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Traditional Indian Medicinal Plants: Synthesis, Characterization and Antibacterial Property of AgNPs against MDR Strains

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Abstract

Nanotechnology is a rapidly developing field, the purpose of which includes manufacturing new materials at nano scale. The current technique of green synthesis of silver nanoparticles (AgNPs) is a progressive method used in medical science. An emerging problem for medical sector is multi drug resistance to antibiotics which is shown by wide range of bacteria pathogens. This study investigates an efficient and sustainable route of AgNPs preparation from 1mM aqueous $AgNO_3$ using leaf extracts of seven plants *Trachyspermum ammi* (ajwain), *Moringa oleifera* (drumstick), *Phyllanthus niruri* (nela usiri), *Tagetes erecta* (marigold), *Pimenta dioica* (jamaica pepper), *Hemigraphis alternata* (red ivy) and *Plectranthus purpuratus* (vicks) well adorned for their wide availability and medicinal property. AgNPs were synthesized by the reaction of 1mM $AgNO_3$ and 10% leaf extract of type of leaf separately. The solutions of AgNPs produced from plant extract were tested for its antibacterial activity. The AgNPs were characterized by UV visible spectrophotometer, X-ray Diffraction (XRD) and transmission electron microscopy (TEM). Fourier transform infrared spectrometer (FTIR) analysis was carried out to determine the nature of capping agents in each of these leaf extracts. AgNPs obtained showed significantly higher antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacteriaceae*. AgNPs from leaf extract of *Trachyspermum ammi* showed highest antibacterial activity on the MDRs used.

Keywords

Synthesis; AgNPs, antibacterial activity, multi drug resistance

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1. INTRODUCTION

Asia is the world's most densely populated continent with sixty percent of the world's people living there. It is one of the largest biodiversity regions in the world and home to some of the richest countries in medicinal and aromatic plant resources. It has diverse plant flora however; species richness is concentrated mainly in tropical and subtropical regions. Six of the world's 18 biodiversity hot-spots: the Eastern Himalayas, the Western Ghats of South India, North Borneo, Peninsular Malaysia, Sri Lanka and the Philippines are part of Asia. The continent has well documented traditional knowledge, a longstanding practice of traditional medicine and potential for socio-economic development of medicinal and aromatic plants in primary healthcare and industrial scale production. Medicinal and aromatic plants constitute the basis of primary healthcare for the majority of the population and are a valuable source of income for rural populations. Approximately 90% of plants are still collected from forests. Only few countries such as China, India, Indonesia, Nepal, Thailand and Vietnam produce medicinal and aromatic plants through cultivation on a commercial scale.

Despite advances in western medicine, ancient traditional systems of medicine are practiced in Asia mainly because of historical circumstances and cultural believes. Medicinal plants are an accessible, affordable and culturally appropriate source of primary healthcare for more than 80% of the Asian population according to the World Health Organization. Indian Ayurveda along with Jammu, Kampo, Sidha, Tibetan, Tribe and Unani systems of medicine are an important source of health and livelihood for millions of Asian people. International and national trade in alternative medicine including herbal products is increasing rapidly. The estimated global trade in medicinal and aromatic plant materials was more than US$60 billion in 2000 and is expected to reach US$5 trillion by 2050 according to the World Bank report. China and India are the world's leading exporters of medicinal and aromatic plant materials. Various international organization such as the Food and Agriculture Organization (FAO), the United Nations Industrial Development Organization (UNIDO), the World Health Organization (WHO), the International Development Research Centre (IDRC) and others have been addressing issues concerning medicinal and aromatic plants through support for research, networking and coordination.

Medicinal plants have been used by mankind for millennia; their use is as old as humanity itself. The range of species used and their scope for healing is vast. Cures as yet undiscovered may exist in plants as yet undescribed. Currently, it is estimated that the number of higher plant species used worldwide for medicinal purposes is more than 50,000 (Schippmann et al. 2002). This equates to approximately 20% of the world's vascular flora and constitutes the biggest spectrum of biodiversity used by people for a specific purpose (Hamilton et al. 2006).

1.1 Traditional medicine

Traditional medicine is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO 2003). In India, Ayurvedic medicine, a system more than 5,000 years old, is based on some 2,000-plant species (Zedan 2002). In China, Traditional Chinese Medicine (TCM) is largely plant-based (80%) and TCM preparations account for 30-50% of total medicinal consumption, rising to 90% in rural areas (WHO 2003). In Sub-Saharan Africa, the ratio of traditional healers to the population is approximately 1:500, while medical doctors have a 1:40,000 ratio to the rest of the population (Richter 2004).

In fact, of the total pharmaceutical drug supply available worldwide, only 15% is consumed in developing countries (Lydecker et al. 1992), supporting the much-quoted WHO's estimate that 80% of people worldwide rely on traditional medicine for their primary healthcare. The majority of these people are in developing countries, where rapid population growth is expected to increase pressures on medicinal plant resources.

The greater part of traditional therapy involves the use of plants. With little or no access to modern pharmaceuticals and a strong cultural preference for traditional medicine, medicinal plants are therefore fundamental to the well-being of billions of people. Demand for traditional remedies is also increasing in so-called developed countries, alongside growing environmental awareness and a desire for natural healing through natural products.

1.2 Modern Medicine

Of course, allopathic or 'modern' medicine also owes a great deal to medicinal plants. *Catharanthus roseus* for example, treats leukaemia and Hodgkin's disease. Morphine and codeine are produced from cultivated opium poppy, *Papaver somniferum*. Aspirin was originally found in willow bark (Salix spp.). Quinine from the cinchona tree has been the primary treatment for malaria for centuries. Digitalin medicines, extracted from the leaves of the common foxglove (Digitalis spp.), are widely used for a variety of heart conditions. Topical steroids for eczema are produced from the yam (Dioscorea spp.) or from sisal (Agave spp.) and the alkaloid Galantamine, sourced from the bulbs of snowdrops (Galanthus spp.) is used to treat Alzheimer's disease, slowing down the progression of dementia.

In fact, as many as 50% of prescription drugs are based on a molecule that occurs naturally in a plant, with some 25% of prescription drugs derived directly from flowering plants or modelled on plant molecules (Foster and Johnson 2006). In many cases modern chemistry cannot offer viable alternatives to active botanical compounds. The compound paclitaxel (found in Taxus spp. and source of the anti-cancer drug, taxol) was described as the kind of molecule that no chemist would ever sit down and think of making; If contemporary chemistry is now allowing us to merely copy such molecules, one can imagine the near impossibility of designing from scratch a molecule with a comparable combination of form and biological function (Capson 2004).

Predictions that advances in chemical sciences and synthetic material development would lessen the need for natural materials have proved to be wrong, and modern medicine depends on the continuing availability of biological materials as an incomparable source of molecular diversity.

Indian greeneries are the chief and cheap source of medicinal plants and plant products. From centuries till date these medicinal plants have been extensively utilized in Ayurveda. Nanotechnology is presently one of the most dynamic disciplines of research in contemporary material science whereby plants and plant products are finding an imperative use of synthesis of nanoparticles. In general particles with size less than 100 nm are referred to as nanoparticles. The two most important area of research as far as nanoparticles are concerned are synthesis and characterization. An effective control over the physical and chemical properties of nanoparticles is provided by the assortment of its size and shape. Gold, platinum, silver and zinc nanoparticles are used in products which are used by humans such as shampoos, soaps, detergents, shoes, cosmetic products and tooth pastes as well as in some medical and pharmaceutical applications.

The nanoparticles used for all the aforesaid purposes, the metallic nanoparticles considered as the most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest for researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains (Khalil et al. 2013). Among the all noble metal nanoparticles, silver nanoparticle are an arch product from the field of nanotechnology which has gained boundless interests because of their unique properties such as chemical stability, good conductivity, catalytic and most important antibacterial, anti-viral, antifungal in addition to anti-inflammatory activities which can be incorporated into composite fibres, cryogenic superconducting materials, cosmetic products, environmental pollution, food industry, agriculture and electronic components (Ahmed et al. 2003; Klaus-Joerger et al. 2010; Bhuyan et al. 2015; Aziz et al. 2016; Uma et al. 2017). For biomedical applications; being added to wound dressings, topical creams, antiseptic sprays and fabrics, silver functions' as an antiseptic and displays a broad biocidal effect against microorganisms through the disruption of their unicellular membrane thus disturbing their enzymatic activities. So, much attention must be given towards developing eco-friendly methods for nanoparticles synthesis that minimizes or completely avoids the use of toxic chemicals. Thus, biological methods of AgNPs synthesis using biological entities like bacteria, fungi and plants were reported to be clean, nontoxic, cost effective and environmentally acceptable when compared to nanoparticles synthesis. Nevertheless, despite these advantages and the FDA approval for clinical use of nanoparticles as transport vehicles nearly two decades back, clinical translation of their theranostic potential has been partially plagued by biotoxicity and incompatibility concerns of the present-day nanoparticles (Prasad et al. 2016).

Synthesis of AgNPs is of much interest to the scientific community because of their wide range of applications. These AgNPs are being successfully used in the cancer diagnosis and treatment as well (Baruwati et al. 2009; Popescu et al. 2010). Generally, nanoparticles are prepared by a variety of chemical and physical methods, which are quite expensive and potentially hazardous to the environment which involve use of toxic and perilous chemicals that are responsible for various biological risks. The development of biologically inspired experimental processes for the syntheses of nanoparticles is evolving into an important branch of nanotechnology. Generally there are two approaches which are involved in the synthesis of AgNPs, either from ''top to bottom'' approach or a ''bottom to up'' approach. Various methods are available for synthesis of AgNPs includes chemical, electrochemical, radiation, photochemical and biological methods (Aziz et al. 2015). Plant mediated green biomemitic synthesis of AgNPs is considered and widely acceptable technology for rapid productions of AgNPs for successfully meeting excessive need and current market demand.

Klebsiella pneumoniae accounts for a significant proportion of hospital-acquired urinary tract infections. *K. pneumoniae* is a Gram-negative, rod-shaped bacillus from the genus Klebsiella and family Enterobacteriaceae. *K. pneumoniae* is facultative anaerobic, oxidase-negative, and produces acid and gas from lactose. It is an enteric bacterium, noted in the intestinal tract of 5% of healthy humans. It can also reside in the skin and mouth. *K. pneumoniae* causes pneumonia in guinea pigs of both sexes and all ages, although there are few reports of natural infections of this animal species with this organism. *Staphylococcus aureus* silently stays as our natural flora, and yet sometimes threatens our life as a tenacious pathogen and it is Gram-positive bacteria (stain purple by Gram stain) that are cocci-shaped and tend to be arranged in clusters that are described as "grape-like." On media, these organisms can grow in up to 10% salt, and colonies are often golden or yellow (aureus means golden or yellow). These organisms can grow aerobically or anaerobically (facultative) and at temperatures between 18 °C to 40 °C. In addition to its ability to outwit our immune system, its multi-drug resistance phenotype makes it one of the most intractable pathogenic bacteria in the history of antibiotic chemotherapy. *Enterobacteriaceae* which produces ESBL show that they are MDR pathogen and are clinically important.

2. MATERIALS AND METHODS

2.1 Selection and Collection of Plant Material

Seven different natural plants were selected for the AgNPs synthesis. They are *Trachyspermum ammi* (ajwain), *Moringa oleifera* (drumstick), *Phyllanthus niruri* (nela usiri), *Tagetes erecta* (marigold), *Pimenta dioica* (jamaica pepper), *Hemigraphis alternata* (red ivy) and *Plectranthus purpuratus* (vicks) were collected in Kerala and authenticated.

The use of plants as the production assembly of AgNPs has drawn attention, because of its rapid, ecofriendly, non-pathogenic, economical protocol and providing a single step technique for the biosynthetic processes. The reduction and stabilization of silver ions by combination of biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpinoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures (Kulkarni and Muddapur 2014).

2.2 Antibacterial Activity of Synthesized AgNPs

The antibacterial activity of synthesized AgNPs was evaluated using agar-well diffusion method. *S. aureus, K. pneumoneae* and *Entereobacteriaceae* strains collected from AIMS hospital, Bellur Cross, Hassan, Karnataka were grown on Mannitol Salt Agar medium to screen MRSA strains. Each bacterial strain was swabbed uniformly on Mueller Hinton Agar plates using sterile cotton swabs. Wells of 6mm diameter were made onto each plate using gel puncture. AgNPs of different concentrations 20, 40, 60 and 80 µl (with a difference of 20µl) were poured into the corresponding wells using sterile micropipette. The antibacterial activity was determined by clear inhibition zone obtained around the sample loaded in the well after incubating the plates at 37 ˚C for 24hrs.

2.3 Synthesis of AgNPs

Silver nitrate, A.R. ($AgNO_3$) used in this study was obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. 1 gram of powdered plant samples were weighed and added into 100 ml of distilled water. Mixed the mixture properly and then kept for boiling in water bath at 90-100 °C for 30 minutes. After the

boiling in water bath the plant extracts were filtered through Whatmann No. 1 filter paper and then centrifuged at 5000 rpm for 15 minutes. The supernatant was then collected and then used for the AgNPs synthesis.

90 ml of aqueous solution of 1mM silver nitrate was prepared in 250 ml of Erlenmeyer flask and used for the synthesis of AgNPs. 10 ml of filtered plant extract solution was mixed with 90 ml of aqueous solution of 1 mM $AgNO_3$. It was then kept for boiling water bath for 20 minutes. During the boiling water bath only we see the change in color in the solution from yellow to dark brown. After boiling water bath the flasks were kept in dark to avoid photoreactions. With this method, we had prepared aqueous solution of AgNPs with all the plant leaves.

2.4 Characterization of Synthesized AgNPs

2.4.1 UV –Visible Spectroscopy

The formation of AgNPs was preliminarily confirmed by visual observation of color change from pale white to reddish brown, after adding 1mM $AgNO_3$ solution to fungal filtrate. Further confirmed by sharp peak given by AgNPs in the visible region using UV-visible spectroscopy (T90+UV-Vis Spectrophotometer) which confirms AgNPs at the absorption range between 390 to 440nm due to Surface Plasmon Resonance (SPR) which is considered to be a reliable and accurate analytical laboratory assessment procedure for the analysis.

2.4.2 X-Ray Diffraction (XRD)

The colloidal suspension containing AgNPs was air dried on a small glass slab and was subjected to XRD which was used to analyze the metallic nature of nanoparticles after bioreduction.

2.4.3 Transmission Electron Microscopy (TEM)

Characterization of AgNPs was done by TEM (Hitachi-H-7500) to know the particle size, shape of a material in nano dimension and study the crystal structure meticulously, TEM is a microscopic technique wherein beam of electron is transmitted through an ultra thin specimen and interacts as electrons waves exiting from the sample to form an image. The samples were prepared by drop-coating the AgNPs solution onto the carbon–coated copper grid and kept under vacuum before loaded onto a specimen holder. TEM micrographs were taken and then sizes, shape of AgNPs were confirmed (Joshia et al. 2008).

2.4.4 Fourier Transform Infrared Spectroscopy (FTIR)

The AgNPs powder was prepared by centrifugation at 10,000 rpm for 15min then the pellets were resuspended in sterile distilled water and again centrifuged at 10,000 rpm for 15min.The collected powder was air dried at room temperature and was used for FTIR analysis. The IR spectrum of the dried sample was recorded (Thermo Nicolet Nexus 670 spectrometer) in the range of 500 to 4000 cm^{-1} at resolution of $4cm^{-1}$.

2.5 Screening for antibacterial activity with antibiotics disc (by agar disc diffusion method)

The Antibacterial activities of synthesized AgNPs was evaluated using each antibiotics disc were determined by Kirby- Bauer disc diffusion method. Two different set of antibiotics disc ring box were purchased from Hi-Media lab. Total 24 different antibiotics (listed in Table 1) were used for screening of MDR. The disk diffusion tests were made according to the following instructions. Plates were dried at 35 °C for 1.5 to 2 hr. Inocula prepared in sterile NaCl at a density adjusted to a 0.5 McFarland turbidity standard were delivered onto Mueller-Hinton agar plates supplemented with 5% defibrinated sheep blood filled with an amount of agar giving a uniform depth of 4 ± 0.5 mm. The disks were added and incubated in a microaerobic atmosphere at 35 ± 1 °C for 48 hr. Sterile blotting paper was used between the lid and plate to inhibit excess moisture during the incubation. Three plates were made for each strain for the testing of susceptibility toward the different antimicrobials from the same inoculum at one measurement time. A maximum of three disks at the same time were applied onto one agar plate to make sure of a proper reading of even large inhibition zones.

Table 1. Results showing resistance and sensitivity towards antibiotics

	Test organisms		
Antibiotic agents	***Staphylococcus aureus***	***Klebsiella pneumonia***	***Enterobacteriaceae***
Azithromycin	Resistant	Sensitive (1.9 cm)	Slightly sensitive (0.9 cm)
Rifampicin	Resistant	Resistant	Resistant
Penicillin	Resistant	Resistant	Resistant
Piperacillin	Resistant	Resistant	Resistant
Augmentin	Resistant	Resistant	Resistant
Ampicillin	Resistant	Resistant	Resistant
Roxithromycin	Slightly sensitive	Slightly sensitive (1 cm)	Resistant
Erythromycin	Resistant	Resistant	Resistant
Ampicillin	Resistant	Resistant	Resistant
Cloxacillin	Resistant	Resistant	Resistant
Amoxicillin	sensitive (2 cm)	Resistant	Slightly sensitive
Vancomycin	Resistant	Resistant	Resistant
Gentamicin	Sensitive (1.5 cm)	Sensitive (1.2 cm)	Sensitive (1 cm)
Netillin	Resistant	Sensitive (2 cm)	Resistant
Nalidixic acid	Resistant	Resistant	Resistant
Kanamycin	Resistant	Sensitive (1.4 cm)	Resistant
Amikacin	Resistant	Highly sensitive (2 cm)	Sensitive (1.2 cm)
Co- trimoxazole	Resistant	Resistant	Resistant
Tobramycin	Resistant	Sensitive (1.3 cm)	Resistant
Clarithromycin	Sensitive (1.8 cm)	Resistant	Resistant
Nitrofurantoin	Sensitive (2 cm)	Resistant	Resistant
Streptomycin	Resistant	Sensitive (2.3 cm)	Sensitive (3 cm)
Oxytetracycline	Sensitive (1.8 cm)	Resistant	Resistant
Furazolidone	Sensitive (1.2 cm)	Resistant	Resistant

Table 2. Results showing of antibiotic resistance by pathogens

Tested plant extracts	**Dose (µl)**	**Test organisms**		
		S. aureus	***K. pneumonia***	***Enterobacteriaceae***
Phyllanthus niruri	10	Resistant	Resistant	Resistant
	20	Resistant	Resistant	Resistant
	30	Resistant	Resistant	Resistant
	40	Resistant	Resistant	Resistant
Pimenta dioica	10	Resistant	Resistant	Resistant
	20	Resistant	Resistant	Resistant
	30	Resistant	Resistant	Resistant
	40	Resistant	Resistant	Resistant
Tagetes erecta	10	Sensitive (1.0 cm)	Resistant	Resistant
	20	Sensitive (0.88 cm)	Resistant	Resistant
	30	Sensitive (1.3 cm)	Resistant	Resistant
	40	Sensitive (1.5 cm)	Resistant	Resistant
Trachyspermum ammi	10	Resistant	Resistant	Border sensitive
	20	Resistant	Resistant	Border sensitive
	30	Sensitive (1.0 cm)	Sensitive (1.1 cm)	Sensitive (1.0 cm)
	40	Sensitive (1.1 cm)	Sensitive (1.0 cm)	Sensitive (1.5 cm)
Moringa olifera	10	Slightly sensitive	Resistant	Resistant
	20	Resistant	Resistant	Resistant
	30	Resistant	Resistant	Resistant
	40	Sensitive (1.2 cm)	Sensitive (1.0 cm)	Resistant
Plectranthus purpuratus	10	Resistant	Resistant	Slightly sensitive
	20	Resistant	Resistant	Sensitive (1.0 cm)
	30	Resistant	Resistant	Sensitive (1.2 cm)

Tested plant extracts	Dose (µl)	Test organisms		
		S. aureus	***K. pneumonia***	***Enterobacteriaceae***
Hemigraphis alternate	40	Resistant	Resistant	Sensitive (1.2 cm)
	10	Resistant	Resistant	Resistant
	20	Resistant	Resistant	Border sensitive
	30	Resistant	Resistant	Border sensitive
	40	Resistant	Resistant	Border sensitive

Table 3. Results showing the Agar diffusion method by plant extract AgNPs

Synthesized silver nanoparticle with plant extracts	Dose (µl)	Test organisms		
		S. aureus	***K. pneumonia***	***Enterobacteriaceae***
Phyllanthus niruri	20	1.5 cm	1.0 cm	1.7 cm
	40	1.8 cm	1.6 cm	2.0 cm
	60	2.0 cm	2.0 cm	2.2 cm
	80	2.5 cm	2.1 cm	2.5 cm
Pimenta dioica	20	1.0 cm	NA	Resistant
	40	1.3 cm	1.3 cm	Resistant
	60	1.7 cm	1.3 cm	Resistant
	80	2.3 cm	1.9 cm	0.9 cm
Tagetes erecta	20	0.6 cm	0.7 cm	0.8 cm
	40	1.7 cm	0.8 cm	1.3 cm
	60	1.9 cm	1.5 cm	1.7 cm
	80	2.0 cm	1.8 cm	1.7 cm
Trachyspermum ammi	20	Resistant	2.3 cm	2.2 cm
	40	1.3 cm	2.3 cm	2.3 cm
	60	1.6 cm	2.4 cm	2.7 cm
	80	2.1 cm	2.5 cm	2.2 cm
Moringa olifera	20	0.8 cm	1.3 cm	1.3 cm
	40	1.0 cm	1.6 cm	1.7 cm
	60	1.6 cm	2.2 cm	2.0 cm
	80	1.7 cm	2.5 cm	2.1 cm
Plectranthus purpuratus	20	1.2 cm	2.0 cm	2.3 cm
	40	1.4 cm	2.2 cm	1.9 cm
	60	2.1 cm	2.2 cm	1.9 cm
	80	2.2 cm	2.4 cm	1.9 cm
Hemigraphis alternate	20	NA	1.1 cm	1.5 cm
	40	NA	1.3 cm	1.7 cm
	60	1.1 cm	1.4 cm	2.2 cm
	80	1.6 cm	1.7 cm	2.6 cm

3. RESULTS AND DISCUSSION

3.1 MDR pathogens were tested for its resistance towards the following list of antibiotics by Disc-Diffusion method

Leaves extract of all the seven plants were tested for antbacterial property by using distilled water as solvent. Following table indicates result of each plant extract on bacteria.

The synthesized AgNP solution of all the seven plants were tested against MDR strains by Agar diffusion methods, following are the results of sensitivity of bacteria against 20 µl, 40 µl, 60 µl and 80 µl of the silver nanoparticle solution of plants.

AgNPs were successfully obtained from bioreduction of silver nitrate solution using *Trachyspermum ammi* (ajwain), *Moringa oleifera* (drumstick), *Phyllanthus niruri* (nela usiri), *Tagetes erecta* (marigold), *Pimenta*

dioica (jamaica pepper), *Hemigraphis alternata* (red ivy) and *Plectranthus purpuratus* (vicks) leaf extracts. Ajwain AgNP was selected for further characterization as it showed the maximum antimicrobial properties among all seven plants. Powdered form of Ajwain AgNPs was sent to Sophisticated Analytical Instrument Facility, STIC, Kochi for TEM, XRD and FTIR tests. Results obtained confirmed the reduction of silver nitrate to AgNPs. Zones of inhibition were formed in the antimicrobial screening, test indicated, that the AgNPs synthesized in this process has the efficient antimicrobial activity against pathogenic bacteria. The biologically synthesized AgNPs could be of immense use in the medical field for their efficient antimicrobial function.

3.2 UV –Visible Spectroscopy

UV-Visible Spectroscopy is one of the techniques to verify the formation of metal nanoparticles. The reduction of silver ions was monitored by measuring the UV-Visible spectrum of the reaction medium at 24 to 48 hrs. The maximum absorbance was observed at 400nm indicating the presence of AgNPs (Fig. 1). To detect AgNPs the absorption range is 400 to 450nm and this is responsible for the striking brown color of AgNPs (Devi et al. 2014). Our results correlate with Vijayraj et al (2012), Shivraj et al (2013) and Prasad and Swamy (2013).

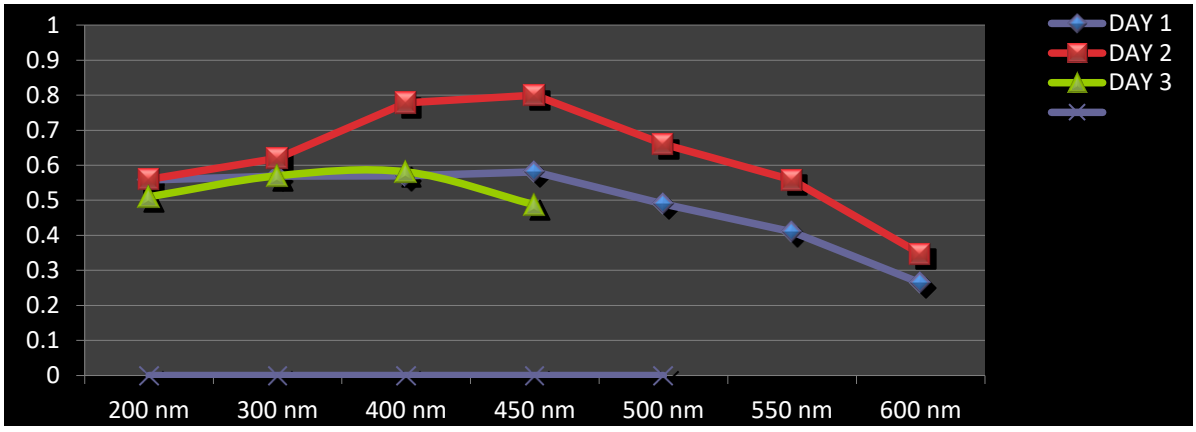

Fig. 1. UV-visible absorption spectroscopy analysis of AgNPs synthesized from *T. ammi* leaves.

3.3 X-Ray Diffraction (XRD) Analysis

The structure of prepared AgNPs synthesized from *Trachyspermum ammi,* has been investigated by X- ray diffraction (XRD) analysis, from SAIF- STIC, Cochin. Kerala, India. The mean particle diameter of AgNPs was calculated from the XRD pattern according to the line width of the plane, refraction peak using the following Scherrer's equation (Balaji et al. 2009).

$$d_c = \frac{k\lambda}{\beta \cos\theta}$$

The equation uses the reference peak width at angle h, where k is the X-ray wavelength (1.5406λ with k0 KV and 35 MA), $b^{1/2}$ is the width of the XRD peak at half height and K is a shape factor. For *T. ammi* leaves extract synthesized silver nanoparticle diffracted intensities were recorded from 0 to 80 (2Θ). Intense peaks corresponding to (110), (111), (200), (211) and (311) were observed. The Braggs peak position and their intensities were compared with the standard JCPDS files. The results were in good agreement with the unit cell of the face centered cubic (FCC) structure (JCPDS file No.04-0783). The observed diffraction pattern coincides with Liesje et al (2009; Aziz et el. 2016).

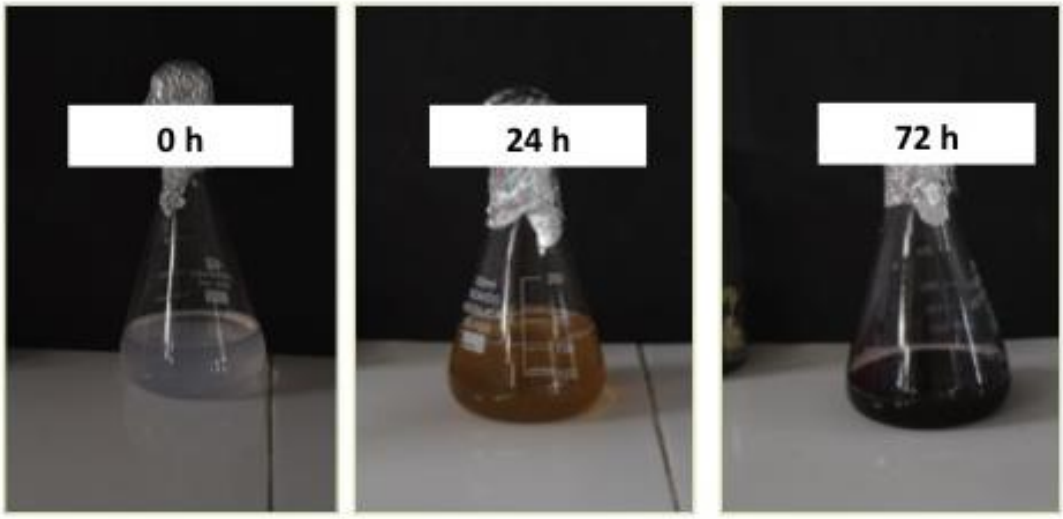

Fig. 2. Optical images of synthesized AgNPs showing a range of colors from colorless (start of biosynthesis) to reddish brown after 24 h incubation

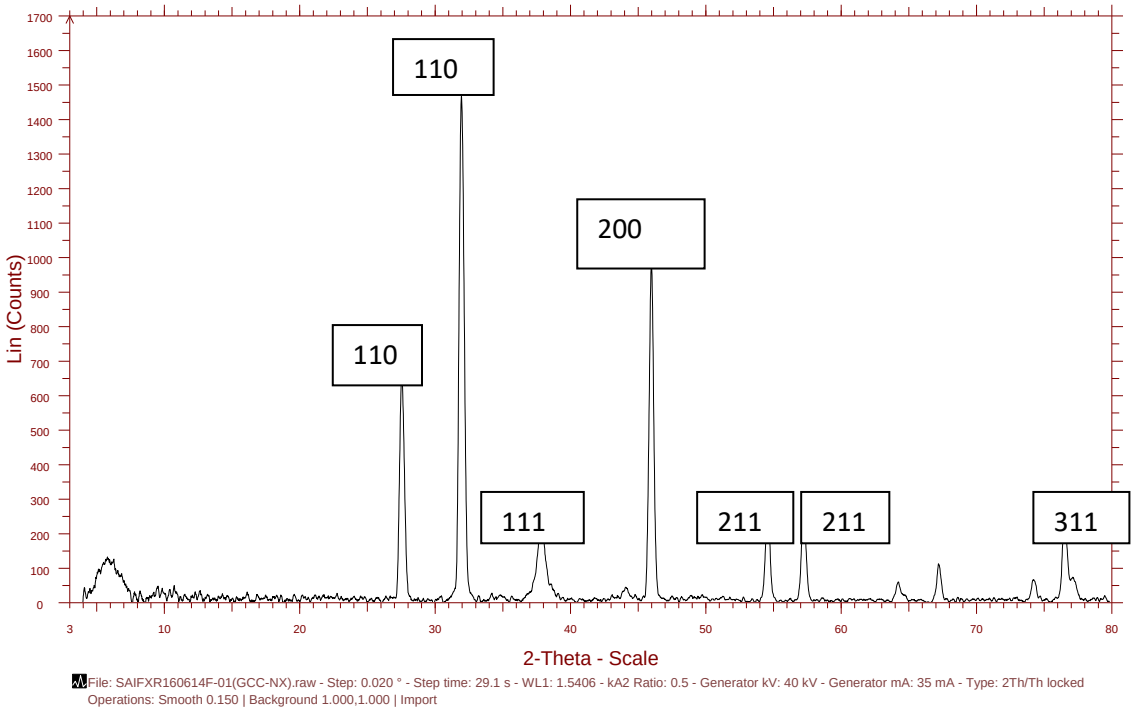

Fig. 3. X-ray diffraction pattern of the AgNPs were synthesized from *T. ammi* leaves.

3.4 Transmission Electron Microscopy (TEM) Analysis

The TEM image reveals that, there is formation of poly disperse irregular shaped particles. It has a smooth surface morphology, the AgNPs with uniform, spherical, dispersed, electron dense with size ranging between shown in 10-30nm (Fig. 4); graph 1. *Pinus elderica* was used by Iravani et al. (2013) to obtain an aqueous extract that was further used to synthesize predominantly spherical AgNPs with diameters between 10 and 40 nm. While Dattu Singh et al. (2013) reported the nanoparticle size between 25 to 30nm produced from *Curcuma longa*. Ingle et al (2009) reported spherical, polydispersed extracellular synthesis of AgNPs in the range of 5-35 nm with average diameter of 16.23nm. Aziz et al (2015) reported on the basis of the different 2θ peaks, we

calculated a range of average crystalline sizes between 5 and 20 nm with an average of ca. 12 nm consistent with the above TEM results. It was also studied the efficiency against multi-drug resistant human pathogenic bacteria *S. aureus, K. pneumonia* and *Enterobacteriacce* and compared to that exhibited by the standard drug. In both cases the antibacterial activity had a dual action mechanism: a bactericidal effect of Ag+ and a membrane- destructive effect caused by the polymer subunits (Veerswamy et al. 2011).

3.5 Fourier Transform Infra-Red Spectroscopy (FTIR) Analysis

The FTIR analysis of powdered AgNPs was carried out through the potassium bromide palate in 1:100 ratios and spectrum was recorded at a resolution of 5 cm^{-1}. IR measurement was carried out to identify the potential biomolecule in enzyme filtrate responsible for the reduction of silver ions and also as capping agent responsible for the stability of the bioreduced AgNPs (Daniel et al. 2012; Joshi et al. 2018). The representative spectra in the region of 4000 to 500 cm $^{-1}$ revealed the presence of different functional groups such as 3386.93 cm^{-1} secondary amide (N-H stretch-bonded), 2921.26 cm^{-1} & 2852.76 cm^{-1}-alkane (C-H stretching), 1616.74 cm^{-1}Amide (C=O stretching), 1544.89-Aromatics (C-C stretching), 1380.22 cm^{-1} and 1022.62 cm^{-1}primary alcohol (C-O stretching) and secondary alcohols- (C-O stretching), 818.92cm^{-1} and 464.91 cm^{-1}-alkene (=C-H bending) respectively (Fig. 4). Proteins present in the extract can bind to AgNPs through either free amino or carboxyl groups (Gole et al. 2001) The release of secondary metabolites including the active peptides/proteins may has a role in the formation and stabilization of AgNPs in aqueous extract (Joshi et al. 2018).

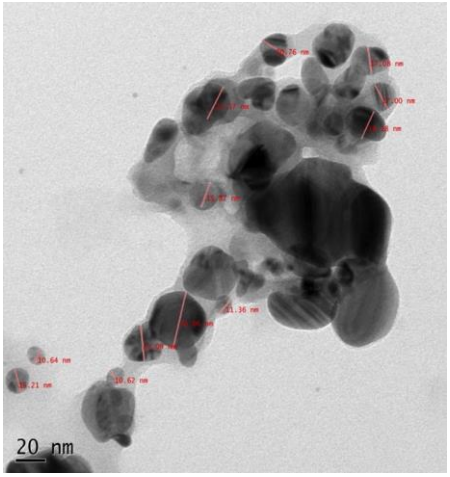

Fig. 4 TEM image of the AgNPs synthesized from *T. ammi* leaves.

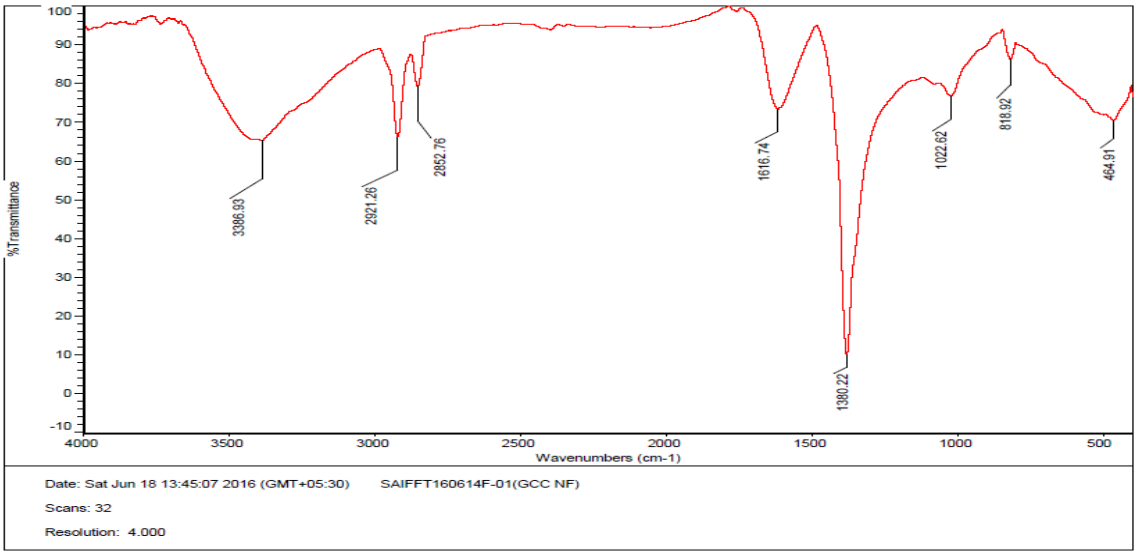

Fig. 5. FTIR spectroscopy of the AgNPs synthesized from *T. ammi* leaves.

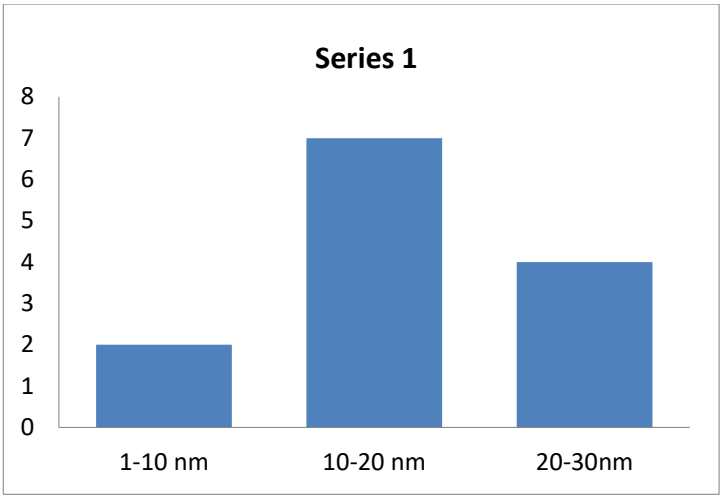

Fig. 6. Shows the various sizes of the AgNPs synthesized from *T. ammi* leaves.

4. CONCLUSION

Silver nanoparticles (AgNPS) were successfully synthesized from bioreduction of silver nitrate solutions using seven different traditional Indian medicinal plants, out of that leaves of *Trachyspermum ammi* (Ajwain) shown potent antibacterial activity compared to others. Ajwain synthesized AgNPs was selected for further characterization as it shown the maximum antibacterial properties, powdered form of AgNPs was analyzed spectroscopically by UV-visible, XRD, TEM and FTIR to know their size, shape, scale and structure. Our results have shown that the ajwain leaves aqueous extract is the easiest, economic and ecofriendly approach to synthesize metallic nanoparticles. It was experimentally evident that the AgNPs found effective against the bacteria like, *S. aureus, K. pneumonia* and *Enterobacteriaceae*. Thus, current scenario of bacterial resistance to modern antibiotics is a serious concern. Recurrence of MDR strains acquired a way to these microbes for their survival and development in a comfortable environment. High percentage of prevalence of MDR infections decreased the effectiveness of even the current third and fourth generation antibiotic treatments causing thousands of deaths. There by there is urgency for an alternate therapy in the form of AgNPs. Our report on synergistic studies of AgNPs with the antibiotic gentamycin against MRSA gives an insight to the researchers for the potential use of nanoparticles as an alternative drug of choice against antibiotic resistant strains in future investigation. Moreover, this plant mediated synthesis method represents a considerable improvement in the preparation of AgNPS, because of various advantages such as reduced reaction time, no need of additional capping agent and better control over their size and shape.

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