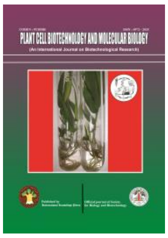


Plant Cell Biotechnology and Molecular Biology

Volume 25, Issue 1-2, Page 53-68, 2024; Article no.PCBMB.11946
ISSN: 0972-2025

Facile Synthesis of Graphene–Metal Nanocomposites with Tephrosia Purpurea for Anticancer and Antimicrobial Activity

Kfait Ullah Khan [a*], G. Malathi [b++], Wongamthing [c], Sanjay Hazarika [d#], Okram Ricky Devi [e#], Th. Nengparmoi [f#], Bibek Laishram [e#] and T Senthilkumar [g†]

[a] Department of Chemistry, University of Lahore, Sargodha Campus, Pakistan.
[b] Krishi Vigyan Kendra, Sandhiyur, Salem - 636 203, Tamil Nadu, India.
[c] Department of Plant Pathology, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur 680656, India.
[d] Department of Entomology, AAU, Jorhat, Assam, India.
[e] Department of Agronomy, AAU, Jorhat, Assam, India.
[f] Department of Agronomy, School of Agricultural Sciences, Nagaland University, Medziphema-797106, India.
[g] Krishi Vigyan Kendra, Pudukkottai, Tamil Nadu-622303, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/PCBMB/2024/v25i1-28597

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.ikprress.org/review-history/11946

Original Research Article

Received: 26/12/2023
Accepted: 01/03/2024
Published: 21/03/2024

[++] *Associate Professor (Horticulture);*
[#] *Ph. D Scholar;*
[†] *Associate Professor (Nematology)*
**Corresponding author: E-mail: kafyniazi@gmail.com;*

ABSTRACT

The research is focused on screening various bioactive extracts from *Tephrosia purpurea* plant leaf for the onward synthesis of nanomaterial and formation of Grephene-Ag nanocomposite for their biological applications. The aerial parts of this plant are comprised of primary and secondary metabolites. Its latex contains many important compounds such as Phenolics, Flavonides, and Terpenes, etc. *Tephrosia purpurea* is used as a traditional medicine to cure different problems of the digestive and respiratory tract. The aerial parts of this plant show immense antifungal and antimicrobial pursuits. Silver nanoparticles were synthesized from methanol extract of aerial parts of the *Tephrosia Purpurea*. These nanoparticles were reacted with lab synthesized Graphene to form Plant-Silver-Graphene nanocomposites. The characterization of plant extract, extract-mediated Ag-nanoparticles, and graphene-metal nanocomposites was carried out by different techniques like FTIR, UV, EDX & SEM. FTIR & UV spectra peaks were found in the ranges already reported. EDX analysis shows the percentage of the elements matching the reported data. SEM shows similar ranges of nanoparticle sizes as found in the research papers. Moreover, anticancer activities of plant-Ag-graphene nanocomposites were done & comparison was made with parent materials. The results were remarkable as plant-Ag-graphene nanocomposites showed better anticancer activities than the nanoparticles and plant extract.

Keywords: Tephrosia purpurea; graphene; nanocomposites; anticancer.

1. INTRODUCTON

Nanoparticle-based drug delivery systems have been established via a lot of hard efforts. They use nanostructured materials created by combining bioactive chemicals with inorganic nanostructured matrices. Metal nanoparticles are widely employed in diverse fields including various medicinal fields and specialized medicinal delivery because of their distinctive characteristics [1]. To induce biomineralization, macromolecules that impersonate carbon-containing molecules of the matrix extracellular can be mixed with nanoceramics that are bioactive [2]. Nanoparticles' antimicrobial action is either direct (interfering with electron transmission through the membrane, penetrating/disrupting the cell envelope, or oxidizing components of a cell) or indirect (creating secondary products) [3]. Because of its affordable price, its handling is simple, and strong resistance to photo-induced breakdown, TiO_2 is one of the most investigated photocatalytic reactions required by semiconductors [4]. In addition to outstanding functioning and long-term stability, nanoparticles are advantageous because of have less cost and nonpoisonous character. Graphene which is a planar layer made of carbon atoms is single atom thick and closely packed in a sieve-like crystalline structure [5,6,7]. The exceptional electromechanical and thermochemical characteristics of graphene have piqued the scientific community's curiosity [8,9,10,11, 37-40]. It is practically transparent, has a lower density than steel, and transmits heat and electricity efficiently [12,13]. This two-dimensional carbon structure's properties and implementations have demonstrated great potential for prospective equipment and software, according to the researchers [14]. Graphene polymer and graphene-nanoparticle composite materials have exceptional thermal, mechanical, chemical, and electrical properties [15,16]. Nanosensors, power storage, catalyst, and hydrogen fuel are only a few of the uses of graphene nanocomposites incorporating metallic nanoparticles [17,18]. Metals and semiconductors have been intercalated with several inorganic nanoparticles were fused into the interface of graphene nanosheets to reduce agglomeration and produce graphene with high personal scattering and electrical conductivity [19,20,21].

Metal nanoparticle attachment to graphene aids in the dry state suppression of agglomeration of the graphene layers that arises. The metal nanoparticles operate as spacers, allowing access towards both faces of the graphene sheet and expanding the space among them [16]. Graphene/nanoparticle hybrid composites have awhile back been used in drug delivery applications like biosensors, biomedical imaging, photo-thermal therapies, Stem cell and bioengineering as well as medicine transport [22].

Tephrosia purpurea which is a member of Fabaceae and due to its anticarcinogenic, antipyretic, antidiabetic, antiviral, antibacterial, and anti-inflammatory properties, it is used as a folk medicine. This plant is a useful folklore medicine for treating hepatic inflammation and enlargement of spleen. It's ability of Cross-linking promotes collagen maturation and repair. Its antioxidants aid in the protection of the body against free radical damage by neutralizing superoxide radicals [23]. Several investigations have shown that this plant is an effective antibiotic against a variety of pathogens associated with wounds and other diseases [24].

Tephrosia purpurea is the scientific name for this plant [25] typical names Sharpunkha, Kingdom of Indigo Plant is a subclass of *Plantae, Tracheobionta* Superdivision*, Spermatophyta* Divison *Magnoliophyta*. *Isoglabratephrin* a and *tephropurpulin*, two unusual prenylated flavonoids were discovered in the aerial extract of Tephrosia purpurea together with glabratephrin a previously found flavonoid. By spectroscopic techniques *Tephropurpulin A*, *isoglabratephrin* and *glabratephrin* were named after ^{1}H–^{13}C Data analysis with spectroscopic analysis of compounds and their structures have been verified by X-ray analysis. The sesquiterpene of peculiar rotundane skeleton, 2-propenylester was assigned to the sesquiterpene of rotundane skeleton. As apollinine spectroscopic analyses were used to determine the structures of the compounds (L. C. Chang *et al*., 2000). *Tephrosia purpurea* leaves extract yielded three new (+)-tephrorins A and B, and (+)-tephrosone flavonoids. NMR spectrum research revealed their structures, and Mosher ester technique established their absolute configurations. Flavanones with an unique Tetrahydrofuron moiety are Compounds 1 and 2.

The pus-forming bacteria Propioni bacterium acnes and Staphylococcus epidermidi which cause acne inflammation have both been discovered. The antimicrobial activity of 12 medicinal herbs was investigated. It was discovered that Tephrosia purpurea had a considerable zone of Propioni bacterium acnes and Staphylococcus epidermidis suppression in the approach of disk diffusion method. For Tephrosia purpurea, the MIC values for respectively 0.675mg/ml and 2.5mg/ml.

2. MATERIALS AND METHODS

2.1 Materials

To prepare metal nanoparticles using the extracts of Tephrosia Purpurea different solvents were used. Silver Oxide nanoparticles were prepared from the plant extract and composites of these nanoparticles were made while reacting with Slunke and Kim model lab prepared graphene. Biological activities of the nanocomposites were checked and compared with the reported bioactivities of plant extracts and plant mediated silver nanoparticles.

2.2 Experimental Work

2.2.1 Scheme of reactions

The reduction method was used to prepare the Ag Nanoparticles suggested by Wen et al. [26].

2.3 Collection and Drying of the Plants

Tephrosia Purpurea plant was collected from the Hilly valley of Musa Khel Mianwali District of Punjab Pakistan and dried in a shade to avoid exposure to direct solar light.

2.4 Grinding of Plant

It was grinded in common house hold grinder, packed in Polythene bag and placed for further use.

2.5 Extraction of Plant Material

05 grams of plant material mixed in lab graded Methanol of 100 mL quantity by stirring on hot plate preheated 40 centigrade temperature for 1 hour [26].

2.6 Synthesis of Plant Catalyzed Silver Nanoparticles

0.017 gram of Silver Nitrate in 100 ml of Distilled Water mixed and stirred for an hour on 90 ^{o}C to 100 ^{o}C and 10ml of *Tephrosia Purpurea* plant extract was added to 90ml $AgNO_3$ soln. It was stirred vigorously on 100 ^{o}C for 1 hour. The steady change in colour from green colour to pale green and after sometimes to brown was noted. The complete change in color was an indicator of Nanoparticles formation. This color change started in 10 to 15 minutes and completed in 45 to 60 minutes. The synthesized nanoparticles were purified by centrifugation at 15000rpm for 20 minutes and redispersed in deionized water. It was left for drying and dried powder was used for different analysis [26].

2.7 Graphene-Metal Nanocomposites Synthesis

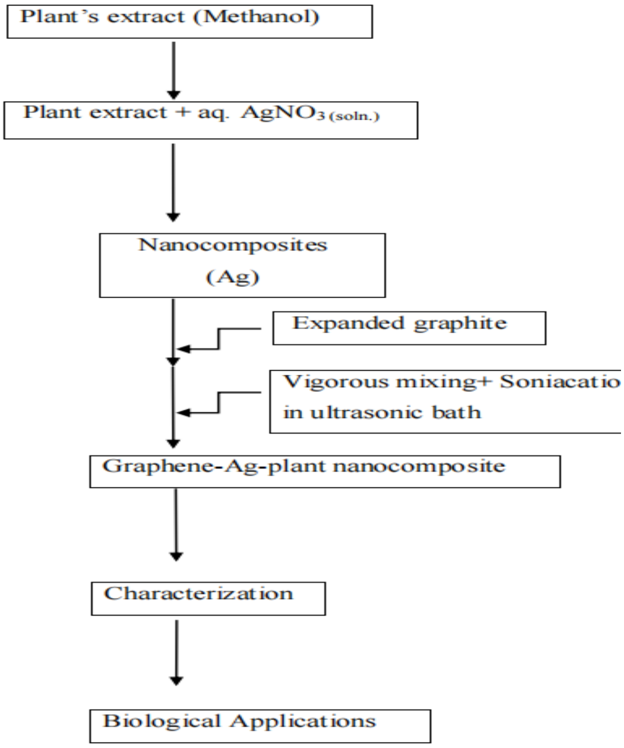
2.7.1 Graphene synthesis

01 gm of graphite was mixed by stirring in 20 mL of H_2SO_4 on room temperature for 45 minutes, 2 gm of $KMnO_4$ was dissolved and stirred for 1 hour at room temperature. After its completed dissolution, it started to become jell like which

was transferred on water bath on 80 °C for 05 minutes. It was slightly gelly like which was vacuum filtered; the filtrate was dried and washed with distilled water uptil it was neutralized. It was dried in oven on upto 70 °C [26,27].

2.8 Graphene-Silver-Plant Nanocomposite Synthesis

01 gram of graphene was dissolved in 100 ml of synthesized plant catalyzed Silver Oxide nanocomposites and sonicated for one day on 60°C. Method of Slunke and Kim was used for synthesis of graphene using plant extract where expanded graphite was added to 100ml of plant extract and the mixture was sonicated for 1 day at 60°C. The temperature of sonication bath was controlled by controlling water level in the bath and covering lid. The synthesized nanocomposites were dried and protected in sample vials for analysis and biological activities.



List 1. Research framework

Fig. 1. *Tephrosia Purpurea* plant leaves

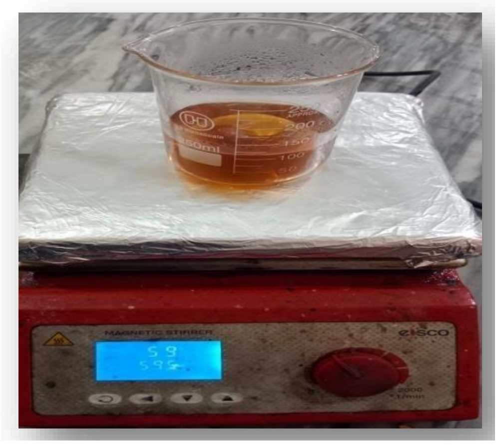


Fig. 2. Synthesized plant catalyzed silver nanoparticles

Fig. 3. Graphene synthesis

Fig. 4. Graphene synthesis

Fig. 5. Graphene synthesis

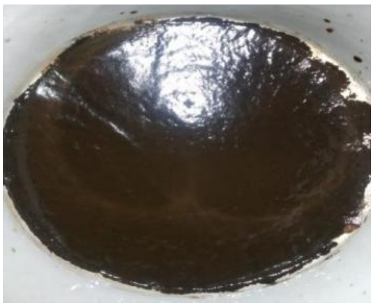


Fig. 6. Graphene synthesis

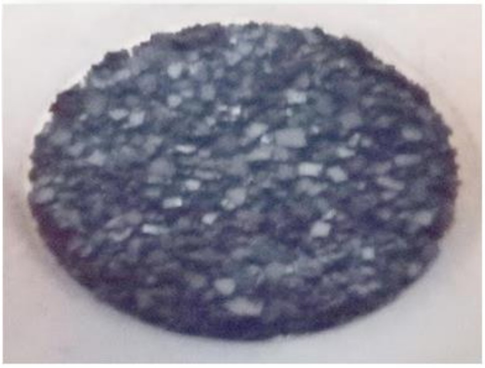


Fig. 7. Synthesized Plant-Ag-Graphene nanocomposites

2.8.1 Charcterizaton

Following techniques were used to characterize the plant extract, Synthesized Silver nanocomposites and Synthesized Graphene-Silver-Plant nanocomposites.

1. Infrared spectroscopy (IR)
2. Scanning Electron Microscopy (SEM)
3. Energy Dispersive X-Ray (EDX)
4. Ultra Violet-Visible (UV-Vis) spectroscopy

2.9 Biological Activities

2.9.1 Anticancer activities

The synthesized derivatives plant-silver nanocomposites, graphene-Ag-plant nanocomposites and plant leaf extract were assessed against human liver tumor cell line (Huh7). Sulphoradamine B Assay was utilized to check viability of the cells. To culture human Huh7 cells, temperature was maintained at 37°C. The Cells those were treated with DMSO used as control in all the experiments. After that 500 µg/mL Sulphoradamine B reagent was added and incubated the cells for 48 h. Results are described as % of cell viability.

3. RESULTS AND DISCUSSION

Silver nanoparticles were formed by mixing *Tephrosia purpurea* extract with aqueous soln. of Silver nitrate, initially color was green changed to yellow, and to brown which showed the reduction of Silver ion. That was used for the synthesis of nanocomposites, whose characterization is discussed below. The scheme of reactions for the synthesis has been drawn above procedure. The mechanism gave information that the Silver metal reacts with the hydroxide group of the flavonoid present in the plants and get reduced and after introduction to graphene these silver nanoparticles form composites with graphene. The possible mechanism of synthesis of nanocomposites from bioactive compounds e.g flavonoids present in the *Tephrosia purpurea* aerial extract is drawn in below Fig. 8 [28,29].

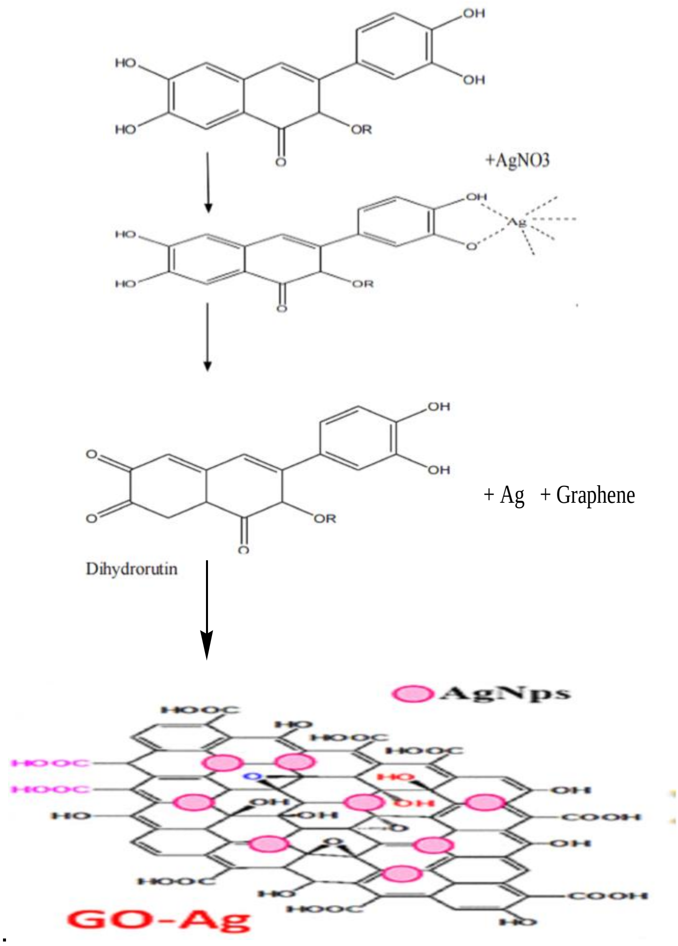


Fig. 8. Mechanism 1

3.1 Characterization

3.1.1 IR spectroscopy

FTIR was conducted on CARY 630 FTIR Spectrophotometer.

3.1.2 IR spectroscopy of plant extract

The spectrum in Fig 9 shows following peaks O-H (stretching) at 3370cm^{-1}, C-H (stretching) at 2924 cm^{-1}, H (stretching) at 2853 cm^{-1}, C=O at 1740 cm^{-1}, HN-CO amide linkage at 1630 cm^{-1}, while C-O bending at around 1000 cm^{-1}.

3.1.3 IR spectroscopy of plant-Ag nanocomposites

The assumed biomolecules responsible for the reduction of the Ag^{+} ions and capping of the bioreduced silver nanoparticles produced by *Tephrosia purpurea* plant leaf extract-Ag nanoparticles were identified using FT-IR. The FTIR spectrum in Fig 10 of the plant catalyzed synthesized Ag nanoparticles is shown in the figure above, with peaks in the range at around 3300 cm^{-1}, C-H at around 2920 cm^{-1}, C-H at around 2812 cm^{-1}, C-O at around 1700 cm^{-1} ,C=C at around 1500 cm^{-1},and of C-O-C around 1000 cm^{-1}. Various functional groups were found in the FTIR spectra, including secondary alcohol (O-H stretching, H-bonded), alkane (C-H stretching), aldehyde (O=C-H stretching), alkene (C=C stretching), aromatic (C=C stretching), alkane (-C-H bending), acid (C-O stretching), ester (C-O stretching), and alkyl halide (C-Br stretching), among others.

The presence of free catechin is shown by the peak at around 3300 cm^{-1}, which indicates the polyphenolic OH group, The peak around 1700 cm^{-1} is attributed to the carbonyl, around 2900cm^{-1} of C-H, bonds , groups of C=C peak at 1500 cm^{-1}. Peaks at around 1700cm^{-1} are carbonyl groups (C=O) from polyphenols including catechin gallate, epicatechin gallate, and theaflavin, while peaks at 1000–1200 cm^{-1} suggest a O–C–O single bond.

3.2 Infrared Spectroscopy of Plant-Silver-Graphene Nanocomposites

The presences of the functional groups, the graphene and Ag involved in the reduction of plant compounds and formation of the nanocomposite can be confirmed from the FT-IR spectra. Fig. 11 illustrates that the strong peak at around 3300 cm^{-1} is assigned to the stretching vibration of – OH groups. There are also bands corresponding to C–H around 2900cm^{-1} ,C=O groups at 1700 to 1800cm^{-1}, C=C groups at 1500cm^{-1}**,** and alkoxy C–O at around 1000cm^{-1} with less intensity and broadness [30].

Loss in intensity around 1000cm^{-1},around 3300cm^{-1},around 1500cm^{-1}, around 2900cm^{-1}, and change in intensity around 1700 to 1800cm^{-1}, clearly confirms the participation and reduction of oxygen-containing functional groups in the synthesis of the Ag-G nanocomposite Besides it also suggests that strong interactions may exist between the AgNps and the remaining surface hydroxide O atoms of GO Hence, FTIR analysis confirms the presence of phenolic compounds, flavonoids, and anthocyanins in *Tephrosia Purpurea* leaf and further it acts as reducing/capping agents for the fictionalization of AgNPs on the surface of “G” or synthesis of Ag-G nanocomposite [31,38].

3.3 Scanning Electronic Microscopy (SEM)

3.3.1 SEM of plant-silver nanocomposite

Scanning Electronic Microscopy was carried out with SEM- ZEIS Model. SEM studies data in Fig.12 shows that nanoparticles were formed in the range upto 200nm. The shape of nanoparticles was circular and disc like. SEM studies were helpful in measuring sizes and variation of shape and sizes, Fig. 12a to 12e shows this variety [39-40].

3.3.2 SEM of plant-silver-graphene nanocomposite

In order to examine the silver nanoparticles loaded on graphene layers, SEM instrument magnification was adjusted accordingly.

From the Fig.13, it can clearly be seen that some granules are spreaded on the layers of the graphene particularly Fig. 6 clearly giving show of the granules (nanoparticles) over the surface of graphene. Meanwhile some nanoparticles were also seen in between the layers of graphene. This confirms the formation of graphene-silver nanocomposites [26].

The comparison of figures makes us able to conclude that the plant-graphene-Ag nanocomposites were formed from the plant-Ag nanocomposites.

Table 1. IR comparative data

Sr No	f.group	Wavenumber cm^{-1} (plant extract)	Wavenumber cm^{-1} (plant-ag nanoparticles)	Wavenumber cm^{-1}(plant-ag-graphene nanocomposits)
1	O-H	3300-3500 sharp	3300-35000 a bit broad	3300 to 3500 fully broadened
2	C-H	2924, 2853 sharp	2920, 2812 a bit broad	2920, 2812 broadened to be mixed with O-H peak
3	C=O	Around1700 less intense	1731 a bit intense	Least intense Around 1730
4	C=C	Around 1500 intense	Around 1500 less intense	Around 1500 least intense
5	O-C-O-	Around 1000 with steep intensity	Around 1000 broader	Around 1000 very small intensity peak

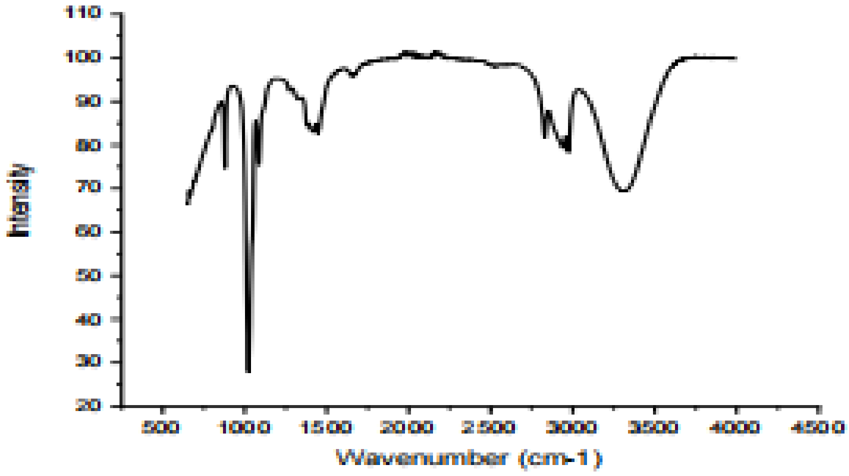


Fig. 9. IR Spectrum of *Tephrosia Purpurea* aerial parts extract

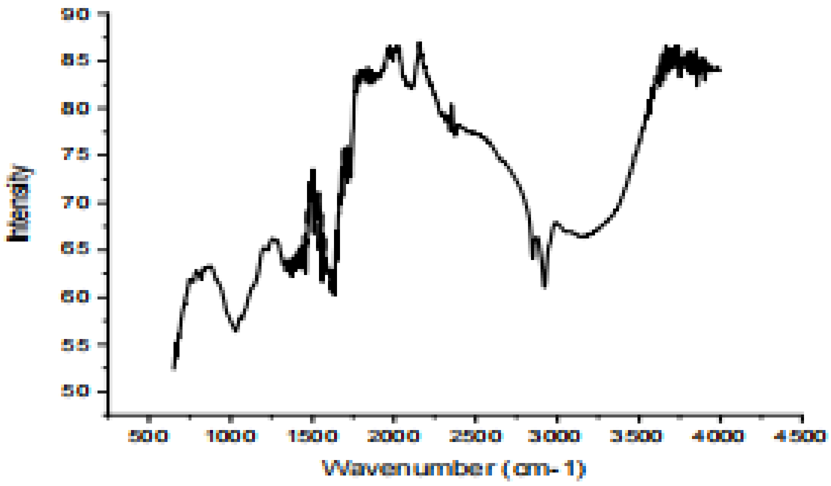


Fig. 10. IR spectrum of *Tephrosia Purpurea* catalyzed Ag Nanoparticles

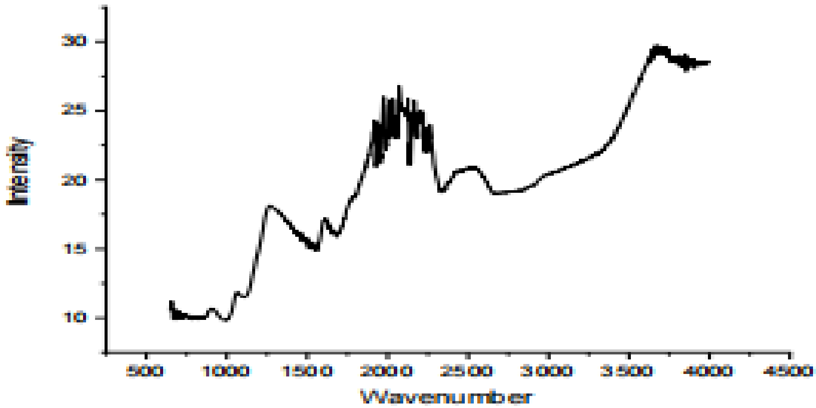
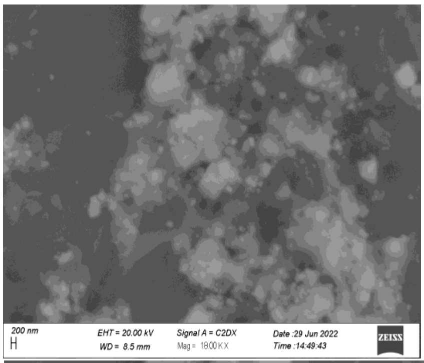
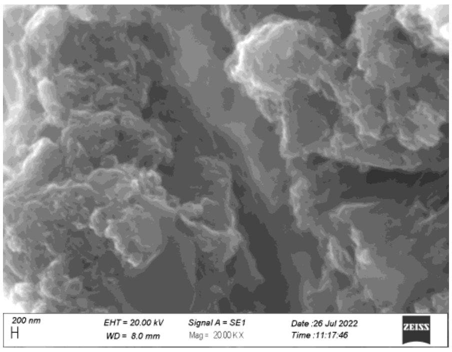
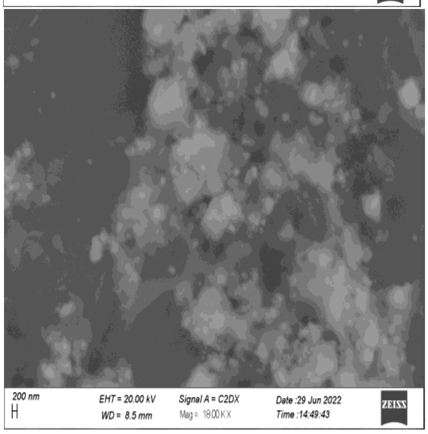
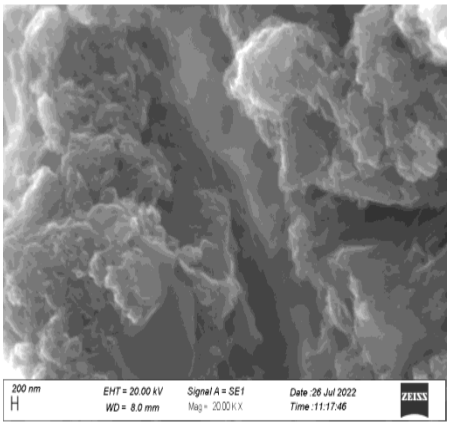
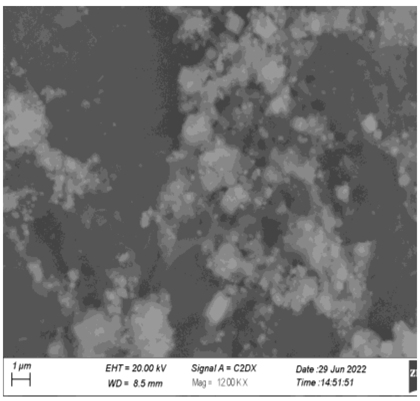
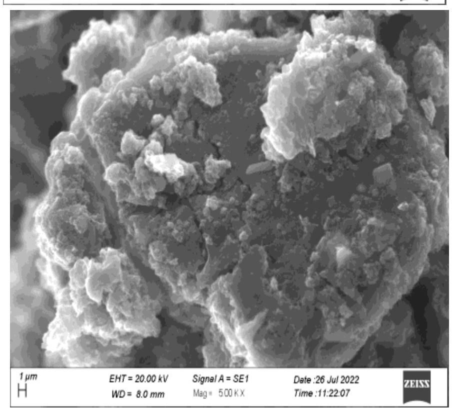


Fig. 11. IR spectra of *Tephrosia purpurea* catalyzed Ag-Graphene nanocomposite

SEM images of Silver-Plant nanocomposite	SEM images of Graphene- Ag-plant nanocomposites
a) 	a) 
b) 	b) 
c) 	c) 

d)

e)

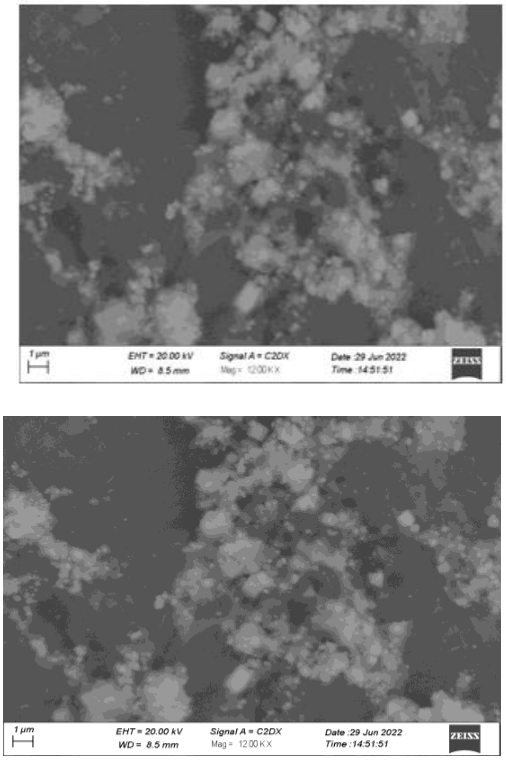


Fig. 12. SEM images of *Tephrosia Purpurea* catalyzed silver nanocomposites

d)

e)

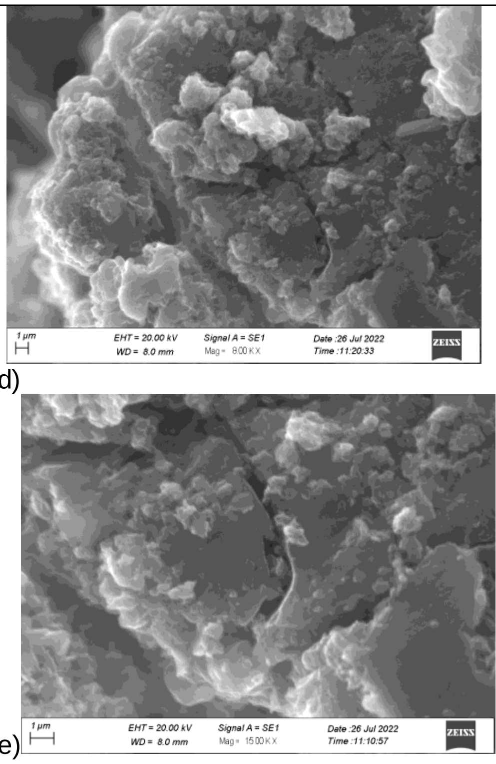


Fig. 13. SEM images of *Tephrosia Purpurea* catalyzed Ag-Graphene nanocomposites

3.4 Energy Dispersive X-Ray Analysis (EDX)

3.4.1 Energy dispersive x-ray analysis (EDX) of plant-silver-graphene nanocomposites

Fig 14 shows the peaks of silver in orange colour with graphene components peaks of Carbon, Sulphur, oxygen, Silicon, Sodium, and Calcium. The presence of silver was confirmed showing the atomic percentage of 18.03% within the Graphene/Silver nanocomposites. Peaks of Ag, C, and O are indicating the formation of graphene-silver nanocomposits. Some Carbon and Oxygen peaks may come from the bimolecules those may be adsorbed on the surface of the nanoparticles and Graphene [26,30].

The EDX data is also shown in Table 2 and the ranges in which the elements lie are also seen. Table No. 2 gives detailed description of a comparison of Plant-Ag-GN nanocomposite data with the reported EDX data of Graphite, Graphene and Silver nanoparticles.

3.4.2 Ultraviolet visible spectroscopy

UV-Visible spectroscopy was carried out with Schmartzo UV-1900i Spectrophotometer while making 1ppm solution of the sample with methanol as solvent.

3.4.3 UV-Vis spectroscopy of plant

The qualitative UV-VIS spectrum profile of plant extract revealed peaks at 290 nm, 360 nm, 410nm.540 nm, and 670 with absorption values of 1.6, 1, 0.6, 0.1, and 0.2, respectively Fig 15 shows the the said peaks.

3.4.4 UV-Vis spectroscopy of plant-Ag nanocomposites

Visual observation was used to document the color change in the reaction mixture (metal ion solution + plant extract). The existence of the synthesized silver nanoparticles was confirmed by sampling the aqueous component two hours after the process and scanning the absorption maxima using a UV-Vis Spectrophotometer on a

Schmartzo UV-1900i Spectrophotometer between 325 and 825 nm [34].

The shape and size of nanoparticles in ppm aqueous solution can be observed in UV Visible spectroscopy. The maximization of the peaks from about 350 nm to 670 nm, those were valuable suggesting polydispersion of nanoparticles. In Fig. 16 the peak shown around 430nm which shows the silver nanoparticles formed in accordance with Mei theory [35]. The flavonoid's peak was present on 290 which were reduced by Silver metal and the peak shifted to around 430nm.

Table 2. EDX data of *Tephrosia Purpurea* catalyzed Ag-Graphene nanocomposites

Sample	C	O	S	Si	Na	Ca	Ag
Graphite	100%						
Graphene	80.44%	18.88%	5.50%	7.54%	13.34%	7.06%	
AgNp		15%	5.5%	1.09%	3.5%		65.35%
Gn/Ag	14.07%	16.37%	5.47%	7.54%	13.34%	14.06%	18.03%

Element	At. No.	Netto	Mass [%]	Mass Norm. [%]	Atom [%]	abs. error [%] (1 sigma)	rel. error [%] (1 sigma)
Carbon	6	9876	59.03	59.03	68.13	8.30	14.07
Oxygen	8	4046	32.54	32.54	28.19	5.32	16.37
Sulfur	16	1385	4.49	4.49	1.94	0.25	5.47
Silicon	14	613	1.82	1.82	0.90	0.14	7.94
Sodium	11	282	0.84	0.84	0.50	0.11	13.14
Calcium	20	106	0.77	0.77	0.27	0.11	14.46
Silver	47	73	0.52	0.52	0.07	0.09	18.03
		Sum	100.00	100.00	100.00		

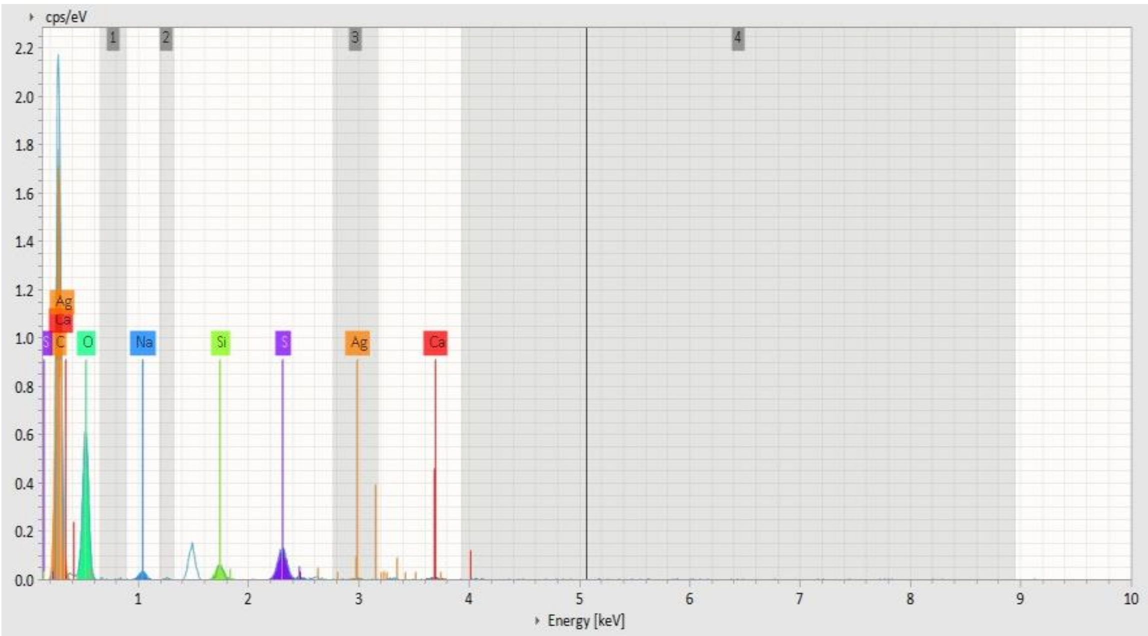


Fig. 14. EDX image of *Tephrosia Purpurea* catalyzed Ag-Graphene nanocomposites

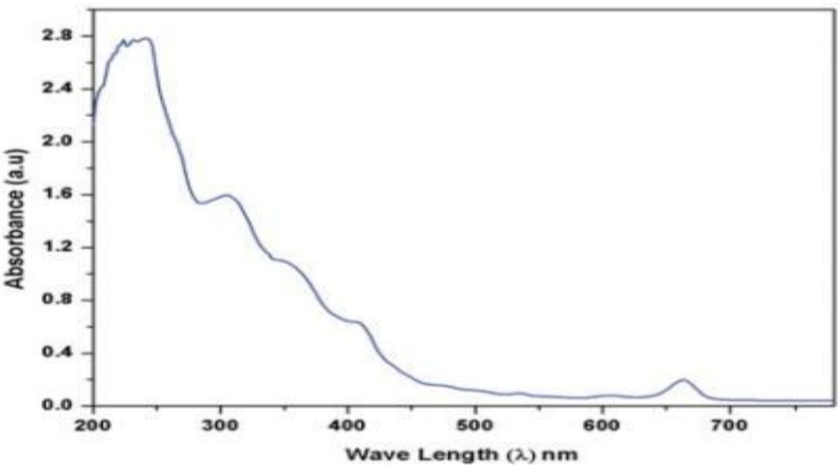


Fig. 15. UV-Vis spectrum of plant extract in methanol

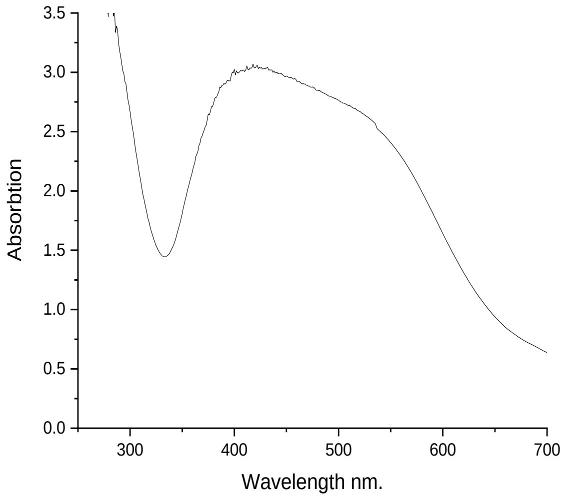


Fig. 16. UV-Vis spectrum of plant-Ag nanocomposites

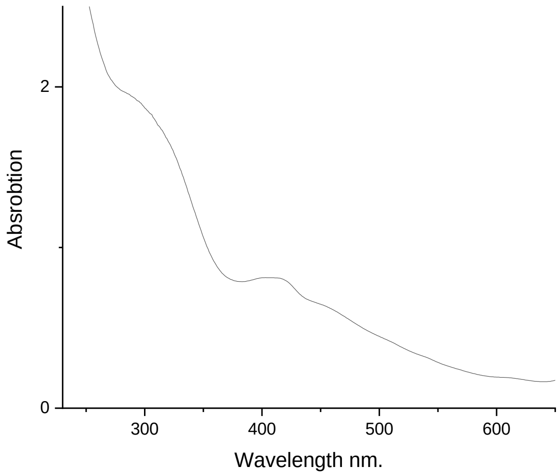


Fig. 17. UV-Vis spectrum of plant-Ag-graphene nanocomposites

3.4.5 UV-Vis spectroscopy of plant-Ag-graphene nanocomposites

After the addition of graphene in plant-Ag nanocomposite, a new peak appeared around 270nm in addition to the earlier peak of 430nm in the UV Vis. Spectrum of the plant-Ag-graphene nanocomposites.

When the peak data of Fig. 16,17 was compared, it was seen that silver nanoparticle formed from the plant extract shown the peak around 430 nm which after addition of graphene,resulting in the formation of plant-Ag-Graphene nanocomposite shown the two peaks at around 270nm and 430nm which indicated the synthesis of plant-graphene-Ag nanocomposites [26].

3.5 Anticancer Activities

The cytotoxic activity of the graphene-silver nanocomposite, plant catalyzed silver nanocomposits and plant extract were assessed. The results for the antiproliferative activity are summarized in Table 3 which revealed that the cytotoxic activity enhanced from plant (*Tephrosia purpurea*) to plant catalyzed silver nanocomposites and more enhanced to plant-silver-graphene nanocomposites. Among all the compounds the viability of the cells treated with 500µg/mL of compound plant-silver-graphene was 56.98925% which was significantly higher than plant-silver nanocomposites) and Tephrosia purpurea plant. After applying different concentrations of compound plant-silver-graphene, 56.98925 cell viability was obtained at 500µg/mL as depicted in Table 3.

Table 3. Anticancer data

Absorbance	1	2	3	%viability	%viability	%viability	Average	S.D
Control	0.93	0.9	0.89	100	100	100	100	0
Plant Extract	0.65	0.67	0.66	69.6	74.6	73.9	72.7	2.73
Plant-Ag nanocomposites	0.62	0.64	0.63	67.1	71.4	71	69.8	2.37
Plant-graphene-Silver nanocomposite	0.53	0.53	0.53	57	58.9	59.4	58.4	1.26

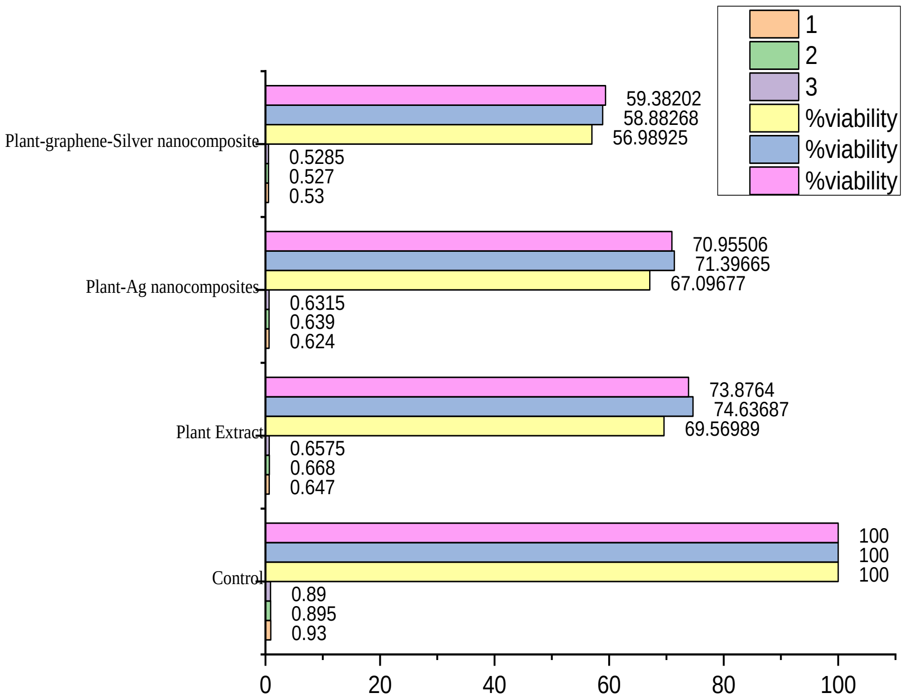


Fig. 18. Anti cancer activities

4. CONCLUSION

In the current study, Graphene-Metal Nanocomposites containing *Tephrosia Purpurea* Plant Leaves are synthesized in vitro from graphite using a novel, straightforward, affordable, and environment friendly method that involves mild sonication and plant extract. Metal nanoparticle deposition produced well-defined separation between graphite sheets. The effective fabrication of graphene/metal nanocomposites was confirmed by UV-Visible spectroscopy, SEM, FTIR and EDX investigation. More cytotoxic activities were displayed by the synthesized GN/Ag nanocomposites than by graphene individually, plant or as a plant-silver nanocomposites.

More green synthesized drugs/nanocomposites with different metals-graphene and bioactive plants can be made by the method used in this project those can definitely open more ways for the researchers to use the chemistry for betterment of humanity. The synthesized graphene/metal nanocomposites may be less harmful, biocompatible, and beneficial for applications such as biomedical ones.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.ikprress.org/review-history/11946