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Biogenic Synthesis of Silver Nano Particles by Aqueous Extract of Leaves Back Tea and its Antifungal Activity

A Reserch

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

نَرْفَعُ دَرَجَاتٍ مَّن نَّشَاءُ

وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ

صَدَقَ اللَّهُ العَلِيُّ العَظِيم

سورة يوسف من الآية (٧٧)

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Hassanien & Bian

Dedications

To…

My father, mother, brothers and sisters

For your support and encouragement, I hope this thesis serves to repay your contributions in some small part….

Summary:

The aim of this study, biosynthesis of silver nanoparticles using aqueous extract of leaves black tea and tested the effected it as antifungal agent .The synthesized nanoparticles were characterized using UV- spectroscopy and Scanning electron microscope (SEM).Results ,show that, silver nanoparticles formation in solution by changing in the color from yellow to brown .The absorption spectrum of silver nanoparticles by uv- visible shown in band of 400 nm ,which signifying the existence of spherical shape nanoparticles . SEM analysis shows that the size of synthesized silver nanoparticles are with range of 10 -500 nm . Also ,the biosynthesis of silver nanoparticles using aqueous extract of leaves black tea effected on growth of yeast *C.albicans* with inhibition zone 9mm compare with leaf extract 2.3mm.

List of contents

List of Figures

List ofTable

Chapter One

Introduction

1-Introduction:

Nanotechnology is manipulation of matter through certain chemical and/or physical processes to create materials with specific properties, which can be used in particular applications. A nanoparticle can be also known as a microscopic particle that has at least one dimension less than 100 nanometers in size (Thakkar.,*et al* 2010). Unlike bulk materials, they have unique optical, thermal, electrical, chemical, and physical properties and, hence, they find a variety of applications in the areas of medicine, chemistry, environment, energy, agriculture, information, and communication, heavy industry, and consumer goods (Mihir *et al*.,2014).whole or partial plant such as fruits, vegetables, (black and green tea) and marine algae extracts have been used to produce nanoparticles. (Luangpipat *et al.*, 2011; Rajesh *et al*., 2012). Biogenic synthesis is useful not only because of its reduced environ-mental impact compared with some of the physicochemical production methods, but also because it can be used to produce large quantities of nanoparticles that are free of contamination and have a well-defined size and morphology (Anastas and Zimmerman, 2007; Biosynthetic routes can actually provide Hutchison, 2008). nanoparticles of a better defined size and morphology than some of the physicochemical methods of production (Raveendran *et al*., 2003). Thus, the objective of present study are:

1-leaves extract of black tea are used for synthesis of silver nanoparticles .

2-Identification and study the characteristics of these nanoparticles using UV-Vis spectrophotometer and scanning electron microscope .

3-Testing the antifungal activity against yeast C.albicans by using disc diffusion method.

Chapter Two

Literature Review

2-Litrature Review:

2-1- Nanoparticles:

Nanoparticle known as a microscopic particle that has at least one dimension less than 100 nanometers in size (Thakkar.,*et al* 2010). Unlike bulk materials, they have unique optical, thermal, electrical, chemical, and physical properties and, hence, they find a variety of applications in the areas of medicine, chemistry, environment, energy, agriculture, information, and communication, heavy industry, and consumer goods (Mihir *et al.*,2014). Among them, the metallic nanoparticles are most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest to researchers due to the growing microbial resistance against metal ions, antibiotics, and the development of resistant strains.

2-2- Synthesis of nanoparticles:

The methods for making nanoparticles can generally involve either a“top down” approach or a“bottom up”approach (Sepeur,2008). In top-down synthesis , nanoparticles are roduced by size reduction from a suitable starting material (Meyers *et al*.,2006). Size reduction is achieved by various physical and chemical treatments . Top down production methods introduce imper- fections in the surface structure of the product and this is a major limitation because the surface chemistry and the other physical prop-erties of nanoparticles are

highly dependent on the surface structure(Thakkar *et al*., 2010) In bottom up synthesis, the nanoparticles are built from smallere ntities, for example by joining atoms, molecules and smaller particles (Mukherjee *et al.,* 2001). In bottom up synthesis, the nanostructured building blocks of the nanoparticles are formedfirst and then assembled to produce the final particle . The bottom up synthesis mostly relies on chemical and biological methods of production. The probable mechanism of nanoparticle synthesis by bottom up approach(Thakkar *et al.*, 2010).

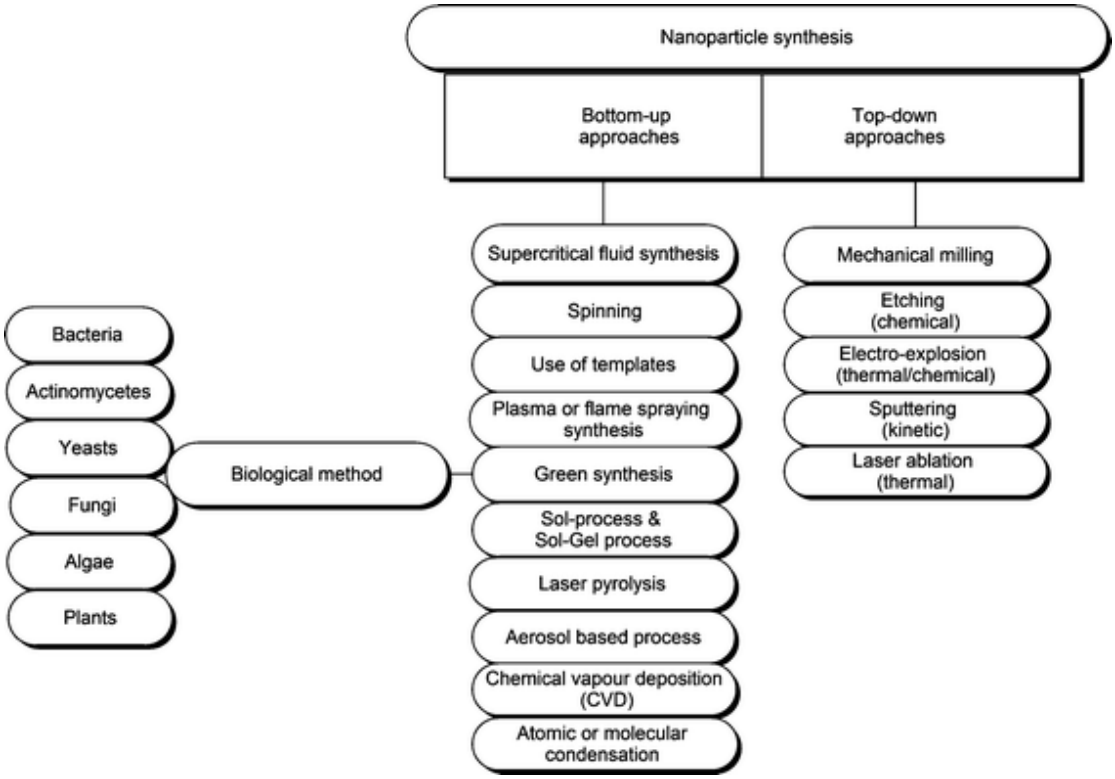
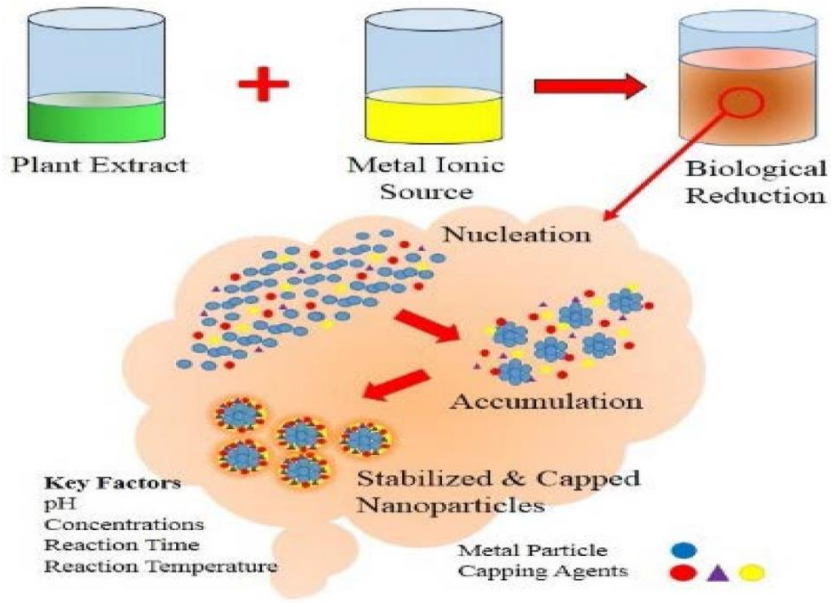


Fig (1) approaches of nanoparticles synthesis

2-3-Synthesis of nanoparticles by using of plant extracts :

In producing nanoparticles using plant extracts, the extract is simply mixed with a solution of the metal salt at room temperature. The reaction is complete within minutes. Nanoparticles of silver, gold and many other metals have been produced this way (Li *et al*., 2011). The nature of the plant extract, its concentration, the concentration of the metal salt, the pH, temperature and contact time are known to affect the rate of production of the nanoparticles, their quantity and other characteristics (Dwivedi and Gopal, 2010) ..Jacob *et al*. (2011)reported the synthesis of silver nanoparticles using Piper longum leaf extracts. The particles had a uniform spherical shape and ranged in size from about 18 to 41 nm. Prathna *et al*. (2011a) reported the synthesis of silver nanoparticles using an extract of Azadirachta indica leaves and a solution of silver nitrate. Increasing the reaction time from 30 min to 4 h resulted in a progressive increase in the particle size from around 10 nm to around 35 nm.

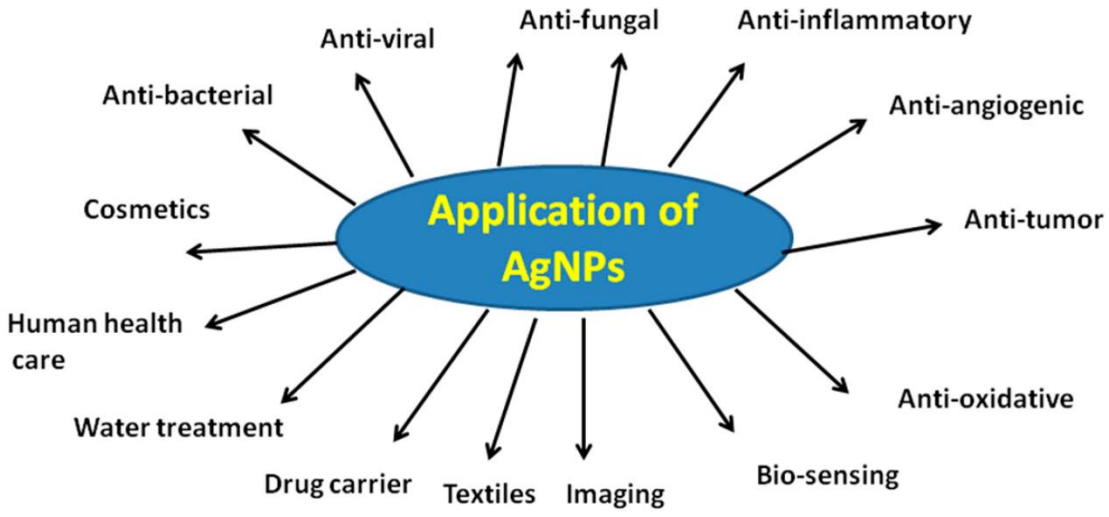


Fig(2)Scheme of biosynthesis of nanoparticles by plant extract

2-4- Silver nanoparticles:

AgNPs are a very important part of nanotechnology mainly because they do not induce modification on living cells and, so, are unable to cause microbial resistance. Recent studies revealed that AgNPs have the ability to attach to cell walls and alter cellular respiration. AgNPs are widely used in biology and medicine especially because of their attractive and unique physiochemical properties. Researches carried out in the late 1970s used silver particles for the treatment of orthopedic In recent years green chemistry and biosynthetic methods have become more attractive ways to obtain AgNPs. These unconventional methods use either biological microorganisms (e.g.: bacteria, fungi, marine algae, yeasts) or different alcoholic or aqueous plant extracts. Green synthesis has multiple

advantages over classical routes: it is cost effective ,ecofriendly and does not require high pressure, energy temperature or the use of toxic chemical reagents. (Wiley,,2007) AgNPs have been used extensively as anti-bacterial agents in the health industry, food storage, textile coatings and a number of environmental applications. It is important to note that despite of decades of use, the evidence of toxicity of silver is still not clear. Products made with AgNPs have been approved by a range of accredited bodies.(Bhattacharya and Mukherjee, 2008).



Fig(3)Application of silver nano particles(Xi-Feng,2016)

2-5-Antifungal activity of silver nanoparticles:

AgNPs play an important role as anti-fungal agents against various diseases caused by fungi. Nano-Ag showed potent anti-fungal activity against clinical isolates and ATCC strains of

Trichophyton mentagrophytes and Candida species with concentrations of 1–7 μg/mL. Esteban-Tejeda *et al.*(2006).Also, the antifungal efficacy of AgNPs was evaluated in combination with nystatin (NYT) or chlorhexidine (CHX) against *Candida albicans and Candida glabrata* biofilms. The results from this investigation suggest that AgNPs combined with either nystatin (NYT) or chlorhexidine digluconate (CHG) showed better synergistic anti-biofilm activity; however, this activity depends on the species and drug concentrations.(Monteiro,2013) Interestingly, AgNPs not only inhibit human and plant pathogenic fungi, but also indoor fungal species such as *Penicillium brevicompactum, Aspergillus fumigatus, Cladosporium cladosporoides, Chaetomium globosum, Stachybotrys chartarum, and Mortierella alpine* cultured on agar media.(Ogar,2015).

Chapter Three

Materials and Methods

3-1-Materials and Methods

3-1-Equipments & Chemical materials:

Table (3-1): Equipment's & Chemical materials used in the laboratory experiments

No.	Equipment	Company	source
1	Incubator	Gallenkamp	(England)
2	Sensitive balance	Gallenkamp	(England)
3	autoclave	Gallenkamp	(England)
4	Hot plate with magnetic stirrer	Gallenkamp	(England)
5	Vortex	Electrothermal	(England)
6	UV Spectrophotometer	Memmert	germany
6	Whitman(No.1)filter paper	Gallenkamp	(England)
7	Centrifuge	Gallenkamp	(England)
8	Silver nitrate	BDH	England
9	Leaves of black tea		orginal
11	Sabuobaud's Dextrose Agar	BDH	(England)

3-2-Methods:

3-2-1- Preparation of Sabouraud dextrose Agar (SDA):

Culture media were prepared according to the instructions of the manufacture companies, the culture media were sterilized by autoclave at 121C under pressure 15 pound /cubic inch for 20 minutes .

3-2-2-Fungal Strain*:*

Candida albicans which obtain from laboratory of microbiology of science college /university of AL-Qadisiyah.

3-2-3-Synthesis of silver nanoparticles :

2 g. of leaves tea put in beaker containing 20ml of DW .the mixture was thoroughly agitated over night by using a magnetic stirrer .Than the mixture was filtered to obtain brown liquor .The liquor was centrifuged and again filtered to remove the impurities .1M of $AgNO_3$ solution was prepared in DW. Silver nanoparticles was prepared by mixed 0.2 ml of $AgNO_3$ solution ,0.1 ml of tea extract and 3.7 ml DW .Then it was kept overnight in dark to stabilize .Change in color was observed on the next day .(Lipi *et.al*.,2014)

3-2-4-UV –spectrophotometer analysis :

Optical characters of the synthesized silver nanoparticles and $AgNO_3$ were studied by analyzing the UV-Vis specter using spectrophotometer at room temperature .

3-2-5-Scanning Electron Microscopy (SEM):

We used Scanning Electron Microscopy (SEM) technique for measuring nanoparticle size of silver.

3-2-6-Antifungal activity :

The Antifungal activity of the extracts was carried out by disc diffusion method(Kim *et.al.*,1995) Circular discs of 5 mm diameter were made from the Whatman No.1 filter paper and sterilized by autoclaving at 15lb/inch2 for 15 minutes. The sterile discs were impregnated with equal volume (100µg/ml) of tea leaf extracts and silver nanoparticles The discs containing each of 25µl samples were aseptically placed on plates containing SDA medium after being spreader with each of the test pathogens, . The plates were incubated at 37 ºC for 24 hours and the zone of inhibition was measured (in mm diameter).

Chapter Four

Results & Discussion

4-Results & Discussion

4-1-Synthesis of silver nanoparticles:

The formation of silver nanoparticles was found to be successful by initial changes in color and that silver nanoparticles exhibit brown color (fig.1) in aqueous solution which output from the surface Plasmon vibration in Ag Nps. Silver nitrate has distinctive characteristics such as good conductivity, catalytic, chemical stability . So the reduce of silver salt (silver nitrate) to metal nanoparticles in present bio reduction agent (leaf tea extract).

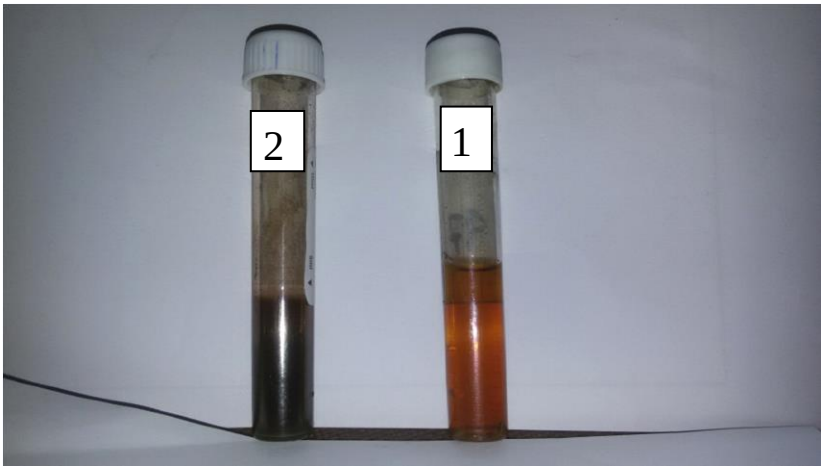


Fig.(4) : (1) aqueous of leaf tea extract ,(2) synthesized Ag Nps

4-2- - U-V-Vis Spectroscopy :

Uv-Vis spectroscopy was used for detection the presence of silver nanoparticles ,particularly absorbance in the range (400-450)nm .(Philip,2010).Results ,in fig(2,3) appearance that the wavelength of synthesized silver nanoparticles in absorption band of 400nm .This results coordinated with Sarah *et.al*.(2014)

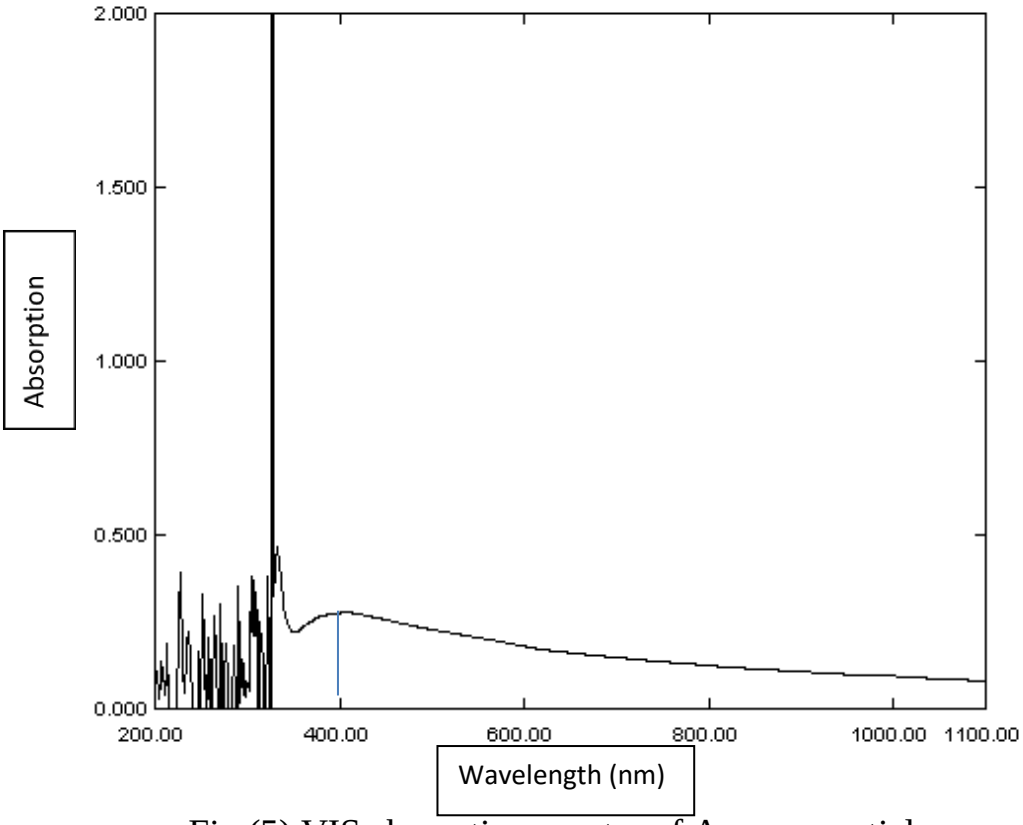


Fig.(5) VIS absorption spectra of Ag nanoparticles

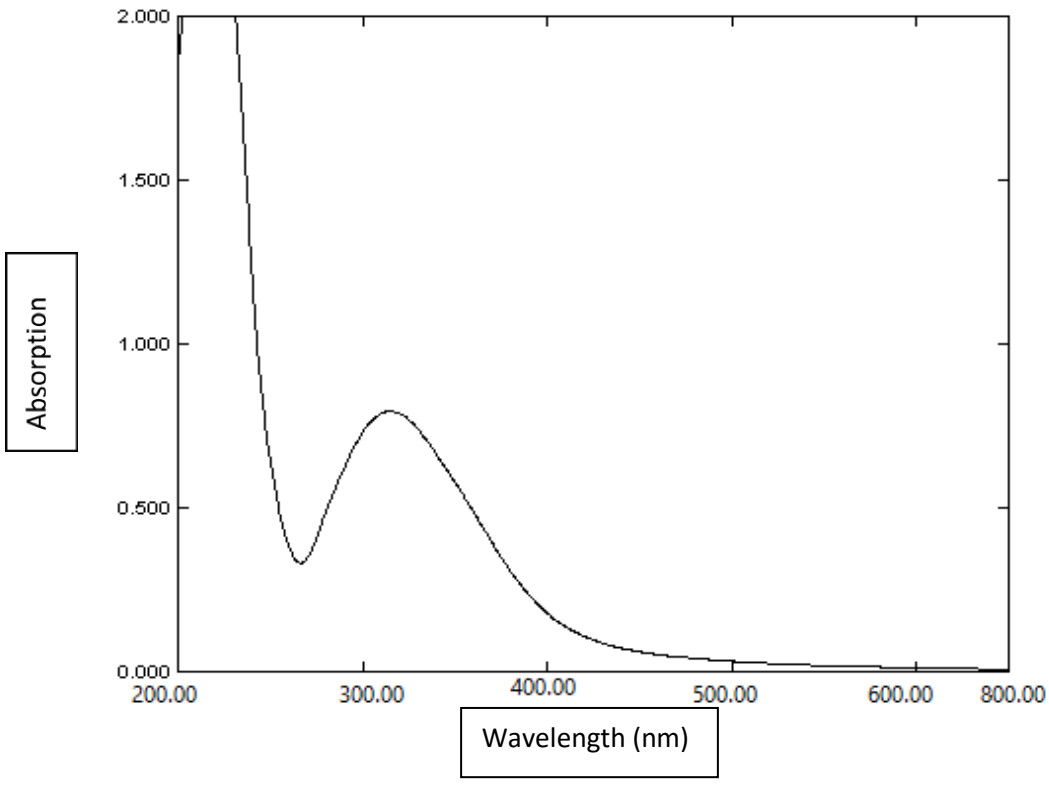


Fig.(6) VIS absorption spectra of $AgNO_3$

4-3- SEM Images:

Fig(4,5,6,7) explain the results of SEM analysis which revealed a characteristics of synthesized silver nanoparticles by leaf black tea extract ,its appear spherical particles with variety size (10,50,100,500)nm .this a coordinated with(Babu & Prabu,2011)

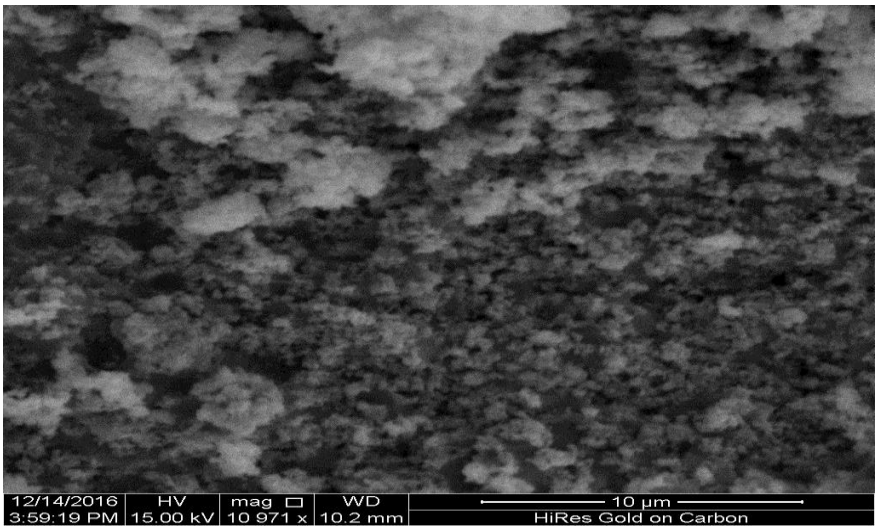


Fig.(7) ESM sections of the biosynthesis of AgNps with size(10nm)

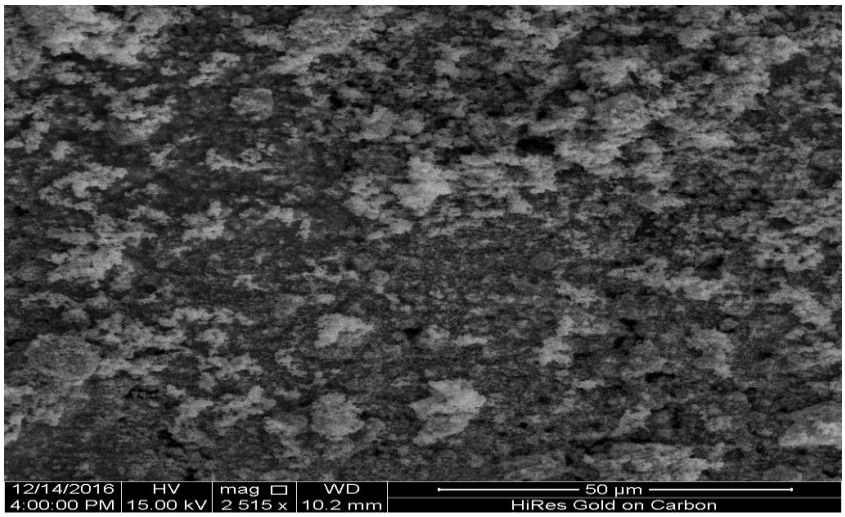


Fig.(8) ESM sections of the biosynthesis of AgNps with size (50nm)

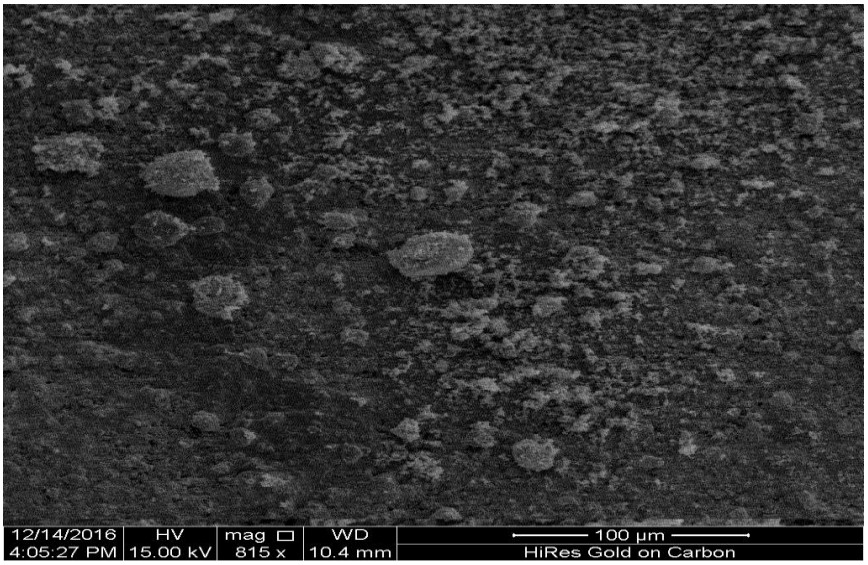


Fig.(9) ESM sections of the biosynthesis of AgNps with size (100nm)

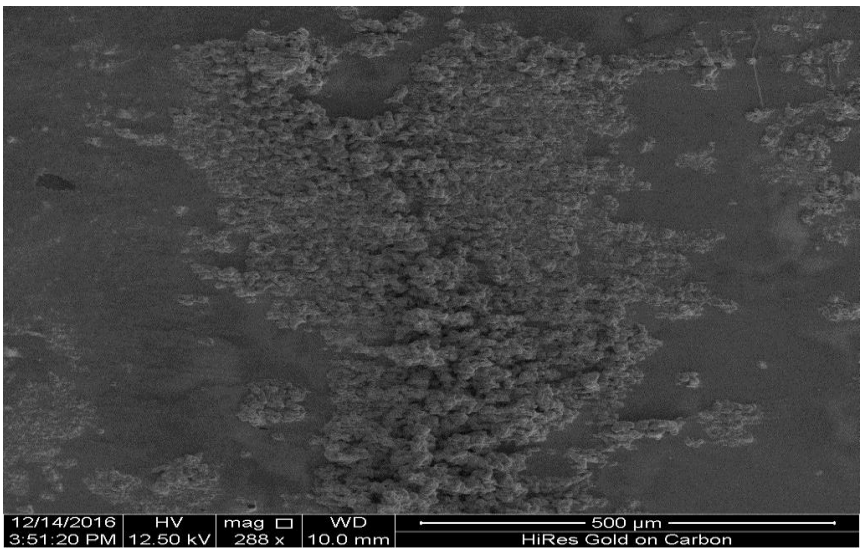


Fig.(10) ESM sections of the biosynthesis of AgNps with size (500nm)

The present results revealed that leaf tea extract at room temperature was able to act as reducing and stability agent .and confirmed the formation of silver nanoparticles . Biogenic synthesis is useful not only because of environmental decline for mental effect (Anastas and Zimmerman .2007) Compared with some physicochemical Production methods, but also because they can be used to produce large quantities of nanoparticles that are free from contamination .The source of plant extract effect

on the characteristics of nanoparticles that these extracts contain different concentration of organic reducing agents(Mukunthan and Balaji, 2012)

4-4 -Antifungal activity:

The antifungal activity of biosynthesis produced was tested on Candida albicans .The inhibition test were due to by disc diffusion method .Fig.(8,9) appearance growth inhibition of yeast by silver nanoparticles compare with aqueous leaf tea extract and emphasis the antifungal activity of AgNps biosynthesis by leaf black tea extract .This results agreement with Vivek *et al*.(2011) which reported that antifungal effects of silver nanoparticles have been demonstrated .Silver nanoparticles are known to affect the permeability of membranes of microbial and other cells (Li *et al*., 2010).

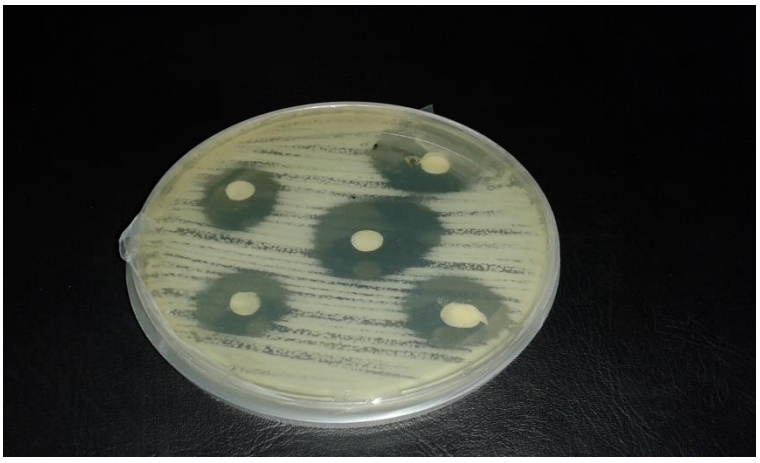


Fig.(11) Effect of biosynthesis of Ag Nps on *C.albicans*(inhibition zone)

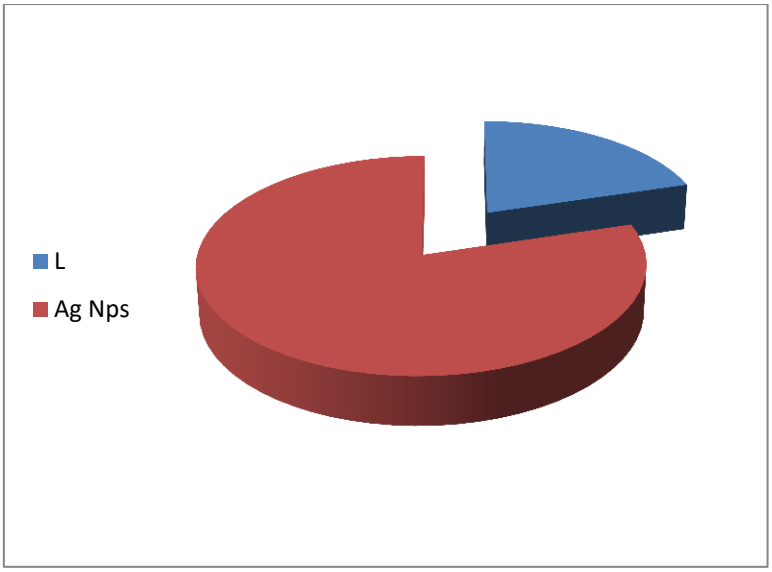


Fig.(12) Effect of biosynthesis of Ag Nps on *C.albicans*

L= An aqueous extract of leaf black tea, AgNps= The biosynthesis Ag nanoparticles

Conclusions & Recommendations

Conclusions:

1-leaf black tea extract at room temperature was able to reducing $AgNO_3$ and produce AgNps .

2-The usage of plant extract considered clean and simpler approaches and less effected on the environment .

3- The UV–visible spectroscopy Scanning electron microscopy used for morphological characterization at the nanometer to micrometer scale .

4-AgNps were most active against the yeast *Candida albicans*

.

Recommendations:

1- We can used aqueous extract of leaves black tea to produce silver nanoparticles in present of silver nitrate.

2-Study Usage aqueous extract of leaves black tea for produce other types of nanoparticles ,which act as a reduction agent of metal salts.

3-Study the effects of biosynthesis silver nanoparticles on *C.albicans* in vivo by using animals lab.

4-Study of possibility to used biosynthesis silver nanoparticles in industrial field.

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