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Theme

# *Synthesis of silver nanoparticles using saussurea costus and study of its antimicrobial and antioxidant properties*

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Before the jury composed of :

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*Academic year 2023/2024*

# Dedication:

*I dedicate this project:*

***To my dear mother,***

***To my dear father,***

*Not every word in the world can express the immense love I have for you. You have instilled in us a sense of responsibility, optimism and self-confidence.*

*Who have never stopped praying for me and supporting me so that I can achieve my goals.*

*Your advices always rank my step towards success. Your patience, your encouragement and your sacrifices are for months the indispensable support that you always have I wear.*

*I hope today to answer the hopes that you have founded in me and embrace at the height May God keep you and provide you with health and happiness so that you set the torch lighting me.*

***To my brothers, FAWZI,ISMAIL,NADIR.***

***To my dear sister "FADIA" and her husband "SLIMEN",***

*For their moral support and valuable advice throughout my studies.*

***To my grandfather.***

*This work is dedicated to my grandfather, who died very early, who always motivated me and motivated me in my study, I hope this work is appreciated and an honorable gesture as a sign of gratitude for a girl who always prayed that his immaculate spirit would culminate in heaven.*

***To all my friends:(KHADIJA, AMINA, BOCHRA, ROKIA, NESRINE, ….).***

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*For her understanding and sympathy.*

***To my dear, DJAWED***

*Who helped and supported me in difficult times.*

***To all my family and my step family***

*Who participated so that I could succeed.*

***NESRINE***

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*I dedicate this hard work to:*

***My father …My role models and my ideals***

***My belloved mother …Example of dedication and giving***

*Words can hardly describe my thanks and appreciation to you. You have been my source of inspiration, support, and guidance. You have taught me to be unique, determined, to believe in myself. I am truly thankful and honored to have you as my parents Thank you for your unwavering love and support along this journey I have taken. I love you both always and forever.*

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*to my friends, with whom this university brought me together, in specially: Rokaya and Karima*

*(Rokaya: I'm more than thankful to your support and encouragement during the time .)*

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***The price of success is hard work, dedication to the work at hand, determination that whether we win or lose, we have done our best to the task at hand and at last thank myself for what I gave throughout this journey.***

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# Table of contents

# List of Figures

## Bibliography Part



## Experimental Part

# List of Tables

## Bibliography Part



## Experimental Part



# List of Histogams

# List of abbreviations:

**Ag:** Silver.
**$Ag^{+}$:** Silver ions.
**$Ag^{0}$:** Metallic silver.
**Ag No3:** Silver nitrate.
**ATCC:** Collection de cultures de type américaines.
**BCE:** Before the Common Era.
**Ca2+:** Calcium homeostasis.
**C.albicans:**Candida albicans.
**C2H6O :** Ethanol.
**$C_6H_5OH$:** phenol.
**C6H8O6 :** Ascorbic Acid.
**DMF:** N, N-dimethylformamide.
**DMSO:** dimethyl sulfoxide.
**DNA:** Deoxyribose Acid Nucleoside.
**DPPH:**2,2-diphenyl-1-picrylhydrazyl.
**D :**Diamter.
**ENPs:** Engineered nanoparticles.
**e:** Electron.
**Etc :** End of thinking capacity
**E.g:** For example
**FCC:** Face centered cubic.
**FT-IR :** Fourier Transformed Infrared Spectroscopy.
**HIV-1:** Human immunodeficiency virus.
**H+ :** Hydrogen cation.
**H2:** Dihydrogen.
**$IC_{80}$:** 80% inhibitory concentration.
**IC50 :** 50% half (50%) inhibitory concentration (IC) of a substance.
**I%:**Inhibition percentages.
**Kr:** Krypton.
**K.pe:** Klebsiella pneumoniae.
**LPS:**lipoproteins, and phospholipids.
**MH:** Muller Hinton.
**ml:** Millilitres.
**min:** Minutes.
**NPs:**Nanoparticles.
**NPs M:** Nanoparticles manufactured.
**Nalco:** sodium hypochlorite.
**NF-KB:** Nuclear factor-kappa B.
**$NaBH_4$:** Sodium borohydride.
**Nm:** nanometer.
**PUF:** Atmospheric particles « ultrafine ».
**rpm :** Revolutions per minute.
**S.costus:**Saussureacostus..
**STL:** Sesquiterpene lactones.
**SPR:** Surface plasmonic resonance.
**TiO2:** Titanium dioxide.
**UV-VIS:** Visible ultraviolet spectroscopy.
**XRD :** X-ray diffraction.
**µl :** Microlitre.
**µg:** Microgram.

# *General Introduction*

## General Introduction:

Saussurea lappa known as vernacular "costus" is a medicinal plant used in traditional medicine. Current research is focused on molecules with biological activities of natural origin that is frequently prescribed in various indigenous systems of medicines especially those of India, Tibet, China and Korea. Its most widespread traditional uses have been for the treatment of inflammation of the lungs, cough, cold, ulcer and rheumatism**[1].** The plant produces a complex enzyme and numerous chemicals such as polyphenols, sterols, flavonoids, triterpenes and proteins that can create metallic nanoparticles, and reducing agents such as sugar (fructose and glucose)**[2].**

Nanotechnology is gaining enormous attention as a new area of research dealing with the development of nanomaterials and nanoparticles (NPs) for their utilization in diverse fields such as catalysis, electrochemistry, biomedicines, pharmaceuticals, sensors, food technology, cosmetics, *etc***[3-5]**. Nanoparticles (NPs) are nanometer-sized (<100 nm) atomic or molecular scale solid particles having some excellent physical properties compared to the bulk molecules depending on their size and morphology **[6, 7]**. Among all types of NPs, metal and metal oxide nanoparticles have been thoroughly examined using science and technology due to their excellent properties such as high surface to volume ratio, high dispersion in solution, *etc* **[8, 9]**. Owing to these, metal and metal oxide nanoparticles display enhanced antimicrobial properties like Zn Oxide, Copper Oxide, Silver nanoparticles **[10-14]**.

Silver nanoparticles have attracted increasing interest due to their chemical stability, catalytic activity, localized surface plasma resonance, and high conductivity. In addition, previous reports showed that the reactive oxygen species formed at the surface of the silver nanoparticles or by the released free silver ions under certain conditions may induce cell death of either mammalian cells or microbial cells, which endows the silver nanoparticles with unique antibacterial and antifungal effects . Based on these effects, silver nanoparticles hold great potential in preventing wound inflammation and hence promoting wound healing in the form of topical administration **[15].**

The synthesis of nanoparticles can be carried out by various methods such as physical, chemical and biological approaches. In general, physical and chemical methods are considered the best for obtaining uniform size nanoparticles with long-term stability. However, these approaches are costly and release materials toxic and dangerous in the environment. Toxic chemicals used in the synthesis of nanoparticles in chemical methods make the nanoparticles obtained less suitable for medical, cosmetic or food applications. Given that several nanoparticles have been widely used in medical products, diagnosis of diseases and cosmetics, it is very important to improve the biocompatibility of nanoparticles **[16].** Therefore, a green synthesis or biological synthesis of nanoparticles using microorganisms and plant extracts **[17]** is desirable to provide a pathway of economic, ecological **[18],** readily available and cleaner **[19].**

Our work is part of the preparation of silver nanoparticule from biosynthesis. The main aim of this work is the chemical and biological study of s.costus and AGNPs extracts. To better understand the interest of bioactive substances of S.costus, it seemed useful to undertake this work which concerns the synthesis of silver nanoparticles using root extract of this plant and the evaluation of their biological activities and antioxidant activity.

Our brief has two parts: the first part is devoted to a bibliographic summary it has two chapters (chapter 01 and 02):

**The first chapter:** aims at the botanical presentation and the chemical composition of the species studied Saussurea costus which is one of the most famous plants in the traditional medicine and introducing their characteristics and multiple uses.

**The second chapter:** which describes a generality about nanoparticles and their origins mainly based on silver nanoparticles their properties and biological synthesis using different biological models such as bacteria, champions and plants and their medical and environmental applications and their biological activities including antibacterial activity.

The second part was devoted to the experimental approach, are covered in two chapters (Chapter 03 and Chapter 04).

**Third chapter:** consists of the methods of synthesis and material used for the development of silver nanoparticles as well as antioxidant and antibacterial tests.

**The fourth chapter:** contains the results obtained from synthesized nanoparticles, where data like infrared and UV-vis and biological activity.

Finally, a general conclusion which is all the results obtained and the proposed perspectives to complete this study.

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# *Bibliography Part*

# *Chapter 1:*

# *Saussurea Costus plant*

## 1-Generality:

Among 47 species of Saussurea reported from India**[1]**, Saussurea costus synonymous with Saussure a lappa C is reputed one of the important plants known as a snow- lotus, from Asteraceae family reported as a critically endangered species of sub-alpine zone of Himalaya **[2][3]**.Cultivation requires a cool and humid climate as in high elevations. A deep rich porous soil is preferred. It is successfully cultivated in semi natural condition in Kashmir and Garhwal, Mature plants rooted out for its highly precious roots which is in great demand in local as well as in international market causing rapid deterioration by population number in wild habitat**[4]**, this family includes about 1000 genera and 30,000 species .

Saussurea costus is well known in Islamic medicine, which enlisted in the Holy A hadith said by Prophet Muhammad (Peace be upon him). It is known in Arab countries as "Al-Kost Al-Hindi" and used by traditional healers since the era of the Islamic civilization **[5]**.

## 2-History and origin:

The saussurea lappa (s.lappa) is widely distributed wild in India at an altitude of 2,500 to 3,000 m among the regions of Himalayas, Kashmir, Jammu, Western Ghats and Kishanganga valley and cultivated in Tamil Nadu, Uttar Pradesh, Kashmir to meet the commercial demand of the market due to over exploitation of the wild**[6].**

In India this plant is endemic in the sub alpine regions of Jammu and Kashmir, Himachal Pradesh and Uttaranchal(fig.1) **[7].**

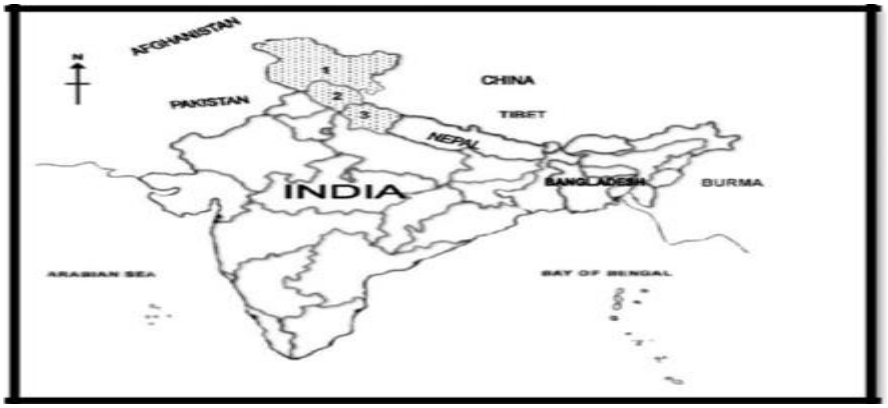


**Fig1:** Map of India showing distribution of Saussurea costus**[8].**

The plant is ancient, well-known about 2,500 years ago and used traditionally in the Indian systems of medicine such as Ayurveda, Unani, Siddha etc and has different names (table 1). **[9].**

**Table1**: the different names of saussurea costus**[10].**

| Language | The name |
|---|---|
| In Arabic | القسط الهندي |
| Hindi and Bengali | Kut, kur, pachak |
| Vernacular name | Costus Indien |
| Scientific name | Saussurea costus |

## 3. Classification and morphological:

### 3.1. Classification:

Saussurea costus belonging to family Asteraceae (table2) **[11].**

**Table2:** Taxonomic Hierarchy of S. lappa C.B. Clarke**.**

| | |
|---|---|
| kingdom | plantae |
| Sub kingdom | Viridae plantae |
| Division | Tracheophyta |
| Subdivision | Spermatophytina |
| Class | Magnoliopsida |
| Order | Asterales |
| Family | Asteraceae |
| Genus | Saussurea DC |
| Species | S. lappa C.B. Clarke |

**3.2. Morphological description:**

Saussurea costus is an erect, robust, pubescent, perennial herb, with a stout simple stem 1–2 m high. Leaves membranous, scaberulous above, glabrate beneath, auricled at base, irregularly toothed; basal ones very large, 0.50–1.25 m long, with a long-winged petiole; upper leaves smaller, subsessile or shortly petiole; two small lobes at the base of these leaves almost clasping the stem. Flower heads stalkless, bluish-purple to almost black, hard, rounded, 2.4–3.9 cm across, often 2–5 clustered together in the axils of leaves or terminal. Involucre bracts many, ovate-lanceolate, long pointed, purple, rigid, hairless. Receptacle bristles very long. Corolla about 2 cm long, tubular, blue-purple or almost black. Another, tails fimbriate. Achenes curved, compressed ca. 8 mm long, tip narrowed, with one rib on each face. Pappus brown, double feathery. Roots are stout, dark brown or grey, up to 40 cm long**[12].**

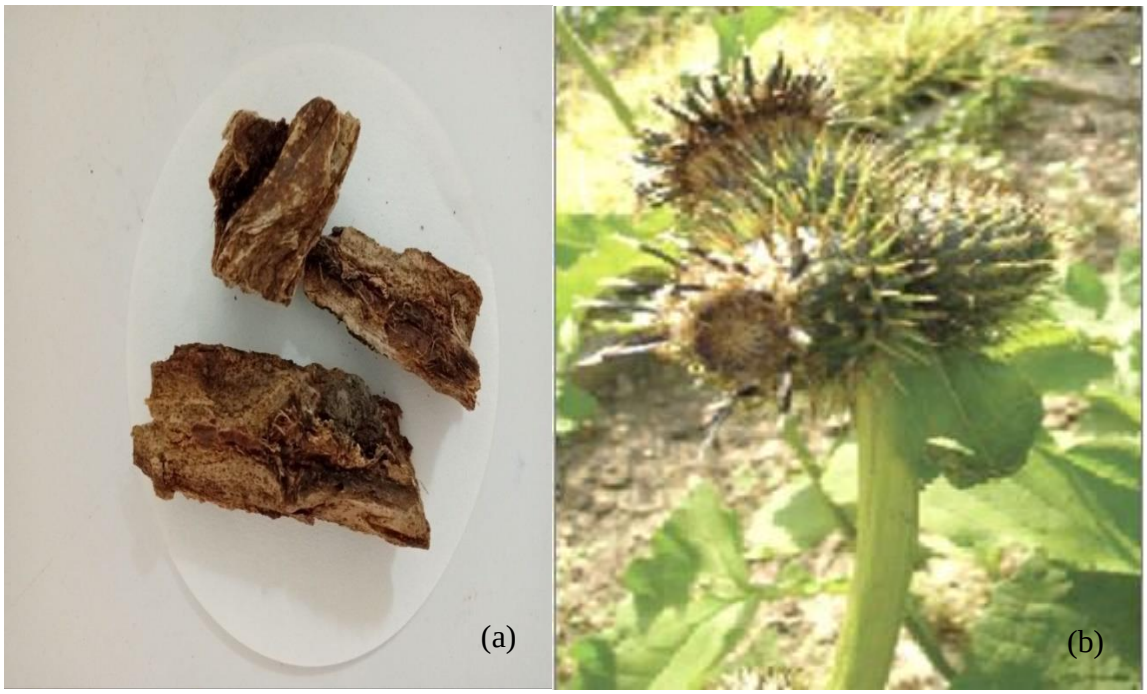


**Fig2:** a) roots of the costus (original photo 2024); b) S. lappa floral part**[3].**

## 4-chemical constituents of S.costus:

Research works on the active compounds of S. costus can be tracked down back to the 1950s, and several molecules have been discovered up to the present day. Terpenes are the primary active components, but it encompasses alkaloids, anthraquinones, and flavonoids. S. costus contains various terpenes, like castanoside and dihydro castanoside, having anti-inflammatory and antitumor characteristics **[13].**

**4.1. Alkaloids:**

Alkaloid, any of a class of naturally occurring organic nitrogen-containing bases. Alkaloids have diverse and important physiological effects on humans and other animals. Well-known alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine. The chemical structures of alkaloids are extremely variable**[14].**

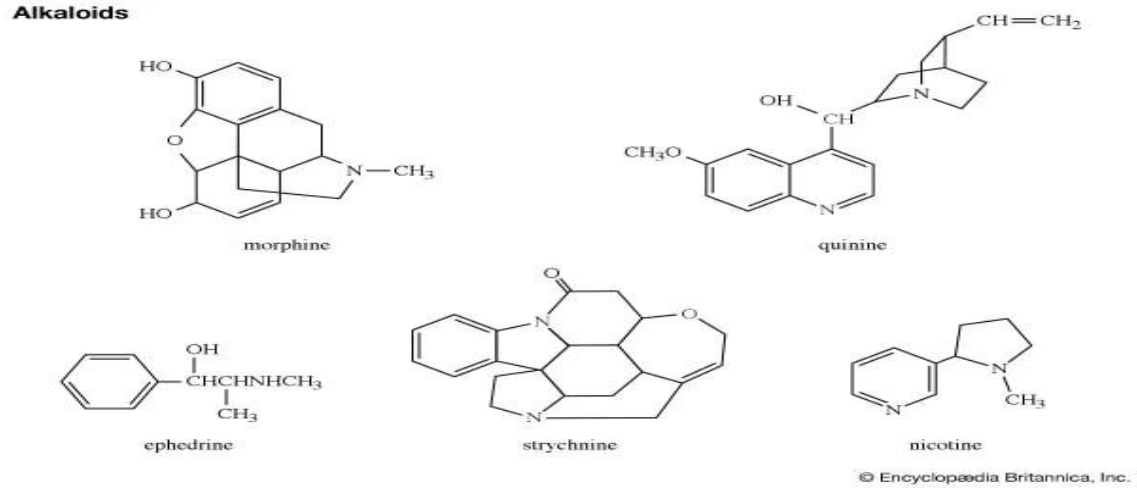


**Fig3:** Chemical structures of alkaloids.

**4.2. Anthraquinones:**

S.Costus has three anthraquinone compounds, namely, Aloe emodin- 8-O-β-d-glucopyranoside, Rhein-8-O-β-glucopyranoside and chrysophanol, which inhibited the activity of protein tyrosine phosphatase **[15]** and chrysophanol or chrysophanic acid is an anthracene derivative with two ketone groups attached to the central benzene ring. It is also known as chrysophanic acid. The molecular formula of chrysophanol is $C_{15}H_{10}O_4$, the molecular weight is 254.2 g/mol. The solubility of chrysophanol in water is poor; the aqueous solution is yellow but turns red on the addition of an alkali or concentrated sulfuric acid**[16].**

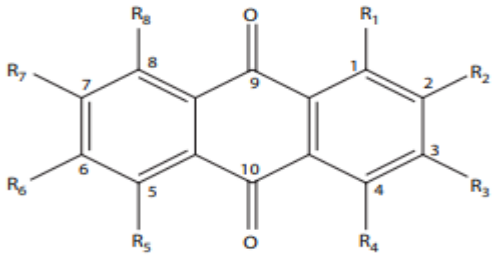

**Fig4:** General structure of anthraquinone**[17].**

**4.3-flavanoids:**

Flavonoids isolated from the roots of S. lappa with one glucoside substituent were Luteolin-7-O-β-D-glucoside, Rutin and Apigenin-7-O-β-D-glucoside**[18].**Whereas flavonoids with large substituents, such as with three glucosides at C3 **[19].**

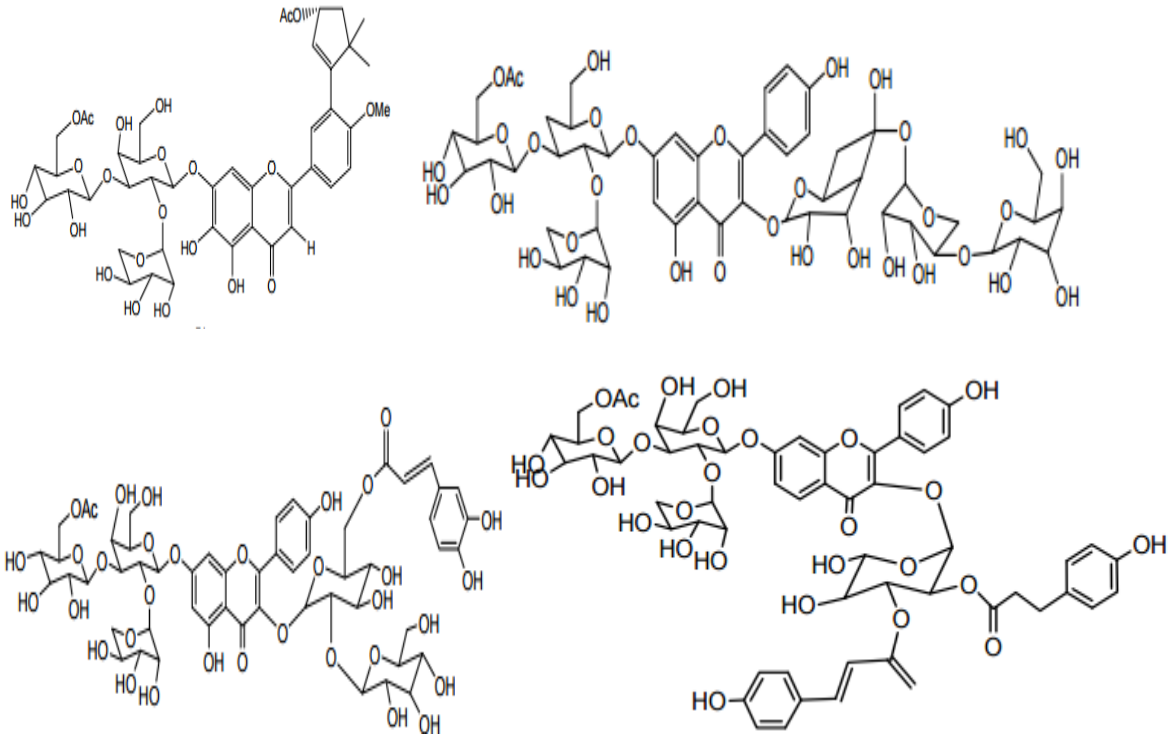

**Fig5:** the chemical structure of flavonoids**[16].**

**4.4-Terpenes:**

Terpenes are natural substances found in a wide variety of plants, including saussurea costus. They are the molecules responsible for the smell and taste characteristic of many plants, and they serve to protect it from insects and pathogens**[20].** Different terpenes include hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sester terpenes (C25), triterpenes (C30), and poly terpenes (>C30). Diverse functional roles of terpenoids have been critically studied and well-accepted now**[21].**

## 5. Medical application:

**5.1. Medicinal plants:**

Medicinal plants have been used for healing purposes throughout human history; Even in today's times, up to 80% of the world's population lives mostly in developing countries and relies on traditional herbal medicine for their primary health care system. Many of these herbal medicines prescribed in traditional medicine have insufficient knowledge and not tested by scientific methods. On the other hand, modern medicine is powerless in the face of the growing phenomenon of antimicrobial resistance, considered a major health problem requiring immediate attention. This crisis has encouraged academics and researchers to develop current antibiotics, synthesize new antibiotics or find new alternatives.

The latter option is preferable because, in nature, plants constitute one of the greatest pharmaceutical factories ever known and plants have been the main source of medicines for humanity since ancient times. Many medicinal plants produce diverse groups of secondary metabolites called phytochemicals, which can suppress microbial growth through different modes of action such as interference with cellular metabolic processes, disruption of the cell membrane, or by modulating transduction pathways signal or gene expression. **[22].**

Saussurea Costus has been quite commonly used for the medicinal properties attributed to these plants, including anthelmintic, antiperiodic, antipyretic, febrifuge, phlegmatic, and antilatent effects. Additionally, these plants are also used to treat nervous problems, skin diseases, coughs, rheumatism, chronic fever, eczema and dyspepsia. **[23,24].**

**5.2. The use of costus roots in medicines**:

The roots of S. costus have a bitter taste with a strong aromatic odor and are reported to have anti-inflammatory, antimicrobial, analgesic, anti-ulcer, anti-cancer and hepatoprotective properties in humans. The main constituents of this plant have the potential to be developed in the form of bioactive molecules.**[25].** A rich treasury of diverse bioactive compounds such as monoterpenes, sesquiterpenes, triterpenes, sterols, cardenolides, flavonoids, coumarins, lignans, phenylpropanoids and alkaloids**[26].**

The costus oil (the oil extracted from the roots of S. costus) has been used in leprosy **[27].**Saussurea costus roots have also been used in many other medical conditions including chronic gastritis, stomach ulcers, rheumatoid arthritis, asthma and bronchitis in traditional medicine and in inflammation-related diseases Saussurea costus is one of the most commercially used herbs in various indigenous systems of medicine (table3)**[28].**

**Table 3:** Showing the ethno medicinal use of the costus.

| Part of the costus plant | Ethno medicinal use | Reference |
|---|---|---|
| Root decoction | Dysentery, ulcer<br>Stomach ache | **[29]** |
| Root / root powder | Malaria, leprosy<br>Persistent<br>Rheumatism<br>Astringent stimulant | **[30]** |
| Root | Stomach ache<br>Toothache<br>Typhoid fever | **[31]** |
| Root | Asthma, disorders skin<br>Teeth ache | **[32]** |
| Root | Rheumatism | **[33]** |

**5.3. Costus application:**

**Table4:**Traditional methods of application of Saussurea costus**[34]**

| conditions | Method of applications |
|---|---|
| stomachache | • Root powder is taken water.<br>• Decoction of root is taken.<br>• Root powder is roasted in mustard oil and the paste is applied on stomach. |
| Headache | • Oil heated with root is applied |
| Cough and cold | • Root powder is taken with warm water |
| Throat infection | • Root is chewed |
| Backache and chest pain | • Root powder is taken with milk/decoction of root powder. |

| | |
|---|---|
| | • Oil heated with root is massaged the affected area. |
| Rheumatism and painful joints | • Root is roasted in ghee/butter.<br>• Powdered and taken with milk.<br>• The above-mentioned ghee/butter is rubbed on the affected area and bandaged to keep warm. |
| Scanty urination | • Jaggery is mixed in the decoction of root powder which is then taken.<br>• Paste of root powder is applied on the stomach below the naval. |
| pustules | • Fine root powder is dusted on the wound.<br>• Mustard oil is heated with root powder and the oil is applied and bandaged. |
| Piles | • Root are taken along with the *Vacha* (Acorus calamus) roots. |
| Epilepsy | • The roots are taken with honey. |
| General weakness | • Root is boiled in milk and the milk is taken twice daily. |
| Scalp scabies | • Essential oil of root is applied. |

## 6. Safety and side effects of S. Costus:

Not much information is available about safety and side effect profile of S. costus. Generally, S. costus root may be safe when taken appropriately by mouth but S. costus may contain a contaminant called aristolochic acid which may have nephrotoxic and carcinogenic effects.

Safety of S. costus has not been ascertained in pregnant or breast-feeding women. Saussure costus may cause an allergic reaction in those who are allergic to Saussurea species, their constituents including the sesquiterpene lactones (STL). Those exposed to STLs, have been observed to develop contact dermatitis. Concerns have been raised about their concerns regarding the genotoxic embryotoxic potential of these compounds. In vivo and vitro assays have reported mutagenic effect of STLs**[35].**

# *Chapter 2:*
# *Silver Nanoparticles*

# 1. Nanoparticles:

## 1.1. Generality to nanotechnology:

Nanotechnology refers to technology that is implemented at the nanoscale **[36]**involved in the design, synthesis, characterization, and application of materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale or one billionth of a meter. At these scales, consideration of individual molecules and interacting groups of molecules in relation to the bulk macroscopic properties of the material or device becomes important, since it is control over the fundamental molecular structure that allows control over the macroscopic chemical and physical properties**[37].**

Unique physical and chemical properties of nanomaterials can be exploited for applications that benefit society. Nanotechnology represents a megatrend and has become a general-purpose technology. The United States instituted the National Nanotechnology Initiative ( NNI ) back in 2000, which was soon followed (2001) by a plethora of projects in nanotechnology in nearly most of the U.S. Departments and Agencies . About 20 Research Centers were subsequently funded by the Nationa1 Science Foundation ( NSF ), an agency responsible solely to the President of the United States and whose mandate is to fund the best of fundamental science and technology projects. NSF was the lead U.S. agency to carry forward the NNI. The word “nanotechnology” soon caught the attention of various media (TV networks, the internet, etc.) and the imagination and fascination of the community at large**[36].**

Nanotechnology is an expanding area of research where we use to deal with the materials in Nano-dimension. The conventional procedures for synthesizing metal nanoparticles need to sophisticated and costly instruments or high-priced chemicals. Moreover, the techniques may not be environmentally safe. Therefore “green” technologies for synthesis of nanoparticles are always preferred which is simple, convenient, eco-friendly and cost effective. Green synthesis of nanoparticle is a novel way to synthesis nanoparticles by using biological sources. It is gaining attention due to its cost effective, ecofriendly and large-scale production possibilities**[37].**

## 1.2-Definition of nanoparticles:

Nanoparticles are defined as particles with at least one spatial dimension of less than 100 nm. This characteristic of nanoparticles is related to the fact that the majority of its atoms are on its surface. This gives the nanoparticle a strong exchange surface (surface-specific) that leads to particular physical and chemical properties. These properties can lead to specific but poorly understood biological effects if they come in contact with living objects. There are a variety of sources of nanoparticles: natural nanoparticles (as produced by volcanic fumes), those produced unintentionally by humans (diesel exhaust particles or particles from welding fumes), and those produced intentionally for new properties (manufactured nanoparticles)**[38].**

In general, the size of a nanoparticle spans the range between 1 and 100 nm (Figure 05). Metallic nanoparticles have different physical and chemical properties from bulk metals (e.g., lower melting points, higher specific surface areas, specific optical properties, mechanical strengths, and specific magnetizations), properties that might prove attractive in various industrial applications. However, how a nanoparticle is viewed and is defined depends very much on the specific application. Of particular importance, the optical property is one of the fundamental attractions and a characteristic of a nanoparticle. For example, a 20-nm gold nanoparticle has a characteristic wintered color. A silver nanoparticle is yellowish gray. Platinum and palladium nanoparticles are black **[39][40].**

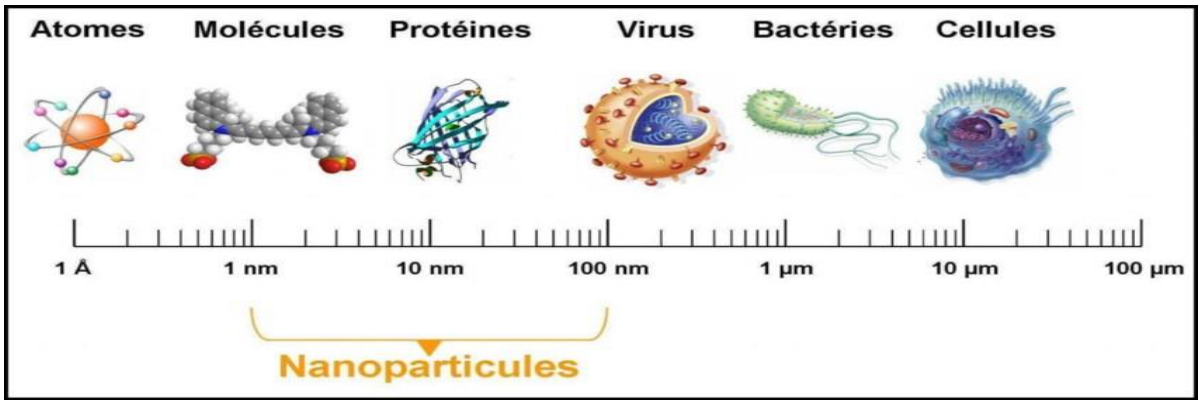


**Fig 6:** size comparison in nanometer scale **[41].**

### 1.3. Origin of nanoparticles:

#### 1.3.1. Natural nanoparticles:

NPs are abundant in nature, and they include ocean spray, forest fire, dust storms, volcanic ash and biological particles such as bacteria and fungi. Humans have long been exposed to other naturally occurring nanoparticles resulting from combustion, and the human body is well adapted to protect itself from these potentially harmful intruders**[42].**

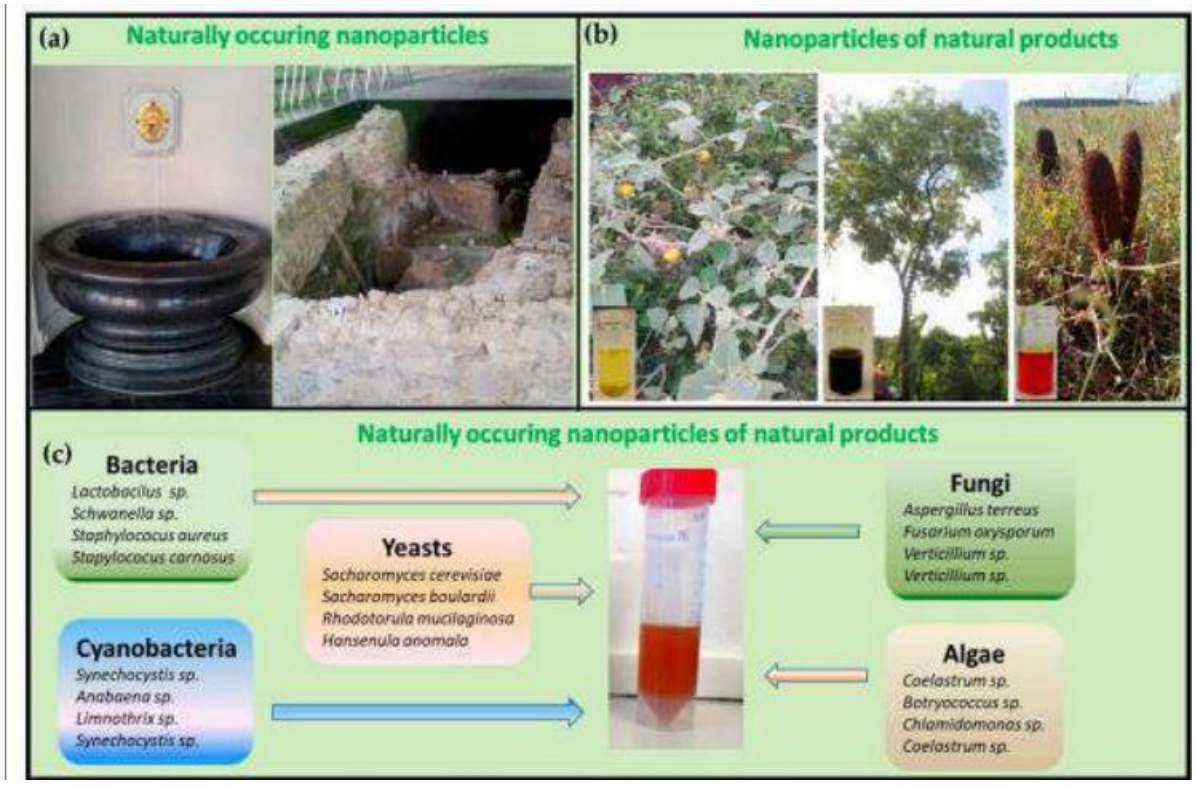


**Fig7:**Examples of natural and biological materials containing particles nanoscopic**[43].**

#### 1.3.2. Anthropogenic nanoparticles:

Anthropogenic nanoparticles are man-made and may result in incidental exposure. These man-made nanoparticles fall into two categories: the first category has no predetermined size and may exhibit undefined chemistry. Examples are combustion particulates, diesel exhaust, welding fumes and coal fly ash. The second category of anthropogenic nanoparticles, also known as engineered nanoparticles (ENPs), exhibit specific size ranging from 1–100 nm. They are pure materials with controlled surfaces. Examples

include fullerenes, carbon nanotubes, dendrimers, quantum dots, TiO2, gold and silver nanoparticles**[42].**

**1.3.2. a. Incidental Nanomaterials:**

These nanoparticles and nanostructured materials are unintentionally produced through direct or indirect human influences or anthropogenic (e.g., mechanical or industrial) processes, such as vehicle exhaust gases, welding gases, solid fuel heating (home heathers), and combustion during cooking. Incidental atmospheric nanomaterials, inadvertently formed during a deliberate procedure, might increase air pollution. Forest fires generate a wide range of nanomaterials (e.g., pigments, cement, fumed silica). It's hard to say when human beings started making incidental nanoparticles, but probably as soon as people started taming fire. Incidental nanomaterials, byproducts of human activities, are generally have poorly controlled sizes and shapes. Incidental nanomaterials have high environmental impacts and must be considered relative to engineered nanomaterials**[44].**

**1.3.2. b. Artificial nanoparticles:**

Engineered particles (ENPs) are artificially produced particles because of their useful properties. They may consist of a single element like C or Si, or a mixture of different elements**.**

The main characteristics of ENPs are high surface area, unusual phase transformation due to their artificial nature, unusual defect stabilization ,high surface strain ,and cryctallographically controlled aggregation (figure 3)**[45].**Very schematically, there are metallic NPs M (titanium dioxide and zinc, for example) and carbon NPs M. Among these, carbon nano-tubes occupy a place important**[46].**

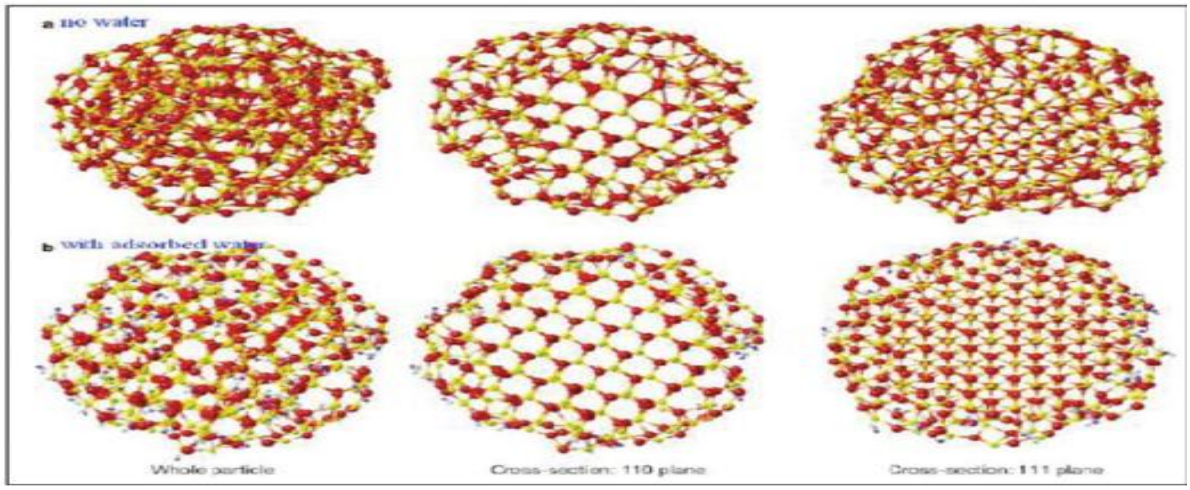


**fig8**:Strained ZnS NPs with and without water molecules**[45].**

**1.4. Advantages and disadvantages of nanoparticles:**

Significant advantages and disadvantages of nanoparticles are given in table5:

**Table5:** advantages and disadvantages of nanoparticles.

| Advantages | Disadvantages |
|---|---|
| 1/Increased bioavailability Dose proportionality.<br>2/Smaller dose form.<br>3/Increased surface area results in a faster dissolution of the active agent in an aqueous environment, such as the human body.<br>4/Faster dissolution generally equates greater absorption and bioavailability.<br>5/Smaller drug doses less toxicity.<br>6/Reduction in fed/ fasted variability**[47].** | 1/NPs can undergo a transformation, because they are thermodynamically are located in the region of high energy local minima. This leads to deterioration in quality, poor corrosion resistance, and the main concern is that conservation of the structure becomes difficult.<br>2/During the synthesis of NPs, training may be aggravated by the environment impure. As NPs are very reactive, there may also be strong chances impure.<br>3/NPs have been reported as toxic, carcinogens and irritants because they become transparent to the dermis cellular.<br>4/Exothermic combustion can cause an explosion because the fines metal particles act like powerful explosives**[48].**<br>5/Nanoparticles due to there their small size cancause inhalation problem and many other fatal diseases by just inhaling for 60 seconds in the air contain nanoparticles can damage lungs easily**[49].**<br>6/It has been shown that NPs, which enter the liver, may induce locally an oxidative stress**[50].** |

## 1.5. Synthesis of nanomaterials:

The nanoparticles are synthesized either bottom-up by combining and assembling the nanosized precursors or by top-down process, where the nanoparticles are ground or derived from the large-sized materials or bulk without atomic control. The methods which have been reported for the synthesis of nanomaterials can be broadly classified as physical methods, chemical methods, and biological methods**[51].**

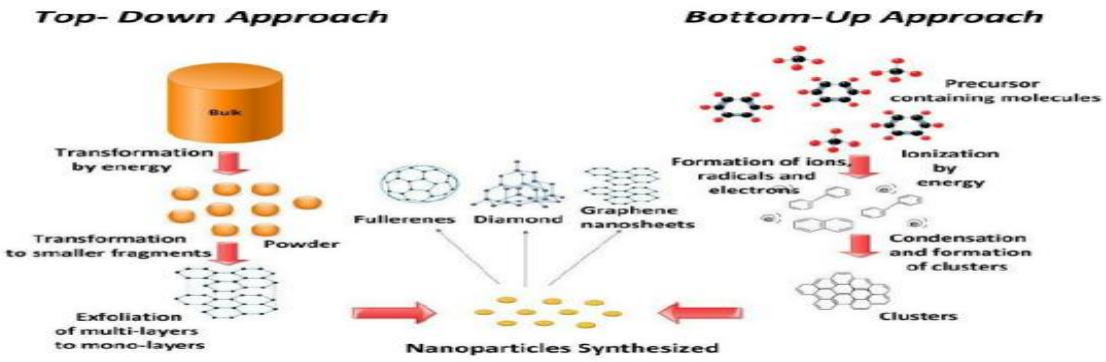


**Fig9:**Synthesis of nanomaterials (NPs) VIA top-down and bottom-up approaches**[52].**

### 1.5.1. The Chemical methods:

Synthesis processes occur by chemical deposition based on deposition reactions (substitution), co-precipitation, oxidation–reduction, thermolysis, hydrolysis, polymerization, and condensation. The control of various variables in a synthetic system plays an important role in controlling particle size and morphology. The products of sedimentary processes under various synthetic conditions range from coarse crystals to nanostructured colloidal particles. Co-precipitation chemical methods allow the synthesis of metal nanoparticles, metal oxides, as well as many metal semiconductor compounds. Also, a wide range of properties and characteristics can be achieved by changing the synthesis conditions. The basis of these methods is the preparation of products from soluble precursors using different systems such as electrochemical equipment, microwave radiation, ultrasound, and high-energy beams**[53].**

### 1.5.2. The physical methods:

The most common physical synthesis techniques used to obtain metal nanoparticles are evaporation–condensation and laser ablation. A physical method involves the presence of a tube furnace and atmospheric pressure. The advantages of a physical approach when compared with a chemical one are the absence of solvent contamination and the uniformity of nanoparticles distribution. However, physical synthesis has its disadvantages: the large size of the tube furnace, the high amount of energy consumed, and the fact that thermal stability is achieved in a long period of time (several tens of minutes in order to reach a stable operating temperature). Small nanoparticles are formed by cooling evaporated vapors, due to the temperature gradient between the vicinity of the heater surface and the tube furnace.

Laser ablation of a metallic part in solution is another physical approach used to obtain silver nanoparticles. The particle characteristics depend on many factors such as laser wavelength, laser pulse duration, ablation time, liquid medium surfactant presence or absence, and laser fluence.

The major advantage of the laser ablation method, compared to other methods, is the absence of chemical reagents in solution, therefore pure and uncontaminated silver nanoparticles are obtained**[54].**

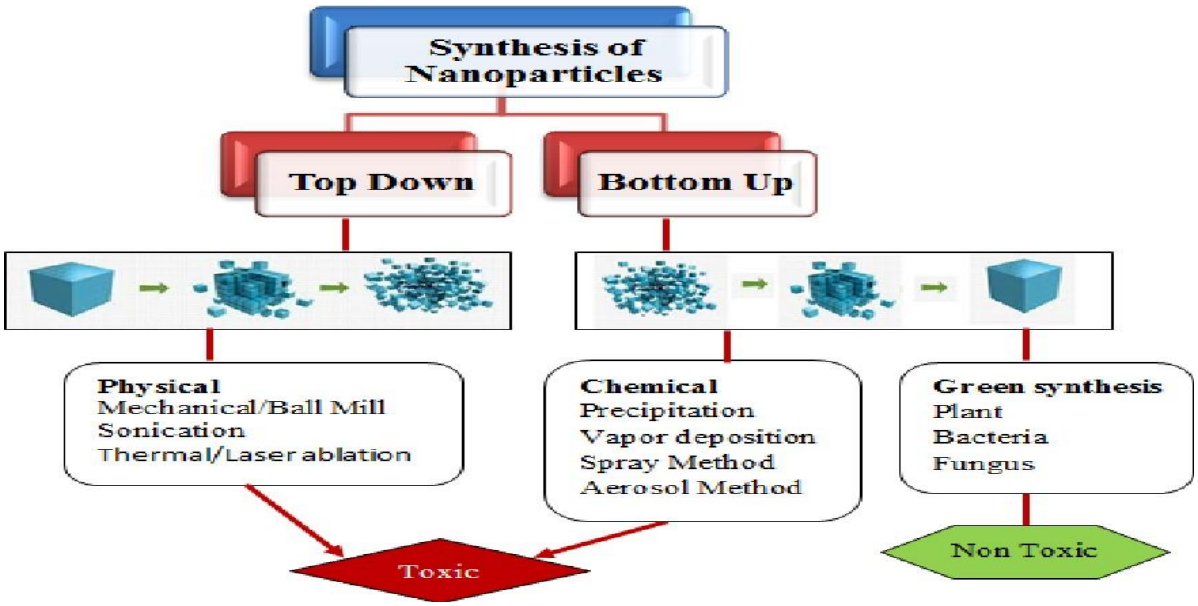


**Fig10:** Approaches for synthesis of metal nanoparticles**[55].**

## 2. Silver nanoparticles:

### 2.1. General presentation of silver:

Silver is a chemical element with symbol Ag and atomic number 47**[56]**in gray-white color**[57]**. Silver is a malleable, ductile, and precious metal that has been known since ancient times (its first debut around 5000 BCE) and is located in group 11 (Ib)and period 5 of the periodic table, between the coinage metal copper (period 4) and gold (period 6). Silver is widely distributed in nature. But its abundance in the earth's crust is very low (0.05 ppm) than other metals. It occurs both naturally in its pure form and in ores, particularly derived from all the sulfur bearing lead, copper, gold, tellurides, and zinc, which is extracted through refining. Silver has the atomic number 47 and atomic weight of 107.880and its ground state electronic configuration is [Kr] $4d^{10}5s^{1}$ just like copper and gold. Mostly, silver can exist in a mixture of isotopes, $^{107}$Ag and $^{109}$Agapproximately occurring in the equal proportions. Agis noticeably diamagnetic, andits magnetic susceptibility is almost independent of temperature from roomtemperature to just below the melting point**[58]**.

**Fig11:** Silver nugget**[59]**

### 2.2. Silver nanoparticles:

Silver nanoparticles or nano-silver are molecules with a size of 20-40 nm, composed of 80% silver atoms and 20% silver ions. They are, in front of the nanotubes of carbon and titanium NPs, the best-selling NPs released into the environment. The AgNPs are highly prized by the pharmaceutical and agri-food industry, particularly for their biocidal property. Nevertheless, the use of these NPs remains controversial due to their risk on the health and environment**[60].**

### 2.3. Properties of silver nanoparticles:

Ag-NPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and non-linear optical behavior**[61]**. Such as mechanical, chemical, magnetic, optical or electric properties compared with bulk materials**[62].**

#### 2.3.1. Optical properties:

Silver nanoparticles are extraordinarily efficient at absorbing and scattering light and, unlike many dyes and pigments, have a color that depends upon the size and the shape of the particle. The strong interaction of the silver nanoparticles with light occurs as the conduction electrons on the metal surface undergo a collective oscillation when excited by light at specific wavelengths known as a surface Plasmon resonance (SPR)(figure 2), this oscillation results in unusually strong scattering and absorption properties. In fact, silver nanoparticles can have

effective extinction (scattering +absorption) cross sections up to 10 times larger than their physical cross section**[63].**

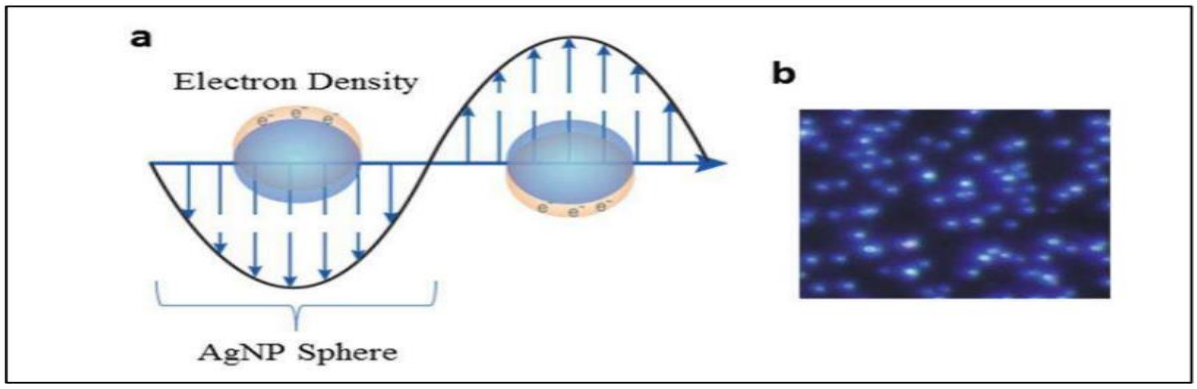


**Fig12:**a) Surface plasmonic resonance (SPR) for AgNPs at one length specific wave; b) AgNPs black field microscopy image**[64].**

**2.3.2. Thermal properties:**

Thermal behavior is an important aspect that is considered in detail in the production or application ofa material. A remarkable property of metal nanoparticles is their low melting temperature due to the thermo-dynamic size effect**[65].**

**2.3.3. Biological properties:**

Silver compounds were shown to be effective against both aerobic and anaerobic bacteria by precipitating bacterial cellular proteins and by blocking the microbial respiratory chain system**[66].**

**2.3.4. Catalytic properties:**

AgNPs have been utilized as effective catalytic agents for the reduction of various dyes such as methylene blue, yellow-12, 4-nitrophenol, Rose Bengal, eosin, and methyl orange. 215–219 AgNPs synthesized using the peach kernel shell method were found to have the capability as a catalyst for the reduction of 4-nitrophenol to 4-aminophenol**[65]**

**2.3.5. Surface chemistry:**

When nanoparticles are, in solution, molecules associate with the nanoparticle surface to establish a double layer of charge that stabilizes the particles and prevents aggregation. Aldrich Materials Science offers several silver nanoparticles suspended in a dilute aqueous citrate buffer, which weakly associates with the nanoparticle surface **[65].**

**2.3.6. Crystalline structures:**

Crystalline structure of AgNPs can be derived from X-ray diffraction patterns, Several studies reported that AgNPs have the cubic structure showing peaks at 38.06°, 44.22°, 64.48° and 77.32°corresponding to the scattering angle 2θ from the(1 1 1), (2 0 0), (2 2 0 ), and (3 1 1), lanes, respectively. In addition, the diffraction pattern of AgNPs occurs at38.5°, 44° and 64.5° (2θ)These patterns can be indexed to the (111),(200),and (220)planes of the face centered cubic (fcc) silver**[65].**

**2.4. Synthesis of silver NPs:**

**2.4.1. Physical method:**

Evaporation-condensation and laser ablation are the most important physical approaches. The absence of solvent contamination in the prepared thin films and the uniformity of NPs distribution are the advantages of physical synthesis methods in comparison with chemical processes. Physical synthesis of silver NPs using a tube furnace at atmospheric pressure has some disadvantages, for example, tube furnace occupies a large space, consumes

a great amount of energy while raising the environmental temperature around the source material, and requires a lot of time to achieve thermal stability. Moreover, a typical tube furnace requires power consumption of more than several kilowatts and a preheating time of several tens of minutes to reach a stable operating temperature. It was demonstrated that silver NPs could be synthesized via a small ceramic heater with a local heating area . The small ceramic heater was used to evaporate source materials. The evaporated vapor can cool at a suitable rapid rate, because the temperature gradient in the vicinity of the heater surface is very steep in comparison with that of a tube furnace**[67].**

### 2.4.2. Chemical method:

The most common approach for synthesis of silver NPs is chemical reduction by organic and inorganic reducing agents. In general, different reducing agents such as sodium citrate, ascorbate, sodium borohydride ($NaBH_4$), elemental hydrogen, polyol process,Tollen's reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers are used for reduction of silver ions ($Ag^+$) in aqueous or non-aqueous solutions. These reducing agents reduce $Ag^+$ and lead to the formation of metallic silver ($Ag^0$), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of metallic colloidal silver particles . It is important to use protective agents to stabilize dispersive NPs during the course of metal nanoparticle preparation, and protect the NPs that can be absorbed on or bind onto nanoparticle surfaces, avoiding their agglomeration. The presence of surfactants comprising functionalities (*e.g.*, thiols, amines, acids, and alcohols) for interactions with particle surfaces can stabilize particle growth, and protect particles from sedimentation, agglomeration, or losing their surface properties**[68].**

### 2.4.3. Biological method:

Uses biological systems such as bacteria, fungi, yeasts, actinomycetes and plant extracts, etc. For the synthesis of metal-based NPs and metal oxides**[69].**Biological methods are safe processes, cost-effective, sustainable and environmentally friendly for nanoparticle synthesis**[78].**The problem with most of the chemical and physical methods of nano-silver production is that they are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks (Table6)**[70].**

**Table 6:**Comparison between biological and chemical synthesis**[71].**

| **Synthesis of nanoparticles** | | |
|---|---|---|
| **Bottom-up approach** | | **Top-down approach** |
| **Green methods** | **Chemical methods** | **Physical methods** |
| • Using bacteria.<br>• Using fungi.<br>• Using plant and their extracts.<br>• Using yeast.<br>• Using enzymes and biomolecules.<br>• Using microorganism. | • Chemical reduction<br>• Sono chemical<br>• Microemulsion<br>• Photochemical<br>• Electrochemical<br>• Pyrolysis<br>• Microwave<br>• Solvothermal<br>• Co precipitin | • Pulsed laser ablation<br>• Evaporation-condensation<br>• Arc discharge<br>• Spray Pyrolysis<br>• Ball milling<br>• Vapour and gas phase<br>• Pulse wire discharge<br>• Lithography |
| **Non-Toxic** | **Toxic** | |

### 2.5. Medical applications of silver nanoparticles:

Silver nanoparticles, due to their unique properties, find use in many day-to-day applications in human life. A few examples include their addition in house cleaning chemicals, in fabric cleaners, as antireflection coatings, to improve the transfer of heat from collectors of solar energy to their fuel tanks, to produce high-performance delicate electronics, and in hundreds of other applications. Though all these are important applications of silver nanoparticles, perhaps their need is most desired in the medical field.

The general aspect of nanoparticles is that the small size of nanoparticles provides for a larger surface area for the particle and hence increases the effect. The nano-size of the particles also increases the penetration potential of the silver particles, hence again aiding in better utilization of the metal properties. Based on the size factor alone, nanoparticles have the ability to penetrate the circulatory system and translocate even the blood–brain barrier in the human system**[72].**

**2.6. Biological activities of silver nanoparticles:**

AgNPs have been received huge attention of the scientists due to their remarkable defense against various pathogenic microorganisms**[73].**

**2.6.1. Antibacterial activity:**

Many studies have shown that NPs have greater activity against Gram-positive bacteria than against Gram-negative bacteria, because the cell wall of Gram-negative bacteria is composed of LPS, lipoproteins, and phospholipids, which form a penetration barrier that allows the entrance of only macromolecules**[74].**In contrast, the cell wall of Gram-positive bacteria includes a thin layer of peptidoglycan as well as teichoic acid and abundant pores that allow foreign molecules to penetrate, resulting in cell membrane damage and cell death. In addition, compared with Gram-negative bacteria, Gram-positive bacteria have a high negative charge on the cell wall surface, which can attract NPs**[75].**the Ag-NPs displayed long-term antibacterial effect as compared with two other disinfectants of sodium hypochlorite (NaClO) and phenol ($C_6H_5OH$)**[76].**The biocidal activity of AgNPs depend on several morphological and physicochemical (e.g., size, shape, and surface) characteristics that influence directly in the success of these compounds as antimicrobial agents**[77].**

**2.6.1.1. Mechanism of antimicrobial activity of silver nanoparticles:**

Currently, the literature supports principally three mechanisms that have been observed together or separately, by which AgNPs exert their antibacterial action **[78].**The first one postulates that AgNPs act at a membrane level as they are able to penetrate the outer membrane, accumulating in the inner membrane where the adhesion of the nanoparticles to the cell generates their destabilization and damage, increasing membrane permeability and inducing leakage of cellular content and subsequently its death **[79].** It is also evidenced that AgNPs can interact with sulfur-containing proteins in the cell wall of bacteria, an interaction that may cause structural damage leading to cell wall rupture.

The second mechanism proposes that nanoparticles not only can break and cross the cell membrane, altering its structure and permeability but can also enter the cell where it has been suggested that, due to its properties, AgNPs will have an affinity to interact with sulfur or phosphorus groups, present in intracellular content such as DNA and proteins altering their structure and functions. In the same manner, they may alter the respiratory chain in the inner membrane by interacting with thiol groups in the enzymes inducing reactive oxygen species and free radicals, generating damage to intracellular machinery and activating the apoptosis pathway. A third mechanism that is proposed to occur in parallel with the two others is the release of silver ions from the nanoparticles, which due to their size and charge, can interact with cellular components altering metabolic pathways, membranes, and even genetic material**[80].**

The main mechanisms at the origin of this antibacterial activity are represented in Figure 13 below. The combined action of nanoparticles and ions released produces, through different pathways, broad-spectrum bactericidal activity**[81].**

The mechanisms at the origin of this activity, especially antibacterial, remain poorly are still being researched. Several studies have shown that the release of silver ions from nanoparticles participates in their antibacterial activity. Silver ions are produced by surface oxidation of nanoparticles .

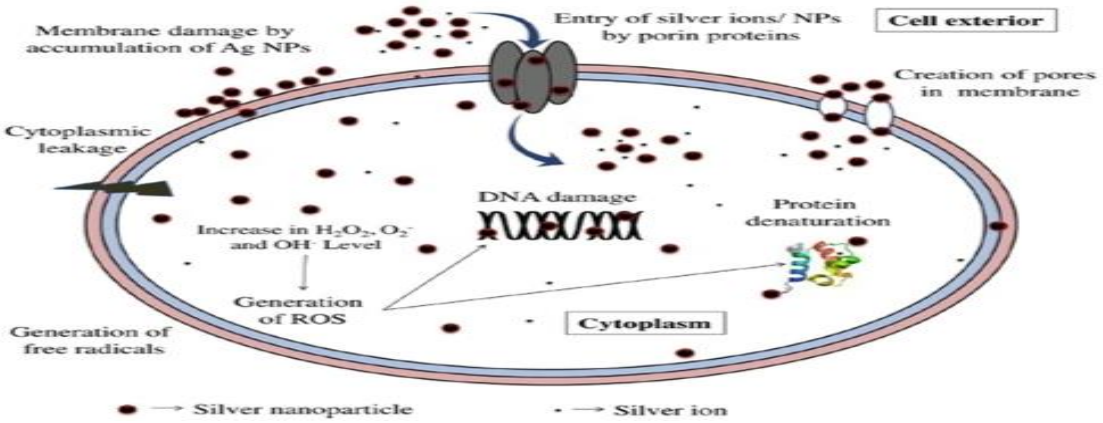


**Fig13:** Main mechanisms of antibacterial action of silver nanoparticles **[82].**

**2.6.2. Other activity:**

**2.6.2.1. Antiviral activity:**

Nanoparticles have strong antiviral potential and because of their multiple interactions with glycoprotein receptor and/or viral envelop, they can inhibit the viral multiplication inside the host cell by preventing the replication or blocking the entry of virus particles inside the host cell. Depending on the interaction and veridical effect, they have huge potential not only to face the challenge posed by viral infections but also enhance the quality of existing antiviral therapies**[77**].Like the human immunodeficiency virus (HIV-1), hepatitis B virus and respiratory syncytial virus**[83].**

**2.6.2.2. Antifungal activity:**

AgNPs can be the appropriate agents for the control of three Candida species, namely, C. albicans, C. tropicalis, and C. krusei. Further, they also demonstrated its application as additive in commercially available dish and hand**[83].**studied the antifungal activities of Ag-NPs against a total of 44 strains of six fungal species from clinical isolates and ATCC strains of *Trichophyton mentagrophytes (T. mentagrophytes)* and *Candida albanicans* (*C. albanicans*). Results showed 80% inhibitory concentration ($IC_{80}$) from 1 to 7 $\mu$g $ml^{-1}$. The antifungal activity of Ag-NPs against *C. albanicans* could be exerted by disrupting the structure of the cell membrane and inhibiting the normal budding process due to the destruction of the membrane integrity**[84].**

**2.6.2.3. Anti-inflammatory activity:**

Studies have shown that exposure to NPs leads to the secretion of several cytokines and chemokines by epithelial cells, leading to the infiltration of macrophages in exposed areas. Then, macrophages initiate the release of inflammatory cytokines, which induce the upregulation of inflammatory mediators such as NF-kB. The inflammatory mediators induce increased production of inflammatory cytokines and chemokines and the migration of neutrophils and monocytes from the circulatory system to the site of inflammation**[85].**

**2.6.2.4. Anti-cancer activity:**

AgNPs can be considered a promising tool in the prevention of various types of cancer cells, such as carcinoma hepatocellular, lung and breast cancer, and cervical carcinoma due to their best penetration, anti-angiogenic and anti-proliferative properties and the ease of their tracking in the body**[86].**

The antiproliferative property in cancer cells is due to their ability to damage DNA, break chromosomes, and produce instability genomics and to disrupt calcium homeostasis (Ca2+) which induces apoptosis and causes instability of the cytoskeleton. The cytoskeletal lesion blocks the cycle and cell division, promoting the anti -proliferative activity of cancer cells**.** Demonstrated that AgNPs (15 nm) may induce apoptosis and improve radiosensitivity on cancer cells**[77].**

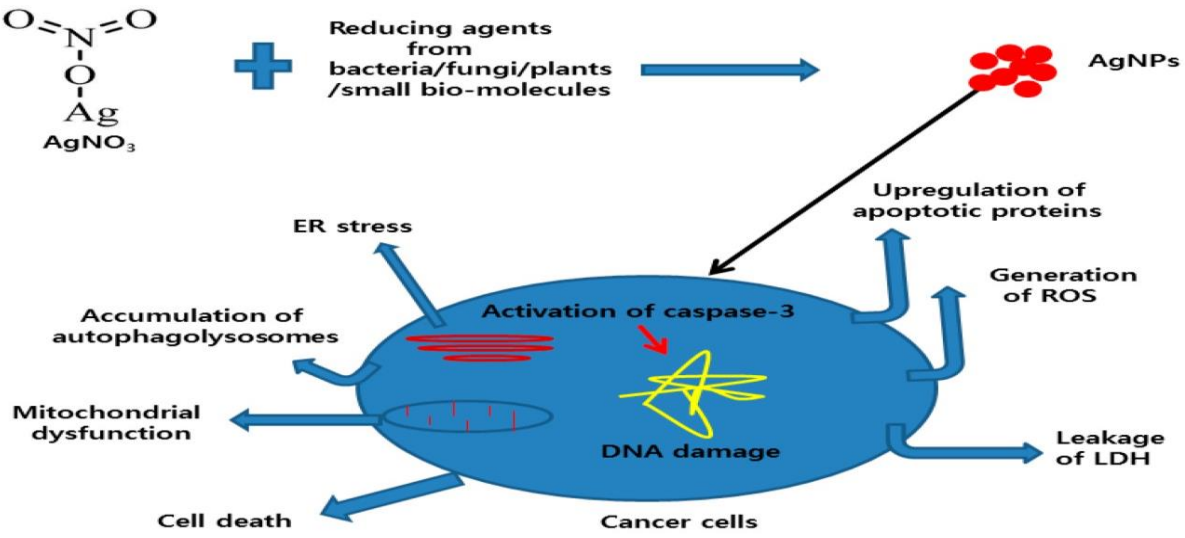


**Fig14:** Possible mechanisms of AgNPs to induce cytotoxicity in cancer cell lines**[89]**

**2.6.2.5. Antidiabetic activity:**

AgNPs trap free radicals and reduce enzyme levels that cause the hydrolysis of complex carbohydrates (α-glucosidase and α-amylase), which increases the rate of glucose consumption**[90].**

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# *Experimental Part*

# *Chapter 3:*

# *Materials and methods*

## 1.Introduction:

Our work consists in synthesizing silver nanoparticles (AgNPs)by bio-reduction of silver ions by bio-molecules of the aqueous extract of the plant sussurea costus to develop new biomaterials. It was realized at the University of Ain-temouchent. the different stages of this chapter summarized in the following diagram:

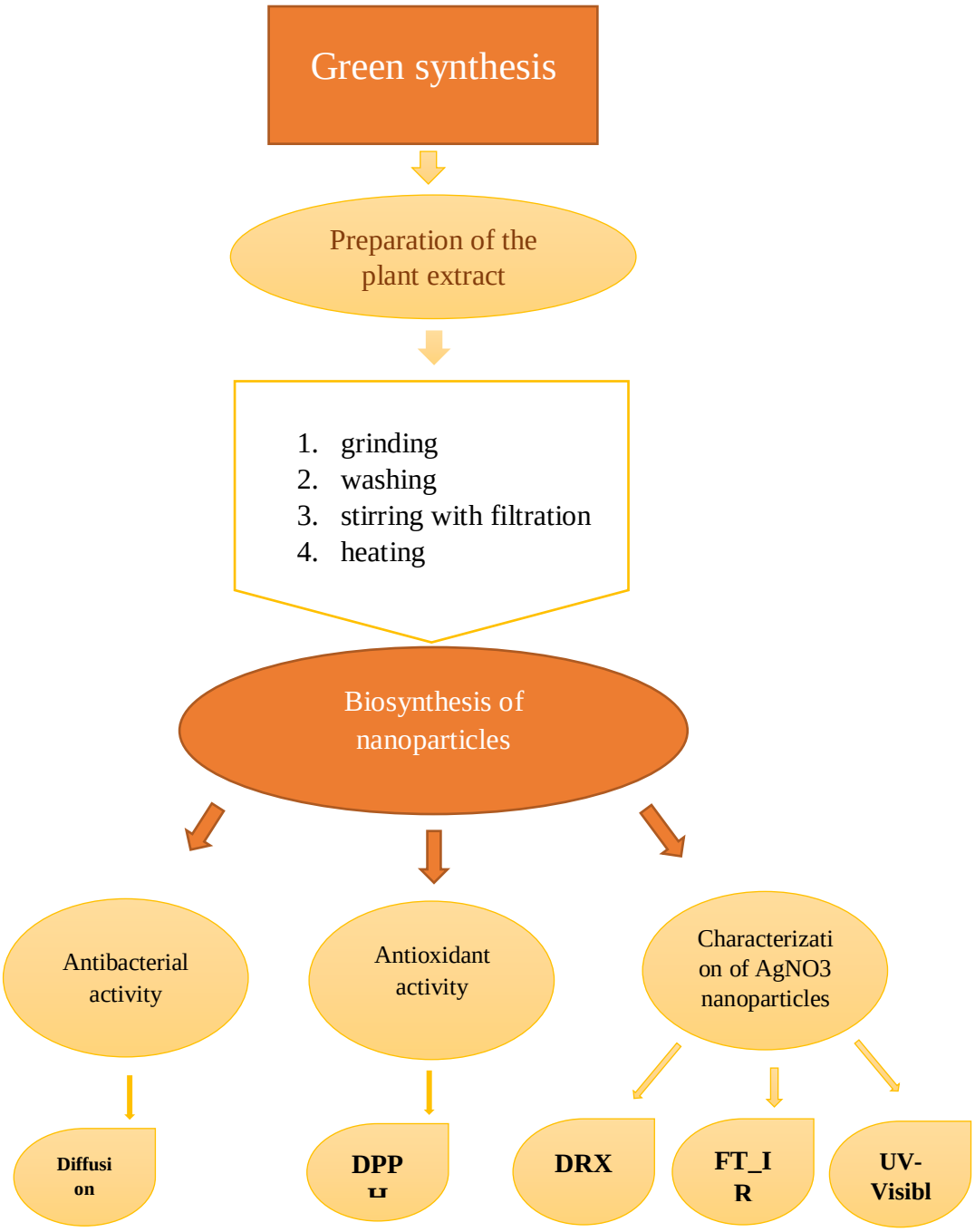



**Fig 01:** Presentation of different stage of this chapter.

## 2.Materials and methods:

In the first part, we will cite the products, materials and laboratory equipment used to realize our work

### 2.1-Plant material:

The plant material used in this study is the saussurea costus plant.

### 2.2. Laboratory Materials and equipments:

- Electric scale
- Magnetic stirrer
- The static oven
- Bath- Marie agitation.
- Centrifuge
- Chemical ultrasonic.
- Ultraviolet UV spectroscopy
- X-ray diffractometry
- Infrared spectroscopy has transformed Furrier (FT-IR).
- Beakers.
- Watch glass.
- Spatula
- Funnel
- Erlenmeyer
- Magnetic bar.
- Filter paper.
- Graduated specimens
- Aluminum foil.
- Micropipette.
- Volumetric flasks.
- Support.
- Buchner.
- Droplets.
- Pestle and mortar.
- The balance of chemical.
- Crystal maker.

### 2.3. Products:

**Table 1**: representation of products:

| **Products** | **Formula** | **The molar mases (g/mol)** | **Originality** |
|---|---|---|---|
| Silver Nitrate | $AgNO_3$ | 169,87 | Purchased from, VWR chemical |
| Ascorbic Acid | $C_6H_8O_6$ | 176,124 | Purchased from, Sigma-Aldrich |
| 2,2-diphenyl-1-picrylhydrazyl | DPPH | 394,317 | Across organics |
| Dimethyl Sulfoxide | DMSO | 78,13 | Purchased from, Sigma-Aldrich |
| Ethanol | $C_2H_6O$ | 46,068 | Sigma-Aldrich |

## 3. Montage and general synthesis protocol:

### 3.1 Green synthesis:

#### 3.1.1. Preparation of saussurea costus aqueous extract:

First, fresh roots were cleaned with tap water several times, then with distilled water, then let them dry well for a few days at room temperature and finely crushed.

Thereafter, we take the powdered root 1,5g were soaked in 100 mL of distilled water, refluxed for 30min (the first 15min with heating at 60 °C and the last 15 min without heating) and then filtered. The obtained aqueous extract was stored at 4°C.

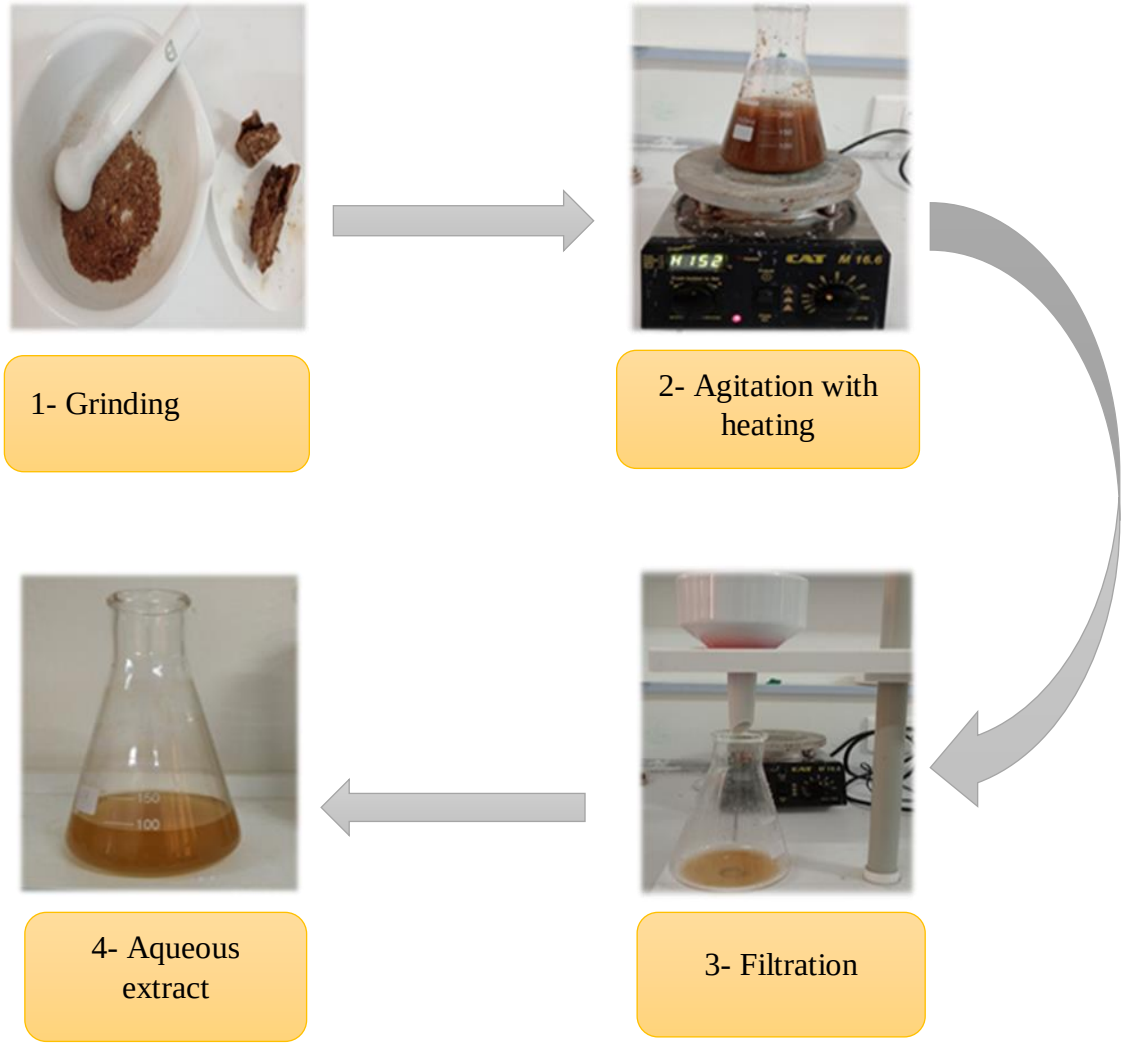



**Fig 02:**Extraction of saussurea costus aqueous extract.

#### 3.1.2. AgNPs biosynthesis:

The aqueous extract 10 ml was added drop by drop to 50 ml of nitrate silver solution with a concentration ($1{,}34.10^{-3}$ )g.mL$^{-1}$ . The reaction mixture was maintained at 60°C for 60 minutes under constant mechanical agitation. The colorless silver nitrate solution is changed to dark brown, indicating the formation of AgNPs and was confirmed by UV-Vis spectroscopy (Figure 03). After the synthesis of AgNPs, the solution containing nanoparticles was centrifuged at 5000 rpm for 5 min to separate the AgNPs, The pellet was washed by several distilled water times to remove impurities to obtain pure Ag NPs powder. The collected pellets of silver nanoparticles were dried in an oven at 50°C.

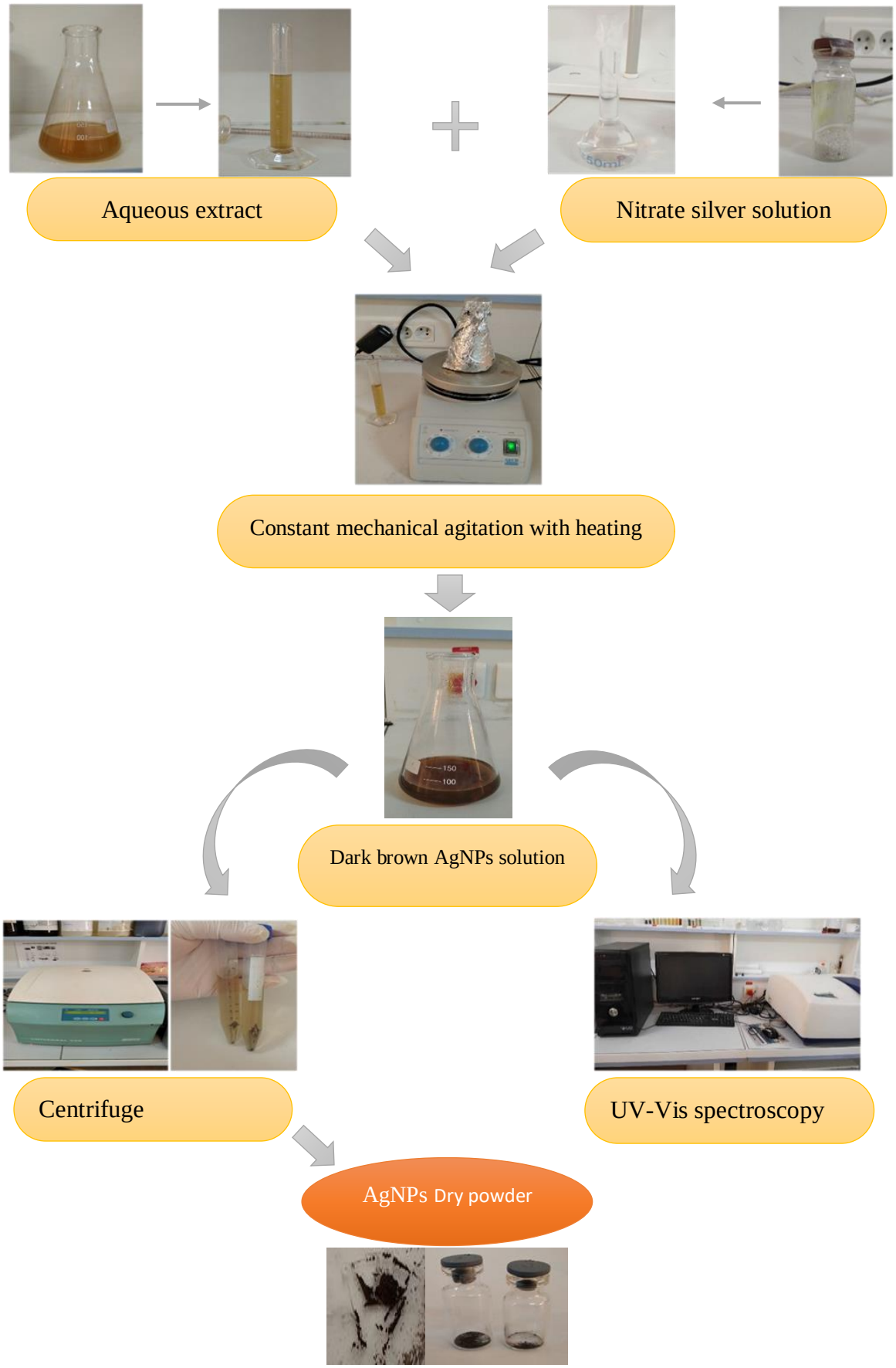



**Fig.3:** The protocol following in the formation of silver nanoparticles.

## 4. Characterization of silver nanoparticles:

### 4.1. Mechanism of formation of silver nanoparticles:

The mechanism of nanoparticle formation is derived from the literature taking into account the fact that the plant extract is very rich in polyphenols. The presence of Ag+ causes oxidation hydroxyl groups to form an intermediate silver complex by Quinone and Ag+ ions, the latter are reduced to metallic Ag in presence of free electrons**.**

The following reaction is the reaction of AgNPs formation mechanism **[1].**

$$2Ag^{+} + 2e^{-} \rightarrow 2Ag^{0}$$

Or

$$2H^{+} + 2e^{-} \rightarrow H_2$$

$$2Ag^{+} + H_2 \rightarrow 2Ag^{0} + 2H^{+}$$

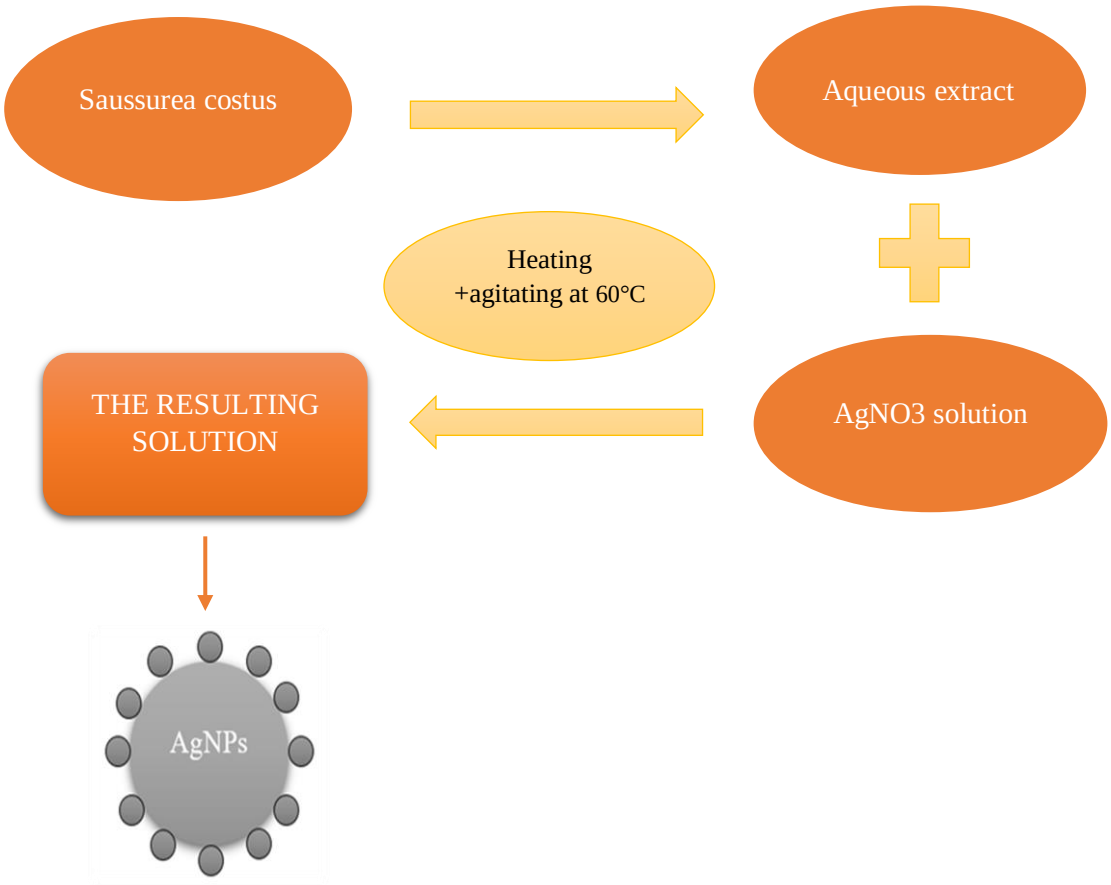



**Fig 4:**Mechanism of formation of silver nanoparticles by saussurea costus.

**4.2. Characterization techniques of silver nanoparticles:**

All the techniques for characterizing silver nanoparticles are grouped in the figure 5.

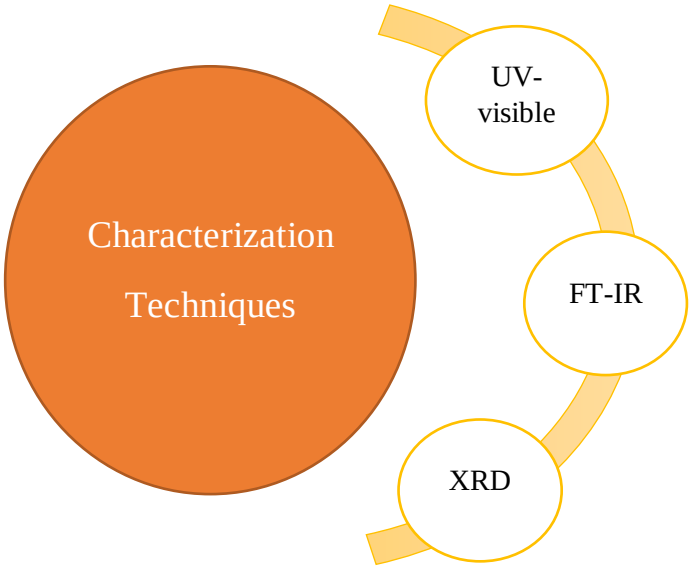



**Fig5:**Characterization techniques of silver nanoparticles.

**4.2.1. UV-visible spectroscopy:**

**4.2.1.1. Method principle:**

UV-Visible spectroscopy is a widely used technique for sorghum and mineral, it is based on the transition of valence electrons from ground to the state excited by an electromagnetic wave. This transition is accompanied by properties rotational and vibrational molecules that requires a fairly strong energy that corresponds to the wavelength of the UV-visible**[2].**

**Fig 6:**UV-visible spectroscopy.

### 4.2.2-Infrared spectroscopy Fourier transform (FTIR)

#### 4.2.2.1-Principle:

Infrared is a widely used method for the characterization and identification of compounds or their functional groups (chemical bonds) in a mixture of extracts. The identification of the bonds is done using the corresponding wave number and the determination of group characteristic by a spectrum of an unknown compound that will be identified by comparison to the library of known compounds. This technique was used to identify the functional grouting present in a sample as well as the bonds developed after the silver nanoparticle formation **[3].**

**Fig.7:** Infrared spectroscopy Fourier transforms (FT-IR).

### 4.2.3. X-Ray Diffraction (XRD):

#### 4.2.3.1. Principle:

The X-ray diffraction technique is a method to determine the phases crystallized from a sample and identify the arrangement of these atoms and their distance inter-atomic. This technique consists in observing the interaction of X-rays with matter, when the material is bombarded by X-rays radiation emitted in all directions with waves of the same phase, of the same wavelength, this diffusion leads interference between the waves diffused by each atomic plane thus forming a wave diffracted whose characteristics depend on the crystalline structure of matter, the diffractogram is recorded as a spectrum. Spectra can be obtained at from a solid fragment or small amount of powder **[4].**


**Fig8:** X-Ray Diffraction.

# 5. Antioxidant activity:

## 5.1. Antioxidants:

Antioxidants are chemical compounds capable of effectively minimizing rancidities, delaying lipid per oxidation, without effect on the sensory and nutritional properties of the food product. They maintain the quality and increase the shelf life of the product. In addition, the ideal food antioxidant must be fat soluble, effective in low doses, and non-toxic does not cause staining, odor, or unwanted flavor, resistant to technological processes, it is stable in fine product**[5].**

## 5.2. Mechanism of action:

In general, an antioxidant can prevent the oxidation of another substrate by oxidizing itself faster than this one. Such an effect results from a structure of donors of hydrogen atom or electrons often aromatic cases of phenol derivatives. In addition, their intermediate radicals are relatively stable due to resonant delocalization and lack of suitable positions to be attacked by molecular oxygen. Antioxidants are actually preventive agents, they block initiation by complexing catalysts, reacting with oxygen, or capable terminators to deflect or trap free radicals, and they act by forming non radical finished products. Others by interrupting the peroxidation chain reaction, reacting quickly with a fatty acid radical before it can react with a new fatty acid. While other antioxidants absorb excess energy from singlet oxygen to turn it into heat**[5].**

## 5.3. Assessment of antioxidant potency:

Many methods have been used to assess antioxidant activity. Most of these methods are based on the staining or discoloration of a reagent in the reaction medium. In our study we used a chemical test that is: the effect of antioxidants on the free radical (2,2 diphenyl 1-1-picrylhydrazyl) (DPPH).

### 5.3.1. Principle:

The chemical 2,2-diphenyl-1-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl) was one of the first free radicals used to study the structure-antioxidant relationship of phenolic compounds**[6].**

DPPH, initially purple, turns to DPPH-H, pale yellow absorbing at 517 nm**[7].**

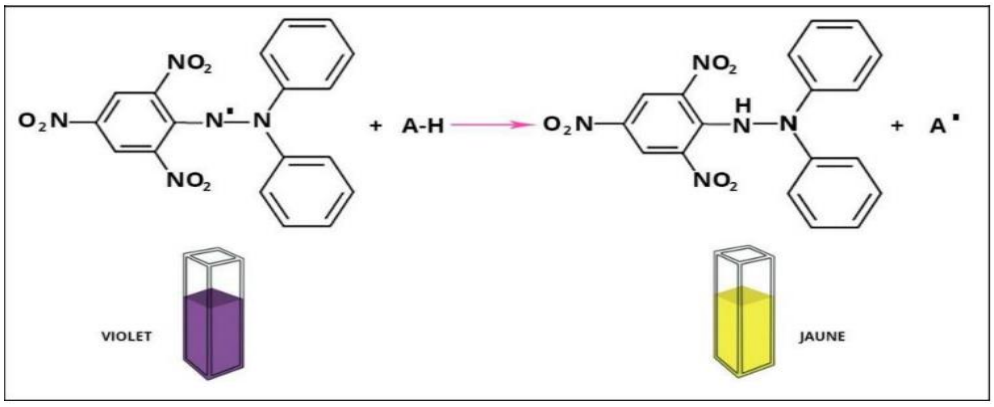



**Fig 9:** Free and reduced form of the DPPH test**[7].**

## 5.4. Ascorbic acid:

Ascorbic acid is a water-soluble antioxidant; it's essential role in compartments is poorly known. It intervenes in its mechanism of action, such as oxydo-reduction reactions between the reduced form of ascorbic acid and its oxidized form (dehydro ascorbate)

## 5.5. Practical implementation:

**5.5.1. Preparation of the DPPH stock solution:**

DPPH is solubilized in Ethanol at a rate of ($2{,}5.10^{-5}$ µg.mL$^{-1}$), under magnetic agitation for 60min away from oxygen and light.

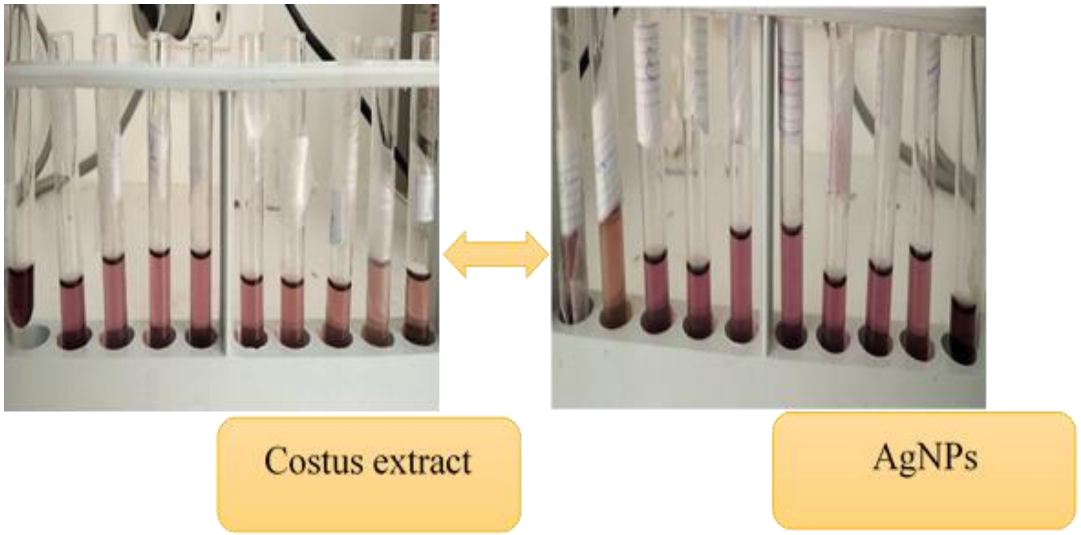
**5.5.2. Preparation of the different concentrations of silver nanoparticle and extract:**

Different concentrations of silver nanoparticle and extract (125, 60, 45, 30, 25,20,15,12,10,5 µg.mL$^{-1}$) in Ethanol were prepared;1ml of the DPPH solution is added to each tube using a micro pipette and the solution is well mixed(Figure10).

This mixture was kept at room temperature for 30 minutes in a dark place. After 45 minutes of incubation, the absorbance of the mixture was taken at 517 nm using the UV-visible Spectro photometer. And for Ascorbic acid was used as the positive control. The results were expressed as a percentage of inhibition compared to control negative control values.

**Fig10:**discoloration of DPPH under the influence of costus extract, AgNPs at different concentrations.

.




**5.5.3. Calculation of inhibition percentages:**

Inhibition percentages were calculated using the following formula:

$$I\% = (Ac - At) / Ac$$

**Ac**: absorbance of control.

**At**: absorbance of test performed.

This parameter is defined as the concentration of antioxidant required to decrease the initial concentration of 50%, it is inversely related to antioxidant capacity.

## 6. Biological test:

### 6.1. Antibacterial activities:

**6.1.1. Bacterial Strains:**

To demonstrate microbial activity, four bacterial strains and one fungal strain were tested against the prepared Nanoparticles, the extract of S. costus. The bacterial strains used are referenced and codified as follows(table 2):

**Table2**: Bacterial strains studied:

| | |
|---|---|
| Gram-negative bacteria | • Escherichia coli ATCC25922.<br>• Klebsiella pneumoniae ATCC 70603. |
| Gram-positive bacteria | • Staphylococcus aureus ATCC25923. |
| Yeast fungal strains | • Candida albicans ATCC10231. |


**Fig11:**The four bacterial suspensions.

### 6.1.2. Culture media:

Mueller-Hinton agar is the only solid culture medium for the study of sensitivity that has been validated by the NCCLS. It is recommended to always use agar Mueller Hinton for agar diffusion tests, depending on the guide lines international standards and the NCCLS. Since the way Mueller-Hinton agar is prepared may affect the results of the disk diffusion procedure, it is very important to refer to Section C below for instructions on preparation and quality control of this environment **[8] [9].**

### 6.1.3. The diffusion method in agar medium:

The aim of the antibiotic is to study the sensitivity or resistance of strains a number of specific antibiotics for each type. For the realize, we have applied the classical method of diffusion of antibiotic disks on Muller Hinton (MH) agar which is a standardized medium for all bacteria except some demanding strains. This method determines the growth inhibitory activity of biocides by measuring the inhibition diameter around a Whatman paper disc impregnated by the different dilutions of the solution to be tested**[10].**

The principle is to deposit discs, loaded with a known dose of antibiotics, on the surface of the MH agar previously seeded with strains studied, and followed by an incubation of boxes in the oven at 37C for 24 h**[11].**

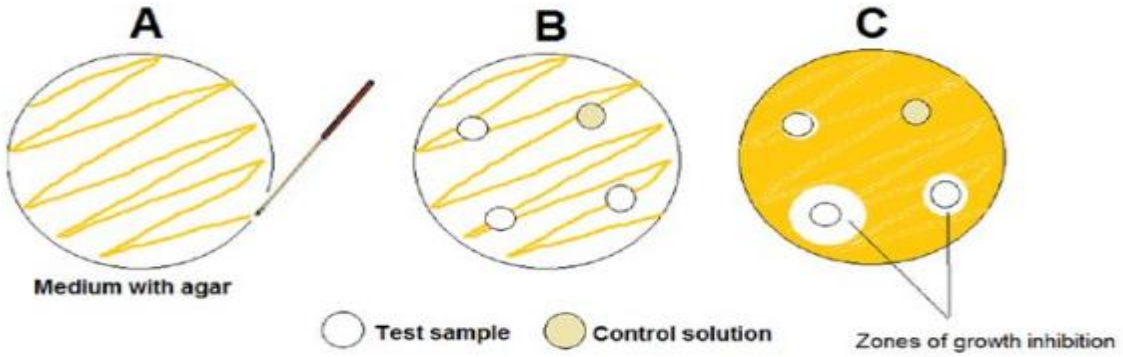



**Fig 12:** Schematic representation of the disk diffusion method **[12]**.

### 6.1.4. Seeding:

Seeding is carried out by sterile swabs on Petri dishes containing MH agar. A swab is soaked in standardized bacterial suspension and then rubbed over the entire gel surface, up and down in tight streaks. The operation is repeated three times by turning the box 60°C each time. The boxes thus seeded were left for 15 minutes.

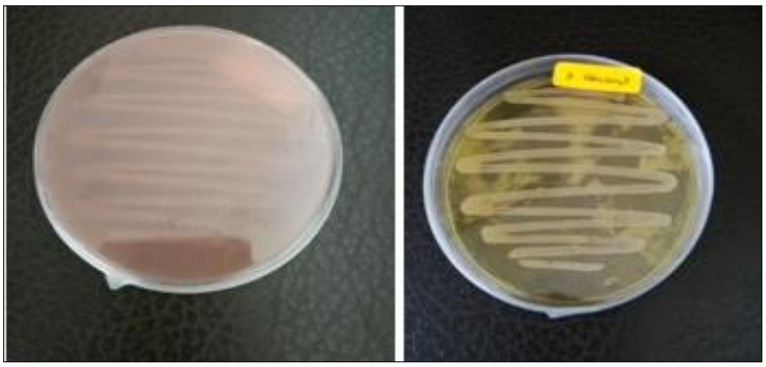


**Fig13**:revefication of E. coli and S. aureus strains.

### 6.1.5. Disk application:

Once the Muller Hinton agars are seeded, the previously prepared watt man paper disks are placed on the surface of the agar under sterile conditions, using a tong sterilized with the Bunsen nozzle, were prepared using a sterilized drill gel. The latter is soaked by the fermented extract of costus until total impregnation of the disc and it is the same for Ag nanoparticles.
For the extract we took different concentrations (100µl,200µl,700µl) which dissolve in 1000µl DMSO. The tests were repeated three times for each strain and for AgNPs take a single concentration that dissolved in 1000µl DMSO. The tests were repeated twice for each strain by marking each time, the names of these products on the lower side of the box. The boxes are closed and left for a certain time at room temperature to allow the diffusion of the solutions. Then, they are incubated in an oven at 37°C for 24 hours as we present on (Figure 14).

Pet rage dishes and the working environment of antibacterial activity

Preparation of the bacterial culture medium

Preparation of dilute extract solutions and nanoparticles

The deposition of the discs and the ambition by the solutions of the different concentrations after the culture medium is seeded with one of the bacterial strain suspensions

incubated in an oven at 37°C for 24 hours.

**Fig 14:** the steps of preparation the antibacterial activity of saussurea costus extract and Ag NPs.

# *Chapter 4:*

# *Results and discussion*

## I.The biosynthesis of silver nanoparticles:

The silver nanoparticles are synthesized using S. costus extract which showed a very dark brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. Prepared silver NPs was characterized by using different characterizations methods as UV-Vis, XRD and FTIR.

### I.1. Characterization of AgNPs:

#### I.1.1. Absorption spectroscopy UV-Vis:

The synthesis of AgNPs was confirmed by the characteristic color change, yellow to brown. The reaction medium changed from light yellow to light brown. Another color change was observed, from light brown to dark brown after stirring 24 hours.

The confirmation of the synthesis of AgNPs particles in solution was controlled by UV-vis spectral analysis for which aliquots of the reaction mixture (after completion of the reaction) were taken and used for UV-vis spectroscopy measurements. In the UV-vis absorption spectrum, a broad and strong peak, located at about 430nm, was observed for nanoparticles synthesized with costus.

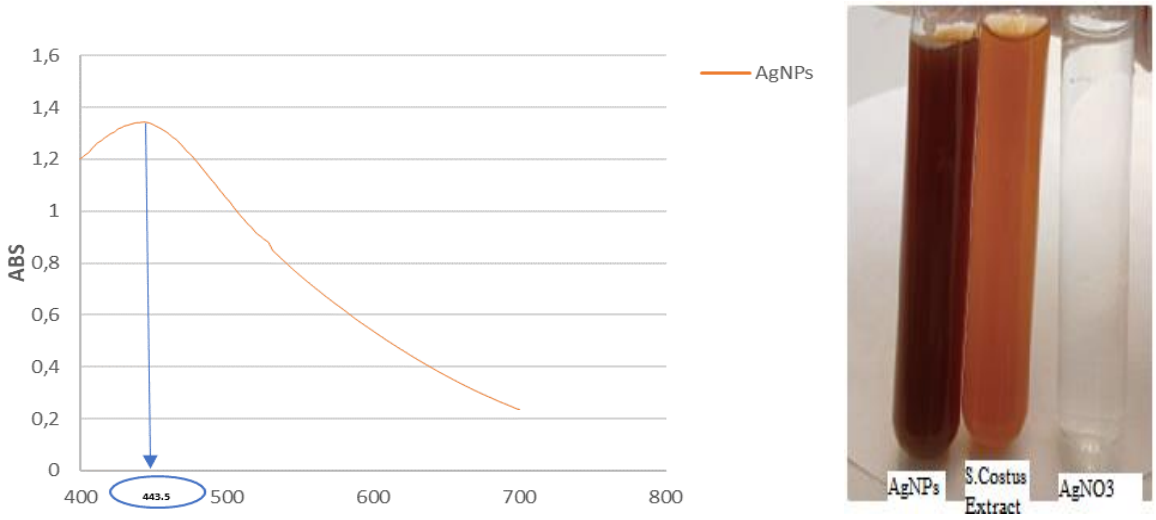



**Fig15**:UV-Vis spectrum of Ag NPs synthesized by aqueous extracts of S.costus

#### 1.1.2. X-ray diffraction (XRD):

Nanoparticles of different sizes were characterized by X-ray diffraction(DRX) to more accurately determine which crystalline phase the nanoparticles. XRD is also used to determine the silver mesh parameter formed, as well as the average size of diffracting domains. To better see the structural properties of our prepared sample the figure below(figure 2) shows the X-ray diffractograms (XRD) of silver nanoparticles. The purpose of this technique is to determine the size of powder formed from silver nitrate nanoparticle .Five different separate diffraction peaks were observed at2 theta =35 , 38,12 , 44,4, 64,3 , 77,3 which could be attributed to diffraction from (101), (111), (200),(220), and (311). The 2 theta values of the XRD peaks matched perfectly with the reference of the face centered cubic structure of the **[13]**.To confirm AgNPs peaks, high value 2 theta =38.12°, In addition, a small peak at 2 theta =78.7°,high intensities reveal high crystallinity in the interval in the second part we find small peaks (lower angles indicating the presence of silver).

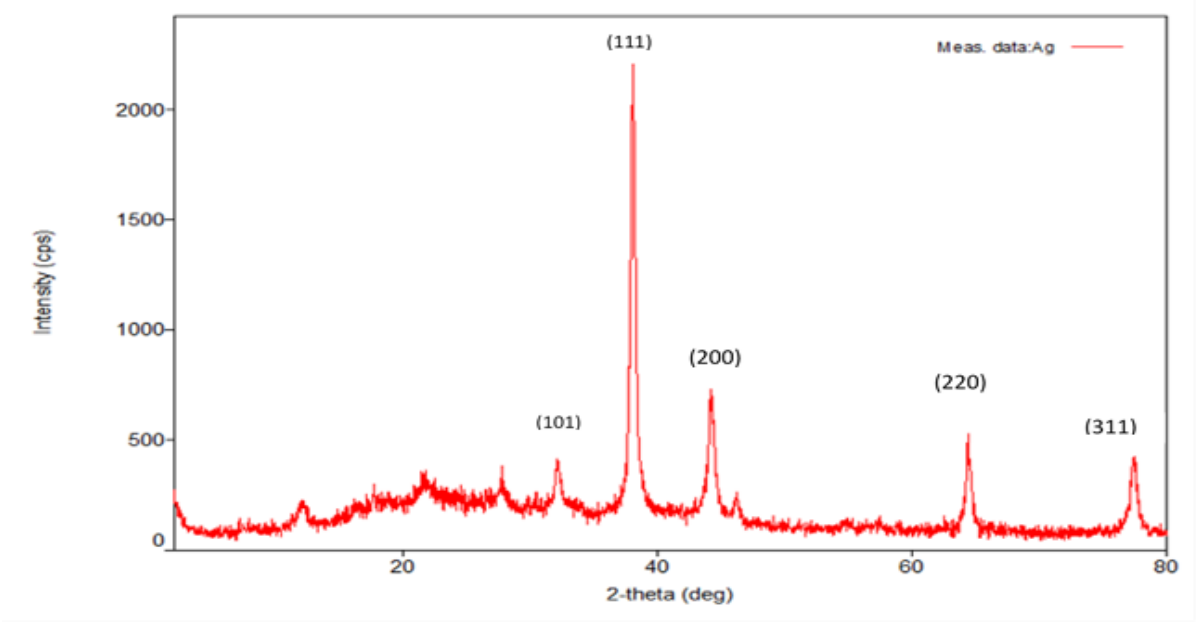



**Fig16:**DRX spectrum of AgNPs synthesized by S.costus extract

### 1.1.3. Fourier Transform Infrared Spectroscopy (FTIR):

The FTIR analysis was performed to identify the potential biomolecule responsible for the reduction of silver ions and the blockage factor of the silver biomolecule synthesized by S. costus. Spectral bands were interpreted to determine the functional groups of organic compounds attached to silver nanoparticles**[14].**the results show significant absorption spectra the band seen at the 4000–450 cm-1(figure17).

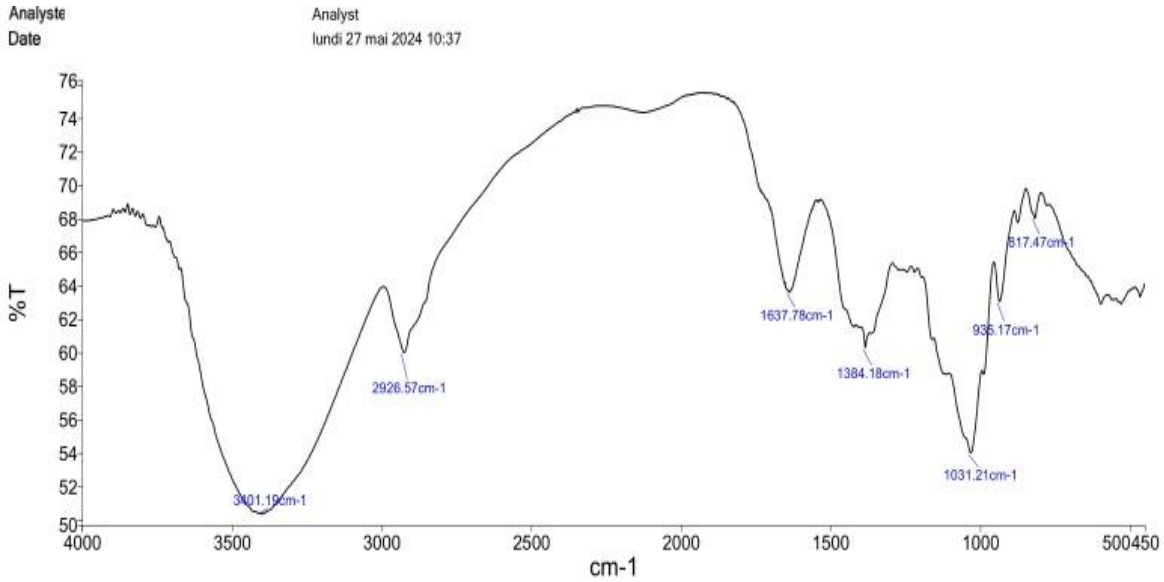



**Fig17:**Ag NPs FTIR spectra

NPs Ag synthesized by S.costus, absorption peaks are observed at 3401.19 , 2926.57 ,1637.78 , 1384.18 , 1031.21 , 936.17 ,817.47, 603 and 517 cm-1.
According to the infrared vibration chart we find:

**Table3:** FT – IR Interpretation:

| Assignment | Wavelength (cm-1) | Intensity |
|---|---|---|
| O-H | 3401,19 | Strong |
| C-H | 2926,57 | Strong |
| C=O | 1637,78 | Strong |
| C-O | 1384,18 | Strong |
| C-C | 1031,21 | Strong |
| C-X | 817,17 and 936,17 | Medium |
| Ag-O | 517 and 603 | Weak |

IR spectroscopy confirmed the formation of silver nanoparticles after the reduction of Ag+ ions by OH and these results are similar to that observed by other study**[15].**

## 2. Evaluation of the antioxidant activity:

Antioxidant activity is one of the main capabilities sought in products natural. For this, we evaluated the antioxidant activity of our silver nanoparticles and costus extract using the DPPH reduction method.

The results obtained are expressed as percentage inhibition of the free radical DPPH and compared to a reference antioxidant ascorbic acid. For this study, the antioxidant activity of Costus extract and AgNPs was evaluated by measuring the level of DPPH after addition of AgNPs at different concentration, the same was with the ethanolic extracts of Saussurea lappa at different concentrations, in comparison with ascorbic acid.

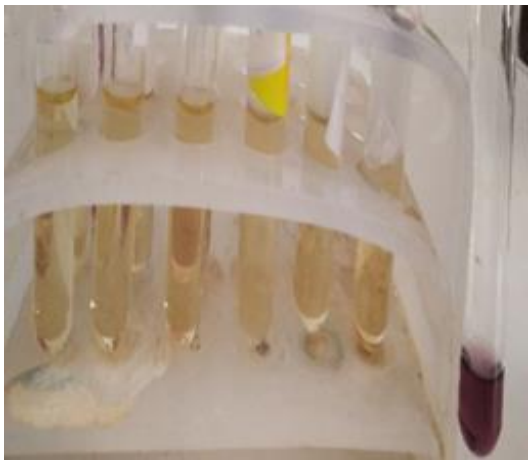


**Fig18:**Discoloration of DPPH under the influence of AgNPs at different concentrations.

According to the results obtained in Figure (18), antioxidant activity was noted in the AgNPs, the color of the solution changed from purple to yellow, this discoloration was confirmed by passing the tubes described above in the absorption rate measurement at $\gamma = 517$ nm, the results are summarized in the table below table (4)results of inhibition of ascorbic acid ,S.costus extract, AgNPs in different concentrations.

**Table 04:** Antioxidant activity of the root extract and its nanoparticles using DPPH assay.

| Concentration (µg/ml) | % Inhibition | | |
|---|---|---|---|
| | Ascorbic acid | Costus extract | AgNPs |
| **15** | 49,51 | 16,99 | 33,52 |
| **20** | 57,65 | 21,52 | 42,09 |
| **25** | 60,78 | 28,25 | 45,85 |
| **30** | 63,6 | 35,01 | 50,65 |
| **45** | 66,42 | 47,77 | 59,45 |
| **60** | 70,69 | 52,01 | 66,97 |
| **125** | 75,13 | 54,12 | 67,6 |

The obtained values allowed drawing curves which means the almost total reduction of the DPPH in its nonradical shape fig 19. From these curves, we can determine the percentages inhibition obtained as a function of the concentrations used and the IC50 value of each extract, ascorbic acid and AgNPs.

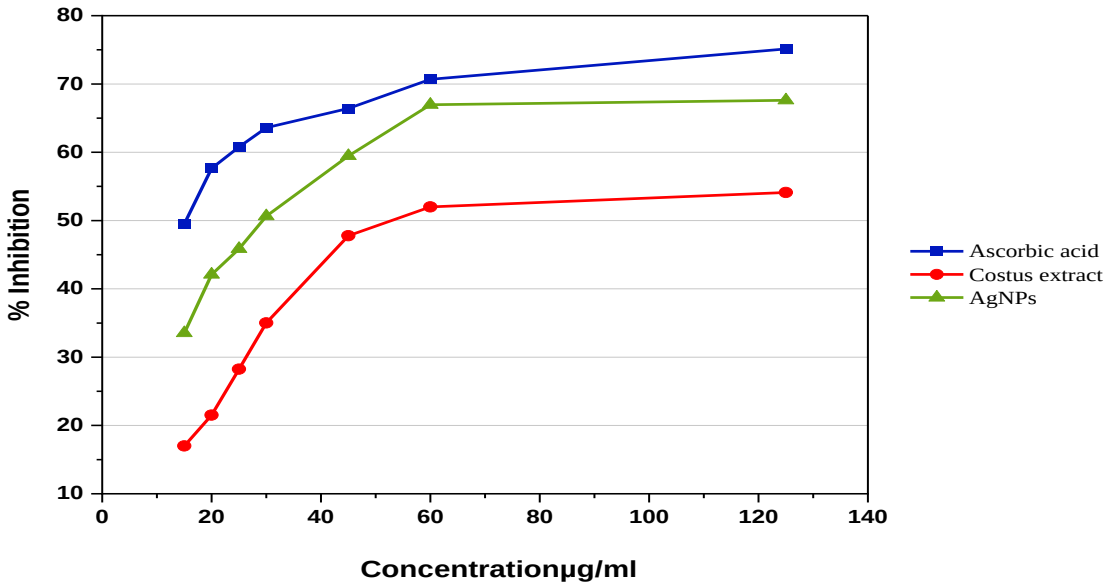



**Fig19:**Percentage inhibition of DPPH based on concentrations of samples of silver nitrate nanoparticles synthesized using the root of costus extract.

According to Table 04 and Figure19, the percentage of extract inhibition and AgNPs between(33,52%) and (67,6%). The highest inhibition percentage in the extract (54,12%) and AgNPs (67,6%) ).Note that the higher concentration, the greater the capacity to trap. These results are compared to the percentage of inhibition by a powerful antioxidant (ascorbic acid)used in this study as a positive control.

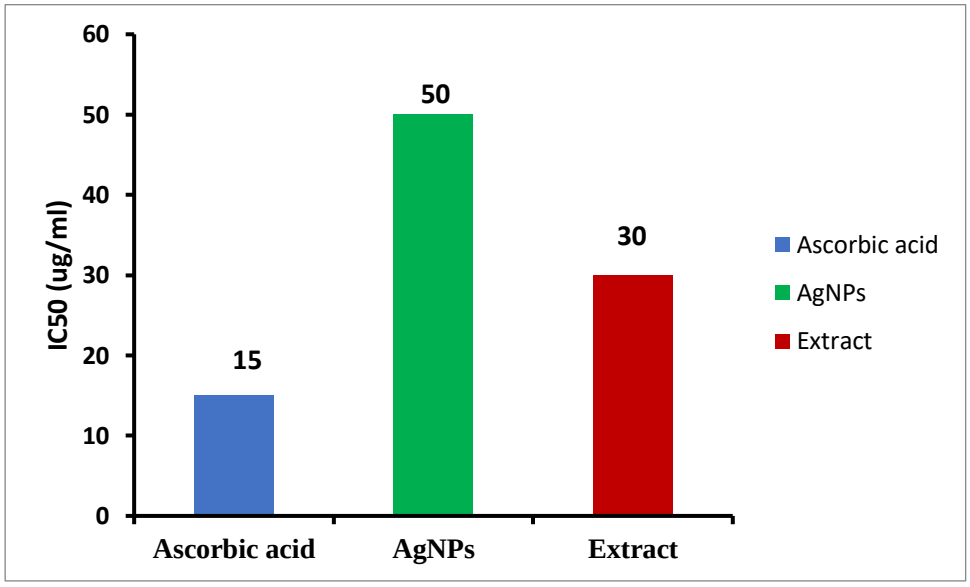
**2.1-The Determination of IC50:**

The inhibition of the DPPH radical is expressed in IC50, this parameter is defined as the effective concentration of the extract capable of trapping 50% of the DPPH radicals in the mixture reaction. The lower the IC50 value, the greater the antioxidant activity of a compound **[16].**

From these curves fig19, we can determine the percentagesinhibition obtained as a function of the concentrations used and the IC50 value ofeach extract ,ascorbic acid and silver NPs.IC50 values are represented in thetable above and in Histogram 01.

**Table5:** IC50 results for the DPPH test.

| | Ascorbic acid | AgNPs | Extract |
|---|---|---|---|
| **IC50 = (µg / ml)** | 15 | 50 | 30 |




**Histogram 01:**Inhibitory concentration (µg/ml) of 50% of DPPH radicals (IC50).

The results reveal that the extract and AgNPs tested as well as the ascorbic acid taken as a reference, are antiradical. However ,AgNPs presented the highest anti-radical activity with 50 µg/ml IC50 followed by ethanolic extract with (IC50 30 µg/ml).

The scavenging activity values of the AgNPs for all tested concentrations were found to be higher than those of ascorbic acid, due to the presence of the anti-oxidant poly-phenolic compounds on their surfaces. Similar to our study**,[17]**a dose dependent increase in the DPPH scavenging was also observed by AgNPs synthesized using leaf extracts of Blighia sapid and reported 72% antioxidant activity for DPPH (125 mg mL1)[18].

## 3- Evaluation of the antibacterial activity:

For the antibacterial study, we tested the antibacterial effectiveness of the AgNPs, as well as the aqueous extract of the studied plant (S. costus) on three bacterial strains, then the

microorganisms were grown and inoculated, which are the positive and negative bacteria. The scale for estimating antimicrobial activity is given by **[19]**classified the diameter of the zones of inhibition (D) of microbial growth as follows:

• Resistant(-): D< 8 mm
• Sensitive (+): 9mm <D <14mm
• Very sensitive (++): 15mm <D< 19 mm
• Extremely sensitive (+++): D >20 mm

**3.1-Antibacterial power of extracts and AgNPs:**

After incubation at 24h at 37°c, we see an increase in the diameters of inhibition zones. AgNPs and extract showed some degree of antibacterial activity as can be seen in the Petri dishes used in the diffusion test (Figure 20). The average size of the inhibition zone measured for all strains is shown in Table (6).

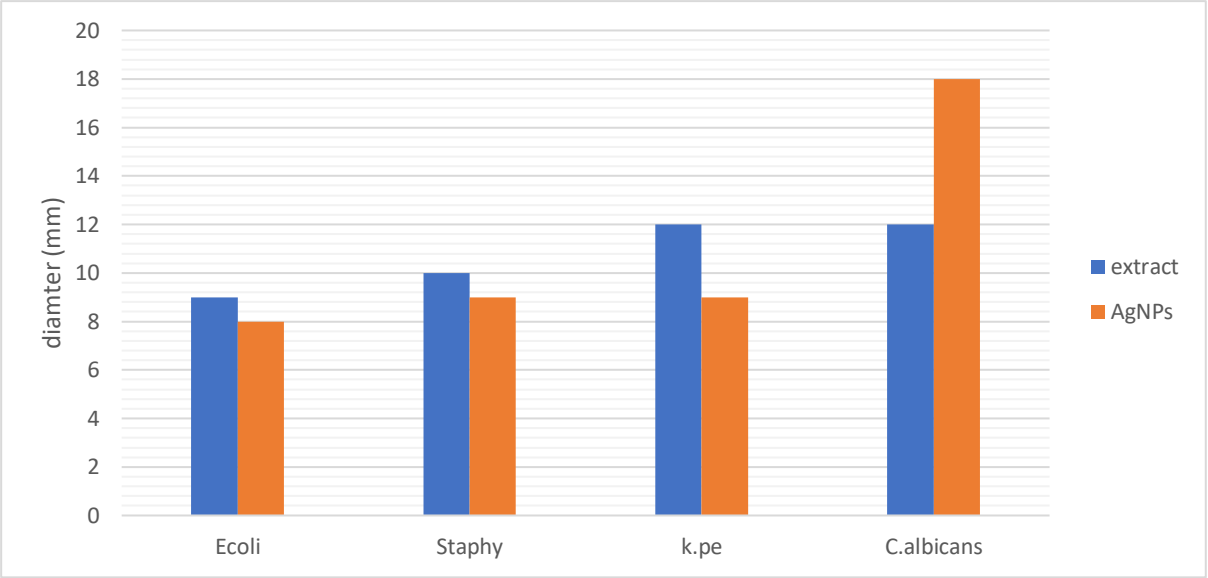
| **Gram-negative bacteria** | | | | **Gram-positive bacteria** | | **Yeast fungal strains** | |
|---|---|---|---|---|---|---|---|
| **Escherichia coli** | | **Klebsiella pneumoniae** | | **Staphylococcus aureus** | | **Candida albicans** | |
| Extract | Ag NPs | Extract | Ag NPs | Extract | Ag NPs | Extract | Ag NPs |
|  |  |  |  |  |  |  |  |

**Fig20:** Antibacterial activity of silver nanoparticles and extract against various strains bacterial pathogens.

The results presented in Figures 20 show that the S.costus extract has good activity against Klebsiella pneumoniae compared to other strains. For AgNPs there is a good result against C.albicans.

**Table6**: Inhibition zone of silver nanoparticles and extract against various pathogenic bacteria.

| | **The zone of inhibition(mm)** | | **Sensitivity** | |
|---|---|---|---|---|
| **The Stump** | **Extract** | **Ag NPs** | **Extract** | **Ag NPs** |
| **Escherichia coli** | 9 | 8 | Sensitive | Resistant |
| **Staphylococcus aureus** | 10 | 9 | Sensitive | Sensitive |
| **Klebsiella pneumoniae** | 12 | 9 | Sensitive | Sensitive |
| **Candida albicans** | 12 | 18 | Sensitive | Very sensitive |




**Histogram02:**Diameter Zone of inhibition produced by biosynthesized AgNPs and saussurea extract against various bacteria.

Silver nanoparticles are well-known as the most universal antimicrobial substances due to their strong biocidal effect against microorganisms and the results of our work presented in the table 6and the histogram 2 shown that the bacteria had a high sensibility against the Ag NPs formed and against the extract especially candida albicans, 18 mm for the AgNPs and 12mm for the Costus Extract. This may be related to the fact that the membrane permeability increases with AgNPs dose; as a result, the cell wall gets damaged.

In contrast, slightly inhibited bacterial growth in E. coli (inhibition zone diameter,8 mm), S. aureus (inhibition zone diameter, 9 mm), and K. pneumoniae (inhibition zone diameter, 9 mm), and this could be probably due to insufficient interaction of AgNPs with the cell membrane of bacteria .

The S. Costus extract showed also a sensitivity bacterial effect against all the tested bacteria with E. coli (inhibition zone diameter,9 mm), S. aureus (inhibition zone diameter, 10 mm), and K. pneumoniae (inhibition zone diameter, 12 mm). These new results confirmed what was obtained in previous studies**[20].** It can be concluded that Gram-negative pathogens and Gram-positive pathogens are sensitive to the as synthesized AgNPs.

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Green Synthesized Silver Nanoparticles Against Selected Gram-negative Foodborne PathogensFrontiers in Microbiology

# *General Conclusion*

## General Conclusion

Nowadays, a large number of aromatic and medicinal plants have very important biological properties that find many applications in various knowledge in medicine, pharmacy, cosmetics and agriculture. This renewed interest comes from the fact that medicinal plants are an inexhaustible source of bioactive substances, and on the other hand the side effects induced by drugs worry users who turn to less aggressive treatments for the body.

The work presented focuses on the development of silver nanoparticles to replace the use of chemical reducers. For this purpose, we have chosen saussurea costus one of the richest medicinal plants in order to put into effect the manufacture of nanoparticles with biological and chemical effect.

The silver nanoparticles prepared from S.costus root aqueous extract using the green synthesis approach. The synthesis of AgNPs was confirmed by the characteristic color change, yellow to brown and was observed under UV–Vis Spectroscopy monitored at 435 nm. AgNPs crystalline nature was confirmed by XRD study, FTIR spectroscopy confirmed the formation of silver nanoparticles after the reduction of Ag+ ions by OH

The antioxidant activity of Costus extract and AgNPs was evaluated by measuring the level of DPPH after addition of AgNPs at different concentrations; the same was with the ethanolic extracts of S Costus at different concentrations, in comparison with ascorbic acid. The results reveal that the extract and AgNPs tested as well as the ascorbic acid taken as a reference, are antiradical. However, AgNPs presented the highest anti-radical activity with 50 µg/ml IC50 followed by ethanolic extract with (IC50 30 µg/ml). this is maybe due to the presence of the anti-oxidant poly-phenolic compounds on their surfaces.

We tested the antibacterial effectiveness of the AgNPs, as well as the aqueous extract of the studied plant (S. costus) on three bacterial strains and one fungal strain, which are the positive and negative bacteria; we have applied the classical method of diffusion of antibiotic disks on Muller Hinton (MH) agar which is a standardized medium for all bacteria. After incubation at 24h at 37°c, we observe an increase in the diameters of inhibition zones.

The results shown that the bacteria had a high sensibility against the Ag NPs formed and against the extract especially candida albicans, 18 mm for the AgNPs and 12mm for the Costus Extract. This may be related to the fact that the membrane permeability increases with AgNPs dose; as a result, the cell wall gets damaged. We noted also slightly inhibited bacterial growth in E. coli, S. aureus, and K. pneumoniae, and this could be probably due to insufficient interaction of AgNPs with the cell membrane of bacteria. It can be concluded that Gram-negative pathogens and Gram-positive pathogens are sensitive to the as synthesized AgNPs.

At the end of this research, it appears that the green synthesis of nanoparticles is an environmentally friendly and cost-effective technique that offers a new and potential to chemically synthesized nanoparticles, decreasing the use of hazardous and toxic chemicals by preserving the environment.

In perspective, it would be Synthesis of silver nanoparticles supplemented with antibiotics, Perform histological sections of the various organs mainly the intestine to see the effect of nano-antibiotics on its membrane structure.

**Abstract:** Recently, silver nanoparticles have gained attention because of their antimicrobial activity which offers the possibility of their use for medical purposes. Metallic nanoparticles can be obtained by physical, chemical or biological methods. Therefore, in the present study, an attempt was made to formulate a cost effective and environment friendly technique for green synthesis of silver nanoparticles. The silver nanoparticles were synthesized using extract of Costus specious with AgNO3 solution. Saussurea Costus is a plant medicinal used in traditional medicine. The as-formed AgNPs were analyzed by (UV-Vis), FT-IR and X-ray diffraction (XRD) . The synthesis of AgNPs was confirmed by the characteristic color change, yellow to brown and was observed under UV–Vis Spectroscopy monitored at 435 nm. Additionally, in the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical antioxidant study, the antimicrobial properties against Gram-positive, Gram-negative pathogens and fungal strain using method of diffusion of antibiotic disks on Muller Hinton (MH) agar of the obtained silver nanoparticles at different concentrations were investigated. The results showed that These FTIR and DRX methods confirmed the presence and molecular interaction of silver nanoparticles and their crystalline nature. AgNPs presented the highest anti-radical activity with 50 µg/ml IC50 followed by ethanolic extract with (IC50 30 µg/ml). the antimicrobial study showed that the bacteria had a high sensibility against the Ag NPs formed and against the extract especially candida albicans, 18 mm for the AgNPs and 12mm for the Costus Extract. This green chemistry is very advantageous for AgNPs biosynthesis, for example, cost-effectiveness and usability for medicinal, pharmaceutical and extensive industrial applications. Furthermore, the bio-recovery unit for plant extracts provides a greater ease of handling, compared to micro-organisms.

**Keywords:** Saussurea Costus, silver nanoparticles, biosynthesis, anti-radical activity, bacterial resistance.

**Résumé :** Récemment, les nanoparticules d'argent ont attiré l'attention en raison de leur activité antimicrobienne qui offre la possibilité de leur utilisation à des fins médicales. Les nanoparticules métalliques peuvent être obtenues par des méthodes physiques, chimiques ou biologiques. Par conséquent, dans la présente étude, une tentative a été faite pour formuler une technique rentable et respectueuse de l'environnement pour la synthèse verte de nanoparticules d'argent. Les nanoparticules d'argent ont été synthétisées à l'aide d'un extrait de Costus spécieuse avec une solution AgNO3. Saussurea Costus est une plante médicinale utilisée en médecine traditionnelle. Les AgNPs tels que formés ont été analysés par (UV-Vis), FT-IR et diffraction des rayons X (DRX). La synthèse des AgNPs a été confirmée par le changement de couleur caractéristique, du jaune au brun, et a été observée sous spectroscopie UV-Vis surveillée à 435 nm. De plus, dans l'étude sur les antioxydants radicaux libres DPPH (2,2-diphényl-1-picrylhydrazyl), les propriétés antimicrobiennes contre les agents pathogènes Gram-positifs et Gram-négatifs et les souches fongiques en utilisant la méthode de diffusion de disques d'antibiotiques sur gélose Muller Hinton (MH). des nanoparticules d'argent obtenues à différentes concentrations ont été étudiées. Les résultats ont montré que ces méthodes FTIR et DRX ont confirmé la présence et l'interaction moléculaire de nanoparticules d'argent ainsi que leur nature cristalline. Les AgNPs présentaient l'activité anti-radicalaire la plus élevée avec une CI50 de 50 µg/ml, suivis de l'extrait éthanolique avec (CI50 de 30 µg/ml). l'étude antimicrobienne a montré que les bactéries avaient une sensibilité élevée contre les Ag NP formés et contre l'extrait notamment candida albicans, 18 mm pour les AgNPs et 12 mm pour l'extrait de Costus. Cette chimie verte est très avantageuse pour la biosynthèse des AgNPs, par exemple en termes de rentabilité et de facilité d'utilisation pour des applications médicales, pharmaceutiques et industrielles étendues. De plus, l'unité de bio-récupération des extraits de plantes offre une plus grande facilité de manipulation par rapport aux micro-organismes.

**Mots clés :** Saussurea Costus, nanoparticules d'argent, biosynthèse, activité anti-radicalaire, résistance bactérienne.

**ملخص:** في الآونة الأخيرة، اكتسبت جزيئات الفضة النانوية الاهتمام بسبب نشاطها المضاد للميكروبات مما يتيح إمكانية استخدامها للأغراض الطبية. يمكن الحصول على الجسيمات النانوية المعدنية بالطرق الفيزيائية أو الكيميائية أو البيولوجية. ولذلك، في هذه الدراسة، جرت محاولة لصياغة تقنية فعالة من حيث التكلفة وصديقة للبيئة للتخليق الأخضر لجسيمات الفضة النانوية. تم تصنيع جزيئات الفضة النانوية باستخدام مستخلص نبات القسط مع محلول AgNO3. Saussurea Costus هو نبات طبي يستخدم في الطب التقليدي. تم تحليل AgNPs المتكونة بواسطة (UV-Vis) و FT-IR و حيود الأشعة السينية (XRD). تم تأكيد تخليق AgNPs من خلال تغير اللون المميز، من الأصفر إلى البني، ولوحظ تحت التحليل الطيفي للأشعة فوق البنفسجية والمرئية الذي تم رصده عند 435 نانومتر. بالإضافة إلى ذلك، في دراسة مضادات الأكسدة الجذرية الحرة DPPH (2،2-ثنائي فينيل-1-بيكريل هيدرازيل)، تم تحديد الخصائص المضادة للميكروبات ضد مسببات الأمراض إيجابية الجرام وسالبة الجرام والسلالة الفطرية باستخدام طريقة نشر أقراص المضادات الحيوية على أجار مولر هينتون (MH). تم دراسة جسيمات الفضة النانوية التي تم الحصول عليها بتركيزات مختلفة. أظهرت النتائج أن طريقتي FTIR و DRXأكدتا وجود وتفاعل جزيئي لجسيمات الفضة النانوية وطبيعتها البلورية. أظهرت AgNPsأعلى نشاط مضاد للجذور بجرعة 50 ميكروجرام/مل IC50 يليها المستخلص الإيثانولي بجرعة 30 IC50) ميكروجرام/مل). أظهرت دراسة مضادات الميكروبات أن البكتيريا لديها حساسية عالية ضد AgNPs المتكونة وضد المستخلص وخاصة المبيضات البيضاء، 18 ملم ضد AgNPsو 12 ملم لمستخلص القسط. تعتبر هذه الكيمياء الخضراء مفيدة جدًا للتخليق الحيوي لـAgNPs ، على سبيل المثال، فعالية التكلفة وسهولة الاستخدام في التطبيقات الطبية والصيدلانية والصناعية واسعة النطاق. علاوة على ذلك، توفر وحدة الاسترداد الحيوي للمستخلصات النباتية سهولة أكبر في التعامل، مقارنة بالكائنات الحية الدقيقة.

**الكلمات المفتاحية**: سوسوريا القسط، جزيئات الفضة النانوية، التخليق الحيوي، النشاط المضاد للجذور، مقاومة البكتيريا