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SYNTHESIS OF SILVER NANOPARTICLES FROM *TRACHYSPERMUM AMMI* SEEDS (AJWAIN SEEDS) AND ASSESSING ITS ANTIBACTERIAL ACTIVITY AGAINST TARGET MICROORGANISM *STAPHYLOCOCCUS AUREUS*.

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Abstract : Nanotechnology is a fast growing field in today's world due to its advantages in the field of biology, biotechnology and biomedical science. The present study reports the antibacterial property of various components found in *Trachyspermum ammi* (Ajwain seeds) used for convenient Green Synthesis method for preparation of silver nanoparticles (AgNPs) from it. The nanoparticles of the plant were efficiently synthesised through mixing the *Trachyspermum ammi* (Ajwain seeds) aqueous extract with silver nitrate solution at room temperature following the predetermined procedures for nanoparticle preparation. The prepared AgNPs were identified and characterised by means of spectroscopic and analytical measurements i.e. UV- visible spectroscopy and FESEM. The Antimicrobial property of extracted silver nanoparticles was determined by performing Agar Well diffusion and MIC methods.

Keywords : Nanotechnology, Silver nanoparticles(AgNPs), *Trachyspermum ammi, G*reen synthesis, UV- visible spectroscopy, FESEM, MIC.

INTRODUCTION

A branch of science and technology known as nanotechnology deals with the creation, synthesis, and manipulation of particle formations having diameters between one and one hundred nanometers. Health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, single electron transistors, light emitters, nonlinear optical devices, and photoelectrochemical applications are just a few of the many fields in which nanoparticles (NPs) are used. (Iravani *et al*. 2014)(Neelu *et al*. 2015.)

According to a literature review, silver nanoparticles can be utilized to fight against a variety of pathogenic organisms, including *Staphylococcus*, *Bacillus subtilis*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Syphilis typhus*, and *E. coli* (Sonam *et al*. 2015).
Silver nanoparticles are valuable in nanomedicine and other fields of nanoscience and nanotechnology. Silver nanoparticles were chosen for the study due to their unrivalled properties, including optical, chemical, electronic, photoelectrochemical, catalytic, magnetic, antibacterial, and antimicrobial activities, as well as their low toxicity towards humans. Various nanoparticles, including gold, silver, copper, iron, palladium, and zinc, are synthesized. Silver ion (Ag^+) has been used in wound dressings to treat severe chronic osteomyelitis and urinary infections, to prevent infections in burn patients, to treat blindness in new borns, to control Legionella bacteria in hospitals, and to enhance the effectiveness of drinking-water filters. It may attach to bacterial cells and enzymes (proteins) at various sites by penetrating specific bacterial DNA and RNA, damaging and preventing them from performing their functions and resulting in cell death. Globally, recent advances in nanotechnology and nanoscience have revolutionized how we identify, treat, and prevent diseases. The study of the antibacterial potential of Silver nanoparticles (AgNPs) appeared to be a natural approach with the development of nanotechnology and the information and data that already existed on the antibacterial

activity of silver. A study of Silver nanoparticles' (AgNPs') antibacterial properties considered to be the natural next step given the advancement of nanotechnology and the prior knowledge and information surrounding the antibacterial properties of silver.

According to earlier studies, green synthesis is the most convenient, economical, and environmentally friendly method. Silver nanoparticles are synthesized using the "Green synthesis method" from *Trachyspermum ammi* (Ajwain seeds). Green synthesis refers to the utilization of microorganisms and plant material in the production of silver nanoparticles. The seeds of ajwain, or *Trachyspermum ammi*, contain potent antibacterial and antifungal activities. Carom (Ajwain) seeds may decrease cholesterol and triglyceride levels, according to animal studies. Heart disease risk factors include having high triglyceride and cholesterol levels. According to some studies, thymol, a key ingredient in carom seeds, may have calcium channel-blocking properties that could aid in lowering blood pressure. It has been demonstrated that carom (Ajwain) seeds have anti-inflammatory properties, which may lessen inflammation in your body.

The biological activity of AgNPs is influenced by a variety of factors, including their size, shape, morphology, and surface chemistry. The physicochemical properties of nanoparticles improve therapeutic agent bioavailability after systemic and local administration. These characteristics, on the other hand, can influence cellular uptake, biological distribution, penetration through biological barriers, and subsequent therapeutic effects. It is critical for many biological applications to create AgNPs with regulated structures that are homogeneous in size, morphology, and function. Following synthesis, precise particle characterization is required because a particle's physicochemical properties can have a significant impact on its biological properties. Before determining the degree of toxicity, it is necessary to consider the distinguishing characteristics of nanomaterials, such as size, shape, and surface area. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), and ultraviolet-visible spectroscopy (UV-vis spectroscopy) have all been used to analyse the synthesised nanomaterials. Numerous credible books and reviews have covered the concepts and applications of various analytical techniques for the characterization of AgNPs.

As novel nano silver is incorporated into an increasing number of FDA-regulated products, concerns about formulation, pyrogenicity, sterility, and sterilisation procedures arise. For instance, little is understood regarding the stability of silver nanoparticles when subjected to the usual sterilising techniques frequently employed in the food sector, as well as for the manufacture of medical devices and medicine formulations. These effects must be thoroughly investigated because any beneficial effects derived from the nanoscale size of silver particles may be reduced or eliminated if their integrity is compromised during autoclave (heat/steam treatment) sterilisation. The antimicrobial property of extracted silver nanoparticles will be determined using the MIC and well diffusion method.

MATERIALS AND METHODS :

1. Extraction of silver nanoparticles and check its antimicrobial activity:

Trachyspermum ammi seed extract preparation

*Trachyspermum ammi (*Ajwain) seeds were purchased from the local market and used for the extraction of its bioactive components. The seeds were dried for 48 hours and were crushed into fine powder with the help of a mechanical grinder. Over 100g of the processed *Trachyspermum ammi* (ajwain) powder was dissolved in 100ml of distilled water and boiled under intense flame for 5 minutes. The concentrated extract was used for characterisation, testing the MIC and then preserved at 4ºC in sealed vials until further use.

Characterisation of prepared aqueous seed extract

UV spectrometry was used to obtain UV-visible spectra of extract using water as a blank.

2. Antibacterial assay for prepared extract :

Well Diffusion method for prepared aqueous seed extract

A bacterial inoculum suspension of Gram-positive *Staphylococcus aureus,* a microorganism causing dental plaque obtained from Medical Microbiological analysis, was Bulk seeded in Mueller–Hinton Agar (MHA) using the pour plate method . After the solidification of the agar, four wells were bored on the plate using a well borer of internal diameter 8mm. Wells were filled with Ampicillin (100 microgram/100ml) as a positive control, Saline as a negative control and extract as test in remaining two wells. The plate was incubated at 37 °C for 24 hrs and the zone of inhibition was measured.

MIC of prepared aqueous seed extract

The following set was prepared while maintaining sterile condition :

1. Media control - 5.0 ml media
2. Test extract - 4.0 ml media + 0.5 ml culture + 0.5 ml Extract
3. Positive control - 4.0 ml media + 0.5 ml culture + 0.5 ml Ampicillin
4. Inhibitory control - 4.0 ml media + 0.5 ml culture + 0.5 ml Ampicillin
5. Negative control - 4.0 ml media + 0.5 ml saline

3. Synthesis of silver nanoparticles using seed extract:

0.01M silver nitrate solution was prepared by adding 100 ml of distilled water with 0.085gm of AgNo3 in 1 amber flask. 0.001M silver nitrate solution was prepared by adding 1 ml of 0.01M AgNo3 to 99 ml of distilled water in another amber flask. The aqueous extract of *Trachyspermum ammi* seeds was used for Green synthesis of AgNP1 and AgNP2 using two molar concentrations by adding (1) 5 ml of extract to 50 ml of silver nitrate(0.001 M) as AgNP2 (2) 3 ml of extract to 25 ml of silver nitrate (0.01 M) as AgNP1. The reaction mixture beakers were covered with Aluminium foils and placed in the ultrasonication unit for 2 hrs and the extract was centrifuged at 2500 rpm for 15 min.

Characterization of synthesised nanoparticles

Supernatant was decanted in the fresh beakers and the pellet was used for UV spectrometry and SEM analysis. The pellet was placed in a Petri plate and kept in the hot air oven for 24hrs to obtain solid silver nanoparticles. 10 ml of distilled water was added to the dried silver nanoparticles and stored at 4ºC for further applications. The antimicrobial testing of prepared silver nanoparticles was carried out using MIC and agar cup method.

4. Antibacterial assay for biosynthesised silver nanoparticles :

Well Diffusion method for biosynthesised silver nanoparticles

A bacterial inoculum suspension of Gram-positive *Staphylococcus aureus*, a microorganism causing dental plaque obtained from Medical Microbiological analysis, was Bulk seeded in Mueller–Hinton Agar (MHA) using the pour plate method . After the solidification of the agar, four wells were bored on each two plates using a well borer of internal diameter 8mm. Wells in one plate were filled with Ampicillin (100 microgram/100ml) as a positive control and Saline as a negative control in the remaining three wells. Extracted silver nanoparticles with 0.01M and 0.001 M concentration of AgNO3 as test were filled in the two wells for each. The plates were incubated at 37 °C for 24 h, and the zone of inhibition was measured.

MIC of biosynthesised silver nanoparticles

The following set was prepared while maintaining sterile condition:

1. Test AgNP 2 - 4.0 ml media +0.5 ml culture+ 0.5 ml AgNP 2
2. Test AgNP 1 - 4.0 ml media + 0.5 ml culture + 0.5 ml AgNP 1
3. Positive control - 4.0 ml media + 0.5 ml culture + 0.5 ml Ampicillin
4. Media control - 5.0 ml media
5. Inhibitory control - 4.0 ml media + 0.5 ml culture + 0.5 ml Ampicillin
6. Negative control - 4.0 ml media + 0.5 ml saline

OBSERVATION :

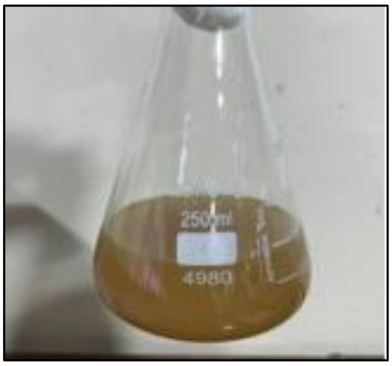


Fig 1. *Trachyspermum ammi* Aqueous seed extract

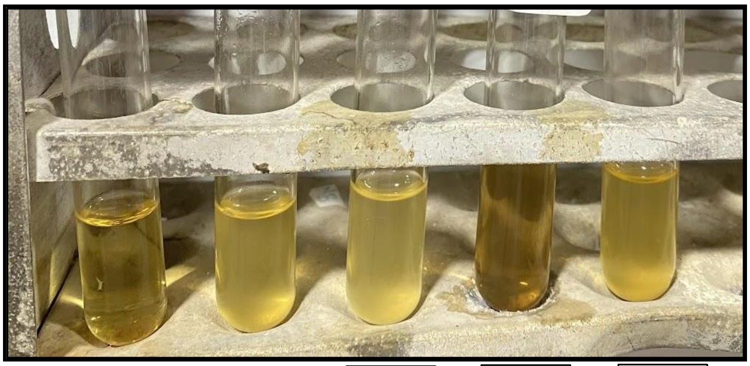


Fig 2. MIC of *Trachyspermum ammi* aqueous seed extract

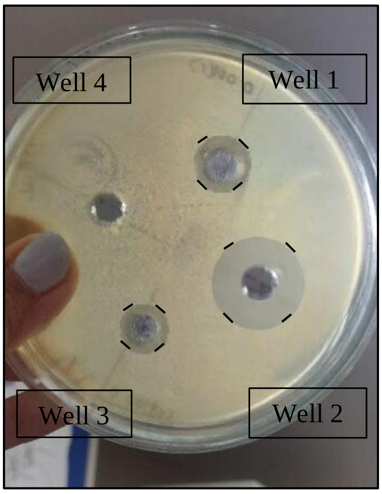


Fig 3. Well diffusion of seed extract

Table 1. Antimicrobial activity of Aqueous seed extract of *Trachyspermum ammi*

	Diameter of zone of inhibition 'x' mm	Diameter of zone of inhibition 'y' mm	Average diameter of zone of inhibition in mm
Seed extract 1	11	9	10
Seed extract 2	11.5	10.5	11
Saline	0	0	0
Ampicillin	23	23	23

Fig 4.1. Before ultrasonication

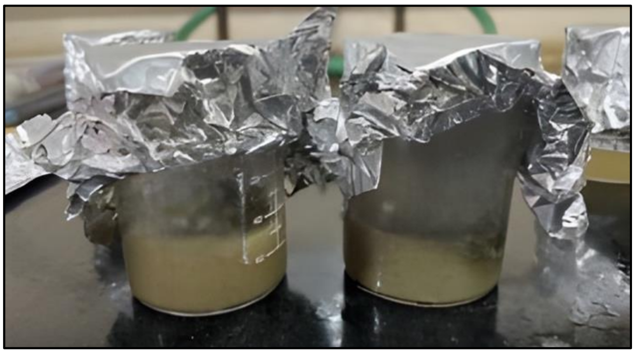


Fig 4.2. After ultrasonication

Table 2. UV Vis spectroscopy of seed extract and biosynthesized AgNP 1 and AgNP 2

Sample	Absorbance at 330 nm	Absorbance at 430 nm	Absorbace at 630 nm
Seed extract	0.68	0.21	0.19
AgNP 1	0.24	0.47	0.32
AgNP 2	0.17	0.29	0.23

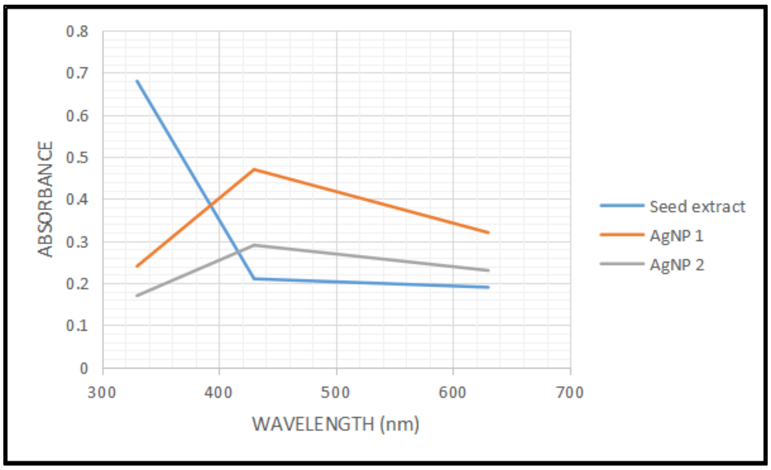
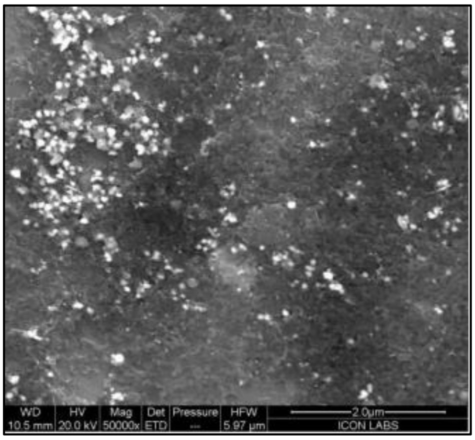
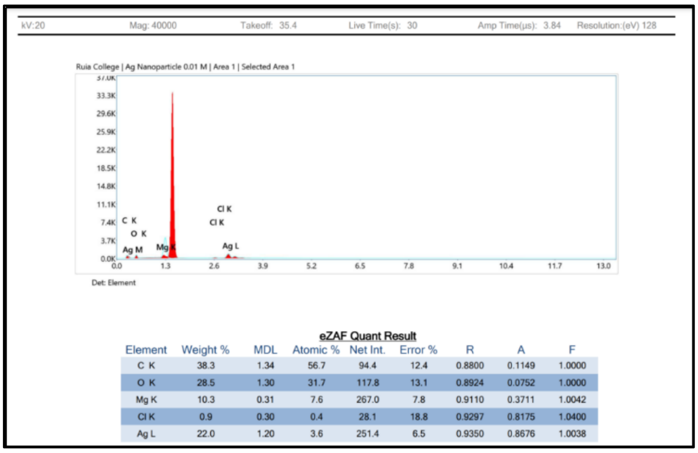


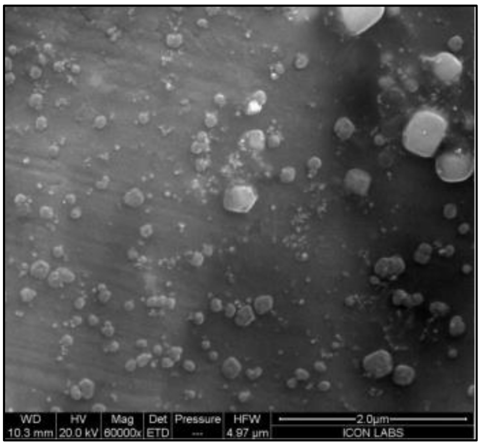
Fig 5. UV Vis spectroscopy

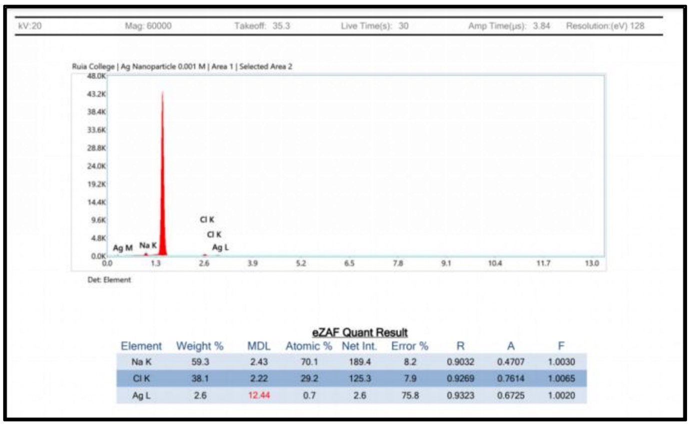




Element	Weight %	MDL	Atomic %	Net Int.	Error %	R	A	F
C K	38.3	1.34	56.7	94.4	12.4	0.8800	0.1149	1.0000
O K	28.5	1.30	31.7	117.8	13.1	0.8924	0.0752	1.0000
Mg K	10.3	0.31	7.6	267.0	7.8	0.9110	0.3711	1.0042
Cl K	0.9	0.30	0.4	28.1	18.8	0.9297	0.8175	1.0400
Ag L	22.0	1.20	3.6	251.4	6.5	0.9350	0.8676	1.0038

Fig 6. FESEM analysis of AgNP 1 with element analysis





Element	Weight %	MDL	Atomic %	Net Int.	Error %	R	A	F
Na K	59.3	2.43	70.1	189.4	8.2	0.9032	0.4707	1.0030
Cl K	38.1	2.22	29.2	125.3	7.9	0.9269	0.7614	1.0065
Ag L	2.6	12.44	0.7	2.6	75.8	0.9323	0.6725	1.0020

Fig 7. FESEM analysis of AgNP 2 with element analysis

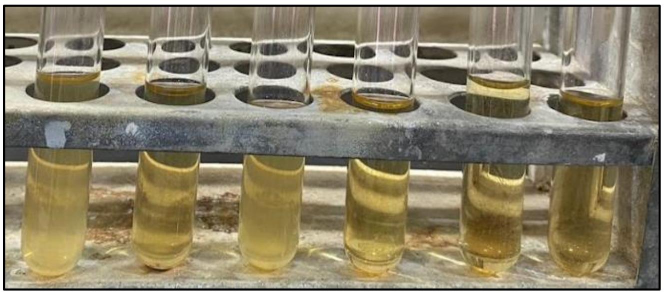


Fig 8. MIC of biosynthesized silver nanoparticles

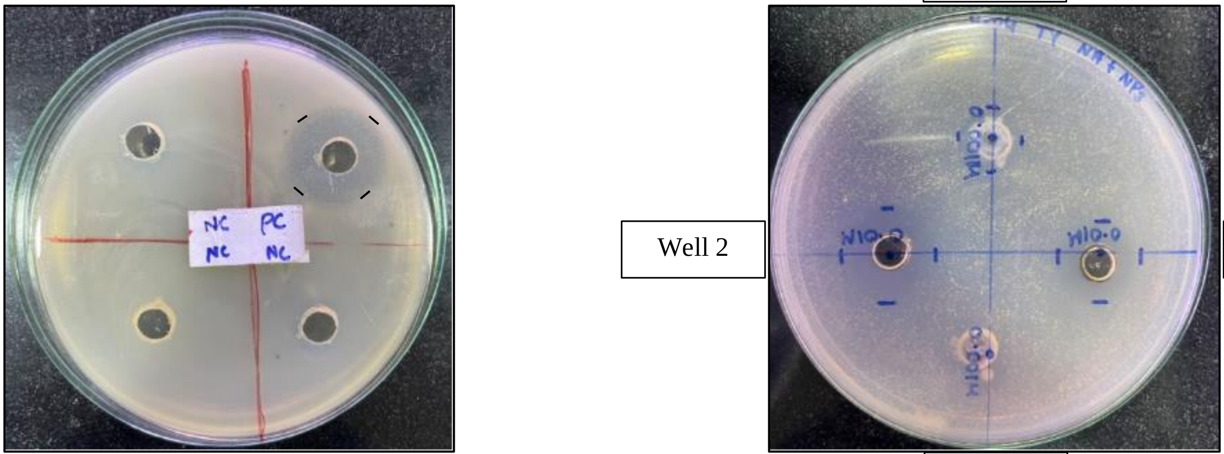


Fig 9. Well diffusion of biosynthesized silver nanoparticles

Table 3. Antimicrobial activity of biosynthesised AgNPs

	Diameter of zone of inhibition ‘x’ mm	Diameter of zone of inhibition ‘y’ mm	Average diameter of zone of inhibition in mm
AgNP 1 (well 2)	19	21	20
AgNP 1 (well 4)	18	18	18
AgNP 2 (well 1)	12	12	12
AgNP 2 (well 3)	0	0	0
Saline	0	0	0
Ampicillin	24	24	24

RESULT :

Characterization of silver nanoparticles :

Nanoparticles synthesis initiates once the *Trachyspermum ammi* seeds extract (Fig. 1) was introduced into 0.01M and 0.001M silver nitrate solution. The gradual colour change of silver nitrate / *Trachyspermum ammi* solution from yellowish brown to dark brown indicates the formation of silver nanoparticles as shown in (Fig. 4.1, 4.2). This colour change is due to decrease in particle size and precipitation by ultrasonication. The formation of AgNPs was further confirmed by using UV-visible spectroscopy and field emission scanning electron microscopy.

Formation of the nanoparticles in the aqueous solution was further confirmed by the UV-visible spectroscopy. The wavelength scale was fixed between 330 and 630 nm, and the solution was scanned within this range(Table 2). Maximum absorbance at 430 nm was observed(Fig. 5), which is characteristic of silver nanoparticles (Bahuguna *et al.*, 2016).

FESEM (Fig. 6, 7) clearly shows the presence of synthesised nanoparticles along with some chlorine, sodium, potassium impurities as result of usage of distilled water instead of double distilled water for dilution of biosynthesised nanoparticles. The nanoparticles were oval, spherical in shape and 53 nm in size . Silver was found in both concentrations with highest abundance in AgNP 1 solution. Most of the nanoparticles were aggregated, and few individual particles were also observed (Suman *et al.*, 2013).

Antibacterial Activity Using Well Diffusion Assay and MIC:

The antibacterial activity of the aqueous *Trachyspermum ammi* seed extract and biosynthesised AgNPs from the same was checked by the well diffusion method. The target microorganism *S. aureus* showed a zone of inhibition in well diffusion for aqueous *Trachyspermum ammi* seed extract which was 11 mm(Table. 1) (Fig. 3) ; The cell wall in Gram-positive bacteria consists of a thick peptidoglycan layer with short peptide cross-linked linear polysaccharide chains leading to a more rigid structure. This increases difficulties in penetration of the silver nanoparticles hence the target microorganism *S. aureus* showed a larger zone of inhibition in AgNP 1 solution i.e. 20 mm than AgNP 2 i.e. 12 mm(Table .3) (Fig. 9). MIC results are in relation to the fact that a larger zone of inhibition corresponds to smaller minimum inhibitory concentration (Mohanty et al. 2010). After confirmation of antimicrobial activity of synthesised AgNPs through well diffusion assay, minimum inhibitory concentration (MIC) of AgNPs against *S. aureus* was determined. Broth dilution method was used to determine the MIC of antimicrobial agents i.e. aqueous *Trachyspermum ammi* seed extract and biosynthesised AgNPs against target microorganism *S. aureus* (Fig. 2). After 24 h of incubation, slight growth of *S. aureus*, in aqueous *Trachyspermum ammi* seed extract. For the MIC of biosynthesised AgNPs, no growth of *S. aureus* in AgNP1 solution & slight growth was observed in AgNP2 solution(Fig. 8) but with less turbidity than that of the aqueous extract. The aqueous seed extract showed antibacterial activity but was found to be lower when applied alone as compared with biosynthesised AgNPs.

CONCLUSION :

Through a green chemistry approach the synthesis of AgNPs using *Trachyspermum ammi* seed extract was performed. The Green Synthesis Technique has several advantages such as an economic, efficient, and eco-friendly process, which is also energy-efficient and cost-effective as there is no chemical reagent or surfactant template required in the process. This results in protecting human health and the environment, healthier workplaces and communities, leading to less waste and safer products.(Hemlata *et al*., 2020). The prepared aqueous extract of *Trachyspermum ammi and* biosynthesised nanoparticles, were studied for assessing their antibacterial activity against Gram-positive *S. aureus*. Synthesised nanoparticles showed antimicrobial activity against Gram-positive *S. aureus* which was investigated by agar well diffusion method. Minimum inhibitory concentration against *S. aureus* was determined by the broth dilution method which was found to be AgNP synthesised through 0.01 M $AgNO_3$ in case of AgNPs with clear solution. But the seed extract showed a slightly turbid solution, indicating less effectiveness of antimicrobial activity than biosynthesised nanoparticles against *S. aureus*.

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