T.C.

TOKAT GAZIOSMANPASA UNIVERSITY

GRADUATE EDUCATION INSTITUTE

DEPARTMENT OF CHEMISTRY

MASTER'S PROGRAM WITH THESIS

GREEN SYNTHESIS, CHARACTERIZATION OF SILVER NANOPARTICLES USING *SALVIA ABSCONDITIFLORA*, *SALVIA HYPARGEIA* AND EVALUATION OF THEIR ANTIOXIDANT ACTIVITY

MASTER THESIS

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Supervisor

PROF. DR. RAMAZAN ERENLER

TOKAT-2023

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TOKAT

JANUARY, 2023

ETHICS CONTRACT

The thesis was prepared according to the thesis writing guide of Tokat Gaziosmanpaşa University Graduate Education Institute. The master thesis titled "Green Synthesis; Characterization of Silver Nanoparticles Using *Salvia Absconditiflora* and *Salvia Hypargeia* and Evaluation of Their Antioxidant Activity" which I prepared under the supervision of Prof. RAMAZAN ERENLER is an original study in accordance with scientific ethical values and rules, and if the contrary is determined, all kinds of legal sanctions will be imposed. I declare that I will accept.

20/02/2023

RILWAN ADESILE OJELADE

JURY ACCEPTANCE AND APPROVAL

The defense examination of the thesis study titled "Green Synthesis; Characterization of Silver Nanoparticles Using *Salvia Absconditiflora, Salvia Hypargeia* And Evaluation Of Their Antioxidant Activity" prepared by Ojelade Rilwan Adesile, was held on the January, 2023 and the following jury voted unanimously with the Tokat Gaziosmanpasa University Graduate Education Institute. It has been accepted as a Master Thesis in the Department of Chemistry.

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ABSTRACT

GREEN SYNTHESIS, CHARACTERIZATION OF SILVER NANOPARTICLES USING *SALVIA ABSCONDITIFLORA* AND *SALVIA HYPARGEIA* AND EVALUATION OF THEIR ANTIOXIDANT ACTIVITY

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Salvia species are the largest genus of the Lamiaceae family. There are about 1000 species in the world and 95 species in Turkey but 45 of these species are endemic. *Salvia absconditiflora* and *Salvia hypargeia* are *Salvia* species grown in Turkey but *Salvia absconditiflora* is an endemic species.

In this work, silver nanoparticles were synthesized using *Salvia absconditiflora* (sa-AgNPs) and *Salvia hypergeia* (sh-AgNPs) with green method. Each plant material (10.0 g) was heated in deionized water (150 mL) at 50°C for 2 hours, after filtration the extract solution was treated with the silver nitrate solution. The solution was centrifuged, then lyophilized to yield the solid silver nanoparticles.

Ultraviolet-visible (UV-2600 Shimadzu) spectrophotometry, Fourier transform infrared (FT/IR-4700 Jasco) spectrometer, Scanning electron microscopes (SEM), X-ray diffraction (XRD), Energy dispersive spectroscopy (EDX), Zetasizer Nano ZSP (Malvern, UK) were used for identification of silver nanoparticles. Antioxidant activity of both plant extracts and silver nanoparticles were carried out using 2,2-Diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) free radical scavenging, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS$^{\bullet+}$) radical cation scavenging, and ferric ions reducing antioxidant power assays.

The nanoparticles synthesized from both *Salvia absconditiflora* (sa-AgNPs) and *Salvia hypergeia* (sh-AgNPs) were determined to be face-centered cubic structures. The average particle sizes were calculated as 26.6 nm and 35.5 nm for sa-AgNPs and sh-AgNPs respectively. The maximum absorptions of sa-AgNPs and sh-AgNPs were defined as 469 nm and 490 nm respectively. DPPH free radical scavenging activity of *S. absconditiflora* extract and sa-AgNPs was described as 21.6±0.3 (IC$_{50}$, µg/mL), 15.8±0.2 (IC$_{50}$, µg/mL) respectively compared to the standard BHT (10.7±0.2, IC$_{50}$, µg/mL). In ABTS radical cation assay, the extract and sa-AgNPs activity was established as 8.4±0.2 (IC$_{50}$, µg/mL) and 4.8±0.1 (IC$_{50}$, µg/mL) respectively in comparison to the standard BHT (7.1±0.1, IC$_{50}$, µg/mL). Reducing power activity of extract and sa-AgNPs was found as 1.73±0.02 (µmol

TE/mg extract) and 4.6±0.1 (µmol TE/mg) respectively with respect to standard BHA (7.9 ±0.2 µmol TE/mg extract). In concerning the *Salvia hypergeia*, DPPH activity of extract and sh-AgNPs was defined as 18.0±0.9 (IC_{50}, µg/mL), 11.8±0.2 (IC_{50}, µg/mL) respectively. ABTS radical cation scavenging effect of extract and sh-AgNPs was signified as 6.7±0.2 (IC_{50}, µg/mL), 3.8±0.1 (IC_{50}, µg/mL) respectively. Reducing power effect of *Salvia hypergeia* extract and sh-AgNPs was determined as 2.4±0.04 (µmol TE/mg) and 6.1±0.1 (µmol TE/mg) respectively.

Keywords: *Salvia absconditiflora*, *Salvia hypargeia*, silver nanoparticles, spectroscopy, green synthesis.

ÖZ

GÜMÜŞ NANOPARTİKÜLLERİN *SALVIA ABSCONDITIFLORA* VE *SALVIA HYPARGEIA* KULLANILAK YEŞİL SENTEZİ, KARAKTERİZASYONU VE ANTİOKSİDAN AKTİVİTELERİNİN DEĞERLENDİRİLMESİ

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Salvia türleri, Lamiaceae familyasının en büyük cinsidir. Dünyada yaklaşık 1000, Türkiye'de 95 tür bulunmaktadır ancak bu türlerin 45'i endemiktir. *Salvia absconditiflora* ve *Salvia hypargeia* Türkiye'de yetişen *Salvia* türleridir fakat *Salvia absconditiflora* endemik bir türdür.

Bu çalışmada gümüş nanopartiküller, *Salvia absconditiflora* (sa-AgNPs) ve *Salvia hypergeia* (sh-AgNPs) kullanılarak yeşil yöntemle sentezlendi. Her bir bitki materyali (10.0 g), deiyonize suda (150 mL) 50°C'de 2 saat ısıtıldı, süzüldükten sonra özüt çözeltisi, 55°C'de 2 saat gümüş nitrat çözeltisi (1.0 mM, 100 mL) ile etkileştirildi. Çözelti santrifüjlendi, ardından liyofilize edilerek katı gümüş nanopartiküller elde edildi.

Ultraviyole görünür (UV-2600 Shimadzu) spektrofotometre, Fourier dönüşümlü kızılötesi (FT/IR-4700 Jasco) spektrometre, Taramalı elektron mikroskopları (SEM), X-ışını kırınımı (XRD), Enerji dağılımlı spektroskopi (EDX), Zetasizer Nano ZSP (Malvern, UK) gümüş nanopartiküllerin karakterizasyonu için kullanıldı. Hem bitki özlerinin hem de gümüş nanopartiküllerin antioksidan aktivitesi, 2,2-Difenil-1-pikrilhidrazil ($DPPH^{\bullet}$) serbest radikal giderme, 2,2'-azinobis-(3-etilbenzotiazolin-6-sülfonat) ($ABTS^{\bullet+}$) radikal katyon giderme ve demir iyonu indirgeyici antioksidan güç yöntemleri kullanıldı.

Hem *Salvia absconditiflora* (sa-AgNPs) hem de *Salvia hypergeia'dan* (sh-AgNPs) sentezlenen nanopartiküllerin yüzey merkezli kübik yapılar olduğu belirlendi. Sa-AgNPs için ortalama partikül boyutları 26.6 nm ve sh-AgNPs için 35.5 nm olarak hesaplandı. sa-AgNPs ve sh-AgNPs maksimum absorpsiyonları sırasıyla 469 nm ve 490 nm olarak belirlendi. *S. absconditiflora* ekstraktının ve sa-AgNPs'nın DPPH serbest radikal giderme aktivitesi, standart BHT'ye (10.7±0.2, IC_{50}, µg/mL) kıyasla sırasıyla 21.6±0.3 (IC_{50}, µg/mL), 15.8±0.2 (IC_{50}, µg/mL) olarak belirlendi. ABTS radikal katyon giderme testinde, standart BHT'ye (7,1±0,1, IC_{50}, µg/mL) kıyasla ekstrakt ve sa-AgNPs aktivitesi sırasıyla 8,4±0,2 (IC_{50}, µg/mL) ve 4,8±0,1 (IC_{50}, µg/mL) olarak belirlendi. Standart BHA'ya (7,9 ±0,2 µmol TE/mg ekstrakt) göre ekstrakt ve sa-AgNP'lerin indirgeme gücü aktivitesi sırasıyla

1,73±0,02 (µmol TE/mg ekstrakt) ve 4,6±0,1 (µmol TE/mg ekstrakt) olarak bulundu. *Salvia hypergeia* ile ilgili olarak, özüt ve sh-AgNP'lerin DPPH aktivitesi sırasıyla 18.0±0.9 (IC_{50}, µg/mL), 11.8±0.2 (IC_{50}, µg/mL) olarak tanımlandı. Ekstrakt ve sh-AgNP'lerin ABTS radikal katyon giderme etkisi sırasıyla 6,7±0,2 (IC_{50}, µg/mL), 3,8±0,1 (IC_{50}, µg/mL) olarak hesaplandı. *Salvia hypergeia* ekstraktının ve sh-AgNP'lerin indirgeme gücü etkisi sırasıyla 2,4±0,04 (µmol TE/mg) ve 6,1±0,1 (µmol TE/mg) olarak belirlendi.

Anahtar Kelimeler: *Salvia absconditiflora, Salvia hypargeia*, gümüş nanopartikül, spektroskopi, yeşil sentez.

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ICONS AND ABBREVIATIONS

Icons	Description
m	Micrometer
g	Gram
s	Second
mL	Milliliter

Abbreviations	Description
NPs	Nanoparticles
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ABTS	2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytolune

CHAPTER ONE

1. INTRODUCTION

1.1 Nanotechnology

Nanotechnology is undoubtedly a novel concept which has helped to bring about substances in the very minute size. Nanotechnology can be defined as the understanding and control of matter at a dimension between 1 and 100 nm where unique phenomena enable novel applications (Akintelu et al., 2020). The word Nanotechnology is the combination of two different words *nano* which means small or minute in the Greek language and *technology* which is the practical application of scientific knowledge, therefore nanotechnology can also be defined as the practical application of synthesizing small substances (Bachheti et al., 2020). Nanotechnology involves the use of both chemical and physical processes to produce materials in the nanoscale (Abd Hamid and Mutazah, 2019). Substances in the nanoscale are gradually improving as they are now used in catalysis process. Materials made of nano elements are now found in large quantity in the market in different forms and shapes. These materials are made of different nano elements such as polymers, fibers, ceramics, and glass (Abdel-Aziz et al., 2014).

There are different advantages associated with the use of nanotechnology, some of these are

1.1.1 The possibility of producing materials with new combinations of properties.

i. Production of devices which requires less energy for their operation and other consumables with extended range of accessibility. Also, their function may be strengthened by reducing their characteristic dimensions,
ii. It can be a means of universal fabrication technology, the apotheosis of which is the personal nano factory (Bordoloi et al., 2020).

Nanotechnology can be used in different fields such as in biomaterials, nanoelectronics, nanomedicines, agriculture, environment, imaging industries and agriculture. It can also be used in the diagnosis and treatment of diseases, formulation of new drugs and drug delivery in healthcare (Burlacu et al., 2019).

1.2 Nanoparticles

Nanoparticles are particles or materials whose sizes are between 1 and 100 nanometers. They are made up of carbon, metal, metal oxides or organic matter (Callegari et al., 2003). Nanoparticles are small in size, and they have large surface to volume ratio. This large surface area to volume ratio helps to enhance their optical properties, making them to confine their electrons, which consequently yield quantum effects can be detected easily (Carlson et al., 2008). There are different types of nanoparticles, and they have various applications in different areas such as in agriculture, textile, medicine, cosmetics, environmental science, electronics, energy generation and optics have been reported. Some of the known nanoparticles are silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnNPs), iron nanoparticles, cobalt nanoparticles (CoNPs) copper nanoparticles (CuNPs) gold nanoparticles (AuNPs), silica nanoparticles (SiNPs), platinum nanoparticles (PtNPs), palladium nanoparticles (PdNPs), magnesium oxide nanoparticles (MgNPs), cerium oxide nanoparticles (CeO_2NPs) and titanium oxide nanoparticles (TiO_2NPs) (El Shafey, 2020). Nanoparticles can be synthesized by numerous methods, some of which are the conventional chemical reduction method, biological method which consist of synthesis from plants and micro-organisms, sonoelectrodeposition, microemulsion and inverse microemulsion, hydrothermal method and the physical and mechanical methods such as laser ablation, Microwave, ball milling, thermal evaporation, lithography and vapour phase (Faraz et al., 2019).

1.2.1 Chemical reduction method of nanoparticle synthesis

Nanoparticles can be synthesized via chemical method. This nanoparticle synthesis method produces nanoparticles which are zero-valent. This method is based on chemical-reducing aqueous salt of metals such as silver nitrate when silver nanoparticles are being synthesized. In this synthesis method, a reducing agent is used to reduce the precursor metal salt which then lead to the production of electrons for metal ions thereby making them zero-valent.

After the reduction process is completed, a stabilizing agent is then added to stabilize the produced nanoparticle. Sometimes, a stabilizing agent can also be used as a reducing agent. An example of a compound which has this ability is citrate of sodium which is used when synthesizing silver nanoparticles (Swihart, 2003; Fierascu et al., 2020).

Examples of reducing agents used in nanoparticle synthesis are ascorbate, borohydride and citrate. An example of stabilizing agent is cetyltrimethylammonium bromide [$(C_{16}H_{33})N(CH_3)_3Br$; CTAB]. The limitations of conventional chemical method of AgNPs synthesis include the health and the environmental pollution caused by the organic solvents used in the reduction and stabiliziation of the nanoparticle. Another limitation of this nanoparticle synthesis method is the high cost of chemicals and reagents used in the synthesis of the nanoparticle. Lastly, chemical reduction method produces products with low yield, high purity and they also require high energy for their synthesis (Kumar et al., 2020; Kumari et al., 2020; Martínez et al., 2021).

1.2.2 Biological method of Nanoparticle synthesis

Nanoparticles can also be synthesized from biological substances using whole plant and plant part extracts and micro-organisms such as algae, yeast, bacteria and actinomycetes (Zafar et al., 2019). Plant extracts and some secondary metabolites could be used in synthesizing NPs, and this has been confirmed to boost the biological effectiveness of NPs via their antifungal, antibacterial, antioxidant and anticancer activities (Zhang et al., 2020). Studies have shown that reducing agents are more concentrated in plant's extracts than the whole plant, making most studies on biological nanoparticle synthesis often make use of plant extract. This can be achieved by mixing the extracts of the plant with an aqueous solution of metal salt, a process which occurs at room temperature and can take some minutes or few hours to be completed (Callegari et al., 2003). This method is eco-friendly as it doesn't pollute the environment and cost-effective. This is achieved by growing microorganisms in culture media where reducing and capping agents are introduced to the media then metal ions are added under controlled conditions (Yin et al., 2003). Metallic NPs synthesis occurs at room temperature in contrast to high temperature required in culture medium of synthesizing NPs. Unlike in culture medium where high temperature is required for heating the reaction mixture for NPs synthesis, such is not required when plant extracts are used as this is performed at room temperature. Also, plants are best for synthesizing NPs on a large scale as plants are abundant and can be easily found. Table1.1 shows a list of some plant extracts and their biomolecules rendering them as capping, reducing and stabilizing agents during AuNPs synthesis (Huang and Yang, 2004).

Research have shown that prokaryotic bacteria have been the most tremendously used for nanoparticle synthesis because they can be easily manipulated. The use of fungi in nanoparticle production as biofactories has advantages as NPs are synthesized in a way in which their sizes and chemical composition are in a monodisperse way. Microorganisms are rarely used in for synthesizing NPs because it takes longer time to culture. Other microorganisms such as Pseudomonas spp. and Escherichia coli, are also toxic and dangerous to handle (Sondi and Salopek-Sondi, 2004). It is worthy to note that some researches have reported that the green synthesis of gold nanoparticle does not show a considerable improvement on preventing environmental pollution as the same trend was observed just like in chemical reduction method (Yin et al., 2004). The aquatic lives are extensively affected using AgNPs which when released into the aquatic ecosystem causes the interaction between the nanoparticle and environmental factors such as inorganic anions, organic compounds and metal cations, which affects the composition and surface of AgNPs. This can lead to the oxidation of the silver ion which are toxic to human health and environment (Balan et al., 2007; Lok et al., 2007).

Table 1.1: Plants and the bio-molecules acting as reducing and stabilizing agents for AuNPs synthesis

S/N	Plants	Plant part	Secondary metabolites
1	Zingiber officinale	Leaf	Flavonoids
2	Wedelia trilobata	Leaf	Polyphenolic
3	Acer Pentapomicum	Leaf	Phenols
4	Polyscias scutellaria	Leaf	Flavonoids
5	Mimosa tenuiflora	Bark	Polyphenols
6	Elaeis guineensis	Leaf	Flavonoids
7	Muntingia calabura	Leaf	Tannin
8	Eupatorium odoratum	Leaf	Alcohol
9	Ocimum tenuiflorum	Flower	Phenol
10	Azadirachta indica	Flower	Alcohol

11	Crinum latifolium	Leaf	Phenol
12	Anthemis xylopoda	Flowers	Polyphenol
13	Allium sativum	Leaf	Protein
14	Ananas comosus	Fruit	Protein
15	Chrysopogon zizanioides	Leaf	Alkaloids
16	Terminalia chebula	Leaf	Tannins
17	Prunus armeniaca	Fruit	Protein
18	Syzygium aromaticum	Leaf	Eugenol
19	Garcinia cambogia	Leaf	Flavonoids
20	Papaver somniferum	Pod	Pectin
21	Cinnamomum zeylanicum	Leaf	Phenol
22	Tiliacora triandra	Leaf	Terpenes
23	Punica granatum	Fruit	Phenols
24	Elephantopus scaber	Leaf	Lactone
25	Dracocephalum kotschyi	Leaf	Phenol
26	Adiantum capillus veneris	Leaf	Alcohol
27	Pteris quadriureta	Leaf	Phenol
28	Genipa Americana	Fruit	Phenol
29	Lansium domesticum	Fruit peal	Carboxylic acid
30	Loeselia Mexicana	Leaf	Terpenes

Metal nanoparticles

Metals are very good for nanoparticles due to their small size and their large specific surface area (Carlson et al., 2008). There are numerous secondary metabolites mainly in the crude extracts of plants. Some of these are phenolic acids, flavonoids, alkaloids and terpenoids. These secondary metabolites are used for the bio-reduction of metal ions which forms the metallic nanoparticles. Metallic nanoparticles have antidiabetic, antioxidative, anticancer and anti-inflammatory properties (Gopinath et al., 2008; Esteban-Tejeda et al., 2009). Synthesis methods such as reverse micelles process, ultrasonic irradiation, and electrochemical synthesis have been used to synthesize metallic nanoparticles (Rai et al., 2009; Shen et al., 2009).

There are several metabolites which can cause the reduction of metallic nanoparticles from metallic precursors in the extracts of plants.

Table 1.2 shows the comparison between chemical synthesis and biological synthesis methods of synthesizing NPs (de Souza et al., 2019).

Table 1.2: Comparison between chemical synthesis and green synthesis method

	Conventional method	Green synthesis method
Stabilizing and reducing reagents	Toxic reducing and stabilizing agents such as sodium borohydride, hydrazine and dimethyl formamide	Non-toxic reducing agents such as citrate, β-D-glucose carboxymethyl cellulose sodium, plant and microorganism extracts
Solvents medium	Synthesis with toxic and/or flammable	Synthesis in aqueous
Energy input	High energy inputs (heat sources such as Water/oil bath and heating mantle)	Low energy inputs (synthesis room temperature)
Recoverability and reusability	Difficult recovery	Easy recovery (e.g using magnetic seaparation for silver or iron oxide composite)

1.2.3 Sonoelectrodeposition

Sonoelectrodeposition is a coupling of ultrasonic vibration to an electrochemical system. This technique makes use of electrodeposition and mechanical waves of ultrasound to produce metallic nanoparticles (Zhu et al., 2001). This method is environmentally friendly, simple and cost effective compared with other nanoparticle synthesis methods nanoparticle (Marquez-Montes et al., 2021).

1.2.4 Microemulsion and Inverse microemulsion

Microemulsion is an isotropic and thermodynamically stable mixture of “oil,” water, and surfactant, or in combination with a cosurfactant. Micelles, which are small drops of aqueous phase may contain salts and or other ingredients with the oil which are the mixture of the surfactants.

There are two types of microemulsions; direct where oil is dispersed in water and the indirect where water is dispersed in oil. The inverse microemulsion method is the simplest way to produce multifunctional nanoparticles by creating the silica matrix in aqueous phase, which will fix the single particles inside (Dung et al., 2016). The reaction to form nanoparticles also can be realized when the micelles mix with each other and the growth of the nanoparticles is controlled by the surfactants in "oil" (López-Quintela and Rivas, 1993). Microemulsion method can be used for synthesizing Fe_3O_4 (Feltin and Pileni, 1997) and can also be functionalized with a silica layer. This method can also be used to prepare Fe_3O_4/Au core-shell nanoparticles to prevent the oxidation of magnetic nanoparticles (MNPs) as well as to form the biocompatibility (Boutonnet et al., 1982).

1.2.5 Hydrothermal method

In this method of NPs synthesis, the crystals of substances which are insoluble at normal temperature and pressure are grown by heterogeneous reaction under high temperature and pressure in an autoclave, a steel pressure vessel. Nutrients and water are then supplied into the solution. The critical condition gives a favorable reaction field for formation of nanoparticles, owing to the enhancement of the reaction rate and large supersaturation based on the nucleation theory. This NPs synthesis method is usually carried out below 300°C and it has been used to synthesize metal oxide NPs in supercritical water (Hayashi and Hakuta, 2010), metal nanoparticles (Kim et al., 2014), and semiconductor nanoparticles (Van Bui et al., 2014).

1.3 Approaches or Techniques for synthesizing Nanoparticles

There are two main approaches for synthesizing nanoparticles. These are the top-down approach and the bottom-up approach.

1.3.1 Top-down approach

In this nanoparticle synthesis technique, molecules of large substances are decomposed or broken down into smaller units which are then converted into suitable nanoparticle. It is often referred to as a destructive approach. In this approach, bulk materials are first converted into powder form after which they are converted into nanoparticles. Top-down approaches are inherently simpler and depend either on removal or division of bulk material or on miniaturization of bulk fabrication processes to produce the desired structure with appropriate properties. Top-down approach is used in chemical etching, mechanical milling, electro-explosion, and sputtering. This approach is employed in physical method of synthesizing nanoparticles. Methods in which top-down approach is used are:

Ball milling: This is an example of mechanicanosynthetic method. In this method, nanoparticles are produced by mechanical attrition from the kinetic energy produced from a grinding medium which causes the reduction in size of the material being reduced. Temperature and pressure created by the interaction between balls, as well as impacts between vessel walls can cause several phase transformations at high temperature. This method of producing nanoparticle is the least expensive way of producing nanomaterials in bulk (Arole and Munde, 2014).

Milling process is used for many purposes such as compressing particle size, agglomeration, blending of two or more phases, modification of the characteristics of a material such as work hardening, density and flowability, growth of particle size, alloying mechanically (solid-state alloying), blending and change in particle structure (Abid et al., 2021).

The ball size, vial volume, rotation speed, overall ball masses, duration of milling, milling environment and the amount of powder determines the outcome of the product when milling is used. The milling process is said to have better impact when the ball to powder weight ratio is high. When the number of balls increases, the number of collisions per unit time increases as well and a smaller amount of powder is in the vial, faster is the velocity of a single ball in the cylinder, the greater the efficiency.

The advantages of milling process are

i. High capacity and universality
ii. It may be used to reduce the thickness of stacked materials
iii. Simplicity in serving, reliability and safety.

The disadvantages of this process are

i. Grinding makes use of lots of energy
ii. It causes noise pollution
iii. Inconvenience and hefty weight

Mills are classified based on the movement of milling balls and vials. Examples include planetary mill, attrition mill, horizontal mill, vibratory and rotatory mills. (Abid et al., 2021).

Thermal methods: these methods involve the use of heat, electricity of solar energy in the process of synthesizing nanoparticles. An example of a process that uses this method is the electrospinning. Arc discharge was the first controlled means of making carbon nanotubes. This method has a limitation in the control of quality and potential upscale.

Lithographic method: Lithography is a planographic method of printing originally based on the immiscibility of oil and water. This is a top-down method in which nanoparticles of micron-size features can be produced. This method is energy intensive and requires expensive equipment and facilities. Compaction and consolidation: this is a method of producing nanoparticles in large scale. Here, nanomaterials are “put back together” to form nanomaterials with enhanced properties. Metallic alloys can be made with this method (Arole and Munde, 2014). An advantage of using this approach includes production of desired size and large quantity of nanoparticles. The disadvantages of this approach are eco-toxicity, high energy consumption. imperfection of surface structure, it is expensive, it is time consuming, and it is ineffective for manufacturing irregularly shaped and extremely small particles. Figure1.1 shows the different methods for synthesizing nanoparticles.

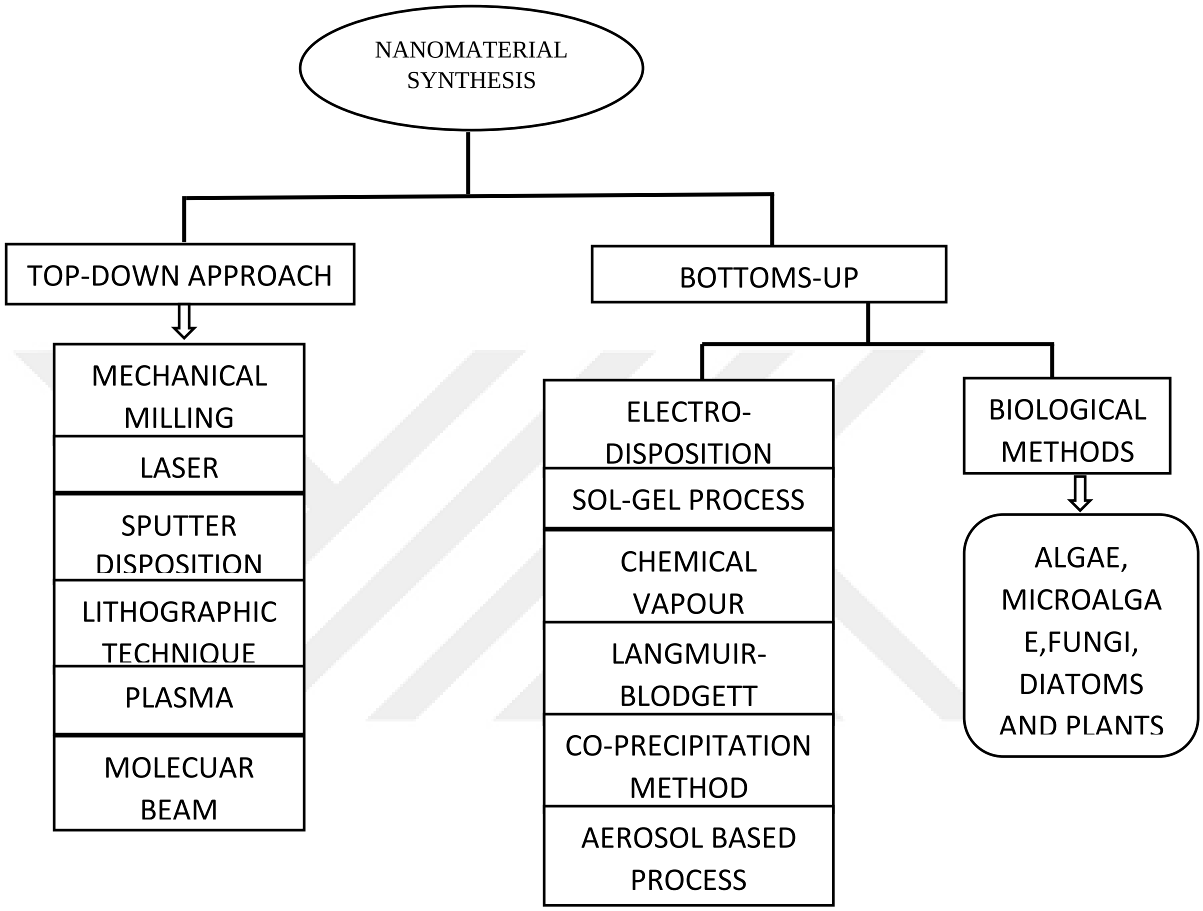

Figure 1.1: Different methods for synthesizing nanoparticles

1.3.2 Bottom-up approach

In this approach, smaller atoms at nanoscale are assembled together to form nano-size particle. This method is usually referred to as the building approach as units are fused together to form a particle which is used in nanoparticle. The bottom-up approach can further be divided into two classes: biological and non-biological approaches. Examples of non-biological approaches include template support synthesis, flame spraying, spinning, laser pyrolysis, atomic condensation, and deposition of chemical vapors. These methods also use toxic chemicals, are expensive and time-consuming, whereas the biological approach uses various biotic resources such as plants, algae, microorganisms, and other biological molecules like starch, egg albumin, and gelatin for the production of different types of NPs (). This method is eco-friendly, simple, reliable, biocompatible, and easy for the synthesis of NPs. The principle of bottom-up is used in chemical and biological method of synthesizing nanoparticles. Bottom-up approach is used in Chemical vapour deposition: this is a gas-phase process in which reactive constituents react over a catalyst or a pre-templated surface to form nanostructure materials. Carbon nanotubes are synthesized economically via this method (Arole and Munde, 2014).

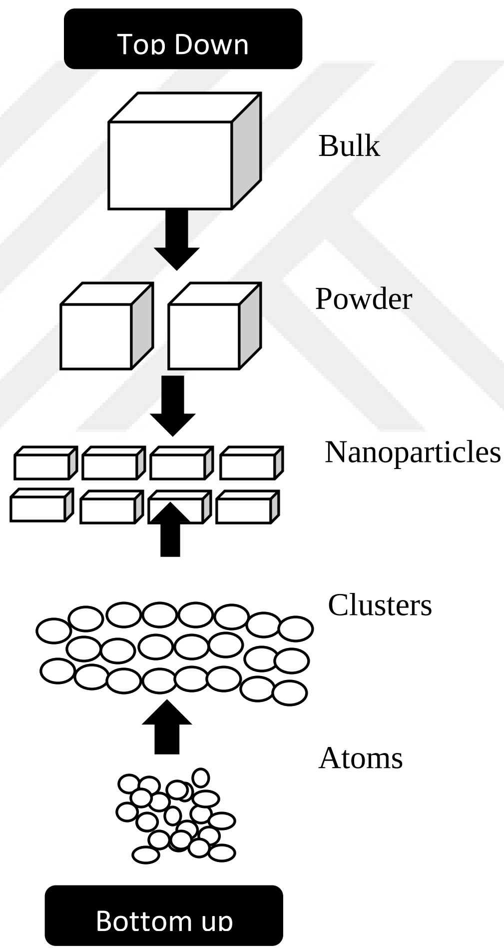

Figure 1.2- Top-down and Bottoms up approach

This occurs when methane or acetylene precursors are passed over Co, Fe or Ni catalyst. This decomposes into carbon and with the catalyst particles, nanotubes are formed. Atomic layer deposition: this is an industrial process that is capable of coating any material with a monolayer or more of a thin film regardless of its size. Other industrial bottom-up processes are molecular beam epitaxy and metal organic chemical vapour deposition.

The advantages of bottom-up approach are

i. It produces NPs with well-defined shape, size and chemical composition. This is obtained through the growth and self-assembly of atoms and molecules as their building blocks (Vaseghi et al, 2020; Kolahalam et al, 2019).
ii. It has the potential of creating less waste therefore, this method is more economical (Kolahalam et al., 2019).

1.4 Scope of the study

It is universally known that Salvia species are good source of chemicals with antioxidant, anti-inflammatory properties. Salvia spp contains biologically active components which can be split into phenolic, monoterpenes, diterpenes and triterpenes.

Studies have shown that Salvia absconditiflora can be used to remove and reduce the effects of oxidant compounds in the body and it has also been found to contain salvianolic acid as its bioactive component.

Salvia hypergeia has been found to have antimicrobial and antioxidant component and its ethanol extract can be used for treatment of diabetic patients because it has biochemical, genotoxic and histopathologic compounds.

This thesis work shows the nanoparticle sizes of Salvia absconditiflora and Salvia hypargeia, details on their antioxidant and analysis on their extract and AgNPs under different conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Salvia Genus

Salvia genus popularly referred to as “sage” are plants that came from the Lamiaceae family which have wide-spread existence and are known for their high medicinal values. The plants have the natural capacity to survive different seasons of the year therefore can be regarded as perennial, biannual and annual plants/shrubs that have simple or compound leaves foliated on the square stems (Uysal, 2019). The insect-pollinated flowers have tubular features with 2 lips and stamens suspended on the terminal inflorescences which can bear nutlet fruits that attract birds like a hummingbird. *Salvia* genus has about 900 plant species globally which can be used in varied proportions for the treatment of diseases such as aches, colds, Alzheimer’s diseases, microbial infections, cancer and heart-related illnesses (Uysal, 2019). *Salvia* species are among the plants with aromatic scents, and high volatile oil content and can be used for economic and medical purposes (Yilar and Kadioglu, 2018). Due to the high level of micro-climate field and the topographic features of Turkey especially in the Kirsehir provinces, over 12,000 plant species survive in the area. Different species of the Salvia plant has different features (some are herbs while some have woody stem) that grow up to 2 feet with sweet-smelling leaves and the species have been found to have a high survival rate in southern Europe (Yılar et al., 2021). In Turkey, the survival rate of the *Salvia* plant is 51% (Poyraz and Koca, 2006). They also reported that the plants under the Lamiaceae family existent in Turkey occur in 742 taxa, 45 genera and 558 species. It has also been identified that the plants grow in Southern, Western and Central Asia, Oceania, the Mediterranean, Europe, America and Africa. Though various species of the *Salvia* plant exist across the globe, this study will present a literature review on the Salvia absconditiflora and Salvia hypargeia.(Demirci et al., 2003)

Salvia species’ essential oils and non-volatile phenolic compounds has been used as preservatives for foods due to their properties such as antioxidants and antibacterial in Europe and many other countries. (Bursal et al., 2019); (Koubaa-Ghorbel et al., 2020).

Scientific research has shown that the extracts from *Salvia* species have anticancer, anti-inflammatory, cytotoxic, hepatotoxic activities. They also have secondary metabolites diterpenes, triterpenes and flavonoids (Kim et al., 2014); (Tayarani-Najaran et al., 2013); (Topcu et al., 2007);(Topcu et al., 2008).

Salvia plants are good sources of phenolic acids. Phytochemical research revealed that the principal secondary metabolites in the roots of *Salvia* species are found to be diterpenoids which are responsible for biological activities. The phenolic acid derivative variety and amount can differ between different *Salvia* species and also between different plant parts and extracts.

Salvianolic acids or yunnaneic acids A-H are phenolic acids which are unique to *Salvia* species. Other phenolics identified in *Salvia* species are benzoic acids such as 4-hydroxybenzoic acid, 3,4- dihydroxybenzoic acid or protocatechuic acid, 3-4methoxy-4-hydroxybenzoic acid or vanillic acid, 2,4-dimethoxybenzoic acid, an ether linked dimer of hexyl 4-hydroxybenzoate, and coumarins; 6,7- dihydroxycoumarin (esculetin), 7-methoxycoumarin (herniarin) (Lu and Foo, 2002). Numerous flavonoids that can be found in *Salvia* species. Flavones, flavonols and their glycosides, proanthocyanidins and anthocyanins are some of the flavonoids.

Caffeic acid and its dimer form rosmarinic acid are the major ones in *Salvia*, which are important for their biochemistry as being the building block of many plant metabolites.

2.1.1 *Salvia* in Turkey

There are about 95 species of *Salvia* plant in Turkey. Eusphace Benth., Hymenosphace Benth., Aethiopis Benth., Plethiosphace Benth., Horminum (Moench) Dumort Drymosphace Benth. and Hemisphace Benth.

Salvia has been used for numerous things in the Anatolia region of Turkey for treating skin diseases, used as antiseptic, stimulant, inflammation and stimulant. It is mostly taken as herbal tea due to the high amount of vesicles in its leaves. *S. absconditiflora* is referred to “tapir, karasalba or karaot” among the people (Yilar and Kadioglu, 2018).

2.1.2 Ethnobotanical Use of Salvia

Laboratory tests have shown that *Salvia* plants have antimicrobial, antioxidant, anti-inflammatory, chemopreventive, hypoglycemic properties. Salvianolic acid B can be isolated from S.miltiorrhiza and has been found to decrease the DMBA (7,12-dimethylbenz (a) anthracene) induced squamous cell carcinoma(Wei et al., 2012). The presence of anti-inflammatory activities of *S. fruticosa*, *S.verticillata*, and *S.trichoclada* by using a carrageenan-induced inflammatory paw edema model that n-butanol extract of Turkish sage with *S.fruticosa* showing the highest activity (Çadirci et al., 2012). The hypoglycemic properties of *S. officinalis* when its methanolic extract was used on diabetic rats. It shows a significant decrease in the level of serum glucose in 3 hours. There was also a significant increase (14%) in liver GST activity when water was replaced by *S. officinalis* tea in the diet of mice and rats for 14 days. Also in these rats, hepatocyte primary culture, they examined increased GSH content and GST activity that shows the high antioxidant potential of *S.officinalis* (Lima et al., 2005)

Danshen, scientifically called Salvia miltiorhiza and alternatively called Chinese Red Sage and Red Rooted Sage. There are about 17 other species of the sage family which may be sold under the same name. It is popularly referred to as supergrade in medicine in China. It has been approved by FDA for clinical tests in USA. It can be used for treatment of myocardinal infraction and angina pectoris. The antioxidant, anticancer and anti-inflammatory properties related to their abitenoids, diterpene quinone pigments especially Tanshinone IIA. Figure 2.1 is a picture of Danshen medicine.

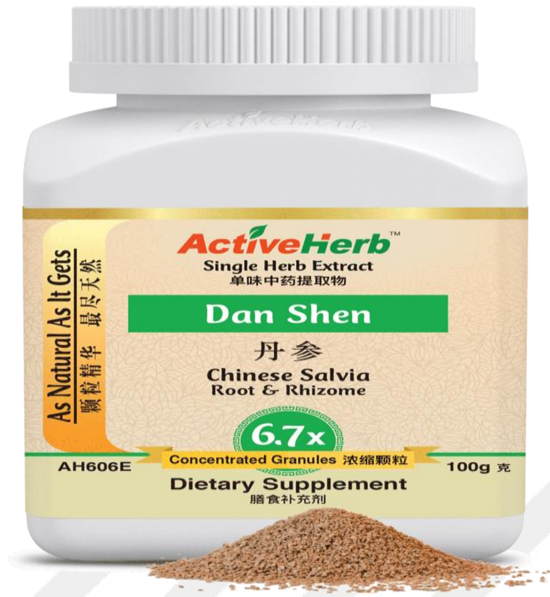

Figure 2.1- Danshen

2.2 Salvia absconditiflora

Salvia absconditiflora can be botanically traced to the plant Kingdom referred to as Plantae, in the Subkingdom of Tracheoblonta, Class of Magnoliopsida, Subclass of Asteridae, the family of Lamiaceae, Genus: Salvia and Specie called Salvia absconditiflora (comprising the genus name and the specie name) (Yılmaz, 2013). The author identified that the plant grows the stony, rocky and uncultivated lands and hillsides of countries. It undergoes floweration between May and August and is usually found by the roadside, limestone slopes and fallowed fields among others (Koysu et al., 2019). Figure 2.2 show S. absconditiflora plant.

Figure 2.2: *Salvia absconditiflora*

Salvia absconditiflora belongs to the Lamiaeae family. This family of plant has 236 genera and 7133 species all over the world. Figure 2.2. shows the regions S. absconditiflora is distributed in Turkey.

Figure 2.3: Salvia absconditiflora distribution in Turkey

Turkey is one of the important gene center of this family of plants. There are 45 genus, 558 species and 742 taxa and the endemism rate is 42.2% ((Belen, 2012). *Salvia* genera are important aromatic pants. They are rich in essential oils which can be used as a natural scent in homemade cosmetics and high-quality natural products. These oils are rich in terpenoid compounds, flavonoids, phenolic compounds and quinoids ((Al-Qudah et al., 2014); (Doğan et al., 2017); (Durling et al., 2007); (Ulubelen et al., 1979). *Salvia absconditiflora Greuter & Burdet* also referred to as *Salvia cryptantha Montbret & Aucher ex Bentham* is a perennial plant. It is an endemic specie in the C group (15). It is found in rocky areas, calcareous hills and high-altitudes (700-2500m) and grows high within the range of 10 to 30cm (İpek et al., 2012) (Ozer et al., 2013).

Salvia absconditiflora has medicinal properties and it can be used as antitumor, antioxidant and wound healing (Ozer et al., 2013); (Süntar et al., 2011))

2.2.1 Medicinal value of Salvia absconditiflora

The origin of man was followed by the emergence of different diseases which compelled man to devise ways of sustaining his existence through healthy living. This led to the use of the herb in the treatment of different illnesses through the trial-and-error method and such error-prone experimentation is usually carried out on animals. Plants with antioxidant elements such as *S. absconditiflora* are used to remove and reduce the effects of oxidant compounds in the body (Akgul et al., 2020). The authors stated that through metabolic processes, the human body produces oxygen species that react with the body of the living organisms which when existent in large quantities harms the living organism. The lack of antioxidants in the human body leads to a health disorder called oxidative stress. Other implications of the insufficiency of antioxidants are cancer, Alzheimer's disease and cardiovascular disorders. Supplementary antioxidants obtained from the plant are used to reduce the level of oxidative stress in humans (Sevindik et al., 2017). This can be done by first determining the oxidant and antioxidant potential of the plant and the index of oxidative stress in the individual. This will guide the proper application of the antioxidant compound in the individual body to address the issues of oxidative stress.

The tremendous increase in the use of alternative medicine in the treatment of diseases has led to global awareness of the pharmaceutical values of *S. absconditiflora*. A plant like *S. absconditiflora* has been found to contain materials that help preserve, expand and sustain human physical and mental health abilities and in the treatment of diseases (Abd Rashed and Rathi, 2021). The authors noted that the plant species under the Lamiaceae family are among the plants mostly studied for medicinal purposes. They further noted that *Salvia* spp contains biologically active components which can be split into phenolic, monoterpenes, diterpenes and triterpenes, despite the high level of studies in the use of the therapeutic plant, few of the components have undergone in-depth studies. It has also been identified that the salvianolic acid content of the plant has recorded more studies in the investigation of its bioactive component. As the name implies, *Salvia* name originated from the “salvare” which is a Latin word that means “to heal” and “to save”. Salvianolic acid is a water-soluble compound, which can be found in varied proportions in the salvia plant species giving the plant species different medicinal values (Ma et al., 2019). They stated that (SaIA) and (SaIB) are the most prevailing salvianolic compounds found in the plant. The plant has been found by many studies to have different phenolic metabolites which are being used as antidiabetic, antioxidants, anti-inflammation, anti-microbial and cytotoxic medical practices (Lu and Foo, 2002), (Zengin et al., 2018).

2.2.2 Chemical composition of Salvia absconditiflora

There are about 36 components in the essential oil of salvia absconditiflora. Some of the main components are camphor (0.52-58.64%), Bicyclo [2.2.1] Heptan-2-one,1.7.7 (21.94-30.16%) and viridifloro (3.42-25.2%) according to GC/MS analysis. The major oil constitiuents of S. absconditiflora are camphor (19.1%), 1,8-cineole(16.4%), borneol (11.9%), viridiflorol (11.5%) and bornyl acetate(2.4%). Table 2.1 shows the chemical composition of the essential oils in salvia absconditiflora (Yılar and Altuntaş, 2017). S. absconditiflora extracts show high anticholinesterase activity (over 70%), it also shows a significant wound healing activity in animals in the circular excision wound model when it is extracted with ethanol (Süntar et al., 2011).

Table 2.1: Chemical composition of essential oil in *Salvia absconditiflora* obtained by GC/MS

No	RT	RR1	Compounds
1	12.510	1387	β-caryophellene
2	12.546	1394	Valencene
3	13.642	1405	Naphthalene
4	12.956	1417	Muurolene < Gamma->

5	13.529	1427	Bisabolene
6	13.540	1428	Gurjunene<Beta-
7	14.765	1429	Camphor
8	14.971	1433	Bicyclo[2,2,1]Heptan-2-One, 1,7,7
9	15.966	1453	Myrtenol
10	17.168	1475	n-eicosane
11	17.265	1478	Sesquisabenene hydrate
12	17.603	1482	Ledol
13	17.609	1484	Cubebol
14	18.799	1508	Germacrene D
15	19.417	1520	Viridiflorol
16	20.052	1532	Bicyclo[4,4,0]dec-1-ene
17	20.092	1534	Longifolene
18	20.315	1534	Alloaromadendrene oxide
19	20.555	1542	Spathulenol
20	20.590	1544	Caryophyllene oxide
21	21.253	1559	Cadinol<Alpha->
22	21.425	1560	Humulene epoxide II
23	21.849	1568	Azulene
24	21.894	1572	Eudesmol<Alpha_>
25	22.117	1576	7-Methoxy-2-Methyl quinolone-5,8-Dione
26	22.264	1580	Eudesmol<Beta-
27	22.758	1588	Valeranone
28	23.245	1598	1H-Cycloprop[A]Naphthalen-6-Ol
29	23.479	1602	Valerenol
30	24.486	1621	Ledene oxide-(II)
31	24.488	1642	Vulgarol A
32	26.569	1663	Manool
33	27.159	1687	1-Naphthanenol
34	27.937	1689	1,2-Benzenedicarboxylic acid
35	30.780	1746	9H-Benziidazol [1,2-d][1,2,3]triazol
36	35.003	1826	Abietal

2.3 *Salvia hypargeia* Fisch. & C.A. Mey

Salvia hypargeia is another specie of the salvia plant in the Lamiaceae family. A study by Ahmet et al, (2011) revealed that like the *Salvia absconditiflora*, *Salvia hypargeia* has antioxidant, antimicrobial and chemical compounds. Figure 2.4 shows *S. hypargeia* plant.

Figure 2.4: *Salvia hypargeia*

The leaves of *salvia hypargeia* can be described to have simple basal, linear or linear-oblong green leaves that have white colouration at the underside. The flowers of the plant species emerge in verticelles containing 4 to 8 flowers in each verticelle (Kandemir, 2003). The author noted that the flower grows from 2.5cm to 3cm in length and has a lavender colouration to purplish-blue colouration at the upper lip of the flower and a lavender-cream colouration at the lower lip of the flowers. In investigating the medicinal value of the *S. hypargeia*, the ethanol extract from the plant has biochemical, genotoxic and histopathologic values and has the potential for the treatment of wounds in diabetic patients, incisional and excisional wounds on diabetic rats (Ozay et al., 2021). It is worthy of note that diabetic patients' wounds are difficult to heal but the use of ethanol compounds from *S. hypargeia* has been found with high wound-healing efficacy. It has been identified that the essential oil extracted from *S. hypargeia* has antimicrobial and antioxidant components that are capable of inhibiting the oxidation processes of lipids or other molecules by delaying the process involved in the initiation of oxidative chain reaction and which is capable of hindering the damage on the body cells' oxygen (Atas et al., 2011). The antioxidation effect of the plant acts by reducing the activities and lessening the radical scavenging abilities of the pro-oxidant metals and also quenches the singlet oxygen (Atas et al., 2011). To achieve healthy living, consuming natural antioxidant phytochemicals contained in the plant has been identified with various health outcomes.

The prevention of diseases can be achieved with natural antioxidants. It can also be used in the retardation of lipid oxidative rancidity. Despite the numerous health benefits of antioxidants, it has been identified that synthetic antioxidants are toxic to the body which demands that further studies are necessary to ascertain the positive and negative health outcomes of the compound to ensure sustainable living (Valentão et al., 2002). The *Salvia* plant has both medicinal industrial and nutritional uses. In medicine, the extracts from the plant are used for antiseptic, anticholinesterase, and antipyretic purposes in addition to the antimicrobial and antioxidant uses of the plant as earlier identified in this work. This is due to the presence of terpenes, phenolics and non-volatile phenolic compounds present in the plant. The study also identified that plant has anticancer and antimutagenic potentials which can help the body combat the genotoxic environment thereby lowering the risk of chronic diseases. The anti-mutagenic effect is seen in the potential to reduce or prevent mutagens from penetrating the human or animal body cells and activate antioxidant enzymes. Recent studies have also revealed that cognitive challenges can be tackled through the anti-cholinesterase present in the plant. Acetyle cholinesterase present in the plant serves as a potent source of health-improving compounds that reduces neuropathological issues (Yadav et al., 2017). Synthetic drugs that have severe or mild side effects can also be controlled through the use of the health properties of the *Salvia hypargeia*.

2.4 Antioxidants

Antioxidants are naturally occurring substances which when present at low concentrations compared with those of the oxidizable substrate, significantly delays or inhibit the oxidation of that substrate. Antioxidants help protect the body from damages caused by harmful molecules called free radicals. Antioxidants exist as vitamins, minerals and other compounds in foods. Antioxidants may improve the functioning of the body’s immune system and perhaps lower the risk for infection, cardiovascular diseases and cancer.

2.4.1 Antioxidants in plants

<u>Free Radicals</u>

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. In essence, a free radical is a chemically reactive molecule that has an unpaired electron. They are produced in living systems as a result of normal metabolic activities such as autooxidation, enzymatic oxidation or respiration or they are generated in the organism when subjected to exogenous sources such as ionizing radiation, drugs which can undergo redox cycling, or xenobiotics that can create reactive species in situ (Freeman and Crapo, 1982).

Free radical chain occurs when a covalent bond between two atoms or molecule is broken which forms a free radical of an atom or a molecule with an unpaired electron. This free radical steals electrons from surrounding cellular structures or molecules which returns the free radical into the ground state (Halliwell and Gutteridge, 1985).

Benefits of antioxidants

They can protect the body against cancer: Lycopenes can help prevent against cancers of the stomach, colon and reticulum, mouth and esophagus and Luteins can may help decrease risk of macular degeneration.

They can help protect against aging: antioxidants can help protect against some of the degenerative effects on the body of age-related diseases that can lead to early death even though it has not been scientifically proven that they can increase lifespan.

They can help boost body immunity: vitamin C can help to reduce the severity of common cold shows that it can boost the body’s immune system.

They can protect against heart diseases: some antioxidants contained in fruits, vegetables and other foods can help the body fight against heart diseases.

2.4.2 Phenolic acid

Polyphenols are the most essential compound usually obtained from salvia species. Salvianolic acids are the most common phenolic acid that has been obtained from Salvia species. The quantity and derivative of the polyphenols obtained from different Salvia species depend on the part in which it is obtained, the extract and the plant species itself.

Some of the phenolic acids that has been identified in Salvia species are benzoyl acids such as 4-hydroxybenzoic acid, 3,4- dihydroxybenzoic acid or protocatechuic acid, 3,4 methoxy-4-hydroxybenzoic acid or vanillic acid, 2,4-dimethoxybenzoic acid, an ether linked dimer of hexyl 4-hydroxybenzoate and coumarins; 6,7- dihydroxycoumarin (esculetin), 7-methoxycoumarin (herniarin) (Lu and Foo, 2002).

CHAPTER 3

METHODOLOGY

3.1 Materials

3.1.1 Sources of plant materials

Salvia absconditiflora Greuter & Burdet

Salvia absconditiflora plant was identified by Dr.Osman Tugay, Selcuk University, Faculty of Pharmacy, Pharmaceutic botany and a voucher specimen was deposited at the herbarium of the department of biology (KNYA-29450).

Salvia hypargeia Fish. & C. A. Mey.

Salvia hypergeia plant was identified by Dr.Osman Tugay, Selcuk University, Faculty of Pharmacy, Pharmaceutic botany. The plant materials were collected from Yozgat province and a voucher specimen was deposited at the herbarium of the department of biology (KNYA-29451).

3.2 Plant preparation

3.2.1 Plant extraction

The leaves and stems of both *S. absconditiflora* and *S. hypargeia* were washed with distilled water and placed to dry under room temperature on a paper. The dried plant parts were then grounded with mechanical grinder to have a product with fine particles.

Figure 3.1a: Ground *S. Absconditiflora*

Figure 3.1b: Ground *S. hypargeia*

3.2.2 Extract and AgNPs preparation

20 g of ground *S. absconditiflora* was mixed with deionized water (300 mL) and heated at 50°C for 2 hours. After filtration of the solution, half of the solution (150 mL) was employed for rotary evaporator to yield the crude extract. The other solution (150 mL) was treated with silver nitrate solution (1.0 mM, 150 mL) at 55°C for 2 hours. The reaction solution was centrifugated for 15 minutes at 5000 rpm, then lyophilized to yield the silver nanoparticles (sa-AgNPs). The same procedure was used for *S. hypargeia* to yield the extract and nanoparticles (sh-AgNPs).

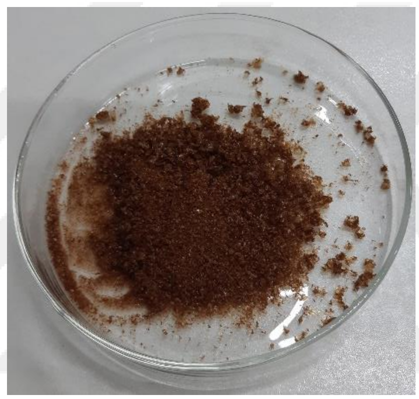

Figure 3.2: AgNPs of *S. absconditiflora*

(i)

(ii)

Figure 3.3: Aqueous solutions of water extracts (A) and AgNPs (B) of *S. absconditiflora* (i) *and S. hypargeia* (ii)

3.3 Antioxidant activity preparation and determination

3.3.1 DPPH assay

DPPH solution was used in determining the free radical scavenging activities of both *S. absconditiflora* and *S. hypargeia* using (Liyana-Pathirana and Shahidi, 2005). DPPH has free electron delocalization which gives a deep violet colour in solution which can be measured with UV-spectrophotometer in the range of 515-520nm (Lin et al., 2013). DPPH is a stable nitrogen free radical. Nanoparticles and extracts of both *S. absconditiflora* and *S. hypargeia* with different volumes (40 mL, 80 mL, 160 mL and 320 mL) of stock solution were added to a test tube, 3 mL of ethanol was then added to the solution. 1.0 mL of DPPH solution was then added and the solution was then stirred with a vortex. This was then stood in dark ambient for 30 minutes. The absorbance values for the different volumes of the sample solution were then read at 517nm with and the free radical scavenging activity value was determined and expressed as IC_{50} from the equation

$$\%\text{Scavenging Radical Activity} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 is the value for absorbance of DPPH and water mixture

A_1 is the value for absorbance of sample (either S. absconditiflora or S. hypargeia) with DPPH

3.3.2 ABTS assay

ABTS solution was used in determining the free radical scavenging activities of the samples. The ABTS cation radical scavenging activity was carried out using the method applied by Re (Re et al., 1999). This analysis was carried out by reacting 2.0 mM of ABTS solution with 2.45 mM of potassium persusfate solution ($K_2S_2O_8$) in a ratio of 1:1 to produce the radical cation of ABTS ($ABTS^{\bullet+}$). The solution was then kept in a dark environment for 6 hours. Extracts and AgNPs of *S. absconditiflora* and *S. hypargeia* together with different volumes of stock solution were added in a test tube to complete 3.0 mL. 0.1M phosphate buffer with pH of 7.4. 1.0 mL of ABTS solution was then added and the mixture was stirred by a vortex. The resulting solution was then kept standing in the dark for 30 minutes after which its absorbance was read at 734nm and the result was expressed as IC_{50}

3.3.3 FRAP assay

Iron ion reducing antioxidant power activity was carried out using the method applied by Oyaizu (Oyaizu, 1986). Three different volumes were taken from stock solution (solutions of *S. absconditiflora* and *S. hypargeia*) and added into a test tube with 0.25 mL of 0.2 M, pH 6.6 of phosphate buffer to make the volume 1.25mL. 1.25mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution was then added to the solution and mixed by a vortex. The solution was then incubated at 50^0C in an oven for 20 minutes. After the solution has cooled to room temperature, 1.25 mL of 10% TCA and 0.25 mL of 0.1% $FeCl_3$ were added to the solution and stirred by a vortex. The resulting solution was then analyzed and the absorbance was obtained at 700nm.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Results and discussions on *Salvia absconditiflora*

4.1.1 Scanning electron microscopes (SEM)

The silver nanoparticles (sa-AgNPs) synthesized from *Salvia absconditiflora* were examined under scanning electron microscope and the average size of the sa-AgNPs was obtained as 26.6 nm (Figure 4.1). The biggest size was calculated as 38.25 nm and smallest was 15.91nm.

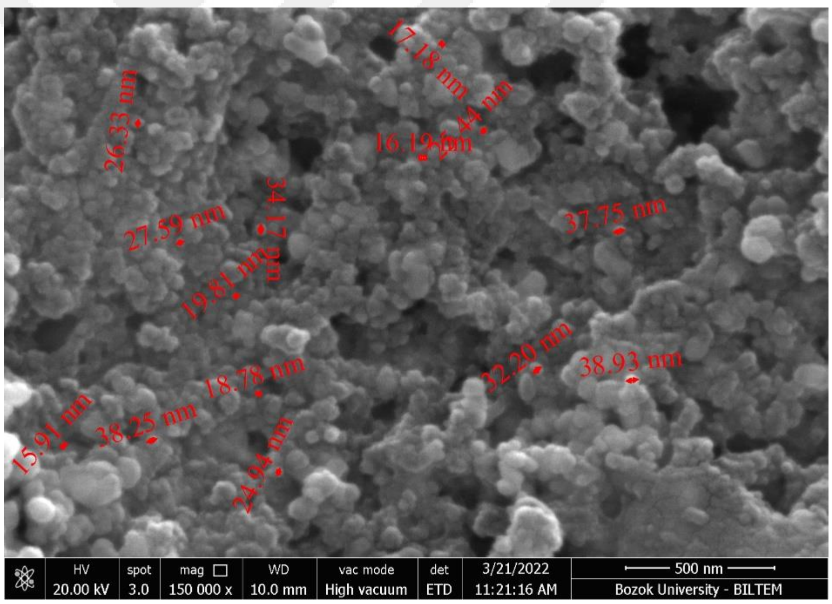

Figure 4.1: The average size of S. absconditiflora AgNPs under SEM

4.1.2 The Fourier-transform Infrared spectroscopy (FTIR) analysis

FTIR analysis was carried out on both extracts and AgNPs of Salvia absconditiflora to identify the molecular composition and functional groups present in the sample.

The extract and AgNPs FTIR spectrum were obtained and shown in figure 4.2. The spectrum for the extract is represented by a while b represents the spectrum for the AgNPs.

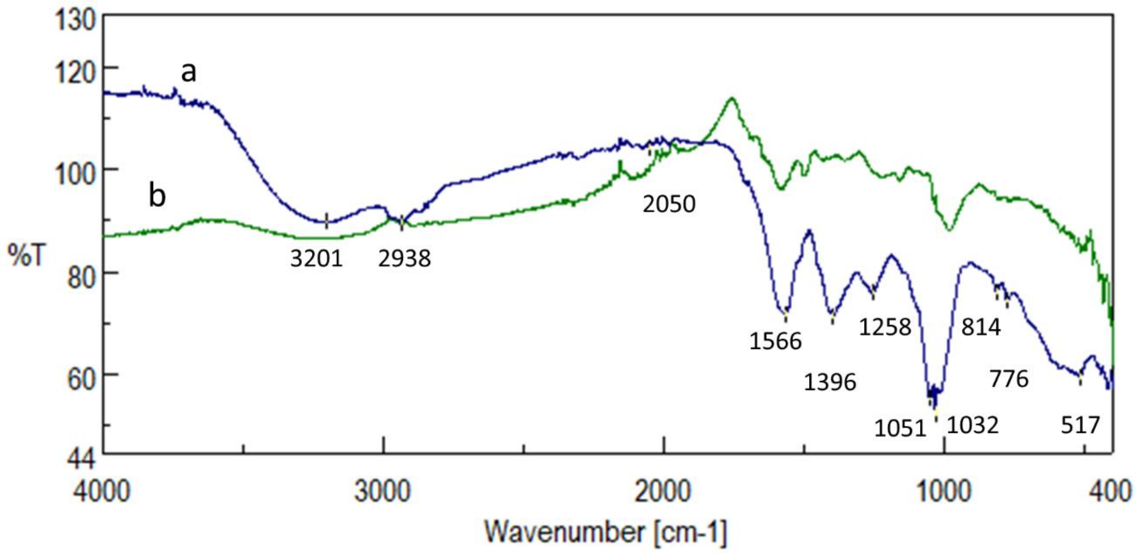

Figure 4.2: FTIR spectrum for *Salvia absconditiflora*

4.1.3 Ultraviolet-visible (UV-Vis) spectrophotometry

The absorbance spectra for both the extract and AgNPs for Salvia absconditiflora were obtained between 300nm to 800nm while the maximum absorbance of the sample was obtained at 471 nm as shown in figure 4.3

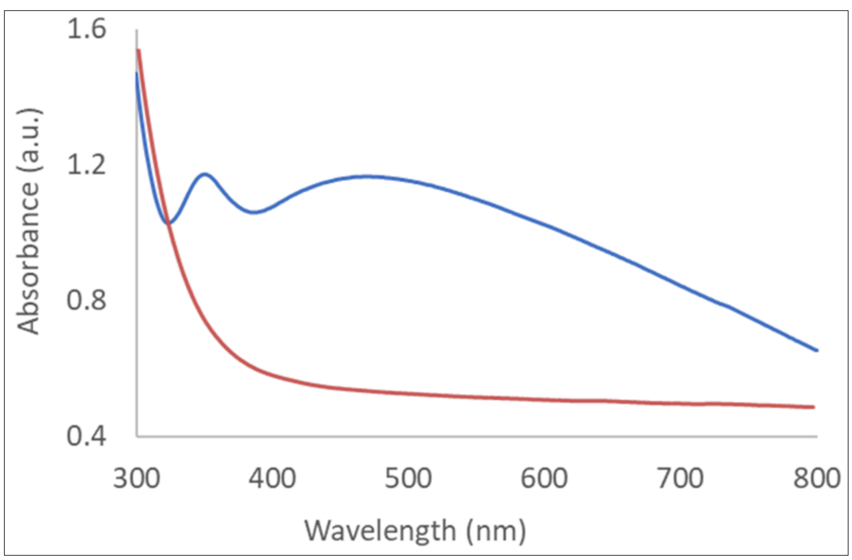

Figure 4.3: Absorbance for Salvia absconditiflora

4.1.4 XRD and EDX analysis

X-ray diffraction (XRD) analysis was carried out to identify the spatial arrangement of the atoms in the crystalline phase of the AgNPs of Salvia absconditiflora.

The energy dispersive x-ray (EDX) analysis was also carried out on its AgNPs to identify the elements from which they were produced. The results were obtained and shown in figure 4.4 and 4.5 respectively.

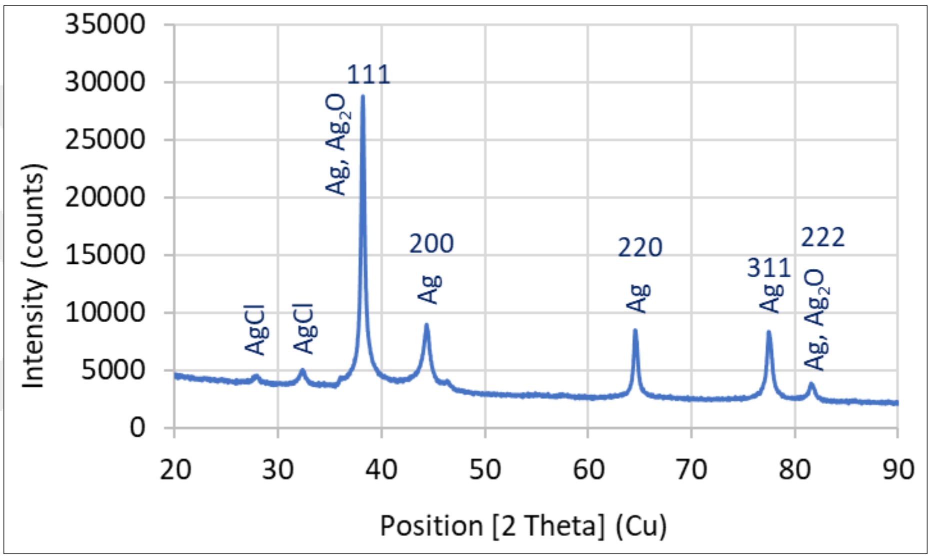

Figure 4.4: XRD pattern of Salvia absconditiflora AgNPs

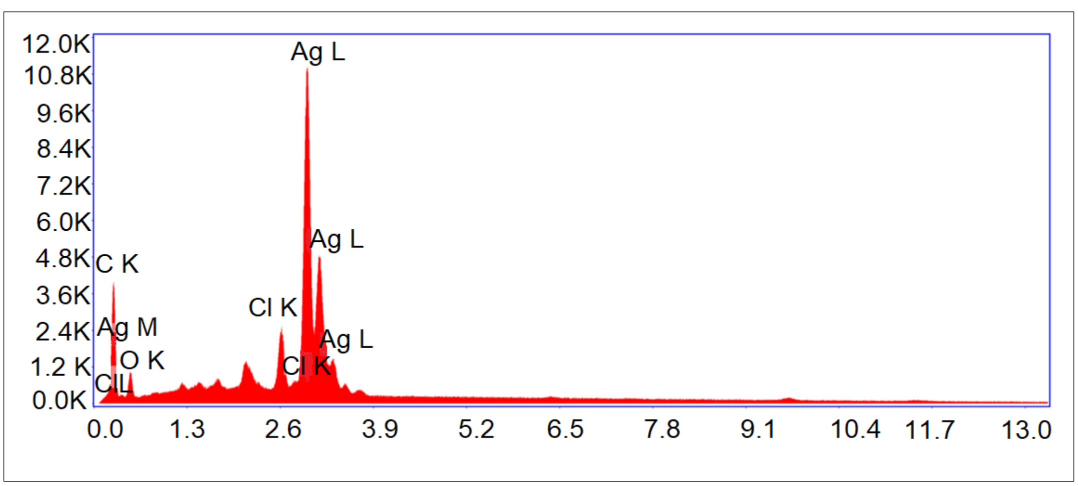

Figure 4.5: EDX spectrum

From the figure 4.4 shown above, it could be seen that there are 4 different diffraction peaks with values of 38.1°, 44.3°, 64.5° and 77.3°. There are also some small peaks respectively at values 27.2°, 33.3° and 82.1° which are insignificant for the result being analyzed.

4.1.5 Free radical scavenging activity

The DPPH scavenging activity and ABTS assay of Salvia absconditiflora extract and sa-AgNPs were compared with butylated hydroxyanisole (BHA), butylated hydroxytolune (BHT) and Trolox. Tables 4.1 show the DPPH activity values (expressed in IC_{50}), ABTS assay values (expressed in μg/mL) and the FRAP activity values (expressed in μmol TE/mg matter) while the values are graphically displayed in figures 4.6, 4.7 and 4.8 respectively.

Table 4.1. Antioxidant activity of *S. absconditiflora* extract and sa-AgNPs and standards.

Samples	*DPPH$^{\bullet}$ effect	*ABTS$^{\bullet +}$ effect	**FRAP
sa-Extract	21.55±0.26^{d}	8.43±0.20	1.73±0.02
sa-AgNPs	15.78±0.22^{c}	4.84± 0.07	4.62 ± 0.07
BHT	10.74 ± 0.20^{b}	7.12 ± 0.10	5.56 ± 0.17
BHA	4.66 ± 0.13^{a}	4.23 ± 0.08	7.90± 0.20
Trolox	4.75 ± 0.13^{a}	5.35 ± 0.11	-

* IC_{50} (μg/mL), ** μ mol TE/mg samples. Different letters indicate the significantly differences. ($p<0.05$).

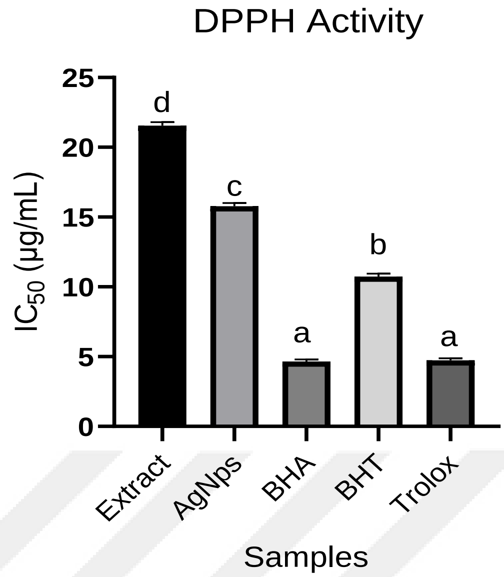

Figure 4.6: DPPH activity for extracts and AgNPs of Salvia absconditiflora

The FRAP activity of the extract and AgNPs of Salvia absconditiflora was compared with BHA and BHT and the result was obtained and as shown in figure 4.8

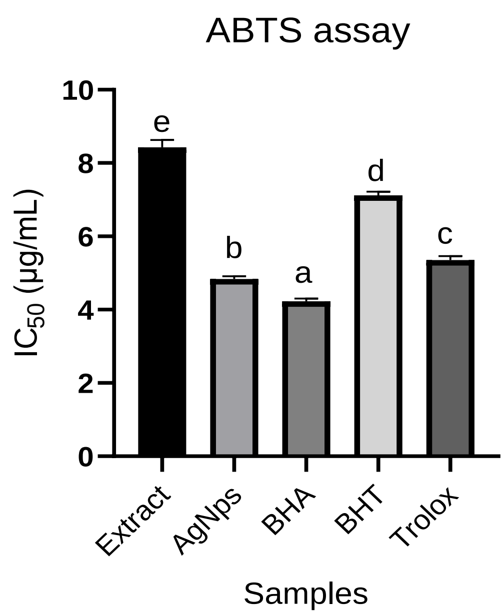

Figure 4.7: ABTS assay for extracts and AgNPs of Salvia absconditiflora

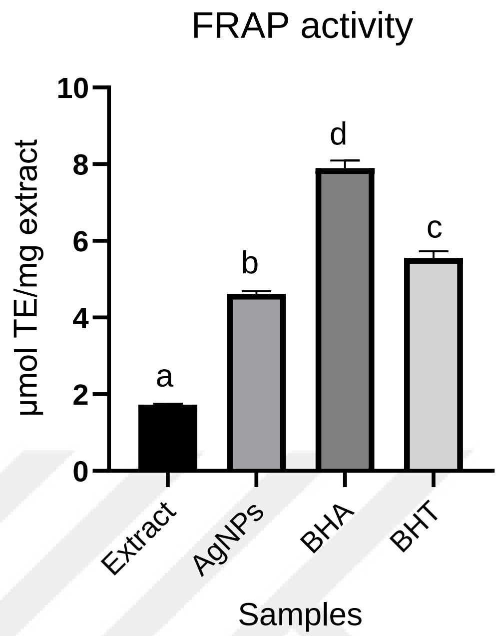

Figure 4.8: FRAP activity for extracts and AgNPs of Salvia absconditiflora

From the DPPH result displayed above, it could be seen that the extract and AgNPs have a very high value as compared to the values obtained from the BHA, BHT and Trolox

From the ABTS assay result, it could be seen that the value obtained for extract is highest and the values for BHT and Trolox are higher than that obtained for the AgNPs. Also, the value obtained for AgNPs is higher than BHA

From the FRAP activity, the BHA value is the highest while the value obtained for extract is the lowest and the value obtained for AgNPs is slightly lower than that for BHT.

4.2 Results and discussions on *Salvia hypargeia*

4.2.1 Size determination of the AgNPs

The AgNPs of Salvia hypergeia was examined under scanning electron microscope SEM and its average size was obtained as shown in figure 4.9

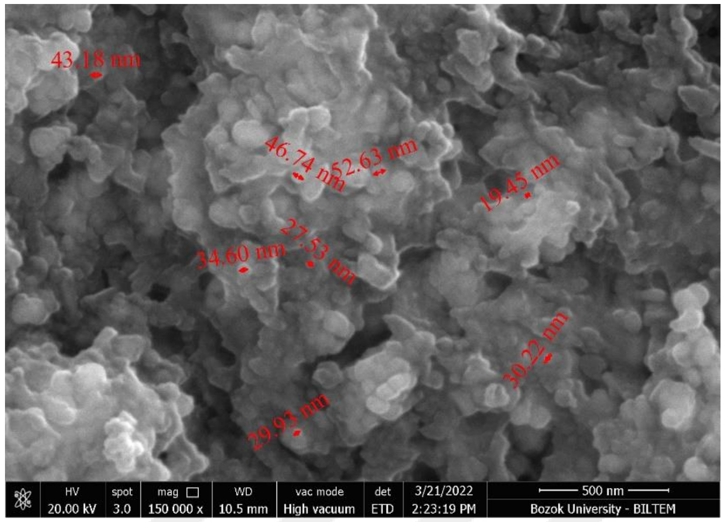

Figure 4.9: The average size of S. hypargeia AgNPs

From the SEM image, it could be seen that the average size of the AgNPs is 35.5 nm with the biggest size being 52.63nm and smallest being 19.45nm.

4.2.2 Discussion of the absorption spectrum of Salvia hypergeia

The extract and AgNPs for Salvia hypergeia were examined and their absorbance were obtained between 300nm to 800nm while the maximum absorbance of the sample was obtained at 490 nm as shown in figure 4.10

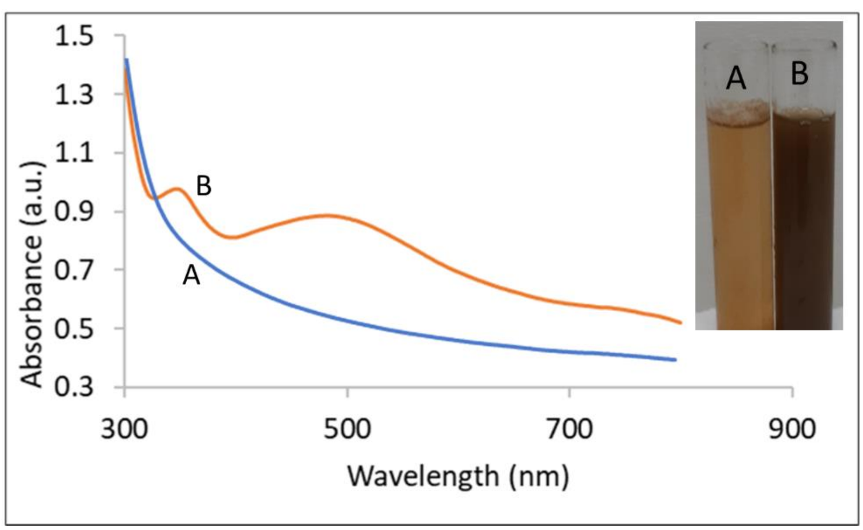

Figure 4.10: Absorbance for Salvia absconditiflora

4.2.3 Infrared analysis of Salvia hypargeia

The extract of the plant was subjected to IR analysis. This was done at different wavelengths and the corresponding values obtained for each of the wavelengths is as shown in figure 4.11

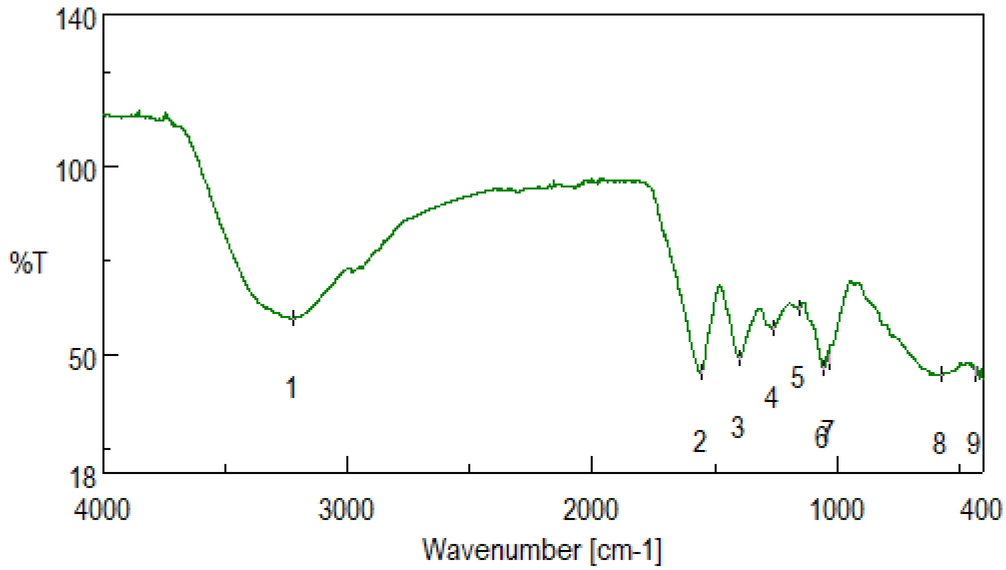

Figure 4.11 Infrared spectra of extract. 1: 3226,

From the result of the spectra obtained above, the value for each of the wavenumber number and the number is tabulated in table 4.2

Table 4.2- Infrared spectra for the extract of S. hypergeia

1	3226
2	1556
3	1397
4	1263
5	1155
6	1054
7	1032
8	574
9	430

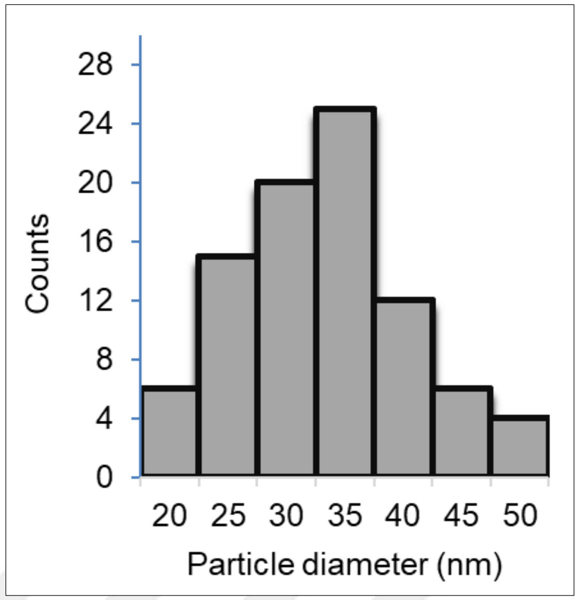

Figure 4.12: the particle diameter chart for the AgNPs

Table 4.3. The elemental composition of S. hypergeia AgNPs

Element	Weight %	Atomic %
C K	14.47	41.66
O K	15.33	33.15
Cl K	4.08	3.98
Ag L	66.12	21.21

From the elemental composition chart displayed above, it could be seen that sample of the S. hypargeia consist of Carbon, Oxygen, Chlorine Silver and the percentage weight and percentage atomic composition was obtained as well.

4.2.4 Zeta analysis of S. hypergeia

The potential difference between the dispersed medium and the accessible surface of the dispersed medium was measured.

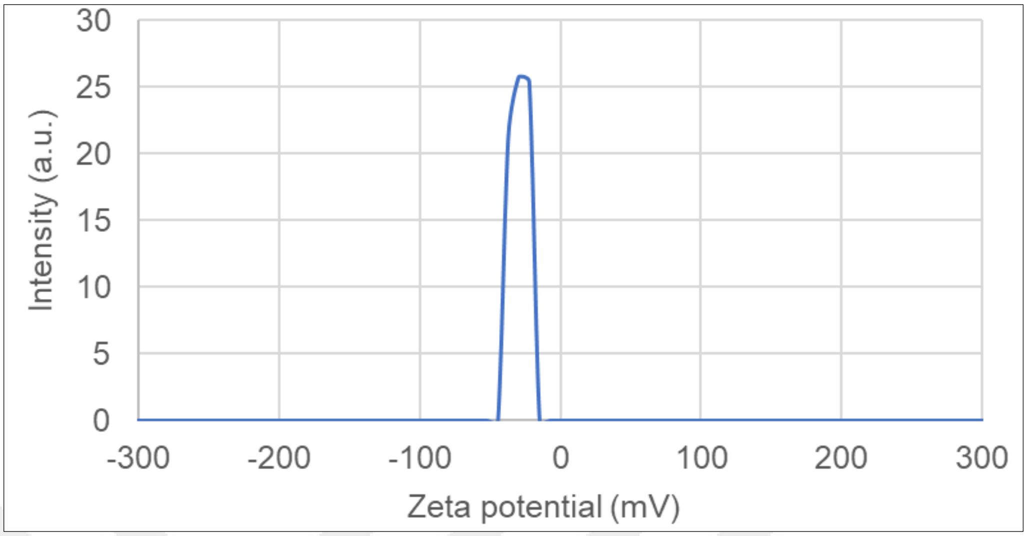

Figure 4.13- Zeta potential analysis of S. hypergeia

From the above diagram, the zeta value was calculated to be -32.8mV. Since the zeta potential value obtained is a negative value, this shows that the sample is anionic, stable for a number of months and no toxicity associated with cell wall disruption.

4.2.5 XRD spectrum

X-ray diffraction (XRD) analysis was also carried out to identify the spatial arrangement of the atoms in the crystalline phase of the AgNPs of Salvia hypergeia and the XRD pattern is shown in figure 4.14

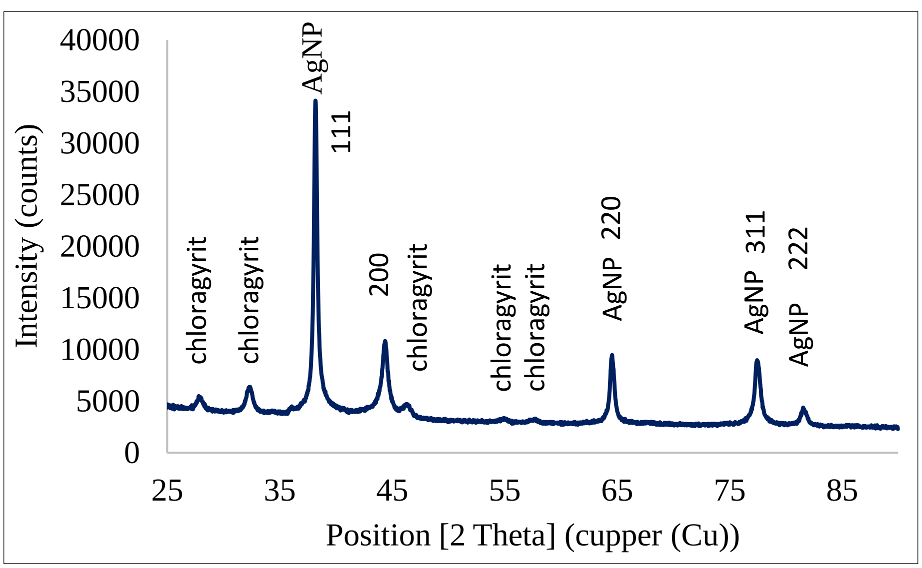

Figure 4.14: XRD analysis of Salvia hypergeia AgNPs

From the figure above, it could be seen that there are 4 different diffraction peaks with values of 38.1°, 44.3°, 64.5° and 77.3°. There are also some small peaks respectively at values 27.2°, 33.3° and 82.1° which are insignificant for the result being analyzed.

4.2.6 Free radical scavenging activity

The DPPH scavenging activity and ABTS assay of S. hypergeia's extract and AgNPs were compared with butylated hydroxyanisole (BHA), butylated hydroxytolune (BHT) and Trolox. Tables 4.4 show the DPPH activity values (expressed in IC_{50}), ABTS assay values (expressed in µg/mL) and the FRAP activity values (expressed in µmol TE/mg matter) while the values are graphically displayed in figures 4.14.

Table 4.4. Antioxidant activity of AgNPs and standards.

Samples	*$DPPH^{\bullet}$ effect	*$ABTS^{\bullet+}$ effect	**FRAP
sh-Extract	18.02±0.89	6.70±0.17	2.45±0.05
sh-AgNPs	11.83±0.22	3.78±0.05	6.10±0.06
BHT	10.74 ± 0.20	7.12 ± 0.10	5.56 ± 0.17
BHA	4.66 ± 0.13	4.23 ± 0.08	7.90± 0.20
Trolox	4.75 ± 0.13	5.35 ± 0.11	-

* IC_{50} (µg/mL), ** µ mol TE/mg samples.

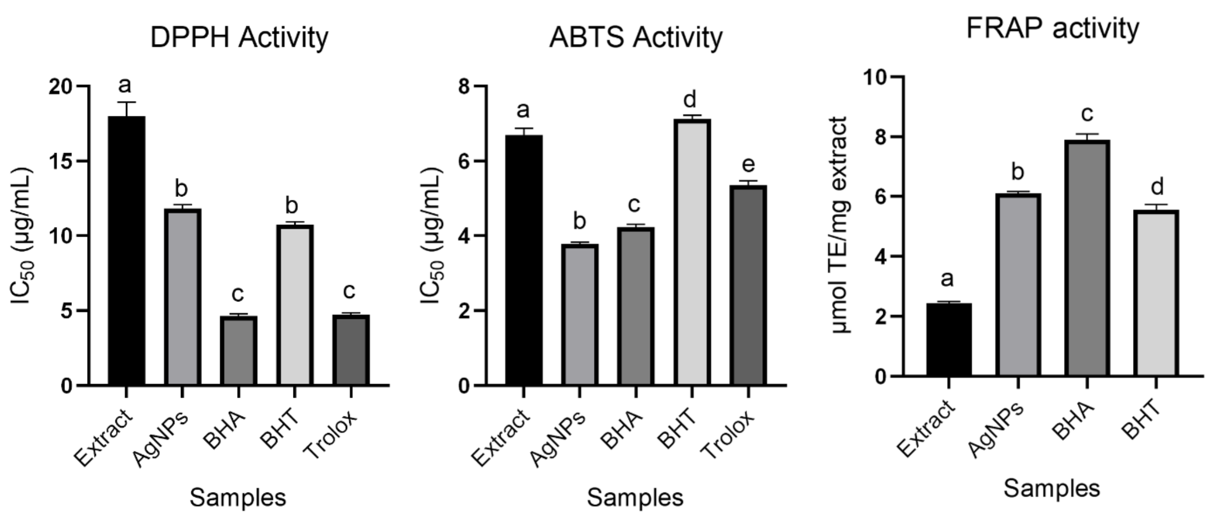

Figure 4.15: Pictorial representation of DPPH, ABTS and FRAP activities of S. hypergeia

From the DPPH result displayed above, it could be seen that the extract and AgNPs have a very high value as compared to the values obtained from the BHA, BHT and Trolox2

From the ABTS assay result, it could be seen that the value obtained for extract is highest and the values for BHT and Trolox are higher than that obtained for the AgNPs. Also, the value obtained for AgNPs is higher than BHA

From the FRAP activity, the value obtained from extract is the lowest while BHA value is the highest. The value obtained for AgNPs is slightly higher than that for BHT.

CHAPTER 5

CONCLUSIONS

From the analysis carried out on the extracts of S. abscoditiflora, it can be concluded that S. Absconditiflora is a good source of polyphenols as found in previous literatures. They are also good source of antioxidant.

From the zeta analysis performed on S. hypergeia, it was observed that its zeta potential value is -32.8mV which shows that S. hypergeia is anionic and stable. This shows that it has the tendency to permeate membranes because the membranes of cells are also negatively charged.

From the experiment and analysis obtained from the AgNPs of S. hypargeia and its average size being 35.5nm, it can be seen that the size of its nanoparticle is small and concluded that the nanoparticle of this plant will be absorbed faster in particles in which it is bound with.

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