesized nanoparticle in wide spectrum of potential areas is presented including targeted drug delivery system, cancer treatment, gene therapy. Nanocarriers with optimized physiochemical and biological properties are taken up by cells more easily than larger molecules, so they can be successfully used as drug delivery tools for currently available bioactive natural molecules. The decreased particles size approx 1-100 nm in nanoscaled; they are used in drug delivery system as a carrier for the various molecules (synthetic as well as natural).In present study bimetallic nanoparticles using bimetallic salts and pulp and peel Musa paradisiaca were prepared and evaluated for antibacterial potency against drug resistant bacterial population.Results indicates the potency of these bimetallic nanoparticles against the selected isolates.*

***KEY WORDS:*** *Bimetallic, Nanoparticles, Antibacterial, Drug resistance*

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## I. INTRODUCTION

*Musa paradisiaca* (banana) selected due to the presence of various phytoconstituents and high nutritive value .Leucocyanidin, quercetic, 3-0-glucoside, sitosterol gentiobioside, and sitosterol myo-inosityl-β-D-glucoside have been isolated from fruit pulp. Bimetallic nanoparticle using phytochemicals can be exploited for ant bacterial potency (Mittal et al,2013). Catecholamine such as norepinephrine serptonin, dopamine, tryptophan, indole compounds and pectin were reported in fruit pulp of banana (Anhwange,2008). Yin et al., 2008 was studied the effect of banana in human and found that significantly reduced of plasma oxidative stress, this effect may be due to the presence of dopamine, ascorbic acid and other antioxidants present in banana. Banana fruit showed good antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Shigella dysenteriae*, *Salmonella paratyphi*, and *Escherichia coli* (Ahmad and Beg, 2001).

## II. MATERIAL AND METHOD

**Fabrication of Zinc and Copper Nanoparticles from banana pulp**

*Musa paradisiaca* fruit collected from local market then fruit pulp was collected in vessel (beaker) and eight was measured. Copper (30 mg) and Zinc (30 mg) were added in form of salt to the pulp (60 g) followed addition of 60 ml of phosphate buffer saline solution (PBS) to the mixture. Mixture was kept on magnetic stirrer for 12-13 hrs continuously with mild heating upto 30-40ºC (Kanchana et al,2018).

**Fig.1: Fruit of *Musa paradisiaca***

**Fabrication of Ferrous and Cobalt Nanoparticles from banana pulp** Banana pulp was collected in beaker. Weight was measured. ferrous (25mg) and cobalt (25mg) were added in form of salt to the pulp(50 g) followed addition of 50ml of PBS to the mixture than mixture was kept on magnetic stirrer for 12-13 hrs continuously with mild heating upto 30-40ºC.

**Fabrication of Zinc and Copper nanoparticles from banana peel** Inner banana peel was collected in beaker. Metal salt Zinc (25mg) and Copper (25mg) were added to the peel (16g) followed of 30ml of PBS mixture. Than mixture was kept on magnetic stirrer for 12-13 hrs continuously with mild heating upto 30-40ºC.

**Fabrication of ferrous and cobalt Nanoparticles from banana peel** Inner banana peel was collected in beaker. Metal salt Ferrous (25mg) and Cobalt (25mg) were added to the peel (30g) followed of 40 ml of PBS mixture. Mixture was than kept on magnetic stirrer for 12-13 hrs continuously with mild heating upto 30-40ºC.

**Characterization of Nanoparticles through Spectrophotometer**

Solution containing nanoparticles was centrifuged at 11000 rpm for 7 minute to separate the nano-formulation and large particles. Supernatant was collected and pellet was discarded. Supernatant was then analyzed through Spectrophotometer. Optical density of supernatant was measured from 200nm to 600nm wavelength and graph was plotted (Siavash ,2011).

**Zeta Potential**

Zeta potential measurement of supernatant for determines surface charge of nanoparticle solution. Nanoparticles was electrical potential at the boundary of the double layer is known as the zeta potential of the particles and has values that typically range from +100 to -100 mV.

**Screening of Antibacterial Activity**

Bacterial cultures were maintained in NAM which is minimal essential media which can provide nutritional requirement to nearly all bacterial isolates This relatively simple formulation provides the nutrients necessary for the replication of a large number of non-fastidious microorganisms.

**Maintence of the culture**

Pure culture of *Staphylococcus aureus, Pseudomonas, Escherichia coli, Citrobacter freundii, Clostridium* and *Klebsiella pneumoniae* were obtained from Department of Biotechnology of Barkatullah University from slants and pure cultures were maintained throughout the dissertation work through subculturing, fresh slants also prepared for storage of pure culture.

## III. RESULTS AND DISCUSSION

Change in color brownish green was first confirmation of metal nanoparticle formulation and for further confirmation, characterization through spectrophotometer analysis and zeta potential studies were performed.

**(A)** **(B)** **(C)**

**Fig2 :-Metal Salt with fruit pulp and PBS; (A) before nanoformulation of Zn-Cu salt, (B) before nanoformulation of Fe-Co salt, (C) after nanoformulation of bi metallic salt.**

Characterization of solution containing nanoparticle was centrifuged at 11000 rpm for 7 min and after the separate the nanoformulation the supernatant was used to measure the absorption spectra. The salt solution was used as a blank solution. The UV-Vis spectra of the solution were measured from 200 to 600 nm wavelength. The optical density of nanoparticle solution was showed characteristic peak between 450-500 nm.

**Table 1: Result showing spectrophotometer analysis of Zn -Cu nanoparticles from *Musa paradisiaca***

| S. No. | Wavelength | Optical density (OD) |
|---|---|---|
| 1. | 200 | 0.108 |
| 2. | 250 | 0.172 |
| 3. | 300 | 0.202 |
| 4. | 350 | 0.256 |
| 5. | 400 | 0.300 |
| 6. | 450 | 0.358 |
| 7. | 500 | 0.326 |

| | | |
|---|---|---|
| 8. | 550 | 0.248 |
| 9. | 600 | 0.192 |

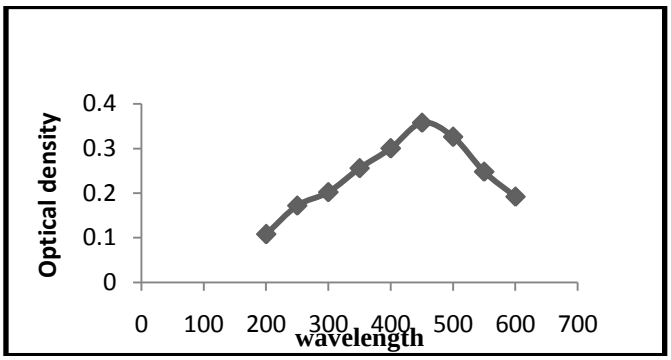



**Fig 3 : Optical density of formulated nanoparticle, showed highest peak between 450-500 nm**

**Table 2 : Result showing spectrophotometer analysis of Fe -Co nanoparticles from *Musa paradisiaca***

| S. No. | Wavelength | Optical density (OD) |
|---|---|---|
| 1. | 200 | 0.028 |
| 2. | 250 | 0.049 |
| 3. | 300 | 0.068 |
| 4. | 350 | 0.178 |
| 5. | 400 | 0.198 |
| 6. | 450 | 0.209 |
| 7. | 500 | 0.238 |
| 8. | 550 | 0.187 |
| 9. | 600 | 0.156 |

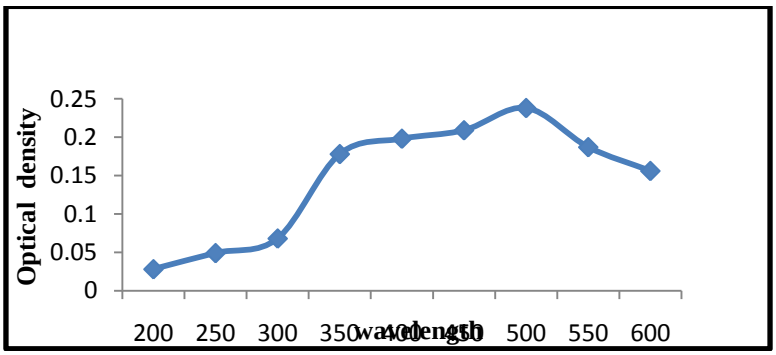
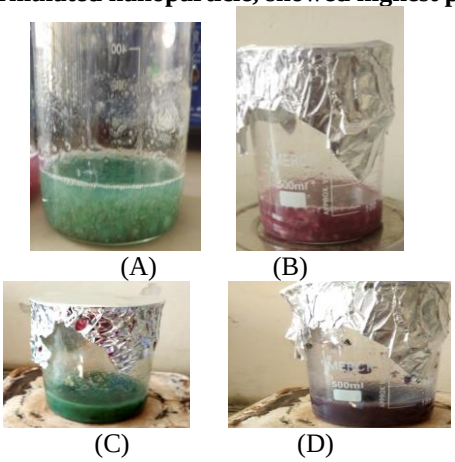



**Fig 4 : Optical density of formulated nanoparticle, showed highest peak between 450-500 nm**



(A) (B)

(C) (D)

**Fig5- Metal salt with fruit peel and PBS; (A) before formulation of nanoparticles Zn-Cu salt, (B) before formulation Fe-Co salt. (C) After formulation Zn-Cu salt, (D) After Fe-Co salt with PBS**

**Characterization** For the study of solution containing nanoparticle was centrifuged at 11000 rpm for 7 min and after the separate the nanoformulation the supernatant was used to measure the absorption spectra. The salt solution was used as a blank solution. The UV-Vis spectra of the solution were measured from 200 to 600 nm wavelength. The optical density of nanoparticle solution was showed characteristic peak between 450-500 nm.

**Table 3: Result showing spectrophotometer analysis of Zn -Cu nanoparticles from *Musa paradisiaca***

| S. No. | Wavelength | Optical density (OD) |
|---|---|---|
| 1. | 200 | 0.049 |
| 2. | 250 | 0.068 |
| 3. | 300 | 0.092 |
| 4. | 350 | 0.120 |
| 5. | 400 | 0.149 |
| 6. | 450 | 0.196 |
| 7. | 500 | 0.215 |
| 8. | 550 | 0.176 |
| 9. | 600 | 0.101 |

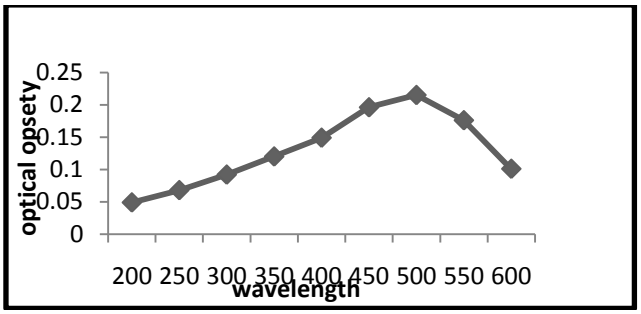



**Fig 6: Optical density of formulated nanoparticle, showed highest peak between 450-500 nm**

**Table 4 : Result showing spectrophotometer analysis of Fe -Co nanoparticles from *Musa paradisiaca***

| S. No. | Wavelength | Optical density (OD) |
|---|---|---|
| 1. | 200 | 0.089 |
| 2. | 250 | 0.099 |
| 3. | 300 | 0.124 |
| 4. | 350 | 0.145 |
| 5. | 400 | 0.177 |
| 6. | 450 | 0.193 |
| 7. | 500 | 0.206 |
| 8. | 550 | 0.186 |
| 9. | 600 | 0.142 |

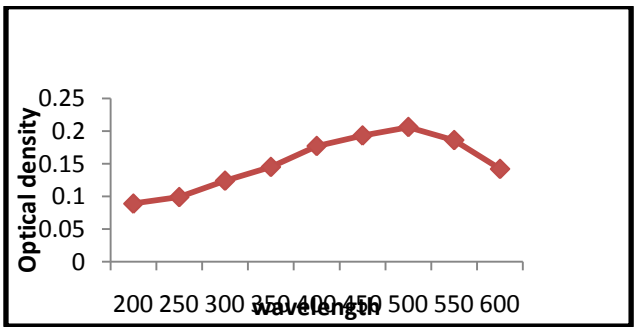



**Fig 7: Optical density of formulated nanoparticle, showed highest peak between 450-500 nm**

It may be concluded that *Musa paradisiaca* may be used as novel and natural source for antibacterial potency. It will further reduce the chances of toxicity and side effects. Magnitude of the zeta potential is predictive of the colloidal stability. Nanoparticles with zeta potential value less than -25vM and greater than +25vM shows high degree of stability. Dispersions with low zeta potential value will eventually aggregate due to Vander waal inter-particles are attractions. Zeta potential difference across phase boundaries between solids and liquids. It's a measure of the electrical change of particles are that are suspended in liquid. Zeta potential of all the formulation was reported to be in between +30vM which indicates the stability of all metal nanoparticles.

**Table 5 : Screening of antibacterial activity of Nanoparticles which is synthesized from *Musa paradisiaca* fruit pulp**

| S. N. | Microorganism | Nanoparticles | Dilution | Area (zone of inhibition in $cm^2$) |
|---|---|---|---|---|
| 1. | *Pseudomonas* | Zn-Cu Nanoparticles synthesized from fruit pulp of *Musa paradisiaca* | 2 µl | 0.2±0.08 |
| | | | 4 µl | 0.78±0 |
| | | | 6 µl | 1.8±0.7 |
| | | | 8 µl | 2.2±0.6 |
| | | | 10 µl | 2.4±0.7 |
| | | | 12 µl | 2.6±0.8 |
| | | Fe -Co Nanoparticles synthesized from fruit pulp of *Musa paradisiaca* | 2 µl | 0.78±0 |
| | | | 4 µl | 1.4±0.61 |
| | | | 6 µl | 1.9±0.8 |
| | | | 8 µl | 2.2±0.7 |
| | | | 10 µl | 2.8±0.6 |
| | | | 12 µl | 3.1±0.2 |
| 2. | *K. pneumoniae* | Zn-Cu Nanoparticles synthesized from fruit pulp of *Musa paradisiaca* | 2 µl | 0.4±0.08 |
| | | | 4 µl | 0.78±0 |
| | | | 6 µl | 1.5±0.1 |
| | | | 8 µl | 1.9±1.2 |
| | | | 10 µl | 2.4±0.7 |
| | | | 12 µl | 2.8±0.8 |
| | | Fe -Co Nanoparticles synthesized from fruit pulp of *Musa paradisiaca* | 2 µl | 1.1±0.3 |
| | | | 4 µl | 1.3±0.61 |
| | | | 6 µl | 1.6±0.8 |
| | | | 8 µl | 2.1±0.6 |
| | | | 10 µl | 2.4±0.7 |
| | | | 12 µl | 2.8±0.8 |
| 4. | *Clostridium* | Zn-Cu Nanoparticles synthesized from fruit pulp of *Musa paradisiaca* | 2 µl | 1.3±0.6 |
| | | | 4 µl | 1.5±0.6 |
| | | | 6 µl | 1.8±0.7 |
| | | | 8 µl | 2.4±0.7 |
| | | | 10 µl | 3.1±0 |
| | | | 12 µl | 3.7±0 |
| | | Fe -Co Nanoparticles synthesized from fruit pulp of *Musa paradisiaca* | 2 µl | 1.1±0.3 |
| | | | 4 µl | 1.4±0.6 |
| | | | 6 µl | 1.6±0.7 |
| | | | 8 µl | 1.8±0.6 |
| | | | 10 µl | 1.9±0.7 |
| | | | 12 µl | 2.2±0.8 |
| 5. | *Escherichia coli* | Zn-Cu Nanoparticles synthesized from fruit pulp of *Musa paradisiaca* | 2 µl | 0.8±0.3 |
| | | | 4 µl | 1.1±0.3 |
| | | | 6 µl | 1.3±0.6 |
| | | | 8 µl | 1.6±0.8 |
| | | | 10 µl | 1.8±0.7 |
| | | | 12 µl | 2.1±0.2 |
| | | Fe -Co Nanoparticles synthesized from fruit pulp of *Musa paradisiaca* | 2 µl | 0.2±0.08 |
| | | | 4 µl | 0.72±0.05 |
| | | | 6 µl | 1.1±0.3 |
| | | | 8 µl | 1.3±0.2 |
| | | | 10 µl | 2.1±0.3 |
| | | | 12 µl | 2.4±0.8 |
| 6. | *Staphylococcus aureus* | Zn-Cu Nanoparticles synthesized from fruit pulp of *Musa paradisiaca* | 2 µl | 1.1±0.3 |
| | | | 4 µl | 1.2±0.1 |
| | | | 6 µl | 1.6±0.3 |
| | | | 8 µl | 1.8±0.7 |
| | | | 10 µl | 2.1±0.6 |
| | | | 12 µl | 2.8±0.4 |
| | | Fe -Co Nanoparticles synthesized from fruit pulp of *Musa paradisiaca* | 2 µl | 0.5±0.02 |
| | | | 4 µl | 0.9±0.06 |
| | | | 6 µl | 1.3±0.1 |
| | | | 8 µl | 1.6±0.8 |
| | | | 10 µl | 2.2±0.6 |
| | | | 12 µl | 2.6±0.4 |

As clearly seen in the table the Zn-Cu nanoformulation of fruit pulp of *Musa paradisiaca* fruit exhibited strong antibacterial activity against *Clostridium* (1.1±0.3cm$^2$, 1.4±0.6cm$^2$, 1.8±0.7cm$^2$, 1.8±0.6cm$^2$, 1.9±0.7cm$^2$, 2.2±0.1cm$^2$) these are the area of zone of inhibition at (2µl, 4µl, 6µl, 8µl, 10µl, 12µl) dilution of nanoformulation respectively. *Escherichia coli* has (0.8±0.3cm$^2$, 1.1±0.3cm$^2$, 1.3±0.6cm$^2$, 1.6±0.8cm$^2$, 1.8±0.7cm$^2$, 2.1±0.2cm$^2$)

*Staphylococcus aureus* (1.1±0.3cm$^2$, 1.2±0.1cm$^2$, 1.6±0.6cm$^2$, 1.8±0.8cm$^2$, 2.1±0.6cm$^2$, 2.8±0.1cm$^2$) *Pseudomonas* has (1.1±0.08 cm$^2$, 1.6±0.3cm$^2$, 2.2±0.3cm$^2$, 2.6±0.1cm$^2$, 3.3±1.4cm$^2$, 3.7±0.1cm$^2$). *Klebsiella pneumoniae* has (0.2±0.08cm$^2$, 0.78±0.1cm$^2$, 1.1±0.01cm$^2$, 1.3±0.6cm$^2$, 1.7±1.4cm$^2$, 2.5±0cm$^2$), *C.freundii* has (0.2±0.01cm$^2$, 1.2±0.6cm$^2$, 1.5±0.4cm$^2$, 1.9±0.81cm$^2$, 2.4±0cm$^2$, 2.8±0.1cm$^2$) these are the area of zone of inhibition at (2µl, 4µl, 6µl, 8µl, 10µl, 12µl) dilution of nano formulation respectively. Also clearly seen in the table the Fe-Co nano formulation of fruit pulp of *Musa paradisiaca* fruit exhibited strong antibacterial activity against *Clostridium* (0.78±0.2cm$^2$, 1.4±0.61cm$^2$, 1.9±0.3cm$^2$, 2.2±0.2cm$^2$, 2.8±0.7cm$^2$, 3.1±0.1cm$^2$) these are the area of zone of inhibition at (2µl, 4µl, 6µl, 8µl, 10µl, 12µl) dilution of nanoformulation respectively. *E. coli has* (1.1±0.3cm$^2$, 1.4±0.6cm$^2$, 1. 6±0.7cm$^2$, 1.8±0.6cm$^2$, 1.9±0.7cm$^2$, 2.2±0cm$^2$), *C.freundii* has (1.1±0.3cm$^2$, 1.4±0.6cm$^2$, 1.8±0.7cm$^2$, 2.2±0.1cm$^2$, 2.4±0.2cm$^2$, 2.6±0cm$^2$) *Staphylococcus aureus* has (0.2±0.01cm$^2$, 1.2±0.6cm$^2$, 1.5±0.4cm$^2$, 2.6±0.8cm$^2$, 3.1±0cm$^2$, 3.5±0.1cm$^2$) as the area of zone of inhibition at (2µl, 4µl, 6µl, 8µl, 10µl, 12µl) dilution of nanoformulatiion showed less effect and showed small zone of inhibition even higher dose at 12µl. So it's cleared that formulated nano formulation was highest effective for *Pseudomonas* and *Clostridium* showed less effect.

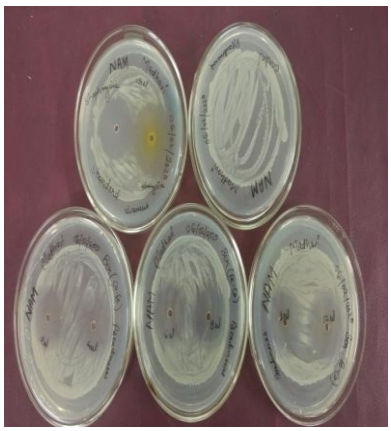


**Fig.8: showing antibacterial activity through zone of inhibition in *Pseudomonas***

**Table 6: Screening of antibacterial activity of Nanoparticles which synthesized from banana fruit peel**

| S. N. | Microorganism | Nanoparticles | Dilution | Area |
|---|---|---|---|---|
| 1. | *Pseudomonas* | Zn-Cu Nanoparticles synthesized from fruit peel of *Musa paradisiaca* | 2 µl | 0.77±0.05 |
| | | | 4 µl | 1.2±0.1 |
| | | | 6 µl | 1.5±0.3 |
| | | | 8 µl | 1.8±0.8 |
| | | | 10 µl | 2.1±0 |
| | | | 12 µl | 2.5±0.6 |
| | | Fe -Co Nanoparticles synthesized from fruit peel of *Musa paradisiaca* | 2 µl | 1.1±0.1 |
| | | | 4 µl | 1.3±0.61 |
| | | | 6 µl | 1.6±0.8 |
| | | | 8 µl | 2.4±0.7 |
| | | | 10 µl | 2.6±0 |
| | | | 12 µl | 3.1±0 |
| 2. | *K. pneumoniae* | Zn-Cu Nanoparticles synthesized from fruit peel of *Musa paradisiaca* | 2 µl | 0.2±0.01 |
| | | | 4 µl | 1.2±0.3 |
| | | | 6 µl | 1.6±0.8 |
| | | | 8 µl | 1.8±0.8 |
| | | | 10 µl | 2.2±0.6 |
| | | | 12 µl | 2.4±0.6 |
| | | Fe -Co Nanoparticles synthesized from fruit peel of *Musa paradisiaca* | 2 µl | 1.3±0.6 |
| | | | 4 µl | 1.5±0.81 |
| | | | 6 µl | 1.7±1.4 |
| | | | 8 µl | 1.9±1.2 |
| | | | 10 µl | 2.2±0.6 |
| | | | 12 µl | 2.4±0.7 |
| 4. | | Zn-Cu Nanoparticles synthesized from fruit peel | 2 µl | 0.78±0.08 |

| | | | | |
|---|---|---|---|---|
| | *Clostridium* | of *Musa paradisiaca* | 4 µl | 1.1±0.02 |
| | | | 6 µl | 1.5±0.4 |
| | | | 8 µl | 1.9±0.2 |
| | | | 10 µl | 2.4±0.6 |
| | | | 12 µl | 3.2±0.1 |
| | | Fe -Co Nanoparticles synthesized from fruit peel of *Musa paradisiaca* | 2 µl | 0.8±0.01 |
| | | | 4 µl | 1.1±0 |
| | | | 6 µl | 1.4±0.5 |
| | | | 8 µl | 1.6±0.8 |
| | | | 10 µl | 1.9±0.2 |
| | | | 12 µl | 2.1±0.3 |
| 5. | *Escherichia coli* | Zn-Cu Nanoparticles synthesized from fruit peel of *Musa paradisiaca* | 2 µl | 0.8±0.01 |
| | | | 4 µl | 1.1±0.3 |
| | | | 6 µl | 1.2±0.2 |
| | | | 8 µl | 1.5±0.8 |
| | | | 10 µl | 1.9±1.2 |
| | | | 12 µl | 2.1±0 |
| | | Fe -Co Nanoparticles synthesized from fruit peel of *Musa paradisiaca* | 2 µl | 1.2±0.2 |
| | | | 4 µl | 1.4±0.1 |
| | | | 6 µl | 1.8±0.6 |
| | | | 8 µl | 2.1±0.3 |
| | | | 10 µl | 2.4±0.7 |
| | | | 12 µl | 2.6±0.6 |
| 6. | *Staphylococcus aureus* | Zn-Cu Nanoparticles synthesized from fruit peel of *Musa paradisiaca* | 2 µl | 0.8±0.1 |
| | | | 4 µl | 1.2±0.1 |
| | | | 6 µl | 1.8±0.6 |
| | | | 8 µl | 1.9±0.2 |
| | | | 10 µl | 2.2±0.6 |
| | | | 12 µl | 2.5±0 |
| | | Fe -Co Nanoparticles synthesized from fruit peel of *Musa paradisiaca* | 2 µl | 0.8±0.1 |
| | | | 4 µl | 1.6±0.3 |
| | | | 6 µl | 1.9±1.2 |
| | | | 8 µl | 2.1±0.6 |
| | | | 10 µl | 2.4±0.7 |
| | | | 12 µl | 2.5±0 |

## IV. CONCLUSION

Bimetallic nanoparticles of both fruit peel and pulp of *Musa paradisiaca* shows potency against the drug resistant bacterial isolates selected for screening . Zetapotential indicates the stability of nanoparticles .Efficiency of *Musa paradisiaca* can further be exploited for antioxidant as well as anti-inflammatory property on various microbial as well as animal cell lines also.

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