



## **Synergistic effect of antibacterial properties of herbal silver nanoparticles on microorganisms**

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### **Abstract**

Nowadays people are adopting our tradition of Ayurveda, not in India but in other foreign countries are also showing their interest in Ayurveda. Herbal medicines are more effective than allopathy or homeopathy. In the field of nanotechnology, we can research the antibacterial characteristics of herbs and improve their effects by working with nanoparticles. In this study, we have synthesised individual and combined silver nanoparticles of swertia chirata, cinnamomum verum, thymol crystal, Tribulus terrestris, curcuma longa, and tinospora cordifolia to examine the antibacterial effects of these plants. We have done characterization methods for silver nanoparticles like UV-VIS, SEM (scanning electron microscope). We have isolated pathogenic bacteria from blood sample and did characterisation method like gram staining to identify microorganism whether it is gram positive or gram negative. We have seen the effect of the antibacterial properties of these herbs on isolated pathogenic bacteria from blood and E. coli.

**Keywords:** Nanoparticle, Herbal silver nanoparticles, Antibacterial, Multiple drug resistance, E.coli, Staphylococcus, UV-vis, SEM.

### **Introduction**

Currently, the most fascinating field of research is nanoparticles. Through hyperthermia, which is the localised heating and tissue destruction of such particles by an alternating magnetic field, these particles can also be utilised to kill cancers. Nanoparticles can be made to improve fluorescence imaging, positron emission tomography (PET), or ultrasound pictures. They have special characteristics due to their increased surface area, which enhances their mechanical, magnetic, optical, and catalytic capabilities and raises their potential for medicinal use (Mauricio et al., 2018), (Borm et al., 2006). There are various herbs in India which have medicinal properties like antibacterial, antifungal, antioxidant, etc. we have chosen 6 variety of herbs for this study, these are swertia chirata, cinnamomum verum, thymol crystal, Tribulus terrestris, curcuma longa, and tinospora cordifolia. swertia chirata, it has multiple properties like antifungal, antibacterial, and it is also useful for sugar patients to control sugar level and Historically used to cure a number of ailments including diabetes, malaria, and liver diseases (Kumar et al., 2016), (Gurib-Fakim et al., 2006). cinnamomum verum, It eases the discomfort of menstruation. According to a study, women should drink a cup of warm cinnamon water every day since it temporarily reduces the pain associated with menstruation and it has been reported to have anti-diabetic, antibacterial, antioxidant, anti-inflammatory, and anticancer effects. (Pathak et al., 2021). Thymol crystal, commonly known as “Ajwain ke phool”. Thymol (2-isopropyl-5-methylphenol) is a white

crystalline substance that gives thyme its strong flavour, pleasant aromatic odour and strong antiseptic property (Lide et al., 2004). *Tribulus terrestris*, also referred to as "Gokshur" *Tribulus*, belonging to .Kingdom: Plantae, Division: Phanerogams, Subdivision: Angiospermae, Class: Dicotyledonae, Subclass: Polypetalae, Series: Disciflorae, Order: Giraniales, Family: Zygophyllaceae, Genus: *Tribulus*, Species: *terrestris* Linn. It is an annual shrub that can be found in countries like India, China, the southern United States, Mexico, Spain, and Bulgaria that have Mediterranean, subtropical, and desert climates(Chhatre et al., 2014),(Nadkarn et al., 1927),(Pharmacopoeia et al., 1996).It is a short, prostrate shrub with silky hair that grows 10 to 60 cm tall. Pinnae range from five to eight pairs and are elliptic to oblong-lanceolate, with opposing, frequently uneven, paripinnate leaves. Yellow is the colour of flowers. Its carpel fruits have a distinctive, stellate shape, are somewhat rounded, compressed, have five corners, and are covered in very pale yellow prickles (Chhatre et al., 2014).

curcuma longa, a flower of curcuma longa commonly known as "Haldi Ka Phool". It is a medicinal plant that is widely used in Ayurveda, Unani, and Siddha medicine as a natural treatment for a number of illnesses, such as sinusitis, rheumatoid arthritis, anorexia, cough, diabetic wounds, hepatic problems, and biliary disorders(Kumar et al., 2014), (Chattopadhyay et al., 2004). *Tinospora cordifolia* extracts have been reported to scavenge free radicals generated during aflatoxicosis(Saha et al., 2012), (Gupta et al., 2011).

## **Material and methods**

### **Chemicals and instruments**

AgNo<sub>3</sub>, 70% ethanol, distilled water, glassware( culture tubes, beaker, funnel, measuring cylinder, centrifuge tubes, glass rod, spirit lamp) , mortar pestle, filter paper, MH agar, NAM agar, bacterial sample,blood sample, incubator, laminar airflow, SEM, double beam. Spectrophotometer XRD.

### **Plant material/ Herbs**

Herbs were collected from Jeevan sharma herbal shop at Chhattisgarh, Mahasamund, pithora. We have used the complete stem and leaves of *swertia chirata*, *cinnamomum verum*, thymol crystal, *Tribulus terrestris*, *curcuma longa*, and *tinospora cordifolia* . all collected samples were in dried form, we have crushed them in a sterilised mortal pestle and stored them in plastic bags at room temperature.

### **Microorganism**

Microorganism and blood samples were collected from a lab at Raipur.

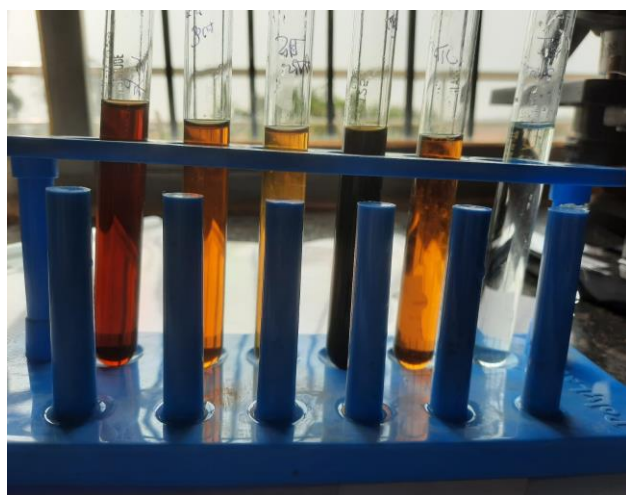
### **Preparation of silver nanoparticles**

To prepare 0.1M silver nitrate, add 1.7gram silver nitrate into 90 ml distilled water and makeup up to 100 ml.

All the steps were performed in the dark to avoid all chemical reactions.

### **Preparation of Plant extract**

2.5 grams herbs were weighed and added into a 6 individual culture tube filled with 50 ml distilled water, mixed properly and soaked all herbs overnight. Next day all herbs were filtered with filter paper and stored in fresh culture tubes. Next plant samples were centrifuged and supernatants were collected in fresh tubes and stored at -4 degree centigrade. Then we mixed aqueous solution of silver nitrate with plant samples in ratio 1:10 and incubated overnight in the dark. All the steps were performed in the dark and samples were stored at -degree centigrade for further use.



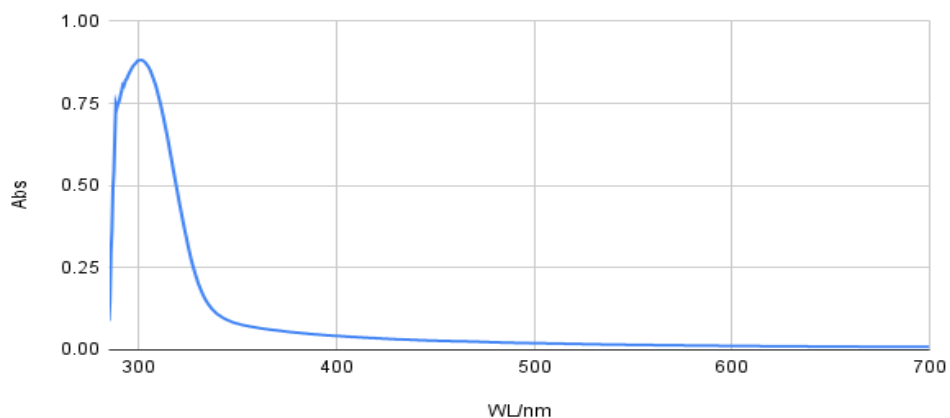
### **Characterisation of nanoparticles**

Characterisation of nanoparticles was done by observation in colour change from light yellow to dark brown, SEM ( scanning electron microscope), and UV- double beam spectrophotometer.

### **UV-Visible absorbance spectroscopy**

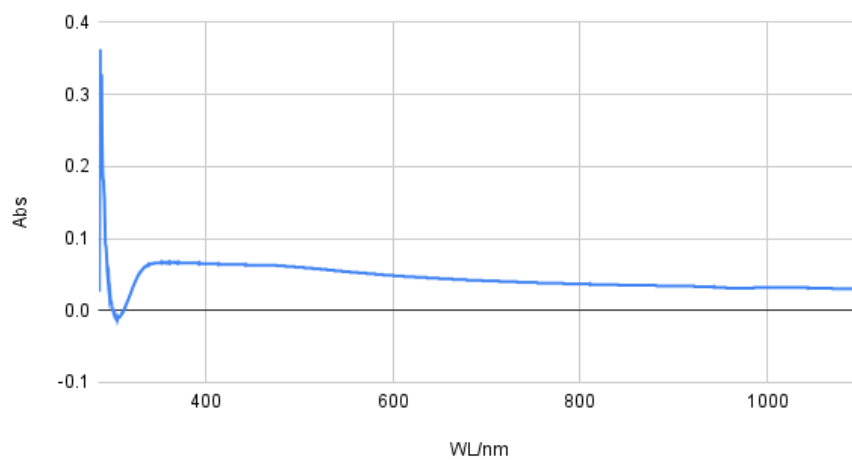
After combining plant extract with silver nitrate for 30 minutes to 24 hours, all samples were examined and all were changed their colour from light yellow/white to dark brown. After 24 hours of incubation, the UV-visible spectrum of the reaction media was measured in order to track the reduction of Ag<sup>+</sup> ions. Each sample was detected between the range of 200 nm and 350nm using the UV-visible spectrum, which was done.

**Abs vs. WL/nm**



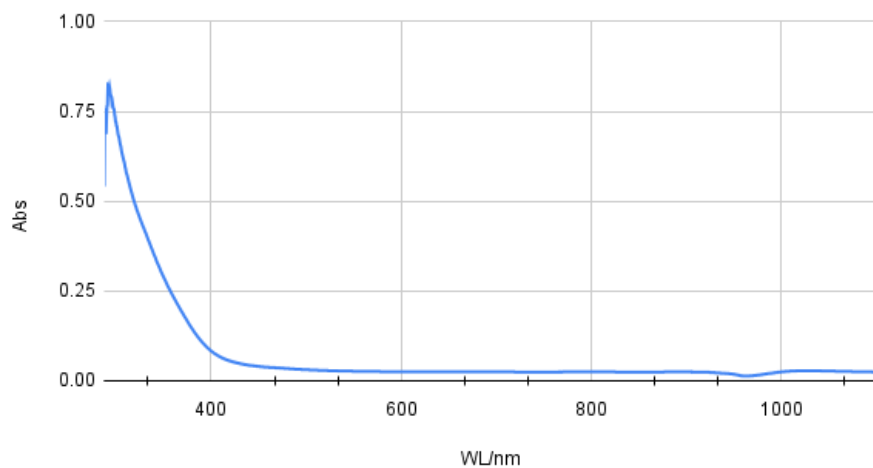
**(Fig.No.1 )Result of UV- visible silver nitrate aqueous solution.**

**Abs vs. WL/nm**



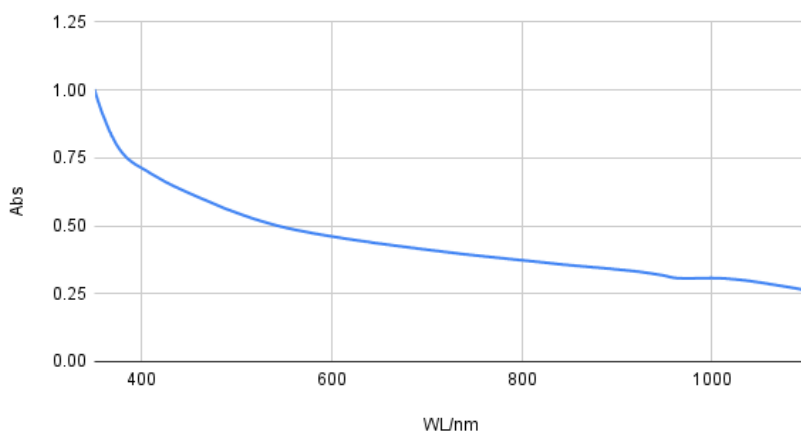
**(fig No. 1.2) result of uv-visible spectroscopy of thymol crystal**

Abs vs. WL/nm

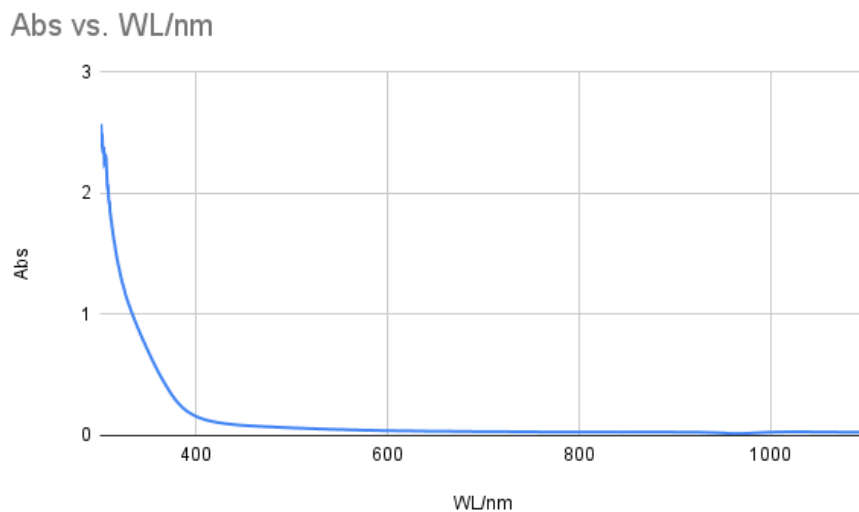


(fig No. 1.3) result of uv-visible spectroscopy of Tribulus terrestris

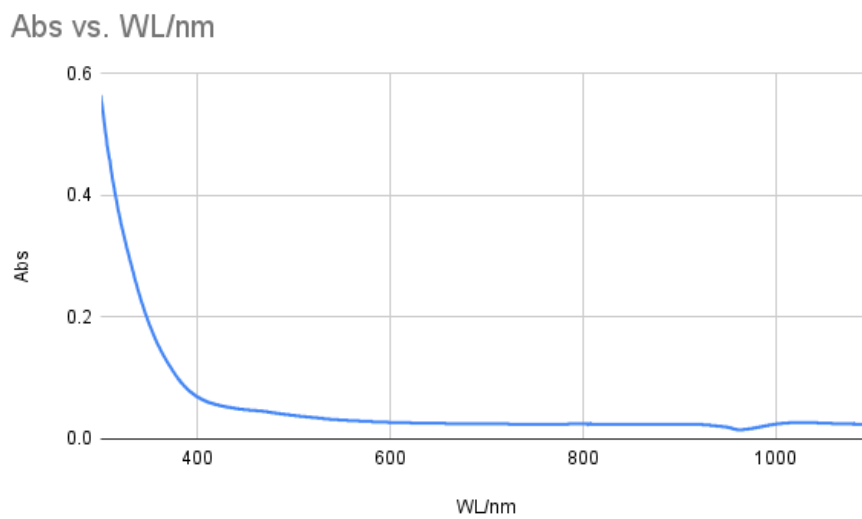
Abs vs. WL/nm



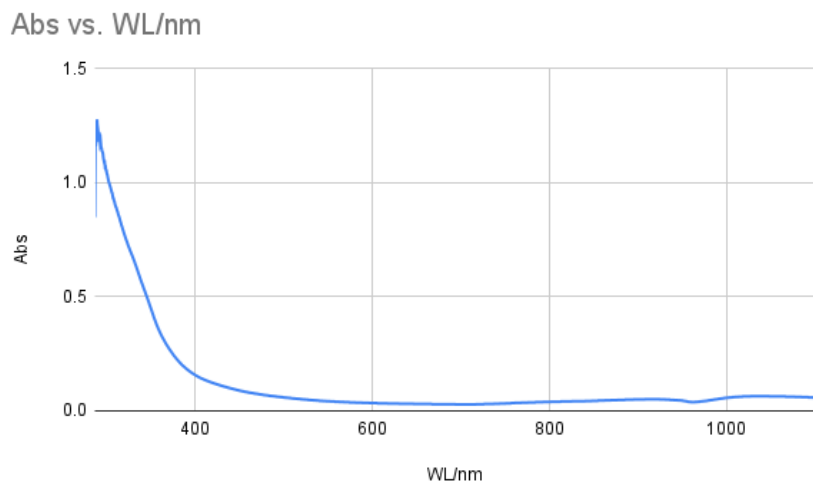
(fig. No. 1.4) Result of uv-visible spectroscopy of cinnamomum verum.



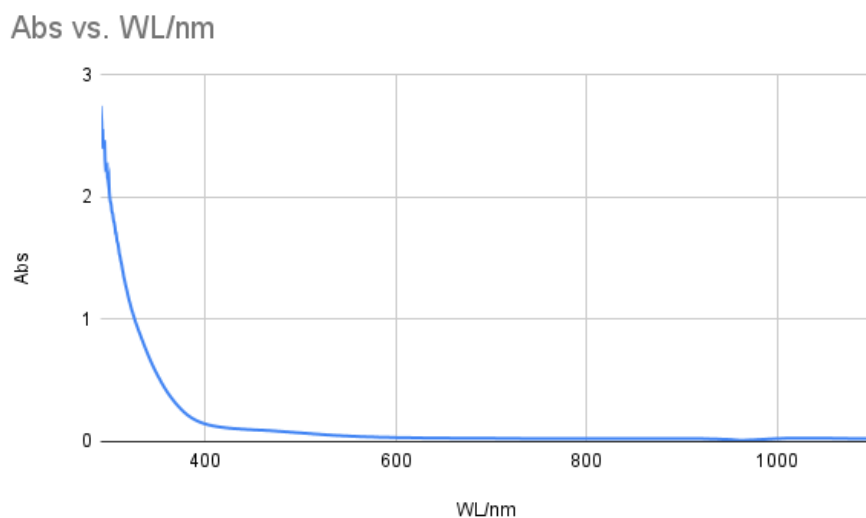
(fig. No. 1.5) result of uv-visible spectroscopy of *Swertia chirayita*.



(fig. No. 1.6) Result of uv-visible spectroscopy of *Curcuma longa* ( turmeric flower)



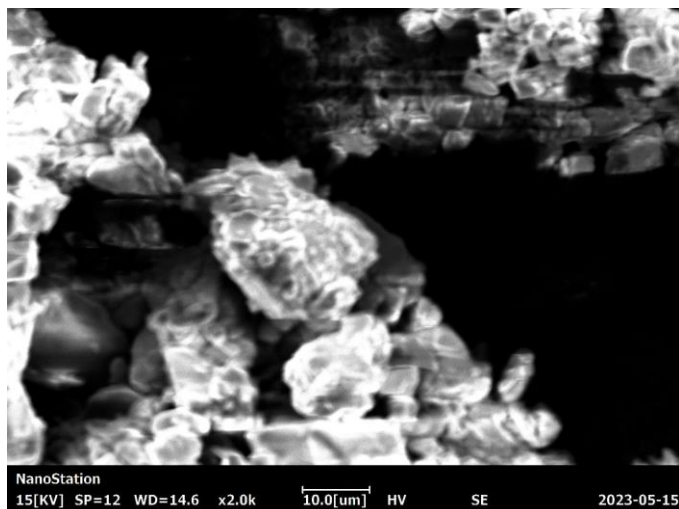
**(fig. No. 1.7) Result of uv-visible spectroscopy of tinospora cordifolia**



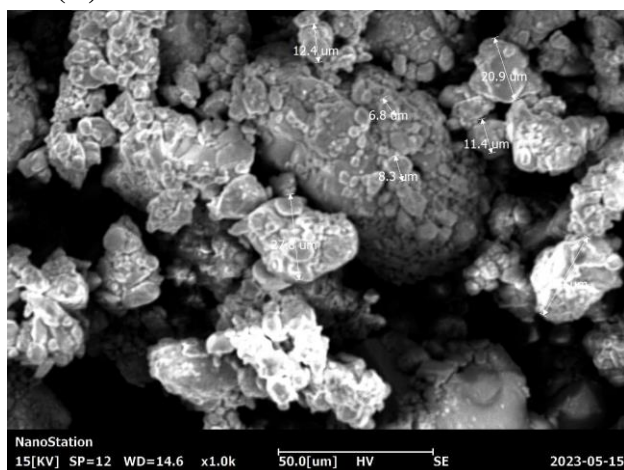
**(fig. No. 1.8) Result of uv-visible spectroscopy of Mixture of all herbs.**

### **SEM ( Scanning Electron Microscope)**

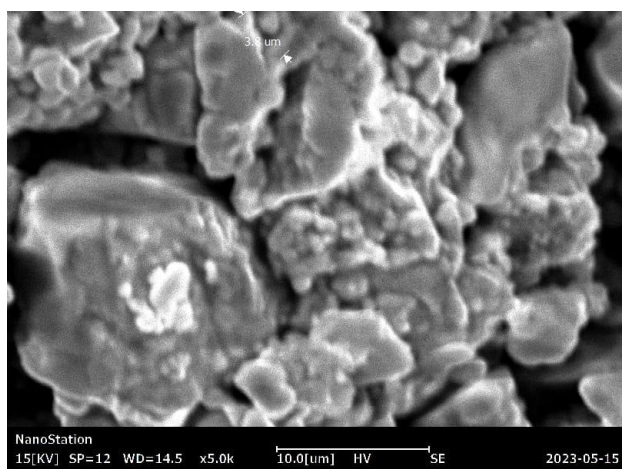
Dry nanoparticle samples were used for the study under the scanning electron microscope. SEM analysis was performed on each sample, and the morphology of each sample was examined.



**(A) *Swertia chirata***

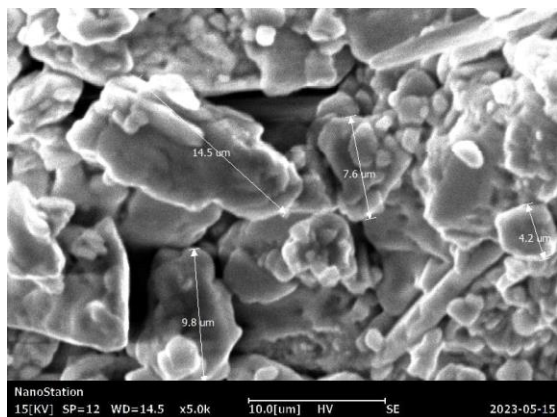


**(B) *cinnamomum verum***

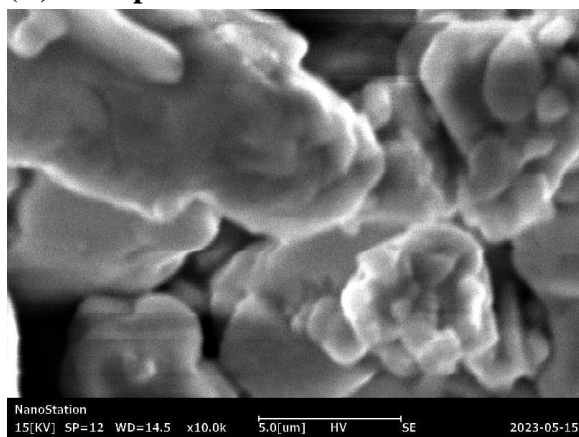


**(C) *Tribulus terrestris***

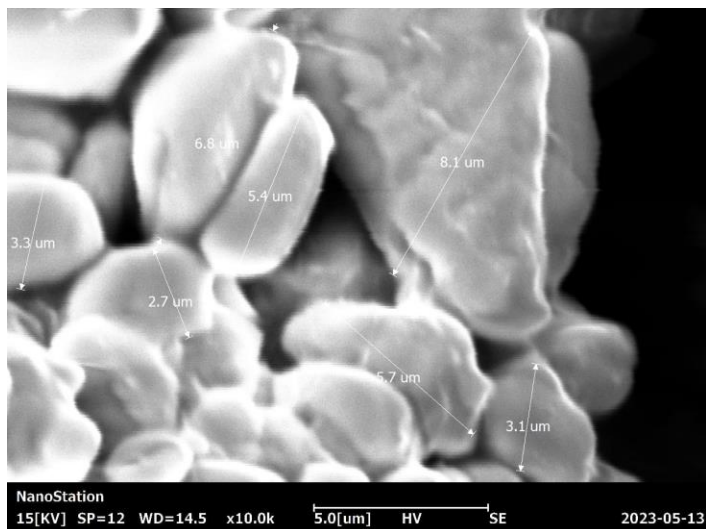




(D) *tinospora cordifolia*



(E) *curcuma longa* ( turmeric flower)



(F) Thymol crystal

### Disc diffusion methods for antibacterial test of herbal silver nanoparticles

First Multiple drug resistant bacteria, E Coli. And staphylococcus were collected from a lab and spread the sample on mueller-hinton agar plates. Disc is prepared by filter paper, disc is treated

with all herbal silver nanoparticles individually and used the disc to test antibacterial activity. All plates were incubated at 37 degree centigrade for 24-72 hours and then observed the plates.

Second pathogenic blood sample is collected from a lab and isolated the pathogenic bacteria from blood and cultured on MH agar. After the isolation of pure culture pathogenic bacteria samples are spread on MH agar plates and treated with herbal silver nanoparticles of herbs individually and incubated the plates at 37 degree centigrade for 24-72 hours then observed the plates

Third, multiple drug resistant bacteria, E coli is spread on MH agar and treated with the mixture of all 6 herbal nanoparticles and incubate it at 37 degree centigrade for 24-72 hours then observe the plate.

## **Observation**

In this observation we got multiple types of results. The impact of all herbal nanoparticles on different types of bacteria has produced different outcomes. We saw an average zone of inhibition in the first test, and a greater zone of inhibition than in the second test.

And in the third test we got a clear plate, no bacterial growth was there in the plate. All data are mentioned in the table below.

<b>(A) Effect of nanoparticles on gram positive bacteria isolated from pathogenic blood</b>		
S. No.	Name of herbal silver nanoparticle	Zone of inhibition (In c.m.)
1	Swertia chirata	1.3
2	Cinnamomum verum	1.5
3	Tribulus terrestris ( fruit )	1.4
4	Tinospora cordifolia	1.4
5	Curcuma longa, (flower)	1.5

6	Thymol crystal	1.5
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<b>(B) Effect of nanoparticles on E. coli.</b>		
S. No.	Name of herbal silver nanoparticle	Zone of inhibition (In mm)
1	Swertia chirata	6
2	Cinnamomum verum	7
3	Tribulus terrestris ( fruit )	10
4	Tinospora cordifolia	10
5	Curcuma longa,( flower)	7
6	Thymol crystal	12

<b>(C) Effect of nanoparticles on Staphylococcus</b>		
S. No.	Name of herbal silver nanoparticle	Zone of inhibition (In mm)

1	Swertia chirata	6
2	Cinnamomum verum	7
3	Tribulus terrestris ( fruit )	7
4	Tinospora cordifolia	9
5	Curcuma longa,( flower)	6
6	Thymol crystal	8

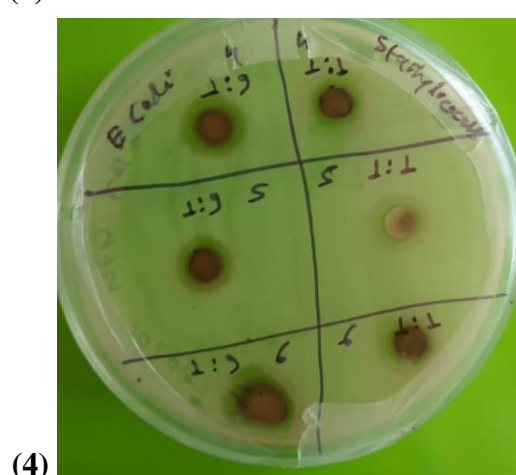
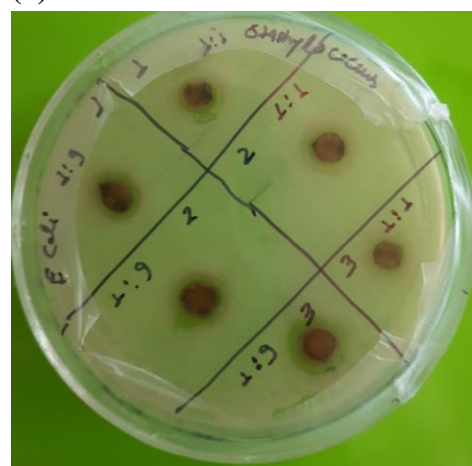
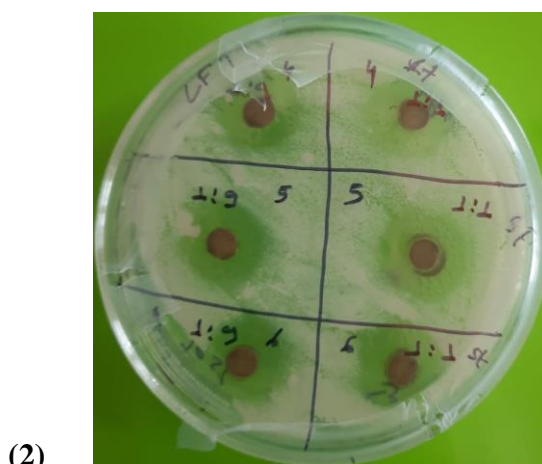
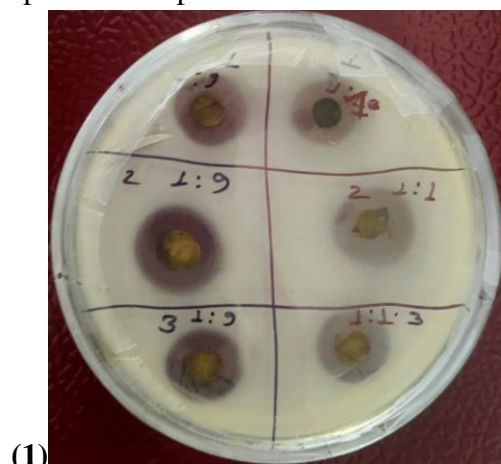
<b>(D) Effect of combination of six herbal nanoparticles on E.coli.</b>		
S. No.	Name of herbal silver nanoparticle	Zone of inhibition
1	Combination of six herbs	No bacterial growth on plates

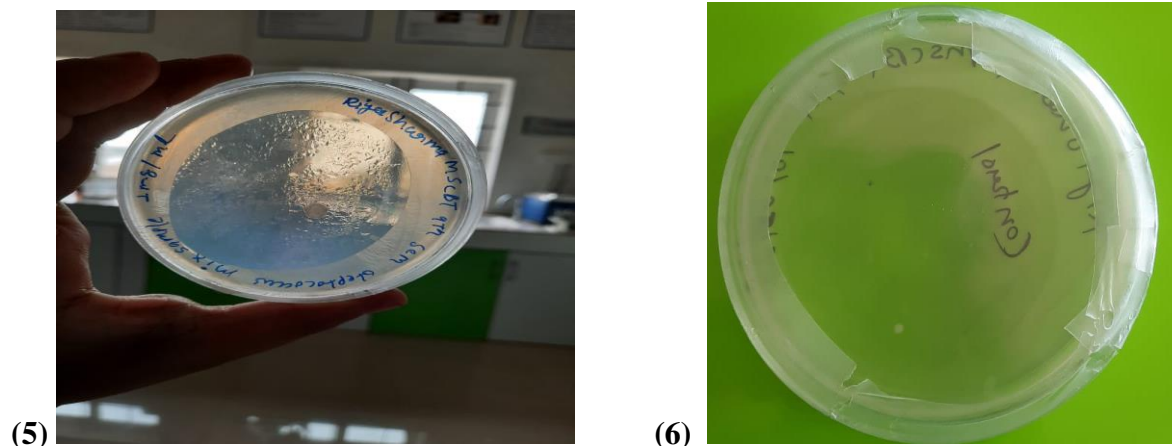
### **Result and discussion**

After mixing plant samples with silver nitrate in order to make silver nanoparticles, all sample colours were changed from light yellow to brown. Then we have analysed swertia chirata, cinnamomum verum, thymol crystal, Tribulus terrestris, curcuma longa, and tinospora cordifolia in UV- vis and observed the absorbance and wavelength of the sample and measured the peak individually. All the peaks of the samples were detected between 200-350 nm of range. After this

we have observed the morphology of swertia chirata, cinnamomum verum, thymol crystal, Tribulus terrestris, curcuma longa, and tinospora cordifolia in SEM and analysed them.

In this study we have analysed six different herbs for their antibacterial properties. We have seen various zones of inhibition of herbs on different bacteria samples. These herbs are very effective on gram positive bacteria and multiple drug resistant bacteria. We have synthesised combined silver nanoparticles of all these six herbs and treated them with multiple drug resistant bacteria, E.coli. And we have observed that there was no growth of bacteria on a plate. There was a clear space on the plate.





(figure No. (1), (2) zone of inhibition of herbal nanoparticles on gram positive bacteria, isolated from blood sample, (3), (4) zone of inhibition of multiple drug resistant bacteria, E.coli and Staphylococcus, (5) effect of combination of six herbs, no growth of bacteria, (6) control).

### Conclusion

On the basis of the result we have obtained the effects of silver nanoparticles of swertia chirata, cinnamomum verum, thymol crystal, Tribulus terrestris, curcuma longa, and tinospora cordifolia. The characterisation is done by change in colour and UV-vis. We have analysed the morphology of all herbal silver nanoparticles, and we observed that the mixture of these all herbs is more effective than individual herbal silver nanoparticles. These all herbal silver nanoparticles are more effective on E.coli. As compared to staphylococcus.

### Acknowledgment

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