Biosynthesis and Antimicrobial activities of Silver Nanoparticles (AgNPs) by using Leaf Extracts of Tagetes erecta (Marigold) and Tridax procumbens (Tridax)

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ABSTRACT
In the last few years, it has been seen that the importance of silver nanoparticles has gained much interest by many chemists and biologists. Therefore, Indian medicinal plants has yet to bring numerous sources of profitable, healthy, mostly reduced and stabilized compounds utilized in the biosynthesis of silver nanoparticle. The main aim of this study is to investigate the viable and sustainable ways for the biosynthesis of AgNPs from 1 mM aqueous AgNO3 using leaf extracts of two widely available plants such as Tagetes erecta (Marigold) and Tridax procumbens (Tridax), which are well known for their wide availability and medicinal property. AgNPs were synthesized by the reaction of 1 mM AgNO3 and 5% leaf extracts of each plant separately. The formations of the AgNPs were confirmed by the colour changes of the mixture solution and duly characterized by UV-Visible spectrophotometric analysis. Further, their antibacterial and antifungal activities were tested against two bacterial strains and one fungal strain. Finally, the AgNPs showing better antimicrobial activity was tested for their water disinfection study against three water samples collected from River, Pond and Cannel. Obtained AgNPs from the two different leaf extracts indicated higher antimicrobial activities against Escherichia coli and Bacillus spp. in comparison to both AgNO3 and the raw plant extracts of Marigold and Tridax. The final results showed that both Marigold and Tridax extract silver nanoparticles are showing significant antimicrobial activities, whereas Marigold has little more antimicrobial activity than Tridax. So the bacterial inhibition zone by the silver nanoparticles prepared from the marigold and Tridax leaves extract show maximum inhibition for Gram +ve S. aureus and K. Pneumoniae and Gram negative E. coli as well as A. niger. These synthesised AgNPs when applied for water disinfection, they became dead to reduce more than 50% of the bacterial growth present in the water samples. At last they synthesized silver nanoparticles were discharged safely in the environment which can be utilized in the processes of pollution remediation.

Keywords- Silver Nanoparticles, Phytochemicals, Plant Exatraction, Antimicrobial Activity

I. INTRODUCTION

Many experimental works has given the data that, the Indian greenerys are the chief and the most cost-effective resources for the production and development of new medicines as well as therapeutics. From many years to till date these medicinal plants are playing a major role in Ayurveda, which is a major source of Indian medication therapy. Currently, nano biotechnology is being considered as the most unique discipline of research in medicinal field whereby plant parts are being utilized in the synthesis of bio-nanoparticles. Generally, in terms of diameter, particles having size between 1nm to 100 nm are considered as nanoparticles. In spite of the bulk structures, these nanoparticles have unique physical and chemical properties due to an increase in the surface area per volume of the particle (Henry et al., 2019). Generally, nanoparticles derived from metals like gold, silver and platinum are all well perceived to have significant applications like detection, catalytic property, surface coating agents as well as antibacterial/antimicrobials properties (Henry et al., 2019). Out of all the bio-nanoparticles developed till now, silver nanoparticles have gained significantly much interest in reducing the antimicrobial and antibacterial activity due to the ease of availability and cost-effectiveness. These are broadly applied in the preparation of shampoos, soaps, detergents, cosmetics, toothpastes and pharmaceutical products (Bhattacharya and Murkerjee 2008; Bhumkar et al. 2007).

“Earlier the silver nanoparticles obtained by the chemical and bio-chemical processes have shown significant anti-fungal as well as antibacterial properties in the field of medical science. But the plant mediated silver nanoparticle is relatively a unique and advanced concept. Nano-biotechnology and the products developed from nano-biotechnology are much exceptional in their treatment philosophy, because of their uniqueness in particle size, physical, biochemical properties and immense scope of utilization. This current emerging field of nano-biotechnology is probably at the primary stage of development due to lack of implementation of cutting-
edge techniques in large industrial scales. Therefore, there is a need to develop and design an economically and commercially suitable as well as environmentally sustainable route for the synthesis of silver nanoparticles in order to meet the growing demand in various sectors” (Banerjee, P. et al., 2014)). Different approaches have been developed for the bio-synthesis of silver nanoparticles like chemical (Sun et al., 2002), electrochemical (Yin et al., 2003), radiation (Dimitrijevic et al., 2001), photochemical methods (Callegari et al., 2003), Langmuir-Blodgett (Zhang, 2006; Swami et al., 2004) and biological techniques (Naik et al., 2002), Langmuir-Blodgett (Swami et al., 2008), biological methods of NPs synthesis using microorganisms (Klaus et al., 1999). In this chase of AgNPs preparation, plant-intervened green synthesis of silver nanoparticle is the most preferred and broadly accepted method for the rapid production of silver nanoparticles, which will also help in fulfilling the top needs and current market requests bringing about a decrease in the employment of this generation of unsafe substances to human wellbeing and nature.

Recent researches have proved that Alfalfa roots can assimilate silver from agar medium and can move it to the plant shoot in a similar condition of oxidation (Gardea-Torresdey et al., 2003), which helps in the development of the plant morphologically, physiologically as well as in the cellular and molecular levels. AgNPs have been already developed by using Aloe vera (Chandan et al., 2006), Acalypha indica (Rishnaraj et al., 2010), Garcinia mangostana (Veerasamy et al., 2010) leaf extracts. Also it has been tested that the Crataegus douglasii fruit extract (Ghaffari-Moghaddam et al., 2014) as well as various other plant extracts (Ghafafari-Moghaddam et al., 2014) act as reducing agents. This investigation was framed out for an eco-friendly and easiest green technique for the synthesis of AgNPs from AgNO3 utilizing leaf extracts of three known Indian therapeutic plants, to be specific, Musa balbisiana (Banana), A. indica (neem) and O. Tenuiflorum (dark tulsi), by microwave irradiation method.

In this analysis, we strongly characterized the plant based AgNPs and the prevailing technologies that can prolong the activity of AgNPs in the recent antimicrobial health issues. The antibacterial/ antimicrobial impacts of the AgNPs prepared from these three Indian therapeutic plant leaf extracts have been assessed by disk diffusion method. A comparable investigation was additionally performed among these three naturally synthesized AgNPs on two Indian pulse plants of the family Fabaceae, to be specific Moong Bean and Chickpea to anatomize the exact effect of these AgNPs mostly on the morphological, physiological as well as cellular and molecular level changes. Then seeds were treated with four different concentrations of AgNPs suspensions to study the germination parameters as well as the oxidative stress in the respective seeds.

Identifying these natural resources for the synthesis of silver nanoparticles are more valuable than the contemporary physical and chemical approaches, as these plant resources are widely available and cost-effective. Results thus obtained from the analysis of antimicrobial property and toxicity of these AgNPs confirms that these are safe and secure to be discharged in the environment and hence fit to be applied for environmental remediation.

II. REVIEW OF LITERATURE

2.1 Principal applications of AgNPs:

Silver nano-particles are firmly known as the best resources to be used in health industry, storage of food, coating of materials and in various other ecological applications. Due to the high antimicrobial and anti-bacterial properties, these are mostly used in the production of new medicines and healthy products. Although these AgNPs produced by the means of physical and chemical processes has been utilised by various industries but the importance of biologically synthesized AgNPs will bring a remarkable change in health as well as food industries. In spite of the decades of its use, the real level of toxicity of silver is not yet clear. Many international accredited organizations such as; US FDA, US EPA, Korea’s testing, SIIA of japan, FITI testing and research institute have been approved the products getting prepared by silver nanoparticles (Veeraputhiran et al., 2003).One of the important components like Silver sulfadiazine creams, which is an antibiotic and is being used to prevent the wound infections.

Silver as a nanoparticle is being used in the production of baby pacifiers, acne creams, and computer’s keyboard, clothing etc due to its antimicrobial properties. The study of reduction of methylene blue by arsine showed that the silver nano-particles have prominent catalytic properties (Kundu et al., 2002). Again the catalytic property of silver nanoparticles was studied from the reduction of phenoasafarine dye (Mallick et al., 2006). The investigation of the use of silver nano particles as antimicrobial agents was studied by developing E. Coli in the agar plate and in the fluid lower broth medium (Sondi et al., 2004). “These nanoparticles were applied to investigate the membrane transport of microbial cells (P. aeruginosa) in real times” (Nancy et al., 2004).

AgNPs fabricated nano-sphere lithography performs more sensitive nano-scale affinity biosensors. These nano-biosensors have all the properties and features of SPR spectroscopy, generally considered as the basic principle behind many colour based biosensor. The changes in the shape and size of the nanoparticles causes the optimization in the refractive index sensitivity. The AgNPs synthesized through green technique have various biomedical applications in controlling pathological organisms. In an investigation, the nano particles
prepared from aqueous *Piper longum* fruit extract as well as the AgNPs prepared through this green technique showed more antioxidant activity in vitro antioxidant assays (Haes et al., 2002).

To investigate the toxic-ness of silver nanoparticles, the human lung fibroblast cells (IMR90) and human glioblastoma cells (U251) were taken and the whole investigation was thoroughly studied. AgNPs have also proven their high antiviral activity against HIV-1 in non-cytotoxic concentrations, but the mechanism of their HIV- inhibitory activity has not been fully simplified. The different modes of their antiviral activity against HIV-1 have been studied via various in vitro assays (Lara et al., 2010). In this study, special interest has been taken on the molecular diagnostics like SNP detection, gene expression and biomarker characterization. Many strategies have been developed to establish nanoscale devices and platform that can be utilized to characterize single molecules of DNA, RNA and proteins at a highly increased rate than the traditional techniques (Goyal et al., 2009).

2.2 Mechanism of Antimicrobial property of AgNPs:

From the beginning of the human civilizations, metals have been used in different sectors for different purposes. People generally use these silvers as ornaments, jewellery and fine cutlery. It was believed that silver as jewellery has innumerable health benefits for the users, such as; it has a long history of its effect as an antimicrobial agent to reduce the microbial contaminations. Silver is considered as a strong antimicrobial agent, which has been proved to be effective against thousands of microorganisms from different species and classes such as: gram +ve and gram –ve bacteria, fungi or viruses etc. Recently the metal has shown many useful properties in producing silver nanoparticles, which can be effective in modern Indian medical system. Earlier, in the ancient Indian medical system (Ayurveda), the effectiveness of silver as a therapeutic agent has been well mentioned.

“In 1884, doctors were advised to use a few drops of aq. Silver nitrate on the new-borns eyes for the prevention of *Neisseria gonorrhoea* from infected mothers. Out of all the metals with antimicrobial properties, silver was found to be mostly effective against the antibacterial actions and was less harmful to the human beings. In a study, it was seen that the silver became a useful medical treatments for the wounded soldiers in the World War I to prevent the microbial growth” (Ankanna et al., 2010). Scientific researches make use of silvers in the form of silver nitrate to reduce the antimicrobial effects, but when the silver nano particles are used, the huge expansion of the surface area allows the microbes to be exposed. AgNPs synthesized from the different plant extracts have been used for analysing the antimicrobial effects against different microbes.

2.3 *Tagetes erecta* (Marigold)

*Tagetes erecta* commonly known as Mexican marigold is an ornamental plant belongs to the family Asteraceae and is being used as garlands for many religious purposes and festivals in many countries including India. It is widely distributed in Mexico, south East Asia including India and Bangladesh. The flowers of these plants are bright- yellow or brownish-yellow or orange in colour. Different parts of these plants are being used as folk medicine as per the traditional beliefs. People suffering from skin infection, wounds and burns, conjunctivitis and poor eyesight, menstrual irregularities, varicose veins, duodenal ulcers, haemorrhoids etc. generally use the extracts of these plants for a primary cure (Wichtl, 1994; Krishnamurthy et al., 2012). These plants also help in treating the bleeding piles and helps in purification of blood (Kirtikar and Basu, 1994; Manjunath, 1969; Ghani, 2003). Essential oils produced from *T. erecta* acts as repellents and proved to be most effective against mosquitoes (Singer, 1987; Wells et al., 1992). In this present work, an attempt has been made to synthesize silver nano particles using aqueous flower extract of *T. erecta*. The characterization was done by using different spectral analysis and the synthesized silver nanoparticles were evaluated for their synergistic antimicrobial activity.

2.4 *Tridax procumbens*

*Tridax procumbens*, commonly known as coat-buttons, is a type of flowering plant belongs to the family Asteraceae of plant kingdom, which is also known for its mythological beliefs as folk medicines, generally used to treat wound healing and sometimes act as anti-coagulants as well as insect repellent. *Bisalya Karani* (*Tridax Procumbens*) has come into lime light from the legendary mythological saga *Ramayana*, when a royal Srilankan physician Sushena prescribed this plant extract for wound healing of arrow to treat laxman. These plants are mostly widespread in the tropical Americas; however it is acquainted with tropical, sub-tropical areas all around the world.

The flowers of these plants contain yellow-centres white with three toothed beam florets, while the leaves are toothed and arrow- head shaped. The fruits of these plants are covered with stiff hairs and having a feathery plume like white pappus towards one side. The Calyx is modified into pappus. The achene like fruits of these plants can catch the wind and can be carried some distance. These plants are broadly available at fields, meadows, croplands, gardens, road- sides etc with tropical or semi- tropical atmosphere. The Federal Noxious weed act has been implemented to list this plant as a noxious weed in United States of America. In the history of Ayurveda, these plant parts are used to treat liver disorders, hepatop- protection, gastritis and heartburn (Wani et al., 2010).

In India people use this plant for the treatment of boils, blisters, and cuts by local healers (Nallella et al., 2013). The effective chemical components like *flavonoid procumbenetin, pentacyclic triterpenes* (Gamboa, 2014; Petchi et al., 2013), fatty acids (Ali et al., 2001) and...
polysaccharides (Pathak et al., 1991) have been separated from the elevated parts of *Tridax procumbens*.

### III. MATERIALS AND METHODS

#### 3.1 Chemicals and Reagents

AgNO₃ was purchased from the Merck India Pvt. Ltd. Nutrient media, Muller Hinton media, antibiotic discs used in the present study were purchased from the Hi-media Laboratory Pvt. Ltd. All the medicinal plants used in this study were collected from the local areas of Bhubaneswar and Cuttack, Odisha. Lyophilised bacterial and fungal cultures were procured from the American type culture collection (ATCC) through Hi-media laboratory Pvt. Ltd., Mumbai, India.

#### 3.2 Preparation of 1mM AgNO₃

0.0849 g of AgNO₃ was added with 400 ml of double-distilled water to prepare 1mM of AgNO₃ solution, and after the complete dissolve of the AgNO₃ in the solution, the volume was maintained to 500 ml. Then the prepared AgNO₃ solution was kept in the freezer for the further use in the experiment.

#### 3.3 Experimental methods:

In this experimental method, the aqueous plant extracts were mixed with an aqueous solution with appropriate metal salt. Then the synthesis of nanoparticles occurred at room temperature involving with the below pathways. (schematic diagram is shown in figure 1).

![Schematic representation of synthesis of AgNO₃ synthesis](image)

Figure 1: Schematic representation of synthesis of AgNO₃ synthesis

#### 3.4 Formation of leaf extracts:

Two commonly available Indian medicinal plants such as *Tagetes erecta* (Marigold) (Fig.2), *Tridax procumbens* (Tridax daisy) (Fig.3) and (Details description was given in Table 1), were collected from the local area of Bhubaneswar and Cuttack, Odisha, India, on the basis of their ancient medicinal history as well as their ease of availability. Firstly, fresh, young and healthy green leaves were collected and washed carefully with tap water followed by distilled water to wipe out all the dust and unwanted particles and then cut into small pieces and allowed to dry at room temperature. Then the dried leaves were finely chopped and 20 g of each plant were weighed separately and transferred into the 250 mL Erlenmeyer flask containing 100 mL distilled water and allowed to heat at 60 °C for about 1 hour (Singhal et al., 2011). Then the extracts were filtered through Whatmann’s No 1 filter paper, which resulted in the elimination of the particulate matters as well as in the collection of clear solutions. Now the clear solutions with proper covering were kept in refrigeration at 4°C for further experiments. The processes of sterilization were maintained throughout the whole experiment to get results of high accuracy level and negligible errors without any contamination.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Plant common names</th>
<th>Odia name</th>
<th>Biological name</th>
<th>Family</th>
<th>Sub family</th>
<th>Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Marigold</td>
<td>Gendu</td>
<td>Tagetes erecta</td>
<td>Asteraceae</td>
<td>Asteroideae</td>
<td>TE</td>
</tr>
<tr>
<td>2</td>
<td>Coatbuttons/ tridax daisy</td>
<td>Bisalya karani</td>
<td>Tridax procumbens</td>
<td>Asteraceae</td>
<td>Heliantheae</td>
<td>TP</td>
</tr>
</tbody>
</table>
3.5 Synthesis of AgNPs:
To synthesize the silver nanoparticles, 45 mL of the 1 mM silver nitrate (AgNO₃) solution was added to 5 mL of each leaf extracts separately in 250 mL Erlenmeyer flasks. The synthesis of AgNPs was carried out separately with each type of plant extract by continuous stirring by a magnetic stirrer in darkness at room temperature. The changes in colour of the solution to brownish were monitored periodically. “The details of time and colour change were recorded along with periodic sampling and scanning by UV-visible spectrophotometry” (Banarjee P. et al., 2014). Then the colour of the solution changed into reddish brown, which confirmed the reduction of AgNO₃ to Ag⁺ ions. The whole process of silver nanoparticle synthesis mediated by plant extract is shown in figure 4. The formation of AgNPs was again confirmed by spectrophotometric scanning in the range of 300 to 800 nm wavelength using a UV-visible spectrophotometer. Then AgNPs colloidal mixture was properly sealed and stored at 4°C for future use and the sterilization was maintained (Medda S. et al., 2014). Synthesis of AgNPs was also carried using the mixture of all two leaf extracts together at equal ratio for evaluate the combined antimicrobial effect of the prepared leaf extract-AgNPs.

3.6 Synthesis of AgNPs with varying AgNO₃ and leaf extract concentration
Synthesis of AgNPs was done with different concentrations of silver nitrate and leaf extract such as 1:9 (i.e. 5 ml leaf extract added to 45 ml AgNO₃), 1:4 (i.e. 10 ml leaf extract added to 40 ml AgNO₃ and 3:7 (i.e. 15 ml leaf extract added to 35 ml AgNO₃).
3.7 UV-visible spectrophotometer analysis:

1 mL of final synthesised AgNPs samples were diluted 10 times with deionized water and then allowed to scan subsequently in UV-visible spectrophotometer in the ranges between 300 to 800 nm wavelengths using a spectrophotometer (Carry, VARIAN).

3.8 Evaluation of antimicrobial activity:

A comparative investigation was performed by taking consideration into the antimicrobial and antifungal activities of the raw leaf extracts and the synthesized AgNPs from the respective extracts against five bacteria such as; Bacillus subtilis (Gram +ve), Escherichia coli (Gram -ve) (Shehzad A et al., 2018), Klebsiella pneumonia (Gm -ve), Salmonella typhimurium (Gm -ve), Staphylococcus aureus (Gm +ve) and one fungus named Asperigillus niger procured from American type culture collection (ATCC). Agar Well-diffusion method was applied for testing each type of plant leaf extract and their respective AgNPs containing solution (Premasudha P. et al., 2015). For this experiment, Petri dishes containing nutrient agar media were prepared by swabbing (using sterile cotton swab) them with the microbial cultures (both bacteria and fungi) and the wells were created on the agar plate using a corer. Then 50 µL of each synthesised AgNPs samples were put into the well on plate separately. The plates were kept in incubation at 37°C for 24 to 48 hour. Then, the maximum zone of inhibition were analysed to measure the antimicrobial property of AgNPs against each kind of taken bacteria and fungi (Sanchooli N. et al., 2018). Again, the antimicrobial action of the above synthesised two AgNPs was also compared with two commercially available antibiotics such as ampicillin (10 mcg, AMP10) and penicillin (10 mcg, P10).

Table 2: Description of bacterial and fungal strains used for antimicrobial test

<table>
<thead>
<tr>
<th>Name of the Microorganism</th>
<th>Sources</th>
<th>Gram staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>ATCC11774</td>
<td>Positive</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>ATCC25922</td>
<td>Negative</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>ATCC BAA-1705</td>
<td>Negative</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>ATCC-14028</td>
<td>Negative</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>ATCC-29213</td>
<td>Positive</td>
</tr>
<tr>
<td>Asperigillus niger</td>
<td>ATCC-6275</td>
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</table>

3.9 Disinfection of contaminated water using synthesised AgNPs

Evaluation of water disinfectant property of AgNPs was carried using all synthesised leaf extract mediated AgNPs against three water samples collected locally from river, pond and canal. This study was carried out by both well-diffusion method and broth-dilution method. In case of well diffusion method, 0.5 mL of each water samples were spread on the nutrient agar plate and all the synthesized AgNPs were tested separately by putting 50 µL of each AgNPs solution to the well and the inhibition zones were measured after 24 hours of incubation at 37°C. Whereas, in the case of broth dilution method, the 0.1 mL of each water samples were added to the test tube containing 10 mL of NB and then 1mL of the the AgNPs mixture solution (Prepared by mixing all two AgNPs at equal ratio) was added to the NB tube. A set of control tubes are also prepared in which no AgNPs were added. All the tubes are incubated at 37°C for 24-48 hr and then the O.D. was taken at 600 nm. The effect of AgNPs regarding the killing of water microbes was evaluated by comparing the O.D. with the control tubes.

IV. RESULTS AND REVIEW

4.1 Silver nanoparticles biosynthesis:

During the process of AgNPs synthesis by the bio-reduction of different leaves extracts, the initial and final colour changes were noted and shown in figure 5 to 6 after the addition of AgNO₃. The colour of the silver nanoparticles gradually changed from watery yellow to deep brownish due to the excitation of silver plasmon resonance and SPR band. As the leaf extracts were added to the aqueous silver nitrate solution, the colour of the solution gradually changed from light yellowish to brownish to reddish brown and lastly to colloidal brown indicating the formation of silver nanoparticles. In this investigation, the final colour of the AgNPs from marigold, tridax were found to be reddish brown. The exact similar changes in colouring have also been observed in early studies and thus confirmed the reaction response between leaf extract and AgNO₃ (Shukla et al., 2010).
4.2 Uv-Vis spectrophotometric characterization of AgNPs

The UV-visible spectrophotometer recorded the reading of final changes in colour of the plant mediated extracts shown in figure 15 to 21. The maximum absorption of AgNPs spectra was noted down in the range of 445 to 455 nm due to surface Plasmon resonance of AgNPs (Sinha, S.N., Paul, D., Halder, N. et al., 2015). As per the readings taken from the spectral analysis of AgNPs, it was observed that the rapid bio-reduction of Ag⁺ happened by using neem leaf extract as reducing agent followed by jatropha and tulsi (Banerjee P et al., 2014). In case of other leave extracts, the formation of AgNPs indicated comparatively slower than the neem, jatropha and tulsi. The above statements was denoted by the broadening of the peak, which specified the formation of poly-dispersed large nanoparticles due to slow reduction rates (Singhal, 2011; Philip et al., 2011). The spectral analysis of UV-visible spectrophotometer confirmed the rapid production of AgNPs.
4.3 Analytical study of antibacterial activity:

With many advanced medicinal properties, the AgNPs thus prepared also indicated some high level specific antibacterial activities that were shown in Table 3 to 9. The obtained inhibition zone size (in mm) indicates maximum antibacterial activity was found higher for prepared AgNPs than the raw leaf extracts. As compared to all the AgNPs, it was found that Jatropha, Marigold, Gaint milk weed are showing more antimicrobial activities than Neem, Tridax daisy, black Tulsi (Table 3). From the results obtained in the earlier studies, we came to a confirmation of the antibacterial potential of AgNPs prepared from these two above plant extracts (Namratha et al., 2013; Rout et al., 2012). The zone of bacterial inhibition by AgNPs were prepared from the two leaves extracts which indicated maximum inhibition for Gram positive S. aureus and K. Pneumoniae and Gram negative E. coli as well as A. niger.

From the antimicrobial results of AgNPs prepared in different ratios of leaf extracts and AgNO₃ solution, it was found that ratio 2:8 and 3:7 of leaf extract and silver nitrate solution was showing slightly higher antimicrobial activities than the 1:9 ratios (table-4 to 8). In case of the antimicrobial properties of the mixture of all leaf extract AgNPs, all the ratio of AgNPs composition are showing well but ratio 1:9 ratio has little more antimicrobial effect (Table-9).

| Table 3: Antimicrobial properties of two different leaf extracts (raw/original) and with AgNPs |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| B. subtilis     | S. typhium      | S. aureus       | K. pneumoniae   | E. coli         | A. niger        |
| Diameter of inhibition zone presented in mm |
| TE-O            | 0               | 3.0             | 2.0             | 1.0             | 0               | 1.0             |
| TE-AgNPs        | 1.0             | 2.0             | 4.0             | 3.0             | 9.0             | 3.0             |
| TP-O            | 0               | 0               | 0               | 0               | 0               | 0               |
| TP-AgNP         | 0               | 4.0             | 4.0             | 0               | 5.0             | 0               |

| Table 4: Antibacterial activity of two AgNPs against Bacillus subtilis |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Silver nanoparticles | Diameter of inhibition zone presented in mm |
| Ratio of leaf extract: silver nitrate solution |
| 1:9             | 2:8             | 3:7             |
| TE-AgNPs        | 1.0             | 6.0             | 7.0             |
| TP-AgNPs        | 0               | 7.0             | 7.0             |

| Table 5: Antibacterial activity of two AgNPs against S. typhimurium |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Silver nanoparticles | Diameter of inhibition zone presented in mm |
| Ratio of leaf extract: silver nitrate solution |
| 1:9             | 2:8             | 3:7             |
| TE-AgNPs        | 2.0             | 3.0             | 4.0             |
Table 6: Antibacterial actions of two AgNPs prepared against *Staphylococcus aureus*

<table>
<thead>
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<th>Silver nanoparticles</th>
<th>Diameter of inhibition zone presented in mm</th>
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<tr>
<td></td>
<td>Ratio of leaf extract: silver nitrate solution</td>
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<td>1:9</td>
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<tr>
<td>TE- AgNPs</td>
<td>4.0</td>
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<td>TP- AgNPs</td>
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Table 7: Antibacterial activity of two AgNPs against *K. pneumoniae*

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<th>Diameter of inhibition zone presented in mm</th>
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<td>Ratio of leaf extract: silver nitrate solution</td>
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<td></td>
<td>1:9</td>
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<tr>
<td>TE- AgNPs</td>
<td>3.0</td>
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<tr>
<td>TP- AgNPs</td>
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Table 8: Antibacterial activity of two AgNPs against *E. coli*

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<th>Diameter of inhibition zone presented in mm</th>
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<td>Ratio of leaf extract: silver nitrate solution</td>
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<td>1:9</td>
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<tr>
<td>TE- AgNPs</td>
<td>9.0</td>
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<tr>
<td>TP- AgNPs</td>
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Table 9: Antifungal activity of two AgNPs against *A. niger*

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<thead>
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<th>Silver nanoparticles</th>
<th>Diameter of inhibition zone presented in mm</th>
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<td></td>
<td>Ratio of leaf extract: silver nitrate solution</td>
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<td></td>
<td>1:9</td>
</tr>
<tr>
<td>TE- AgNPs</td>
<td>3.0</td>
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<tr>
<td>TP- AgNPs</td>
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Figure 9: Comparison of antimicrobial activity between synthesised two AgNPs and commercial antibiotics (Ampicillin and Penicillin)
Figure 10: Evaluation of antimicrobial activities of synthesised two AgNPs from two leave extracts through well diffusion method.
4.3 Evaluation of water disinfectant property of AgNPs:

The killing power of the mixed AgNPs prepared from all two leaf extracts against water microbes present in three different water samples (river, pond, and cannel) are shown in Table 10 and Fig. 23. It was shown that while adding the AgNPs mixture to the water samples, the growth of the total bacterial population was decreased more than 50% as compared to the control (without addition of AgNPs) (Figure-23).

<table>
<thead>
<tr>
<th>Silver nanoparticles</th>
<th>Canal water</th>
<th>Pond water</th>
<th>River water</th>
</tr>
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<tbody>
<tr>
<td>TE- AgNPs</td>
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<td>0</td>
<td>2.0</td>
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<tr>
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<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 10: Water disinfection test results of all two AgNPs

V. DISCUSSIONS

The synthesized AgNPs using leaf extracts of two widely available medicinal plants such as *Tagetes erecta* (Marigold) and *Tridax procumbens* (Tridax) have shown well antimicrobial properties against five bacteria and one fungal strain. Silver nanoparticles formation was confirmed by the colour changes of the two mixture solutions as well as the absorption peak obtained from 445-455 nm during UV-Vis spectrophotometric analysis. *Tagetes erecta* (Marigold) is showing more antimicrobial activities than *Tridax procumbens* (Tridax). The maximum antimicrobial properties of prepared AgNPs from two leaves extracts was shown against Gram positive *Staphylococcus aureus*, Gram negative *Klebsiella pneumonia* and Gram negative *Escherichia coli* as well as *Aspergillus niger*. The synthesized AgNPs when applied for water disinfection; they can able to reduce more than 50% of the bacterial growth present in the water samples.

VI. CONCLUSIONS

Silver nanoparticles (AgNPs) were successfully obtained from bio-reduction of AgNO₃ solutions of *Tagetes erecta* and *Tridax procumbens*. Basing upon the different properties of these two plant species, AgNPs obtained from these also varied in size and antimicrobial properties. From the present investigation, it was conclude that the AgNPs prepared from these two
important medicinal plant leaves can successfully be applied for killing bacteria and fungus in the field of health science and water treatment processes.

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