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Green Route Synthesis and Characterization of Silver Oxide Nanoparticles using *Ocimum Gratissimum* Leaf Extract

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Abstract: Green synthesis of metal nanoparticle is a welcome development considering its eco-friendliness, less hazard and cost effectiveness. This paper reports green synthesis and characterization of silver oxide nanoparticles (Ag_2O-NPs) using methanolic extract of *Ocimum gratissimum* leaf as reducing and capping agent with silver nitrate ($AgNO_3$) as precursor. The synthesized Ag_2O-NPs were characterized by subjection to UV–Vis spectroscopy, Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), Energy Dispersive X-ray Spectroscopy (EDX) and Fourier Transform Infrared Spectroscopy (FTIR). UV–Vis spectroscopy revealed the reduction of $AgNO_3$ to Ag_2O-NPs by the methanolic leaf extract. From SEM analysis, the Ag_2O-NPs synthesized were spherical with size range of 20-100 nm. X ray diffraction (XRD) analysis shows that the Ag_2O-NPs were of face-centered cubic structure. From Energy dispersive X ray spectroscopy, elemental compositions of the Ag_2O-NPs were Ag (80%), O (10.267%) and C (9.74%). Analysis of stretching and bonding in the Ag_2O-NPs using FT-IR showed Ag-O bonding at 475.51- 415.14 cm^{-1} thus confirming the formation of Ag_2O-NPs.

Keywords: *Ocimum gratissimum; plant extract; green synthesis; silver nanoparticles; characterization*

1. Introduction

Metal nanoparticles (MNPs) have received great attention due to their applications in different fields of technology including pharmaceuticals (Salvioni *et al.*, 2017; Barakat *et al.*, 2013; Suleiman *et al.*, 2013), water treatment including heavy metal removal (Awodi *et al*, 2023) as water bodies can be polluted by heavy metals (Okon *et al.*, 2022). Other uses are in electronics (Chen *et al.*, 2009), photonics (Zhang *et al.*, 2012; Aldwayyan *et al.*, 2013), therapeutics (Jain *et al.*, 2009) and antimicrobial productions (Ahmed *et al.*, 2016). Amidst the novel metal nanoparticles, silver has gained popularity in biomedical, food, textile, and other industries due to its antimicrobial properties

(Ahmed *et al.*, 2016). Silver nanoparticles (AgNPs) are believed to exert antimicrobial effect by releasing Ag^+ ions. Although ionic silver itself has antimicrobial properties, AgNPs seem to be more versatile in terms of providing long-lasting effect and better penetration into microbial cells due to their nano-size and large surface area to volume ratio (Prabhu and Poulose, 2012). The use of leaf extract for the green synthesis of metal oxide nanoparticles has been illustrated by Ekwumemgbo *et al*. (2023) and kpega *et al*. (2023) when iron oxide nanoparticles (Fe_3O_4-NPs) was synthesized from *Prosopis Africana* leaf extract and when zinc oxide nanoparticles was synthesized from *Corchorus olitorius* Leaf Extract. These successful green syntheses have contributed to what informed the choice of green synthesis as a method of metal oxide nanoparticles synthesis in this study.

Ocimum gratissimum (clove basil, African basil) is an herbaceous plant belonging to the Lamiaceae family; in Nigeria, it is commonly known as scent leaf and is found in the savannah and coastal areas (Kumar and Lal, 2022). Its medicinal value makes it to be used in traditional medicine for the treatment of different diseases such as upper respiratory tract infections, diarrhea, headache, conjunctivitis, toothache fever, and as mosquito repellents (Igbinosa *et al.*, 2013). Oil from the leaves have been found to possess antiseptics, antibacterial and antifungal activities (Alexander, 2016; Elmsellem *et al.*, 2019).

Recently green synthesis of Ag_2O-NPs has been studied with various plants leaf extract such as *Parieteria alsinaefolia* (Ullah *et al.*, 2023**)**, orange leaf extract (Sultan *et al.*, 2023), *Lawsonia inermis* (henna) Leaf extract (Fayyadh and Alzubaidy, 2021) and *Stachys lavandulifolia*, (Shahriary, 2018), but report on the use of *Ocimum gratissimum* extract for the synthesis of Ag_2O-NPs is not common. It has been established from phytochemical screening that *Ocimum gratissimum* (clove basil, African basil, scent leaf) contains phytochemicals such as saponins, flavonoids and phenols (Usunomena and Eseosa 2016; Venuprasad *at al* 2014). This makes it an auspicious plant for the synthesis of nanoparticles following the presence of these phytochemicals that can serve as reducing and stabilization agents in green synthesis of metal oxide nanoparticles (Singh *et al*., 2019). This study therefore aimed at green synthesis and characterization of silver oxide nanoparticles (Ag_2O-NPs) using *Ocimum gratissimum* leaf extract as the reducing agent.

2. Methodology

2.1 Materials

Silver nitrate ($AgNO_3$) and sodium hydroxide (NaOH) were obtained from Sigma-Aldrich Chemicals. Distilled water was used throughout the synthesis. All reagents used in the study were of analytical grade. Fresh *Ocimum gratissum* leaves were bought from a vendor at Samaru market, Zaria, Nigeria. Identification and authentication were carried out at the herbarium unit of Ahmadu Bello University, Zaria and were taken to the chemistry laboratory for preparation and stored until needed for study.

2.2 Preparation of plant extract

The *Ocimum gratissimum leaves* were washed with tap water for three times to remove dust and other dirt materials, there were then rinsed with distilled water. The leaves were air-dried for two weeks, ground to powder using mortar and pistol and 15.00 g of it were weighed out for analysis. Afterwards, the powdered sample was macerated in 80% methanol by mixing carefully 2.00g of powdered sample

with 100 cm^3 of methanol in a beaker in order to obtain a hydro-alcoholic crude extract at room temperature for 72 hours, after which the filtrate was separated from the marc by using filter paper (Whatman No.1). The methanol was allowed to evaporate from the filtrate with mild heating in dry oven at 40 °C for 10 minute.

2.3 Green Synthesis of Silver Nanoparticles

A 1.00 cm^3 of the *Ocimum gratissimum* extract was added into a clean Erlenmeyer flask and then 9.00 cm^3 of 0.10 M aqueous $AgNO_3$ solution was added into the extract. The mixture was stirred for 30 minutes using a magnetic stirrer. While stirring, sodium hydroxide (1.00 M) was added in drops in order to adjust the pH to 10 and to increase the yield of Ag_2O-NPs produced. A color change from somewhat yellowish brown to dark brown was observed as the stirring continued. The change in colour from yellowish brown to dark brown (**Figure 2**) confirmed the formation of the Ag_2O-NPs. After stirring the mixture for 10 minutes, the mixture was stored at room temperature for two hours to allow the particles to settle. After the complete formation of the nanoparticles, which was in form of dark brown coloured aqueous colloidal mixture the solution was then filtered using Whatman No 1 filter paper to separate out the Ag_2O-NPs (Allafchian *et al.*, 2016). The dark brown colloidal product was dried absolutely and calcined at 400 ◦C for 3 h after which a black Ag_2O-NPs (**Figure 3**) was obtained. After the calcination, the synthesized Ag_2O-NPs were characterized using UV–Vis spectroscopy, Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), Energy Dispersive X-ray Spectroscopy (EDX) and Fourier Transform Infrared Spectroscopy (FTIR).

Figure 1. Plant extracts

Figure 2. Formation of Ag_2O-NPs

3. Results and Discussion

3.1 UV–visible spectroscopy of the green synthesized Ag_2O-NPs

Figure 4 represents the UV–visible absorption spectrum of the green synthesized Ag_2O-NPs from *Ocimum gratissimum.* The UV–visible Spectroscopy was carried out using an Agilent Cary 300 U.V

spectrophotometer in spectra range of 200–800 nm. The formation of silver oxide nanoparticles started when *Ocimum gratissimum* leaf extract was added to $AgNO_3$ solution. The visible color change (light yellow to dark brown) of the solution and spectral analysis confirmed the formation of silver oxide nanoparticles as shown in **Figure 4**.

Figure 3. Green synthesized Ag_2O-NPs after calcination

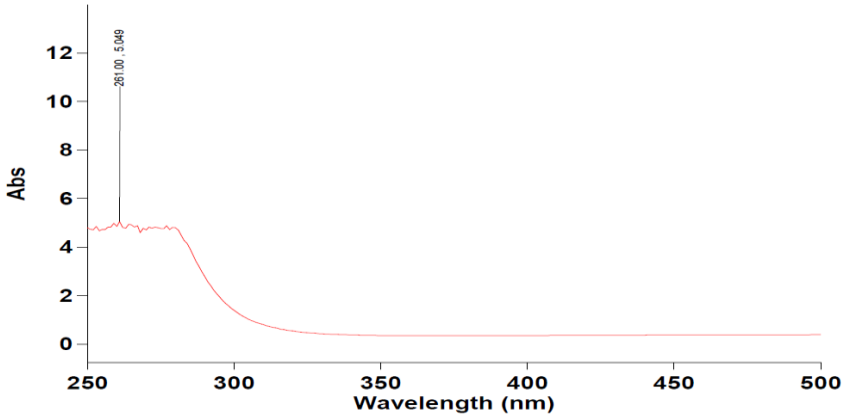

Figure 4. UV-Visible spectroscopy of silver oxide nanoparticle.

It was observed that the absorbance peak centered around 261 nm due to surface plasmon resonance, which corresponds to nanoparticles production, and this indicates the reduction of $AgNO_3$ to Ag_2O-NPs. The 261 nm observed is lower than the 425 nm and 430-433 nm reported by Fayyadh *et al.* (2021) and Ullah *et al*. (2023). This could result from the difference in particle size of the Ag_2O-NPs formed as the absorption maxima depend on the particle size of Ag2O NPs (Shume *et al*., 2020). Apart from nanoparticle characterization, UV–visible Spectroscopy can also be used for Characterization of synthesized complexes such as NI(II) complex of L-leucine (Oladunni *et al*., 2023)

3.2 Scanning electron microscopy (SEM)

The surface morphology and size of the Ag_2O-NPs were examined using a Scanning Electron Microscope that is coupled with EDX detector, Model Supra TM 35 VP (Carl Zeiss instrument, United

Kingdom). The SEM image shows the high density of the synthesized Ag_2O-NPs. SEM micrographs at multiple magnifications as presented in **Figure 5** show that the Ag_2O-NPs are spherical in shaped and well distributed without aggregation. The spherical shape observed is in line with the reports of Sultan *et al.* (2023) and Adamu *et al*. (2021) when green synthesis of Ag_2O-NPs and silver nanoparticles were synthesized and characterised. The SEM analysis revealed that the synthesized nanoparticle sizes ranged from 20 – 100 nm.

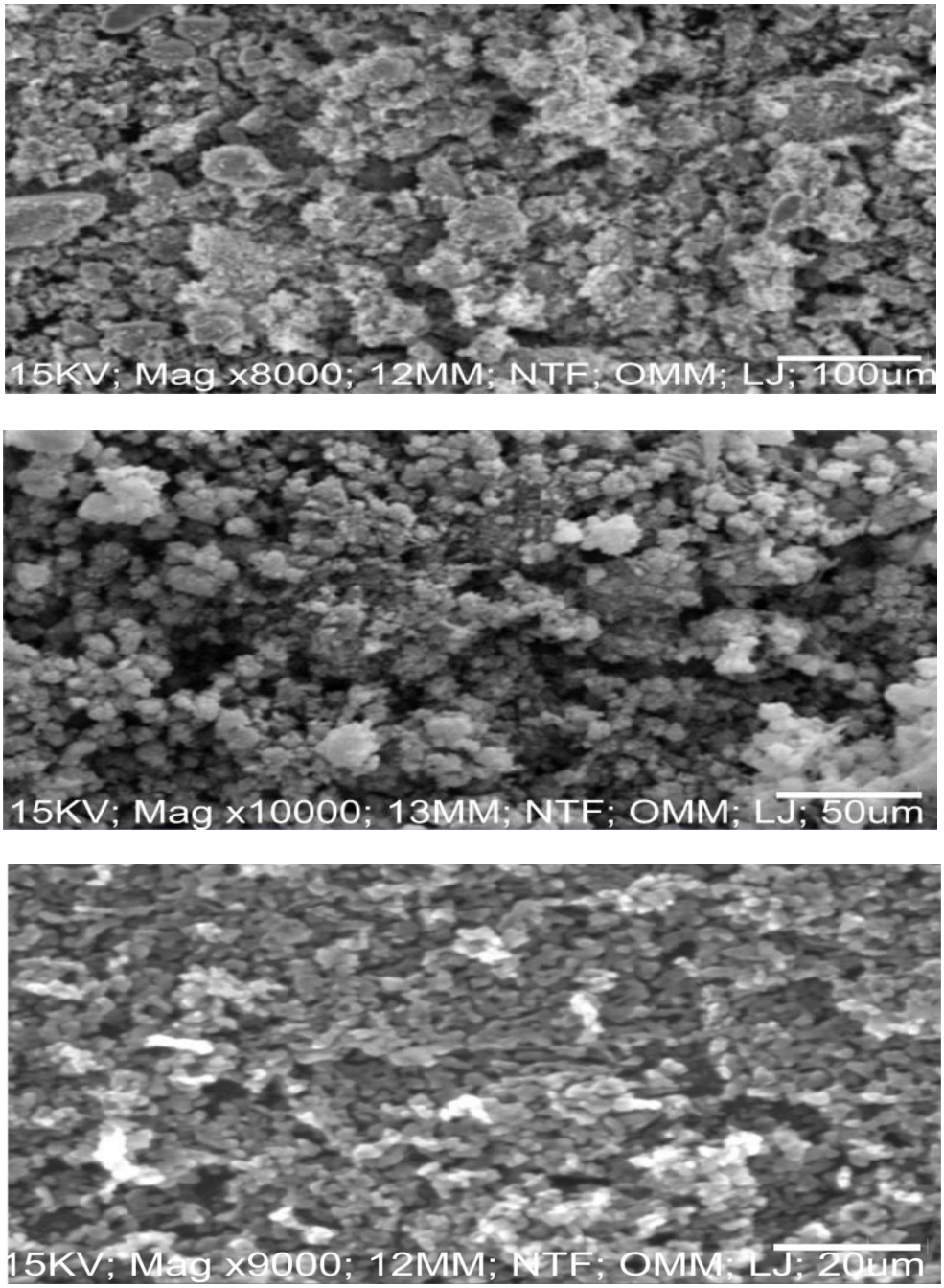

Figure 5. SEM micrograph of the green synthesized Ag_2O-NPs at multiple magnifications

3.3 X-ray diffraction (XRD) analysis

X-Ray diffraction (XRD) analysis was carried out using X-Ray Diffractometer, Model Miniflex 600 (Rigaku Corporation, Japan) with Cu kα radiation (λ=0.15406 nm) over the scanning range of $2\Theta = 5^0$-80^0 to establish the crystalline nature of the synthesized nanoparticles. The XRD pattern of the green synthesized Ag_2O-NPs is presented in **Figure 6**. XRD is used to determine the structural properties and size of NPs. XRD gives characteristic x-ray spectra for each material because every crystalline substance has a characteristic atomic structure (Vaishnav *et al*., 2017). XRD gives information on average particle size (D) when Debye Scherer formula is applied as $D = (0.9\ \lambda)/(\beta \cos \Theta)$. Where, λ= wavelength of X-ray, β=Full width at half maximum (in radians), Θ= Bragg's diffraction angle **(**Khan *et al*., 2017a, Khan *et al*., 2017b, Vaishnav *et al*., 2017). The XRD pattern of the green synthesized Ag_2O-NPs as presented in **Figure 6** shows several peaks where the two main peaks located at 33.93^0 and 38.61^0 corresponds to (100) and (101) planes of the face centered cubic structure of the green synthesized Ag_2O-NPs. The diffraction peaks of the XRD correspond with those recorded in Joint Committee on Powder Diffraction Standards (JCPDS, card No. 04-0783) which confirms the formation of Ag_2O as the peaks tally with the characteristic face centered cubic structure of Ag_2O-NPs. Fayyadh et al. (2021) also observed face centered cubic structure for Ag_2O-NPs when green-synthesis of Ag_2O nanoparticles for antimicrobial assays was studied. From the Debye Scherer formula, the average crystallite size of the synthesized Ag_2O-NPs was 26 nm. This is within the average range (20 -100 nm) observed from the SEM analysis.

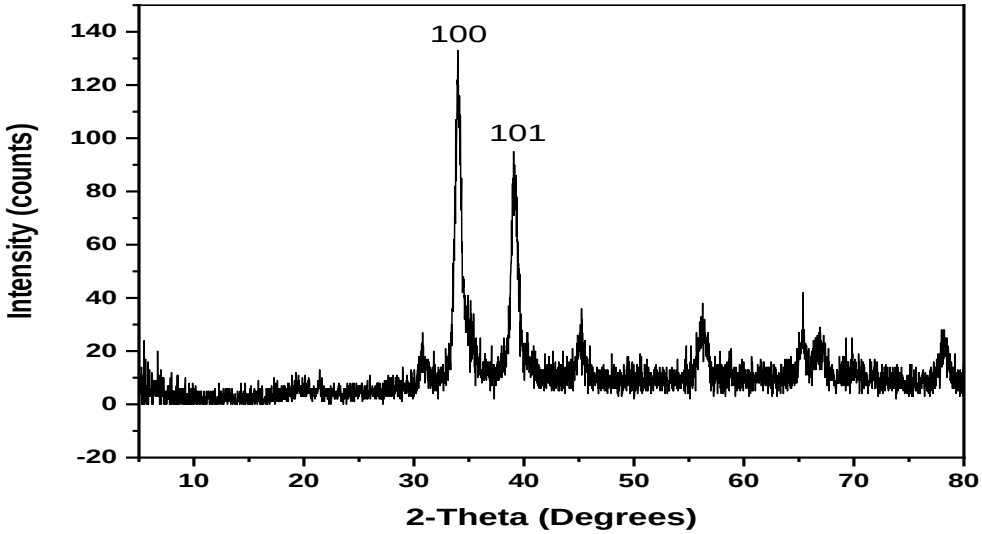

Figure 6. XRD pattern of the green synthesized Ag_2O-NPs

3.4 Energy dispersive X-ray analysis (EDX)

EDX analysis gives qualitative as well as quantitative status of elements involved in formation of nanoparticles. **Figure 7** presents the EDX spectra of the green synthesized Ag_2O-NPs, it shows the elemental composition of the green synthesized Ag_2O-NPs. From the spectra, the elemental content is

silver (Ag), Oxygen (O) and carbon (C), with an atomic composition of 80%, 10.26% and 9.74% respectively. This elemental composition is an indication that the green synthesized Ag_2O-NPs is of high purity. The carbon present as an element in the sphere of the green synthesized Ag_2O-NPs is an organic capping agent bound to the surface of Ag_2O-NPs. This carbon is associated with phytochemicals including phenols, saponins and flavonoids present in the *Ocimum gratissimum* leaf extract (Usunomena and Eseosa 2016; Venuprasad *at al* 2014), that was used as reducing and stabilizing agent during the green synthesis. The high amount of Ag is an indication that the synthesized nanoparticle could be a good source of Ag metal in plants through absorption from soil if dumped on soil where plants grow and can find its way into food chain like other heavy metals like lead, zinc, cadmium, iron and nickel (Okon *et al*., 2023a; Okon *et al*., 2023b).

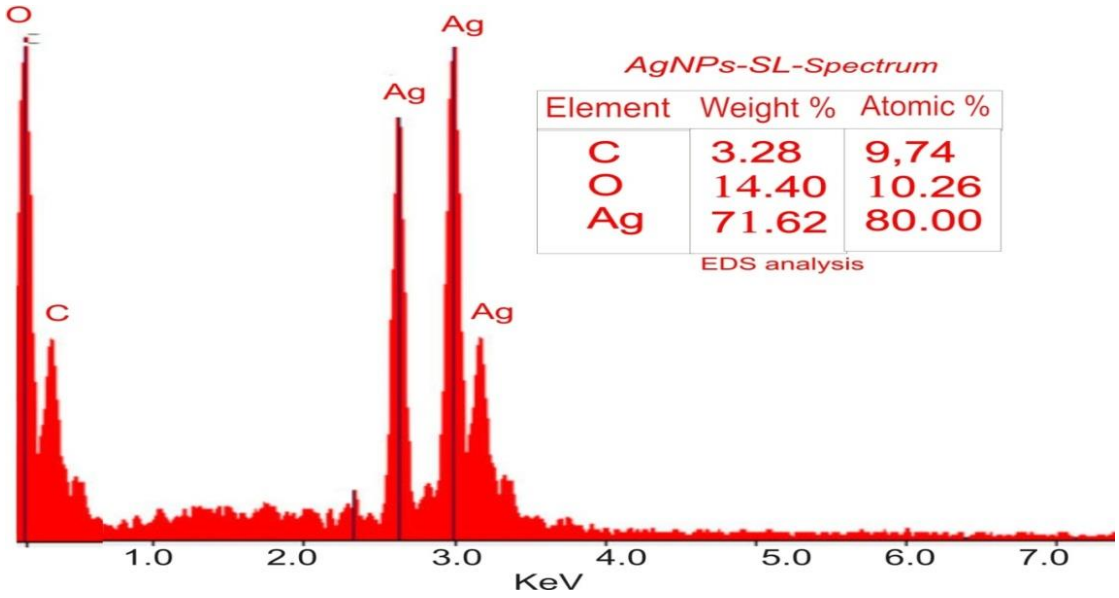

Figure 7. EDX spectra of the green synthesized Ag_2O-NPs

3.5 Fourier transforms infrared (FTIR) spectroscopy

The completely dried powdered sample was placed in the sample holder in FTIR spectrophotometer (Agilent Cary 630) and FTIR spectra were recorded in the range 4000-400 cm^{-1} as shown in **Figure 8**. The FTIR spectra reveal functional groups of the compounds on nanoparticles surface and in synthesis solution and also indicates the presence of Ag_2O-NPs nanoparticles. The Fourier transform infrared spectroscopy (FTIR) typical bands at 3686–3626 cm^{-1} correspond to O-H stretching of alcohols, 3140 correspond to O-H stretching of carboxylic acids, 2000.67 cm^{-1} correspond to C-H bending of aromatic compounds. 1614.56 cm^{-1} correspond to N-H bending of amine, 1310.30 indicates C-N stretching of aromatic amine. 797.73 cm^{-1} corresponds to C-Cl stretching of alkyl halides. The multiple peaks at 475.51-415.14 cm^{-1} correspond to Ag-O stretching, thus confirming the presence of Ag_2O-NPs. These characteristic absorption peaks suggested the presence of phytochemicals in the extract that were responsible for capping and efficient stabilization of silver nanoparticles thus protecting them from aggregation (Bichi *et al*., 2022).

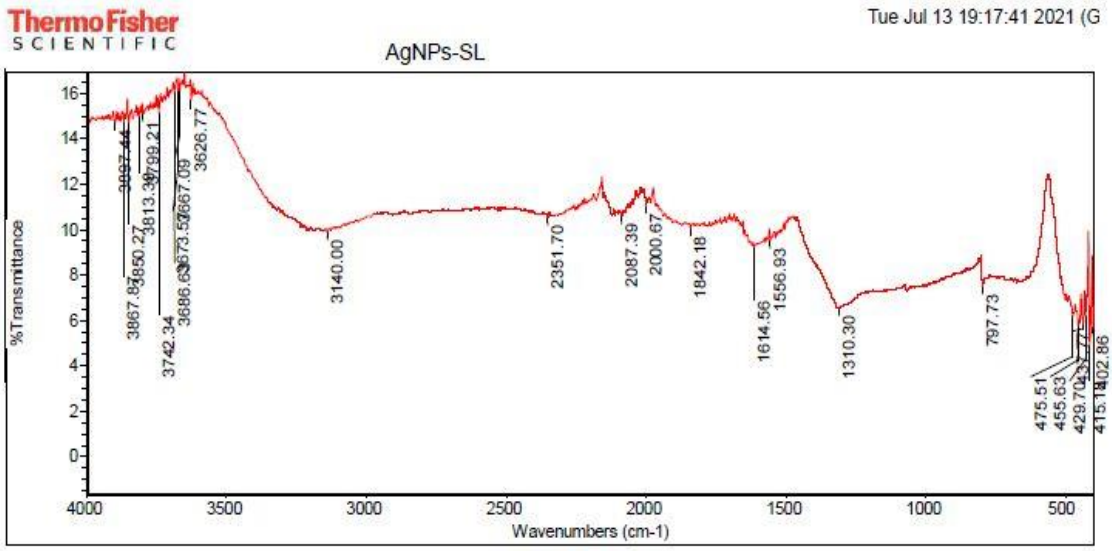

Figure 8. FTIR spectra of the green synthesized Ag_2O-NPs

Conclusion

Green synthesis of silver oxide nanoparticles (Ag_2O-NPs) nanoparticles using methanolic extract of *Ocimum gratissimum* provides an environmental friendly, simple and efficient route for synthesis of nanoparticles. This work reports green synthesis and characterization of Ag_2O-NPs from *Ocimum gratissimum* leaf extract and Silver nitrate ($AgNO_3$) as the precursor. UV–Vis spectroscopy, Scanning Electron Microscopy (SEM), X-Ray diffraction (XRD), Energy Dispersive X-ray Spectroscopy (EDX) and Fourier Transform Infrared Spectroscopy (FTIR) were deployed in the characterization of the green synthesized Ag_2O-NPs. UV-vis indicated the reduction of $AgNO_3$ to Ag_2O-NPs by the leaf extract. SEM revealed that the sizes of the synthesized ZnO-NPs was in the range of 20-100 nm. The XRD pattern revealed face centered cubic structure of the synthesized Ag_2O-NPs. Elemental composition given by EDX showed that the Ag_2O-NPs is mainly composed of Ag (80%), O (10.267%) and C (9.74%). Investigation into stretching and bonding in the Ag_2O-NPs using FT-IR showed Ag-O bonding at 475.51- 415.14 cm^{-1} thus confirming the formation of Ag_2O-NPs.

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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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