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Formulation and Evaluation of geranium oil Loaded carbon dots from the essential oil of pelargonium graveolens

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ABSTRACT

This research explores the development of carbon dots (CDs) loaded with geranium essential oil extracted from Pelargonium graveolens. The study involves the extraction of essential oil through hydro distillation, followed by characterization and preliminary analysis of the oil's physical and chemical properties. The work emphasizes the potential of these bio-derived carbon dots for various applications, leveraging their unique optical and chemical stability, biocompatibility, and luminescent features. The comprehensive evaluation includes extraction yield, physicochemical constants, phytochemical screening, and preliminary formulation assessments. The findings suggest that geranium oil-loaded carbon dots exhibit promising properties for use in sensing, bioimaging, and other nanotechnology-based applications, highlighting their potential as sustainable and biocompatible nanomaterials.

Keywords: Geranium oil Loaded carbon dots, pelargonium graveolens, Formulation, Evaluation, Plant characterization, Plant-based nanomaterials.

1. INTRODUCTION

"Nanoscience" is the emerging science of objects that are intermediate in size between the largest molecules and the smallest structures that can be fabricated by current photolithography; that is, the science of objects with smallest dimensions ranging from a few nanometers to less than 100 nanometers.(1) Further it refers to research and technology development at the atomic, molecular, and macromolecular scale, leading to the controlled manipulation and study of structures and devices with length scales in the 1- to 100 nanometers range.

Carbon Dots:

One of the most prevalent elements, carbon, has been extensively studied since the 19th century and has seen an ongoing increase in research.[19] For the most part made of carbon (mainly sp2 with some sp3 character), hydrogen, oxygen, and

nitrogen moieties, CDs are quasi-spherical nanoparticles with a typical size between 1 and 10 nm. The chemical constitution of the precursors, however, allows for customization of their composition. The physico-chemical and optical features of carbon dots, an emergent class within the family of carbon allotropes, have drawn a lot of attention.[20] They have potential for sensing because of their distinctive electrical, fluorescent, photoluminescent, chemiluminescent, and electrochemiluminescent features.[21] Materials made of carbon have a number of desirable qualities, including great electrical and thermal conductivity, high strength and stability, and good flexibility. Carbon fibers, fullerene, porous materials, carbon nanotubes and carbon quantum dots are some of the members in the carbon-based family.Further, excellent optical and chemical stability and biocompatibility of CDs have recently been shown to be more important than those of semiconductor quantum dots and organic dyes. [(22)]
Due to their distinct luminous features carbon dots (CDs) have come into prominence. A wide variety of technological applications, such as photocatalysis, optical sensing, bioimaging, or tribology among others, have been thoroughly investigated. Additionally, CDs exhibit tremendous potential, particularly for biological applications, due to their capacity for scavenging reactive oxygen species.[23] Due to their significant uses in photoelectric devices, biomedicine, etc., fluorescent nanomaterials such semiconductor quantum dots, carbon dots, polymer dots, fluorescent nanodiamonds, and fluorescent nanoclusters have been widely used by scientists. Particularly, the attention given to carbon-based nanomaterials is growing as a result of their clear advantages in terms of sustainability and stability.[24] Due to their intriguing and adaptable character in terms of their varied and variable precursor sources and inbuilt robustness in performance capabilities, carbon quantum dots have attracted a lot of attention in recent years.

2. COLLECTION OF PLANT MATERIAL:

The plant *pelargonium graveolens* (Family: *Geraniaceae*) was chosen for the current study and was harvested in the Maharashtra district of Sangli.

3. AUTHENTIFICATIONOFPLANTMATERIAL:

Yashwantrao Chavan College of Science, Karad, identified and certified the chosen plant.

4. EXTRACTION OF PLANT MATERIAL:

Double Distillation Method:
Hydro distillation by Clevenger:
For the extraction of essential oils from leaves of *pelargonium graveolens* by hydro distillation under optimal operating conditions, a quantity of 100 g of leaves was added to 800 ml of distilled water in a 2-liter flask. The set was placed in a balloon heater attached to a refrigerator to ensure condensation of essential oils for 3 hours. At the end of the distillation, two phases were observed, an aqueous phase (aromatic water) and an organic phase (essential oil), less dense than water. The essential oil was collected, dried under anhydrous sodium sulphate, and stored in sealed vials in the dark, at 4° C, until used. Experiments were conducted twice for each condition.

Yield of Essential Oils:-
The yields of essential oil of rosemary were expressed in g relative to 100 g of dry vegetable matter; it was calculated according to Equation

$$\text{Yield (\%)} = \frac{\text{Amount of extracted oil (g)}}{\text{Amount of dry vegetal matter mass (g)}} \times 100$$

$$= 50/200\times100 = 50\text{gm}$$

5. PREFORMULATION STUDY

Miscibility:
For additional analysis, the pure geranium oil's miscibility was examined. To determine the miscibility of the geranium oil, it was diluted in several solvents.

Boiling Point:
The capillary method, which involves putting an inverted capillary within the liquid of interest and heating it, can be used to figure out the boiling point of geranium oil. The air in the capillary escapes as the temperature rises, and is replaced by the liquid's vapour. The three readings were averaged, and the results were compared to the literature.

Determination of Wavelength:
10 milligrams of geranium oil were precisely weighed and added to a volumetric flask holding 100 ml. Add 100 ml of ethanol to the prepared 100 g/ml solution (Stock I) in this volumetric flask. After that, a sonication process was used to create a clear solution. To create a 10 g/ml solution (Stock II), 1 ml of this Stock solution was taken out and put into another volumetric flask with 10 ml of ethanol to make up the volume. This 10 g/ml solution was tested against ethanol as a blank solution using a UV-Visible spectrometer in the wavelength range of 200 to 800 nm.

Preparation of calibration curve of Pure Geranium Oil:
Pipette out 0.1, 0.2, 0.3, 0.4, and 0.5 ml of the aforementioned stock II solution (10 g/ml) and ethanol 10 ml to create a 1–5 g/ml solution, respectively. This solution underwent filtering and UV-visible double beam spectrometer analysis between 200 and 800 nm. Afterward, determine the slope, intercept, and correlation coefficient of the standard calibration curve. [93].

Macroscopical Characteristics:
Macroscopical studies were carried out by using organoleptic evaluation method. The shape, size, colour, odour, taste. Base, texture, margin, apex of leaves and stem were observed. *Pelargonium Graveolens* fresh plants were observed for their intricate macroscopic characteristics, which included unique traits like colour, size, and shape. *Pelargonium graveolens* is an erect, multi-branched shrub, that grows up to 1.5 m and has a spread of 1 m. The leaves are deeply incised, velvety and soft to the touch (due to glandular hairs). The flowers vary from pale pink to almost white and the plant flowers from August to January. The leaves may be strongly rose-scented, although the leaf shape and scent vary. Some plants are very strongly scented and others have little or no scent.

6. ROOTS

Colour: Pale grey to brown
Shape : Shallow, compact nature, window box staple, top choice for hanging basket
Leaves:
Shape: Rounded or hand-shaped, triple-loaded
Colour: Green
Size:longer than 5 cm, long petiolate
Stem:
Size: 6 Meter Height
Colour: Green
Shape: Thick

Figure 1: Macroscopical Characteristics of *Pelargonium Graveolens* Plant

Flowers:
Size: upto 1.5 mandhas a spread of 1m
Colour: Pink to almost white
Shape: Rounded clusters that look like umbelin florescences

7. MATERIALS AND METHODS

Results of Determination of Physical Constants:
As shown in table no. ., the results of determination of physical constants of powder of *Pelargonium Graveolens* plant shows 10.66% total ash value, 1.50% acid insoluble ash value and 9.20% water soluble ash value.

Table 1: Determination of Physical Constants

Sr. No.	Physical Constituents	Percentage
1.	Total Ash Value	10.66%
2.	Acid Insoluble Ash Value	1.50%
3.	Water Soluble Ash Value	09.20%

Table 2: Result of Preliminary Phytochemical Screening And Evaluation of Alcoholic powder of *Pelargonium Graveolens:*

Sr. No.	Test	Observation	Inference
1	Test for Alkaloids		
	(A)Mayer's Test: Test solution + Mayers reagent(Potassium Mercuric Iodide)	Cream precipitate	Alkaloids Present
	(B)Dragendorff Test: Test solution + Dragendorff reagent	Reddish precipitate	Alkaloids Present
	(C)Wagner's Test: Test solution + Wagner's reagent	Brown precipitate	Alkaloids Present
	(D)Hager's Test: Test solution + Hager's reagent	Yellow precipitate	Alkaloids Present
2.	Test for Protein and Amino Acid		
	(A)Modified Millon's Test: 1ml Extract+1ml10%Mercuric Sulphate+10%H2SO4,boiledforhalfmin	Yellow Precipitate	Ammonia Present
	(B)SulphurTest: 2ml Extract+40%NaOH+10drops of 2 % Lead Acetate, boiled for amine and cool	Black Precipitate	Ammonia Present
	(C)Biuret Test: Equal volume of 5% NaOH + 1% Copper Sulphate	Pink to Purple Colour	Protein and Free Amino Acids Present
	Tests for Tannin and Phenolic Compound		
	(A) 5% Ferric Chloride Test: Extract + Ferric Chloride	Green colour disappeared	Tannins Present
	(B)Gelatin Test: 1% Test Solution + NaCl	Precipitate Formed	Tannins Present
	(C)Acetic Acid Test: Test Solution + Acetic Acid Solution	Precipitate Formed	Tannins Present

Microscopical Characteristics:

Leaf: In cross section, the petiole has a rounded shape. The parenchymatic cortex, which contains the calcium oxalate druses, is the second layer after the epidermis. The pericycle, which is made up of a few layers of collenchymatic cells, delimits the central cylinder at its exterior side. The principal collateral vascular bundles, which are located below the pericycle and are separated from one another by strings of parenchyma, are arranged on a circular contour. The inferior portion of the lamina corresponds to the central vein and has a well-developed prominence. The two epidermis, or the, preserve the lamina's bifacial anatomical structure on both sides.The mesophyll, which has a parenchymatic structure and a lot of calcium oxalate druses, can be found between the two.

Stem: The transverse section of *P. graveolens* stem is shown in fig. The epidermis was single thick wall layered; the cuticle was surrounded with dark pink colored oval shape cells. The cork cells consisted of 3–5 layers, the vascular bundles were surrounding the polygonal lignified parenchymatous cells. The cortex was of several layers of thin-walled tangentially elongated cells; cluster crystals of calcium oxalate and starch grains were present. The central pith was very small and vascular bundles were arranged in a ring form. The vascular bundle consisted of secondary phloem and secondary xylem; secondary phloem consisted of sieve tubes, companion cells and phloem parenchyma; secondary xylem consisted of lignified trachea, tracheids, fibres and vessels. Xylem fibres were pitted, elongated and moderately thickened. Root: As a result of the cambium and phellogen's activity, the sectioned organ displays a secondary structure. The suber, which is composed of 4-5 layers of overlapping cells that are tangentially elongated and have suberified walls, is found on the outer side. It is seen that the secondary phloem is positioned in a circular arrangement, facing the exterior. Two parenchymatous strings formed by the cambium, the secondary medullary rays, disrupt the secondary xylem, which makes up the majority of the root's centre. Vessels placed in radial strings and fibres make up the secondary xylem. The section's principal xylem is located in the middle. A secondary structure can be seen in the radicle. The suber cells tangentially

overlap each other to encircle it on the outside. Crystals of calcium oxalate can be found inside the cortex. The secondary xylem, which has the secondary phloem above it, takes up most of the space in the center of the section.

Powder Characteristics:
Powdered drug examined under compound microscope showed presence of needle shaped structures whichwere indicates confirmation of *pelargonium greveolens*Crystals of prismatic calcium oxalate, unicellular covering trichomes, and parenchymatous cell fragments were all present in the leaf-specific powder.

Carbon Dots Prepared by High Temperature Pyrolysis method: I
Fresh rhizomes of ginger were purchased from local market, peeled and cut out in tosmall pieces and make the fine paste.Then 25ml of pulpfree juice of ginger, 10ml 0.01M NaOH and 15ml of double distilled water were taken in100ml beaker and carbonized at 200˚c for 3hrs over hotplate under ambient air covered byaluminum foil. From this one pinch of carbonized material was diluted in 150ml of distilledwater and then sonicated at 27ºctempature firstly for 20min and then for 10min after shaking.Then the solution is agitated for high-speed homogenization at 500rpm for 1hr and the high-pressure homogenization for maximum cycle. Then the solution is dialyzed through dialysismembrane with porosity 37.70mm in dialysis bag with size 10KDa for 8 hrs. to get a browncarbon dots solution by placing it in a beaker containing 6.8PH phosphate buffer on magneticstirrer.

Preparation of Carbon Dots: Method II
The oil was heated at 240°C temperatures and time intervals of 12 hrs. After heat treatment, the samples were then sonicated, centrifuged for 30 minutes at 7000–8000 rpm and filtered. The samples were then examined under a UV lamp of long-range, wherein suitable fluorescent radiation could be seen. The synthesized nanoparticles were further validated using particle size, zeta potential, UV, infrared spectroscopy and transmission electron microscopy.

Formulation Of Gel :
A gel formulation comprising nanospheres was created in this experiment for efficient skin application and improved penetration. Hydrophilic gels made of carbopol and HPMC K were tested for their ability to disperse these nanospheres. After refining the CD approach, a topical gel containing carbon dots made of geranium oil was created utilizing a dispersion swelling methodology. Following the weighing of all excipients as per Table . . , Carbopol 934 and HPMC K4M were concurrently dissolved in water and let to stand for 4 hours in order to appropriately swell the polymer. In order to prevent air bubbles, CD powder (equal to 0.5 mg/g) was then continuously agitated in one direction while being polymer gel. Time the geranium oil alsothe formulation. The crosslinking of the Carbopol 934 polymer and HPMC K4M to create a gel required the addition of triethanolamine to the gel mixture. The gel's viscosity a was then balanced by the addition of propylene glycol, and its permeability was increased by the addition of azone and methyl paraben as preservative. Finally, the pH was brought down to 7.4 0.1, the skin pH, by the addition of 0.1 N NaOH.

Evaluation of Gel:
1) Determination of visual appearance and clarity :
Different formulations were evaluated for basic physicochemical properties. The prepared gel's colour, transparency, homogeneity, and appearance were all visually evaluated. The appearance of carbon dots loaded geranium oil is fogy/cloudy and white in colour. The clarity of all formulations was assessed visually against a black and white backdrop, as shown in figure.

2) Determination of pH :
A pH metre (made by Mettler Toledo, Switzerland) was used to determine the pH of the 1% aqueous solution of the gel. A calibrated pH metre was used to determine the pH of the created gel system after all the ingredients had been added. Each formulation's assessed three times, with the average value being determined. The pH of formulated gel is in between 6-7 which is well suited for skin.

3) Viscosity :
How long the medication lingers at the location is significantly influenced by its viscosity. After the prepared solutions gelled at physiological temperature, their viscosity was assessed. The decision was made to use the average of three determinations.

4) Gelation Tempreture :
The solution was placed in a test tube and submerged for two minutes in a water bath that was at room temperature to determine the gelation temperature. The temperature at which the fluid began to gel was measured using a thermometer inside a test tube. For formulations containing carbopol, phosphate buffer solution pH 6.8 was added to a test tube and the formulation was placed within. Prior to being placed in the water bath, this had been well combined. Up to 60°C was used as the highest gelation temperature test. It was observed that the gel formed when the container was turned upside down and there was no formulation flow.

5) Gelation Time :
The solution's gelation time was determined by placing it in a 50 ml beaker and heating it in a water bath.

6) Spreading Coefficient :
A ground glass slide set on a wooden block makes up the device. Each formulation, weighing about 2 grams, was placed on this ground slide and analysed. Then, a second slide with the same dimensions as the fixed glass slide was sandwiched between this one and a gel preparation. The second slide has a hook on it. Lay a 1 gram weight on top of the two slides for 5 minutes to remove air and create a uniform layer of gel between them. The measured weight is placed on a pan connected to the pulley with the aid of a hook. It was timed how long it took the top slide to disengage from the bottom slide. A larger spreading coefficient (S) corresponds to a smaller interval.

$$S = ML/T$$

M- Weight tied to upper slide, L- Length of glass slides, T- Time taken to separate the slides

7) % Drug Release :-
% Drug release of gel compromising geranium oil carbon dots is performed by dialysis bag. Dialysis membrane was soaked in water for 12 hours before the experimentation. Then phosphate buffer solution having pH 7.4 taken in beaker. Then gel is filling in dialysis bag and then it is mounted in beaker containing phosphate buffer. After that at 100rpm for 37℃. Then the 5ml of sample is and the sample is analyzed on UV spectroscopy at wavelength of 204.8nm.
Characterization of geranium oil carbon dots

Preparation of calibration Carbon dots solution:
10 milligrams of carbon dots were precisely weighed and added to a volumetric flask holding 100 ml. Add 100 ml of distilled water to the prepared 100 g/ml solution (Stock I) in this volumetric flask. After that, a sonication process was used to create a clear solution. To create a 10 g/ml solution (Stock II), 1 ml of this Stock solution was taken out and put into another volumetric flask with 10 ml of distilled water to make up the volume. This 10 g/ml solution was tested against distilled as a blank solution using a UV-Visible spectrometer in the wavelength range of 200 to 800 nm.

Preparation of calibration curve of carbon Dots Solution:
Pipette out 0.1, 0.2, 0.3, and 0.4 of the aforementioned 10 ml to create a 1–5 g/ml solution, respectively. This solution underwent filtering and UV-visible double beam spectrometer analysis between 200 and 800 nm. Afterward, determine the slope, intercept, and correlation coefficient of the standard calibration curve.

Particle size:
The zeta-sizer device (Horiba Scientific Instruments, SZ-100 Series) is utilised for the light scattering method analysis of the vesicles diameter and PDI of Geranium oil carbon dots. The formulation was soluble in DW, and the analysis was performed using a quartz micro cuvette at 25℃, followed by three iterations of the instrument run to ensure precise analysis.

Zeta potential
For the electrophoretic mobility of the vesicles, zeta potential was used. The SZ100 series from HORIBO Scientific was used to assess the zeta potential. Filtered distilled water was used to dilute the carbon dots suspension before it was used for further investigation. In order to improve analysis, each sample was run in triplicate at 25℃ for 2 minutes at a time.

FT-IR of Geranium oil carbon dots:
The FT-IR measurement determines whether geranium oil carbon dots are compatible with the pure medication. The attenuated total reflectance (ATR) method was used to determine the IR spectrum of the medicine, excipients, and formulation. Under the spectrophotometer probe, a known quantity of the material was used in the ATR method's analysis, which used a 4 cm-1 resolution and a wavelength of 4000-400-1. When compared to the formulation of geranium oil carbon dots, the IR spectra peak of the medication and excipient were examined. Data was translated into standard values based on the outcome.

(XRD):
investigate of a structure, chemical species are resolved, crystallinity is measured, etc. using the XRD technique. Any substance that absorbs X-ray radiation will reflect it in a variety of diffraction patterns, each of which reveals a physico-chemical characteristic of the crystal structure. The physico-chemical characteristics were reflected by the light that was diffracted from the powder sample in this case. Inorganic catalysts, biomolecules, and polymers, among other things, can have their structural characteristics analysed using the XRD technique. Using the X-ray diffractometer (XRD), one can assess phase identification, undertake quantitative substance analysis, and identify sample structure flaws. Additionally, this was true for physico-chemical characterisation of nanoparticles. Utilising an x-ray diffractometer, X-Ray diffraction patterns were examined.

Scanning Electron Microscopy (SEM) :
Drug distribution requires vesicles with a specific diameter and shape. The morphology of the particle's surface and modifications to factors like biocompatibility and drug encapsulation are what determine these properties. In addition, they analyse drug release and excretion patterns as well as trapping effectiveness. The determination of a nanoparticle's surface morphology is now done using scanning electron morphology (SEM). SEM technology provides information on particle dispersibility and requires less time for analysis.

Transmission Elctron Microscopy (TEM):-
The form and size of the carbon dots may be verified using TEM. A spherical morphology may be seen in the TEM images. The histogram was created by counting 100 particles with a 0.32 nm crystal lattice spacing. It has been determined that carbon dots are typically spherical in shape and 4-5 nm in size**.**

Entrapment Efficiency:
technique waasussedtoo achieve thhe entrapment efficiency of geranium oil carbon dots. The samples were spun at 20,000 revolutions per minute (rpm) for one hour at a temperature of 40 degrees Celsius while being stored in a centrifuge machine.The obtained supernatants were filtered using Whatman filter paper with a pore size of 0.22 mm after centrifugation. The amount of un-entrapped carbon dots was then calculated using this method after the supernatant was dissolved in methanol to prepare the proper dilution.Utilising a UV-Visible spectrometer at 200 nm, the un-entrapped carbon dots was investigated. [110,111]. Applying the following calculation, the percentage of entrapment efficiency was determined.

$$\text{Entrapment efficiency} = \frac{\text{Amount of total drug} - \text{Amount of free drug}}{\text{Amount of total drug}} \times 100$$

8. RESULTS AND DISCUSSIONS

Preformulation Study
Miscibility:
A) Geranium Oil:
For further analysis, the miscibility of pure geranium oil was established. Geranium oil contains different pharmaceutical ingredients which show the distinct pharmacological activities. Geranium oil is miscible in methanol, ethanol, chloroform, acetone, butanol, toluene, acetic acid and immiscible inwater, Ethyl acetate.Miscibility ofgeranium oil was shown in Table.1. respectively.

WhereasA-Methanol, B- Acetone, C-Butanol, D-Toluene, E- Ethyl Acetate, F-Water and G -Acetic Acid

Table 3: Solubility of Geranium Oil in Different Solvents

Sr. No.	Solvent	Solubility
1.	Methanol	+
2.	Acetone	+
3.	Butanol	+
4.	Toluene	+
5.	Ethyl acetate	-
6.	Water	-
7.	Acetic Acid	-

Whereas, (+) indicate sign soluble and (-) indicate sign insoluble.

B) Geranium Oil Loaded Carbon Dots:
Geranium oil loaded carbon dots comes under the nanoformulation. Carbon dots are soluble in methanol, ethanol, chloroform, acetone, butanol, toluene, acetic acid and insoluble water. Solubility of geranium oil loaded carbon dots were shown in Fig.2 and Table 4 respectively.

Table 4: Solubility of Geranium Oil Loaded Carbon Dots in Different Solvents

Sr. No.	Solvent	Solubility
1.	Methanol	+
2.	Acetone	+
3.	Butanol	+
4.	Toluene	+
5.	Ethyl acetate	+
6.	Water	-
7.	Acetic Acid	-

Whereas, (+) indicate sign soluble and (-) indicate sign insoluble.

Boiling point:

The Pelargonium graveolens plant yields geranium oil. Numerous high-quality cosmetic items, including soaps, detergents, creams, lotions, and balms, are also made with it. It is occasionally and is sold for use in aromatherapy and massage therapy. Theboiling point of geranium oil was found to beat 250-258°Cwhich was shown in Fig.3.

Determination of λmax:

The Geranium oil solution 10 µg/ml in ethanol was prepared and analyzed at 200 to 800 nm inUV spectrometer against ethanol as blank solution. The λmax Geranium oil solution was found to be at 204.8 nm. The λmax of Geranium oil was shown in Fig.4.

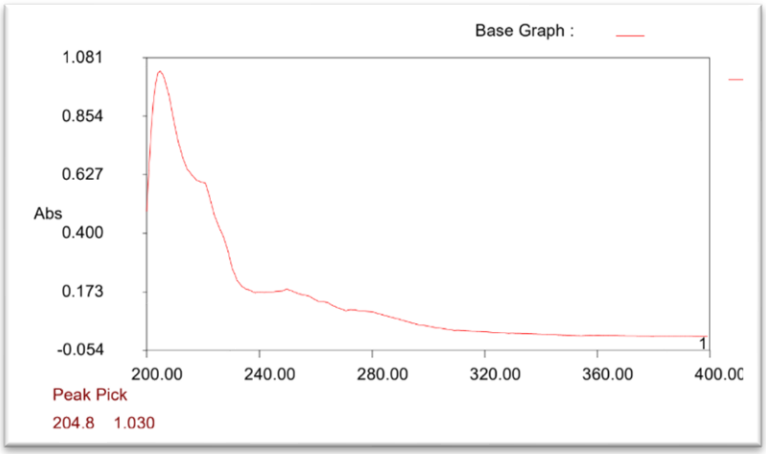


Figure 2: λmax of Geranium Oil in Ethanol

Calibration Curve of Pure Geranium Oil:

The calibration curve of pure geranium oil was performed as per standard procedures by preparing 2-10 µg/ml concentration was analyzed at 204 nm. The abs was recorded at various concentration shows straight line that crosses the origin. The calibration curve was followed the Beer's-lambert law. The value of the coefficient of correlation was observed to be 0.9536. The different concentration absorbance and the calibration curve shows in Table 5. And Figure respectively.

Table 5: Absorbance of Geranium Oil At Different Concentrations

Sr. No.	Concentration	Absorbance
1	1	0.16
2	2	0.32
3	3	0.474
4	4	0.57
5	5	0.865

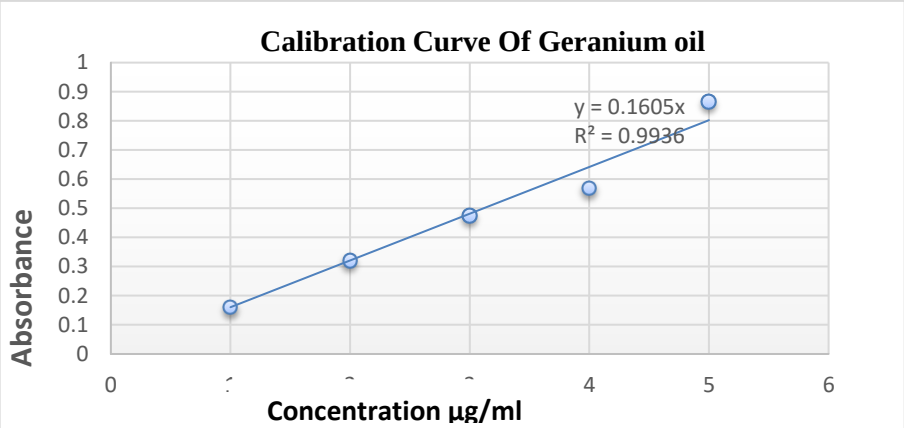


Figure 3: Calibration curve of pure Geranium Oil and correlation coefficient

FT-IR of Pure Geranium oil

The FT-IR analysis was performed by Jasco FT-IR at wavelength 4000–400^{-1} at 4 cm^{-1} resolution. The FT-IR spectra of Geranium oil shows intense peak at 3401.48 cm^{-1} of stretching vibrations of phenolic -OH group. The absorbance band at 2919.92 cm^{-1} of stretching vibrations of C-H aromatic atom. The sharp absorbance band at 1719.08 cm^{-1} shows stretching of C=O (anhydride) group. The absorbance band at 1429.97–1511.97 cm^{-1} indicated stretching vibration of C=C olefinic and aromatic C=C group. The 1376.49 cm^{-1} absorbance band stand for bending vibration of phenolic C-O group. While the absorbance band at 1173.25 cm^{-1} is stretching vibration of aromatic ester (C-O) group. Fig.6. represent FTIR spectra of Pure Geranium oil and Table.12. shows interpretation of FT-IR data.

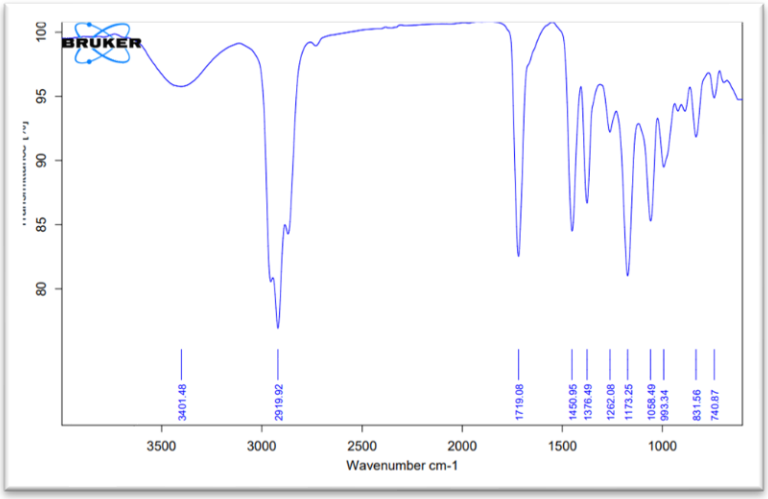


Table 6: FT-IR Interpretation of Geranium Oil

Sr. No.	Wave Number (cm -1)	Vibration	Functional Group
1	3401.48	Stretching	O-Haromatic
2	2919.92	Stretching	C-Haromatic
3	1719.08	Stretching	C=O
4	1450.95	Bending	C-Haromatic
5	1376.49	Bending	Phenolic C-O
6	1173.25	Stretching	C-O

Gas Chromatography Mass Spectroscopy of Geranium Oil :-

The essential oil of *Pelargonium graveolens* was studied using two-dimensional gas chromatography-mass spectrometry (GC-MS), which revealed 21 components. Citronellol (33.65%), cyclohexane (2.74%), caryophyllene (2.92%), and citronellyl tiglate (1.44%), 6-octane-1-ol,3, 7-dimethyl-, formate (12.70%), linalool (2.74%), Cyclohexanone, 5-methyl-2-(1-methyl)-, (10.69), (1R,3aS,8aS)-7-Isopropyl-1,4-dimethyl-1,2,3 (12.58) were the eight substances that were most prevalent, which supports this hypothesis.

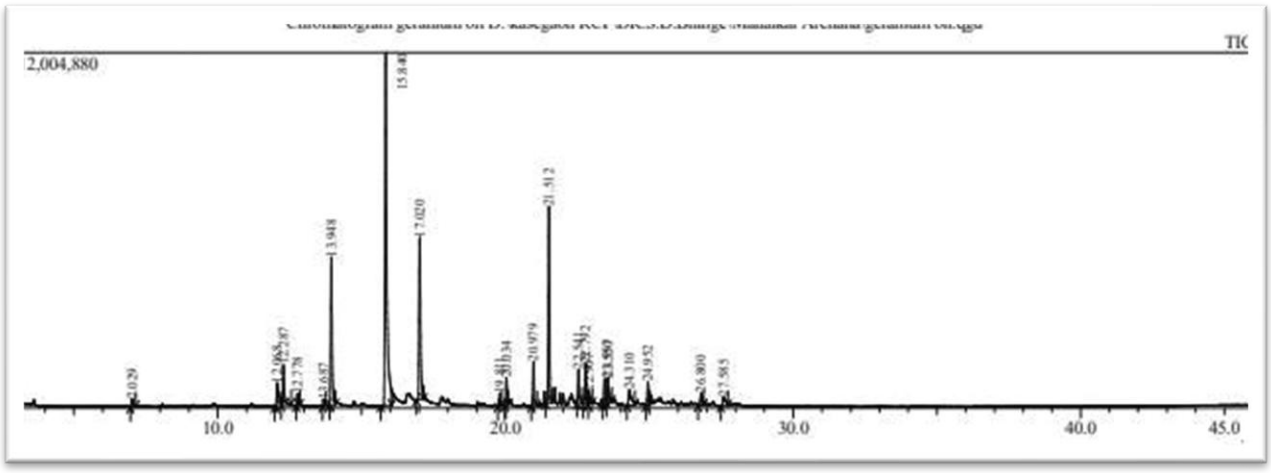


Figure 5: GC-MS of Geranium Oil

Table 7: GC-MS Interpretation of Geranium Oil

Sr.No.	Name of The Compound	Area (%)	R. Time
1	Citronellol	33.65	15.84
2	Linalool	2.74	2.061
3	caryophyllene	2.92	20.97
4	citronellyl tiglate	1.44	26.80
5	6-octane-1-ol,3, 7-dimethyl-, formate	12.70	17.02
6	Cyclohexanone, 5-methyl-2-(1-methyl)-	10.69	13.94

Differential Scanning Calorimetry of Geranium Oil:
Figure.13 displays the analysis's findings utilising a differential scanning calorimeter (DSC). The DSC of geranium oil was determined endothermic peak with melting point. The DSC graph of geranium oil have low endothermic peak at 132.68º C temperature due to hydrophobic nature of Geranium oil and this was represented in Fig.13. According to the findings, geranium oil's differential scanning calorimetry does not exhibit a significant peak. Geranium oil's melting peak has drastically decreased. Additionally, it showed new peaks when the effect of geranium oil's heat absorption decreased.

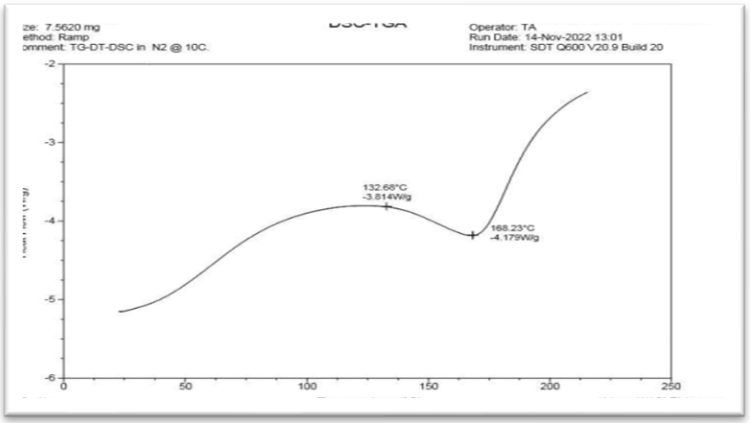


Figure 6: DSC graph of Geranium Oil

Thermal Gravimetric Analysis of Geranium Oil:
In this investigation, the geranium oil was exposed to a controlled temperature program and atmosphere. A method for calculating the weight of a substance in relation to heat or time is called thermogravimetric analysis. The TGA graph of geranium oil is shown in fig:14. The preparation's thermal strength comes from the interaction of the active ingredient and excipients. The weight loss of the sample was carried in that same percentage if the sample was damaged at high temperatures. [13]

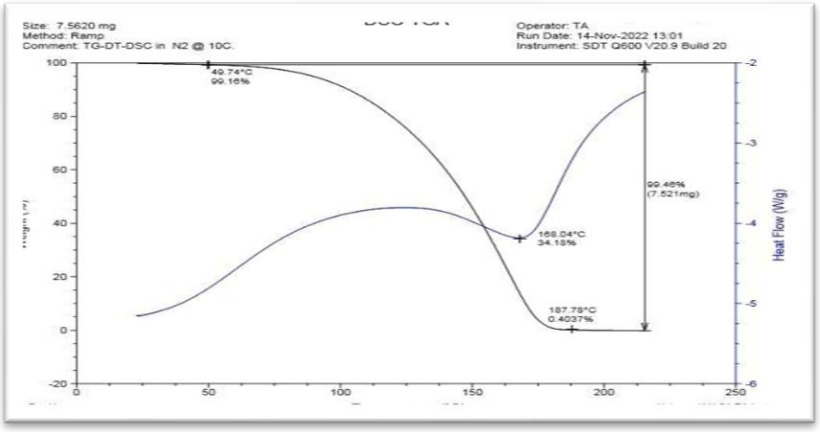


Figure 7: TGA graph of Geranium Oil

Lyophilization of Prepared Formulation:

Mannitol was added in modest amounts throughout the lyophilization process to ensure that the resulting product dried properly. Following lyophilization, it was found that dried formulations were more stable in liquid ones.Fig. 8 depicted the dried Carbon Dots formulation.

Figure 8: Lyophilized Carbon Dots

Formulation of Gel:

Figure 9: Geranium Oil Loaded Carbon Dots Gel

Recent interest in encapsulating lipophilic functional chemicals has boosted the usage of nanosystems to create formulations, such as gels comprising oil/water. This is an alternate method of formulating essential oils because it makes them more physically stable, shields them from environmental influences, and reduces their volatility. Additionally, nanoformulations may increase the retention of active ingredients in the vaginal mucosa, encouraging a longer residence duration, greater interaction between the oil and the bacteria, optimising the transport of active ingredients, and ultimately increasing therapeutic efficacy. Phase analysis and microscopic observation were used to analyse an extremely viscous and translucent gel that forms very diluted water-rich zone.

Free radicals and lipid peroxidation in human bodies are responsible for a number of disorders, including cancer, atherosclerosis, and inflammation. The primary component of many plants' essential oils, monoterpenes are known for their inherent anticancer properties. as evidenced by the anticancer characteristics that have been observed. Monoterpenes

make up the majority of the active ingredients in geranium essential oils. In rodent models, monoterpenes have demonstrated a protective effect against stomach, liver, lung, and breast malignancies.

Evaluation of Gel:

a. Determination of visual appearance and clarity:

The formulations were visually inspected for clarity and appearance using a white and black background to check for the presence of any particulate debris.

Table 8: Assessment of appearance

Sr. No	Formulation	Appearance of gel
1	F1	Cloudy
2	F2	Cloudy
3	F3	Cloudy
4	F4	Transparent
5	F5	Cloudy
6	F6	Transparent
7	F7	Cloudy

Results in Table No. 9 reveal that the formulations with low concentrations of Carbapol 934 were transparent and clear, while the formulations with high concentrations of Carbapol 934 had foggy appearances. According to the above mentioned finding, formulations with a higher concentration of Carbapol 934 are cloudier by nature due to a change in concentration and hydrogen bonding.

pH: The pH of each formulation was assessed in triplicate at room temperature using a digital pH meter. The results are displayed in the table below.

Table 9: Assessment of pH

Sr. No	Formulation	pH
1	F1	7
2	F2	7.3
3	F3	6.8
4	F4	7.5
5	F5	7
6	F6	7.8
7	F7	6.9

The formulas mentioned above have a pH between 6.8 and 7.4. Formulations F1, F6, and F7 produced pH that was close to skin pH. pH below the 4 may cause irritate to skin. As carbapol 934 concentration rises, the pH shifts to an acidic state. From the facts above, it can be inferred that the formulations F1, F6, and F7 have pH levels that are appropriate and won't irritate users when administered.

a. Viscosity :-

Gel formulations of geranium oil loaded carbon dots had their viscosities evaluated revolutions per minute in triplicate before and after gelation. It is depicted as the subsequent observation.

Table 10: Assessment of Viscosity

Sr. No	Formulation Code	Viscosity Of Gel (cps)
1	F1	1220
2	F2	1111
3	F3	1118
4	F4	1320
5	F5	1530
6	F6	1260
7	F7	1220

b. Gelation Temperature :-

The formulations of skin gel with geranium oil loaded carbon dots were studied for gelation temperature. In the range of 15 to 25 °C, it was estimated. It is shown in the observation Table below.

Table 11: Assessment of Gelation Temperature

Sr.No.	Formulation Code	Gelation Temperature (°C)
1	F1	22
2	F2	21
3	F3	19
4	F4	18
5	F5	23
6	F6	24
7	F7	25

c. Gelation Time :-

The research of the gelation period of geranium oil loaded carbon dots gel was conducted. It is established in the observation table that follows.

Table 12: Assessment of Gelation Time

Sr. No	Formulation Code	Gelation Time (sec)
1	F1	122
2	F2	80
3	F3	90
4	F4	140
5	F5	150
6	F6	115
7	F7	95

d. % Drug Release :-

Figure No. 8.24 displays the formulations' release profiles. In-vitro drug diffusion research was conducted on formulation F5, the optimised batch that was selected after consideration of several evaluation criteria. In drug release experiments, the formulation F5 performed better and maintained the drug's activity for 8 hours. This might be as a result of the F5 Batch's optimal Carbapol 934 and HPMC K4 concentrations. The findings support the hypothesis that increased viscosity extends the time that drugs take to leave formulations.

Table 13: Assessment of % Drug Release

Sr. No.	Time (min)	Drug Release (%)
1	00	40
2	15	43
3	30	50
4	45	60
5	60	64
6	75	70
7	90	74
8	105	78
9	120	80
10	135	83
11	150	85
12	165	88
13	180	90

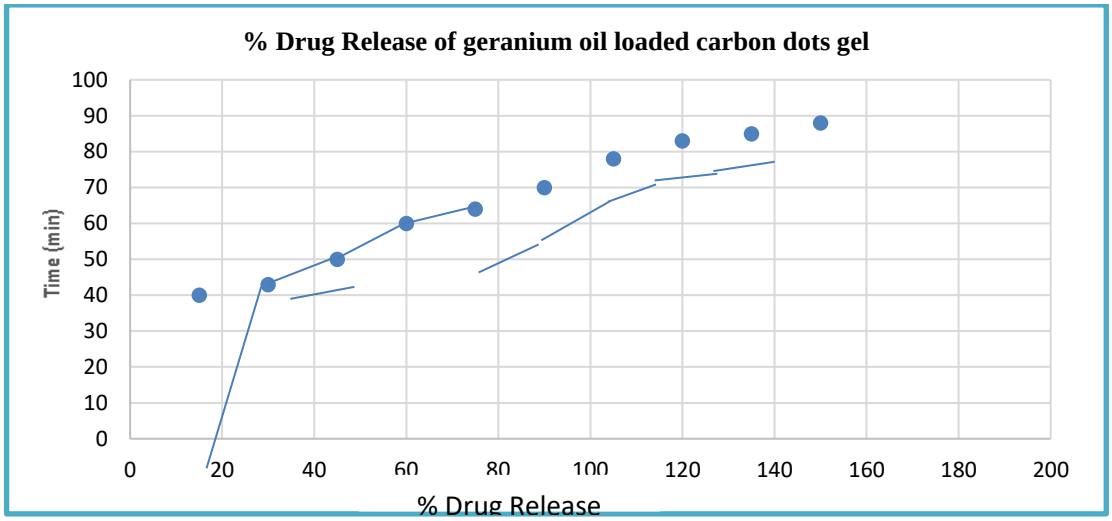
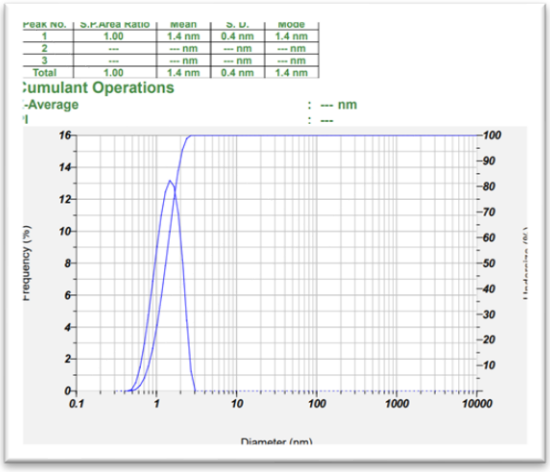


Figure 10: Graphical Representation of Viscosity of Geranium Oil Loaded Carbon Dots Gel

Characterization of Geranium carbon dots:

Particlessize

The stability and release of the active ingredients that were utilised in formulation are affected by the scattering of particle size, which is crucial. The poly dispersive index (PDI) was used as an analysis for the least amount of particle agglomeration. Vesicles should have a PDI value of 0.3 or below for lipoidal drug delivery systems.[14] So, the particle size and PDI of pure geranium oil was found to be 538.0nm and 0.478 while, the particle size and PDI of plain carbon dots was observed to be1.4 nm and 0.363subsequently.



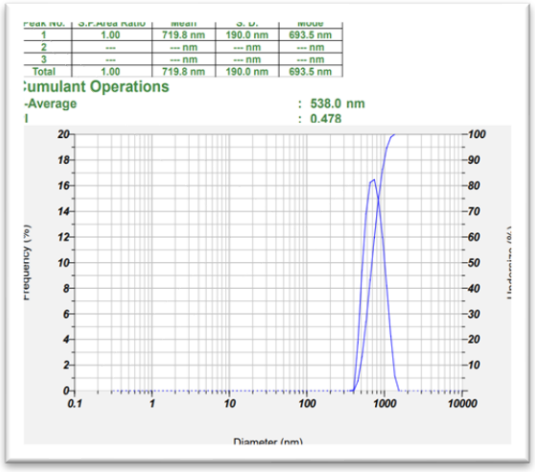
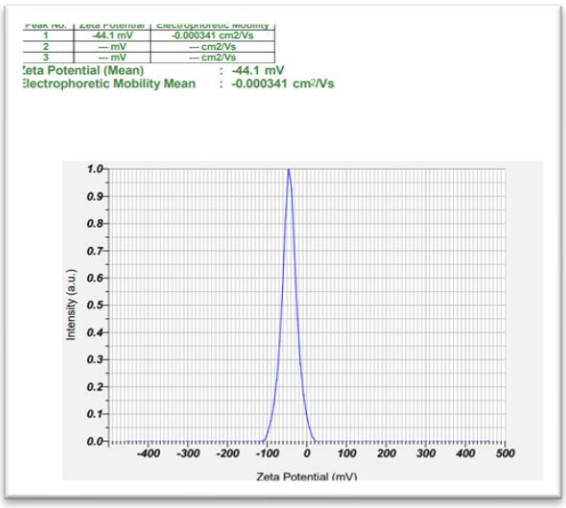


Figure 11: Particle size of Geranium Oil Carbon Dots **Figure 12: Particle size Geranium Oil**

Zetapotential

Zeta potential is used to determine the electrical charge on particles and is an effective indicator of stability. For a capping agent that coats the surface of geranium and consists primarily of negatively charged groups, negative values of zeta potential are indeed achievable. Since all particles in suspension have high zeta potentials, either negative or positive, they repel one another and prevent particle sedimentation. [141]. The zeta potential value of pure geranium oil was found to be -44.1mV. While, Carbon dots carbon dots were projected to be safe and stable for future use based on the explanation above.



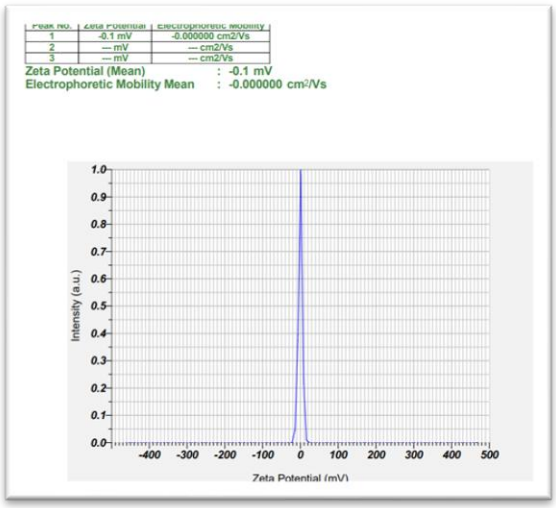
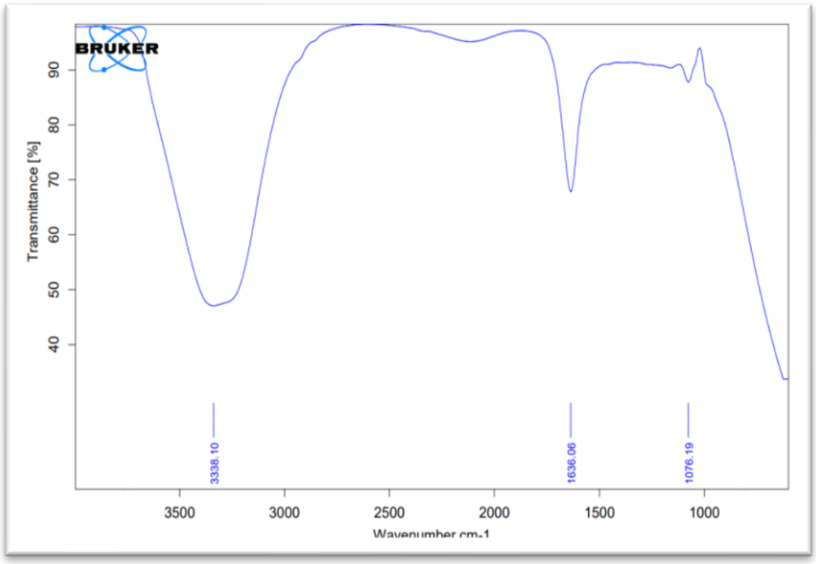


Figure 13: Zeta potential of Geranium Oil **Figure 14: Zeta potential of Carbon Dots**

Figure 15: FT-IR of Carbon Dots



FTIR of Carbon Dots:

Table 14: FT-IR interpretation of Carbon Dots

Sr. No.	Wave Number (cm^{-1})	Vibration	Functional Group
1	3338.10	Stretching	O-Haromatic
2	1636.06	Stretching	C=C
3	1076.19	Stretching	C=O

X-Ray Diffraction of Geranium Oil Loaded Carbon Dots:

The crystallisation of a dry sample is investigated by XRD patterns. The Carbon dots was stabilised in amorphous state and solubilized in lipid matrix. Figure: 5 displayed the carbon dot XRD patterns. Peaks at 2ɵ of 14º,27º,31º,45º and 56ºwere seen in the XRD diffraction, which indicates that the material is crystalline. As a result, the carbon dots display sharker peaks, demonstrating the sample's high degree of amorphousness and purity.

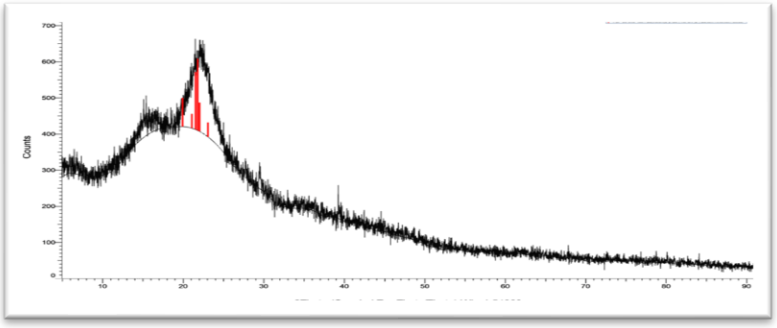


Figure 16: XRD graph of Carbon Dots

Scanning Electron Microscopy of Carbon Dots:
Analysing the surface morphology and particle size is crucial to improve the biological activity of nanosized formulations. The matrix structure of the produced trapped carbon dots can be identified using SEM investigations.Fig.16. represent SEM pictures of Carbon dots that are enclosed. SEM result reveals that uniforms particle distribution and Spherical crystalof carbon dots. Moreover, all carbon dots particles have roughsurface and all particles shown porous matrices. The particles are easily distributed in formulation because to their porosity nature.

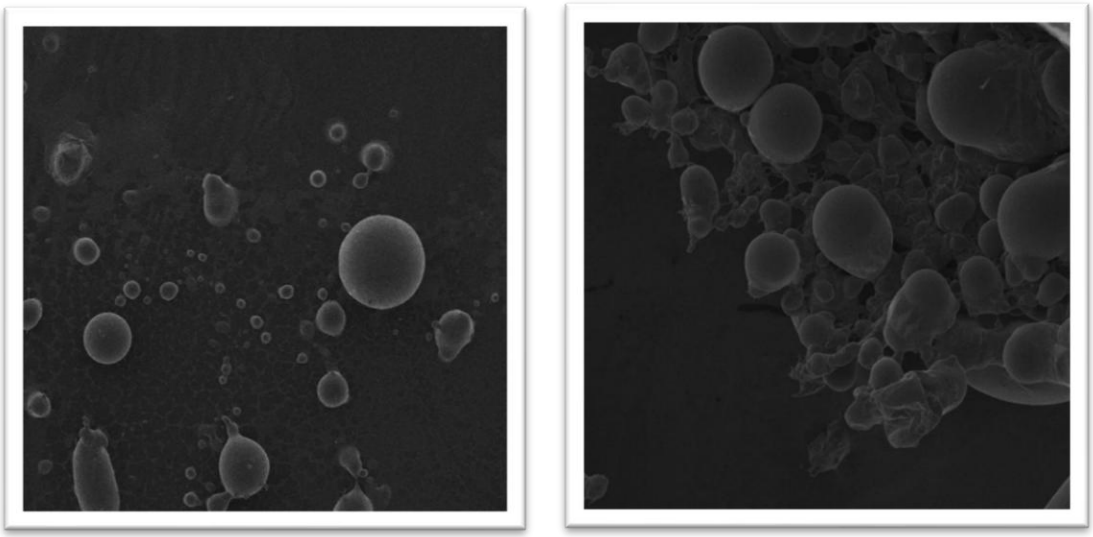


Figure 17: SEM images of Carbon dots

TEM:
The average CD size of 20 nm was found by TEM to be crystalline. Using TEM, the acquired CDs' morphological examination was completed.

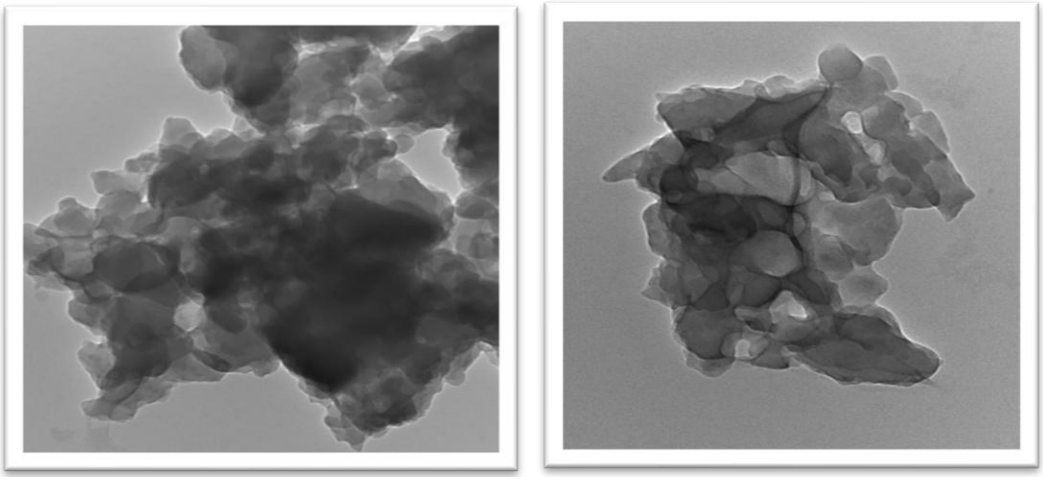


Figure 18: TEM of Carbon Dot

9. CONCLUSION

The hydro distillation method effectively yielded geranium essential oil from *Pelargonium graveolens* leaves, with consistent extraction procedures. The physicochemical analyses, including physical constants and spectroscopic studies, confirmed the quality and purity of the extracted oil. The plant powder exhibited notable phytochemical constituents, including alkaloids, proteins, amino acids, and tannins, indicating a rich phytochemical profile that supports its medicinal and industrial potential. The study highlights the promising features of carbon dots, particularly their optical stability, biocompatibility, and functional versatility, which make them suitable candidates for applications such as sensing, bioimaging, and biomedical devices. The detailed characterization of *Pelargonium graveolens* and its essential oil provides a solid foundation for developing geranium oil-loaded carbon dots, aiming at harnessing their combined properties for innovative nanotechnological and biomedical applications.

10. CONFLICT OF INTEREST Declared None

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