Article

The Biogenic Synthesis of Reduced Graphene Oxide using *Hibiscus sabdariffa L.* as Natural Precursor

Emmellie Laura Albert [1], Eddie Khay Ming Tan [2], Huey Fang Teh [3], Theresa Ng Lee Mei [4] and Che Azurahanim Che Abdullah [1,4,5]*

[1] Institute of Advance Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.; emmellielaura@gmail.com
[2] Technospex Pte Ltd, Block 1092 Lower Delta Road #04-01, 169203, Singapore; eddie@technospex.com
[3] Sime Darby Technology Centre, Lebuh Silikon, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; teh.huey.fang@simedarbyplantation.com; theresa.ng.lee.mei@simedarbyplantation.com
[4] Department of Physics, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; azurahanim@upm.edu.my
[5] UPM-MAKNA Cancer Research Laboratory Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; azurahanim@upm.edu.my
* Correspondence: azurahanim@upm.edu.my; +603-97696775

Abstract: Owing to their extraordinary properties, carbon-based nanomaterials are gaining traction in biomedicine. Green synthesis is the cost-effective method for fabricating carbon-based nanomaterials due to its rapidity, renewable nature, and sustainability. This study emphasis on the graphene oxide (GO) reduction using a simple one-pot technique that does not require the use of toxic reducing agents. This article reports the green synthesis of reduced graphene oxide (RGO) using *Hibiscus sabdariffa L.* calyxes extract as the natural reducing agent. Additionally, this article also provides analysis RGO using X-ray diffraction (XRD), UV-Visible spectroscopy (UV-Vis), and Raman spectroscopy. XRD result showed that the GO peak at 11^o diminished, and a new hump appear at 22^o indicating that the GO is fully reduced when it is refluxed for 6 hours, at 100^{o}C with 1:3 ratio of GO:PE. The UV-Vis data indicated absorption peak of GO (237 nm) and RGO (265 nm) at distinct locations. This finding shed new light on the enormous potential of *Hibiscus sabdariffa L.* calyxes extract for green GO reduction. As a result, this environmentally friendly method can help reduce dependence on chemical materials.

Keywords: Graphene oxide, Green synthesis, Reduced graphene oxide, *Hibiscus sabdariffa L.* calyxes extracts

1. Introduction

Graphene is one of the most sought after nanoparticles and it has been dubbed a 'miracle materials' due to its extraordinary physical and chemical properties [1] . Graphene is a hexagonal crystalline single layer of graphite with a C-C bond with distance of 0.142 nm. It is made up of a tightly bonded carbon allotrope by an individual electronic cloud [2]. Owing to its special electrical, thermal, and mechanical properties, it can be used in a variety of applications, including energy storage [3], catalysis [4], sensors [5], nanoelectronics [6], solar cells [3], biomedicine [7], and functional nanocomposites [8].

Graphene can be synthesized via chemical vapor deposition [9], mechanical exfoliation [10], and annealing single crystal SiC [11]. However, these techniques have a number of disadvantages, including high power consumption, limited output, the use of explosives materials, high manufacturing costs, and instrument restrictions that prevent them from being used on a wide scale. A simple, low-cost, and sustainable method of manufacturing graphene is to oxidise graphite, then exfoliate and reduce it, yielding reduced graphene oxide (RGO).

RGO is GO which had undergo reduction process. It form a graphene-like sheets, and the oxygen functional groups is eliminated, whereas the conjugated form is restored [12]. There are a number of reduction processes for GO, such as chemical [13], electrochemical [14], thermal [15], solvothermal [16], photodeposition, and green [17] techniques. Currently, the common reduction method of GO is from the chemical method which uses a poisonous and toxic substance namely hydrazine hydrate. It is highly volatile and poisonous, rendering it dangerous to be control and used for mass production of RGO. Additionally, if its escape into the disposal waste system, it will create a detrimental environmental issue and also present a health hazard [18–20]. Owing to its harmful nature, the quest for a more environmentally friendly reductant is on demand [1].

Green synthesis is a growing paradigm of graphene which offers economic and environmental advantages over traditional chemical and physical approaches. Nontoxic, environmentally sustainable, and biosafe reagents are used in this process. The aim of green synthesis of nanomaterials is to reduce waste and incorporate sustainable methods. Green processes that use mild reaction conditions and nontoxic precursors have been emphasized in nanotechnology in recent years to promote environmental sustainability. In recent year, various types of plants were used to reduced GO such as Chrysanthemum extract [21], palm leaves [22], *Salvadora persica L.* root (Miswak) [23], Rose [24], Aloe vera [25], and many more.

One of the plants attempted to reduce GO is Roselle (*Hibiscus sabdariffa* L.). *Hibiscus sabdariffa L.* is a Malvaceae flowering plant that can be found in Asia, South Asia, Malaysia, Africa, Australia, and Latin America. It's part of a plant family that includes spices, shrubs, and trees. In several nations, this fleshy, bright red flower is used as a common medicine. The bioactive compounds in plants enabled them to be used as reducing agents in green synthesis. Anthocyanins, L-ascorbic acid, citric acid, and tartaric acid are abundant in its petals that make up the majority of the calyxes extract's constituents [26].

The benefit of drinking *Hibiscus sabdariffa L.* juice is to treat diarrhea, dysentery, hypertension, hypercholesterolemia, and urinary tract infections because of its high phenolic content (mostly anthocyanins), polysaccharides, and organic acids [27]. In addition, numerous studies have discovered that the *Hibiscus sabdariffa L.* calyxes may be used to synthesize nanoparticles such as silver nanoparticles [28] and Platinum nanoparticles [29]. Previous studies attempted to use *Hibiscus sabdariffa L.* s as the reduction agent using the ultrasonication method; however the GO is not fully reduced [30]. Hence, this project aims to investigate the effect of the temperature, time, and ratio of GO to the *Hibiscus sabdariffa L.* calyxes extract (GO:PE) ratio to fully reduced the GO through a safer method that does not require harmful substance.

In this unpredictable and intensely competitive environment, traditional trial-and-error methods are insufficient to address the demands of reducing GO in a timely manner. Hence, this study also investigates the research on the practical application of the Taguchi technique for optimizing the stated parameter. This is due to the practicality and durability of the Taguchi approach which has been widely used in experimental design for issues involving numerous factors. We demonstrate the reducing ability of *Hibiscus sabdariffa L.* extract in the synthesis of RGO in this work. The as-synthesized RGO samples are characterized morphologically, structurally, and elementally

2. Materials and Methods

The green synthesis of RGO only required two main materials such as GO (5 mg/mL) which is purchased from GO advanced and dried *Hibiscus sabdariffa L.* calyxes which were bought from *Hibiscus sabdariffa L.*'s producer in Johor, Malaysia. The dried *Hibiscus sabdariffa L.* calyxes is illustrated in **Figure 1.**

Figure 1. Image of *Hibiscus sabdariffa L.* calyxes

2.1 *Preparation of Hibiscus sabdariffa L. calyxes extract*

Plant extraction was prepared using modified method from previous research [31]. The *Hibiscus sabdariffa L.* calyxes was weighed (5 g) (Mettler Toledo, Switzerland) and mixed with deionized water (DI) (50 mL) which is (10 % w/v). The solution was stirred with a hotplate magnetic stirrer (Cimarec$^+$, ThermoFisher Scientific, USA) set to 60 °C for 1 h. The extract was filter using milk cloth strainer filter followed by filtration through Whatman filter paper (Grade 1; 11μm) to remove any remaining trace solids and stored in refrigerator (8 ^{o}C) until further used.

2.2 *Synthesis of reduced graphene oxide*

The GO was exfoliated using probe sonicator (Cole-Parmer, USA) at 40% of 20 kHz frequency for 1 h . The exfoliated GO was added into *Hibiscus sabdariffa L.* calyxes extract and reflux at different condition. There are 3 variables; time (1, 3, and 6h), temperature (60, 80, and 100^{o}C), and ratio of GO to *Hibiscus sabdariffa L.* calyxes extract (v:v) (1:1, 1:2, and 1:3) as shown in **Table 1**. The parameter was inserted inside Minitab Software (Minitab® version 19.2020.1) following Taguchi method for design of experiment. The Taguchi approach is a versatile modelling methodology that is commonly used in industries because it can increase processing efficiency, reduce the number of trials, reduce processing variance and maintenance, and encourage quality stability [32]. The Taguchi method is a powerful tool of optimization when faced with adversaries such as limited funding, time, and resources. There are 9 different parameters established using Taguchi Method as shown in **Table 1.**

Table 1. Parameter suggested by the Taguchi method.

Temperature (^{o}C)	Time (h)	Ratio GO:PE (w:w)
60	1	1:1
60	3	1:2
60	6	1:3
80	1	1:2
80	3	1:3

80	6	1:1
100	1	1:3
100	3	1:1
100	6	1:2
100	1	1:3
GO	NA	NA

2.3. *Sample Characterization*

The synthesized nanoparticles were characterized using UV-Vis spectroscopy (PerkinElmer, Lambda 35, US), X-ray diffraction (XRD, Smartlab, Rigaku, Japan), and Raman spectroscopy (uRaman Microscope Module, Technospex, Singapore). All sample were ground before it was sent for characterization.

2.3.1. UV-Vis spectrophotometer

The light absorbing and scattering properties of a sample are measured with a UV-Vis spectrophotometer. The sample were dispersed using bath sonicator (Apex Scientific, for 1 h. Then, about 2 mL of the sample dispersion was inserted in the quartz cuvette. The light in the scanning range will be absorbed by the spectrophotometer, between 200 and 500 nm.

2.3.2. X-Ray Diffraction

XRD is a way to analyze crystal structure and atomic spacing. For the preparations, all powdered samples will be placed on a glass plate and exposed to CuKα radiation with a wavelength of 1.5406 nm. They will then be tested at ambient temperatures ranging from 5° to 80° with a steady phase of 0.02° per 4 seconds. The data collected will be processed with the aid of Xpert Software. In addition, the d-spacing was calculated using the online calculator [33].

2.3.3. Raman Spectroscopy

Raman analysis can provide information about the degree of graphitization and defects in the sp^2 carbon structure of the samples. GO and rGO were characterised in this study using a Raman spectroscopy machine equipped with a 532 nm and 785 nm laser. Samples was then put on a normal glass slide, and it is irradiated with a 30 mW, 785 nm laser. Raman signals were acquired at an intergration time of 1 sec over a spectral range of 500-3000 cm^{-1} with smoothing polynomial order 3.

3. Results

3.1. *UV-vis spectroscopy*

GO reduction was first assessed by UV spectra absorption of RGO, as shown in **Figure 2**. The reduction of GO was asses at different temperature, time, and ratio of GO to PE as shown in **Table 1**. The maximum for raw GO is located at 237 nm. The GO did not undergo reduction at 60 ^{o}C for the first 1 h and 3 h of reflux as shown by the small red-shift from 237 nm to 239 nm for both sample with GO:PE ratio at 1:1, and 1:2. As the reflux process increases to 6 h, at 60 ^{o}C there is a weak reduction of GO as can be seen in the red-shifted of the GO peak to 259 nm, when the amount of PE was increase by 1:3 ratio of GO to PE.

As the temperature increases to 80 ^{o}C, there is a partial reduction of GO observed by the slight increase of the lambda max from 237 nm to 245 nm for both the samples undergoing reflux at 1 and 3 h with GO:PE ratio of 1:2, and 1:3 correspondingly. When the reflux period increases to 6 h at 1:1 ratio, the reduction ability slightly increases in the lambda max to 251 nm.

However, as the reflux temperature was set to 100 °C, the reduction of GO improves slightly by the increase in the lambda max to 248 nm at 1 h of reflux process with GO:PE ratio of 1:3. The reflux period was lengthened to 3h and 6h with ratio of GO to PE at 1:1 and 1:2 respectively. It is observed that the lambda max is becoming closer to the lambda max of rGO at 270 nm found in the previous works [34].

At first, the Taguchi variable is only up to 100 °C, 6 h at 1:2, GO:PE ratio however the GO is only partly reduced. Taguchi method is used to know the best temperature, time, and ratio however it can only determine the best temperature and time which are 100 °C and 6 h, as the lambda max is the nearest to 270 nm. The GO:PE ratio is modified to 1:3 at 100 °C for 6 h because the GO is only partly reduced when the GO:PE ratio is 1:2. The lambda max is 265 nm when the GO was reflux at 100 °C, for 6 h at 1:3, GO:PE ratio which is the closest to RGO synthesis from chemical route, suggesting the successful reduction of GO.

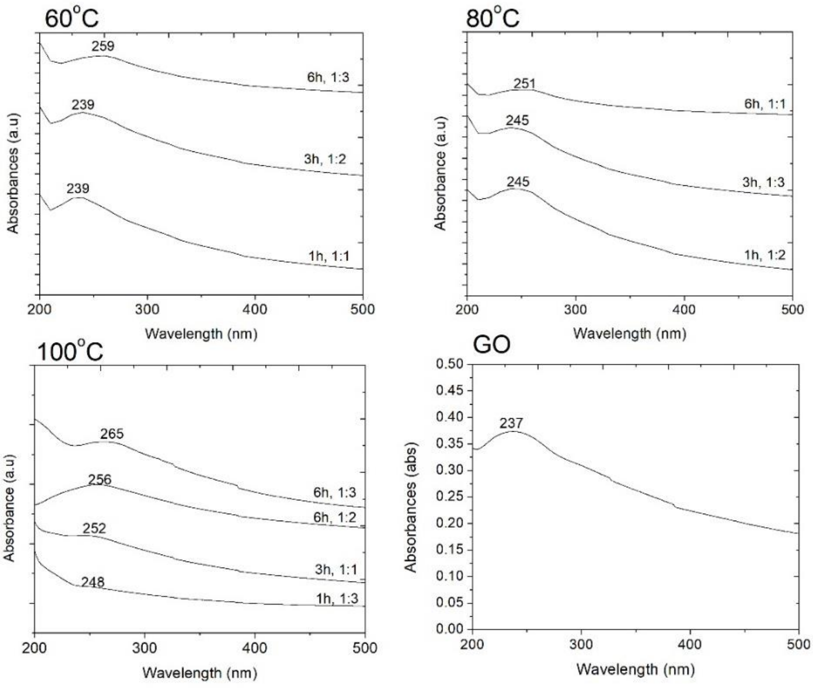

Figure 2. UV spectrum of GO and thermally reduced GO at 60, 80, and 100°C.

3.2. *X-ray diffraction*

The GO and RGO XRD patterns exhibited in **Figure 3** were evaluated for structural information and confirmed removal of the oxygen-contained groups. There is a diffraction peak at about 11 ° for GO, corresponding to an interlayer d-spacing of 0.80 nm. The sharp diffraction peak of exfoliated GO diminished after reduction, and a wide hump at approximately $2\theta = 21^{\circ}$ was detected, suggesting completely reduced GO and the formation of RGO. **Figure 3** shows that the peak intensity reduces drastically, and a new hump develops at around 21 ° which correlate to (002) with 0.40 nm d-spacing. The nearly invisible peak at 11 ° and the subsequent strong peak at around 22 ° suggest that GO is partly reduced to graphene sheet below 100 °C. However, when the temperature increases to 100 °C, the GO sheet is further reduced as indicated by the disappearance of the peak at 11 ° and the wide peak at 22° remained. This is an indicative that the GO has been fully reduced as compared to the previous reported studies which only manages to partly reduces the GO using the same type of plant [30].

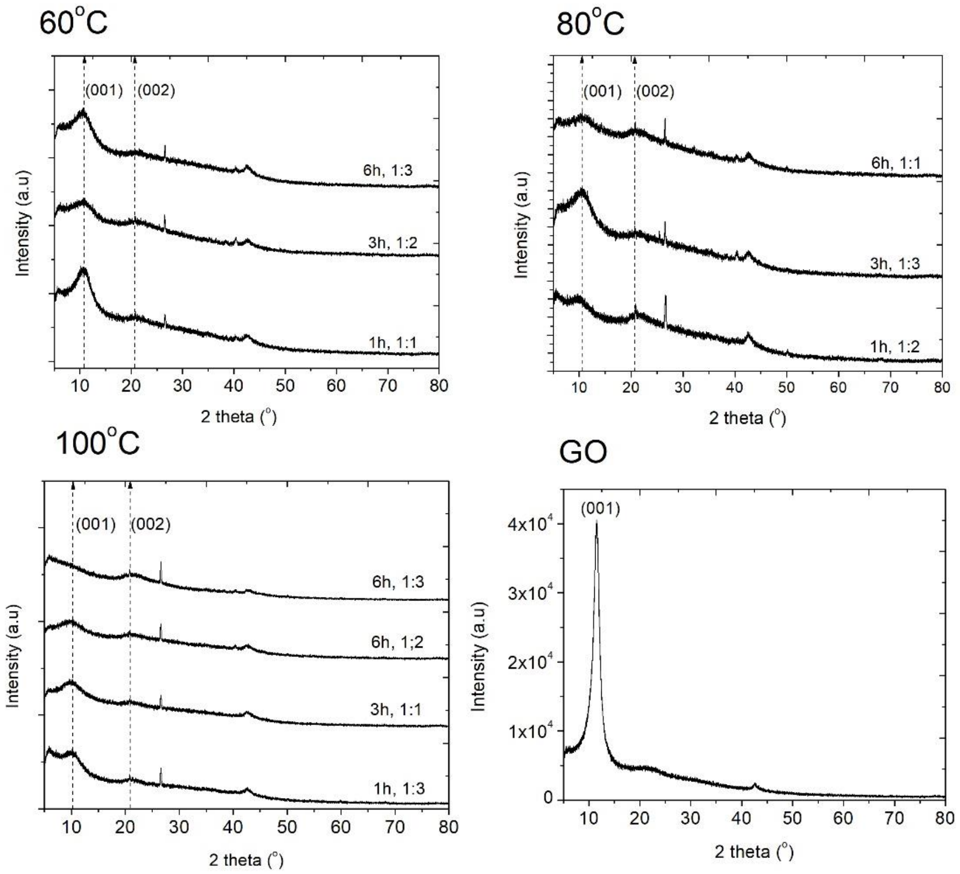

Figure 3. XRD spectra of GO and thermally reduced GO at 60, 80, and 100^{o}C.

3.3. *Raman spectroscopy*

The graphene structure is determined via Raman spectroscopy. Generally, two fundamental peaks observed at frequencies less than 2000 cm^{-1} namely the D peaks (around 1300 cm^{-1}) and the G peaks (around 1500 cm^{-1}) [35]. The G band is often associated with the E2g phonon of C sp^{2} atoms, which is the in-phase the graphite lattice vibration, and it indicates the graphitization degree. The graphite edges yield the D band, which is a breathing mode for K-point phonons with A1g symmetry. Its intensity is proportional to the edge's degree of chirality. The ratio of the intensities of the D and G bands (ID/IG) demonstrates the extent of disorder, such as flaws, ripples, and edges. Additionally, it can be used to determine the size of the sp^{2} domain in a carbon structure comprising of sp^{3} and sp^{2} bonds.

The Raman spectra of GO had a wide G peak at 1593 cm^{-1}, correlating to lattice of graphite oxide in-phase vibration and a D band at 1314 cm^{-1}, as seen in **Figure 4**. **Table 2** is the ID/IG ratio of GO and the thermal reduced graphene oxide at different temperature (60, 80, and 100 ^{o}C). According to **Table 2**, GO is fully reduced at 100 ^{o}C reflux at 6 h, using 1:3 ratio of PE and GO, resulted in the increased ID/IG ratio of 1.355, which was substantially greater than GO (1.145) and the rest of the samples. The ID/IG intensity ratio increases with the treatment period during reduction process due to the restoration of the sp^{2} network. Furthermore, the graphene sheet disordered increased, as evidenced by the increased in the RGO intensity ratio, and the in-plane sp2 domain size shrank following reduction.

Table 2. ID/IG ratio of GO and thermally reduced GO at 60, 80, and 100 ^{o}C.

Samples	ID/IG

60oC, 1h, 1:1	1.196
60oC, 3h, 1:2	1.273
60oC, 6h, 1:3	1.327
80oC, 1h, 1:2	1.142
80oC, 3h, 1:3	1.251
80oC, 6h, 1:1	1.207
100oC, 1h, 1:3	1.331
100oC, 3h, 1:1	1.286
100oC, 6h, 1:2	1.351
100oC, 6h, 1:3	1.355
GO	1.145

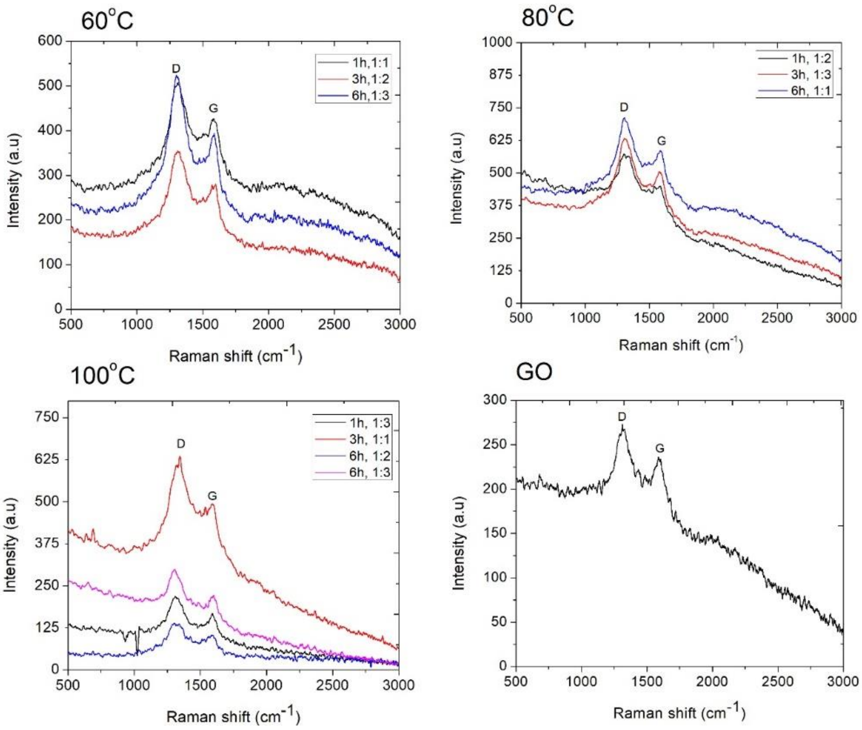

Figure 4. Raman spectra of GO and thermally reduced graphene oxide at 60, 80, and 100oC

4. Discussion

Extensive research is being conducted on the biosynthesis of inorganic nanoparticles utilising plants and their derivatives. The bio-reduction of nanoparticles is due to the presence of a variety of bioactive molecules such as amino acids, aldehydes, phenols, carboxylic acids, ketones, and nitrogen-containing chemicals that help to reduce, cap, or stabilize the NPs during their production [29].

For instance, Kaffir lime extract (citrus hystrix) [36], Chrysanthemum extract [21], Lotus Garcinii leaf extract [37] and many more were employed in the synthesis of RGO. Chrysanthemum extract includes flavonoids such as diosmetin, luteolin, apigenin, and glucoside, all of which have a strong antioxidant action. When heated in the presence of GO solution at 95 ^{o}C, flavonoids are prone to be oxidized by the oxygen-containing functional groups on the GO sheets into benzoquinone-type compounds, releasing hydrogen ions in the process. Additionally, hydrogen ions can function as catalysts in the dehydration of GO, leads to water molecules and sp2 bonded RGO [21].

Nevertheless, research on RGO biosynthesis is relatively scarce. As a result, researchers are continuously investigating innovative methods for quickly fabricating RGO from various plant components such as, root, calyxes, bark, leaf, fruit, or by-products such as gum. The production of RGO, like that of other inorganic nanoparticles, is aided by bioactive chemicals found in plants. As a result, biosynthesized RGO reduction and stabilization processes must be well understood.

Hibiscus sabdariffa L. 's fleshy red calyxes are frequently utilized in the creation of non-alcoholic soft beverages and tonics such as wine, juice, jam, jelly, and syrup, as well as dried and boiled into spice and tea. It is abundant in riboflavin, carotene, ascorbic acid, calcium, niacin, vitamin C, and anthocyanins. Past studies had discovered that the dehydrated *Hibiscus sabdariffa L.* calyces had a total polyphenol content (TPC) of approximately 683.13 mg which is gallic acid equivalent (GAE)/100 g while the total anthocyanin content (TAC) is 361.99 mg CGE/100 g. Thus, *Hibiscus sabdariffa L.* is an excellent source of anthocyanins [38].

Anthocyanins are a type of flavonoid found in polyphenols that has strong antioxidant and free radical scavenging activities. Previous research, discover that the anthocyanins diminished after the reduction of GO, indicating that the *Hibiscus sabdariffa L.* ’s anthocyanins were used up in the reduction process. Although the substitute groups were engaged in the reduction, it does not cause any ring-opening reaction. Due to the electron removal of the ring structure, hydroxyl group anthocyanins became acidic. The H^+ ions were easily dissociated from the anthocyanins, and the linkages were open that might chelate with metal ions. Before the reduction process, flavonoid is presence however during the reduction, the phenolic group are consumed. The phenolic groups in the anthocyanins in the aqueous extract interacted with GO, but the ring structures in the anthocyanins did not [30].

In this project, the ratio of GO:PE investigated are 1:1, 1:2, and 1:3. The amount of *Hibiscus sabdariffa L.* calyxes extract must be three times the amount of GO in order to fully reduced the GO. The amount of anthocyanins are expected to increased when the amount of *Hibiscus sabdariffa L.* calyxes increase, hence improving the reduction of GO. Furthermore, as shown by the XRD data, poor reduction environment was obtained when the temperature is set lower than 100^{o}C or a shorter reduction period of 1 and 3 h was used. Hence, the time taken to achieved full reduction process via *Hibiscus sabdariffa L.* calyxes extract must be 6h. **Figure 5** showed proposed mechanism for the reduction of graphene oxide.

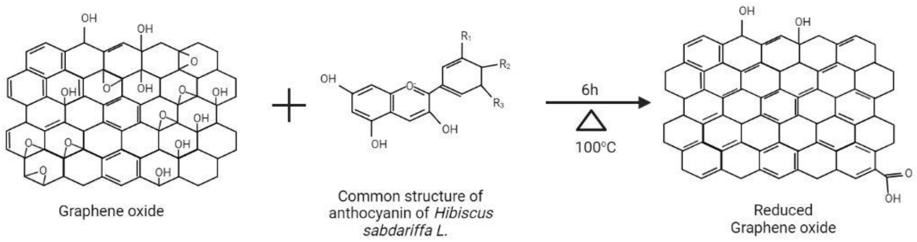

Figure 5. Proposed schematic mechanism of rGO using *Hibiscus sabdariffa L.* calyxes extract.

5. Conclusions

In conclusion, RGO were successfully synthesized by using *Hibiscus sabdariffa L.* calyxes extract. The primary benefits of our environmentally friendly green RGO reduction procedure are that it is non-toxic, efficient, simple, and cost-effective. Data from XRD, UV-vis, and Raman confirmed the successfully reduction process. XRD verifies that the RGO is formed throughout the synthesis process since the peak at 11^{o} vanished and only the broad hump at 22^{o} remained hence verified that the GO had been completely reduced. Besides that, the XRD spectrum indicates the loss of functional groups in GO decreases the distance between the RGO layers, resulting in a decrease in d spacing. Raman spectroscopy confirmed the deoxygenation of the RGO and the formation of abnormalities. Additionally, given that the ID/IG intensity ratio is inversely proportional to the average size of the sp2 domains, a rise in the ID/IG intensity ratio implies the formation of smaller in-plane sp2 domains demonstrating an effective decrease of the size for the RGO sp2 domains. As a result, this method represents a viable alternative to conventional chemical reduction methods for avoiding the use of hazardous chemicals while also meeting the future demand for graphene. All of the results demonstrated the efficacy of using *Hibiscus sabdariffa L.* calyxes extract as a substitute reducing agent in place of chemical agents in the preparation. These distinctive properties of GO, and RGO may open up new opportunities for meeting requirements in a variety of biological applications.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Conflicts of Interest: Declare conflicts of interest or state “The authors declare no conflict of interest.” Authors must identify and declare any personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results. Any role of the funders in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript, or in the decision to publish the results must be declared in this section. If there is no role, please state “The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results”.

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