PHYTOCHEMICALS, ANTIOXIDANT STUDIES OF *Deinbollia pinnata* (Poir) Schumach. & Thonn. AND ITS USES IN THE SYNTHESIS OF TITANIA NANOPARTICLES

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DEDICATION

This Thesis is Dedicated to Humankind.

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Al-hamdu lilah. Certainly, all praise is due to Allah. We praise Him and seek His help and forgiveness. We seek protection with Allah from our souls' evils and our wrong conducts, He whom Allah guide, no one can misguide; and he whom He misguides, no one can guide.

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ABSTRACT

Sapindaceae is a tropical and sub-tropical continental plant. Phytochemical studies on *D. pinnata*, its antioxidant activity and application in the photodegradation of methyl orange dye were conducted. The sonication/agito-sonication method was used for leaves and root bark samples. Optimal sonication conditions were obtained at a temperature of 45°C, a time of 35 mins and a solvent ratio MeOH:H_2O (1:1) for a yield of 83.01%. Fractionation and purification on the extracts of the aerial parts and root bark using chromatographic methods produced eighteen compounds. The compounds were identified spectroscopically and compared with literature data. These compounds include pyrogallol**,** methyl gallate, ethyl gallate, methyl-β-*D*-glucopyranoside, squalene, γ-sitosterol, lupeol, stigmasterol, taraxasterol and stigmasta-5,22-diene-3-ol acetate, ethyl oleate, ethyl stearate, stearic acid, palmitic acid, myristic acid, phytyl palmitate, and 4,8,12,16-tetramethylheptadecan-4-olide. Alkaloids, sugars, phenolics, tannins and steroids were present in all the plant parts. Saponins, terpenes and flavonoids were found in leaves and root bark, while root bark and heartwood possessed quinones. The methanol extracts demonstrated total phenolic content at 75.93 ± 2.05 GAE (mg GAE/g dw) and total flavonoid content at 88.41 ± 0.56 QUE (mg QUE/g dw). The extracts showed good DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity with a percentage inhibition of more than 70% at 125 ppm. The methanol extracts showed excellent antioxidant activity towards (ABTS) (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) assay with SC_{50} value of 13.68 µg/mL. The methanolic extracts also exhibited an equivalent FRAP (Ferric reducing antioxidant power) value of 3.45 ± 1.30 mM. Methyl gallate displayed the highest antioxidant radical scavenging activity towards DPPH assay with an SC_{50} value of 0.19 µg/mL and pyrogallol showed the highest ABTS activity with an SC_{50} value of 7.44 µg/mL. Ethyl gallate showed good potential as a ferric ion reducer from 0.69 ± 0.00 to 3.22 ± 0.03 mM FRAP, respectively. The TiO_2 NPs was prepared using *D. pinnata* leaves extracts of different polarities. X-ray diffraction (XRD) pattern confirmed the synthesized TiO_2 consisted of anatase phase in high purity, with average crystallite size in the range of 19 - 21 nm. Characterization using field emission scanning electron microscopy (FESEM) showed uniform semi-spherical shape in the size range of 33 - 48 nm. The energy dispersive X-ray (EDX) spectrum shows two peaks for the main elements of Ti (61 wt.%) and O (35 wt.%). The 3.2 eV band-gap energy was determined using UV-Vis spectroscopy. Nitrogen sorption analysis showed that the type V isotherm was obtained, with a Brunauer-Emmett-Teller (BET) surface area of 31.77 m^2/g. The photocatalytic activity of TiO_2 NPs was evaluated for methyl orange (MO) photodegradation under UV light irradiation. TiO_2 NPs synthesized with ethyl acetate extract of *D. pinnata* leaves showed the most effective photodegradation performance, achieving 98.7% of MO conversion within 150 mins. The application of plant extracts using TiO_2 successfully produced crystalline TiO_2 anatase with superior photocatalytic activity in the process of photodegradation of organic dyes.

ABSTRAK

Sapindaceae merupakan tanaman benua tropika dan sub-tropika. Kajian fitokimia ke atas *D. pinnata*, aktiviti antioksidan dan aplikasinya dalam fotodegradasi pewarna metil jingga telah dijalankan. Kaedah sonikasi/agito-sonikasi digunakan bagi sampel daun dan kulit akar. Keadaan optimum sonikasi diperoleh pada suhu 45°C, masa 35 min dan nisbah pelarut MeOH:H_2O (1:1) untuk berat hasil 83.01%. Pemeringkatan dan penulenan ke atas ekstrak keseluruhan bahagian dan ekstrak kulit akar menggunakan kaedah kromatografi menghasilkan lapan belas sebatian. Sebatian tersebut dikenal pasti secara spektroskopi dan dibandingkan dengan data literatur. Sebatian ini merangkumi pirogallol, metil gallat, etil gallat, metil-β-*D*-glukopiranosida, skualena, γ-sitosterol, lupeol, stigmasterol, taraksasterol, dan stigmasta-5,22-dien-3-ol asetat, etil oleat, etil stearat, asid stearik, asid palmitik, asid miristik, fitil palmitat dan 4,8,12,16-tetrametilheptadekan-4-olid. Alkaloid, gula, fenolik, tanin dan steroid hadir di dalam semua bahagian pokok. Saponin, terpena dan flavonoid ditemui pada bahagian daun dan kulit akar, sementara kulit akar dan kayu mengandungi quinona. Ekstrak metanol menunjukkan jumlah kandungan fenolik pada 75.93 ± 2.05 GAE (mg GAE/gdw) dan jumlah kandungan flavonoid pada 88.41 ± 0.56 QUE (mg QUE/g dw). Ekstrak menunjukkan aktiviti pengautan DPPH (2,2-difenil-1-pikrilhidrazil) dengan peratus perencatan lebih daripada 70% pada 125 ppm. Ekstrak metanol menunjukkan aktiviti antioksidan yang baik terhadap ujian ABTS (asid 2,2'-azino-bis-3-etilbenztiazolin-6-sulfonik) dengan nilai SC_{50} 13.68 µg/mL. Ekstrak metanol juga mempamerkan nilai setara FRAP (keupayaan antioksidan penurunan ferik) 3.45 ± 1.30 mM. Metil gallat menunjukkan aktiviti pengumpulan radikal antioksidan tertinggi terhadap DPPH dengan nilai SC_{50} 0.19 µg/mL dan pirogallol menunjukkan aktiviti ABTS tertinggi dengan nilai SC_{50} 7.44 µg/mL. Etil gallat menunjukkan potensi yang baik sebagai penurun ion ferrik masing-masing antara 0.69 ± 0.00 hingga 3.22 ± 0.03 mM FRAP. TiO_2 NP disediakan menggunakan ekstrak-ekstrak daun *D. pinnata* dengan kekutuban yang berbeza. Corak pembelauan sinar-X (XRD) mengesahkan TiO_2 yang disintesis terdiri daripada fasa anatase berketulenan tinggi dengan ukuran kristal dalam julat 19 - 21 nm. Pencirian menggunakan mikroskopi elektron pengimbas pemancaran medan (FESEM) menunjukkan bentuk separa sfera yang seragam dalam julat ukuran 33 - 48 nm. Spektrum serakan tenaga sinar-X (EDX) menunjukkan dua puncak bagi unsur utama Ti (61 wt.%) dan O (35 wt.%). Tenaga luang jalur 3.2 eV ditentukan menggunakan spektroskopi UV-Vis. Analisis erapan nitrogen menunjukkan isoterma jenis V diperolehi dengan luas permukaan Brunauer-Emmett-Teller (BET) 31.77 m^2/g. Aktiviti fotopemangkinan TiO_2 telah dinilai untuk fotodegradasi metil jingga (MO) di bawah penyinaran cahaya UV. TiO_2 NP yang disintesis dengan ekstrak etil asetat daun *D. pinnata* telah menunjukkan prestasi fotodegradasi yang paling berkesan, mencapai 98.7% penukaran MO dalam 150 min. Penggunaan ekstrak tumbuhan menggunakan TiO_2 berjaya menghasilkan TiO_2 anatase kristal dengan aktiviti pemangkinan foto yang unggul dalam proses fotodegradasi pewarna organik.

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LIST OF ABBREVIATIONS

1D	-	One Dimensional
2D	-	Two Dimensional
ABTS	-	2,2'-azinobis(3-ethyl-benzothiazoline-6-sulfonic) Acid
A.A	-	Ascorbic Acid
Ace	-	Acetone
BBD	-	Box–Behnken Design
BET	-	Brunauer-Emmett-Teller
BHA	-	Butylated Hydroxyl Anisole
BHT		Butylated Hydroxyl Toluene
BJH		Barrett-Joyner-Halenda
C		Carbon
CC		Column Chromatography
CoA		Coenzyme A
DB		Data Base
DDT		Dichloro Diethyl Trichloroethane
DF		Degree of Freedom
DI		Deionized Water
D.P.		*Deinbollia pinnata*
DPPH		2, 2’- Diphenyl-2-Picrylhydrazyl
DTA		Differential Thermal Analysis
DPLH		*D. pinnata* Leaves Hexane Extracts
DPLEA		*D. pinnata* Leaves Ethyl Acetate Extracts
DPLMT		*D. pinnata* Leaves Methanol Extracts
DPRBEA		*D. pinnata* Root Bark Ethyl Acetate Extracts
DPRBMT		*D. pinnata* Root Bark Methanol Extracts
EDX		Energy Dispersive X-Ray
ESI		Electrospray Ionization
FESEM		Field Emission Scanning Electron Microscopy
FRAP		Ferric Reducing Antioxidant Potential
FTIR		Fourier Transform Infrared Spectroscopy

GAE	Gallic Acid Equivalents
GA	Gallic Acid
GC	Gas Chromatography
Glc	Glucopyranoside
HIV	Human Immunodeficiency Virus
IR	Infrared Spectroscopy
LC	Liquid Chromatography
LDL	Low-Density Lipoprotein
MA	Macroscopic Abnormalities
MIC	Minimum Inhibitory Concentration
MO	Methyl Orange
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance
TLC	Thin Layer Chromatography
UV	Ultraviolet
VLC	Vacuum Liquid Chromatography

LIST OF SYMBOLS

α	-	Alpha
Å	-	Angstrom
β	-	Beta
2θ	-	Braggs Angle
γ	-	Gamma
δ	-	Chemical Shift
δ_H	-	Chemical Shift for Proton
δ_C	-	Chemical Shift for Carbon
J	-	Coupling Constant
d	-	Doublet
IC_{50}	-	Inhibition Concentration at 50%
kg	-	Kilogram
H_Z	-	Hertz
dd	-	Doublet of Doublets
%	-	Percentage
%I	-	Percentage Inhibition
%D	-	Percent of Degradation
cm^{-1}	-	Per Centimeter
Eg	-	Band Gap Energy
eV	-	Electrovolt
h^+	-	Positive Hole
h	-	Hour
kV	-	Kilovolt
m/z	-	Mass to Charge
m	-	Multiplet
M^+	-	Molecular Ion
µL	-	Microliter
µm	-	Micrometer
mA	-	Milliampere
mg	-	Milligram

min	-	Minute
mL	-	Milliliter
mm	-	Millimeter
mp	-	Melting Point
nm	-	Nanometer
psi	-	Pound Per Square Inch
ppm	-	Part Per Million
R_f	-	Retention Time
s	-	Singlet
SiO_2	-	Silica Gel
t	-	Triplet
λ	-	Wavelength

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Plants have provided a source of inspiration for novel drug compounds as plant-derived (Walker, 2012), medicines have made large contributions to human health and wellbeing. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world like Africa and Asia (Chikezie *et al*., 2015). Traditional herbal medicine is often used side by side with modern medicine, while herbal medicine has taken the upper hand when the cost of modern medicine is beyond reach (Busia, 2005).

Herbal plants and their preparations have been reported for antimicrobial, antimalarial, anti-inflammatory, antidiabetic, anthelmintic, antiparasitic, anti-obesity, anticancer, and antiviral activities (Shakya, 2016) Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently in all parts of the world (Lalitha *et al*., 2012). They are naturally synthesized in all parts of the plant body which include barks, leaves, stems, roots, heartwoods, flowers, fruits, and seeds. They have been in use which continues today as medicines.

Extraction techniques are widely accepted as a prerequisite for analytical determination of both organic and inorganic analytes in a small variety of samples. Response surface methodology (RSM), originally described by Box and Wilson (Giacometti *et al*., 2018), enables evaluation of the effects of several processing variables and their interactions on responses. Thus, RSM is a collection of statistical and mathematical techniques that has been successfully used for developing, improving and optimizing processes (Lucas, 2017). Many factors such as solvent ratio or composition, extraction time, extraction temperature (Soraya *et al*., 2017), solvent to solid ratio (Le *et al*., 2019) and extraction pressure (Cace & Mazza, 2002) among others, may significantly influence the extraction efficacy. Thus, Box-Behnken design was chosen in this work to optimize high yield. Purification of phytochemical constituents in plant sample account for isolation (Bucar *et al.,* 2013) of pure compounds.

The nature of nanostructured materials has prompted researchers' attention in the field of natural products. Their naturally occurring oxide of titanium (IV) oxide or titania with the chemical formula (TiO_2) is of unique optical and electronic properties (Ahmadu *et al*., 2017). The surface chemistry of nanostructures in terms of smaller size and uniqueness have already been benefited in medicine, nutrition, energy (Agarwal *et al*., 2018; Thang *et al*., 2018) and in the biosynthesis of TiO_2 NPs with metabolites present in plants extract to reduces and stabilizes bulk metal into elemental forms (Mohamad *et al*., 2014). Plants are a major segment of biodiversity affected by many living and non-living components that haven medicinal values, due to the presence of certain chemicals/active ingredients used for the treatment of disorders, with low cost, eco-friendly, easy availability in the forest area (Dk, 2015). Stable biological nanoparticles are achieved with appropriate choice of solvents polarity for plant extraction (reducing agents) and as toxic-free material (Abdul Jalill et al., 2016). Fungi, actinomycetes, bacteria and plant extracts (Pantidos, 2014) have replaced the chemical agents (O. L. Kang *et al*., 2016) to green synthesis. This could enhance their photocatalytic and pharmacological properties. Bio-mediated TiO_2 NPs have also been obtained from *Morinda citrifolia L*. (Sundrarajan *et al.,* 2017), *Tamarindus indica* (Hiremath *et al.,* 2018)*;*and *Azadirachta indica* (Thakur *et al.,* 2019) extracts etc.

1.2 Sapindaceae Family

The Sapindaceae (soapberry family) is a predominantly tropical trees, shrubs, lianas and vines (Acevedo et al., 2010), with few species extending to temperate zones with about 140-150 genera and 1400-2000 species worldwide (T.O. Adeyemi *et al*., 2013). The majority of species are native in Asia, although there are a few in South America, Africa and Australia (Temitope Olabisi Adeyemi & Ogundipe, 2014). They occur in temperate to tropical region through the world with about 18 to 26 genera in west tropical Africa and 13 to 18 species in Nigeria (T.O. Onuminya & Ogundipe, 2016). Sapindaceae are recognizable by their often spiral, alternate, simple, or more commonly pinnately compound leaves that often have sub-opposite leaflets and a terminal rachis tip. Most often they are pollinated birds or insects, with a few species pollinated by wind (Temitope Olabisi Onuminya & Ogundipe, 2014). However, a total of one hundred and four taxa were identified belonging to twenty six genera with their percentage abundant following Muller and Leenhouts' method listed; *Allophylus* (15.1%), *Aporrhiza* (2.8%), *Blighia* (2.8%), *Cardiospermum* (2.8%), *Chytranthus* (9.4%), *Deinbollia* (11.3%), *Dodonaea* (0.9%), *Eriocoelum* (5.7%), *Ganophyllum* (0.9%), *Glenniea* (0.9%), *Haplocoelum* (0.9%), *Harpullia* (0.9%), *Laccodiscus* (1.9%), *Lecaniodiscus* (1.9%), *Lepisanthes* (0.9%), *Lychnodiscus* (3.8%), *Majidea* (0.9%), *Melicoccus* (0.9%), *Nephelium* (0.9%), *Pancovia* (9.4%), *Paullinia* (0.9%), *Placodiscus* (14.2%), *Radlkofera* (3.8%), *Sapindus* (1.9%), *Schleichera* (0.9%) and *Zanha* (0.9%) (T.O. Adeyemi et al., 2013; Takhtajan, 1980). Members of this family are widely reported for pharmacological activities (Begumand & Kalaiarasan, 2016; Chinnaveeramani Veeramani & Pugalendi, 2010; Simpson *et al.*, 2010). Ethnobotanical reports indicated that plants in Sapindaceae family are used for treating ulcer, boils, pain, dermatological problems, wound healing, diarrhea and dysentery (Aliyu Adebayo Lasisi *et al.*, 2016; Sotubo *et al.*, 2016).

1.3 Genus *Deinbollia*

Deinbollia is a major group of *Angiosperms* (flowering plants). *Genus Deinbollia* are usually dioecious trees or shrubs; leaves paripinnate with leaflets in 2-11 pairs and the fruits contain cocci with subspherial indehiscent (Cheek & Etuge, 2009). *D. pinnata* reaches a height of 11 m with compound leaves 12 cm long and has tomentose, orange, sub globose drupe-like fruits, which are between 1.3 - 1.5 cm in diameter (Onana, 2010). Roots and leaves of *D. pinnata* extracts are used in folk medicine as remedy for febrifuge, analgesic, bronchiasis intercostals, intestinal pains, jaundice, cough, asthma, infections (Borokini.T.I *et al*., 2013; Fatokun *et al*., 2016; A A Lasisi *et al*., 2016) and foetus positioning during child birth (Borokini.T.I *et al*., 2013). *Deinbollia* species are among the non-popular plants in African traditional medicine. However, it has been in used by hunters and aged traditionalist for the treatment of rare diseases that form basis for further research to determine their unique phytochemicals, pharmacological activities and application. The plant and their parts were shown in **Figure 1.1** and **Figure 1.2**

Scientific Classification of *D. pinnata* are as follows:

Family:	Sapindaceae
Genus:	*Deinbollia*
Species:	*pinnata*

Figure 1.1: *Deinbollia pinnata* (Poir) Schumach. & Thonn. Plant

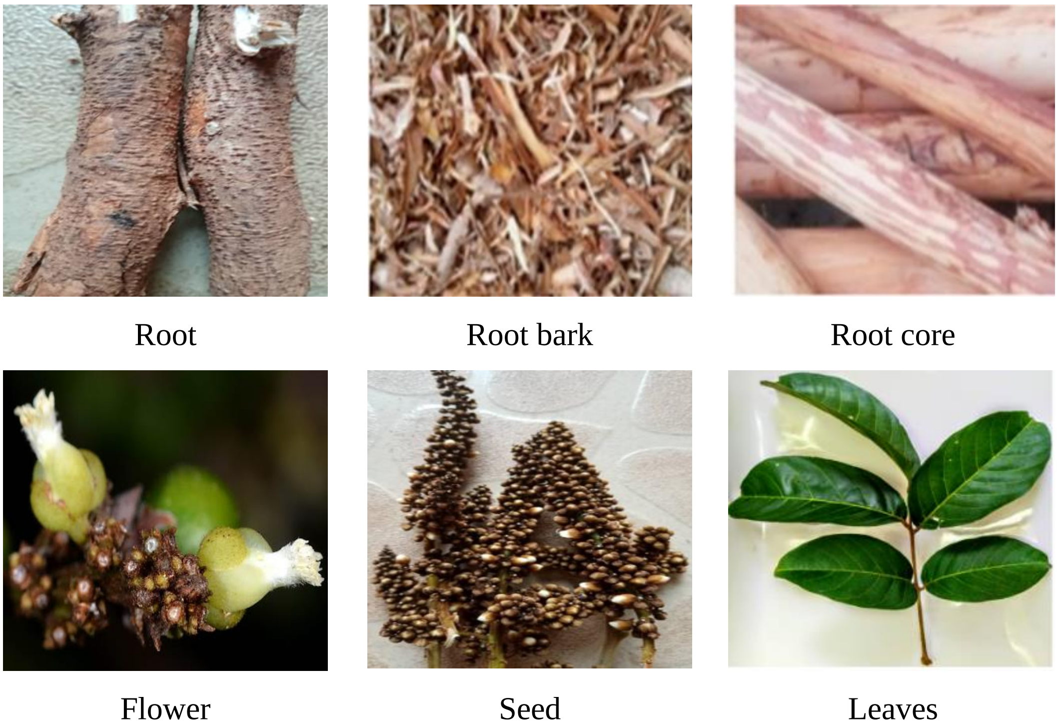

Figure 1.2: *Deinbollia pinnata* (Poir) Schumach. & Thonn. Plant Parts

1.4 Problem Statement

To date, *D. pinnata* species belonging to Sapindaceae family has been neglected with little attention been given to its phytochemicals, biological activities and their applications. Very little of secondary metabolites, or small molecular weight constituents have been reported with respect to their isolation, elucidation of compounds and their extracts used in the field of green synthesis of nanoparticles with applications. Thus, it is possible that *D. pinnata* will become extinct before their phytochemicals and various biological benefits is investigated and documented. These medicinal uses highlighted necessitate to further research on the phytochemicals, bioactivity, and its application of extracts from *D. pinnata* leaves and root bark.

The extraction of bioactive phytochemicals from plant matrices using conventional techniques such as soxhlet (heat) and maceration (cold) methods has been practiced for decades. As such, the need to extract leaves and root bark of *D. pinnata* with solvents of different polarities using extraction methods with best optimization conditions became necessary. Thus, ultrasound irradiation from high-intensity ultrasonic processors opens another door to new perspectives concerning bulk analytes research.

1.5 Significance of the Research

Plant is known to have been existed before the creation of mankind as their main sources of food, shelter, contribute to human health and wellbeing. Although synthetic drugs developments tend to offer great promise with respect to human medications. Their side effect led to the appearance of new health challenges such as allergies, cancer, heart disease, and hepatitis. However, chemist and pharmacist have prepared compounds from various plant with pharmaceutical activity to treat these incurable diseases, but sometimes failed to yield appraisable result. Thus, it became noteworthy to state their ethnomedicinal uses, extraction of various parts, phytochemical analysis, isolation of phytochemicals and green nanoparticles synthesis from *D. pinnata* plant which will contribute substantially to alternative potent medicine and their industrial application.

1.6 Research Objectives

The objectives of this study are:

1. To extract the leaves and root bark of *D. pinnata* with solvents of different polarities using sonication methods with best optimization conditions.
2. To isolate, identify and elucidate structures of pure compounds by chromatographic and spectroscopic techniques and evaluate antioxidant activities for some pure phytochemicals along with the extracts.

3. To synthesize TiO_2 NPs from *n*-hexane, ethyl acetate and methanol extracts in the form of powder and characterize the best with crystal phase, crystallinity and peak sharpness.

4. To evaluate photocatalytic activity of the selected TiO_2 NPs synthesized for photodegradation of methyl orange dye.

1.7 Scope of Study

This study investigates the phytochemicals in leaves, root bark and root core of *D. pinnata* plants. The air-dried powdered samples were extracted with *n*-hexane, ethyl acetate and methanol in order of increasing polarity ultrasonic assisted extraction conditions which include extraction time, temperature and solvent to sample ratio; for high extraction yield using RSM software design. The crude extracts were fractionated and purified by chromatographic techniques such as vacuum liquid chromatography (VLC) and column chromatography (CC). The structures of isolated compounds were elucidated using combined spectroscopic methods which include, 1D NMR, IR, EIMS, and UV. Both crude extract and pure compounds were subjected to antioxidant activity test. The various extracts (*n*-hexane, ethyl acetate, and methanol) were further subjected to green synthesis of TiO_2 nanoparticles with titanium isopropoxide. The physiochemical properties of synthesized TiO_2 NPs were analysed by Fourier transform infrared (FTIR) spectroscopy for the determination of functional groups, field emission scanning electron microscopy (FESEM) for morphology studies, energy dispersive X-ray (EDX) for the sample elemental composition analysis, X-ray diffraction (XRD) for crystallinity studies, N_2 adsorption-desorption analysis which include (Brunauer-Emmett-Teller BET) and pore size distribution (BJT) for the porosity and surface area determination, photoluminescence (PL) for the quality of surfaces and interfaces, UV- Vis spectroscopy for the optical study and the calculation of band gap energy, TGA and DSC for decomposition temperature/ heat flow. The TiO_2 NPs were calcinated at 500°C and applied to methyl orange dye for evaluating its percentage photodegradation performance under UV-light irradiation.

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LIST OF PUBLICATIONS AND CONFERENCES

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