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Theme

Study of the biological activities of Novel green and red clay nanoparticles synthesized by *Aristolochia longa* aqueous extract

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Abstract

The term "clay" may refer to a clayey substance used in various industries, which mainly consists of silica and aluminum minerals. Clay materials are known for their unique properties, such as the ability to absorb and retain water, and the bonding between particles to form a fine structure. The aim of this study is to use aqueous extract of *A. Longa* to prepare red and green clay nanoparticles and investigate their antioxidant and anti-inflammatory properties. Phytochemical screening of *A. longa* aqueous extract showed the presence polyphenols, flavonoids, tannins, carbohydrates, and saponins. Total phenolic and flavonoids content in *A. longa* roots was found to be 31.274± 0.440 mg GAE/g 1.6647 ±0.0966 mg QE/g. The nanoparticles were characterized using various analytical techniques including UV-Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), scanning electron microscope (SEM), Energy Dispersive X-Ray Analysis (EDX), light Microscope and X-ray diffraction (XDR). The antioxidant activity of *A. Longa* and red and green clay nanoparticles was evaluated using the FRAP assay, while anti-inflammatory and hemolysis assays were also performed. The obtained results show that the minerals that make up the clay were transformed into nano-sized less than 170 nm in irregular crystalline shape and that the main minerals were silica carbon, calcium and magnesium. Results of Antioxidant activity show that both *A. Longa* and red and green clay were exhibited reducing capacity, with IC50 values 0.563, 3.012 and 1.44 mg/mL, respectively. Furthermore, both elements demonstrated an anti-inflammatory ability, with IC50 values 0.034, 0.0374 and 0.0015 mg/mL, respectively. In addition, the study indicated the photocatalytic and sonocatalytic efficiency of red and green clay nanoparticles against methylene blue (MB), achieving degradation efficiencies of (93.14% and 87.13%) (84.47% and 49.32%), respectively, under UV light irradiation and without. In conclusion, This study suggested a the phytosynthesis of the clay nanoparticles have a potential antioxidant and anti-inflammatory activities and high catalytic activity in photonic and sonotic conditions which indicate that our new products can be used in pharmaceutic and environmental field.

Key words:

Aristolochia longa, Nanoparticle, Green clay, Red clay, Characterization, Biological activities, Catalytic activity.

دراسة الأنشطة البيولوجية للجسيمات النانوية الجديدة من الطين الأخضر والأحمر التي تم تصنيعها بواسطة المستخلص المائي لل*Aristolochia longa*

الملخص

قد يشير مصطلح "الطين" إلى مادة طينية تستخدم في صناعات مختلفة ، والتي تتكون أساسًا من معادن السيليكا والألمنيوم. تُعرف مواد الصلصال بخصائصها الفريدة ، مثل القدرة على امتصاص الماء والاحتفاظ به ، والترابط بين الجزيئات لتشكيل بنية دقيقة. الهدف من هذه الدراسة هو استخدام المستخلص المائي لعنصر *A. Longa* لتحضير جزيئات الطين الأحمر والأخضر النانوية والتحقيق في خصائصها المضادة للأكسدة والمضادة للالتهابات. أظهر الفحص الكيميائي النباتي لمستخلص *A. longa* المائي وجود البوليفينول والفلافونويد والعفص والكربوهيدرات والصابونين. تم العثور على إجمالي محتوى الفينول والفلافونويد في جذور *A. longa* 31.274 ± 0.440 مجم GAE / جم 1.6647 ± 0.0966 مجم QE / جم. تم تمييز الجسيمات النانوية باستخدام تقنيات تحليلية مختلفة بما في ذلك التحليل الطيفي للأشعة المرئية وفوق البنفسجية ، والتحليل الطيفي للأشعة تحت الحمراء لتحويل فورييه (FTIR) ، ومجهر المسح الإلكتروني (SEM) ، وتحليل الأشعة السينية المشتتة للطاقة (EDX) ، والمجهر الضوئي ، وحيود الأشعة السينية (XDR). تم تقييم النشاط المضاد للأكسدة لـ *A. Longa* والجسيمات النانوية من الطين الأحمر والأخضر باستخدام اختبار FRAP ، بينما تم أيضًا إجراء فحوصات مضادة للالتهابات وانحلال الدم. أظهرت النتائج المتحصل عليها أن المعادن التي يتكون منها الطين قد تحولت إلى نانو بحجم أقل من 170 نانومتر في شكل بلوري غير منتظم وأن المعادن الرئيسية هي كربون السيليكا والكالسيوم والمغنيسيوم. أظهرت نتائج النشاط المضاد للأكسدة أن كلا من *A. Longa* والطين الأحمر والأخضر أظهروا نشاط مهم، مع قيم التركيز المثبط 0.563 و 3.012 و 1.44 مغ / مل على التوالي. علاوة على ذلك، أظهر كلا العنصرين قدرة مضادة للالتهابات ، بقيم تراكيز التثبيط 0.034 و 0.0374 و 0.0015 مغ / مل على التوالي. بالإضافة إلى ذلك ، أشارت الدراسة إلى الكفاءة التحفيزية الضوئية و الصوتية لجزيئات النانو للطين الأحمر والأخضر ضد أزرق الميثيلين (MB) ، محققة كفاءات تحلل (93.14٪ و 87.13٪) و(84.47٪ و 49.32٪) على التوالي تحت إشعاع الأشعة فوق البنفسجية و بدون اشعاع ايضا. في الختام ، اقترحت هذه الدراسة أن التركيب النباتي لجسيمات الطين النانوية له أنشطة مضادة للأكسدة ومضادة للالتهابات ونشاط تحفيزي عالٍ في الظروف الضوئية والصوتية مما يشير إلى أن منتجاتنا الجديدة يمكن استخدامها في المجال الصيدلاني والبيئي.

الكلمات المفتاحية :

Aristolochia longa ، الجسيمات النانوية ، الطين الأخضر ، الطين الأحمر ، التوصيف ، الأنشطة البيولوجية ، النشاط التحفيزي.

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Abbreviation list

A.longa: *Aristoloshia longa*

AgO NPs : Silver oxide nanoparticles

AlCl3: aluminum chloride

BSA: bovine serum albumin

CH_3COOH: acetic acid

CEC: cation exchange capacity

CeO2 : Cerium Oxide

Cu2O NPs: cuprous oxide nanoparticles

Cuo: copper oxide

DNA : deoxyribonucleic acid

EDTA: Ethylenediaminetetraacetic acid

EDX : Energy-dispersive X-ray spectroscopy

Fe^{+2}: Ferrous

Fe^{+3} : Ferric

Fe2O3 NPs: iron oxide nanoparticles

FeoNPs: iron oxide nanoparticles

fe3o4: Iron Oxide

FRAP: Ferric Reducing Antioxidant Power

FTIR: Fourier-transform infrared spectroscopy

FeCl3: ferric chloride

FC: Folin-Ciocalteu

GAE: Gallic acid equivalent

H : atom

H2SO4: Sulfuric acid

HCL: hydrochloric acid

IC50: Inhibitory Concentration of 50%

MB: methylene blue

NPs : Nanoparticles

Na: Sodium

NiONPs: nickel oxide nanoparticles

Na2CO3: Sodium carbonate

OH : hydroxyl groups

PBS: Phosphate-buffered saline

QEq: Quercetin equivalent

RNA: Ribonucleic acid

RE : Rutin equivalent

SDS : sodium dodecyl sulfate

SiO2 : Silicon Dioxide

SEM : Scanning Electron Microscopes

Tio2 : titanium dioxide

TCA : Trichloroacetic Acid

Tem : transmission electron microscopy

TPC: Total Phenols compounds

TFC: total flavonoids compounds

UV-vis : UltraViolet-Visible

XDR : x-ray diffraction

ZnONPs : zinc oxide nanoparticles

Summary

Chapter III: Nanoparticles

second Part: Experimental Part

Chapter I: Materials & Methods

Chapter II: Results& Discussion

Introduction

Introduction

In the field of geology clay is a size-based term for very fine-grained minerals with an estimated spherical diameter <2.0 µm and approximate density of 2.65 g/cm3 (Williams & Haydel 2010). Clays are sources of major elements (e.g., Fe, Ca, Mg, Na, etc.) which are essential for the regular functioning of the human body. Clays also have adsorbent, emollient and soothing properties; making them suitable as cheap traditional antacids and cosmetic (Olajide-Kayode et al., 2023). Nowadays, nanotechnology is expected to be the basis of many biotechnological innovations in the 21st century and regarded as the upcoming industrial revolution. Nanomaterials have been called 'a wonder of modern medicine' and elicited much interest over the past few decades (Gunalan et al., 2012). Nanobiotechnology is the merger between biotechnology and nanotechnology for developing biosynthetic and ecofriendly technology for the synthesis of nanomaterials. Various studies suggested that plants seem to be the superior candidate and are proper for large scale biosynthesis of nanoparticles where the rate of synthesis is faster than that in the case of other organisms. In addition, the nanoparticles produced through plants are more various in shape and size in comparison with those produced by other organisms such as bacteria, fungi and algae (Ramesh et al., 2014). Forasmuch, many bioactive constituents in plants such as alkaloids, terpenoids, flavonoids, amino acids, enzymes, vitamins, proteins (Makarov et al., 2014), in contrast to other methods that require the usage of costly and harmful chemicals as reducing, capping, and some stabilizing agents for the synthesis of NPs, green synthesis utilizes the plant itself to produce such chemicals that work as natural reducing and stabilizing agents (Shaikh et al.,2018).

In recent years, with the development of nanoscience and dissociation technique of clay minerals, the nanostructured clay minerals are widely used in food, environment, agriculture, catalysis and many other fields due to their abundant reserves, non-toxicity, low price, good stability and unique morphology. *Aristolochia longa* (*Aristolochiaceae*) is a species commonly used in traditional Algerian medicine (Cherif et al., 2014). Another possible application could be its utilization in the nanobiotechnology field., also known as Berrostom in Algeria, has been reported as antioxidant anti-inflammatory (Samir et al.,2018) .

So, the aim of this study was to use *Aristolochia longa* roots aqueous extract in order to prepare red and green clay NPs by an eco-friendly method; characterization of NPs using some physical technics and also to evaluate the antioxidant and anti-inflammatory activities of aqueous extract of *Aristolochia longa* and both red and green clay NPs.

First part:
Bibliographic synthesis

Chapter I: the clay

1. Definition of clay

The terms clay can assume different meanings for different groups of people (Do Nascimento, 2016). Clays are ubiquitous materials whose study as fine-grained rocks and soil components is critical for the understanding of global sedimentary (Srodon, 1999), biological (Moreno-Maroto & Alonso-Azcárate,2018) and environmental (Singer, 1984) processes. Clay a group of natural minerals with plastic properties at appropriate water contents that will harden when dried or fired. Are primarily composed of hydrous-layer silicates of aluminium, occasionally containing magnesium and iron particles of smaller size, in other words, less than 2 μm (7.9 × 10−5 inch). Hence, in broader terms, clay minerals practically involve minerals of the above-cited particles size. These are essentially composed of silica, alumina, magnesia, iron and water with varying degree of potassium, sodium and calcium (Khurana et al., 2015).

2. Geological origin of clay minerals

The interpretation of the origin of clay minerals is one of the most interesting aspects of clay mineralogy. Clays and clay minerals occur under a fairly limited range of geologic conditions. The environments of formation include soil horizons, continental and marine sediments, geo thermal fields, volcanic deposits, and weathering rock formations. Most clay minerals form where rocks are in contact with water, air, or steam. Recall that the nature of clay formed during the weathering process depends upon many factors including the mineralogical and textural composition of the parent rock, the composition of the aqueous solution and the nature of the fluid flow (i.e., rate of water flow and pore network). The contact of rocks and water produces clays, either at or near the surface of the earth. Rock +Water → Clay (Al-Ani & Sarapää, 2008).

3. Clay mineral

Clay minerals are crystalline substances that essentially derive their origin from the chemical alteration of certain minerals that make up the parent materials (Harrat, 2007), with particle size lower than 0.002 mm with majorly clay minerals (Akisanmi, 2022). The clay material is formed from chemical alteration processes on the earth’s surface and accounts for approximately 40% of the first-class grained sedimentary rocks (mudrocks) which incorporates dust stones, clay stones and shales (Ombaka ,2016). The formation of clayey minerals depends on the physical-chemical conditions of the environment of the immediate altering environment, the nature of the raw materials and other related external environmental factors (Nesbitt et al.,1996). As such resulting in different types of clay materials. Hence, the potential for application of any clay mineral type in nature will depend on its will depend on its chemistry, structure and other intrinsic properties. (Wilson, 1999).

4. Structure of clay mineral

4.1. The tetrahedral layer

The tetrahedral layer of phyllosilicates is made up of tetrahedra with four oxygens at their vertices and a central cation (Figure 1). This cation is mainly Si4+, frequently Al3+, and rarely Fe3+. The tetrahedrons are linked together by the pooling of three vertices, the basal oxygens, the fourth remaining free is called the apical oxygen (Hachemi, 2020).

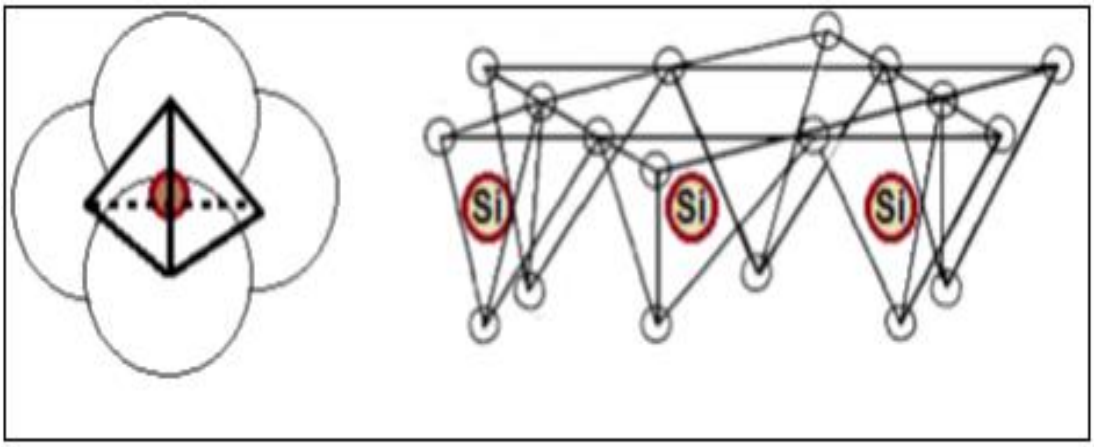


Figure 01: Tetrahedral layer (Hachemi, 2020)

4.2. The octahedral layer

The octahedral layer is formed by contiguous octahedra whose vertices are either oxygens or hydroxyls. These anions are bound to a central trivalent (Al3+, Fe3+) or bivalent (Fe2+, Mg2+) cation in coordination 6 (Hachemi, 2020). (Figure 2).

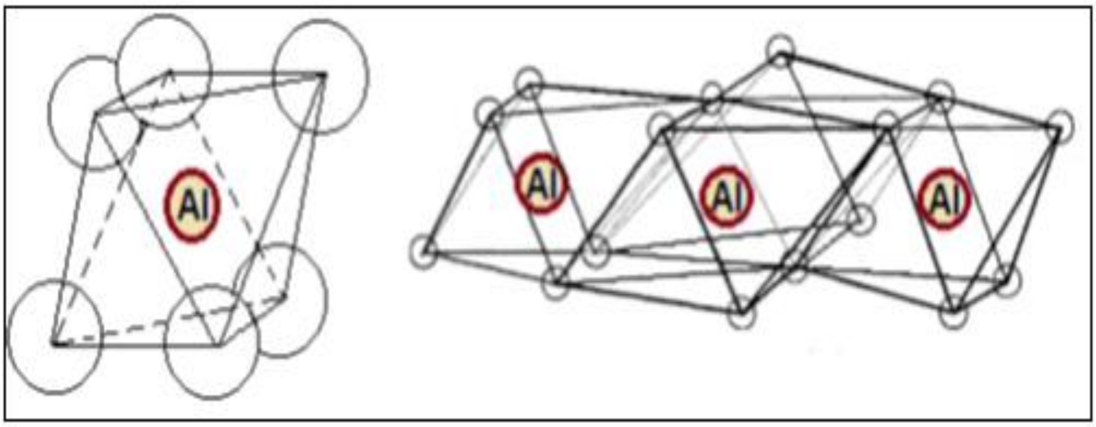


Figure 02 : Octahedral layer (Hachemi, 2020)

5. Classification of clay minerals

Clay minerals are generally classified into three-layer types based upon the number and arrangement of tetrahedral and octahedral sheets in their basic structure (Barton & Karathanasis ,2002).

5.1. Clay Minerals 1:1

The 1:1-layer minerals contain one tetrahedral and one octahedral sheet in their basic structural unit. This two-sheet mineral type is represented by the kaolin group with the general formula Al2Si205 (0H) 4. Kaolinite, the most common mineral in this group, is dioctahedral, exhibiting Al3+ octahedral and Si4+ tetrahedral coordination. The sheets are held together by van der Waals bonds between the basal oxygens of the tetrahedral sheet and the hydroxyls of the octahedral sheet. Layers are held together tightly by hydrogen bonding, which restricts expansion and limits the reactive area to external surfaces. Isomorphic substitution for Si4+ and Al3+ in this mineral is negligible. As such, soils dominated by 1:1 mineral exhibit a low capacity for adsorbing cations and have low fertility. The serpentine group, with the general formula Mg3Si2O5 (OH) 4, represents the trioctahedral version of the 1:1-layer minerals (Barton & Karathanasis, 2002).

5.2. Clay Minerals 2:1

The joining of two tetrahedral sheets (one from each side) to one octahedral sheet produces a three-sheet mineral type, which is called 2 :1 and is represented by the mica, smectite, and vermiculite groups. Talc [Mg3Si4Oio (OH)2] and pyrophyllite [Al2Si4Oio (OH)2] are typical representatives of electrically neutral 2 :1 type mineral in which adjacent layers are joined to each other by van der Waals bonds. Although these two minerals are found infrequently in soils (2), their structure serves as a model for discussing transitions leading to the formation of other more common 2:1 clay mineral (Barton & Karathanasis ,2002).

5.3. Clay Minerals 2:1:1

Chlorites are a group of minerals that exhibits a basic 2:1 layer structure similar to that described for talc or pyrophyllite, but with an interlayer brucite- or gibbsite like sheet, which forms a 2:1:1 structural arrangement. Isomorphic substitutions within the interlayer hydroxide sheet create a net positive charge that balances the negative charge arising from the 2:1 layer. A typical formula for the interlayer sheet is (MgFeAl) (OH)g; however, a variety of cation species may exist in these brucite or gibbsite-like islands that contribute to a large number of mineral species within this group. There is no water adsorption within the interlayer space; thus, chlorites are considered no expansive minerals. (Barton & Karathanasis ,2002).

6. Composition and Properties of Red and Green Clay

The red clay, which is composed of a mixture of natural minerals known as "clay minerals" such as quartz, feldspars, iron oxide, oxidizes titanium, earthy oxide, etc., contains clay minerals with higher contents of silica (quartz, cristobalite, tridymite), oxides and hydroxides of aluminum (corundum, gibbsite, diaspore, boehmite...), carbonates (dolomite, diobertite, siderite, calcite, aragonite...), and iron minerals (lepidocrocite, maghemite, etc.) (Rakhila et al, 2018). On the other hand, the green clay is a metallic clay that is rich in remnants of decayed plants, as well as numerous other minerals such as calvpur, zinc, copper, magnesium, among others. This type of clay is used in alternative medicine to alleviate various health and skin problems (Asthana et al., 2021).

7. Properties of clays

7.1. Cation exchange capacity (CEC)

A remarkable property of clays is their ability to interact with the chemical species present in an aqueous solution. These chemical species, which can be both ionic and molecular, will be able to be adsorbed on the external surface of the clay particles or on the internal surfaces. It is this property of adsorption or absorption of certain chemical species present in solution which is called the cation exchange capacity (CEC). This capacity is measured in the number of charges fixed on the surface of the clay and, more precisely, in the number of moles of ionic charges per 100 g of dried clay (milliequivalent/100 g) (Le Pluart, 2002).

7.2. Hydration capacity

The processes of water absorption in the structure of clays are complex and can be described from the dry state to the hydrated state or from the hydrated state to the dry state. But these two sequences do not describe the same phenomenon and it follows that the mechanisms are different when studying hydration or dehydration. The study of adsorption isotherms highlights the existence of hysteresis, which shows that the mechanisms of hydration and dehydration are not completely reversible (Salles, 2006).

8. Application pharmaceuticals

The pharmaceutical applications of clay minerals are promising, and further research is warranted to fully understand their potential benefits. With their natural origins, low toxicity, and versatile properties, clay minerals have the potential to revolutionize modern medicine. Clay minerals have been utilized for their medicinal properties for centuries. Clay minerals are known for their adsorption capability, which makes them useful for removing impurities and toxins from the body. In addition, they also possess antimicrobial properties that have been found to be effective against a wide range of bacteria and fungi. Recent studies have suggested that clay minerals have the potential to treat various medical conditions such as skin infections, diarrhea, and gastric ulcers. One study (Carretero, 2002). Highlighted the beneficial effects of clay minerals on human health. The authors reported that clay minerals have been shown to improve the immune system, reduce inflammation, and even prevent cancer. Clay minerals have also shown promise as drug delivery agents. (Choy et al., 2007) .

Chapter II: Aristolochia Longa

1. Definition of *Aristolochia longa*

Aristolochia longa is a plant belonging to the genus *Aristolochia,* family *Aristolochiaceae* (Madani et al., 2022), that contains about 500 species. This family has been reported in the forest of America, Asia, Africa, Europe, and rarely in other countries (El Idrissi et al., 2021). It is widely distributed in the tropical and temperate regions. *Aristolochia s*pecies contain secondary metabolites that have well-known beneficial effects. *A. longa* locally known as "Barraztam" (El-Omari et al.,2019). The plant is widely used in traditional medicine in Algeria (Benarba et al., 2014). Several investigations have shown numerous biological properties, including antibacterial, antifungal, anti-inflammatory, antioxidant, and anti-cancer activities (Madani et al,2022).

2. Geographical location

In this context, the monophyletic botanical genus belongs to the *Aristolochiaceae* Juss family (Piperales). Around 400 plants are distributed in the tropical regions of South America, Asia, and Africa. In the flora of Brazil 2020, the genus *Aristolochia* is represented by 83 species, highlighting the Atlantic Forest, with 39 species, the Cerrado, with 36 species, and the Amazon, with 35 species (Mueller et al., 2022).

Algeria it grows in the medal and in west up the mountains of Tlemcen, Cherea Medea, and in Reghaia (Blida). It also grows in Tizi ouzou and Laghout. In Jijel, it is found in the mountains of Djimla (Benarba, 2013).

3. Description and Botanical classification

The root is spindle-shaped from 5cm to 3 dm in length, about 2 cm in thickness, fleshy, very brittle, greyish externally, brownish-yellow inside, bitter and of strong disagreeable odour when fresh. Its root is bitter in taste. Leaves are long, branches are thin, flowers are violet coloured (Sana,2020). The table 1 shows the classification of the plant *A. longa*:

Table 1: Scientific classification of *Aristolochia longa L* (Ojo et al., 2022)

Kingdom:	**Plantae**
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Magnoliids
Order:	Piperales
Family:	*Aristolochiaceae*
Genus:	*Aristolochia*
Species:	*A. longa*
Binomial name:	*Aristolochia longa L*

4. Chemical composition

It is well known that there is a close relation between the chemical composition of the plant and its biological activities. There are only a small number of studies carried out on the chemical composition of *Aristolochia longa*. They have shown the presence of polyphenols, flavonoids, cheterosides, carbohydrates, and saponins in the aqueous extract of the plant. Also, it has been reported that the flavonoid glycosides are the major compounds in the extract of the plant (Ibtissam et al., 2021). *Aristolochia L*. like the other members of the genus is rich with terpènes. Previous study aimed to identify the phenolic compounds of *Aristolosia Longa* root extracts and evaluate their antibacterial activity on multi-resistant strains. Plant chemical analysis revealed the presence of flavonoids, tannins, terpenoids and alkaloids. HPLC-DAD analysis of *A. longa* extracts showed the presence of many active biocompounds such as virulic acid, 4-hydroxycinamic acid, citric acid and kenic acid. Rent spread method used to test allergies (El Omari et al., 2020).

5. Traditional therapy

Aristolochia species have well-known beneficial effects. The active compounds produced during secondary metabolism are generally responsible for the biological properties (El Yahyaoui El idrissi et al., 2018). *Aristolochia longa* have been used as medicinal herb since ancient time in different cultures. It has multiple applications and virtues, and used in traditional medicine. It has been reported that the most widely uses of *A. longa* in Algeria are in cancer treatment. In Morocco, the rhizomes of this plant are used to treat acute toxicities (Benarba, 2015). A variety of traditional uses for species of the genus *Aristolochia* were found in the literature. The traditional uses of this plant are cited, the most common uses are anticancer, antioxidants, snake anti-venom, anti-inflammatory , abdominal pain, antiparasitic , insecticide an predator protection ,antimalarial, skin diseases , fever , headache (Lerma-Herrera et al,2022), upper respiratory tract infections (El Omari et al,2019). Also, it is used in weight loss diets, arthritis prevention (El Omari et al., 2020).

Chapter III: Nanoparticles

1. Nanotechnology

The term "Nano" comes from the Greek word dwarf which reveals the particle size to lie in the range of 1 to 100 nm (Gebremedhn et al., 2019). It is the most important dynamic exploration regions in the current of material science. It has strengthened of an important of nanotechnology pathway, which includes better control of the shape and size of various nano-applications (Chinnapaiyan et al., 2022). In the diverse field of nanotechnology, nearly all aspects of the industry, from medicines to machines, are undergoing tremendous advancements. In healthcare area, nanotechnology opens new limits in the life sciences industry. Nanotechnology has great promise in manipulating things at the atomic level to change many parts of medical treatment, such as diagnosis, monitoring for diseases,operating equipment, regenerative medicine, developing vaccines, and medication delivery (Raffa et al., 2010; Cormode et al., 2009). In medicine, extensive research is ongoing into best practices and methodologies, including nephrology, cardiovascular disease therapeutic gene, and cancer therapy. There is a significant development in the traditional treatment and the quality of nanoparticles and nanotechnology have improved and shown encouraging results. (Vishwakarma et al., 2013 ; Keskinbora & amp, 2018). Nanotechnology makes it possible to overcome the limitations of conventional treatment, such as particle distribution in the body and insufficient membrane trafficking (Szewczyk et al., 2022).

2. Nanoparticles

Nanoparticles have special properties due to their nano-size (1–100 nm), with one nanometer equaling one billionth of a meter (Ibrahim et al., 2023). Metallic-element (such as copper, silver, and gold) nanoparticles have many applications in biotechnology and biomedicine (Vijayakumar et al., 2021). Nanoparticles have fascinated many researchers because they have unique characteristics such as size, shape, distribution, and morphology compared to bulk materials (Pannerselvam et al., 2020 ; Shah et al., 2015; Chung et al.,2017) .In all science fields, including the therapeutic field, metallic nanoparticles are being utilized. They are still alluring scientists to investigate new scopes due to their small sizes. These metal nanoparticles have advantages due to their small sizes, large surface areas, excellent chemical and optical properties, and good electrical conductivities (Saranya et al., 2020).

We can classify the nanoparticles into many types according to many properties

Carbon based Nanoparticles, Polymeric Nanoparticles, Ceramics Nanoparticles, Semiconductor Nanoparticles, Metal Nanoparticles and Lipid based Nanoparticles etc (Mughal, 2022).

3.Synthesis of nanoparticles

Nanoparticles are synthesized by a number of methods and those are broadly grouped into physical, chemical and biological methods (Begum & Jayawardana, 2023). Synthesis of nanoparticles by all these techniques has been reported. Even though all three methods are in practice over the years, application of physical and chemical means at large scale has been limited in agriculture and health sectors (Herrera-Marín et al., 2023). This is due to the risks and challenges associated, such as the use of potentially toxic chemicals (Some et al., 2019), requirement of expensive equipment and machinery (Hossain et al., 2019), requirement of larger laboratory space, rigorous processing conditions like elevated temperature, pressure (Ahmad & Jaffri, 2019) and high energy, formation of harmful side products (Kowshik et al., 2002). Furthermore, the time consumed (Kausar et al., 2022, Some et al., 2019), high cost involved (Kausar et al., 2022), harmful effects on the environment (Hamouda et al., 2019) exacerbates the challenges associated with their synthesis. As a result, the need for an alternative means of synthesis being particularly environmentally friendly is pronounced and has opened new avenues for the development of green technologies in synthesizing them. (Herrera-Marín et al., 2023).

The synthesis of nanoparticles using biological methods has attracted interest in recent years due to the structure of the nanoparticles obtained, which exhibit unique electronic, catalytic, antibacterial, and antimicrobial properties, in addition to supporting environmental conservation (Ahmed et al., 2016; Orabi et al., 2022; Ahmad et al., 2020; Ghoshal & Singh, 2022).

Biosynthesis using plant extracts is a good choice because of the ease of preparation, its lower cost, its environmentally friendly nature, and the purity and high quality of the product obtained (Crisan et al., 2021; Bayat et al., 2021).

4. Green synthesis

In recent times, the biological method otherwise termed the green synthesis method has received increased attention due to the growing need to develop an environmentally benign technology in nanoparticle synthesis. Due to its simple processes and cost effectiveness (Mustapha et al., 2022). In the recent past, the evaluation of green chemistry or biological techniques for synthesizing metal nanoparticles from plant extracts has drawn the attention of many researchers (Khan et al., 2022). Green synthesis is an emerging and novel technology for the synthesis of hybrid nanoparticles (NPs) in an eco-friendly or non-hazardous way to decrease toxic wastage, and energy and using environmental solvents like water, ethyl acetate, ethanol, etc (Krishnan et al., 2021) . The "green syntheses" or "Green approaches" simply referred to the metal nanoparticle synthesis with plants, plant body plant extracts, flowers, and leaves, as an alternative to chemical and physical procedures. By combining various materials organic-inorganic or organic-organic (Rahman et al., 2022).

5. Characterization of nanoparticles

There are different techniques for the characterization of nanoparticles. These techniques are atomic force microscopy, particles size analysis, scanning electron microscopy, X-ray diffraction (XRD), transmission, electron microscopy Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy and Raman spectra (Salame et al.,2018) . Characterization is the study of many facts like structure of nanoparticles (Shin et al., 2016). Facts like structure of nanoparticles, the material from which nanoparticles can be formed and many other properties (Chirayil et al., 2017). SEM and TEM are the essential techniques. SEM is depends upon electron scanning method (Saeed & Khan, 2016) . TEM is depends on electron transmittance properties (Khlebtsov & Dykman, 2010). XRD is based on the structural properties (Khan et al., 2017).

6. Applications

Nanotechnology's benefits are rapidly expanding in a variety of sectors. Many new technologies use nanoparticles, including sun protection and moisturizers, desalination, inks, sun filters, blemish clothing, agribusiness and pharmaceuticals, finished fabrics, or wound treatments are all examples of products that are manufactured. Nanoparticle's characteristics have sparked a lot of interest in biomedical research. Drug delivery, theranostic, cancer treatment, antibacterial, and implants & wound healing are some of the biological applications of nanoparticles (Pandit et al., 2022). Nanoparticles, especially metal nanoparticles and metal oxides, have been widely used by medical consumers and manufacturers. Nanoparticles, display unique, physical and chemical properties and represent an increasingly important material in the development of novel nanodevices which can be used in numerous physical, biological, biomedical and pharmaceutical applications (Thema et al.,2015;Shaneza et al., 2018). Silver nanoparticles can have anti-microbial properties such as bacteria,either by inhibiting the growth of biofilms or by directly interacting with the bacterial or viral genome (DNA or RNA) (Derouiche et al., 2022). Antioxidant and anti-inflammatory activity of CuNPs (Ouidad et al., 2020), anticancer activity (Ma et al., 2023). It is clear that nanomaterials have tremendous promise in the food industries, and many interesting uses remain to be explored. The beneficial uses derive from their physicochemical properties, characteristic of their nanoscale, enabling them to be used as antimicrobials, oxygen scavengers, and water repellents, or as reinforcing materials providing gas barriers and increased mechanical properties. Currently investigated materials are metal and metal oxides like TiO2, CeO2, SiO2, ZnO, Au and AgNPs,carbon-based nanomaterials like carbon nanotubes and graphene, and nano cellulose. Nanosized products such as nano-liposomes or nano-capsules carrying active nutraceuticals (e.g. nanofood) arealso used to improve the functional properties of food (Mustafa & Andreescu, 2020).

second Part:
Experimental Part

Chapter I:
Materials & Methods

I.Materials

I.1.Plant material

The plant used in this work is the rhizomes of *Aristoloshia longa*, was purchased from local market, washed several times, air dried for 48-92 hours and pulverized with a blender until a fine powder is obtained. *Aristoloshia longa* powder is stored at the room temperature in airtight containers in the arbi da bright light until the beginning of the experiment (figure 03).

Figure 03: *Aristolochia longa* rhizomes (original photo)

I.2.Clay materials

I.2.1. Geographical location of the Wilaya El-Oued

The Wilaya of El-Oued is located in the northeast of the Algerian Sahara. It is bordered:

- ✓ To the north, by the Wilayas of Tebessa, Khenchela and Biskra .
- ✓ TO the west, by the Wilayas of El M'Ghair and Touggourt.
- ✓ To the south and by the Wilaya of Ourgla.
- ✓ and to the east by Tunisia.

Figure 04: Geographic location of El-Oued

I.2.2. Studied clay sample area

Clay was taken from the "EL Hamraia " It is 110 km north of El Oued Province as shown in the figure 05 showed that the soil of the EL Hamraia region contains clays.

Figure 05: Clay sampling area

Figure 06: career of clay

I.3.Reagents and products used

Wagner reagent, ferric chloride ($FeCl_3$), chloroform, aluminum chloride($AlCl_3$), Folin-Ciocalteu (FC),acetic acid(CH_3COOH),quercetin, ascorbic acid, gallic acid , Fehling's liquor, ammoniac, Sulfuric acid (H_2SO_4), hydrochloric acid (HCL), Trichloroacetic Acid (TCA), Sodium carbonate (Na_2CO_3), bovine serum albumin (BSA) , Diclofenac, ethanol, methanol .

II.Methods

II.1. Method of preparation of the aqueous extract

25g of the dry *A.longa* plant with 250ml of distilled water was boiled over low heat for 2h. After maceration at room temperature for 24 hours, then filtered, the filtrate was then evaporated using a rotary evaporator (Majhenic et al., 2007).

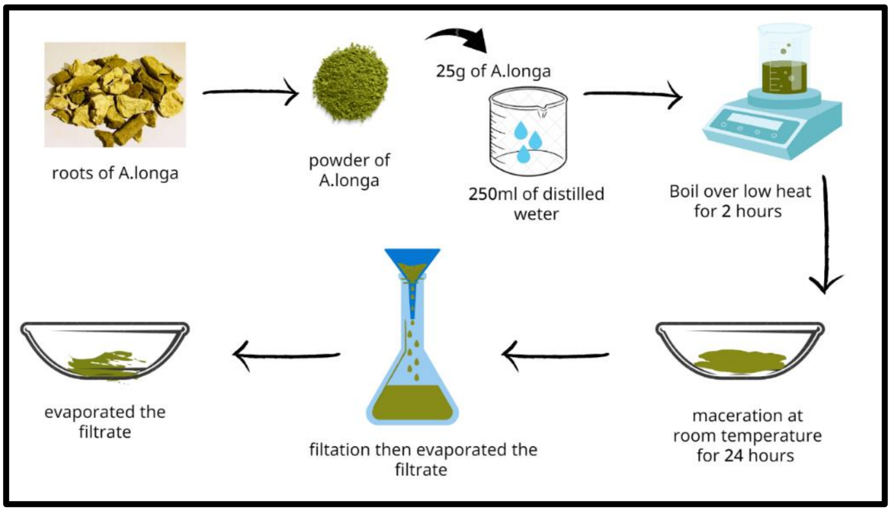


Figure 07: Preparation protocol for the aqueous extract of the *A.longa* plant

II.2. Phytochemical analysis

The phytochemical analysis was carried out on the aqueous extracts prepared from the plant by qualitative characterization method according to **(according to standard protocols).**

II.2.1. Phenols

Introduce 5 ml of extract in a test tube and drops few of natural 5% ferric chloride solution. A dark green color indicates the presence of phenolic compounds.

II.2.2. Flavonoids

In a test tube, introduce 5ml of extract, 5ml of diluted ammoniac and 1ml of H_2SO_4. The appearance of a yellow color indicates the presence of flavonoids.

II.2.3. Alkaloids

1 ml of aqueous extract were treated with a few drops of hydrochloric acid then 1–3 drops of Wagner reagent were added. The appearance of brown precipitate reveals the presence of alkaloids in the sample.

II.2.4. Tannins

In a test tube, introduce 5 ml of extract and add 1 ml of a 2% aqueous solution of ferric chloride ($FeCl_3$). The presence of tannins was indicated by a greenish or bluish-blackish coloration.

II.2.5. Terpenoids

The formation of a reddish-brown color indicates the presence of terpenoids, through the addition of chloroform (2ml) and concentrated sulfuric acid (3 ml) to 5 ml of plant extract.

II.2.6. Reducing compound

Add Fehling's liquor (1ml of reagent A and 1ml of reagent B) to the extract and incubate the whole in a boiling water bath, the appearance of a brick-red precipitate indicates the presence of reducing sugars.

II.2.7. Saponins

In a test tube, introduce 5ml of extract, mixed with 5ml of distilled and with vigorous manual agitation. The formation of a steady foam indicates the presence of saponins.

II.2.8. Steroids

For 1ml of plant extract, add 0.5ml of acetic acid solution, followed by 0.5ml of concentrated H_2SO_4. If the solution does not give any green color, it proves the presence of unsaturated steroids. In a second tube, the same volume of H_2SO_4 was added. The presence of the red color indicates the presence of steroid derivatives.

II.3. Total phenols and flavonoids compounds

II.3.1. Total phenols

Determination of the total polyphenols was carried out according to the Folin-Ciocalteu (FC) method (Boizot & Charpentier, 2006): 100 µl of *Aristoloshia longa* extract are mixed with 500 µl of the FC reagent and 400 µl of Na_2CO_3 at 7.5% (w / v). The mixture is stirred and incubated in the dark and at room temperature for ten minutes and the absorbance is measured at 760 nm by a UV spectrophotometer. The results are expressed in mg gallic acid equivalent/ g of dry vegetable material with reference to the calibration curve of gallic acid. Calibration curve is carried out by gallic acid at different concentrations (20 - 40 - 60 - 80 - 100 - 120 µg/ml) under the same conditions and the same steps of the assay. The results are thus expressed in milligrams of gallic acid per gram of dry extract (mg of EAG / g). All measurements are repeated 3 times.

II.3.2. Total flavonoids

The determination of total flavonoids was carried out according to the method described by (Dehpour et al., 2009): 500 µl of each extract, 100 µl $AlCl_3$, 100 µl of 1 M sodium acetate and 2.8 ml of distilled water. The mixture is stirred and then incubated in the dark and at room temperature for 30 minutes. The blank is made by replacing the extract with 95% methanol and the absorbance is measured at 415 nm using a UV spectrophotometer. The results are expressed in mg equivalent quercetin / g of dry vegetable material with reference to the quercetin calibration curve. The quercetin calibration curve is performed by quercetin at different concentrations (20 - 40 - 60 - 80 - 100 – 120 µg/ml) under the same conditions and the same steps of the assay.

II.4. Synthesis of clay nanoparticles (NPs)

For the synthesis of Clay nanoparticles, we followed an experimental protocol according to studies of (Raj & E, J. 2015; Dhuha et al.,2018) with some modifications. Briefly, 200 mg of the Clay were added to distilled water 100 ml and then add 1 ml of aqueous extract of *Aristolochia longa* under agitation for 10 minutes. It was then placed in a magnetic stirrer for 3 hours. The precipitate was removed and washed with distilled water and then with ethanol to remove impurities. Then, a powder of clay nanoparticles was obtained after drying at 60 C° in the oven overnight .

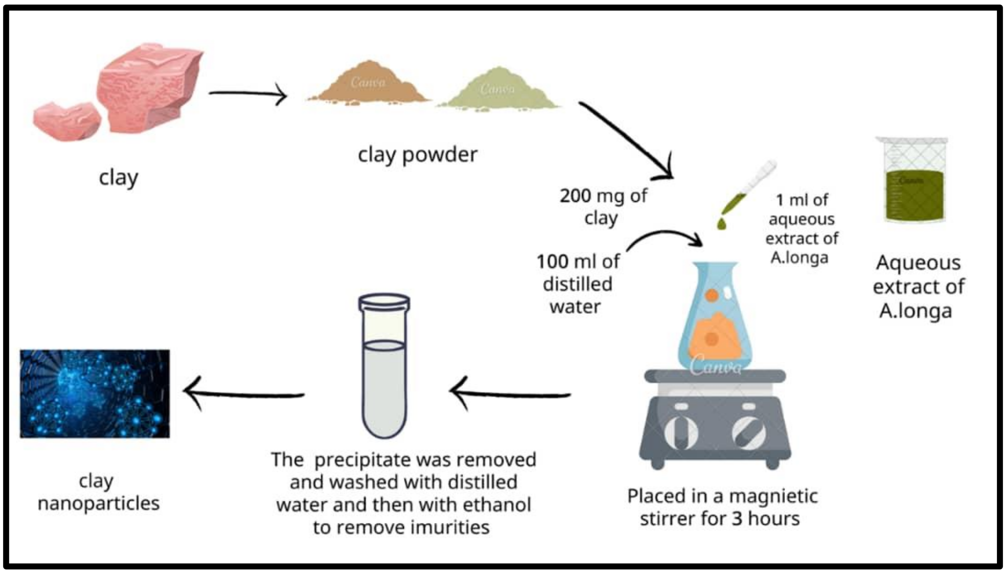


Figure 08: Preparation protocol for the clay nanoparticles

II.5. Characterization of clay NPs

The red and green clay nanoparticles synthesized using *A. Longa* through a biological method were characterized using various techniques including Ultraviolet-visible spectroscopy (UV-VIS), Fourier transform infrared spectroscopy (FTIR), X-ray powder diffraction (XRD), scanning electron microscope (SEM), and light microscope analysis. The UV-Vis spectroscopy technique allowed for easy observation of the synthesis of nanoparticles solution. FTIR spectroscopy utilized the Fourier transform mathematical process to identify the presence of organic and inorganic compounds in the sample. XRD analysis provided information on unit cell dimensions, allowing for phase identification of a crystalline material. Lastly, SEM imaging utilized a high-energy beam of electrons in raster scan patterns to analyze the sample.

II.6. Biological activity

II.6.1. Antioxidant activity

II.6.1.1.Ferric Reducing Antioxidant Power Assay, FRAP

Take 500µl of sample and Add 1.25ml of the buffer solution (0.2 M, PH = 6.6). Add to 1.25 potassium fericianure.Then Incubation during 20 min in a water bath at 50 ° C. After cooling, add 1.25ml of the aqueous TCA solution (10%) to stop the reaction. Centrifugation at 3000 rpm for 5 minutes. Then take1.25 ml of supernatant are then mixed with 1.25 ml distilled water and 250 µl $FeCl_3$ (0.1%). The absorbance was measured at700 nm against a blank.The results expired by IC50, after calculating of the inhibition percentage values according to(Oyaizu,1986) as follows:

$$\text{FRAP (\%)} = 100 - \frac{\text{OD controle}}{\text{OD sample}} \times 100$$

II.6.2.Anti-inflammatory activity

II.6.2.1.Hemolysis assay

Hemolysis assay was done as described by study of vinjamuri et al., (2015). 5mL of blood was collected from healthy volunteers in the tubes containing 5.4 mg of EDTA to prevent coagulation and centrifuged at 1000 rpm for 10 min at 40 ºC. Plasma was removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4 for 5min. Washed erythrocytes were stored at 40C and used within 6 h for the hemolysis assay. 50 µL of 10 erythrocytes were stored at 40C and used within 6 h for the hemolysis assay. 50 µL of 10 dilutions (100 uL Erythrocytes suspension: 900 µL 1XPBS) of erythrocytes suspension was mixed with 100 µL of test samples (48µg/mL), 100 µL of 1XPBS was used active control and 100 uL of 1% SDS as positive controls. Reaction mixture was incubated at 37ºC water bath for 60 min. Volume of reaction mixture was made up to 1 mL by adding 850 µL of 1XPB. Finally, it was centrifuged at 300rpm for 3min and the resulting hemoglobin in supernatant was measured at 560 nm by spectrophotometer to determine the concentration of hemoglobin. Percentage haemolysis was calculated as follows:

$$\text{Hemolysis inhibition (\%)} = 100 - \frac{\text{OD sample}}{\text{OD control}} \times 100$$

II.6.2.2. Protein denaturation assay

❖ **Principal**

The anti-inflammatory activity is measured of protein denaturation inhibition in presence of the anti-inflammatory compound, which is studied through in vitro assay. The measured turbidity at 660 nm is proportional to the concentration of anti-inflammatory compound present in the sample (Vennila et al .,2018).

❖ **Procedure**

1. Add different concentrations (10–50 µg ml-1) of the sample to bovine serum albumin (BSA) solution (1%).

2. Incubation during 30 min at room temperature.

3. The pH of the solution was adjusted to 2 using dropwise addition of concentrated HCl.

4. After incubation, the mixture is heated at 72 °C for 30 min.

5. The all tubes are cooled for 10 min.

6. The turbidity is measured at a wavelength of 660 nm. Diclofenac is used as standard.

The results expired by IC50, after calculating of inhibition percentage (IP) as follows:

$$IP\ \% = (A\ Sample – A\ Control)\ 100$$

II.7. Catalytic activity of nanoparticules

II.7.1. Photocatalytic Degradation of Dye

Typically, 10mg of methylene blue dye was added to 1000mL of double distilled water used as stock solution. About 10 mg of biosynthesized clay nanoparticles was added to 100mL of methylene blue dye solution. A control was alsomaintained without addition of clay nanoparticles. Before exposing to irradiation, the reaction suspension was well mixed by being magnetically stirred for 30min to clearly make the equilibrium of the working solution. Afterwards, the dispersion was put under the sunlight and monitored frommorning to evening sunset. At specific time intervals, aliquots of 2-3mL suspension were filtered and used to evaluate the photocatalytic degradation of dye. The absorbance spectrum of the supernatant was subsequently measured using UV-Vis spectrophotometer at the different wavelength. Concentration of dye during degradation was calculated by the absorbance value at 660 nm (Vanaja et al.,2014).

Percentage of dye degradation was estimated by the following formula:

$$\%\ Decolourization = 100 \times (C0 - C)/C0$$

where $C0$ is the initial concentration of dye solution and C is the concentration of dye solution after photocatalytic degradation.

II.7.2.Sonocatalytic

Sonocatalytic Degradation Reaction Procedures was performed according to study of (Chauhan *et al.*, 2020). To evaluate the sonocatalytic activity of clay NPs and to further understand the degradation mechanism of the dyes, batch experiments were performed at room temperature. Before exposing to ultrasonic waves, the suspensions (dye+clay NPs) were kept in dark conditions and stirred for 10 minutes to get the adsorption-desorption equilibrium of the dye on the clay NPs. Therefore, the effect of adsorption during sonocatalysis could be calculated accurately. These suspensions were placed in an ultrasonic bath apparatus (Magnetic stirrer) for a definite interval of time. After ultrasonic irradiation, percent degradation was calculated by measuring the change in the absorbance of dye using UV-Vis spectrophotometer

Percentage of dye degradation was estimated by the following formula:

$$\%\ Decolourization = 100 \times (C0 - C)/C0$$

where $C0$ is the initial concentration of dye solution and C is the concentration of dye solution after Sonocatalytic degradation.

Chapter II:
Results & Discussion

I.Results

I.1.Qualitative phytochemical analysis

Results of phytochemical essays shows that aqueous extract of *A. longa* rich on different important chemical compounds such as flavonoïds, phenols, Alkaloids,Tannins,Terpenoids Reducing compound,Saponins,Steroids.

Table 02: phytochemical compounds for aqueous extract of *A. longa*

Compounds	Phenols	Flavonoids	Alkaloids	Tannins	Terpenoids	Reducing compound	Saponins	Steroids
Aqueous extract of *A. longa*	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ + +

(+): Present.

I.2. Dosage of polyphenols

I.2.1. Total Phenols and flavonoids assay

Table 03 shows the total polyphenols content of *A. longa* calculated by equation $y = 0.0073x + 0.0409$ with $R^2 = 0.957$ obtained by a calibration curve based on Gallic acid as a standard. Similarly, the flavonoid content in this plant was calculated by equation $y = 0.0158x + 0.0557$ with $R^2 = 0.9962$, obtained by a calibration curve based on quercetin as standard.

Table 03: Total Phenols compounds (TPC) and total flavonoids compounds (TFC) concentration in aqueous extract of *A. longa*

Compounds	**TPC (**mg GAEq/g of extract**)**	**TFC** (mg QEq/g of extract)
aqueous extract of *A. longa*	31.247±0.440	1.6647±0.0966

I.3. Characterization of clay NPs

I.3.1. UV-Vis Spectroscopy Analysis

Nanoparticles prepared via biological and green methods were studied in the range of 250-410 nm at room temp., and the spectra are shown in Figure 09. The maximal absorption peak of the red Clay nanoparticles and the green clay nanoparticles in the UV–Vis spectrum range is up to 300 nm.

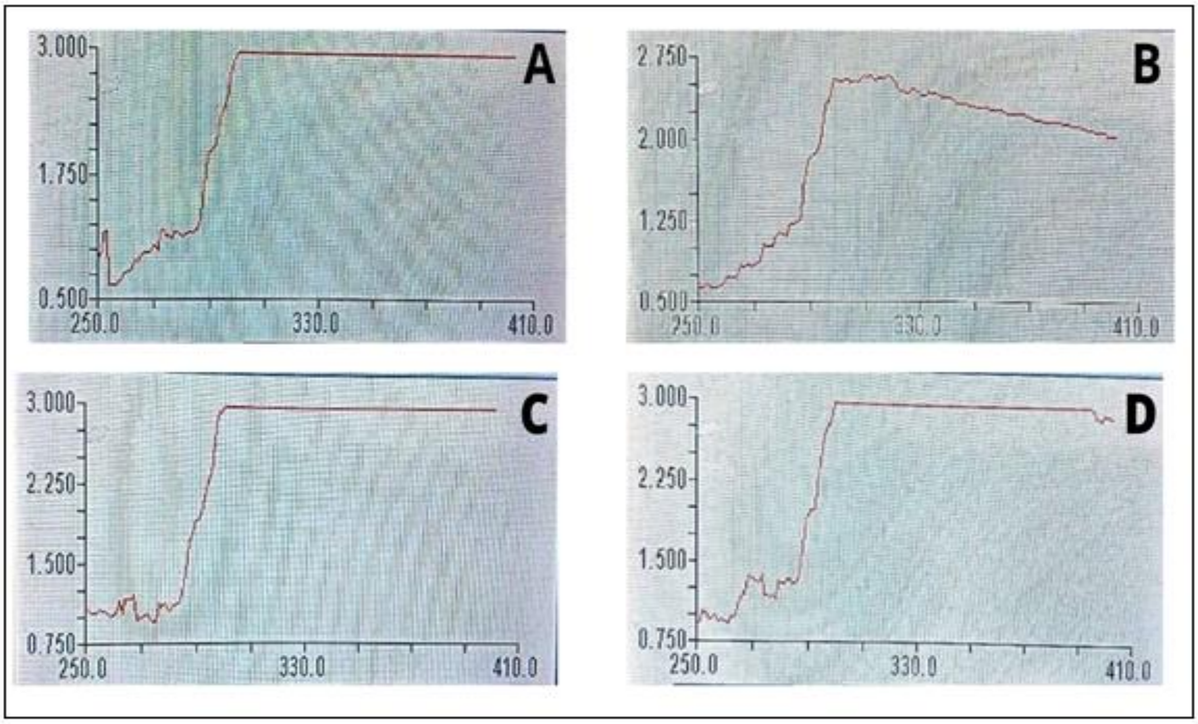


Figure 09: UV-Vis Spectroscopy of green clay (A), green clay NPs (B), red clay (C) and red clay NPs (D)

I.3.2. FTIR spectroscopic analysis

The FTIR spectroscopic analysis of *A. longa* plant extract and green clay NPs and Red clay NPs revealed the presence of several vibrations; the vibrations belong to the functional groups of the organic compounds that participated in the synthesis like vibrations at 3294 cm^{-1}, at 2919 cm^{-1}, at 563 cm^{-1} and the vibration at 798 cm−1 (Figure 10).

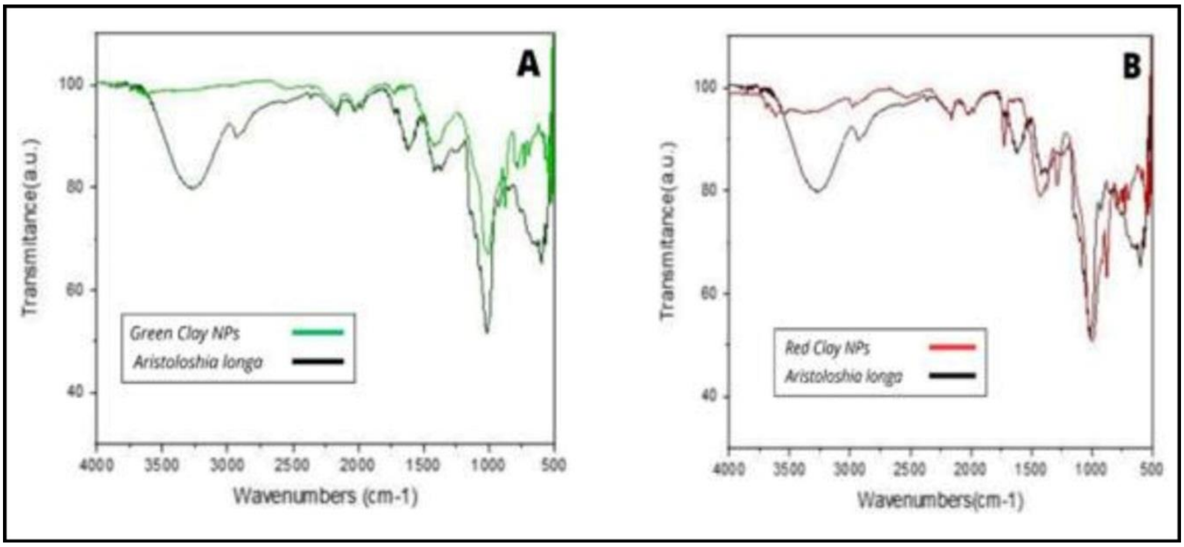


Figure 10: FTIR spectroscopic of green clay NPs (A), red clay NPs (B) obtained from aqueous extract of *A. longa*

I.3.3. X-ray diffraction (XRD) analysis

Figure 11 shows the XRD pattern of red clay NPs and green clay NPs. The nanoparticles synthesised by green method are characterized by using this technic in order to confirm the size and the presence of some elements as nanoparticles and to know the structural information.

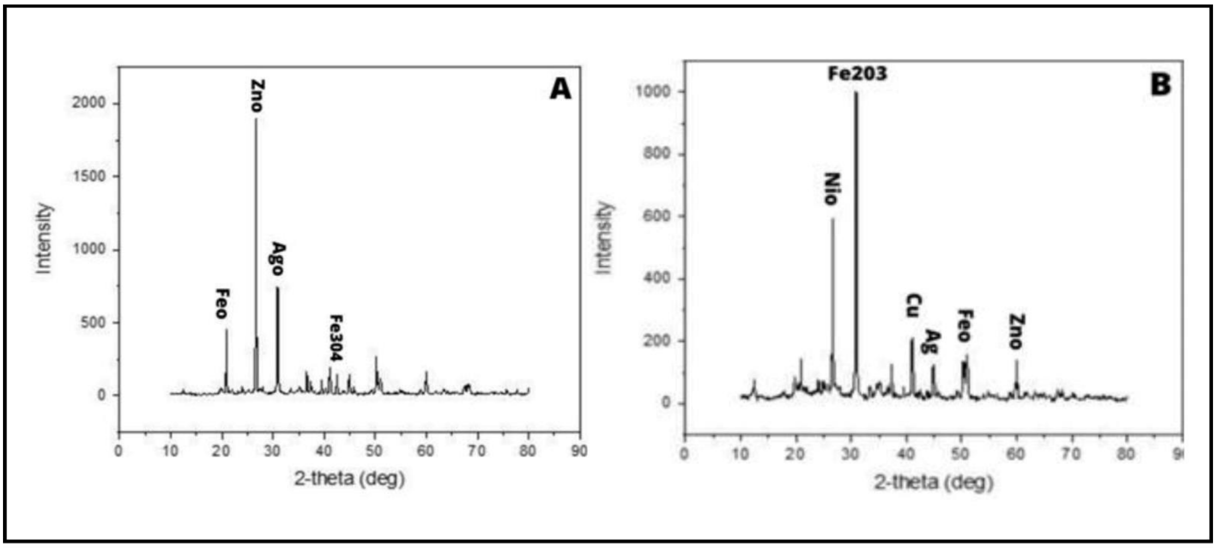


Figure 11: X-ray diffraction (XRD) of green clay NPs (A), red clay NPs (B) obtained from aqueous extract of *A. longa*

I.3.4. Light microscopy analysis

The results of the light microscopy analysis, as presented in both Figure 12 and Figure 13, exhibit a marked contrast between the different elements present in the two types of clay, which suggests the existence of red and green clay nanoparticles in the sample.

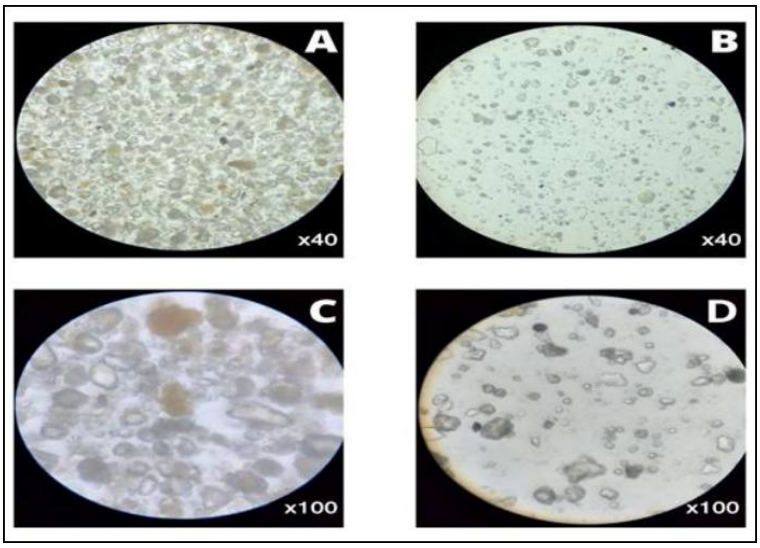


Figure 12: Light microscope analysis of green clay (Ax40, Cx100), green clay NPs (Bx40, Dx100)

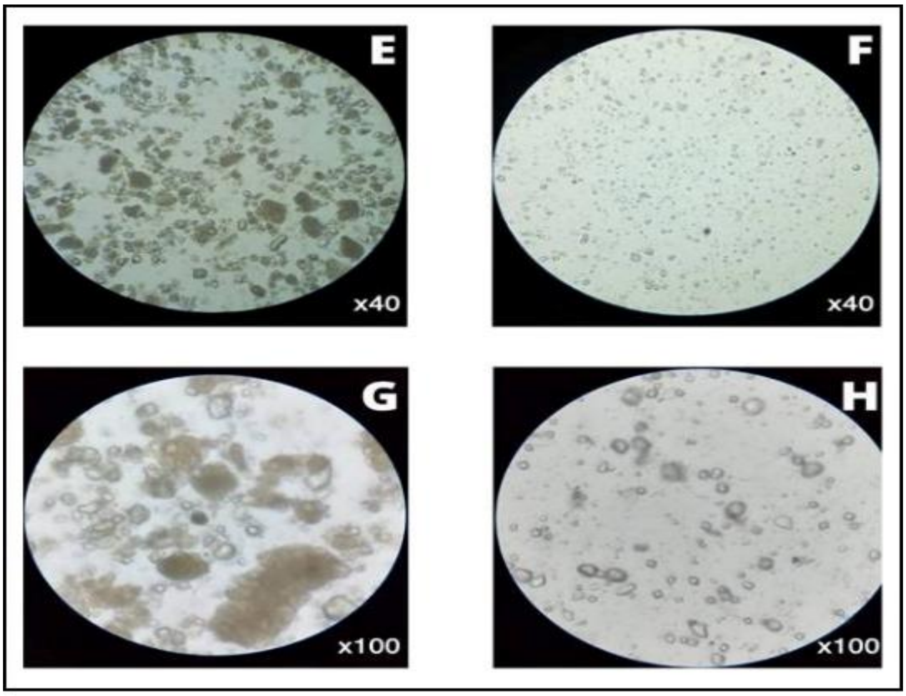
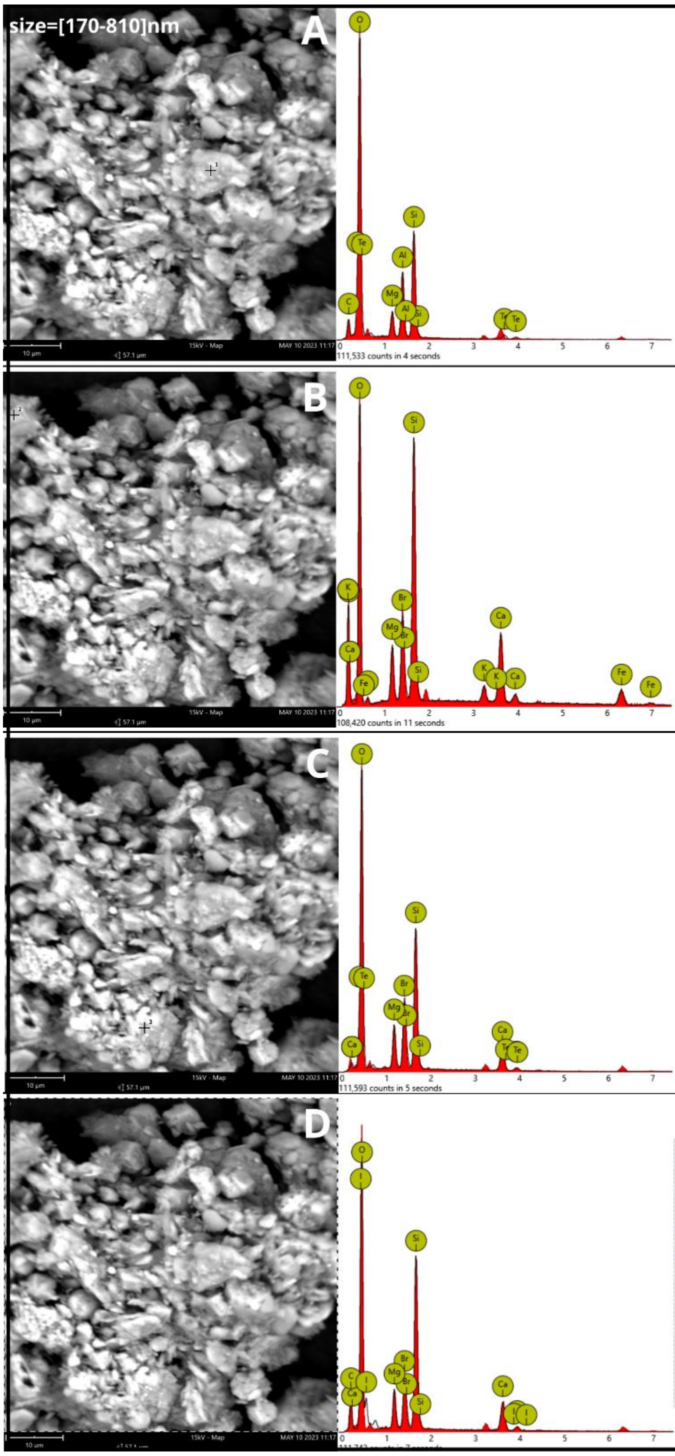


Figure 13: Light microscope analysis of red clay (Ex40, Gx100), red clay NPs (Fx40, Hx100)

I.3.5.Electronic microscope analysis

The images of clay NPs biosynthesized by *A. longa* aqueous extract obtained from SEM determined that this nanoparticle owned irregular crystalline shape with the size 170-810nm Moreover, EDX analysis of the obtained red and green clay NPs shown in figure 14 and 15 confirms the presence of elements in different proportions, with oxygen having the highest percentage, followed by carbon, silicon, carbon, Aluminum, Magnesium, Bromine, iron, Potassium, Tellriun, calcium.



A

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	64.00	57.53
14	Si	Silicon	8.54	13.48
6	C	Carbon	19.02	12.83
13	Al	Aluminium	5.34	8.09
52	Te	Tellurium	0.66	4.73
12	Mg	Magnesium	2.44	3.33

B

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	41.72	36.26
6	C	Carbon	41.18	26.87
14	Si	Silicon	7.82	11.92
35	Br	Bromine	2.26	9.83
20	Ca	Calcium	2.98	6.49
26	Fe	Iron	1.66	5.04
12	Mg	Magnesium	1.79	2.36
19	K	Potassium	0.58	1.24

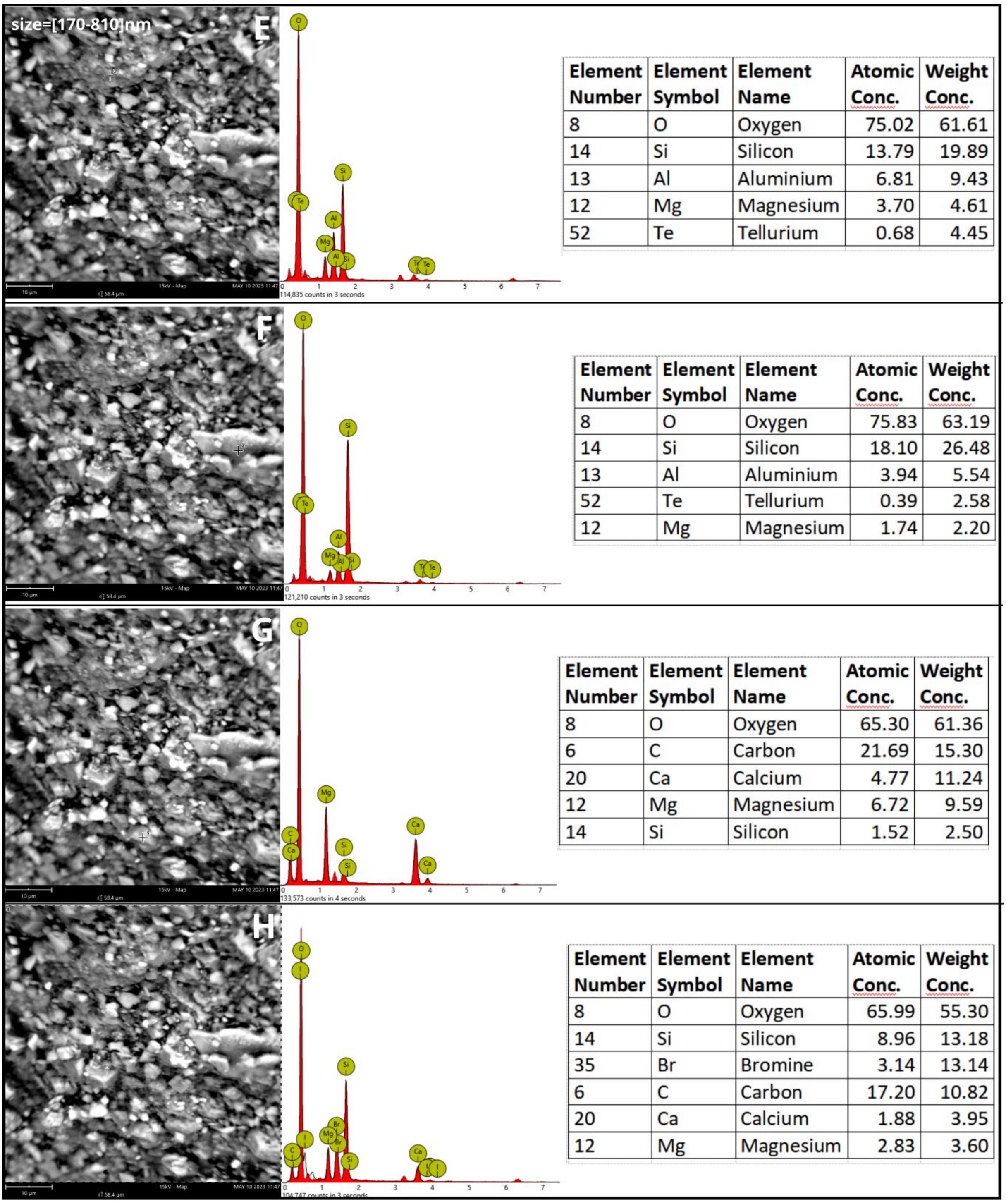
C

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	74.87	54.62
35	Br	Bromine	5.10	18.59
14	Si	Silicon	13.35	17.10
20	Ca	Calcium	2.83	5.16
12	Mg	Magnesium	3.80	4.21

D

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	56.56	49.81
6	C	Carbon	27.91	18.45
14	Si	Silicon	8.89	13.74
35	Br	Bromine	2.37	10.42
20	Ca	Calcium	2.15	4.74
12	Mg	Magnesium	2.12	2.84

Figure 14: Scanning electron microscope and EDX image of green clay NPs



Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	75.02	61.61
14	Si	Silicon	13.79	19.89
13	Al	Aluminium	6.81	9.43
12	Mg	Magnesium	3.70	4.61
52	Te	Tellurium	0.68	4.45

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	75.83	63.19
14	Si	Silicon	18.10	26.48
13	Al	Aluminium	3.94	5.54
52	Te	Tellurium	0.39	2.58
12	Mg	Magnesium	1.74	2.20

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	65.30	61.36
6	C	Carbon	21.69	15.30
20	Ca	Calcium	4.77	11.24
12	Mg	Magnesium	6.72	9.59
14	Si	Silicon	1.52	2.50

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	65.99	55.30
14	Si	Silicon	8.96	13.18
35	Br	Bromine	3.14	13.14
6	C	Carbon	17.20	10.82
20	Ca	Calcium	1.88	3.95
12	Mg	Magnesium	2.83	3.60

Figure 15: Scanning electron microscope and EDX images red clay NPs

I.4. Biological activities

I.4.1. Antioxidant activity

The FRAP assay was used for the direct assessment of the total antioxidant activity of a sample. Based on the inhibition percentage versus concentration curve equation we found the following equation y = 0.0323x + 3.364 (R^2 = 0.9792) for green clay NPs, y = 0.0152x +

4.2033 (R^2 = 0.9472) red clay NPs, y = 0.0224x + 37.389 (R^2 = 0.9616) for *A. longa* and y = 0.8735x + 15.124(R^2 = 0.9028) for vitamin C.

We found variation in the IC50 values, where the IC50 value of green clay NPs was lower than red clay NPs and *A. longa* had the lowest IC50 value among them all. This is in comparison to the standard IC50 value, which is vitamin C (Figure 16).

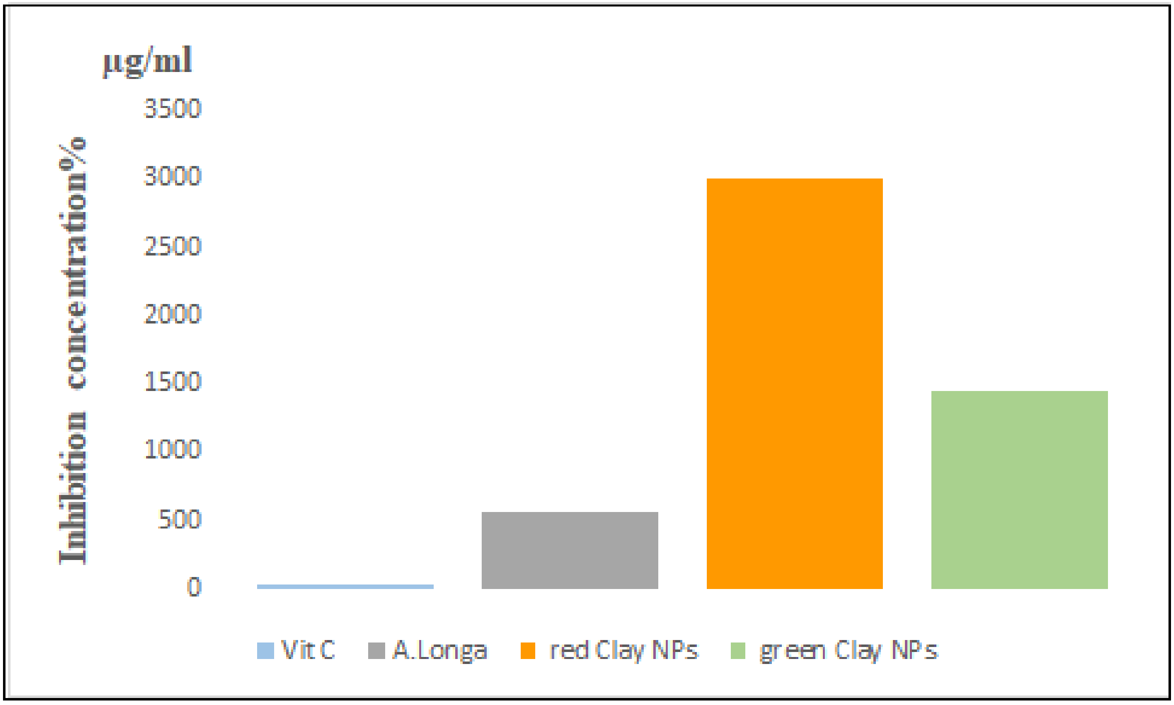


Figure 16: FRAP assay for aqueous extract of *A.longa* and red clay NPs and green clay NPs and vitamin C

I.4.2. Anti-inflammatory activity

I.4.2.1. Hemolysis assay

Dehydration and delayed proton equilibria of human blood erythrocyte membrane mediated by phosphate buffer (XPBS, pH= 7.4) induces membrane damage and subsequently hemolysis. The antihemolytic activity of aqueous extract of *A. longa* and clay red NPs and clay green NPs on human blood erythrocytes are presented in Figure 17 Based on the Protection percentage versus concentration curve equation we found the following equation of Antihemolytic activity y = 0.2775x + 2.5732 with high regression coefficient (R^2 =0.9803) (for clay green NPs), y = 0.0926x - 3.0271 in aqueous extract of *A.longa* with regression (R^2 = 0.9461), y = 0.1694x + 4.0263 in clay red NPs with regression(R^2=0.9043) and y = 0.0394x + 48.646 for diclofenac with regression (R^2=0.9947). Our results found the variation in the IC50 values, where the IC50 value of red clay NPs was lower than that of *A.longa* and green clay NPs had the lowest IC50 value among them all. This is in comparison to the standard IC50 value, which is diclofenac.

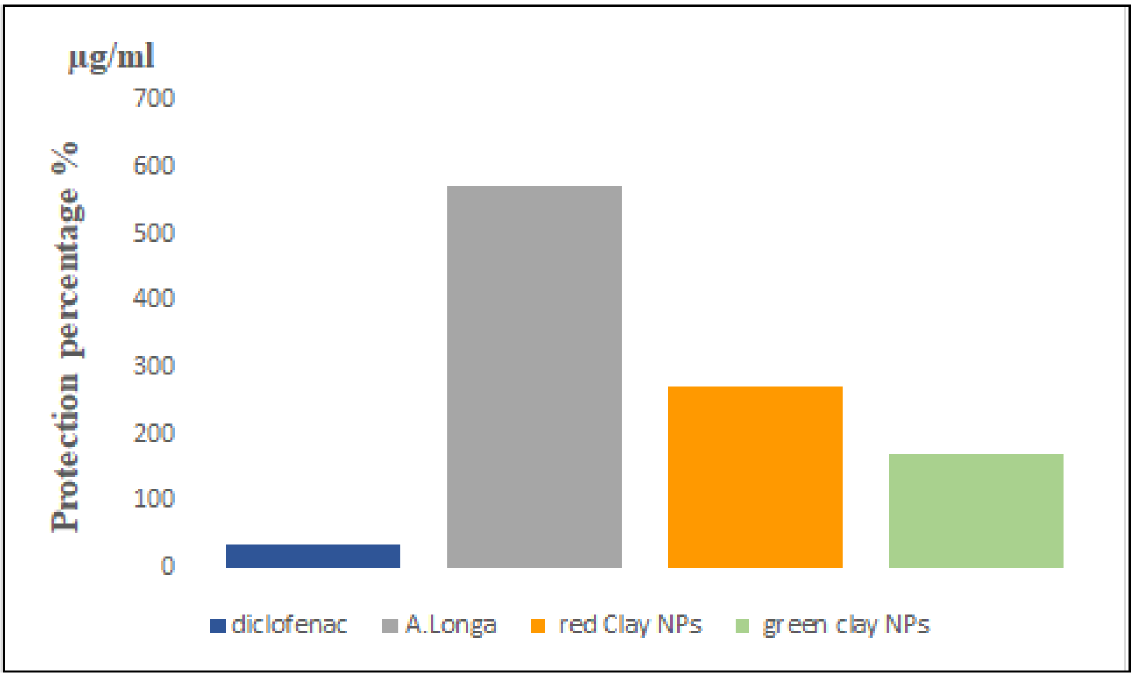


Figure 17: Antihemolytic activity of aqueous extract of *A.longa* and red clay NPs and green clay NPs and diclofenac

I.4.2.2. Protein denaturation assay

Based on the equation of inhibition percentage versus concentration curve we found the equation of anti-inflammatory activity y = 0.9048x + 48.647 with high regression coefficient (R^2 = 0.9945) for green clay NPs, y = 1.3261x + 4.9052 for *A.longa* with regression (R^2 =0.9669), y = 1.1509x + 6.9252 for red clay NPs with regression (R^2=0.9544) and y = 2.4035x + 15.643 for diclofenac with regression (R^2=0.9159).

Based on these results, we found different values of IC50, where we observed that green clay NPs had a lower IC50 value, indicating that it had a higher anti-inflammatory activity compared to the other samples tested Then, we observed that the IC50 values of red clay NPs and *A. longa* were close to each other, indicating that they had similar anti-inflammatory activity. This was in comparison to the standard IC50 value of diclofenac (Figure 18).

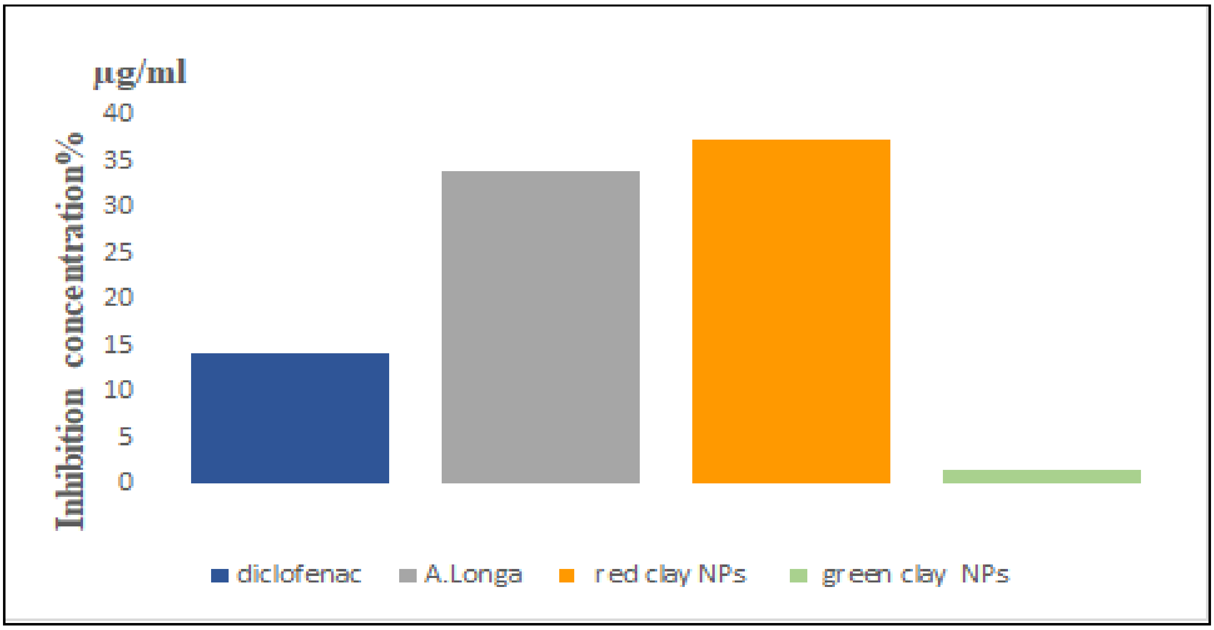


Figure 18 : Anti-inflammatory activity of aqueous extract of *A.longa* and red clay NPs and green clay NPs and diclofenac

I.5. Catalytic activity of nanoparticles

I.5.1. Photocatalytic Degradation of Dye

Results Represent the plot of % degradation (% Y) of the red and green clay NPs Phyto-synthesized by using *A .longa* . Visual observation of color change from blue to colorless indicates the degradation of methylene blue dye at different time intervals (A) 1 h, (B) 2 h, (C) 3 h, (D) 4 h, (E) 5 h, (F) 6 h and (G) at 7h. The photocatalytic degradation process makes it abundantly evident that the absorbance steadily lowers with increasing exposure time. The decolorization percent of methylene blue dye under irradiation are displayed in (Figure 19).

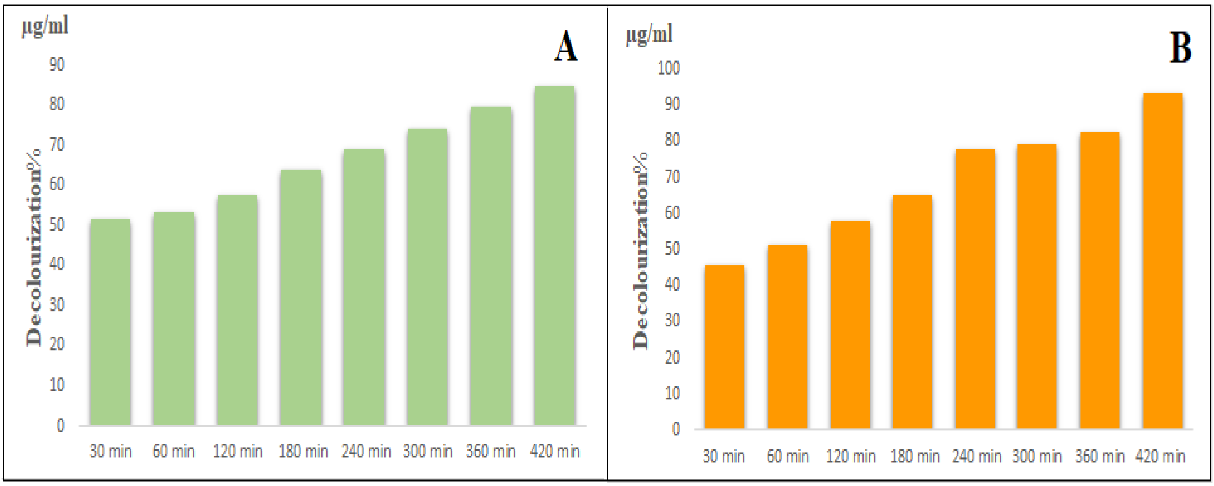


Figure 19: Photocatalytic activity of green clay NPs (A) and red clay NPs (B)

I.5.2. Sonocatalytic

The efficacy of red and green clay nanoparticles in sonocatalysis was assessed through the degradation of MB dye under ultrasound irradiation. Visual observation of color change from blue to colorless indicates the degradation of methylene blue dye at different time intervals figure 20 displays the sonocatalytic degradation of the MB solution, indicating a reduction in MB dye concentration with increasing irradiation time.

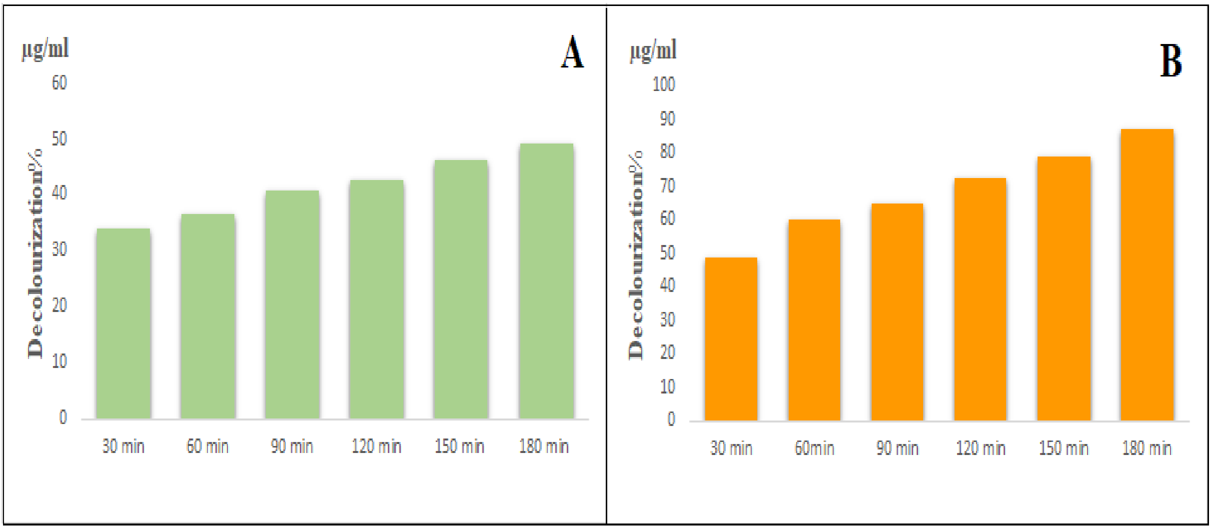


Figure 20: Sonocatalytic activity of green clay NPs (A) and red clay NPs (B)

II.Discussion

Clays have played an important role in medicine from the dawn of mankind, ranging from oral ingestion for therapeutic purposes (geophagy) to wound healing and hemorrhage inhibition (Joshi et al., 2009; Mousa et al., 2018). Clays are still widely applied as active ingredients in pharmaceutical formulations, typically administered either orally as antacids, gastrointestinal protectors, and anti-diarrheic or topically as dermatological protectors and anti-inflammatories (Ding et al., 2016). Clay nanoparticles, composites and hydrogels are emerging as a new class of biomaterial with exciting. The objective of the present work is to study the effectiveness of herbal *Aristolochia longa* on reduction of metals of clay for obtaining its nano form of clay. In addition, nanoscale compounds have various therapeutic advantages, including low side effects. for this we chose clay NPs.

II.1. Phytochemical analysis

The phytochemical analysis proved the presence of bioactive compounds such as phenols, flavonoids alkaloids, Tannins, Terpenoids, Reducing compound, Saponins and Steroids These compounds have an antioxidant activities which could play a major role in the capture of free radicals, The identification of alkaloids, saponosides, tannins, flavonoids and the dosage of polyphenols have been implemented because the majority of the pharmacological effects of plants are attributed to these substances (Faye et al .,2022) .The tannins have antioxidant or even healing properties, they will be able to chelate the metals (Desmier, 2016), Terpenoids form an essential part of direct and indirect defense systems against herbivores and pathogens (Bohlmann & Keeling, 2008). Alkaloids can be used as antimalarial, analgesic, antispasmodic, bactericidal and These molecules very often cause acute toxicity when they are present in high concentration (Rujjanawate et al., 2003). The results of the study of Ibtissam *et al*., (2021) indicate that treatment with *A longa* root powder Reduces liver damage caused by nickel and nickel accumulation. These compounds have an interest in the prevention and treatment of various diseases including cancer, inflammatory diseases, diabetes, osteoporosis, vascular and neurodegenerative disease (Keerthi et al., 2014).

The presence of phenols and flavonoids in the root of *A. longa* According to El Omari et al., (2020), the presence of Saponins in the root of *A.longa* corroborated by several work that has been demonstrated by the rich extracts of *A. Longa* in compounds, this is the case of the work published by Benarba & Meddah , in 2014 and Benarba et al., in 2016, the presence of Alkaloids, Tannins and Terpenoids These results are in agreement by Bouteldja et al., (2019), Merouani et al., (2020) ,the presence of Reducing compound, Steroids according to study of (Attou et al., 2020) .

II.2. Total polyphenols and flavonoids

Results of quantitative analysis showed the presence of polyphenols and flavonoids in a large amount in the roots of *A. longa*. The existence of the important amount of the total polyphenols and flavonoids. It can be said that the plant is an important source of total phenols. The concentration of polyphenols and flavonoids of our extract is widely higher compare to other studies. In a recent study of Djeridane et al., (2006) reported that the aqueous fraction of *A.longa* from root contains 1.47 ± 0.02 mg GAE/g of polyphenols and 0.81 ±0.02 mg RE (Rutin equivalent)/g of flavonoids. The finding of Ibtissam *et al.*,(2021) reported that total phenolics and flavonoids content in *A. longa* roots are 9.20 ± 0.05 mg GAE/g and 2.17 ± 0.04 mg RE/g, respectively. Previous studies reported that the aqueous extract from *Aristolochia longa* contains a high amount of phenols and flavonoids. The difference in the concentration of bioactive compounds may be caused by several factors like the extraction method and the change in the environmental conditions also the soil composition can be a factor (Treutter, 2010). The phenolic and flavonoid compounds of plants can influence glucose metabolism by several mechanisms such as inhibition of carbohydrate digestion and absorption of glucose in the intestine, Stimulation of insulin producing cells, modulation of glucose release from the liver, activation of glucose uptake in insulin-sensitive tissues, and modulation of hepatic glucose production (Derouiche et al., 2020) .

II.3. Characterization of clay nanoparticles

3.1. X-ray diffract meter (XRD)

XRD is one of the most important characterization techniques to reveal the structural properties of NPs (Khan et al.,2019). The XRD pattern of the photosynthesis red clay nanoparticles indicate the presence of a large number of sharply pronounced reflections, which are indicative of the crystallinity of the synthesized particles structures (Shutov et al., 2017). The presence of major sharp peaks around 2Ɵ = 27.12 and 30.60 and 42.3 and 44.37 and 52.59 and 62.91 implies the presence of NioNPs Fe2O3NPs, Cu2ONPs, AgO NPs and FeO NPs and ZnO NPs, respectively. Our results are in agreement with other reports that point to similar outcomes by using XRD analysis (Zhang et al., 2021 ; Bibi et al., 2019 ;Guzman et al., 2022;Anandalakshmi et al., 2016 ; Liu et al., 2020).

Concerning the green clay nanoparticles, the sample was analyzed by XRD. the appearance of diffraction peaks in a pattern of ranges at 21.6°, 28.32°, 32.35°, 43.48°, The peak positions with 2θ values are in good agreement with those of FeONPs, ZnONPs, AgOINPs, Fe3O4 respectively. The ecofriendly method of nanoparticles synthesis is confirmed by XRD technique as showing in study of (Senapati et al.,2013 ; Lakshminarayanan et al., 2021 ; Mehta et al., 2017 ; Mahdavi et al., 2022).

3.2. Fourier transforms infrared spectroscopy (FTIR)

Results of FTIR analysis show a broad peak at 3294 cm−1 could be attributed to the O–H stretching vibration (Ajitha et al., 2015). The two low-intensity peaks at 2919 cm−1 and 2879 cm−1 were the symmetric and asymmetric C–H of the alkane group (Álvarez-Chimal et al., 2022). The peak at 1612 cm−1 is attributed to C=C stretching vibration (Jayachandran et al., 2021), while the C–O- vibrations appeared around 1035 cm−1 (Dobrucka and Długaszewska, 2016). These vibrational frequencies suggest the availability of different biologically active Phyto molecules on the nanoparticles that are available for adsorption, which can mediate the synthesis of the nanoparticles from their respective metal clay. The FTIR nanoparticles' spectra showed a new peak at approximately 798cm−1(Ibrahim et al,.2023) and 563 cm-1 (Bhattacharya et al,.2014), which was characteristic of Cu2+ nanoparticles and FeO respectively. The phenolic compounds of *A. longa* extract contain υ (OH) hydroxyl groups that can interact with the surface metal ions, stabilizing the green-synthesized nanoparticles (Murthy et al,.2020). The spectra of the red clay NPs and green clay NPs show the presence of peaks identified in the spectrum of the plant extract but with a slight shift in the positions of their appearance and this could be a result of the interaction of the functional groups with the nanoparticles (Jobe et al., 2022).

3.3. UV-Vis Spectroscopy

NPS has unique optical properties that are sensitive to the size, composition, concentration, state of agglomeration, and refractive index near the surface of the NP, making the UV-vis a valuable tool for the identification, characterization, and study of biological nanomaterials NPS Red Clay NPS Green Clay NPS was monitored by the Spectrophotometer Toolkit UV from 250 to 410 nm The maximum absorption peak of the clay nanospheres in the spectral nanoparticles in UV is up to 300 nm. Moreover, these sharp peaks are due to the presence of particles within the resulting system. It may also be indicative of a wide distribution of autosomes.

Several studies confirm our findings like study of Abbas,(2019) which has found the peak absorption of the ZnO nanoparticles amounted to 376 NM and Schwaminger et al., 2017 in the study of Iron Oxide amounted to 300 nm. and the UV–Vis's spectra revealed absorption copper nanoparticle in the study of Vardhana et al .,(2022) peaks 350 nm.

3.4. Microscopic techniques

The LIGHT microscope technique was used for visualizing the particles and shapes of red clay NPs. The biosynthesized and capped of red clay NPs and green clay NPs using the *A.longa* structural morphology and crystallinity were better supported by LIGHT analysis. It is evident from the images that the particles are nearly spherical with a uniform size distribution. the morphology and size of NPs may be depended on the interactions between metal atoms and reducing biomolecules (Abbasi et al., 2023), which in this study is *A.longa*. The image showed a flake-like appearance due to the aggregation of several thousand clay particles. The flakes are so dense and interconnected that there are no clear boundaries between each other.

In the other side, SEM and EDX (Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy) were used to analyze morphological properties, size and initial composition of clay nanoparticles made from *A. longa* extract. An assessment of SEM images of clay nanoparticles is given. SEM analysis revealed irregular crystalline shape clay nanoparticles with an average particle size of 170 to 810 nm. The growth and basic character of clay nanoparticles in the spectrum has been confirmed. EDX images of uncounted clay nanoparticles using biosynthesis appeared and showed that they are the highest for oxygen, just as different peaks of carbon and NPs elements appear in variable ranges. This accumulation may be due to the existence of secondary metabolites in the plant separately (Abbas & Fairouz 2022). Comparable to many other studies, research of Meshkatalsadat et al., (2022), which prepared a new biosynthesis of CaO nanoparticles through a green and environmental method by using *Crataegus pontica C. Koch leaf* extract as a green agent to reduce and stabilize the calcium oxide nanoparticles with size ranging from 40 to 65 nm,. In addition, the study of Jadhav et al., (2022), show that the CaO NPs were produced by using *Moringa oleifera* aqueous extract. Structural description of spherical schemes obtained using SEM-EDS indicates the presence of a spherical nanomaterial essentially assembled at an average size of 32.08 nm observed. Also, in the study Derouiche *et al*., (2020), the SEM analysis of MgO showed that Size 50-400 nm with specific binding energies. In the study of Önal *et al*.,(2019) , SEM images revealed that FeNPs were synthesized by leaves Aqueous extract of *E. japonica* , they obtained the particle’s size of 89 nm for the synthesized FeNPs .

II.4. Antioxidant activity

Antioxidant activity evaluation of NPs is one of the most important elementary studies in nano science and nanotechnology due to antioxidants has an important impact on the operational of all biological systems (Ghosh et al.,2022).

Antioxidant activity was observed for *A. longa* aqueous extract, clay NPs, based on the observed in their IC50 values. The high antioxidant potential given by the clay NPs may be correlated to the number of groups (OH) involved in the structure indicated by the FTIR analysis. In fact, phenolic groups facilitate the conversion of clay-to-clay NPs due to its electron-donating ability (Salari et al., 2019 ; Essghaier et al., 2022). The lower IC50 value indicates a stronger ability of the extract to act as an antioxidant, while the higher IC50 value indicates a lower antioxidant activity (Salari et al.,2019). The percentage of inhibition ratio of *A.longa* and NPs compared to synthesized using of *A. longa* aqueous extract with that of ascorbic acid as a standard compound in a dose-dependent manner in FRAP assay. As IC50 value for *A.longa* and NPs was more than that for ascorbic acid, so a higher concentration of the *A.longa* and NPs is needed to produce a similar effect (Pyrzynska& Sentkowsk 2021).

The anti-oxidant activity result studies have reported that *A. longa*. plant extract carries a strong antioxidant effect. Our result showed that *A. longa* is a good source of phenolic compounds and flavonoids. Phenolic and flavonoids have been reported to be the most important phytochemicals responsible for the antioxidant capacity (Salari et al.,2019).

The data collected by the polyphenol dosage and the measurement of the antioxidant potential of extracts of *A. Longa* showed a very good correlation between the total polyphenol content and the antioxidant activity (Ouedraogo et al.,2015). Flavonoids also serve as natural antioxidants as they contain multiple hydroxyl groups as the main antioxidant active groups. The hydroxyl groups on the benzene ring form stable semi-quinone radicals after losing the H atom, terminating the chain reaction of free radicals. Therefore, the number of hydroxyl groups in the flavonoid extract and their positions closely correlate to their antioxidant activity (Huang et al.,2023). The richness of our extracts in total phenols and flavonoids, suggests that any biological activity depends on the presence of certain metabolites inside the tissues of the plant (Zbadi et al,.2018).

Biological methods of nanoparticle synthesis, often termed green synthesis offer a potential alternative due to non-toxic materials, milder reaction conditions, and energy utilization (Vinayagam et al.,2023). Our results indicated that, the main constituents of *A. longa* roots were terpenes, flavonoids, saponins, tannins, and alkaloids, phenols, Reducing compound.

Several studies indicated that the clay is an element contained complex of minerals like iron silice zinc and copper and many other metals according to region and type of clay (Sipos et al.,2008). Green synthesis of clay nanoparticles based on *A. longa* maybe lead to formation of some number of metals oxide like copper oxide, zinc oxide and iron oxide.

In general, metallic nanoparticles display remarkable and extraordinary antioxidant impacts (Zhang et al., 2021). The results obtained in our study are in line with many studies in which effect monitored the antioxidant capacities in NPs.

The reaction mechanism for the formation of zinc oxide nanoparticles in clay nanoparticles is as a result of the reaction of the zinc ions present in the clay solution with the polyphenols present in *A. longa* such as the tannins, glycosides, and flavonoids forming complexation.

This is followed by hydrolysis reaction to form zinc hydroxide due to the presence of the hydroxyl group in the polyphenol. Calcination and decomposition reaction shortly follows to form zinc oxide nanoparticles (Basnet et al.,2018) Also, the synergistic reaction of all the phytochemical components present in the extracts (terpenes, flavonoids, saponins, tannins, glycosides/carbohydrates, and alkaloids) act as both reducing and stabilizing agent by reducing Zinc to the 0 valence state (Basnet et al.,2018).In previous studies, use of tomato extract to obtain zinc oxide nanoparticles was effective due to the presence of ascorbic acid which acted as a reducing and capping agent (Sutradhar et al.,2016) while the flavonoids, and phenols played the vital role of reduction of zinc oxide when *A. longa* roots extract were used (Hashemi et al.,2016). On the other hand, the flavonoids such as flavone, glycosides, and flavonols present in chamomile flower extract acted as the reducing and capping agents for synthesis of zinc oxide (Fouad et al.,2018).

Among several metallic-element nanoparticles in biosynthesized clay nanoparticles, copper nanoparticles have been under an exceptional spotlight. Plant-based molecules are of great interest to the scientific community because they have a wide range of sizes and structures with various biological functions (Vijayakumar et al.,2021). Therefore, extract namely *A.longa*, have been used as a reducing and capping agent to biosynthesize copper nanoparticles in clay with a wide variety of applications. Redox reaction takes place with the help of biomolecules viz flavonoids, terpenoids, polyphenols, alkaloids, tannins, in the plant extracts, and the metal ions were reduced to produce respective nanoparticles and also assist in maintaining uniform stability. Chung et al., 2017 in the study of nanoparticles (CuNPs) by mixing copper acetate solution with leaf extract of *Eclipta prostrata* the study showed an antioxidant capacity in CuO NPs.

Iron is the main compound of both type of clay which can be transforming on nano-size by biological method, Iron nanoparticles (FeNPs) are among the most promising metallic nanoparticles (Nahari et al.,2022). The high oxidation tendency of phenols, especially in an alkaline condition, can facilitate the initiation of the nucleation process and helps in the reduction of metal salts to metal nanoparticles. Plants are capable of reducing metallic ions on their surface as well as different tissues. Plants with a higher capacity of reduction and accumulation of metal ions are used for metal-based nanoparticle synthesis. In the green synthesis approach, the phytocompounds present in the extract of the plant are involved in the reduction of the metal ions, they also act as stabilizers of the clay metals including iron nanoparticles. Polyphenols, terpenoids, and flavonoids in the plant act as both a reducing agent and a capping agent resulting in the production of nanoparticles FeNPs (Batool et al.,2021).

II.5. Anti-inflammatory activity

Extracting plant materials is the first major step towards testing the biological activities of this plant. When a whole extract is used, there is a good chance for synergism between active components that might be lost when each of these components is isolated. Such it was discovered in several medicinal tests, including those for anti-inflammatory activity (Ouidad et al.,2020). In the clay, the metals are transferred into nanoparticles and gain potent anti-inflammatory activity. The results obtained show that our plant extract have significant anti-inflammatory capacities and are comparable to the protective effect of the diclofenac as a control. These results are confirmed by study of Khenchil& Bensam, (2018) which found that the plant exhibited a significant anti-inflammatory effect in vivo by reducing the size of the inflamed paw with 80.31%.

In the other hand, our results show that clay NPS (red and green) have a significant capacity to fight inflammation. the nanoparticles of clay have moderate anti-inflammatory power. The richness of metals nanoparticles in clay are probably responsible for the anti-inflammatory activity of clay nanoparticles like FeO, ZnO, CuO…. This study may be in agreement with the study of Bhattacharya et al (2014) which indicate the anti-inflammatory role of Fe_2O_3 /C nanoparticles and with the study of Ouidad,et al in (2020) which confirm that CuO NPs have a greet anti-inflammatory activity.

II.6. Catalytic activity

Photocatalysis is a viable option for the effective management of the degradation of dyes (Bustos-Guadarrama et al,.2023), due to its effectiveness in producing hydroxyl free radicals which able to degrade numerous dyes (Almehizia et al., 2022; Hegazey et al., 2020).

Sonocatalytic Ultrasound passing through aqueous solution can facilitate breaking chemical bonds via sonolysis, resulting formulation of free radicals (Abdurahman et al., 2021).

The effectiveness of photodegradation and sonocatalytic of the red and green clay nanoparticles was investigated using methylene blue (MB) dye as a model. These dyes were selected by different colors before and after degradation, which is helpful to confirm the completion of the reaction. Methylene blue belongs to a category of cationic thiazine dye which is applied in several fields including chemical and biomedical applications. Methylene blue demonstrates deep blue color in its oxidized state in the aqueous solution and is colorless when reduced (Al-Hamoud et al., 2022). Semiconductor metal oxide nanoparticles such as TiO2, ZnO, CuO etc, show great potential against these contaminated dyes (Tahir et al., 2020), Our XRD characterization showed presence these particles that were the reason for decomposing the dye over time, silver nanoparticles as a photo-catalyst in the study (Ringwal et al.,2021). In the photocatalytic and acoustic stimulation processes, the percentage of dye degradation in the photocatalysis was higher than its sum in the photocatalytic process. Photocatalysis was always faster than the related single processes due to the formation of more reactive radicals.

Organic dyes play vital roles in the textile industry, while the discharge of organic dye wastewater in the production and utilization of dyes has caused significant damage to the aquatic ecosystem. so that the mechanisms of photocatalysis, sonocatalysis, and sonophotocatalysis in the treatment of organic dye wastewater and the recent advances in catalyst development (Wang & Cheng,2023). The ability of nanoparticles to degrade toxic dyes by the process of photolysis and sonolysis in the treatment of polluted water in an environmentally friendly way makes it more efficient against organic and inorganic pollutants, heavy metals, microbes (Tahir et al., 2020) and detoxification (MeenaKumari & Philip, 2015).

Conclusion & Perspective

❖ Conclusion

The study of the pharmacological effect of clay and phytoformulation of nanoparticles is a fascinating and emerging area of research in the field of pharmacology. Clays have been used for medicinal purposes for centuries in traditional medicine, and recent research has revealed their potential as a carrier for drug delivery.

Our work has been for the objective to evaluate the effect of *Aristiolochia longa* on the synthesis of Clay nanoparticles and the biological activities of these nanoparticles , Phytochemical analysis shows that *Aristolochia longa* is very rich in polyphenols, flavonoids, tannins, alkaloids, saponins, terpenoids and reducing sugars. This allows us to discover a new source of bioactive molecules have an important therapeutic effect against several pathologies.

Results of characterization of nanoparticles indicate that IR, UV-Vis, SEM and XRD results confirmed the presence of pic related the different metal oxide like ZnO, CuO, FeO… which strongly linked to the role of plant extract on the reduction effect of metals of clay on the oxide form this thanks to the chemical compounds of the *Aristolochia longa* aqueous extract.

Study of the biological activity of aqueous extracts of *Aristolochia longa* and Clay nanoparticles has shown that both molecules possess a remarkable antioxidant power. These results could therefore to use these compounds against several diseases induced by pro-oxidant agents.

Anti-inflammatory activity has also been investigated, and the results have shown that both clay and plant extracts have anti-inflammatory properties which can be used in the preparation of many new products used in pharmaceutical and medical field.

Photocatalytic and sonocatalytic degradation of dye has been studied, and the results have shown that clay nanoparticles can be used in the catalyze of dyes degradation. These results open a new way to use the clay nanoparticles in the detergent field.

❖ Perspective

Through the results of this study, we can benefit the clay nanoparticles synthesized by biologically methods in the following areas:

1) The combination of clay nanoparticles and phyto-compounds of plant presents an exciting opportunity to develop innovative drug delivery systems that can increase their therapeutic effects and decrease the adverse effects.
2) Discovering new pharmacologically active compounds, which can be extracted from natural sources (*A. longa*) and used to develop new drugs.

3) Studying the pharmacological effect of clays nanoparticles and the plant composition is a promising area of research that has the potential to revolutionize drug delivery and advance the of pharmacology, nutrition and other fields.

However, there is a need for more in-depth analyzes to know the other components of the clays and to study other biological efficacy and therapeutic effects of the clays as well as other uses in various fields of life.

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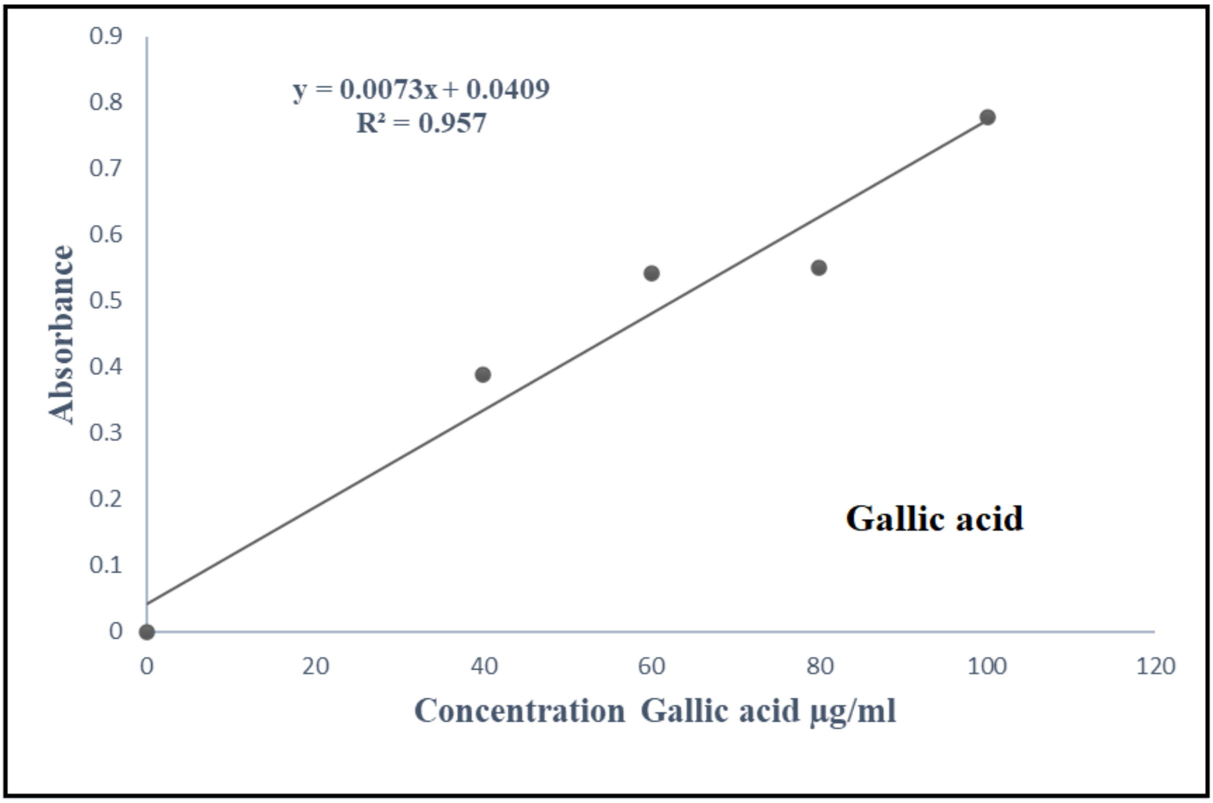
Annexes



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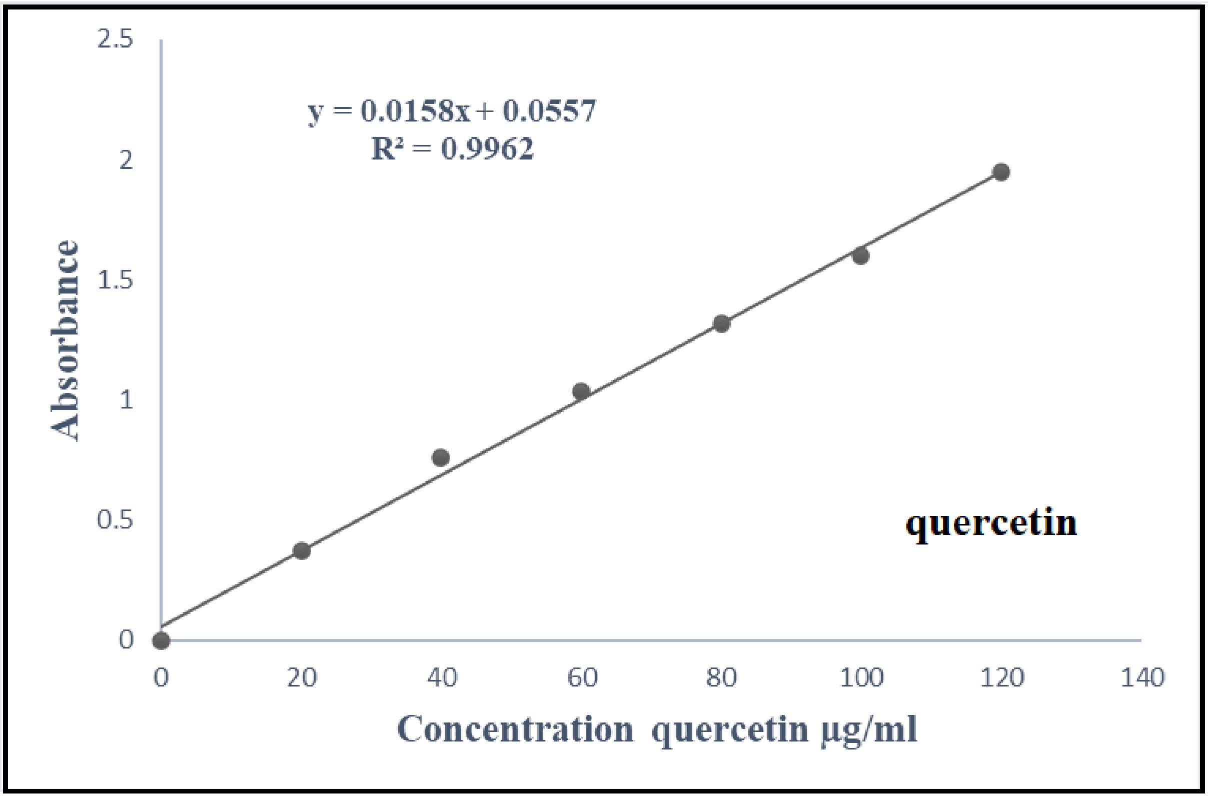

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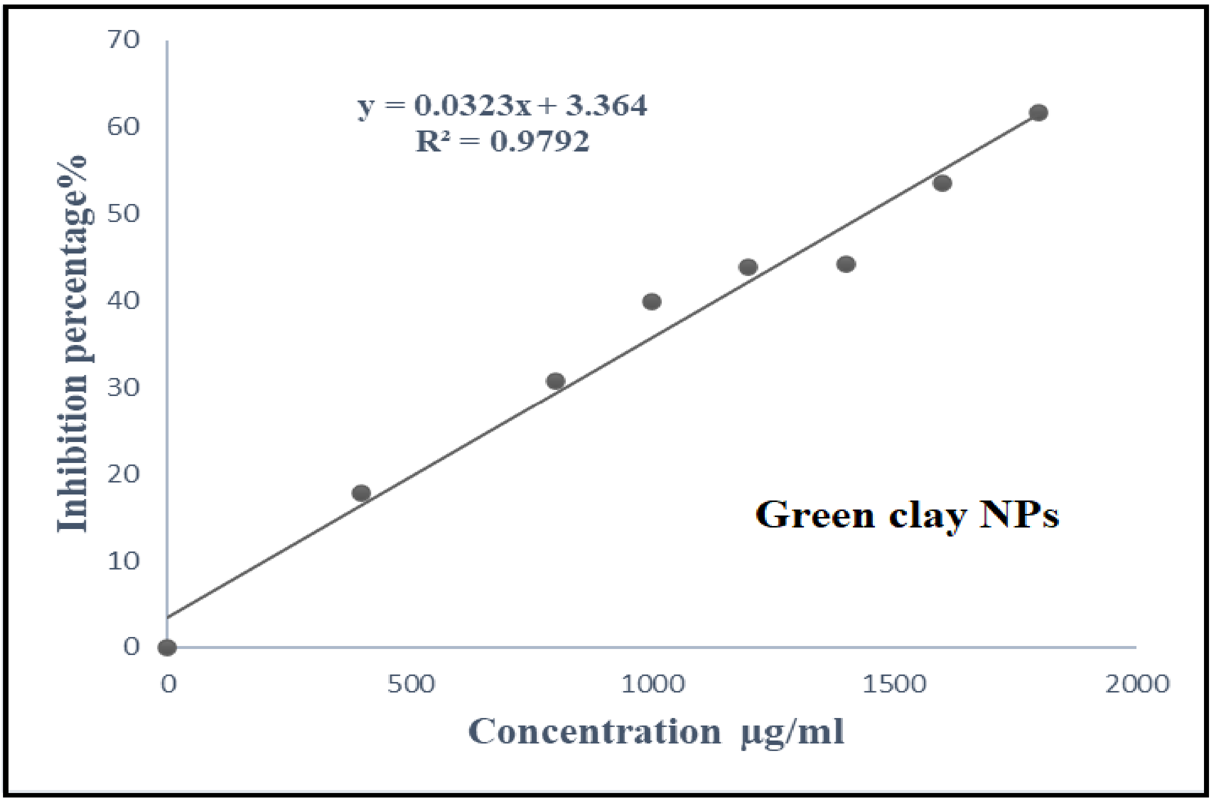

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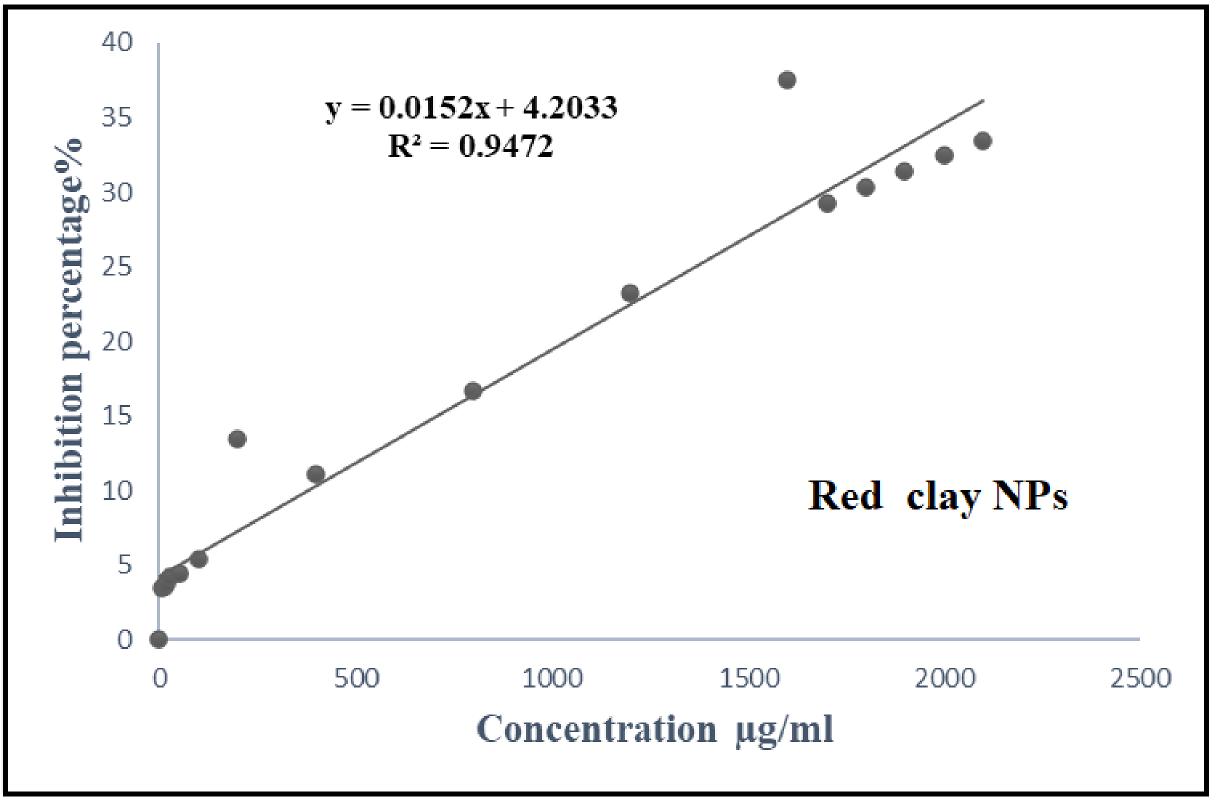

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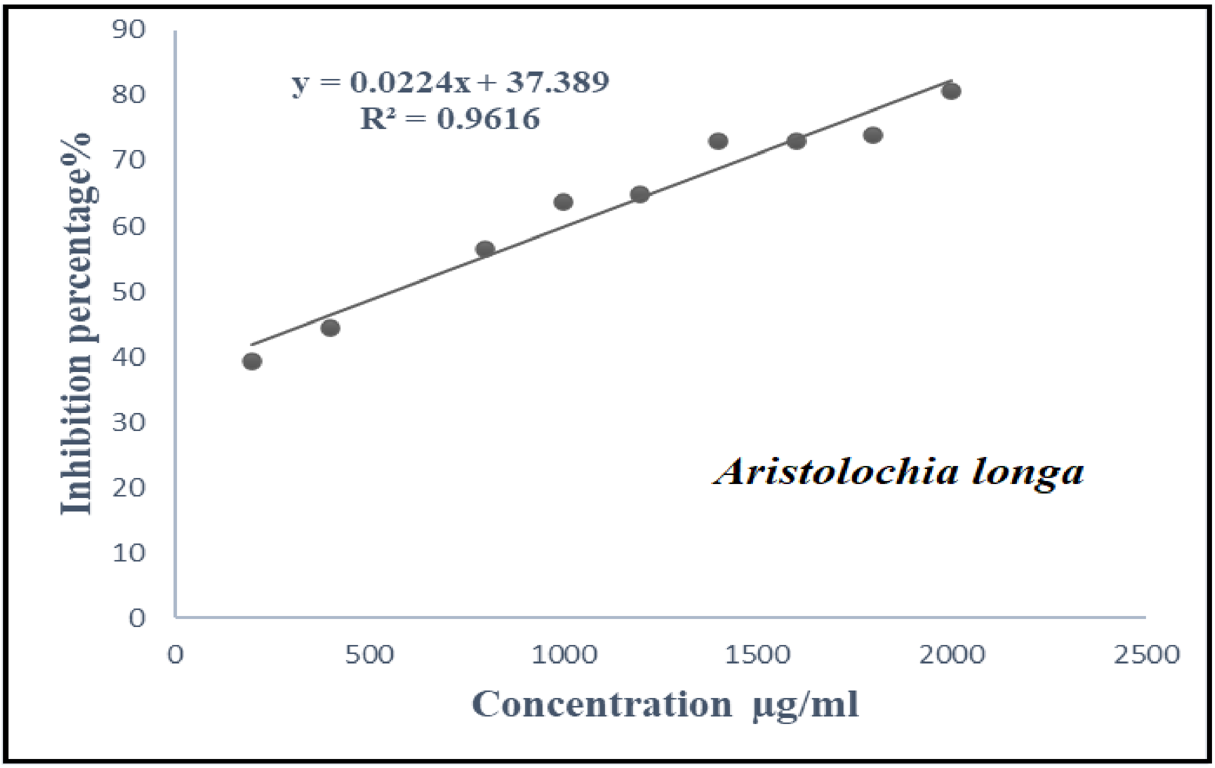

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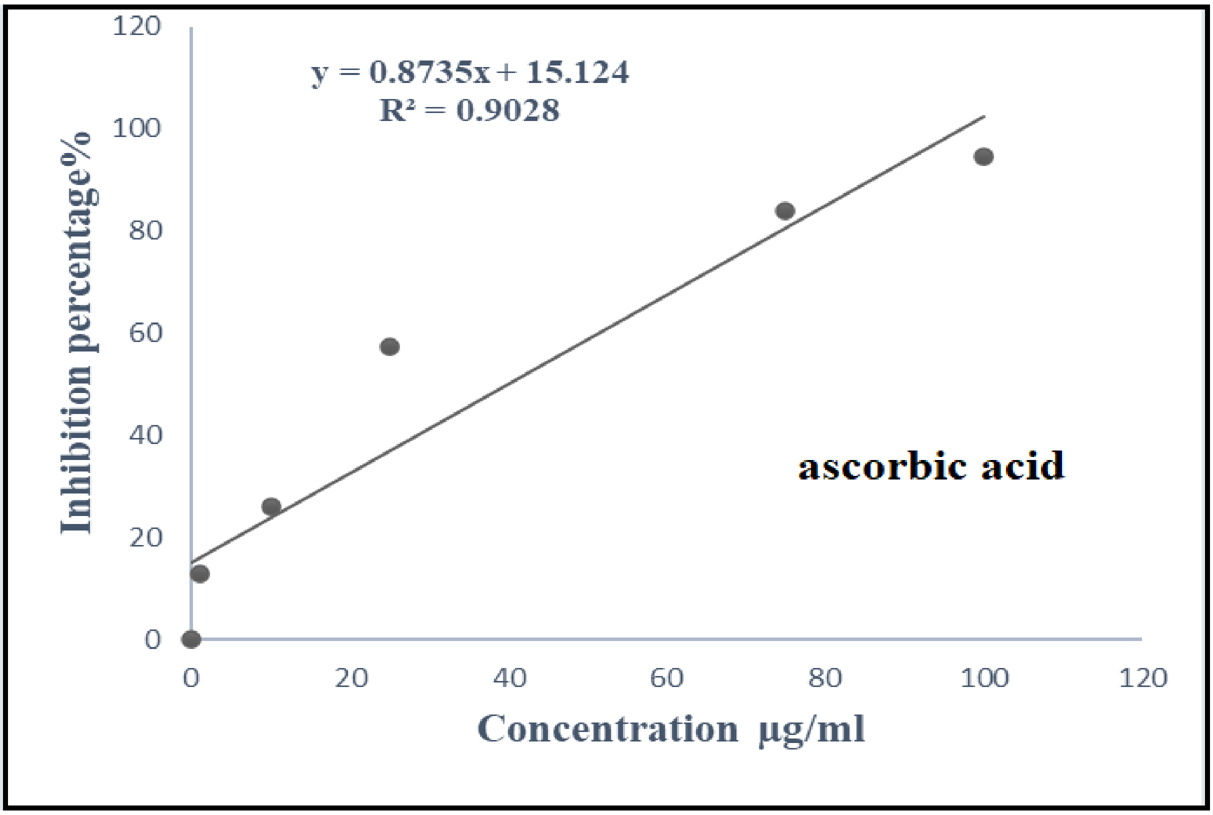

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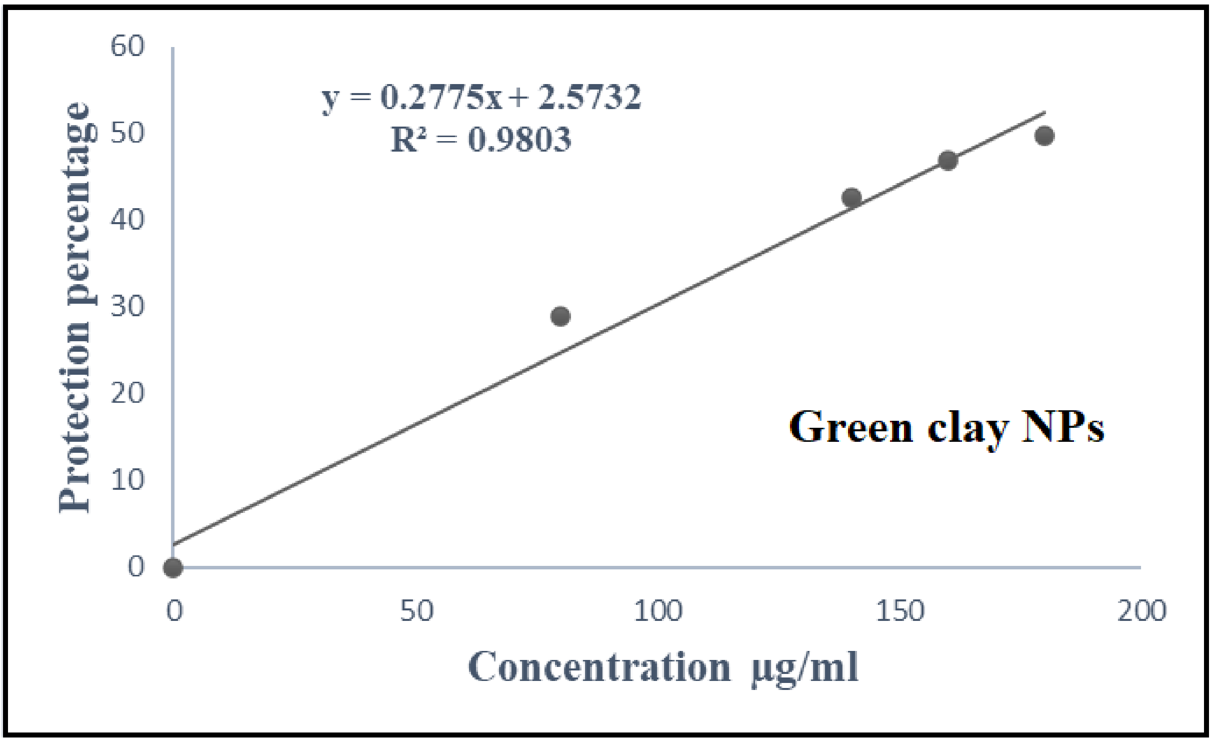

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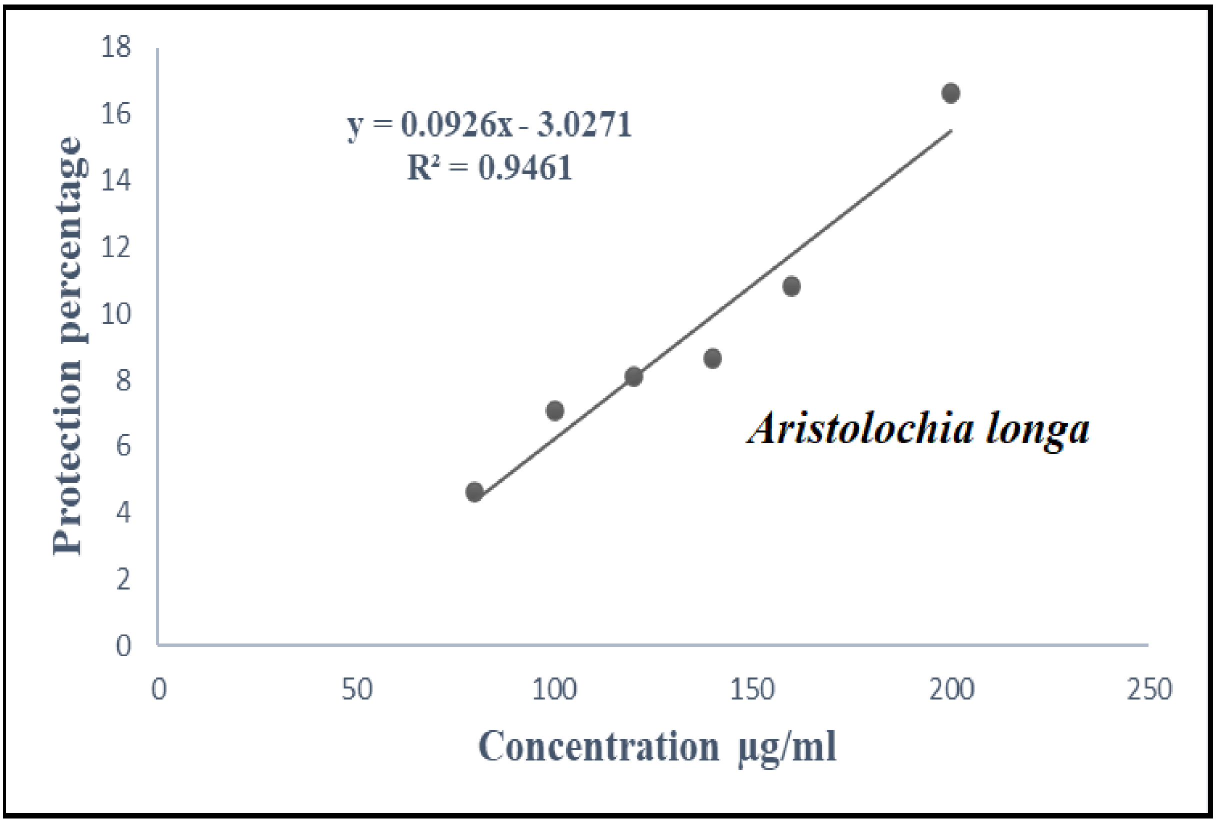

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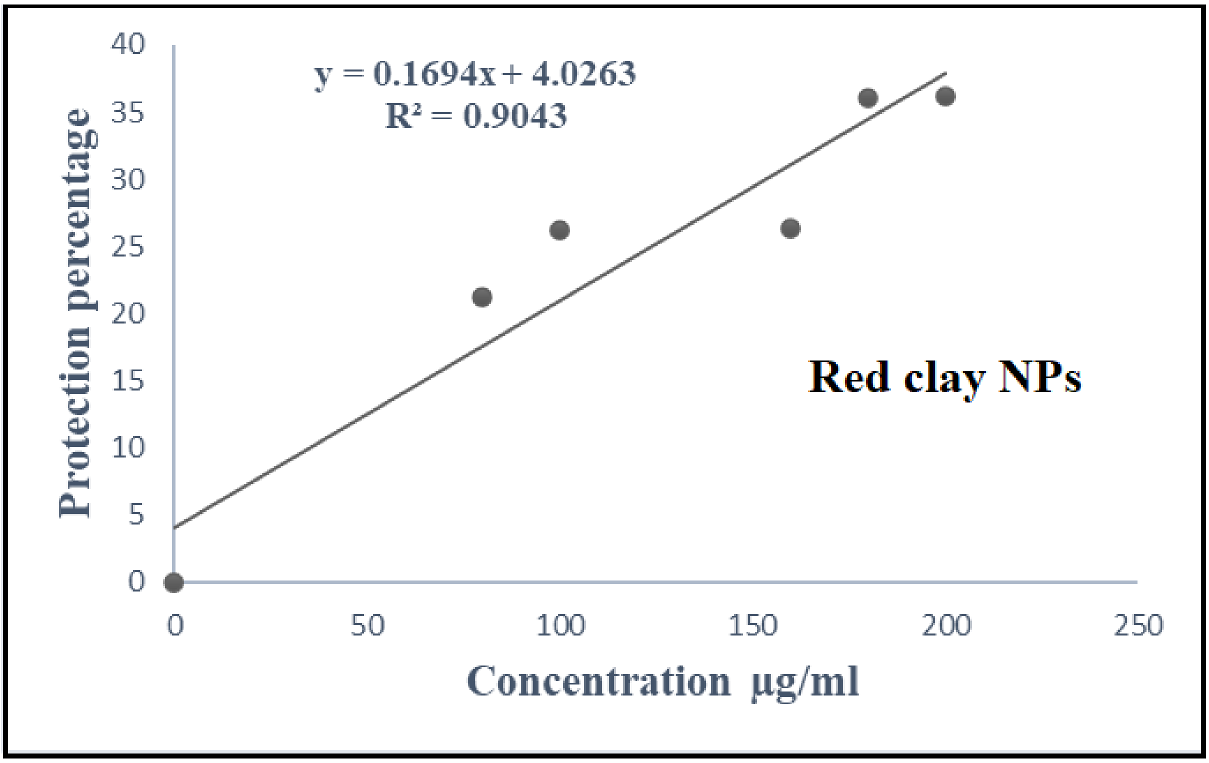

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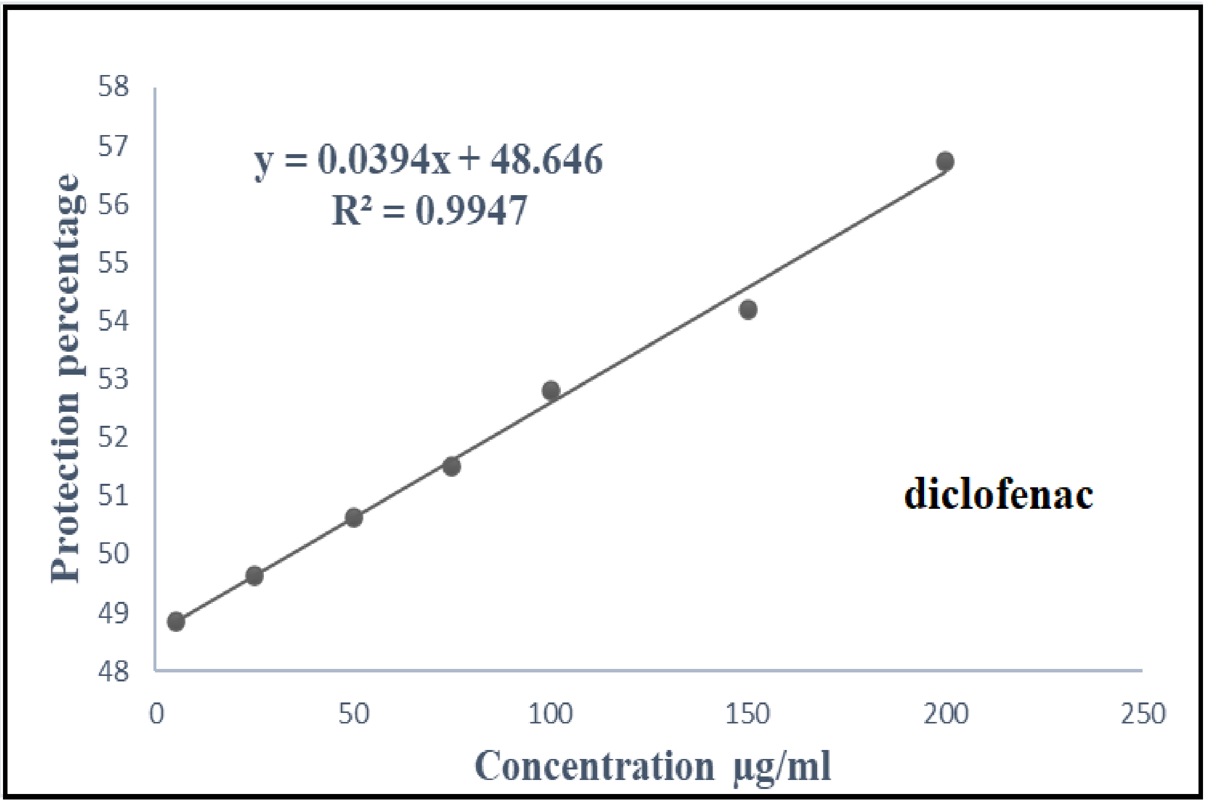

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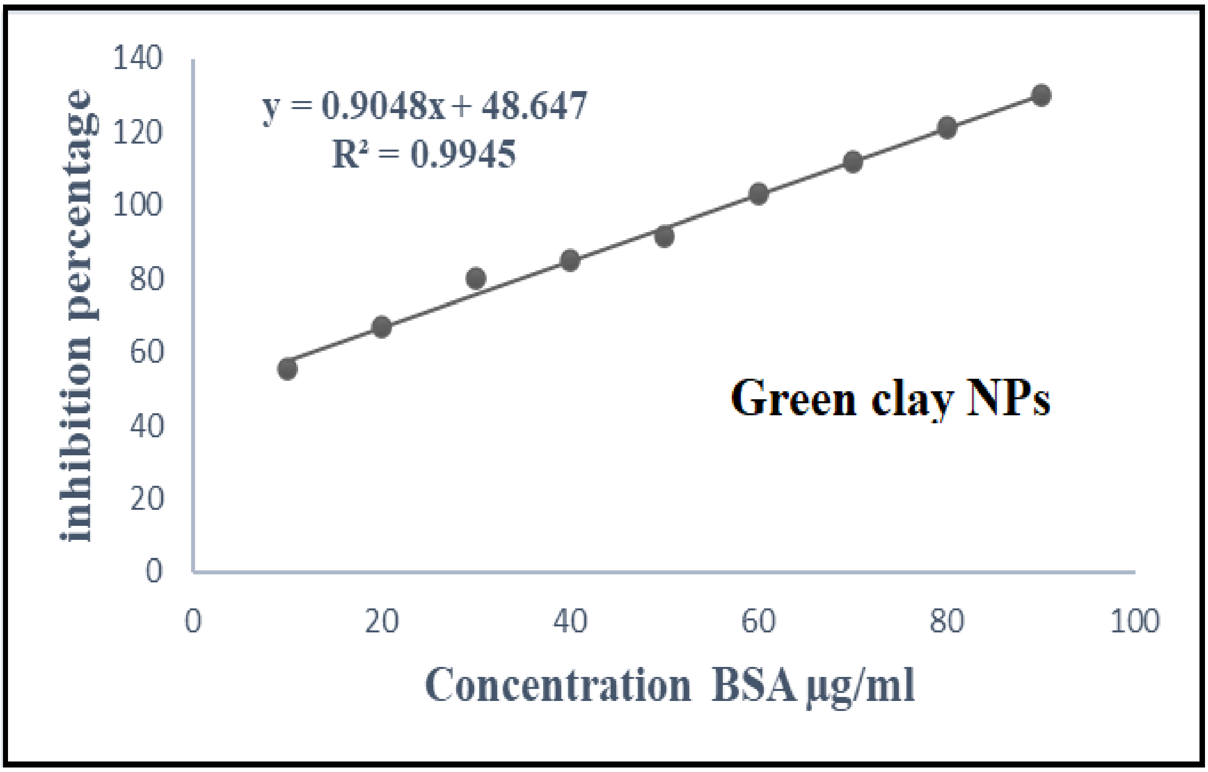

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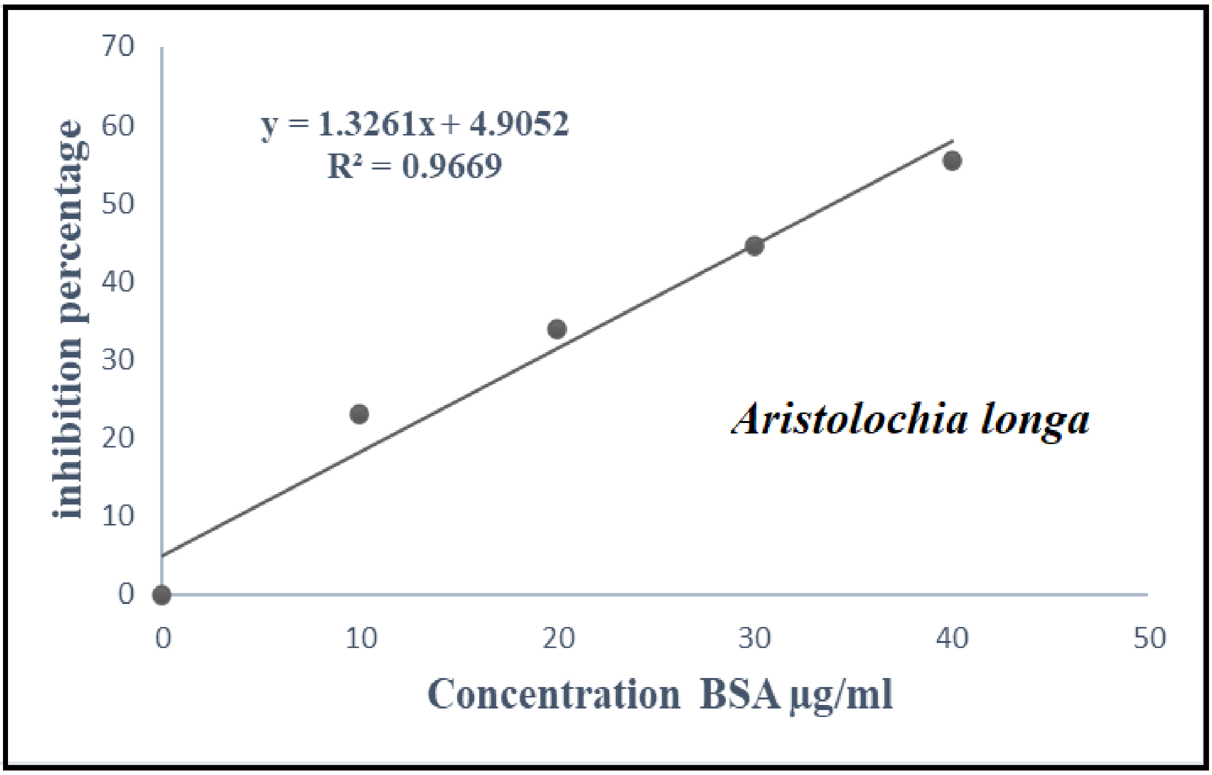

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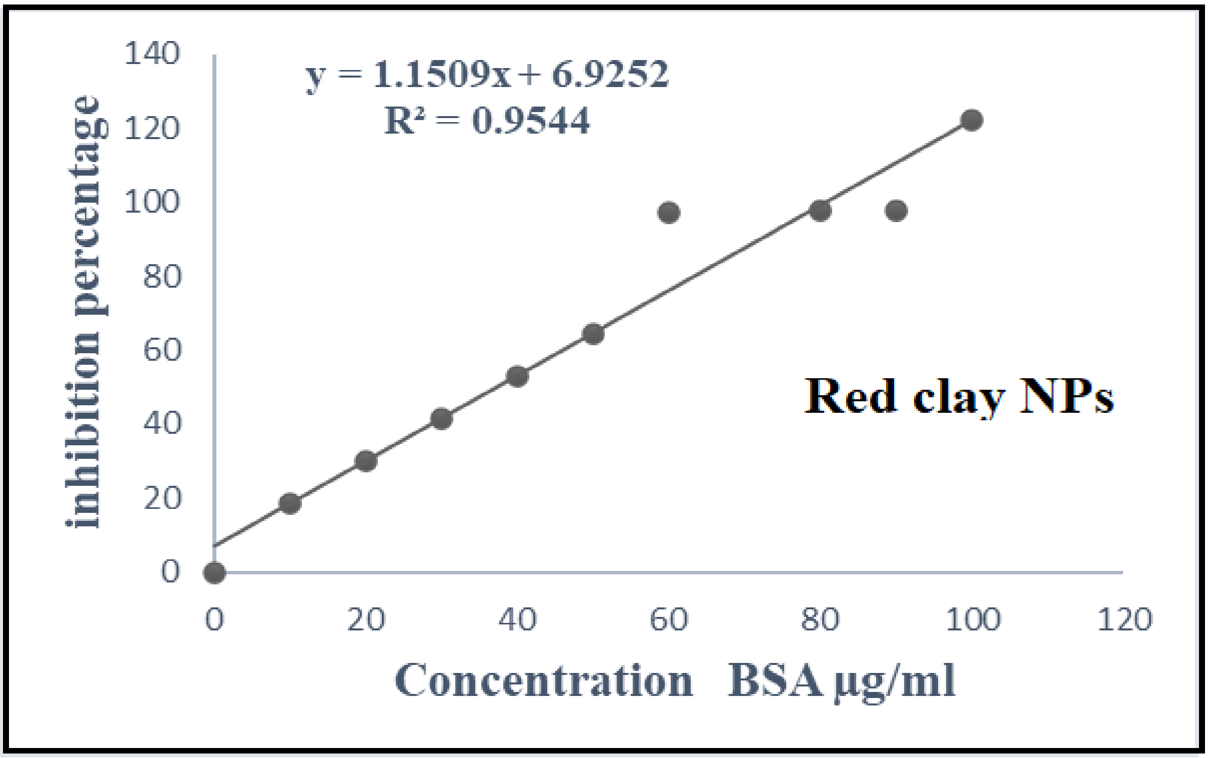

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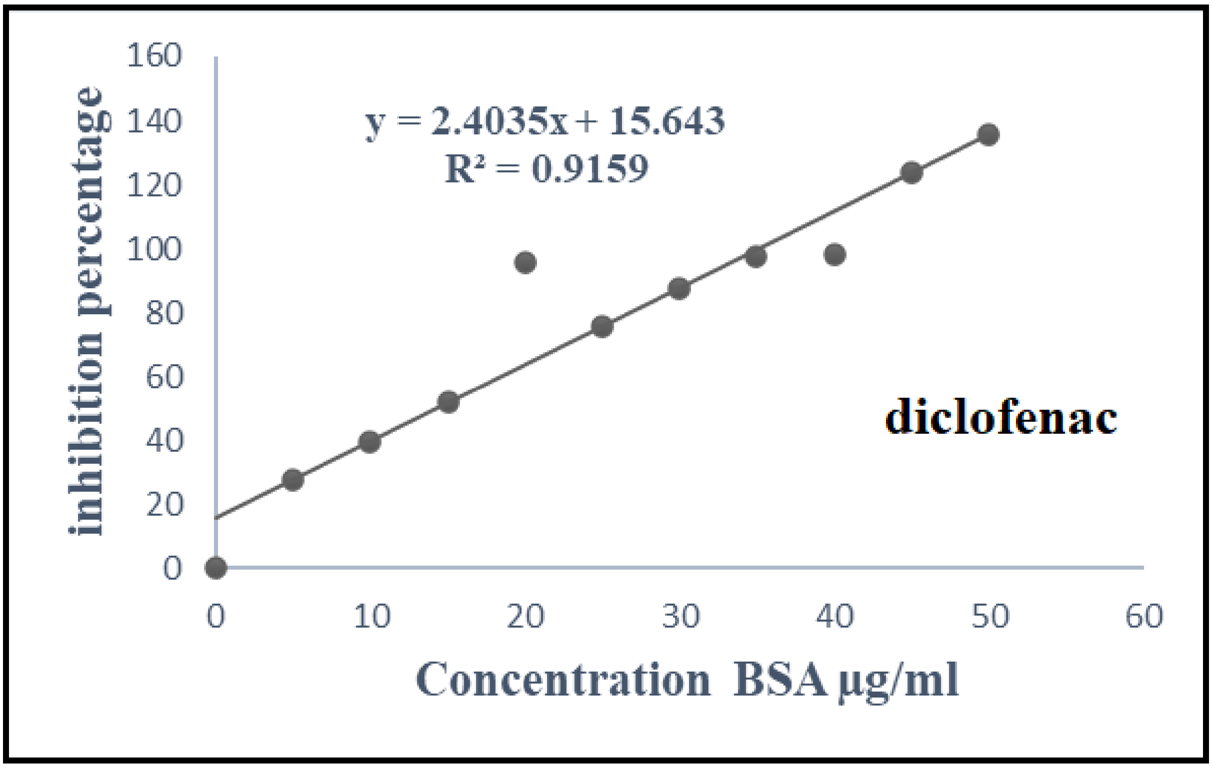

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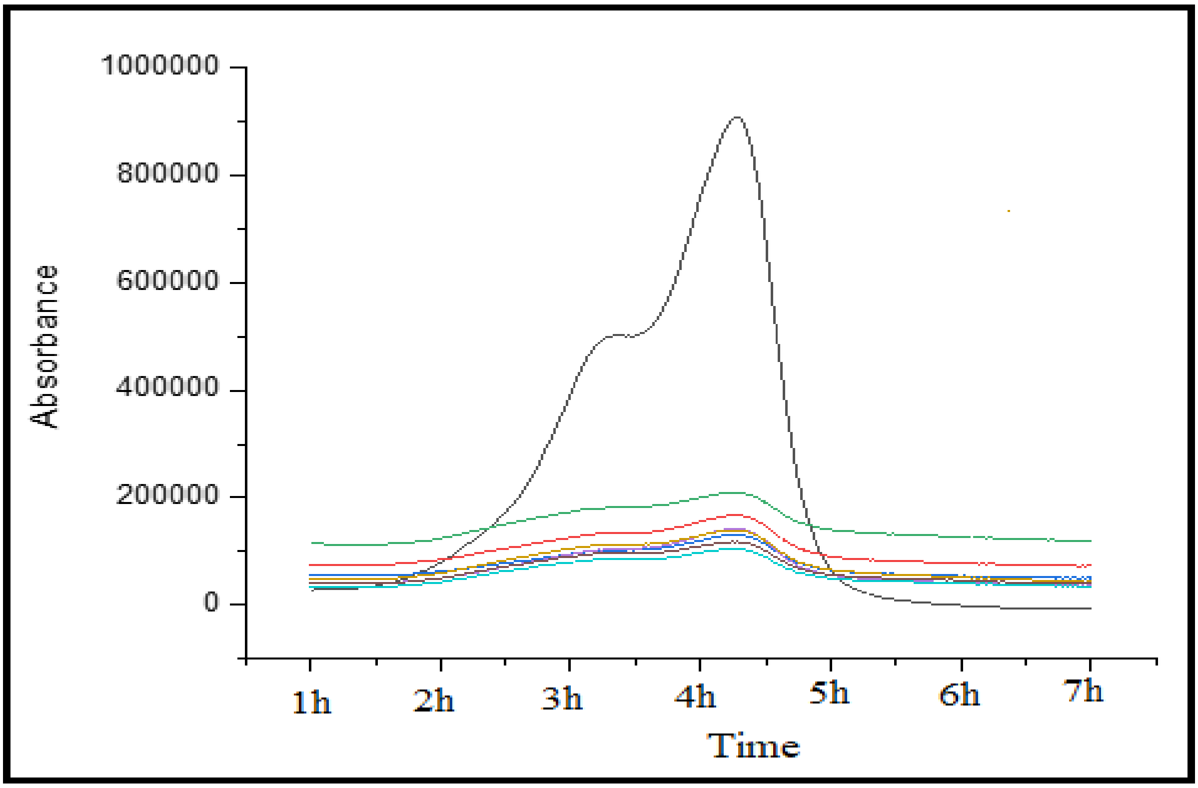
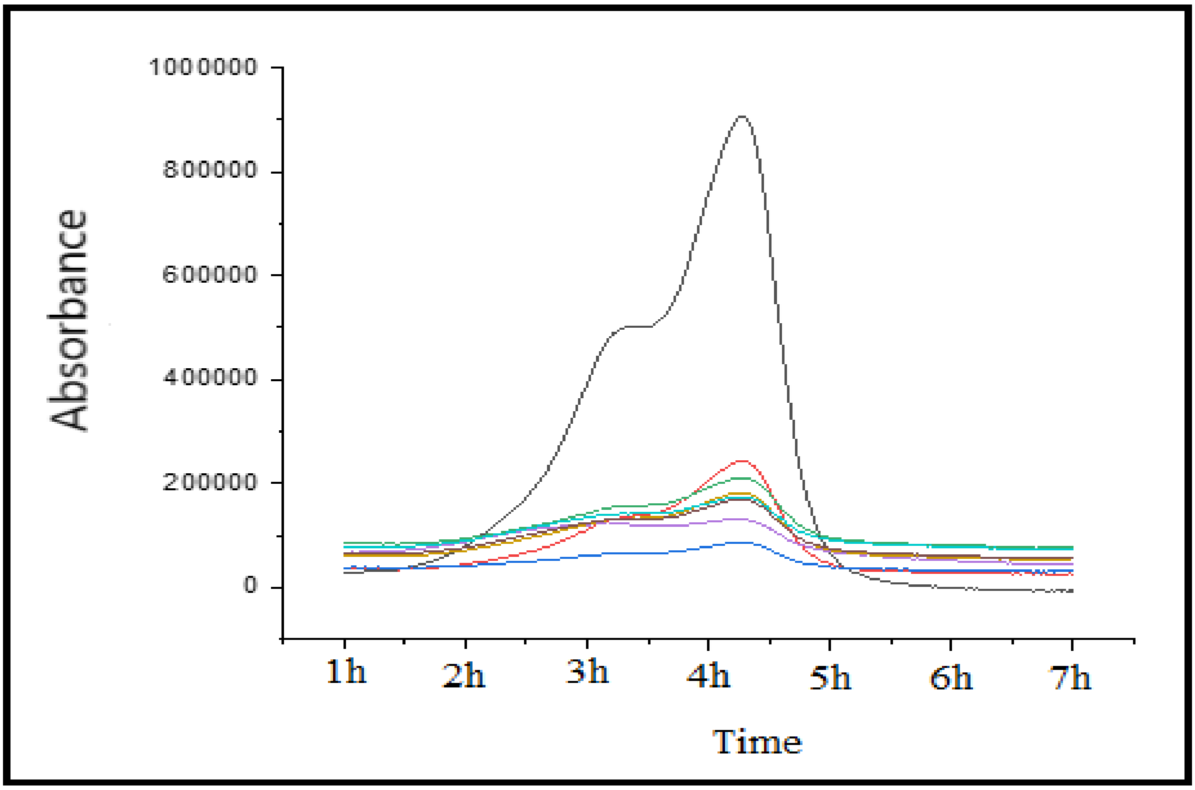


Annexe 15 : Absorbance of methylene blue in photocatalytic condition red clay NPs.



Annexe 16: Absorbance of methylene blue in photocatalytic condition green clay NPs.

RYAD

المعهد الوطني الجزائري للملكية الصناعية

INSTITUT NATIONAL ALGÉRIEN
DE LA PROPRIÉTÉ INDUSTRIELLE

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الجمهورية الجزائرية الديمقراطية الشعبية

RÉPUBLIQUE ALGÉRIENNE
DÉMOCRATIQUE ET POPULAIRE

R2-FO-03
E1

Nature de la demande de protection *

Brevet d'invention ☑ Extension de la demande internationale selon le PCT ☐ Certificat d'addition ☐

[71] - DEPOSANT[S] : *Nom, Prénom, [dénomination], et Adresse complète*

Université Echahid Hamma Lakhdar - El Oued
BP 789 le nouveau groupement * El Oued * Algérie * 39000

Nationalité du ou des déposants Algérienne

[72] - INVENTEUR[S] : *Nom, Prénom, Adresse*

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SEBAA, Achouak, cité Al-Sahn, El Oued, 39000, Algérie
HAFRI, Asmaa, Belle vue, Hamraya, El Oued, 39000, Algérie
CHETEHOUNA, Sara, Cité belle vie, El Oued, 39000, Algérie
BOULAARES, Islam, cité sidi Abdallah, El Oued, 39000, Algérie
DEROUICHE, Samir, Pôle Universitaire, Cité Echott, El Oued, 39000, Algérie

[54] - TITRE DE L'INVENTION :

Crème naturelle à base d'argile rouge pour les soins de la peau

[30] – REVENDICATION DE PRIORITE (S)

[31] - N°[s] de dépôt	[32] - date[s] :	[33] - pays d'origine	Nature de la demande

Numéro de dépôt	Date de dépôt	Heure
221053	21 DEC. 2022	13:21

N° de la demande internationale et date internationale de dépôt

Visa

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El Hafsi
Service Dépôt
D.Z.P
Chef de Service