

# Combining Studies on Preparation of Metal Nanoparticles from Plant Extracts as Nano-Antibiotic

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**Abstract:** Nano antibiotics are an arising antibiotics particularly arranged for the multidrug resistance, the infection basically occurred by the medically valuable strains that have genetically altered and become unaffected to the antibiotics. The application of ions of metals and salts being unchanged but precisely affect the bacterial resistance mechanisms. The plants contain antimicrobial activity can be employed for the complementary therapies as stuff for the nanoparticle creation. The nanoparticles have powerful destination action on the pathogen as its working efficiency can be altered as per requirement. The nanoparticles can be used as tablets and also in suspensions. The multi drug resistant pathogens would be hindered and its strain could be altered for our aim. The employment of altered specimen with the salts can be grouped as per the wanted dosage for the bacterial pathogens.

**Keywords:** Nanoparticles, Nanoantibiotics, Phytocompounds, Phytochemicals, Plantshifts

## 1. Introduction

Plants are the major source of the phytocompounds which shows many medicinal properties. These compounds help to hinder challenger, predators or pathogens. The name originates from Greek, where phyto means plant. Some of the phytochemicals employed as toxins and others as common drugs. Phytochemicals is broadly employed to explain shifts of plants that are under analysis with well-established results on health and are not scientifically explained as essential nutrients. Phytochemicals under analysis can be classified into major divisions, such as carotenoids and polyphenols, which carry phenolic acids, flavonoids, and sibenenes/lignans. Flavonoids can be grouped as epicatechins, catechins, and proanthocyanidins. Phytochemicals acquired from fruits, vegetables, spices, herbs and medicinal plants, such as, terpenoid, alkaloids and other phenolic stuffs, have been accessed to hinder developmental tumor genesis in different organs in pre-clinical models. Most of oxidation inhibition activity is ascribed to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, catechin etc. oxidation inhibitory based drug formulations are serviced for the avoidance and treatment of complex diseases like stroke, diabetes, Alzheimer's disease and cancer. The biological molecules those are present in the plants shifts are serviced to minimize metal ions to nanoparticles in a single-step green synthesis process. This biogenic devaluation of metal ion to base metal is absolutely accelerated, immediately managed at room temperature and pressure, and comfortably scaled up. Synthesis conciliated by plantshifts is environmentally benign. The reducing agents involved the various solvent or water soluble plant metabolites (e.g. alkaloids, phenolic compounds, terpenoids) and co-enzymes. Extracts of a diverse range of plant species have been successfully used in making nanoparticles.

## 2. Material and Methods

### 2.1 Sample collection

The plant samples were collected and then separated into different parts of the plants

**Table 1:** Three different samples plant samples were collected

| S no. | Scientific name              | Common name     | Location                  |
|-------|------------------------------|-----------------|---------------------------|
| 1.    | <i>Calendula officinalis</i> | Marigold orange | Gomti Nagar, Lucknow (UP) |
| 2.    | <i>Calendula officinalis</i> | Marigold yellow | Gomti Nagar, Lucknow (UP) |
| 3.    | <i>Matricaria chamomilla</i> | Chamomile       | Gomti Nagar, Lucknow (UP) |

### 2.2 Microbial strain and culture preparation

*Salmonella typhi* (St), *Pseudomonas aeruginosa* (Pa) and *Bacillus subtilis* (Bs) are some bacterial pathogens which are available at the MRD LifeSciences (P) Ltd. Lucknow. The pathogens were subcultures and then broth was prepared for further testing's.

### 2.3 Extract preparation

The samples were separated in different parts of plants, and then properly washed with double distilled water then air dried. For organic extract preparation the samples were dipped in polar and nonpolar solvents in the ratio of 1:10, and incubated at room temperature for 48-96 hours. For aqueous extract preparation the sample were boiled in same ratio at 75°C-100°C for 90 minutes. Then the extracts were filtered and collected for nanoparticles preparation.

## 2.4 Nanoparticle preparation

The nanoparticles preparation was based on bottom up synthesis approach, where the plant extracts and the metals in 100mM concentration are taken in three different ratios, such as 1:1, 1:4 and 4:1 at 80°C in hot plate magnetic stirrer. The confirmation is done by the color change of the sample extract. The nanoparticles were incubated for drying and then collected for further use.

## 2.5. Antibiotic Sensitivity Tests:

The test is based on agar well diffusion method, where the pathogens were firstly spread over the sterilized nutrient agar media and the samples were loaded to the well. Incubated at 37°C for 24 hours and then zone of inhibition was calculated.

## 2.6. Minimum Inhibitory Concentration test:

The prepared nanoparticles were serially diluted in sterilized nutrient broth and then the bacterial pathogens were inoculated to the respective test tubes. Incubated at 37°C for 24 hours at 121 rpm and then OD was taken at 620 nm.

## 2.7 Phytochemical Analysis:

### a) Flavanoids

- 10% lead acetate/ lead nitrate was prepared.
- 1 ml of extract was added with 1 ml of lead nitrate.
- A yellow precipitate was observed that determined positive result for flavonoids.

### b) Saponin

- 1ml of extract with 3 ml of distilled water was taken, mixed.
- Froth denotes positive result for saponin

### c) Tannin

- Few drops of lead nitrate was added in 1 ml of extract.
- Precipitate was observed for positive result.

### d) Steroids

- 1 ml of extract was added with 2 ml of Chloroform and 2 ml of H<sub>2</sub>SO<sub>4</sub>.
- Reddish brown interface shows the positive result.

### e) Terpenoids

- 0.1ml of chloroform was added with 0.1 ml of extract.
- 0.1ml of H<sub>2</sub>SO<sub>4</sub>.
- Few drops of acetate shows Red color indicating positive result.

### f) Carbohydrates

- 0.1ml of extract was added with 0.1 ml of Fehling A.
- 0.1 ml of Fehling B was added in the solution.
- A red precipitate indicates positive result.

## 3. Results

### 3.1 Sample Collection



Figure 1: (a) *Matricaria chamomilla* (b) *Calendula officinalis* (C) *Calendula officinalis*

### 3.2 Antimicrobial sensitivity test of nanoparticles of aqueous extracts of samples

Table 2: Antimicrobial test of the nanoparticles against the *P. aeruginosa*, *S typhi* and *B. subtilis*

| S no. | Nanoparticles  | Zone of Inhibition (mm) |                |                    |
|-------|--|-------------------------|----------------|--------------------|
|       |  | <i>P. aeruginosa</i>    | <i>S typhi</i> | <i>B. subtilis</i> |
| 1     | <i>Calendula officinalis</i> (Yellow Flowers)- Flowers |                         |                |                    |
| a     | ZnSO <sub>4</sub> (1:1)                                | 30                      | 32             | 29                 |
| b     | FeSO <sub>4</sub> (1:1)                                | 22.5                    | 22.5           | 26.5               |
| c     | CuSO <sub>4</sub> (1:1)                                | 30                      | 24             | 24.5               |
| 2     | <i>Calendula officinalis</i> (Yellow Flowers) - Roots  |                         |                |                    |
| a     | ZnSO <sub>4</sub> (1:1)                                | 29                      | 30             | 34                 |
| b     | FeSO <sub>4</sub> (1:1)                                | 26.5                    | 27             | 25.5               |
| c     | CuSO <sub>4</sub> (1:1)                                | 13                      | 15             | 12.3               |
| 3     | <i>Calendula officinalis</i> (Yellow Flowers)- Stems   |                         |                |                    |
| a     | ZnSO <sub>4</sub> (1:1)                                | 23                      | 21.2           | 14.2               |
| b     | FeSO <sub>4</sub> (1:1)                                | 18                      | 17.4           | 14.9               |
| c     | CuSO <sub>4</sub> (1:1)                                | 20.2                    | 29             | 30                 |
| 4     | <i>Calendula officinalis</i> (Orange Flowers)- Flowers |                         |                |                    |
| a     | ZnSO <sub>4</sub> (1:1)                                | 19.7                    | 20.6           | 21.1               |
| b     | FeSO <sub>4</sub> (1:1)                                | 16.9                    | 13             | 14                 |
| c     | CuSO <sub>4</sub> (1:1)                                | 24.5                    | 19.8           | 20.5               |
| 5     | <i>Calendula officinalis</i> (Orange Flowers)- Roots   |                         |                |                    |
| a     | ZnSO <sub>4</sub> (1:1)                                | 12.3                    | 21             | 13.9               |
| b     | FeSO <sub>4</sub> (1:1)                                | 22                      | 19             | 14                 |
| c     | CuSO <sub>4</sub> (1:1)                                | 31                      | 12             | 23                 |
| 6     | <i>Calendula officinalis</i> (Orange Flowers)-Stems    |                         |                |                    |
| a     | ZnSO <sub>4</sub> (1:1)                                | 14.5                    | 13.1           | 20.1               |
| b     | FeSO <sub>4</sub> (1:1)                                | 18.6                    | 19             | 13                 |
| c     | CuSO <sub>4</sub> (1:1)                                | 20                      | 21             | 19                 |
| 7     | <i>Matricaria chamomilla</i> Flowers                   |                         |                |                    |
| a     | ZnSO <sub>4</sub> (1:1)                                | 20                      | 23             | 21                 |
| b     | FeSO <sub>4</sub> (1:1)                                | 23                      | 29.1           | 28                 |
| c     | CuSO <sub>4</sub> (1:1)                                | 28.1                    | 26             | 21                 |
| 8     | <i>Matricaria chamomilla</i> Stems                     |                         |                |                    |

|   |                                    |      |      |      |
|---|------------------------------------|------|------|------|
| a | ZnSO <sub>4</sub> (1:1)            | 13.2 | 17.5 | 18   |
| b | FeSO <sub>4</sub> (1:1)            | 21   | 20   | 22   |
| c | CuSO <sub>4</sub> (1:1)            | 23   | 24.2 | 22   |
| 7 | <i>Matricaria chamomilla</i> Roots |      |      |      |
| a | ZnSO <sub>4</sub> (1:1)            | 15   | 13.4 | 19   |
| b | FeSO <sub>4</sub> (1:1)            | 19   | 18   | 22   |
| c | CuSO <sub>4</sub> (1:1)            | 23.4 | 25.6 | 22.5 |
| 8 | <b>Controls</b>                    |      |      |      |
| a | Norflox                            | 13   | 11   | 10.9 |
| b | ZnSO <sub>4</sub>                  | 11   | 12.4 | 13.5 |
| c | FeSO <sub>4</sub>                  | 10.9 | 14.3 | 13.1 |
| d | CuSO <sub>4</sub>                  | 15   | 11   | 10.8 |

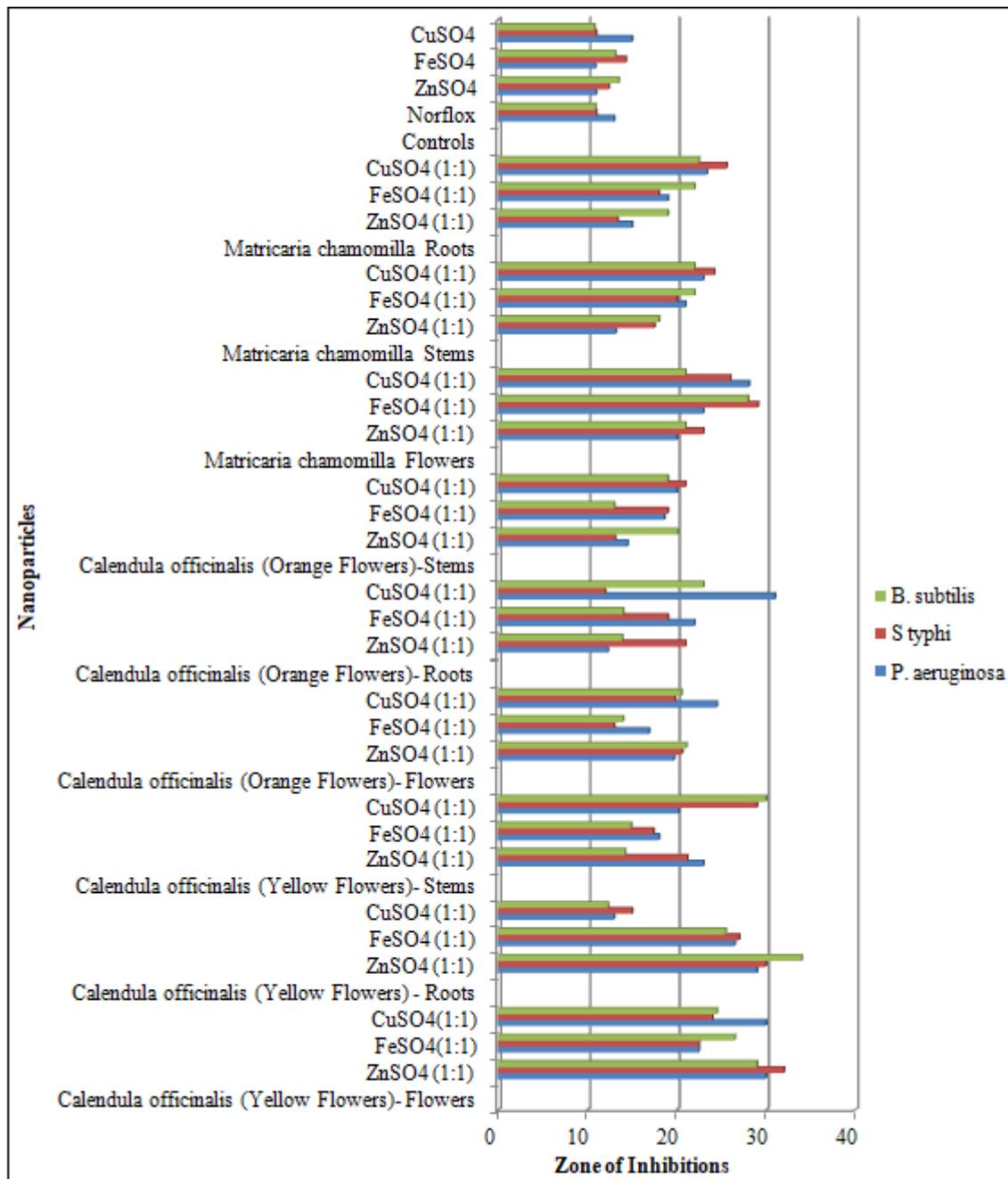


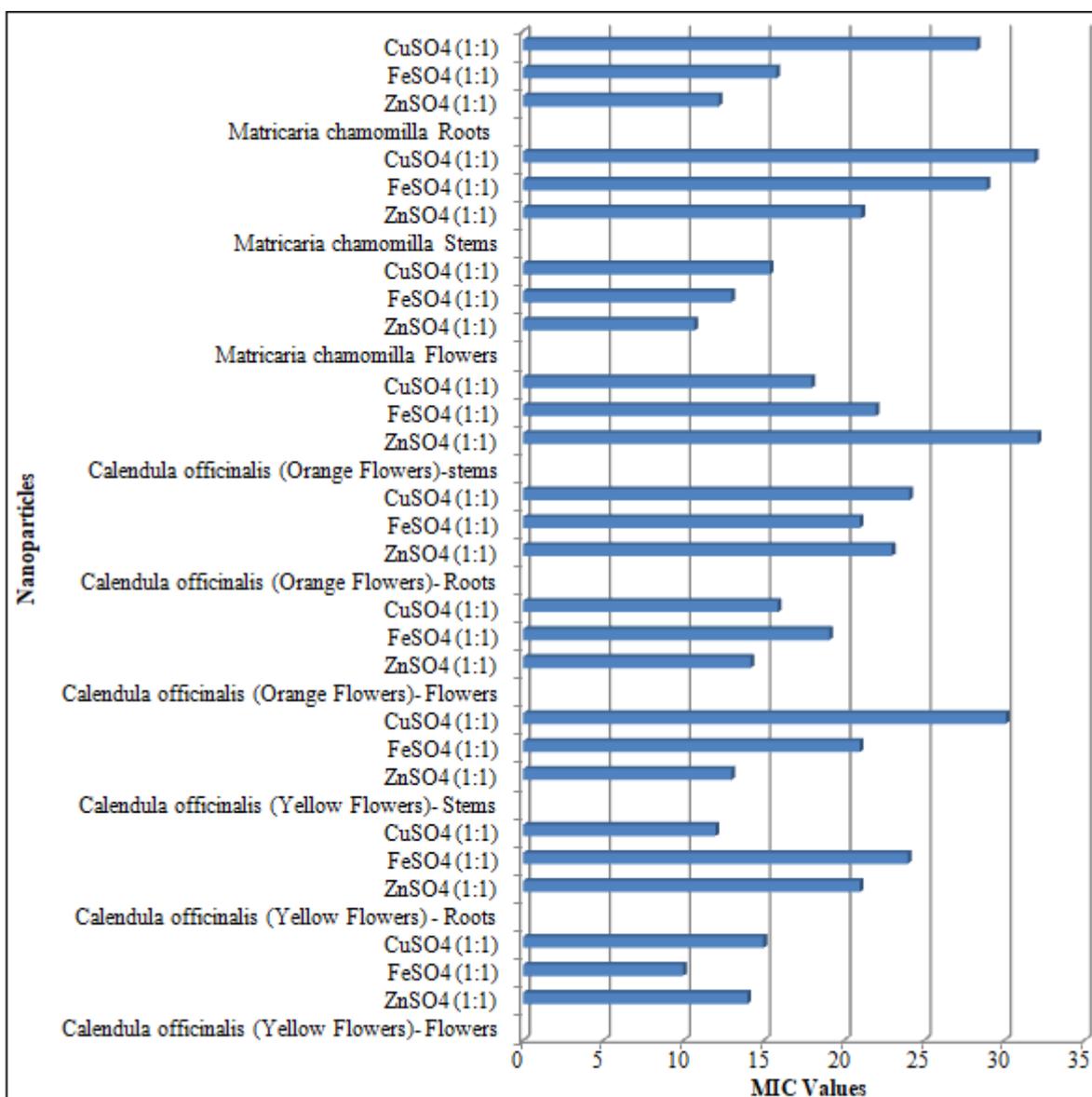
Figure 2: Graphical representation of the antimicrobial test for nanoparticles against bacterial pathogens

3.3 Minimum Inhibitory Concentration Test

**Table 3:** MIC value calculated for nanoparticle of calendula officinalis (yellow flower), of calendula officinalis(orange flower), Matricaria chamomilla flower against B subtilis

| S no.    | Nanoparticles  | MIC (mg/ml) |
|----------|--|-------------|
| <b>1</b> | <b>Calendula officinalis (Yellow Flowers)- Flowers</b> |             |
| A        | ZnSO <sub>4</sub> (1:1)                                | 14          |
| B        | FeSO <sub>4</sub> (1:1)                                | 10          |
| C        | CuSO <sub>4</sub> (1:1)                                | 15          |
| <b>2</b> | <b>Calendula officinalis (Yellow Flowers) - Roots</b>  |             |
| A        | ZnSO <sub>4</sub> (1:1)                                | 21          |
| B        | FeSO <sub>4</sub> (1:1)                                | 24          |
| C        | CuSO <sub>4</sub> (1:1)                                | 12          |
| <b>3</b> | <b>Calendula officinalis (Yellow Flowers)- Stems</b>   |             |
| A        | ZnSO <sub>4</sub> (1:1)                                | 13          |
| B        | FeSO <sub>4</sub> (1:1)                                | 21          |
| C        | CuSO <sub>4</sub> (1:1)                                | 30.1        |
| <b>4</b> | <b>Calendula officinalis (Orange Flowers)- Flowers</b> |             |
| A        | ZnSO <sub>4</sub> (1:1)                                | 14.2        |
| B        | FeSO <sub>4</sub> (1:1)                                | 19.1        |
| C        | CuSO <sub>4</sub> (1:1)                                | 15.9        |

|          |  |      |
|----------|--|------|
| <b>5</b> | <b>Calendula officinalis (Orange Flowers)- Roots</b> |      |
| A        | ZnSO <sub>4</sub> (1:1)                              | 23   |
| B        | FeSO <sub>4</sub> (1:1)                              | 21   |
| C        | CuSO <sub>4</sub> (1:1)                              | 24.1 |
| <b>6</b> | <b>Calendula officinalis (Orange Flowers)-stems</b>  |      |
| A        | ZnSO <sub>4</sub> (1:1)                              | 32.1 |
| B        | FeSO <sub>4</sub> (1:1)                              | 22   |
| C        | CuSO <sub>4</sub> (1:1)                              | 18   |
| <b>7</b> | <b>Matricaria chamomilla Flowers</b>                 |      |
| A        | ZnSO <sub>4</sub> (1:1)                              | 10.7 |
| B        | FeSO <sub>4</sub> (1:1)                              | 13   |
| C        | CuSO <sub>4</sub> (1:1)                              | 15.4 |
| <b>8</b> | <b>Matricaria chamomilla Stems</b>                   |      |
| A        | ZnSO <sub>4</sub> (1:1)                              | 21.1 |
| B        | FeSO <sub>4</sub> (1:1)                              | 28.9 |
| C        | CuSO <sub>4</sub> (1:1)                              | 31.9 |
| <b>9</b> | <b>Matricaria chamomilla Roots</b>                   |      |
| A        | ZnSO <sub>4</sub> (1:1)                              | 12.2 |
| B        | FeSO <sub>4</sub> (1:1)                              | 15.8 |
| C        | CuSO <sub>4</sub> (1:1)                              | 28.3 |



**Figure 3:** Graphical representation of MIC values of nanoparticles against B subtilis

### 3.4 Phytochemical Analysis

**Table 4:** Test for different phytochemical analysis was done and results were observed.

| S no. | Samples  | Steroids | Flavonoids | Tannin | Terpenoids | Carbohydrate | Saponin |
|-------|--|----------|------------|--------|------------|--------------|---------|
| 1.    | <i>Calendula officinalis</i> (Yellow Flowers)- Flowers | +        | +          | +      | +          | +            | +       |
| 2.    | <i>Calendula officinalis</i> (Yellow Flowers) - Roots  | -        | -          | -      | +          | +            | +       |
| 3.    | <i>Calendula officinalis</i> (Yellow Flowers)- Stems   | +        | +          | -      | -          | +            | -       |
| 4.    | <i>Calendula officinalis</i> (Orange Flowers)- Flowers | -        | -          | -      | -          | +            | -       |
| 5.    | <i>Calendula officinalis</i> (Orange Flowers)- Roots   | -        | -          | -      | +          | +            | +       |
| 6.    | <i>Calendula officinalis</i> (Orange Flowers)- Stems   | -        | -          | +      | +          | +            | +       |
| 7.    | <i>Matricaria chamomilla</i> Flowers                   | +        | +          | +      | +          | +            | +       |
| 8.    | <i>Matricaria chamomilla</i> Roots                     | -        | -          | -      | -          | +            | -       |
| 9.    | <i>Matricaria chamomilla</i> Stems                     | +        | -          | +      | -          | +            | -       |

### 4. Discussion

Nanotechnology can be explained as technology that can overwhelm the simple mechanisms of drug release and modified antibiotic work action that would aid to kill the mutated bacteria. The sample used Marigold orange, Marigold yellow, and Chamomile have all important photochemical that hinder the growth of pathogens. Metal Nanoparticles prepared by ZnSO<sub>4</sub> and flower of Marigold yellow against gave best results *S.typhi*. The nano- drug creation was done with preparing various concentrations of the salts of metals combined with various concentrations with the desired extract and hold for incubation of 24hrs . the shifts and the metal ions after reaction were then admitted for magnetic stirrer methods and centrifuge method. The magnetic stirrer method was fruitful as it employed rotations for time period also the specimen was heated, the color alteration before and after the use of magnetic stirrer expressed the increased kinetic energy and so the color alteration depicts the precipitation of the nano particles in the extract. The discriminative studies of the best concentrations of our antibiotic were examined with the marketed drugs. One of which overcome the market drug.

### 5. Conclusion

The multi drug resistance are a new complication basically in the medical sectors, the probability of the infections are huge there. The newly altered and developed pathogens have the tendency to unaffected from various antibiotics. The entry of the antibiotics is made easy with the tiny sized particles. The employment of compound that has antimicrobial activity can be employed also its working efficiency can be intensified. The nano particles were constructed through magnetic stirrer methods. The application of these methods was to easy to examine the preparation of nanoparticles. The assessment was analyzed by using the antimicrobial susceptibility test of the prepared nanoparticles. The best concentrations were analyzed against with the marketed drugs. Prepared nanoparticle drug defeated norfloxacin and gave best ZOI against the pathogen .The MIC also depicted the better concentrations against pathogens. The medically essential strains are susceptible to the phytochemicals that's why sample were analysed for the phytochemical tests.

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