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GREEN SYNTHESIS AND CHARACTERIZATION OF GRAPHENE QUANTUM DOTS FROM *ROSA GALLICA* PETAL EXTRACT

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Abstract

Graphene quantum dots (GQDs) have high capability in biological imaging due to their bright visible photoluminescence ability. But its preparation, procedures and its toxicity of GQDs is which that limits their usage in the biological field. The GQDs are synthesized by green synthesis method. & is characterized by using the UV-Vis, XRD, FTIR spectroscopic studies. We have also evaluated the antifungal activity and toxicity studies of GQDs using zebrafish model. The GQDs synthesized sized between 250 - 350/ nm and was confirmed by XRD spectroscopy analysis, corroborating that the green synthesis is an eco-friendly method to produce graphene.

Key words: Quantum dots, graphene, GQDs, green synthesis, antifungal activity.

Introduction

Graphene is one of the crystalline forms of carbon and is considered as a revolutionary and innovating product. The nanoparticles are labeled with graphene and is one of the advanced application of graphene in diagnosis specifically in radiotherapy coupled with radioisotopes. The quantum dots have significant characteristic that includes solubility in water, less toxicity, high stability to chemicals. As it has all the above unique features, they are majorly used in the biological research fields such as biotechnology, delivery of drug, bioimaging etc (Shen and all in the year 2012; Wang & all in the year 2013). The properties of optics shall be used in controlling size, doping and applications in biological research studies such as in drug and gene delivery, sensing and catalysis (Nekoueian *et al*., 2019).

The arrangement of important principles that eliminates the usage of hazardous compounds in the manufacture, design and synthesis of less toxic or less hazardous chemicals, solvents etc is called green chemistry (Varma 2014, 2016). The medicinal value of plants are reported several years back but its scientific evidence are recently reported (Masek *et al.,* in the year 2017). The single step hydrothermal treatment using *Tamarindus indica* leaves has been used for green synthesis of carbon dots and studied its application in bio imaging, bio sensing, diagnosis of diseases etc. (Bano *et al*., 2018). For production of fluorescent carbon dots pine apples and calamansi that is *Ananas comosus and Citrofortunella microcarpa* wastes was treated by hydrothermal method. The *Escherichia coli* with carbon dots fluoresced act as very effective probe of bio imaging. The generation of H- bonds are the major connection for the attachment of bacteria and quantum dots (de Yro *et al*., 2019).

The compounds such as favonols, phenolic acid & anthocyanins etc. of flowers have high antioxidant properties and has several beneficial biological characters (Mohebitabar *et al*., 2017). *Rosa gallica* belonging to the member of Rosaceae family commonly known as Rosa species are very rich in medicinal properties (Bitis *et al*., 2017). *Rosa gallica* has been used for preparation of herbal medicines since 13th century (Ueno *et al*., 2019).

The synthesis of quantum dots by sustainable methods has lots of advantages as less cost and non-toxic raw materials, renewable and straight forward post processing steps. The nanoparticles that are synthesized by the above mentioned method are an assurance for biomedical and clinical sciences. The advancement of research in metallic nanoparticles (NPs) since earlier times has resulted in its utilization of NPs as antibacterial, antiviral, antifungal and anti-inflammatory agents (Brandelli *et al*., 2017). NPs in particular have demonstrated broad-spectrum

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antimicrobial properties against both pathogenic and drug resistant microbes.

Toxicity testing of new compounds is essential for any drug discovery and development process. The toxicity of embryos of zebra fish model are used for toxicology assessment as it is transparent, less cost and its genetic redundancy to humans (Jayasinghe and Jayawardena, 2019). Embryos of zebra fish represent an alternative model for preclinical studies for early stage drug discovery systems. Hence, this study is focused on synthesizing graphene quantum dots from *Rosa gallica*. The anti-fungal activity was determined and toxicity studies were also carried out using zebrafish embryos.

Materials and Methods

Sample preparation and processing

1g of fresh petals of *Rosa gallica* were collected, washed with sterile distilled water and shade dried until the moisture level was less than 14% and the temperature range was maintained between 28 to 35°C. The dried petals were minced with wooden knife and crushed in a mixer-blender. The powder was stored in an airtight container in room temperature until further use. 0.425 g of crushed petals were boiled with 1 milli litre of distilled H_2O and heated for 5 min and then filtered in a filter paper, the filtrate was used as extract and the residues were discarded.

Phytochemical screening

The *Rosa gallica* petal extract was tested for different phytochemical constituents, the standard procedures followed for the experimentation were listed in table 1.

Green synthesis of graphene quantum dots from *Rosa gallica*

Pyrolysis method

20 g of petal extract of *Rosa gallica* was mixed in equal volume of sterile distilled H_2O and was filtered using whatmann filter paper with a diameter of 110 mm. 25 ml of the filtrate was heated to 100°C in a mantle for 90 minutes until the citric acid in the extract gets altered to form an orange liquid. This liquid was then centrifuged at 10,000 rpm for 30 min, filtered and the resultant product with GQDs was refrigerated for further studies.

Table 1: Preliminary phytochemical evaluation tests performed with crude extract of *Rosa gallica.*

Phyto-constituents	Procedure followed
Alkaloids	Mayer's test
Terpenoids	Salkowski test
Flavonoids	Shinoda test
Coumarins	Sodium hydroxide test
Steroids	Liebermann Burchard test
Saponins	Foam test
Tannins	Gelatin test
Phenols	Ferric chloride test
Cardiac glycosides	Keller-Killiani's test
Resins	Ammonia test

Hydrothermal method

0.1 g of the *Rosa gallica* extract and 1 milli litre of hydrazine hydrate were mixed in 10 milli litre of sterile water, boiled at in an ultrasonic bath for 30 minutes and then let to cool for few minutes. The solution transferred to an autoclave and heated to 200°C and kept for 6 to 10 hours. The solution was let to cool at 20°C and the product with GQDs soluble in water was filtered using whatmann filter paper with a pore size of 0.22 mm to remove insoluble carbon products. The filtrate was dialyzed in a dialysis bag for 2 days for the unfused small molecules to get removed. The purified black GQDs were dried at 80°C in hot air oven and was refrigerated for structure characterisation studies.

Spectroscopic studies of green synthesized GQDs

The green synthesized graphene quantum dots were spectrally characterized using UV-Vis, XRD & FTIR studies. GQD sample was scanned from 200 to 800 nm and spectrum was obtained with a Jasco UV-Vis spectrophotometer V-650. The crystalline phases of samples were studied from XRD pattern of the synthesized GQDs in a Bruker/D8 advanced diffractometer in the 2θ range from 5° to 80° by 0.04° steps using CuKá (λ=0.15406 nm) radiation. Bruker, Alpha T, Germany FTIR instrument is needed to determine the functional groups present of sample. A little quantity of sample name extract was mixed in dry KBr. The mixture completely mixed by using a mortar & for the formation of KBr thin disc is pressed at a pressure of 6 bars within 2 min. Later the disc was kept in a sample cup of reflectance accessory for noting the diffusion. The sample was scanned from 4000 to 400 cm^{-1}. The values in the peaks of the UV-Vis and FTIR spectra were recorded.

Antifungal Assay of synthesized GQDs

The antifungal activity of the green synthesized graphene quantum dots were tested against infectious fungi *Microsporum gypseum* and *Trichophyton equinum* using disc diffusion assay. Amphotericin-B discs (20 µl/disc) were used as positive control. Fungal cultures were swabbed in separate Sabouraud Dextrose agar (SDA) plates and sterile discs loaded with 20 µl (1mg/ml) of sample was placed on it. The culture plates, then

incubated at 28°C for a day and zone of inhibition were measured and recorded.

MIC determination

The MIC of GQDs towards the infectious fungi were studied by suspending fungal inoculum of 2×10^8 CFU/ml of *Microsporum gypseum* and *Trichophyton equinum* in a tube with 0.5 Mc Farland standard in the ratio of 1: 150. The inoculum was diluted and adjusted so that each tube contains approximately 5×10^5 CFU/ml of the fungal cultures which was used for the assay. 1 mg/ml concentration of sample was obtained by dissolving in DMSO and used for MIC determination. 1 ml of sterile SDA broth was taken in sterile tubes and to this 1 ml of sample was added to tube 1. 1 ml from tube was then transferred to tube 2 and serially diluted until tube 8. 100 µl of the fungal inoculums were added from tubes 1 to 8 and incubated at 37°C for 24 hours. Fungal growth was observed with the turbidity in the tubes and MIC is the concentration of higher dilution tube in which there was no fungal growth.

Toxicity studies

In vivo toxicity assessment of the synthesized graphene quantum dots were carried out in zebrafish embryos (OECD, 2013).

Results and Discussion

Rosa gallica was well known for its pharmacological activities such as anti-diabetic, cholesterol reducing property, analgesic, anti-inflammatory, hepatoprotective, anti-oxidant and cytotoxic properties. The phytochemical constituents present in petal extract of *Rosa gallica* was analysed using standard procedures and terpenoids, flavonoids, coumarins, saponins, tannins, phenolics, cardiac glycosides and resins were found to be present. Alkaloids and steroids were absent in the extract.

The natural carbon compounds are highly attracted by the world as it has several unique properties and excellent application. Innumerable methods are available to synthesize GQDs, comprising ultrasonic, microwave, solution chemistry, hydrothermal and electrochemical methods, as well as solvo-thermal route. An ideal source for the preparation of carbon dots are plants as they are sustainable, renewable and less cost (less than USD 0.1 to produce 100 mg of GQDs). Leaves of neem (*Azadirachta indica*) and Fenugreek (*Trigonellafoenum graecum*) have been used to produce GQDs, with a high QY of 41.2% and 38.9% respectively. The green synthesized GQDs from *Rosa Gallica* flower by hydrothermal method were characterized using ultra violet-visible absorption spectroscopy. Fig. 1 displays the

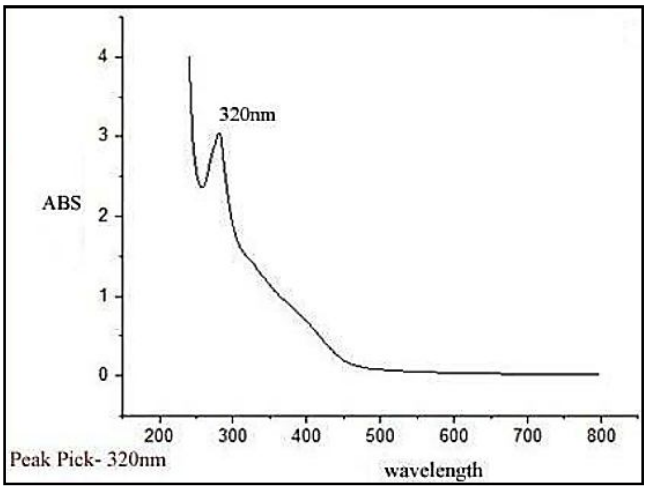

Fig. 1: Ultra Violet-Visible absorption spectrum of GQDs synthesized *Rosa gallica* showing maximum absorption at 320 nm.

ultra violet -visible absorption spectrum with a peak point range at 320 nm, showing the presence of GQDs.

The XRD pattern of GQDs *Rosa gallica* is shown in Fig. 2. The broad reflection seen at 2 theta = 38.63p shows graphite nature of GQDs.

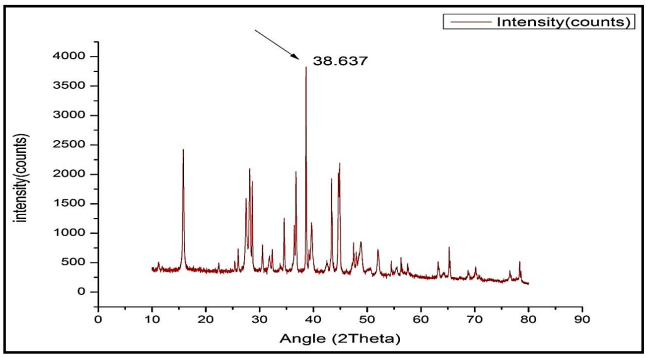

Fig. 2: X-Ray diffraction pattern of GQDs synthesized from *Rosa gallica*.

The molecular stretching obtained from FTIR consisted of an aromatic C-H bond at 2850 cm^{-1}, H-C-H vibration at 2850 and 3003 cm^{-1} and O-C=O stretch of a carbonyl group bound to an ester group at 1737 cm^{-1} the

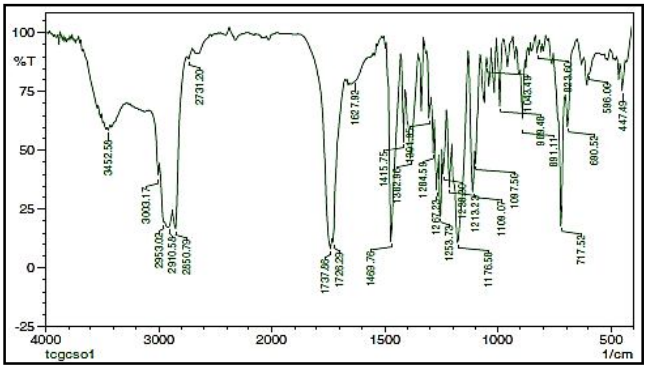

Fig. 3: FTIR Spectrum of biosynthesized graphene quantum dots.

results are depicted in the Fig. 3. Vibrations at 1213 cm^{-1} shows the presence of C-O stretch. A plane bending peak at 1627 cm^{-1} shows the presence of C=C. The result confirms a strong interaction between the chemical components and the extract from petals of *Rosa gallica* table 2.

Table 2: Characteristic peaks in FTIR spectrum of GQDs synthesized from *Rosa gallica.*

Peak values Frequency, cm^{-1}	Bond	Functional groups
1737	O-C = O stretch	Carbonyl group bound to ester
1627	C = C stretch	Olefin group
2850	Sp3-CH stretch	Alkane C-H
3003	Sp2-CH stretch	Alkene C-H (Aliphatic)
1213	Sp2 C-O stretch	Carbonyl single bond stretch

The antifungal activity of the green synthesized graphene quantum dots were determined using disc diffusion assay against two pathogenic fungi *Microsporum gypseum* and *Trichophyton equinum*. The nanoparticle was found to exhibit antagonistic activity against both the study pathogens. The minimum inhibitory concentration of GQDs against the infectious fungi were also studied and both the pathogens *Trichophyton equinum* Fig. 4 and *Microsporum gypseum* Fig. 5 showed MIC at >1000 µg/ml and increased concentrations

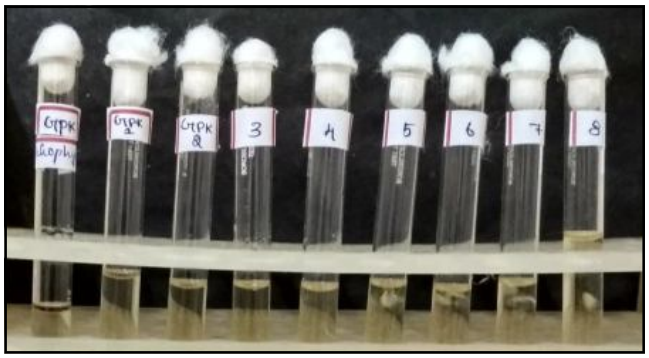

Fig. 4: MIC of *Trichophyton equinum* in the GQDs sample tubes.

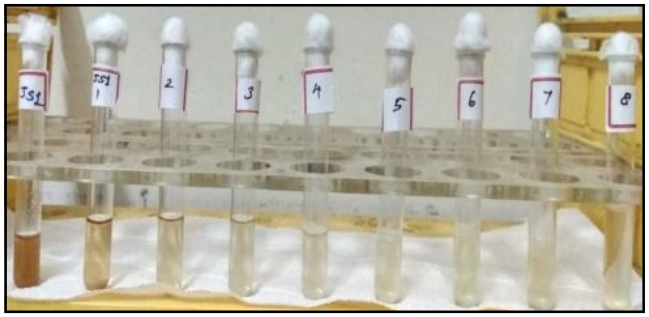

Fig. 5: MIC of *Microsporum gypsuem* in the GQDs sample tubes.

Table 3: MIC determination of synthesized GQDs against *T. equinum and M. gypseum*.

Dilution	Concentration (µg/ml)	*Trichophyton equinum* Absorbance (nm)	*Microsporum gypseum* Absorbance (nm)
1	1000	0.121	0.132
1:1	500	0.164	0.142
1:3	250	0.227	0.187
1:7	125	0.306	0.198
1:15	62.5	0.307	0.380
1:31	31.25	0.374	0.889
1:63	15.6	0.620	1.033
1:127	7.8	0.642	1.051

table 3.

The toxicity of embryo of zebra fish model is more suitable for nanoparticles as the test can be done with less quantities & is transparent also detects the effects of compounds on various organs, including the heart, brain, intestine, pancreas, cartilage, liver, and kidney without complicated processing (Parng *et al*., 2002). In order to identify a non-toxic and safe antimicrobial nanoparticle, a biomedical screening in zebrafish embryos were carried out and their phenotypic toxicity assessments were studied. The zebrafish embryo based whole organism toxicity analysis was performed for the synthesized graphene quantum dots. Embryos were monitored for organ deformities over a period of 96 hours after treatment. The particles were treated in zebrafish embryos in different concentrations (5, 10, 20 µg/ml) in 1 days post fertilized (dpf) embryos. The GQDs were not lethal upto the tested concentration during 24 hours post

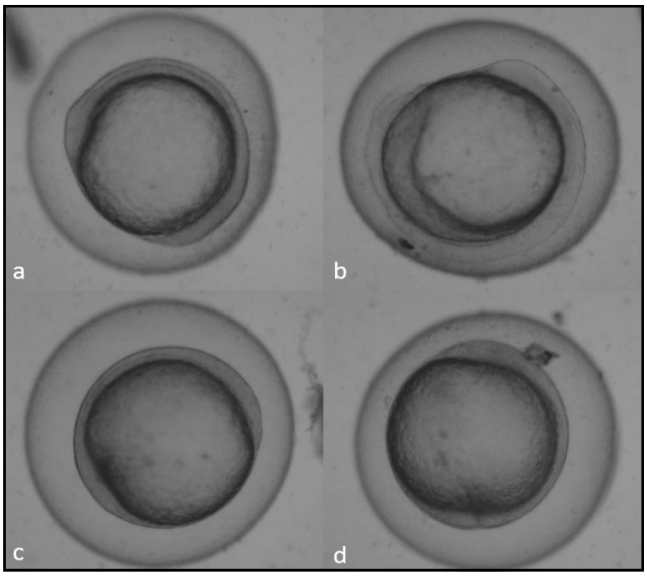

Fig. 6: Organ toxicity effect of GQDs at 24 hours post treatment at different concentrations a. control b. 5 µg/ml c. 10 µg/ml d. 20 µg/ml.

treatment. The treated embryos at 78 hours showed phenotypic deformities and revealed pericardial bulging and elongation in the yolk sac region at the tested concentration of 10 µg/ml Fig. 6. No notable phenotypic abnormity was observed in 5 µg/ml whereas the nanoparticles were found to be lethal at 20 µg/ml. At 96 hours post treatment, the zebrafish embryos showed pericardial bulging at 5 µg/ml. Concentrations of gold nanoparticle at and above 10 µg/ml were found to be lethal.

Conclusion

The luminescent GQDs are interesting nanomaterials, emerging with immense applications in the fields of chemical sensor, bioimaging, nanomedicine, drug delivery, and electrocatalysis. In order to syntheses the GQDs from *Rosa gallica* extracts, two different methods such as green synthesis and hydrothermal methods were employed. UV-vis, XRD, FTIR characterization of the graphene quantum dots were used to perform the application studies. The XRD pattern of GQDs showed broad reflection seen at 2 theta = 38.63p , which confirms the graphite nature of GQDs. The formation of GQDs are confirmed due to the noticeable UV absorption peaks at 250-350 nm. The functional groups present in the GQDs synthesized from *Rosa gallica* were identified using FTIR. Biological assays such as anti-fungal activity and MIC were determined and GQDs samples showed good results. The anti-fungal activity of these green synthesized GQD samples revealed that they could inhibit the growth of microorganisms.

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