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Green Synthesis and Characterization of Silver Nanoparticles from Thymus Vulgaris Leaf Extract

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Abstract: In this present study thymus vulgaris leaf were collected from home town(Nilgiris). The fresh leaves are taken in the round bottom flask and refluxed for around 1 hour until the aqueous colour changes. The extract was collected and 1mM silver nitrate solution was added to it after the addition of silver nitrate the pale yellow extract changes its color to dark brown. The change in colour indicates the reduction of particle size in the thymus vulgaris leaf extract. The characterization of green synthesis of silver nanoparticles were done by using U-vis, XRD, FTIR, SEM and SEM EDAX. This studies explains the physical characterization of the silver nano particles produced.
Keywords: Thymus vulgaris, green synthesis, silver nanoparticles reflux

I. INTRODUCTION

Nanotechnology is the act of manipulating compounds at very tiny scales at the levels of atoms and molecules. Commonly nanotechnology explain their structures and size between1-100 nanometer in one dimension, and involves the development of materials or devices within that size[1]. General nanometer is compared one millionth of a millimeter approximately 100,000 times smaller than the diameter of a human hair. The broad and inter disciplinary area of research and development activity in Nanoscience and Nanotechnology has been growing very fast worldwide in the last few years[2]. A nanoparticle is defined as nano object with all three external dimension in the nanoscale, whose longest and the shortest do not differ significantly[3]. A nano fiber has two external dimension in the nanoscale, with nanotubes being hollow nanofibres and nano rods being solid nanofibers[4]. Nanostructured materials are often categorized by what phases of matter the contain.a nanocomposite is a solid containing at least one physically or chemaically distinct region,or collection of regions, having at least one dimension in the nanoscale[5]. A nanocrystalline material has a significant fraction of crystal grains in the nanoscale[6]. The nano particle size ,shape and surface of the morphology were play pivotal role in controlling the chemical, physical, , optical, and electronic properties of these nanoscopic materials[7]. Looking at the nanoparticles, silver nanoparticles are alluring particularly for antimicrobial cleansing. Consequently, a few plants have been examined in the combination in silver nanoparticles. This load of examinations are confined to plants of earthly beginning, however not with beach front plants. Subsequently, the current review was made to combination the antimicrobial silver nanoparticles by utilizing leaf and callus concentrates of the beach front *Thymus Vulgaris* plant[8].

II. MATERIALS AND METHODS

A. Plant Extract Preparation

The fresh leaves of *Thymus vulgaris* were collected from Medical Plant Development Area (MPDA) which is located in Cinchona village, Doddabetta post, Ooty, The Nilgiris , Tamil Nadu, India. The collected fresh leaves were washed several times with distilled water until the dust particles are removed. The leaves of *Thymus vulgaris* were measured around 20 gram and cut into small pieces then it was taken in 500ml of round bottom flask. A mixture of leaf along with 200ml of distilled water was made to reflux around 2 hours.Initially the extract is green color. After refluxing the extract color changes to pale yellow until extract color changes to pale yellow. The extract was collected and filtered using Whats Mann No.1 filter paper. After filtering,the extract was stored in room temperature for further process.

B. Green Synthesis of Silver Nanoparticles

Silver Nitrate ($AgNO_3$) was purchased from Spectrum Reagents and Chemical Pvt.Ltd Edayar, Cochin, India. 100ml of *Thymus vulgaris* leaf extract and 200ml of 0.01mM solution was added. The mixture was stirred around 20-30 minutes until the color changed from pale yellow to dark brown and was allowed to rest for precipitate. Then the precipitate was washed with distilled water twice to remove soluble impurities and was collected and centrifuged around 1000 rpm for about 30 minutes.

The obtained precipitate was taken in the petri dish glass plate and it was shade dried until the moisture was removed. The powder samples were then collected for further characterization techniques.

C. Characterization of Silver Nanoparticles

All the characterization studies were done in SAIF cochin. Spectra were recorded with UV Vis NIR spectro photometer agilent cary 5000 in the region 200nm to 3000nm DRS(Diffuse Intergrating Sphere diameter 150mm angle of incidence 8^0) **[9]**.The X-Ray Diffraction(XRD) measurement was performed on X-ray diffractometer(Bruker D8 Advance) operated at 30 Kv and 100 MA and spectrum was recorder by cukα radition with wave length of 1.506 A^0 in the range 200-800nm[10].The surface morphology and size were characterized by Scanning Electron Microscope (SEM)(Joel 6390LA)[11]. Energy Dispersive X-ray (EDX) oxford XMX N operated at 0.5 kV to 30 kV at magnification 300000 and EDX resolution at 136 Ev[12]. FTIR analysis was using thermo Nicolet Avtar)370 range between 4000cm^{-1} to400cm^{-1}[13].TGA studies was recorded by TGA-DTA Hitachi STA7000 instrument up to 1500 deg [14].

III. RESULTS AND DISCUSSION

A. UV-VIS ANALYSIS RESULT

UV–visible spectroscopy is mostly used to analyse the techniques for structural characterization of nanoparticles[15]. The biosynthesized silver nanoparticles were measured by UV–visible spectroscopy at different time intervals to study the change in light absorption and increase in intensity. The UV spectra of *Thymus vulgaris* silver nanoparticles synthesized displayed a strong broad absorbtion peak around 350nm due to the formation of AgNps as shown in Fig(1).

This peak corresponded to the surface plasmon resonance of the synthesized AgNps. After 48 hours the prepared mixture of AgNps is stored in the dark room the mixture of solution change into pale yellow to dark brown colour[16].

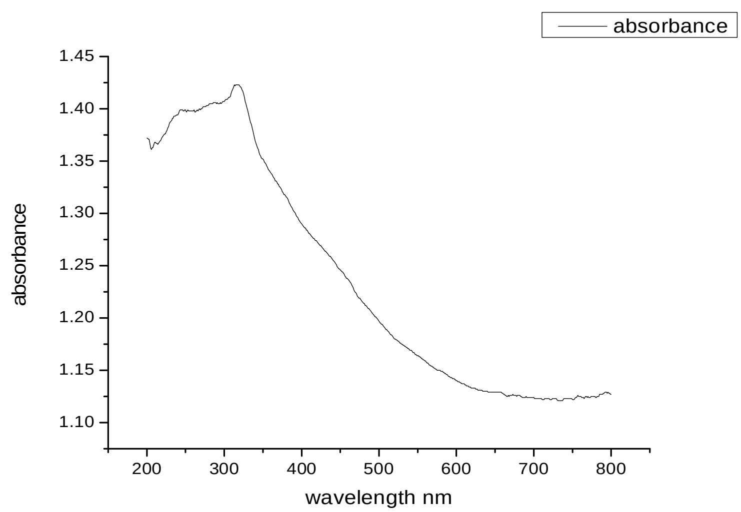


Fig .1 UV-Vis spectra of *Thymus vulgaris* $AgNP_S$

B. XRD ANALYSIS RESULT

The *XRD* analysis was carried out to determine the known phase of the silver nanoparticles. XRD pattern of the dried synthesized $AgNP_S$ Nano particles prepared by *Thymus vulgaris* leaf extract.

$$D= K\lambda / \beta\cos\theta \text{ nm}$$

The above given is the scherrer formula.Where D – is the mean size of the ordered crystalline domains –is a dimensionless shape factor, with a value also to unity. The shape factor has a typical value of about 0.9 λ –is the X-ray wavelengthβ –is the line broadening at half the maximum intensity (FWHM)θ –is the Bragg angle[17][18]. The obtained data was matched with the Joint Committee on Powder Diffraction Standards (JCPDS) file No.030921. The XRD spectrm fig(2) showed five distinct diffraction peaks at 32.118 ,38.007,44.177 and 64.344 77.242which are indexed the (111),(200),(220), (311) of the fcc silver nanoparticles[19][20].The average grain crystalline size of AgNps was prepared from *Thymus vulgaris* was calculated to be 13nm[21].

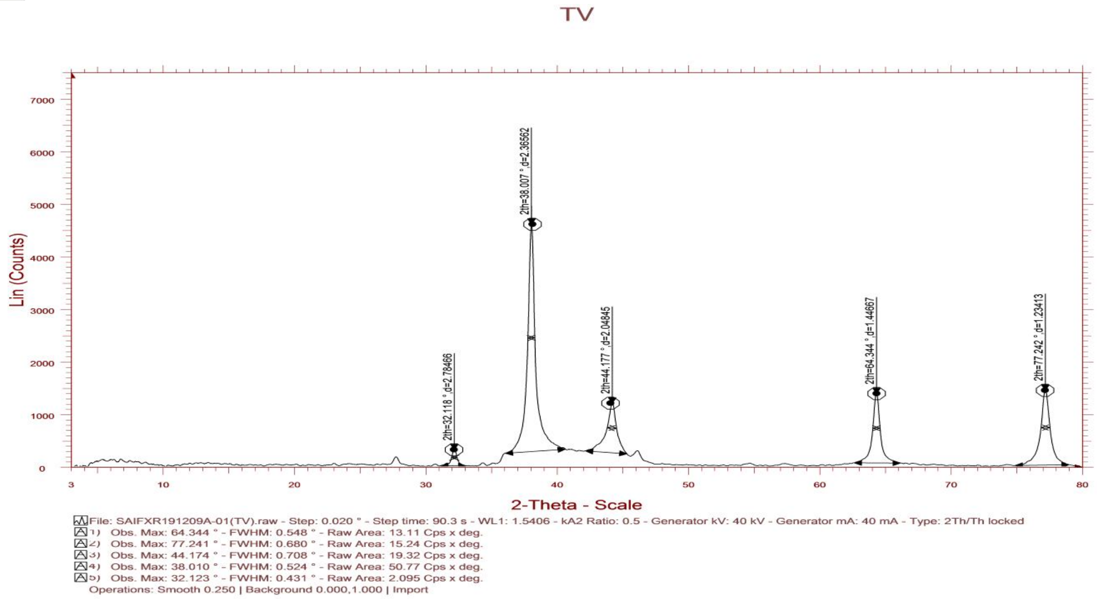
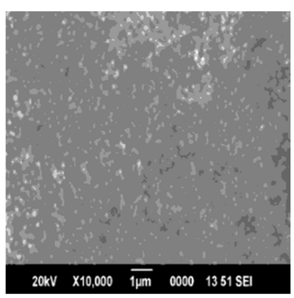
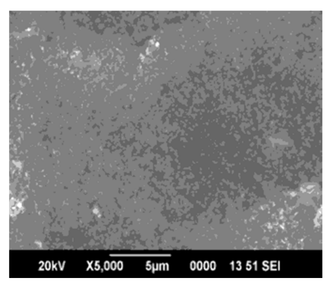


Fig .2 XRD pattern of greensynthesized silver nanoparticles

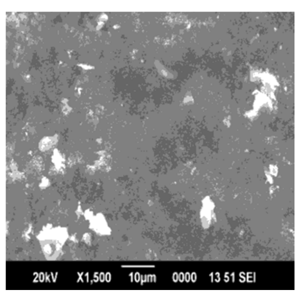
C. SEM Analysis Result

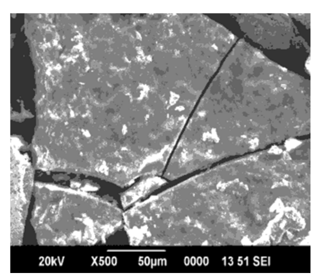
The surface morphology of the AgNps was studied using Scanning Electron Microscope analysis. SEM image exhibited that the biosynthesized silver nanoparticles were mostly in spherical shapes. The size of the nanoparticles were within the range of 1-50nm.It is also noticed that the nanoparticles are in direct contact with each other .The capping agent noticed in the nanoparticles gives the stabilization for the particle. The caping agent may be due to the sediments in the leaf extract. [22].





(a) (b)





(c) **(d)**

Fig 3. SEM analysis of (a) silver nanoparticles in 1µm and (b) silver nanoparticles in 5µm(c) silver nanoparticles in 10µm (d) silver nanoparticles in 50µm

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D. EDX Analysis Result

EDX technique is used to determine the elemental analysis and quantitative determination of available minerals in the nanoparticles. EDX spectra of the synthesized silver nanoparticles from Thymus Vulgaris leaf extract confirmed the presence of elemental silver in the sample [23]. The EDX profile of the AgNps prepared using thymus vulgaris extract showed the presence of silver(fig4),additional elements also observed C,O,P,S,Cl,K,Ca,Ag [24]. Quantitative analysis proved 3% silver contents in the examined samples.The mass % of silver in the sample is 34.65 and atom % is 6.39[25].

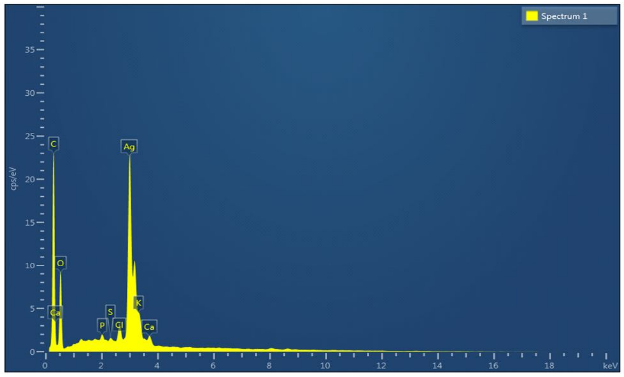


Fig 4 EDX spectrum recorded for prepared silver nanoparticles and different X- ray emission peaks are labeled

E. FTIR Analysis Result

Fouier transform infrared spectroscopy analysis method uses infrared light to scan test samples and observe chemical properties and characterization of the *Thymus vulgaris* leaf extract and the functional groups of present silver nanoparticles[26]. The FTIR spectrum of stabilized silver nanoparticles is shown in (fig 5). FTIR showed absorption 3451.92cm^{-1}, 1621.85 cm^{-1},1494.54 cm^{-1},1248.24 cm^{-1},1166.52 cm^{-1},1058.01 and cm^{-1},511.51 cm^{-1} as shown in (Fig 5). In this spectra the peak 3451.92cm^{-1} absorption corresponds to the strong stretching vibrations of OH functional group[25]. 1621.85 cm^{-1} cm−1 due to the stretching vibration of C=C[27]. 1494.54 cm^{-1} corresponds to C-Cl stretching mode and it indicated alkyl halides groups.[28] The peak absorbed in1248.24 cm^{-1} and 1058.01 cm^{-1} is remarked to C–N stretching possible due to the presence of amines group[29]. 1166.52 cm^{-1} corresponds to C-N stretching alcohols[30].

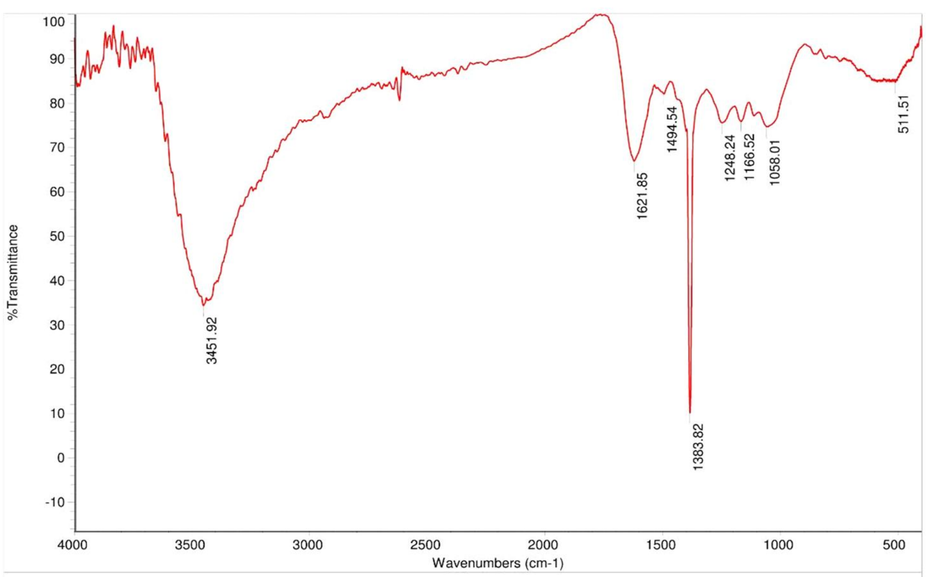
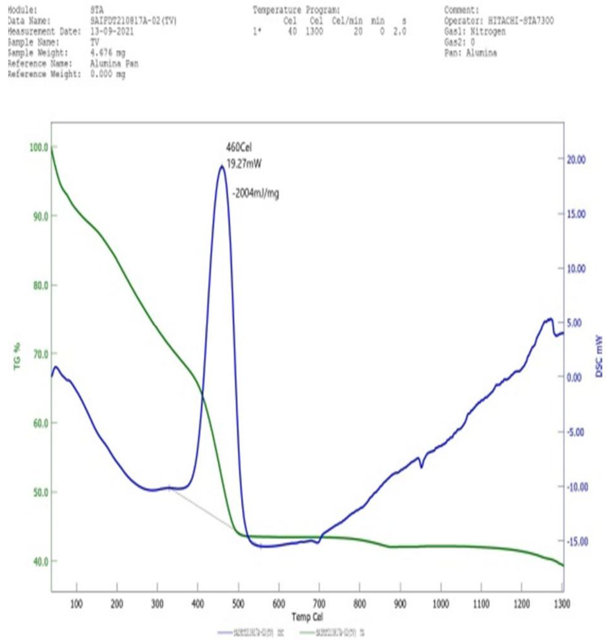


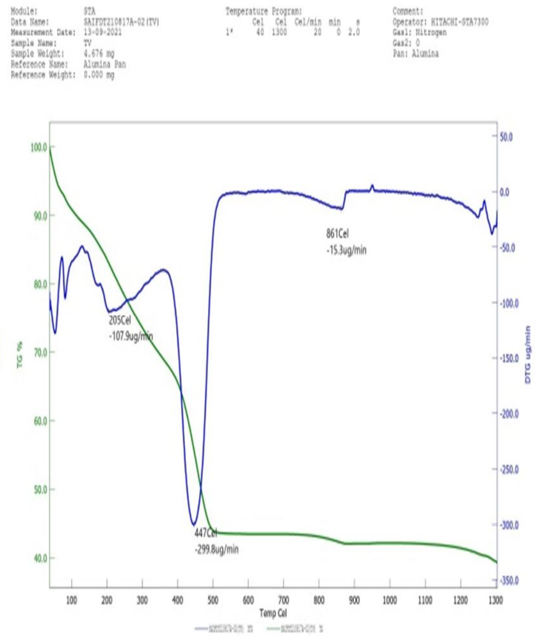
Fig.5 FTIR spectra of the *Thymus vulgaris* silver nanoparticles

F. Thermo Gravimetric Analysis (TGA)

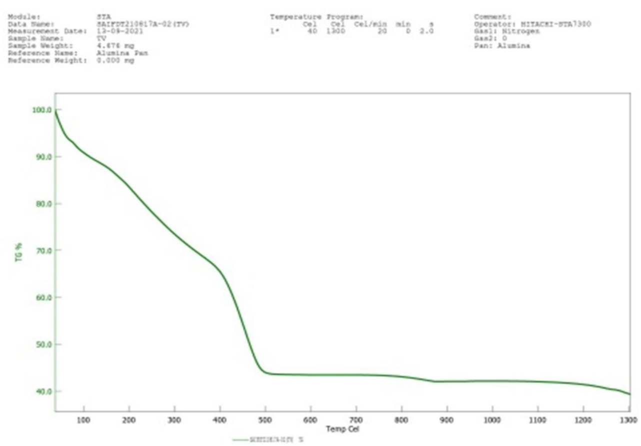
TheAgNps sample prepared using *Thymus vulgaris* is anaysed was for Thermo Gravimetric property. When the temperature gradually increased the sample.



(a)



(b)



(c)

Fig 6, TGA of *Thymus vulgaris* silver nanoparticles

G. Antimicrobial Studies

1) Antibacterial

The antibacterial activity of biosynthesized silver nanoparticles using Thymus vulgaris silver nanoparticles ,ciprofloxacin, was studied against Gram-positive (S. aureus and B.subtilis) and Gram-negative K.pneumoniae and E. coli) bacteria using the agar well diffusion assay, and the zone of inhibition was tabulated in Table 1. The synthesized AgNPs showed good efficient antibacterial activity against for both Gram-negative and Gram-positive bacteria. The silver nanoparticles synthesized by Thymus vulgaris silver nanoparticles showed the maximum zone of inhibition around 14 mm for S.aureus for and 13 nm for B.subtiliswhich were followed by K.pneumoniae (5 mm) and E.coli (8 mm). When compared with the standard ciprofloxacin the synthesized $AgNp_s$ shows 72 % of inhibition against E. coli, 38% against K.pneumoniae, 46% against B.subtilis and 42% against S. aureus. The positive control

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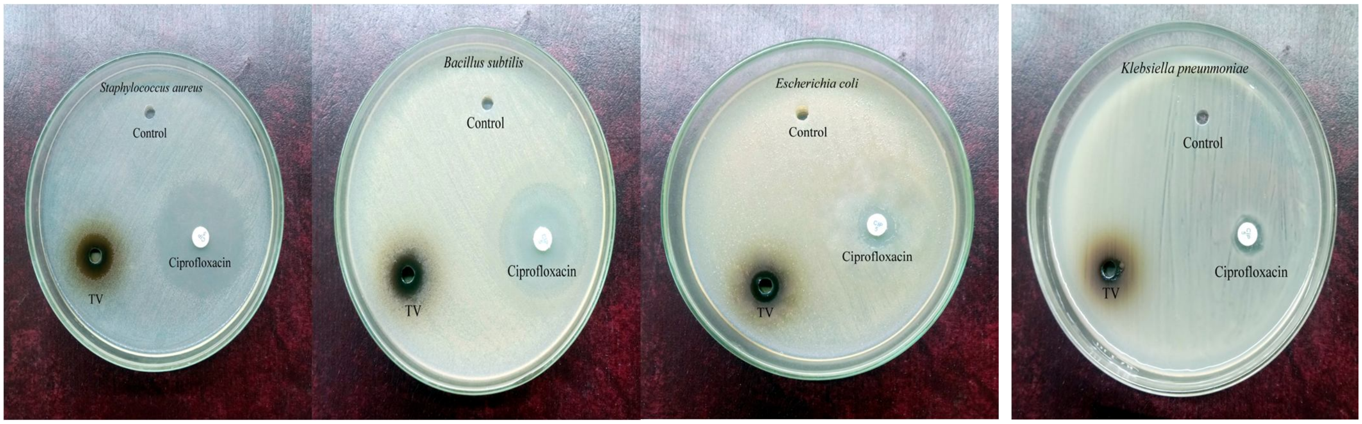
(AgNO3) displayed antimicrobial activity against all tested microorganisms.[29][6]



A b c d

Figure 7. Antibacterial activity against a)S.aureus b)B.subtilis c)K.pneumoniae d)E.coli

Table 1 The results of antibacterial activity with zone of inhibition

	Microorganisms	Control	TV	Ciprofloxacin
		Zone of inhibition in mm		
1.	*Staphylococcus aureus*	-	14	33
2.	*Bacillus subtilis*	-	13	28
3.	*Klebsiella pneumoniae*	-	05	13
4.	*Escherichia coli*	-	08	11

2) *Antifungal*

In the agar disc diffusion method, the concentrations of greener synthesized Thymus vulgaris silver nanoparticles and inhibition of ketoconazole is shown in the fig 7. Inhibition zone around discs incorporated with nanoparticles. The synthesized silver nanoparticles (positive control) showed significant inhibition of fungi. A broad spectrum of antifungal activity of silver nanoparticles was observed against a wide range of wood-degrading fungi. Moreover, the biosynthesized silver nanoparticles exhibited strong antifungal activity against the *Aspergillus niger(10mm), Aspergillus flavus(10mm), Candida albicans(7mm). Penicillium sps(6mm)* were showed in table 2

a b c d

Figure 8.Antifungal activity against (a) A. niger, (b) A. flavus and (c) C albicans. (d) Penicillium sps

Table 2 The results of antifungal activity with zone of inhibition

S.No.	Microorganisms	Control	TV	Ketoconazole
		Zone of inhibition in mm		
1.	*Aspergillus niger*	-	10	15
2.	*Aspergillus flavus*	-	10	12
3.	*Candida albicans*	-	07	10
4.	*Penicillium sps*	-	06	15

IV. CONCLUSION

In this study the silver nanoparticles were successfully synthesized using the extract of *Thymus vulgaris* leaf for the first time. The biosynthesized AgNPs were characterized using UV-Vis, FTIR, SEM, EDX, and XRD,TGA have confirmed characteristic features of *Thymus vulgaris* silver nanoparticles. Furthermore *Thymus vulgaris* silver nanoparticles were made without any impurities. The results were within approximate ranges of each other and the values reported in previous studies. The UV-Vis analysis confirmed the AgNPs at 320 nm. The average size of the AgNPs prepared was calculated in the range of 14 nm using XRD pattern. The spherical shape and plate surface shape of AgNPs was observed by SEM image. Energy dispersive x-ray (EDX) shows the ED spectrum of the synthesized silver nanoparticles strong silver signal along with weak chlorine (Cl), carbon(C), oxygen (O) peak was observed. The FTIR analysis related the presence of various functional groups. Here in we reported eco-friendly and low cost process for synthesis of AgNPs. The plant source may act as capping agents which control the size and capping agents which control the size and shape of the silver nanoparticles.

V. ACKNOWLEDGMENT

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