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# Green synthesis of silver nano particle and study the antimicrobial activity from *Mentha arvensis* Leaf's

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**Abstract**
In this study shows a presence of secondary metabolites in the crude extract of methanolic from *Mentha arvensis* such as steroids, reducing sugar, sugars, alkaloids, phenolic compound, flavonoids, saponins, tannins and amino acids but the absence of Catechins. These secondary metabolites are used for different therapeutic applications.For example tannins possess several mechanisms: antioxidative, antiviral, antitumor, antithrombogenic and anti-inflammatory properties.Reduction of silver ions in aqueous solution of silver complex during the reaction with plant extract have been confirmed by color changes. The UV spectral analysis of silver about the SPR bands at 330nm is the absorbance is emitting from silver nanoparticles is shown.The SPR bands are corresponds to surface Plasmon vibration resonance in the AgNPs, whereas the peak range are occurs due to excitation of SPR bands formation.Figure 5 shown the absorption peak ranges in FTIR spectra analysis of synthesized AgNPs from plant extract. The peak of reduced silver at 1699 cm−1.The presence of bonds due to C=C stretching (around 1621cm−1) which predicts the presence of alkenes, Stretching of Polyols C-O (around 1159cm−1),Stretching of 1° amine N-H (around 3377 cm−1),Stretching of carbonyl C=O (1735 cm−1). The bio constituents are interacting with silver ions and mediate the reduction process with their functional groups. In SEM micrograph shown a particle size of silver particle from extract are formed at 1000 nm to 10000 nm. The comparative study of antimicrobial activity and antibiotic activity of synthesized silver nanoparticle from *Mentha arvensis.*The antimicrobial activity are more active against the tested microorganisms shown in.Possess a better inhibition while comparing to the tested antibiotics.Finally, here we concluded that extract could be consider as an antibiotic.Instead of antibiotic drugs, this plant extract having the capacity to cure a disease.

**Keywords:** Mentha Arvensis, Uv Spec, FTIR, Nano Particle

## Introduction
In modern medicine, around a quarter of the drugs prescribed to patients are derived from medicinal plants, and they are rigorously tested. The Food and Agriculture Organization estimated in 2002 that over 50,000 medicinal plants are used across the world. A medicinal plant is used to maintain a health and is to be administered for a specific condition or both, whether in modern medicine or in traditional medicine.
The Royal Botanic Gardens, few 30,000 plants for which a use of any kind is documented. The World Health Organization formulated a policy on traditional medicine in 1991, and since then has published guidelines for them, with a series of monographs on widely used herbal medicines. The use of plant-based materials including herbal or natural health products with supposed health benefits are increased in developed countries.
This brings attendant risks of toxicity and other effects on human health, despite the safe image of herbal remedies. The botanical survey of India has prepared a provisional list of threatened plants which includes large number wild (or) wild relatives of food, horticultural, medicinal and aromatic plants. In some cases it becomes difficult to grow them (or) it may not even survive. In certain other cases if survives and grows but may not be producing the desired traits. An understanding of the biological and ecological back ground of the species in their normal habitat is also essential to understand their conservation biology as well as to predict their behavior under artificial cultivation.

## Mentha Species
***Mentha*** is known as **mint**, derived from Greek word *míntha*. It is a genus of plants in the Lamiaceae family (mint family).It is estimated that 13 to 18 species exist, and the exact distinction between species is still unclear. Plants from genus *Mentha* are used for antimicrobial, antiviral and insecticidal activity (Johnson *et al*., 2011) [8]. Hybridization between some of the species occurs naturally. The genus has a sub cosmopolitan distribution across Europe, Africa, Asia, Australia, and North America (Biswas *et al*., 2017). Mints are aromatic and they have wide-spreading underground and over ground stolons and erect, square and branched stems. Mints will grow 10–120 cm tall and can spread over an indeterminate area.

### *Mentha arvensis*
Mentha arvensis is a species of flowering plant in Lamiaceae family.Its common names include Field Mint, Wild Mint or Corn Mint; however, it is better known by its scientific name Mentha arvensis. They are also found in the eastern regions of Himalaya, various regions of North America and Eastern Siberia. These plants grow well at riverbanks, edges of marshes and moist prairies. It is quite easy to grow these plants from seeds. These seeds can be sown during fall; however, one can also sow them in spring after refrigerating them for 2 to 3 months.

### Scientific Classification
Kingdom: Plantae

Clade: angiosperms
Clade: Eudicots
Clade: Asterids
Order: Lamiales
Family: Lamiaceae
Genus: Mentha
Species: M. arvensis
Binomial name: *Mentha arvensis*

**Fig 1**

**Health Benefits of *Mentha arvensis***

Many health benefits can be derived from the Field Mint plants and their essential oil.The Mint leaves can work as blood cleanser with their antibacterial and antiseptic properties.It can alleviate mouth ulcers, toothache and swollen gum. Fresh leaves can cure headache and dizziness.Mint leaves can relief arthritis and joint pains.The leaf infusion helps in curing health disorders like dysmenorrheal, stomachache and diuresis.These plants have antispasmodic and anesthetic properties.

- **Objectives**

1. The preliminary phytochemical screenings of methanolic extract from Mentha arvensis are performed.
2. Present study is focused on synthesize the silver nanoparticle from this plant methanolic extract and its characterization study is done by using UV visible Spec, FTIR, and SEM.
3. Investigate the antimicrobial activity.

**Material and Methods**

**Plant material collection and preparation of extract**

*Mentha arvensis* fresh leaves was collected from common market. The selected fresh leaves could be taken without any infection and it was dried in shadow for six days.After six days, weigh the dried leaves and its weight is 47 grams. The dried leaves are grounded like a fine powder.

**Chemical used**

**Methanol**

The fine powder of *Mentha arvensis* was dissolved in 250 ml of methanol solvent. The whole extraction process was done by using soxhlet apparatus (hot method). Packed a cotton with inside the sample holder.The dissolved fine powder was taken in the bottom flask and begins the soxhlet extraction.The solvent was evaporated and dried the extract for 15 days. After 15 days, the sample was used for further study.

**Preliminary phytochemical studies**

The Soxhlet crude extract of methanolic solvent from *Mentha arvensis* was used for preliminary phytochemical studies. The screening of preliminary phytochemical components is followed by using standard method are given below in Table1 (Brindha *et al*., 1981)

**Synthesis of silver nanoparticles**

**Chemical used**

The preparation of 1 mM aqueous extract of silver nanoparticle.For the synthesis of silver nanoparticle, 2 ml of dissolved methanolic extract and 8 ml of 1 mM silver nitrate was taken in 15 tubes and one tube was maintained for control without adding silver nitrate solution. It was incubated for 24 hrs at light condition. The formation of synthesized silver nanoparticles from plant extract was used for centrifuge at 5000 rpm. Collected the pellet for further study and discard the supernatant. The pellet was dried to evaporate the solvent and kept in micro oven.Finally collected the pellet as a semisolid in nature.The silver nanoparticles was confirmed by color changes and it is characterized by UV Visible Spectrophotometer, FTIR for chemical analysis and SEM

**Uv visible spectroscopy**

The UV-Visible Spectroscopy is the most valuable tool for identifying, characterizing and studying nanomaterial. It is measures the extinction (scatter absorption) of light by passing through a sample. Nanoparticles must have unique optical properties that are sensitive to the size, shape, concentration, agglomeration state, and refractive index near the nanoparticle surface.

**Ftir**

Fourier Transform Infrared Spectroscopy is otherwise known as FTIR analysis or FTIR spectroscopy. FTIR is used to identify the organic material, inorganic material and polymeric substances. It was used to scan test samples by using infrared light and observe its chemical properties.

**Sem**

SEM is known as Scanning Electron Microscope. Scanning electron microscope magnification ranges from 15x to 200,000x and their resolution power is 50Å.SEM provides high resolution images of the sample by rastering a focused electron beam across the surface and detecting secondary or backscattered electron signal.It gives the 3-D structure of objects to reveal the structure.

**Antimicrobial activity**

The synthesized silver nanoparticle was used for the investigation of antimicrobial activity.The antimicrobial

assay was done on human pathogenic bacteria like gram Positive bacteria and gram negative bacteria. Gram negative bacteria are *Klebsiella Pneumoniae, E.Coli* and Gram positive bacteria are *Staphylococcus aureus, Bacillus subtilis* are used by the disc method.Nutrient broth medium was prepared in 50 ml conical flask. *Klebsiella Pneumoniae, E.Coli Staphylococcus aureus* and *Bacillus subtilis* these bacteria was subcultured in nutrient broth at overnight at $37^0$c

Prepared the Nutrient agar medium and poured on sterile petriplates. The sub cultured bacteria was inoculated by swab on the nutrient medium surface by spread out the four bacteria (*Klebsiella Pneumoniae, E.Coli, Staphylococcus aureus, Bacillus subtilis)*.Place the sterile disc on nutrient medium plate.After load four different concentrations of synthesized AgNPs from plant extract such as 20µl, 40µl, 60µl, 80µl on respective sterile discs and it was incubated at 37 °C for 24 hrs. The minimum inhibitory zone was formed after incubated and one was measured.

## Results

### Extraction

The most of the components are eluted out from methanolic extract of *Mentha arvensis.Thick green colour was formed.*The crude extract was collected

**Fig 2:** Preliminary phytochemical Screening of Methanol Extract

**Table 1**

| S.NO | CHANGES OF COLOR | Methanol Extract |
|---|---|---|
| 1. | Steroids: Brown ring formed | +ve |
| 2. | Reducing sugars: red or orange | +ve |
| 3. | Sugars: green or purple | +ve |
| 4. | Alkaloids: white precipitate or turbidity | +ve |
| 5. | Phenolic compound: blue color | +ve |
| 6. | Catechins: pink color | -ve |
| 7. | Flavonoids: red or orange | +ve |
| 8. | Saponins: foamy leather formed | +ve |
| 9. | Tannins: white | +ve |
| 10. | Amino acids: blue or violet | +ve |

### Synthesized of Silver nanoparticle

The mixture of silver nanoparticle solution and *Mentha arvensis* extract was dark green before the reaction occurs.After that added a 1mM of silver nitrate solution into the extract.The reaction of mixture became pale yellow to dark brown within 24 h when treated with the leaf extract of *Mentha arvensis* at room temperature.The formation of dark brown color shows a AgNps extract.

**Fig 3**

### Uv-vis spectroscopy

The synthesis of silver nanoparticles from *Mentha arvensis* extract was primarily characterized by UV-Visible spectroscopy analysis. The size of the synthesized silver nanoparticles was identified by using the spectral analysis. Shown the stable level of absorbance peak at 330 nm in the sample

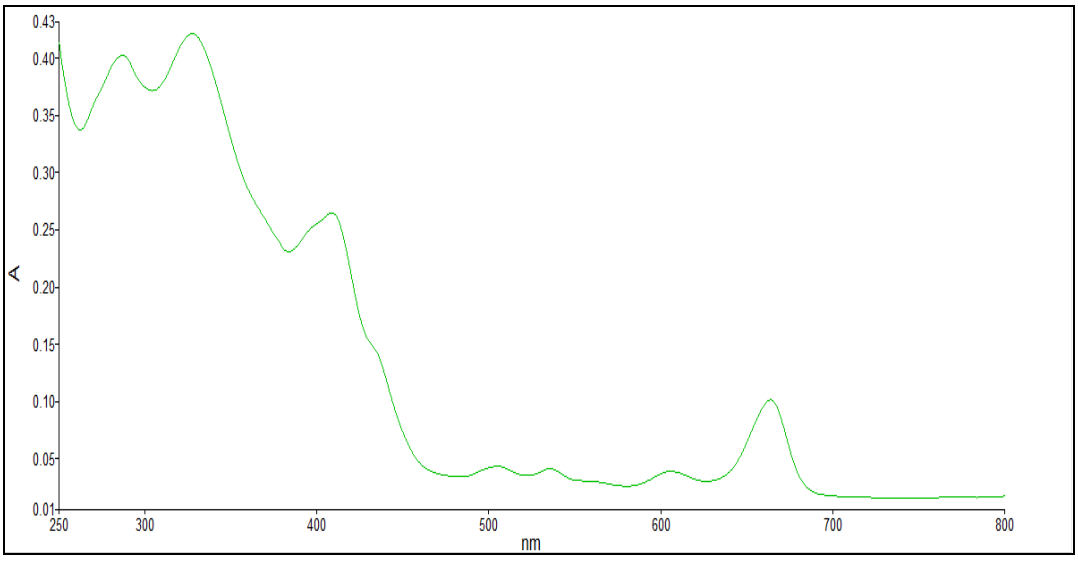


**Fig 4:** UV Visible Spectral analysis of Synthesized AgNPs from Mentha arvensis extract.

**Ftir**

The FTIR measurement is used to identify a major compound which is responsible for the biological reduction of silver ions into synthesized AgNPs from the Mentha arvensis extract.

Fourier transform infrared spectroscopy analysis showed an absorption peaks of reduced silver at 1699 cm−1.The stretching vibration of C=C obtained at 1621cm−1 and the single absorbance peak located at 1159 cm−1 is Assigned to C-O Polyols, while 3377 and 1735 cm−1 corresponds to N-H and C=O stretching vibration shows in peak.

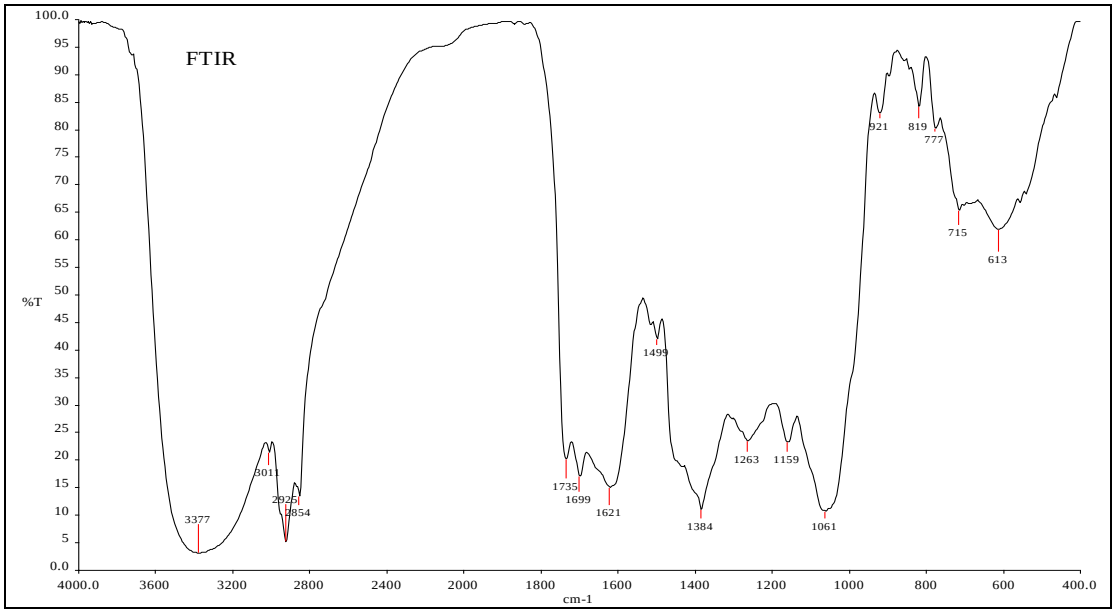


**Fig 4:** FTIR analyses for AgNo3 synthesis of *Mentha arvensis* extract.

- **Sem**

SEM analysis represents the better particle size in 1000nm.In 1µm shown at 30000 x magnifications, 3µm shown at 15000 x magnifications. The crystal like structure of silver nanoparticle are formed in SEM analysis shows.

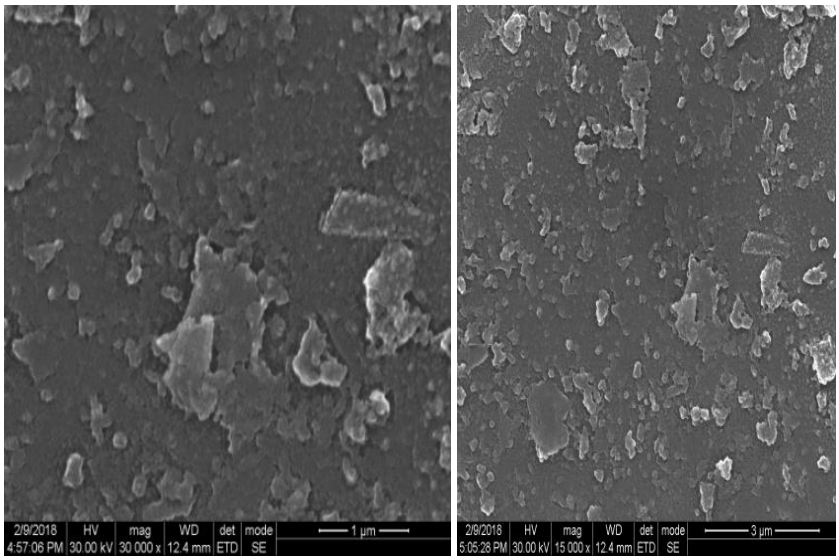


**Fig 5:** The SEM Micrograph of synthesized silver nanoparticles from *Mentha arvensis* extract (A)1µm of 30000x magnification (c) 3 µm of 15000x.

- **Antimicrobial activity**

The antimicrobial activity has been studied against the gram positive and gram negative bacteria organism at various concentration of sample.In *Klebseilla pneumoniae*, shown formation of zone inhibition and their concentration level on 19 mm in 80 µl, 14 mm in 60µl,11mm in 40µl, 10 mm in

**Table 2**

| S.No | Microorganisms | Concentration level (µl) | | | |
|---|---|---|---|---|---|
| | | 2 80 | 4 60 | 40 | 20 |
| 1. | *Klebsiella pneumoniae* (M1) | 19 | 14 | 11 | 10 |
| 2. | *E.coli* (M2) | 19 | 14 | 11 | 10 |
| 3. | *Staphylococcus aureus*(M3) | 12 | 15 | 12 | 10 |
| 4. | *Bacillus subtilis* (M4) | 19 | 15 | 19 | 10 |

20µl. In *E.Coli*, shown formation of zone inhibition and their concentration level on 19 mm in 80 µl, 14 mm in 60µl, 11mm in 40µl, 10 mm in 20µl. In *Staphylococcus aureus*, shown formation of zone inhibition and their concentration level on 12 mm in 80 µl, 15 mm in 60µl, 12mm in 40µl, 10 mm in 20µl. In *Bacillussubtilis*, shown formation of zone inhibition and their concentration level on 19 mm in80µl, 15 mm in60µl, 10mm in40µl, 10mm in20ul

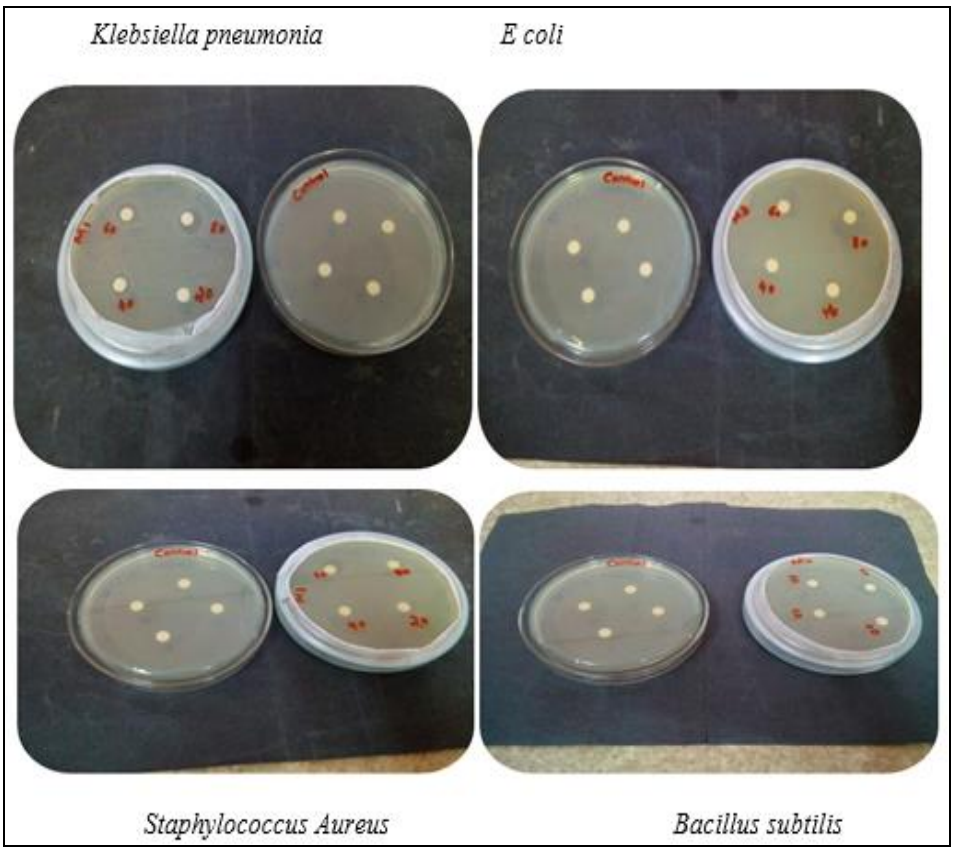


**Fig 6**

The inhibition zone diameter of synthesized AgNPs from *Mentha arvensis* in antibacterial activity of a) *Klebsiella pneumoniae* b) *E.Coli* c) *Staphylococcus aureus* d) *Bacillus subtilis* was observed.

- **Antibiotic activity**

The antibiotic activity were tested against *Klebsiella pneumoniae*(M1),*E.Coli*(M2) *Staphylococcus aureus*(M3), *Bacillus*(M4) by using streptomycin, tetracycline ampicillin,penicillin are inhibited the growth of microorganisms.The zone inhibition diameter are shows in Table6.In E.Coli,shows a better zone of inhibition in *streptomycin* and *tetracycline* and its inhibition zone diameter is 19mm compare to Klebsiella pneumonia,*Bacillus subtilis* bacteria are active against *streptomycin* and *tetracycline* and its zone diameter is 12 mm to 14mm.The *Staphylococcus aureus* are active against *streptomycin* only with 15 mm zone formation. There is no zone formation in *tetracycline*.In these four bacterial cultures are not active against *ampicillin* and *penicillin* antibiotics.

**Table 3**

| Microorganism | High level of common Antibiotic zone | High level zone of synthesized AgNPs | Microliter of synthesized AgNPs | Common high level zone form Antibiotics |
|---|---|---|---|---|
| Klebsiella pneumoniae (M-1) | 14mm | 14mm | 60,80 | Streptomycin |
| E. Coli-(M-2) | 19 mm | 19mm | 80 | Streptomycin,Tetracycline |
| Staphylococcus aureus (M-3) | 15mm | 15mm | 60 | Streptomycin |
| Bacillus (M4) | 16mm | 19,15mm | 19(80µl) 15mm (60µl) | Streptomycin |

**Table 4:** The inhibition zone diameter of antibiotic activity.

| S.no | Micro organisms | SPN | TCN | AMP | PEN |
|---|---|---|---|---|---|
| 1. | *Klebsiella pneumoniae* (M1) | 14 | 12 | NZ | NZ |
| 2. | *E.coli* (M2) | 19 | 19 | NZ | NZ |
| 3. | *Staphylococcus aureus*(M3) | 15 | NZ | NZ | NZ |
| 4. | *Bacillus subtilis* (M4) | 16 | 13 | NZ | NZ |

NZ-No Zone formation

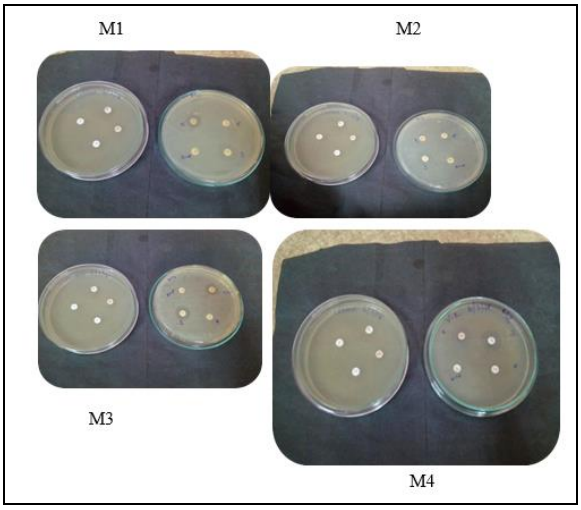


**Fig 7**

**Comparative study of antimicrobial and antibiotic activity**

The antimicrobial activity of green synthesized silver nanoparticles extract was given a better zone of inhibition against those particular four bacterial microbes while comparing the antibiotic drugs inhibition. That the extract can act as an antibiotic drug. Instead of standard common antibiotic drugs like Streptomycin, tetracycline, ampicillin and penicillin, so we can use this extract for tested against bacterial cultures causing diseases. The comparison of antimicrobial activity and antibiotic activity zone formation were shown

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