Development of eco-friendly green and chemical routes for exfoliation of graphite as effective antibacterial agent

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Abstract

Liquid phase exfoliation (LPE) has emerged as an effective technique for producing large scale 2D nano-sheets. In present study, graphite has been exfoliated by using chemical and green routes. The adopted methods are cost effective, eco-friendly, time saving and utilize less toxic solvents for producing high quality exfoliated graphite (EG). Herein, graphite exfoliation has been achieved by using NaOH, HCl, natural acids (lemon juice) and plant extract (mint extract). In chemical route, exfoliation of graphite using NaOH has given excellent results as compared to HCl. By using NaOH, d-spacing value has been increased from 3.30 to 3.37 nm and sheets with average thickness of 14.8 nm have been achieved. In green route, mint extract gives better result as compared to lemon juice. The bioactive constituents of plant extracts penetrate between graphitic layers results in weakening of van der waals forces which facilitates exfoliation. Using mint extract, d-spacing value has been increased from 3.30 to 3.36 nm. Thin sheets with average thickness of 10.79 nm has been obtained. Overall exfoliation using NaOH and mint extract gives excellent result. The higher ID/IG ratio for exfoliated graphite using NaOH and mint extract is 0.77 and 0.88, respectively as compared to pure graphite which confirmed the degree of exfoliation. Exfoliation of graphite enhances the surface properties of nano-sheets leading to the enhanced antibacterial activity against Pseudomonas Aeruginosa as compared to pure graphite. The exfoliated graphite using mint extract showed the best antibacterial activity.

1. Introduction

2D nanomaterials have attained worldwide attraction because of their remarkable properties arises from dimensionality and structures in recent years [1–3]. The benefits of 2D nanomaterials have opened promising prospects for wide applications at technological and scientific level [4]. Generally, 2D layered materials are characterized by their weak van der Waals interactions and strong in-plane bonding. Therefore, the main factor during exfoliation of layered material into individual layers is mainly based upon the interruption of non-covalent inter-layer bonding [5, 6] and how to deal with interactions in order to achieve high quality and defect free sheets. Till now, a large variety of 2D layered materials have been reported which categories into various groups based upon structural resemblances [7–10]. Graphite is simple layered crystal comprises of sp² hybridize hexagonal lattice. In each graphitic layer, carbon atoms are aligned in an infinite and regular arrangement. The carbon atoms experience two type of interactions among one another. Firstly, each carbon atoms are bounded by three other carbon atoms and arranged in a network of regular hexagon. This regular arrangement continues in 2D to produce a hexagonal and horizontal ‘chicken wire’ array. In addition, weaker forces hold the regular arrangement of layers termed as stacking interactions. Between layers, atoms of carbon are organized in hexagonal framework with 0.142 nm separation. The inter planar distance is 0.335 nm [3].
Until now, several methods have been reported for graphite exfoliation including micromechanical exfoliation [11], chemical exfoliation [12], electrochemical synthesis [13], and liquid phase exfoliation [14–17]. Micromechanical cleavage is basically the utilization of scotch tape which led Geim and Novoselov in 2004 to earn Nobel Prize in Physics [16]. Furthermore, graphite has been exfoliated by consequent reduction of graphene oxide. In 1859, Brodie and co-worker [18] first prepared graphite oxide (graphitic acid or graphitic oxide) by reacting graphite with a mixture of fuming nitric acid (HNO₃) and potassium chloride (KClO₃).

Staudenmaier [19] in 1898 improved this technique using sulfuric acid. Hofmann [20] utilised concentrated nitric acid, KClO₃ and concentrated sulfuric acid. The widely known Hummers method used a mixture of sulfuric acid, potassium permanganate (KMnO₄) and sodium nitrate (NaNO₃) [21]. Chemical exfoliation depends upon the intercalation of various molecules or ions in graphite such as 1-pyrene carboxylic acid [22, 23], tetra butyl ammonium cations [24, 25] or alkali metals in N-methyl Pyrrolidone (NMP) [26] spontaneously and readily exfoliate the intercalated graphite and produces reduced graphene sheets. Lastly, exfoliation of graphite has been achieved by using solvents governed by sonication under optimal operational conditions. The most commonly utilized solvents were water alcohol mixtures [27], N-methyl pyrrolidone [26], 1-hexyl-3methyl-imidazolium hexa-fluoro phosphate [28], cyclohexanone, benzyl benzoate [29], fluoro benzene (C₆F₆), uoropyridine (C₅F₅N) and fluorobenzo nitrile (C₆F₅CN) [30]. The above stated approaches have advantages as well as disadvantages. For instance, mechanical exfoliation technique can yield low quantities of graphene sheets. The graphene oxide obtained as a result of chemical reduction can yield graphene on a large scale but with some defects. Consequently, people are still committed in finding most efficient, high yield and low cost methods for producing high quality graphene nano-flakes.

Liquid phase exfoliation (LPE) method has attained considerable attention as an efficient, cost-effective and versatile method. This method is cheaper, simple and environment friendly. This method does not utilize hazardous chemicals as strong acids. LPE technique includes different approaches that exfoliate bulk graphite into exfoliated graphite in the presence of suitable liquid medium [31–33]. This method comprises the production of few or multilayer graphitic nanoflakes under high stress or sonication [34, 35] to layerd material the in presence of specific stabilizing liquids (e.g polymer solution, surfactant, biomolecules or appropriate solvent). In most cases, interaction between nanosheets and liquid interface decreases the final exfoliation energy and sustains the nanoflakes against aggregation [36, 37].

The speedy boost in bacteria arbitrated infectious diseases has been one of the most impending fears to human health and the cause of upsetting millions of people around the world [38]. The rushed mishandling of conventional antibiotics have heading for the pathway to develop many drug-resistant bacteria, leading to the irremediable infections. For combating MDR strains, new antimicrobial materials are urgently needed. Scientists have dedicated to discover the activity of graphene based materials against the kingdom of bacterial cells [39]. The antibacterial tests on the majority of graphene based materials were applied mainly against E. coli and S. aureus. Therefore, it is very important to investigate the materials with other pathogenic species to demonstrate a broad bactericidal range and keep the account of increasing antibiotic resistance among various bacteria and their association as a serious threat to worldwide public health [40]. In present work, a novel, eco-friendly, less toxic and low cost strategy has been developed for liquid phase exfoliation of graphite. Liquid phase exfoliation has been achieved by utilizing chemical and green routes. Consequently, benefits of both routes have also been studied. Solvents used in chemical routes are NaOH and HCl are less toxic as compared to strong oxidizing acids and organic solvents. Plant extracts have been considered as valuable materials for synthesis of many nanomaterials. In this regard, utilization of plants extract is promising new approach. In case of green route, mint leave extract and lemon juice (a natural acid) have been used as exfoliation media (solvent). Mint leave extract and lemon juice are rich sources of phytochemicals and citric acid, respectively which effectively employed in exfoliation because of their remarkable reducing and stabilising properties. Both routes are highly cost effective, environment friendly which effectively exfoliate the graphite. In chemical route, NaOH gives efficient exfoliation as compared to HCl. In green route, mint leaves have found more effective for exfoliation as compared to lemon juice. Exfoliated graphite comprises of layered structure, large in plane spacing and high volume that can be utilised in various applications. Exfoliation of graphite dramatically affects the surface activities which lead to many applications such as in energy storage devices, photo catalysis and antibacterial activity. The antibacterial activity has been explored using exfoliated graphite against clinical ophthalmic strain of Pseudomonas Aeruginosa.
2. Experimental procedures

2.1. Materials
Graphite powder, Sodium hydroxide (NaOH), Hydrochloric acid (HCl), Mint leaves and Lemon juice. All the chemicals were purchased from Sigma Aldrich. Distilled water has been utilized throughout the experimental procedure.

2.2. Synthesis of exfoliated graphite
Liquid phase exfoliation of graphite has been achieved by using chemical and green routes. In chemical route, NaOH and HCl solutions have been utilised for exfoliation. In green route, mint and lemon extract have been employed for exfoliation process.

2.3. Chemical route
2.3.1. Exfoliation using NaOH solution
Initially 0.5 M solution of NaOH has been prepared. Then 3.0 g of graphite powder is added to NaOH solution. This mixture had stirred continuously for 10 h at 500 rev/min. Then sonication was performed in bath sonicator for 45 min. After this, resulting dispersion was washed several times in order to remove un-exfoliated part and impurities. Finally the obtained dispersion was dried in oven at 60 °C.

2.3.2. Exfoliation using HCl solution
Firstly 0.5 M HCl solution has been prepared. Then 3.0 g of graphite powder is added to HCl solution. This mixture had stirred continuously for 10 h at 500 rev/min. Then sonication was performed in bath sonicator for 45 min. Then resulting dispersion was washed several times in order to neutralize pH and removed un-exfoliated part and impurities. Lastly, the obtained dispersion was dried in oven at 60 °C. The reaction steps of chemical route for exfoliation are shown in figure 1(A).

2.4. Green route
2.4.1. Exfoliation using mint extract
For making mint extract, 10.0 g of mint leaves were heated in 200 ml of distilled water for 2 h at 120 °C. The resultant solution was filtered in order to get mint extract. Then 3.0 g of graphite powder is stirred in mint extract.

Figure 1. Flow sheets of graphite exfoliation using chemical route (A) and green route (B).
for 8 h and left soak for 3 days. As bioactive molecules were slow in chemical reaction as compared to chemicals. So soaking was done in order to enhance the activity of bioactive molecules present in mint leaves extract and lemon juice, respectively. Then sonication was performed in bath sonicator for 45 min. Then resulting dispersion was washed several times in order to neutralize pH and removed un-exfoliated part and impurities. Lastly the obtained dispersion was dried in oven at 60 °C.

2.4.2. Exfoliation using lemon extract
3.0 g of graphite was added to 30 ml fresh lemon juice. The dispersion was stirred for 8 h and left soaks for 3 days. Then sonication was performed in bath sonicator for 45 min. Then resulting dispersion was washed several times in order to neutralize pH and removed un-exfoliated part and impurities. Lastly, the obtained dispersion was dried in oven at 60 °C. The reaction steps of green route are shown in figure 1 (B).

2.5. Antibacterial activity
Antibacterial activity of synthesized exfoliated graphite using chemical and green methods was evaluated by agar well diffusion method against clinical ophthalmic pathogen *Pseudomonas Aeruginosa*. For antibacterial activity, a lawn of *Pseudomonas Aeruginosa* is made using overnight culture on King B medium. A well of 4 mm is bored on pre inoculated culture plate using sterile borer and 100 μg ml⁻¹ of sample material was placed inside the wells. Then the inoculated plates were incubated at 37 °C for 24 h and observed for zones of inhibition around well containing our synthesized exfoliated graphite. The antibacterial activity was measured in mm using scale.

2.6. Catalyst characterization
The crystallinity and crystal phase composition of as synthesized exfoliated graphite was determined through X-ray diffraction in range of 20°–80° (2θ) by using Rigaku D/MAX 2550 X-ray spectrophotometer (Cu Ko1 radiation, λ = 0.154056 nm) operated at 100 mA and 40 kV. Fourier transform infrared (FT-IR) spectroscopy was performed by employing Nicolet 740 spectrometer equipped with KBR beam splitter and TGS detector. The morphology of samples and their composition was examined using Scanning Electron Microscopy (JEOL. JAD-2300). Thermo gravimetric analysis was performed on a Perkin Elmer Pyris Diamond thermo gravimetric analyser. Raman analysis was performed by using Lab Ram III. Dong Woo Optron spectrometer. AFM was carried out by using Agilent Technologies spectrometer model N9451A.

3. Results and discussion

3.1. X-ray diffraction
The XRD spectra of pure and exfoliated graphite using NaOH and HCl solutions are shown in figure 2(A). Both pure and exfoliated graphite show characteristic peaks at 2θ = 26.4° and 2θ = 54.5°. As shown in figure 2(A), peak intensity of pure graphite at plane (002) is high with sharp and distinct peak as compared to exfoliated graphite. A sharp peak with high intensity at 002 exhibit typical graphitic material. A intensive decrease in peak intensity at (002) of exfoliated graphite is due to increase in inter layer separation determine from Bragg’s Law (2dsinθ = nλ). The spacing is increased from 3.30 to 3.37 Å using NaOH solution and from 3.30 to 3.36 Å using HCl solution which confirms exfoliation [41, 42]. XRD spectra of pure and exfoliated graphite using lemon juice
Table 1. Average sheet thickness and d-spacing values of exfoliated graphite using NaOH, exfoliated graphite using HCl, exfoliated graphite using mint extract, exfoliated graphite using lemon juice.

| Samples                              | Average sheet thickness (nm) | d-spacing(A) |
|--------------------------------------|------------------------------|--------------|
| Exfoliated graphite using NaOH       | 14.8                         | 3.30         |
| Exfoliated graphite using HCl        | 25.0                         | 3.37         |
| Exfoliated graphite using mint extract | 10.7                         | 3.36         |
| Exfoliated graphite using lemon juice | 17.5                         | 3.36         |

3.2. Scanning electron microscopy

The morphology and structure of pure and exfoliated graphite using NaOH and HCl solutions were investigated by SEM analysis which is shown in figures 3(A)–(C). In SEM image of pure graphite (figure 3(A)), there is compact stacking of graphitic sheets with irregular pointed edges. In figure 3(B), compared to pure graphite, exfoliated graphite (using NaOH) exhibits multi-layer, thin, semi transparent graphitic sheets which confirms exfoliation. Highly defined edges of exfoliated graphite make it promising to determine the size of exfoliated flakes or sheets. Sheets have smooth surface with well defined edges having average thickness of 14.8 nm. Restacking and clustering of graphitic flakes might be due to aggregation during drying process. Figure 3(C) shows
the morphology of exfoliated graphite (using HCl solution). Compared with pure graphite, exfoliated graphite consist of multi-layer, stacked graphitic sheets with average thickness of 25 nm. The flakes were partially twisted and folded with sharp edges which confirmed the exfoliation. The lateral size of exfoliated graphite has been significantly reduced after exfoliation process as compared to pure graphite. The smaller lateral size could be due to breakage of graphitic particles around their defects or weak points. This is because of receiving intense energy during stirring and sonication process. The exfoliation of graphite is good with the utilisation of NaOH as compared to HCl [45]. Figures 3(D), (E) shows the SEM image exfoliated graphite using mint extract and lemon juice. From figure 3(D), compared to pure graphite, exfoliated graphite (using mint) comprises of multi-layer, piled up thin sheets with average thickness of 10.79 nm. Highly defined edges of exfoliated graphite make it promising to determine the size of exfoliated flakes or sheets. The sheets are well noticeable, well defined and clear around edges. Figure 3(E) shows the SEM image of exfoliated graphite (using lemon juice). Compared to pristine graphite, exfoliated graphite exhibit multi-layered, thin, semi transparent graphitic sheets with average thickness of 17.5 nm which confirms exfoliation. It is well noticeable from figure 3(E), lateral size of exfoliated sheets has been greatly reduced compared to pristine graphite [46–48]. In chemical route, NaOH shows efficient exfoliation as compared to HCl. Because of larger size of Na\(^+\) ions, it intercalated more easily than H\(^+\) ions which weakens the interlayer interaction and facilitates exfoliation. Average sheet thickness was found to be 14.8 nm using NaOH solution. In green route, mint leaves have found more effective for exfoliation as compared to lemon juice. The phytochemical constituents of plant extracts penetrate between graphitic layers which facilitates exfoliation. Average sheet thickness was found to be 10.7 nm using mint extract. Table 1 shows the average thickness and d-spacing values of as prepared samples.

### 3.3. Fourier transform infrared spectroscopic

The degree of functional groups present in exfoliated graphite was investigated by FTIR spectroscopy as shown in figure 4. The exfoliated graphite prepared from all four routes indicates the absence of functional groups which exhibits high purity of exfoliated graphite. The absence of any functionalities indicates that absorption bands are less than detection level of IR spectroscopy. The results showed that exfoliated graphite exhibited well defined exfoliation with absence of any impurities, functional groups and oxides [43, 49].

### 3.4. Thermo gravimetric analysis

Thermogravimetric analysis was carried out in order to examine the weight loss as function of time in as prepared samples. The examination was carried out using 3.074 mg of pure graphite, 3.66 mg, 2.182 mg and 3.99 mg of exfoliated graphite prepared from NaOH solution, mint extract, lemon juice, respectively under atmosphere of nitrogen gas (flow rate of gas = 40 mL min\(^{-1}\)) in the presence of 10 °C heating rate from 70 to 600 °C for sample 1, from 40 to 500 °C for sample 2 and from 40 to 600 °C for sample 3, respectively. In figure 5(a), mass loss of 3.0% is observed in case of pure graphite. From figure 5(b), in case of exfoliated graphite prepared by using NaOH solution (sample 1) initially there was mass loss of 0.015 mg upto 400 °C. After that there was slightly increase in mass due to absorption of humidity from envirnment upto 550 °C. After 550 °C, the residual mass
left behind is 3.666 mg. Only 1.0% mass loss is seen. This minute mass loss signified that solvents were significantly removed from sample during exfoliation process. From figure 5(c), exfoliated graphite prepared from mint extract (sample 2), there was mass loss of 0.22 % only till 600 °C which signifies that exfoliated graphite which is extremely free from residual impurities. From figure 5(d), exfoliated graphite prepared from lemon juice, there is gradual decrease in mass from 40 to 600 °C. After analysis, only 3.0% mass loss is studied which depict that as prepared exfoliated graphite is almost free from residual impurities [50].

3.5. Raman spectroscopic analysis
Raman spectroscopy is powerful technique for analysing exfoliated graphite [51]. A characteristic Raman spectra gives useful information about various types of bondings present between carbon atoms as well as interaction between graphitic layers [52]. Figure 6 showed a Raman spectra of exfoliated graphite using NaOH and mint extract. The spectra consist of three distinctive peaks at D around 1359 cm$^{-1}$, G around 1600 cm$^{-1}$ and 2D around 2759 cm$^{-1}$ for exfoliated graphite using NaOH. The spectra consist of three distinctive peaks at D around 1364 cm$^{-1}$, G around 1600 cm$^{-1}$, 2D around 2759 cm$^{-1}$ for exfoliated graphite using mint extract. D peak around 1350 cm$^{-1}$ is referred to disordered carbon structure. G peak around 1580 cm$^{-1}$ is referred to graphitic structure or whiskers like carbon. 2D band is referred to the stacking order of graphene layers. The occurrence of 2D peak around 2700 cm$^{-1}$ designated to the presence of multilayer exfoliated graphite [53]. The $I_D/I_G$ ratio for exfoliated graphite using NaOH and mint extract is 0.77 and 0.88, respectively as compared to pure graphite which is 1.31 [54]. The higher ratio indicated the presence of some defects around the edges of graphitic layers and degree of exfoliation.
3.6. AFM analysis
The surface topography and morphology of as prepared exfoliated graphite (prepared by using NaOH) was characterised by Atomic Force Microscopy. Figure 7 showed as distinctive height profile of exfoliated graphitic nanoflakes drop casted on silica substrate at 15 and 20 micron range, respectively [55]. AFM study after exfoliation showed surfaces with wide size distribution. We found that the sheets have thickness of about 20–50 nm which exhibit multi-layer. These sheets are thin and folded. The sheets have well defined edges of exfoliated graphite to determine the size of exfoliated flakes or sheets.

3.7. Mechanism for chemical route
The principle of LPE of graphite is basically depends upon delamination of graphitic layers. LPE of graphite is multistep process. The electrostatic repulsion is a key factor during exfoliation process. Increasing electrostatic repulsion inhibits the restacking of graphitic layers in NaOH dispersion. Initially the polarizable π electrons in graphitic layers facilitate the aggregation of cations (Na⁺, H⁺) at the corners of graphite sheet and then form [graphite]⁻ Na⁺, H⁺. Anions (OH⁻) insert themselves between interlayer spaces and increase the distance between graphite layers. Thus it opens a network for continuous ion insertion. At higher pH, the graphite surface is negatively charged due to proton extraction from OH⁻. The negatively charged anion OH⁻ can be partially isolated by cations (Na⁺, H⁺) which drops the energy barrier of anion insertion. More cations insertion takes places between graphite layer thus forming exfoliated graphite. The weakly confined graphite layers are then separated by ultra-sonication and centrifugation in order to obtain final exfoliated product [33, 56, 57]. In case of hydrochloric acid, the polarizable π electrons initiate the aggregation of cations (H⁺) at the edges of graphitic sheets and then form [graphite]⁻ H⁺. The negatively charged anion Cl⁻ inserts between interlayer spaces and
makes bonds with carbon atoms which increases the distance and weakens the bonds between graphitic layers. The exfoliation of graphite using NaOH and HCl has shown different results. Due to larger size of Na⁺ ions compared to H⁺, Na⁺ effectively intercalated between the graphitic layers, weakened the van der Waals interaction and facilitated exfoliation. Overall exfoliation using NaOH gives excellent result as compared to HCl. The proposed mechanism of exfoliation using chemical route is represented in figure 8.

3.8. Mechanism for green route

In the present study, a simple new, eco-friendly and economical strategy has been demonstrated with the utilization of plant extracts. The exfoliation of graphite has achieved by plant extracts (lemon juice and mint extract). The mint plant mainly comprises of phytochemicals such as flavonoids, steroids, alkaloids, proteins, anthraquinones and phenols [58]. Lemon juice is rich source of citric and ascorbic acid (vitamin C) [59]. Mint leaves and lemon are easily available. Their extracts have good potential in reduction and stabilization of graphitic sheet. The function of plant extract during exfoliation process may be considered similar to surfactants. As both mint and lemon juice have good reducing properties (anti-oxidants), adsorption of plant extract at the edges of graphitic sheets enhanced more negative charge. Thus it creates a barrier in aggregation of sheets permitting graphite to exfoliate slowly. The bioactive constituents of plant extracts combined with sonication process allow green molecules penetrate between graphite layers imparting electrostatic repulsion between layers. This results in weakening of van der Waals forces which facilitates exfoliation process [53]. The proposed mechanism of exfoliation using green route is represented in figure 9.

3.9. Antibacterial activity

We explored the antibacterial activity of exfoliated graphite in P. Aeruginosa which is a gram- negative bacterium that causes various diseases in animals and humans. Figure 10 depicts the antibacterial activity of pure graphite in comparison to exfoliated graphite using NaOH and mint extract, respectively. The antibacterial activity mechanism of exfoliated graphite involves both physical and chemical approaches of action. Physical damages are mostly common and induced by direct contact of the sharp edges of exfoliated graphite with bacterial membranes. The chemical modes of action are related with stress generated by charge transfer between graphitic layers [56]. There are several remarkable studies enlightening the potential mechanisms of graphitic sheets (graphene oxide, reduced graphene oxide, graphene) cytotoxicity e.g. the interaction of sharp edges of graphene-flakes with the cell wall. Surprisingly, we have found antibacterial activity of as prepared exfoliated graphite against bacterium P. Aeruginosa which was not stated previously. This effective antibacterial activity has been credited to membrane stress prompted by sharp edges of exfoliated nanosheets, which might resulted in physical...
destruction to cell membranes, leading to the leakage of RNA and the damage of bacterial membrane integrity [61]. Surface charges and charges at the edges of graphitic sheets produced may also cause disrupting interaction with bacterial cell membrane [62].

Interestingly, we have found antibacterial activity of as prepared exfoliated graphite against *P. Aeruginosa* which was not reported earlier [40]. The anti *P. Aeruginosa* activity was noticed by our green and chemically synthesized exfoliated graphite. A (Pure graphite) showed minimum activity (18 ± 0.32 mm diameter) followed by B (exfoliated graphite using NaOH) produced moderate activity with a zone of inhibition (15 ± 0.22 mm diameter). The maximum activity (10 ± 0.24 mm diameter) was shown by C (exfoliated graphite using mint extract) by agar well diffusion method.

4. Conclusions

In present study, an innovative, eco-friendly, less toxic and low cost strategy has been developed for liquid phase exfoliation of graphite. Liquid phase exfoliation of graphite has been accomplished by utilizing chemical and green routes. Solvents employed in chemical routes are NaOH and HCl are less toxic as compared to strong oxidizing acids and organic solvents. In case of green route, utilization of plants extract is a promising new approach. Mint leave extract and lemon juice (a natural acid) have been used as exfoliation media (solvent). Mint leave extract and lemon juice are rich sources of phytochemicals and citric acid, respectively which effectively employed in exfoliation because of their remarkable reducing and stabilising properties. Both routes are highly cost effective, environment friendly, effectively exfoliate graphite. In chemical route NaOH gives efficient exfoliation as compared to HCl. By using NaOH d-spacing value has been increased from 3.30 to 3.37 nm. Nano-sheets with average thickness of 14.8 nm has been achieved. In green route, mint leaves has found more effective for exfoliation compared with lemon juice. Using mint extract d-spacing value has been increased from 3.30 to 3.36 nm. Thin sheets with average thickness of 10.79 nm have been obtained. Inclusively graphite
exfoliation using NaOH and mint extract gives excellent result. The higher $I_D/I_G$ ratio for exfoliated graphite using NaOH and mint extract came out to be 0.77 and 0.88 respectively compared with pure graphite confirmed the extent of exfoliation. Exfoliation improves the surface activation of graphitic nanoflakes leading to applications in antibacterial activity. Pure graphite showed minimum activity and exfoliated graphite using NaOH produced moderate activity. The maximum activity was achieved by exfoliated graphite using mint extract.

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