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Comparative studies on synthesized silver nanoparticles using *Artemisia vulgaris* Linn., and *Cinnamomum zeylanicum* Nees., for their antifungal activity

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Abstract:-

The present study aims to compare the anti-fungal activity of green synthesized Silver nanoparticles by using the two indigenous plant *Artemisia vulgaris* Linn. and *Cinnamomum zeylanicum* Nees.,. Silver nanoparticles (AgNPs) are one of the most vital and fascinating nanomaterials among several metallic nanoparticles that are involved in biomedical application. The green synthesized AgNPs can be used in the field of medicine due to their smaller particle size, increased activity and less toxicity. Artemisia, being the largest and widely distributed genus of the plant family Asteraceae encompasses more than 400 species. *Artemisia vulgaris.* Linn (Mugwort) is used by many tribes throughout India to treat varieties of conditions including colds, allergies and pain. Indian Mugwort is also used as women's medicine. It also posses anti-bacterial, anti-inflammatory, anti-microbial, anti-fungal activities. The genus Cinnamomum (family Lauraceae) contains more than 300 evergreen aromatic trees and shrubs. *Cinnamomum zeylanicum* Nees bark were used for the study. Cinnamomum, commonly used as spices, contain many antibacterial compounds. The prepared nanoparticles were characterized by UV-VIS spectroscopy, SEM, particle size analysis and zeta potential. Further antifungal activities were determined. Fungicidal activity was done by using standard agar well diffusion method against human pathogenic fungi (*Candida albicans, Aspergillus flavus ,Fusarium oxysporum,*). A comparative study was done between *Artemisia vulgaris.* Linn and *Cinnamomum zeylanicum Nees* using three different microorganisms and found that *Cinnamomum zeylanicum* Nees have better antifungal activity.

Key Words: *Artemisia vulgaris* .Linn , *Cinnamomum zeylanicum* Nees, AgNPs, antifungal activity, MIC, Kirby Bauer Method

1. INTRODUCTION

The current research field includes nanoparticles as the main concern of scientists as the nanoparticles have specific site of action, and the small size give enhancement to the permeability of immune response through the membrane of the cells[1] and the small size increases the surface to volume ratio and the gradational effects on the particles are also reduced[2]. Nanoparticles size ranges from 1nm to 900nm, and size range between 1-100nm have higher microbial activity [3].

The silver nanoparticles are vigorously involved in the anti-microbial activity against a lot of disease causing food borne and water born pathogenic bacteria's and fungus. Chemical synthesis methods in the preparation of nanoparticles lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical application [4]. Synthesis of nanoparticles by using microorganisms, enzyme and plant or plant extract, have been proposed by many authors. In the biological methods, it is found that the extracts of living organisms act both as reducing and capping agents in the synthesizing process of the nanoparticles[5]. Green synthesis of nanoparticles was more preferred than the chemical as well as synthetic methods since it is used in the reduction of metal using hazardous chemical and moreover they are non toxic. Naturally, plant posses both primary and secondary metabolites to carry out the green synthesis. In the green synthesis the silver nanoparticles were widely used for many applications such as antidiabetic, antimicrobial, antioxidant, anticancer, and it is also used in industries like biomedical,magnetic, energy space and aerospace[6,7]. Biologically synthesized silver nanoparticles (Ag-NPs) have wide range of applications due to their remarkable physical and chemical properties and the method is simple, rapid and also cost effective[8, 9]. The literature on the extra cellular biosynthesis of Ag-NPs using plants and pure compounds from plants are highly insignificant[10] In the past decades green nanoparticle synthesis has evolved into an important branch of nanotechnology because of its potential application in the biomedical, magnetic, energy science and aerospace industries.

More than 90% of therapeutic classes were derived from natural prototypic product and 2/3 of the total population relive on herbals for basic pharmaceutical care. Natural herbs posses a number of medicinal properties and are excellent source of pharmaceutical and health care products. The majority of the population in the developing countries depends on natural resources, particularly medicinal plants species to treat variety of diseases. Increasing awareness towards green chemistry and other biological processes has led to a desire to develop an eco-friendly approach for the synthesis of nanoparticles[11]

Artemisia vulgaris [12] belongs to Asteraceae family which is commonly known as mugwort. Traditionally it is used as anti-septic, anti-pyretic, diuretic, analgesic, diaphoretic, anthelmintic, hypoglycemic, antispasmodic, expectorant and tonic. This species is also effective in treating colic, depression, asthma, rheumatism, cancer, dyspepsia, epilepsy, cough, diarrhoea, headache, hemorrhage and

inflammation. Additionally anti-nociceptive and hepato-protective effects have also been reported in this plant species. They are also used in gastric diseases, malaria, anti-fungal, anthelmintic, sedative agents. *Artemisia vulgaris*[13] is a tall aromatic perennial herb which grows in hilly district of India in areas upto 2400m elevation .

Cinnamomum zeylanicum Nees is a small, tropical, evergreen tree known for its bark, which is commonly known as spice, cinnamon. The common name of cinnamon is Ceylon cinnamon. Ceylon is an extinct term originated from Sri Lanka, the native country of *Cinnamomum zeylanicum* Nees [14,15]. C. *zeylanicum* bark is widely used as a spice. It is principally employed in cooking as flavoring agent and as well as condiment. In ancient medicine, it acts like other volatile oils and were used for the treatment of colds. It has also been used to treat diarrhoea and other problems involved in the digestive system. C. *zeylanicum* bark is also high in antioxidant activity [16,17]. The essential oil of cinnamon also has antimicrobial properties, which helps in the preservation of certain foods. Terpenoids are believed to play an important role in silver nanoparticle biosynthesis through the reduction of silver (Ag) ions [18].

Silver was used for many infectious diseases since olden times before the emergence of antibiotics as an antibacterial agent [19]. Similarly, silver nanoparticles are widely used for antibacterial, antifungal, anti-HIV activity [20] and in controlling plant pathogens [21]. In addition, antimicrobial nanoparticles offer various distinctive advantages in reducing acute toxicity, overcoming resistance, and lowering cost, when compared to conventional antibiotics [22]

There is an essentiality of new anti-microbial agents because more microorganisms becoming resistant to the available drugs in the market. New anti-microbial drugs have to be discovered with better pharmacokinetic and pharmacodynamic properties with minimal side effects [23]. Worldwide researchers are trying to synthesize new drugs especially from plant origin [24]. WHO has listed that 80 % of population still relays on plants for diseases [25].

The present study deals with the formulation and characterization of silver nanoparticles using aqueous leaves extract of *Artemisia vulgaris* and *Cinnamomum zeylanicum* Nees and to compare their antifungal activity. Human pathogenic fungi (*Candida albicans, Aspergillus flavus ,Fusarium oxysporum*) were used in the study[26].

2. MATERIALS AND METHODS

2.1. Preparation of leaf extract of *Artemesia vulgaris* Linn.

The collected fresh leaves of *Artemisia vulgaris* Linn were washed thoroughly with deionized water for 3 times. The leaves were grinded using an electric motor.

The solution was prepared by taking 10 g of grinded leaves with 100 ml of distilled water in a 250 ml of Erlenmeyer flask and the mixture is boiled at 60 ^{o}C for 5 minutes. Cool the extract at room temperature and the clear solution were decanted [27].

2.2. Preparation of *Cinnamomum zeylanicum* Nees Plants Barks Extract:

The extract was prepared by adding 2.5 g of *Cinnamomum zeylanicum* Nees plants barks powder into 100 ml of distilled water and boiled for 5 minutes in 500 ml flask. After cooling it was filtered using whatman No.1 filter paper. The final extract was kept at 4°C [28, 29].

2.3. Preparation of Silver nanoparticles

2.3.1 Preparation of Silver nanoparticles of Artemesia vulgaris Linn:

The 12 ml of this extract solution was added to 88 ml of 1 mM aqueous $AgNO_3$ solution. Then the solution was heated at 60^{o} C for 5 minutes. The resulting solution which was brown in color and the extract was filtered through Millipore hydrophilic filter (0.22µm) and used for further experiments [1].

2.3.2 Preparation of Silver nanoparticles of Cinnamon zeylanicum Nees.

1ml of Cinnamon zeylanicum plants barks extract was added to 50 ml of 1mM aqueous silver nitrate ($AgNO_3$) solution and kept at room temperature for 8 hrs to produce silver nanoparticles. The solution initially appeared yellowish in color and upon reduction of nitrate from silver Ag+ to free reduced form change to dark color [30].

2.4 Characterization of Silver nanoparticles

2.4.1 UV-VIS spectroscopy

UV-VIS spectroscopic studies were carried out on a Shimadzu UV-VIS double beam spectrophotometer over a wavelength range of 200-400 nm to study the presence of nanoparticles.

2.4.2 Zeta Sizer and Zeta Potential

The average particle size and zeta potential of the prepared AgNPs was evaluated with the help of Malvern Zeta-sizer nano ZS90 at 25.1^{o}C after suitable dilution with distilled water.

2.4.3 SEM Studies

Scanning electron microscopy analysis was used to study the surface morphology and size of the nanoparticles.

2.4.4 Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) of the test substances against *Candida albicans, Aspergillus flavus and Fusarium oxysporum* was determined by liquid broth method using two fold serial dilution technique [31] . In this assay, the minimum concentration of each test substance required to inhibit the growth of microorganism was determined. For this assay, a series of assay tubes were prepared containing uniform volume (1ml) of sterile Sabarose Dextrose broth and equal volume of known concentration of test substance was added. The test substance in the first tube was serially diluted in twofold decreasing concentrations through the sixth tube and seventh tube was left without test substance as positive control. The tubes with the test substance i.e. from one to seventh were inoculated with 1 ml of inoculum ($1x10^6$ CFU per ml). The final concentration of test substance ranged from 1000 to 125 µg per ml. Solvent control and sterility controls were maintained in the experiment. The tubes were incubated at 28^{0}C for 48hrs. Standard

ciprofloxacin was tested as standard drug at concentrations ranging from 1000 to 125µg per ml. The tubes were inspected visually to determine the growth of the organism as indicated by turbidity (In fact, turbidity of the culture medium is indicative of the presence of a large number of cells), the tubes in which the antibiotic is present in concentration sufficient to inhibit fungal growth remain clear. In experimental terms the MIC is the concentration of the drug present in the last clear tube, i.e. in the tube having the lowest concentration in which growth is not observed.

2.4.5 Zone of inhibition using well diffusion method

Potato Dextrose Agar (PDA) was prepared for cultivation fungi respectively. Approximately 20 ml of molten and cooled media was poured in sterilized Petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. Then under an aseptic condition, placed a sterile swab into the broth culture of a fresh overnight grown cultures of the fungi then gently removed the excess liquid by gently pressing or rotating the swab against the inside of the tube and spread it on potato dextrose agar containing petriplates respectively. With a help of sterile borer, 5mm wells punched in the solid agar medium and then different concentration of the solution (25µl, 50 µl, 75 µl, 100 µl) containing nanoparticles, 1 mM silver nitrate was inoculated in these wells and the plates were incubated at 28^0C for 2 or 3 days for fungi respectively. Further, the plates were examined for evidence of zone of inhibition, which appear as a clear area around the wells surrounding fungi growth. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

3. RESULT AND DISCUSSION

Initially the prepared nanoparticles were confirmed by observing the solution colour changing from pale yellowish green to brown colour. The colour change in the reaction mixture indicated the formation of silver nanoparticles (AgNPs). During the visual observation *Artemisia vulgaris* L. *and Cinnamon zeylanicum* is mixed with silver nitrate showed colour changed from pale yellowish green to brown colour due to the reduction of silver ion; which is indicated the formation of silver nanoparticles. This colour arises due to excitation of surface plasmon vibration in AgNPs. They are the surface-active molecules that play an awfully necessary role in reducing and stabilizing process of silver nanoparticles. The changing of color solution was measured at every 1hrs. The prepared nanoparticles were illustrated in fig: 1 and fig: 2.

3.1. Characterization of silver nanoparticles (AgNPs)

3.1.1. *UV visible spectra*

The preliminary characterization of AgNPs was analysed by UV-VIS spectroscopy. The absorbance was studied over the range of 200 to 400nm.

UV spectra of A*rtemisia vulgaris* L. (Fig: 3) showed a maximum peak at 264 nm and *Cinnamon zeylanicum* Nees., (Fig: 4) at 300 nm. A single maximum peak was observed in both Ag nanoparticles which indicated the presence of uniform distribution of nanoparticles.

3.1.2. *Zeta sizer*

The synthesized silver nanoparticles using A*rtemisia vulgaris* L. and *Cinnamon zeylanicum* were characterized with Zeta sizer to measure the average particle size which was shown in the table 1. From the fig: 5, it was observed that the AgNPs synthesized using A*rtemisia vulgaris* L. showed the average particle size 190.8 nm. Whereas from the fig: 6, AgNPs synthesized using Cinnamon zeylanicum showed the average particle size 104.5 nm.

3.1.3. *Zeta potential*

The zeta potential of the prepared nanoparticles was carried out and compared (table 2) to study the surface charge and stability. From the fig: 7 it was observed the zeta potential of the synthesized AgNPs using A*rtemisia vulgaris* L. was found to be -18.5Mv which indicated that the prepared AgNPs were moderately stable in nature. From fig: 8 it was observed the zeta potential of the synthesized AgNPs using *Cinnamon zeylanicum* was found to be -16.3Mv which also indicated that the prepared nanoparticles were moderately stable in nature. However the AgNPs using *Cinnamon zeylanicum* Nees,. was more stable than A*rtemisia vulgaris* L,. Further the stability of nanoparticles will be included by using stabilizing agent.

Zeta potential is the physical property of the particles in colloidal solution. The zeta potential of A*rtemisia vulgaris* L. was found to be -18.5 mV. The Zeta potential of *Cinnamon zeylanicum* AgNPs was found to be -16.3mV. Magnitude of Zeta potential gives an indication of potential stability of the prepared nanoparticles.

Cinnamon zeylanicum AgNPs have better zeta potential than A*rtemisia vulgaris* L AgNPs. Thus *Cinnamon zeylanicum* was found to be more stable than A*rtemisia vulgaris* L.

3.1.4. *Scanning Electron Microscopy (SEM) analysis*

The morphology of prepared silver nanoparticles was observed by scanning electron microscopy (fig: 9 & 10). Both the extracts were investigated for the surface morphology.

3.1.5. *Minimum inhibitory concentration*

The minimum inhibitory concentration of the prepared silver nanoparticles of A*rtemisia vulgaris* L. (Fig: 11.1, 11.2 & 11.3) and *Cinnamon zeylanicum Nees* (Fig: 12.1, 12.2 & 12.3) were illustrated in table 3.

Based on the results cinnamon was found to show maximum inhibition at lowest concentration of 125 µg/ml compared to Artemisia which is of concentration 250 µg/ml when tested against *Candida albicans.* Similar is the case in *Aspergillus flavus and Fusarium oxysporum* organisms too as *Cinnamon zeylanicum Nees* showed a promising antifungal activity against *Artemesia vulgaris* Linn.

3.1.6. *Zone of inhibition*

Well diffusion method was employed for determining the zone of inhibition of the prepared silver nanoparticles. The prepared nanoparticles were inoculated in the agar plates filled with microorganisms and kept for 3 days. The result was remarkable with *Cinnamon zeylanicum* Nees silver

nanoparticles (fig: 13.1, 13.2 & 13.3) showing a larger zone of inhibition than *Artemesia vulgaris* Linn silver nanoparticles (fig: 14.1, 14.2 & 14.3). The results were tabulated in table 4.

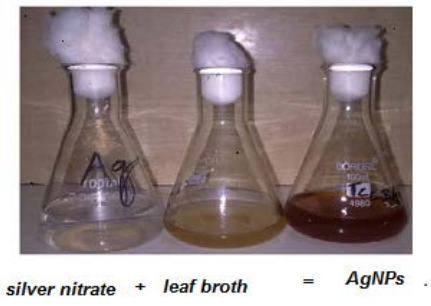

Fig: 1: preparation of silver nanoparticles of *Artemisia vulgaris* L.

Fig: 2: preparation of silver nanoparticles of *Cinnamon zeylanicum* Nees.,

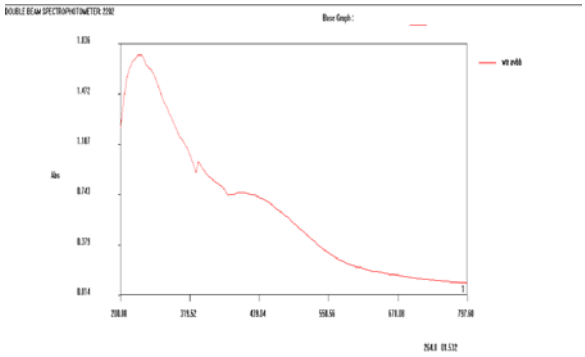

Fig 3:- UV spectra of A*rtemisia vulgaris* L

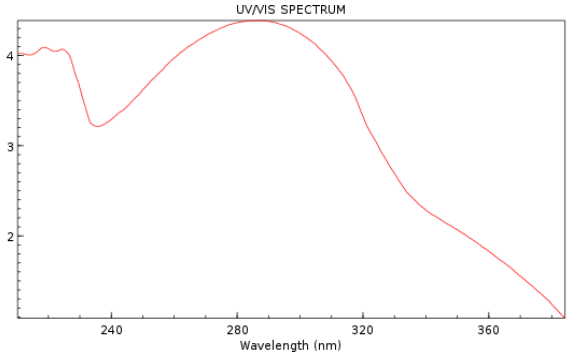

Fig 4:- UV spectra of *Cinnamon zeylanicum* Nees

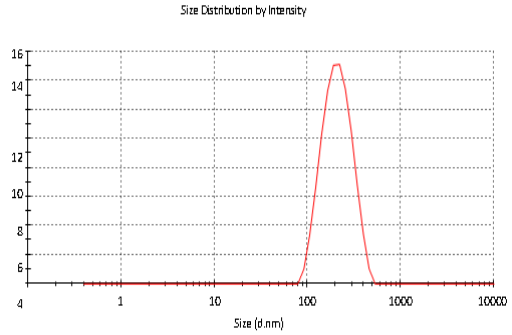

Fig 5:- Size distribution of A*rtemisia vulgaris* L.

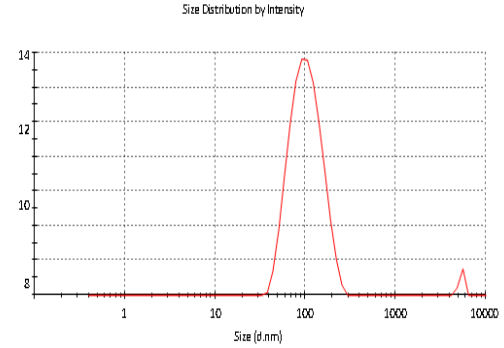

Fig 6: Size distribution of *Cinnamon zeylanicum*

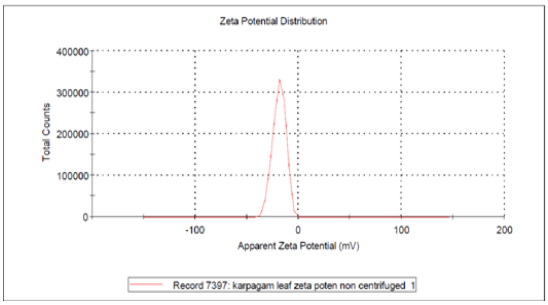

Fig 7:- Zeta potential of A*rtemisia vulgaris* L.

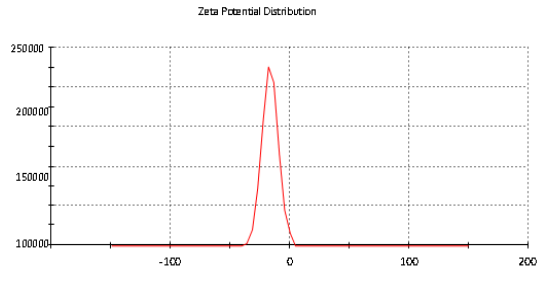

Fig 8:- Zeta potential of *Cinnamon zeylanicum* Nees

Table 1: Particle size distribution of formulated Silver Nanoparticles

FORMULATED SILVER NANOPARTICLES	PARTICLE SIZE DIZTRIBUTION
A*rtemisia vulgaris* L. AgNPs	190.8 nm
Cinnamon zeylanicum AgNPs	104.5 nm

Table 2: Zeta Potential of formulated Silver Nanoparticles

FORMULATED SILVER NANOPARTICLES	ZETA POTENTIAL
A*rtemisia vulgaris* L. AgNPs	-18.5 mV
Cinnamon zeylanicum AgNPs	-16.3mV

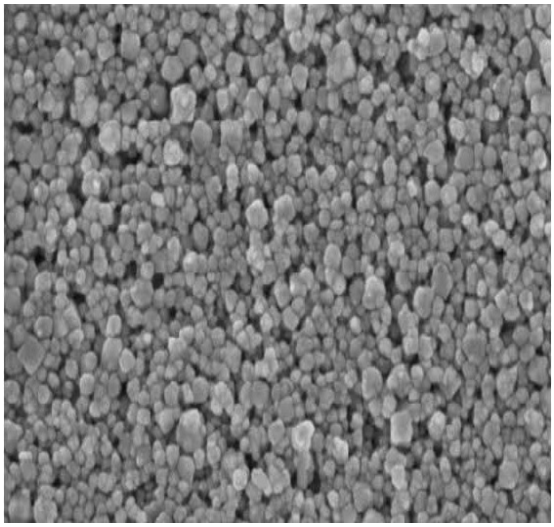

Fig 9:- SEM of A*rtemisia vulgaris* L. AgNPs

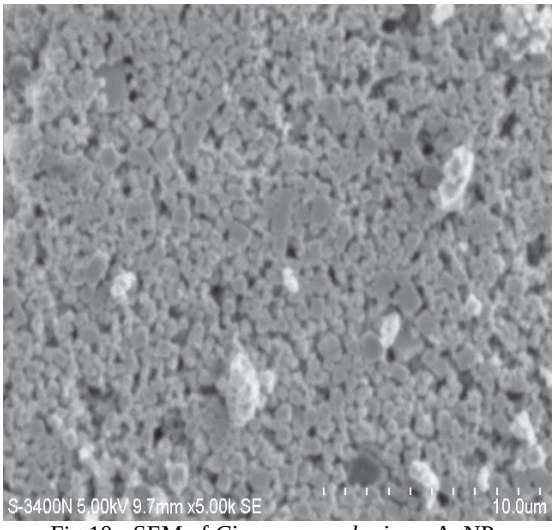

Fig 10:- SEM of *Cinnamon zeylanicum* AgNPs

Table 3: Comparison of Minimum Inhibitory concentration of the formulated Silver nanoparticles

S.No.	Micro-organisms	MIC of Standard Ketoconazole µg/ml	Minimal inhibitory concentration µg/ml
	***Cinnamon zeylanicum* Nees**		
1	Candida albicans	<3.25	125
2	Aspergillus flavus	3.9	250
3	*Fusarium oxysporum*	12.5	500
	***A.vulgaris* Linn**		
1	Candida albicans	<6.25	250
2	Aspergillus flavus	12.5	500
3	*Fusarium oxysporum*	25	500

Table 4: Zone of Inhibition of formulated Silver Nanoparticles

Sl . no	Microorganisms	Positive control (Ketocona-zole)	*Cinnamon zeylanicum* Nees silver nanoparticles				Positive control (Ketocon-azole)	*Artemesia vulgaris* Linn silver nanoparticles			
			100 µl	75 µl	50 µl	25 µl		100µl	75 µl	50 µl	25 µl
1	*Candida albicans*	25	21	18	16	14	26	18	16	14	13
2	*Aspergillus flavus*	20	16	12	10	09	22	14	12	11	07
3	*Fusarium oxysporum*	21	15	14	11	08	20	12	10	09	08

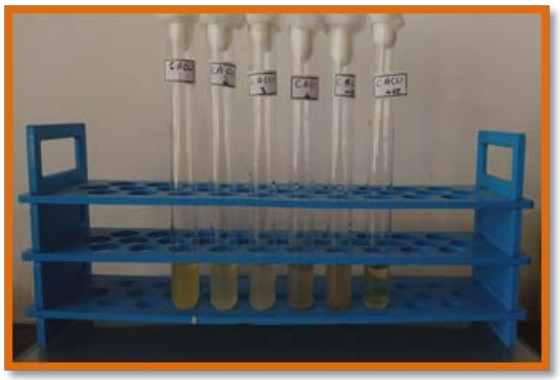

Fig 11.1:- MIC of *Cinnamon zeylanicum* Nees silver nanoparticles against *Candida albicans* at different concentrations

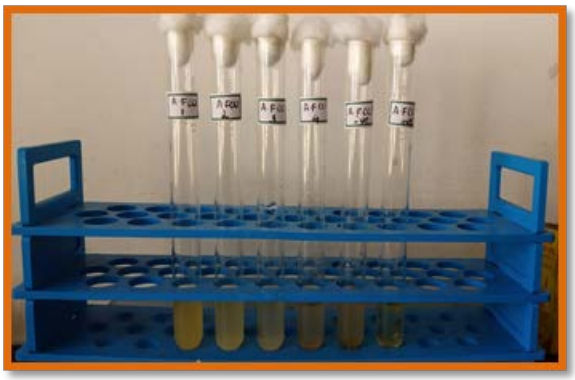

Fig 11.2:- MIC of *Cinnamon zeylanicum* Nees silver nanoparticles against *Aspergillus flavus* at different concentrations

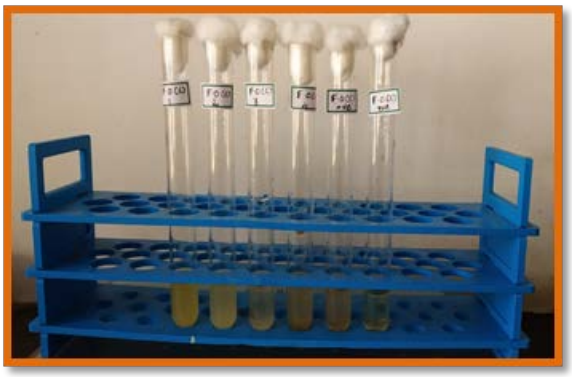

Fig 11.3:- MIC of *Cinnamon zeylanicum* Nees silver nanoparticles against *Fusarium oxysporum* at different concentrations

Fig 12.1:- MIC of *Artemesia vulgaris* Linn silver nanoparticles against *Candida albicans* at different concentrations

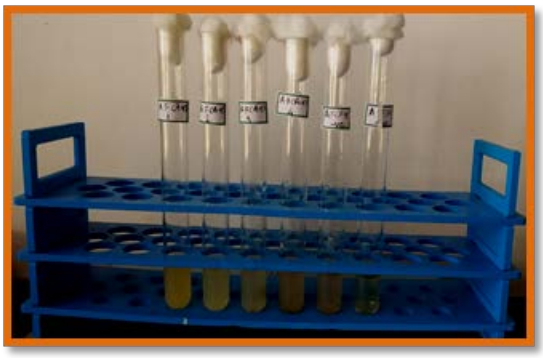

Fig 12.2:- MIC of *Artemesia vulgaris* Linn silver nanoparticles against *Aspergillus flavus* at different concentrations

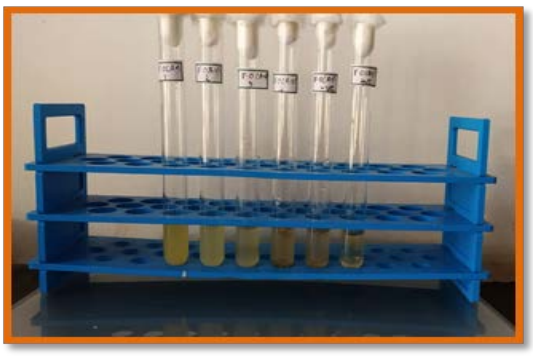

Fig 12.3:- MIC of *Artemesia vulgaris* Linn silver nanoparticles against *Fusarium oxysporum* at different concentrations

Fig 13.1:- Zone of inhibition of *Cinnamon zeylanicum* Nees silver nanoparticles against *Candida albicans* at different concentrations

Fig 13.2:- Zone of inhibition of *Cinnamon zeylanicum* Nees silver nanoparticles against *Aspergillus flavus* at different concentrations

Fig 13.3:- Zone of inhibition of *Cinnamon zeylanicum* Nees silver nanoparticles against *Fusarium oxysporum* at different concentrations

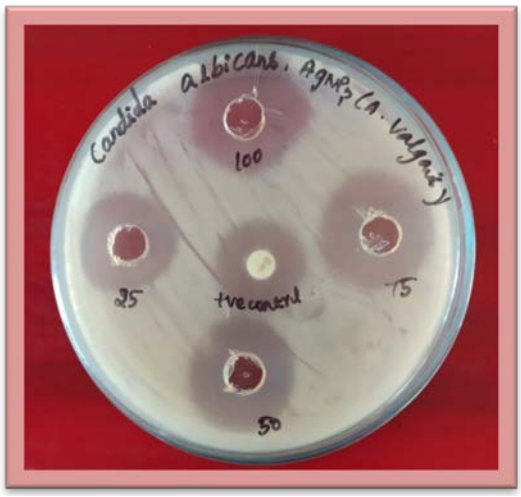

Fig 14.1:- Zone of inhibition of *Artemesia vulgaris* Linn silver nanoparticles against *Candida albicans* at different concentrations

Fig 14.2:- Zone of inhibition of *Artemesia vulgaris* Linn silver nanoparticles against *Aspergillus flavus* at different concentrations

Fig 14.3:- Zone of inhibition of *Artemesia vulgaris* Linn silver nanoparticles against *Fusarium oxysporum* at different concentrations

4. CONCLUSION:

Silver nanoparticles possess unique properties with a wide range of applications such as antimicrobial, anticancer, larvicidal, catalytic, and wound healing activities. Green synthesis of silver nanoparticles using plants with its pharmacological and other potential applications are gaining momentum owing to its assured benefits. Silver nanoparticles have received great attention due to their physical, chemical, and biological properties owing to its catalytic activity as well as bactericidal effects. It even found its applications in nanobiotechnological research. They are used as antimicrobial agents in wound dressings, as topical creams to prevent wound infections, and as anticancer agents.

The rapid biological synthesis of silver nanoparticles using aqueous leaves extract of *Artemisia vulgaris* L and *Cinnamomum zeylanicum* Nees bark provide eco-friendly, simple and efficient route for synthesis of nanoparticles. Hence, silver nanoparticles provide a promising goal for the treatment of anti microbial activity and have attracted wide attention of researchers.

In this study, silver nanoparticles using aqueous leaves broth of *Artemisia vulgaris* L and *Cinnamomum zeylanicum* Nees bark as reducing agent were prepared using silver nitrate solution as precursor. The nanoparticle formation was identified by the change of colour from pale yellowish green to brown. The prepared nanoparticles were then analysed by UV-VIS spectroscopy. The nanoparticles showed its peak within the range of 200-400nm indicating small particle size. *Artemisia vulgaris* L silver nanoparticles showed its maximum peak at 264nm and *Cinnamomum zeylanicum* Nees bark showed at 300nm. Further characterization was done by Zeta sizer, Zeta potential and SEM analysis. *Cinnamomum zeylanicum* Nees silver nanoparticles had smaller particle size of 104.5nm and zeta potential of -16.5Mv than *Artemisia vulgaris* L with particle size of 190.8nm and zeta potential of -18.7mV. SEM analysis proved that both the nanoparticles have a perfect morphology which is almost round in shape. Anti fungal activity was carried out using three human pathogenic fungi such as *Candida albicans, Aspergillus flavus and Fusarium oxysporum.* The results were remarkable with better zone of inhibition and minimum inhibitory concentration for *Cinnamomum zeylanicum* Nees rather than *Artemisia vulgaris* L. Hence the study proved an excellent antifungal activity of *Cinnamomum zeylanicum* Nees rather than *Artemisia vulgaris* L.

Conflict of Interest

No conflict of interest

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