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Biosynthesis of Silver Nanoparticles using Plants

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Abstract – In the present work attempt has been made to synthesise the silver nanoparticles using neem (*Azardirachta indica*), mango (*Mangifera indica*), lemon (*Citrus limon*) and combination of all the three leaves. It is an easy, eco-friendly and cost effective method for the synthesis of silver nanoparticles (AgNPs) and to evaluate its antimicrobial activity. Bio synthesised silver nanoparticles were characterized by using UV – Visible spectrophotometer and scanning electron micrograph (SEM) and it shows the maximum absorbance peak at 420 nm in UV – Visible spectrophotometer. Antimicrobial activity of silver nanoparticles were also studied against *Escherichia coli* (*E. coli*, gram negative) and *Streptococcus aureus* (*S. aureus*, gram positive), and it showed the good antimicrobial activity towards both the bacteria.

Key Words: Biosynthesis, Silver Nanoparticles, Antimicrobial Activity.

1 INTRODUCTION

Nowadays, nanotechnology is regarded as a distinct field of research in modern science and technology with multidirectional applications (1). Useful application of nanotechnology in medicinal purposes is currently one of the most fascinating areas of research, metallic nanoparticles have also been receiving considerable interest in biomedical applications (1). Silver nanoparticles in particular, are finding applications to the researchers as tools for antibacterial and antifungal, anti – inflammatory, wound healing, radio – imaging, retinal neovascularization, antiviral and anti – oxidant agents, and also as novel cancer therapeutics, capitalizing on their unique properties to enhance potential therapeutic efficacy (1). Nanoparticles are known as particles with a size smaller than 100 nm (3). Many methods are adopted for nanoparticles synthesis like physical, chemical, and biological methods (3). Biological method of synthesis of silver nanoparticles was achieved by using bacteria, fungi, algae and plants (3). The use of plants as the production assembly of silver nanoparticles has drawn attention, because of its rapid, eco – friendly, non – pathogenic, economical protocol and providing reduction and stabilization of silver ions by combination of bio molecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannis phenolic, saponins, terpinoids and vitamins which are already established in the plant extract having medicinal values and are environmental benign, yet chemically complex structure (4) and these helps in the reduction of silver ions to silver nanoparticles and act as capping and stabilizing agent (3).

2 MATERIALS AND METHODOLOGY

2.1 Synthesis of Silver Nanoparticles by Using Plants

Different leaves of plants (neem, mango and lemon) was used for the synthesis of silver nanoparticles. Fresh leaves were collected from JSS University. The leaves were cleaned with running water followed by distilled water to remove the debris and other contaminants from the leaves. About 100 gm of leaves were weighed and grinded well, then the grinded leaves were filtered by using what man's filter paper which is of grade 1 and the obtained filtrate was boiled for about 10 – 15 minutes to get the plant extract. The plant extract was filtered again to remove the solid part in the extract. About 1 ml of plant extract was taken and it was added to 50 ml of 1mM silver nitrate solution to get or to synthesise the silver nanoparticles. The change in colour from light yellow to dark brown or reddish brown indicates the synthesis of silver nanoparticles (reduction of Ag^+ ion to Ag^0).

2.2 Preparation of Adsorbent by Combination of Leaves

Combination of three leaves were used for the synthesis of silver nanoparticles. Fresh leaves of neem, mango and lemon were collected from the JSS University. All the three leaves were washed thoroughly with the distilled water to remove the debris and other organic contaminants. About 100 gm of leaves were weighed with the help of weighing balance and the weighed leaves were grinded with the help of domestic grinder and the grinded leaves were filtered by using by using whatsman filter paper of grade one. The filtrate was boiled for about 10 – 15 minutes to get the plant extract. The obtained extract was filtered again so has to remove the solid part from the extract. About 1 ml of combination

of leaves extract was taken and it was added to 50 ml of 1 mM silver nitrate solution to get the silver nanoparticles. The change of colour from yellow to red indicates the synthesis of silver nanoparticles.

2.3 Preparation of Silver Nitrate Solution ($AgNO_3$)

Silver nitrate which bears the chemical formula of $AgNO_3$ and molecular weight 169.87 g / mol. It was procured from Merck specialities private limited, and it was used for the synthesis of silver nanoparticles by various leaves of plants (neem, mango, lemon, and combination of all the three leaves).

2.4 Nutrient Agar

Nutrient agar is a nutrient medium which is used for the cultivation of micro – organisms. Nutrient agar can grow a variety of bacteria and fungi because it contains many nutrients which is needed for the growth of micro – organisms. It was procured from Himedia Laboratories, Mumbi. It is used in the antimicrobial activity of synthesised silver nanoparticle against micro – organisms.

2.5 Bacterial Strain

Bacterial strain was procured from ISCTS, Bangalore, India. It is used in the antimicrobial assay of silver nanoparticles.

To study the optimum factors for silver nanoparticles synthesis, the experiment were carried out at different conditions are silver ion concentration (1, 2, 3, 4 and 5 mM), pH (4, 5, 6, and 7), and temperature of (20, 25, 30, 35, and 40 degree celsius). The pH of the reaction mixture was adjusted by using 0.1 N sodium hydroxide and 0.1 N Hydrochloric acid. The effect of these parameters on the synthesis of silver nanoparticles was monitored by UV – Visible spectrophotometer.

2.6 Characterization of silver nanoparticles

The reduction of silver ions to silver nanoparticles was spectrometerically identified by UV – Visible spectrophotometer, at different wavelengths (400 – 700 nm). The size and shape of the synthesised silver nanoparticles was analysed by scanning electron micrograph.

3 RESULTS AND DISCUSSION

3.1 Visual Observation

Formation of silver nanoparticles was preliminarily well known by changing of colour from yellow to reddish brown colour while adding leaf extract with silver ion solution due to excitation in the nanoparticles (3). The colour formation was occurred within few minutes after the addition of leaves extract. Metal nanoparticles exhibits different colours in solution due to their optical properties and silver nanoparticles were characterized by forming of brown colour (3) and it is shown in Figure- 1.

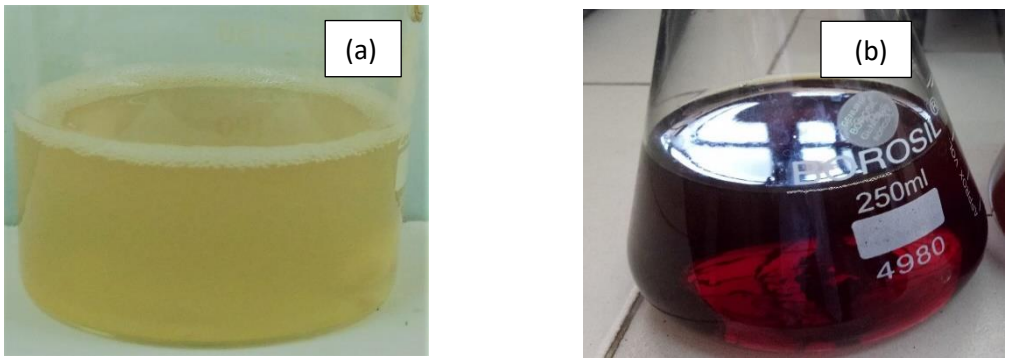


Figure- 1: Change of colour from yellowish (before the synthesis of AgNPs(a)) to reddish brown (after the synthesis of AgNPs (b))

3.2 Effect of Silver Ion Concentration

The UV – Visible spectrum (Figure - 2) shows the effect of silver nitrate concentration on the synthesis of silver nanoparticles which was synthesised by using neem, mango, lemon and combination of all the three leaves. 1mM concentration shows narrow band with increased absorbance. The adsorption was increased while increasing the concentration of silver nitrate from 1mM to 5 Mm. In 1mM concentration the nanoparticles synthesis and reduction in size was started quickly due to the more availability of functional groups in the leaf extract (3). Thus, the optimization study showed the significant effect of silver nitrate concentration on the synthesis of silver nanoparticles and this research concludes that, 1Mm of silver nitrate concentration is optimum for the synthesis of silver nanoparticles in all the cases (neem, mango, lemon and combination of all the three leaves). Similarly, increasing in the intensity indicated the increasing in the nanoparticles concentration, higher concentration of silver nitrate leads to the formation of larger sized nanoparticles (3).

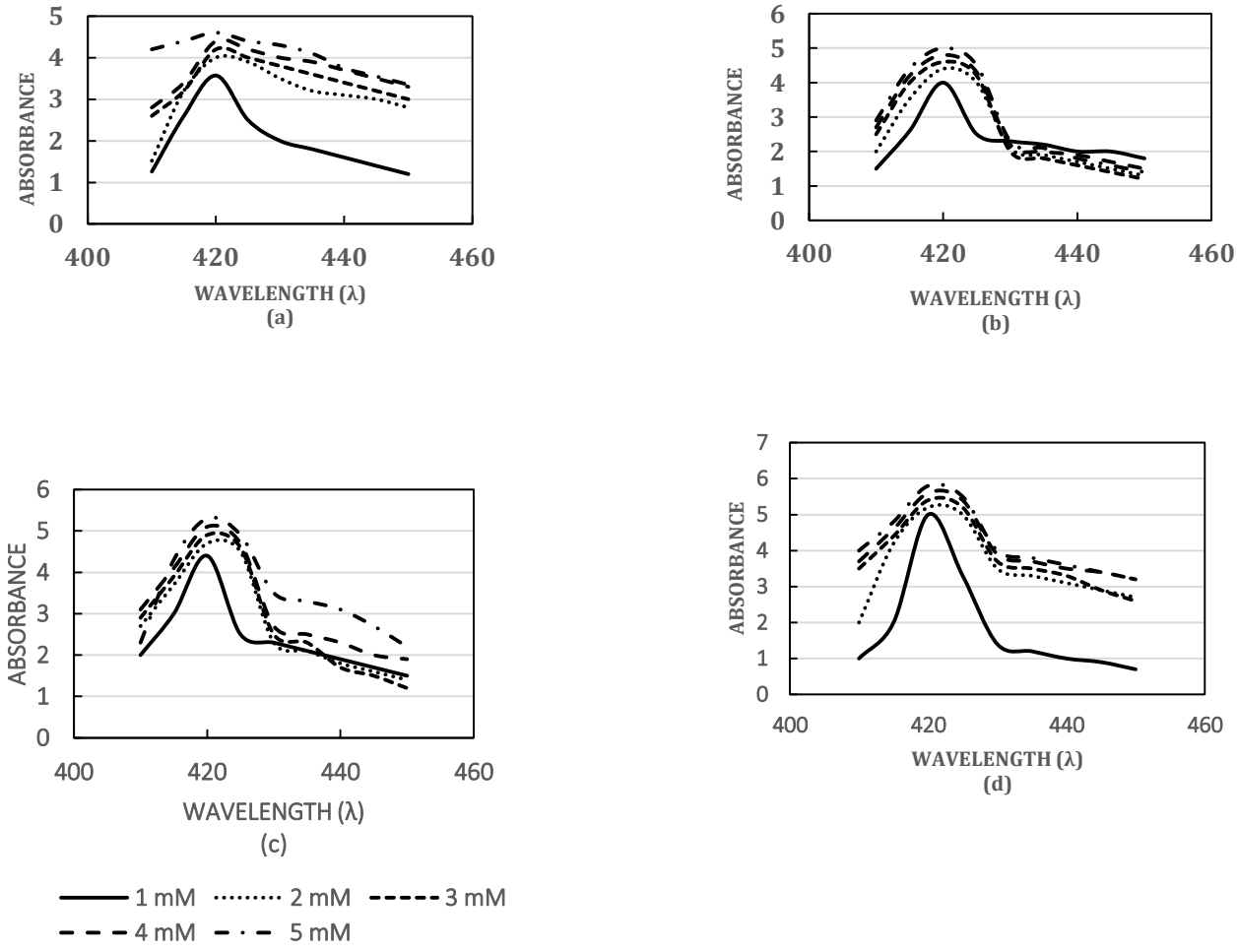


Figure- 2: UV – Visible spectra showing absorbance with different concentration of AgNO3 for neem (a), mango (b), lemon (c) and combination of all the three leaves (d).

3.3 Effect of Plant Extract

The effect of biomass concentration on the synthesis of silver nanoparticles was studied by exposing 1ml to 5 ml of plant extract in 1mM of silver nitrate solution and it was monitored by using UV – Visible spectrophotometer. 1ml of plant extract in all the cases (neem, mango, lemon and combination of all the three leaves) showed the narrow band with increased in absorbance. The adsorption was increased while increasing the biomass concentration from 1ml to 5 ml. When 1ml of plant extract (neem, mango, lemon and combination of all the three leaves) was added to 1 Mm of silver nitrate solution, the synthesised silver nanoparticles were well dispersed, but when the concentration of biomass

increased 2ml and up to 5 ml the so formed silver nanoparticles showed the agglomeration. Higher the concentration of biomass leads to the formation of larger sized nanoparticles (3). The results are shown in Figure - 3.

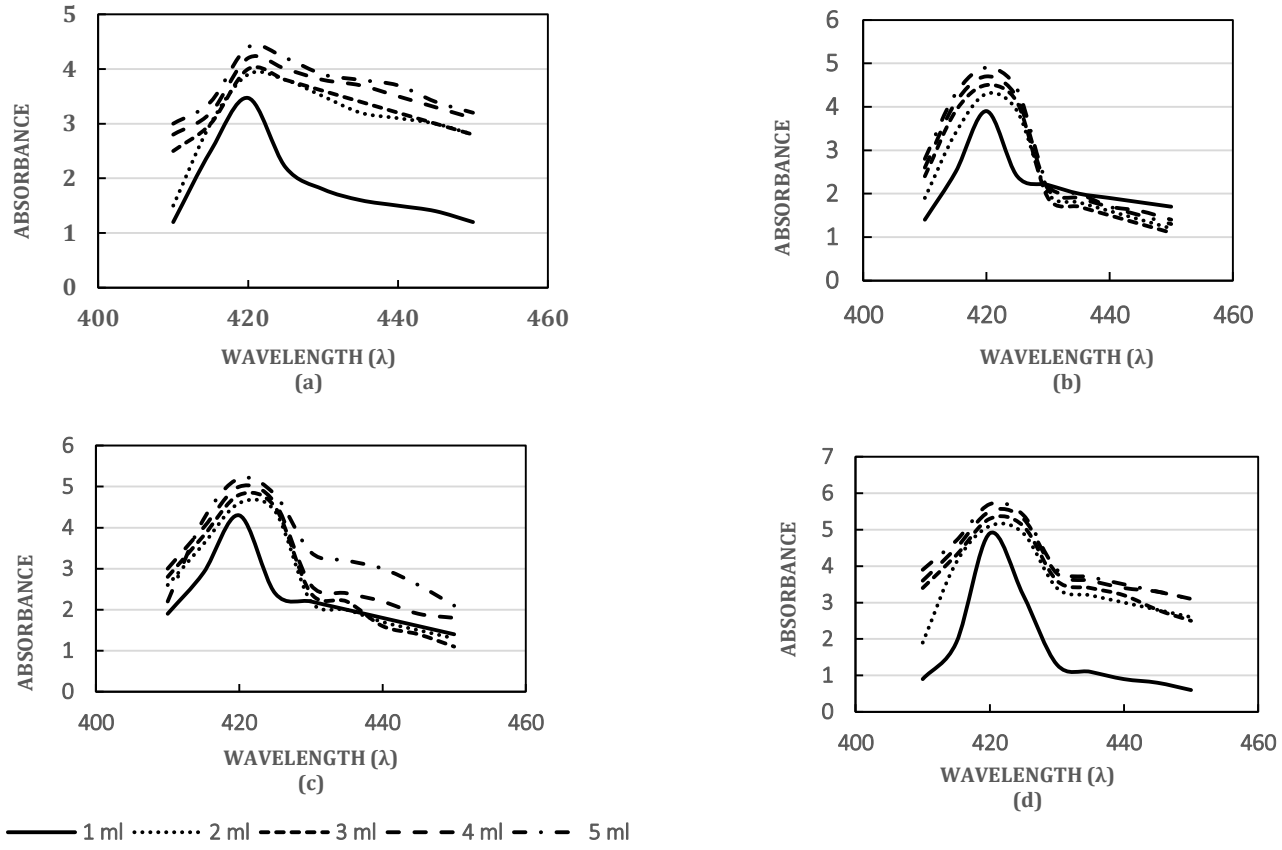


Figure- 3: UV – Visible spectra showing absorbance with different concentration of biomass for neem (a), mango (b), lemon (c) and combination of all the three leaves (d).

3.4 Effect of pH

pH play a very important role in the synthesis of silver nanoparticles (3). The effect of pH on the synthesis of silver nanoparticles using leaves extract (neem, mango, lemon and combination of all the three leaves) was investigated by keeping biomass concentration (1 ml) and silver nitrate concentration (1mM) constant, under different pH of the reaction mixture by the leaf extract and it is depicted from Figure - 4. The maximum synthesis of silver nanoparticles were obtained at the pH of 7 (in all the cases of plants) and the highest colour intensity was observed at this ph. At the lower pH the aggregation of nanoparticles is observed this may be due to the reduction in the activity of functional group of the biomass which reduces the stability of the capping agent (5). Thus, the optimization study shows that pH 7 is suitable for the synthesis of silver nanoparticles by using 1 ml of plant extract (neem, mano, lemon and combination of all the three leaves) and 1mM of silver nitrate concentration.

3.5 Effect of Temperature

Temperature play an important role in the synthesis of silver nanoparticles. Figure – 5 shows the effect of temperature on the synthesis of silver nanoparticles using plants (neem, mango, lemon and combination of all the three leaves) and it was carried out t different temperatures from 25 degree Celsius to 40 degree Celsius. The maximum production of silver nanoparticles was obtained at 25 degree Celsius. When the temperature was increased to 30 and up to 40 degree Celsius there no much increase in the production of silver nanoparticles this is because at the higher temperature the activity of functional group of plants reduces and which is nothing but the reduction in the activity of capping agent (5). Thus, the optimization study indicates that 25 degree Celsius is optimum for the synthesis of silver

nanoparticles using 1 ml of plants (neem, mango, lemon and combination of all the three plants) and 1mM of silver nitrate solution.

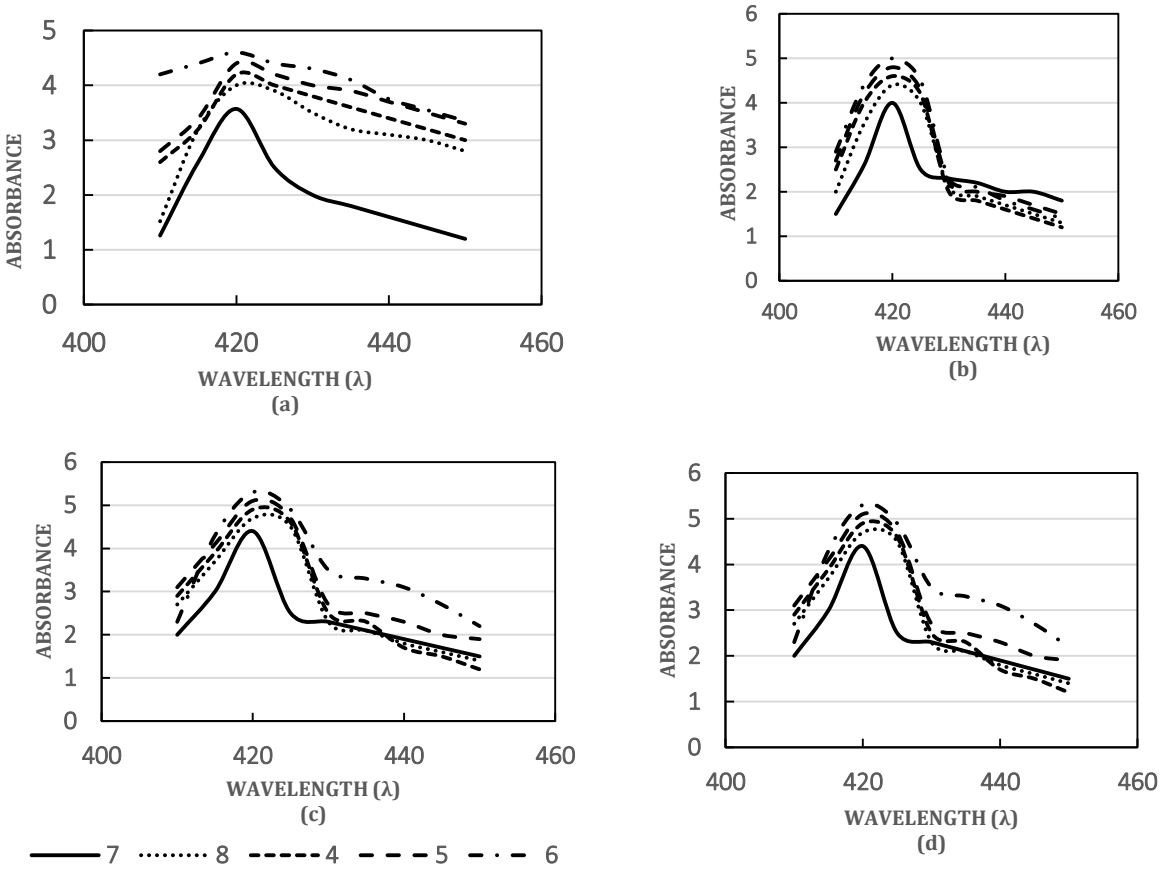
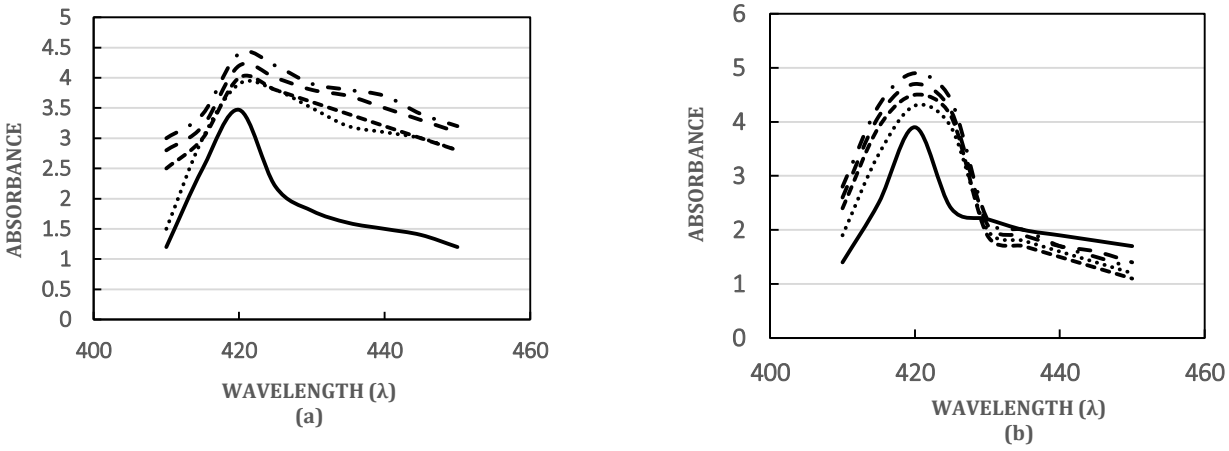


Figure- 4: UV – Visible spectra showing absorbance at different pH for neem (a), mango (b), lemon (c) and combination of all the three leaves (d).



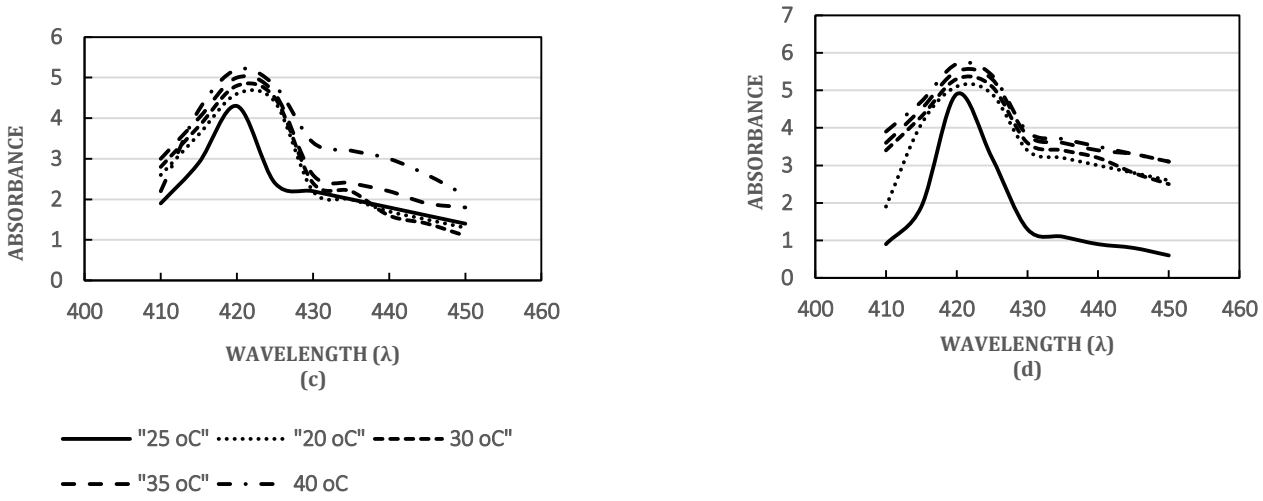


Figure- 5: UV – Visible spectra showing absorbance at different temperature for neem (a), mango (b), lemon (c) and combination of all the three leaves (d).

3.6 Characterization Studies for Silver Nanoparticles

The synthesis of silver nanoparticles using plants (neem, mango, lemon and combination of all the three leaves were analysed using UV – Visible spectrophotometer. Reaction solution was monitored at the regular interval of time and it showed the maximum absorbance peak at 420 nm and it is shown in Figure- 6, the silver nanoparticles were characterized using scanning electron micrograph (SEM). The results of SEM revels that the size of silver nanoparticles synthesised using neem, mango, lemon and combination of all the three leaves were 20, 100, 200, and 50 nm in size.

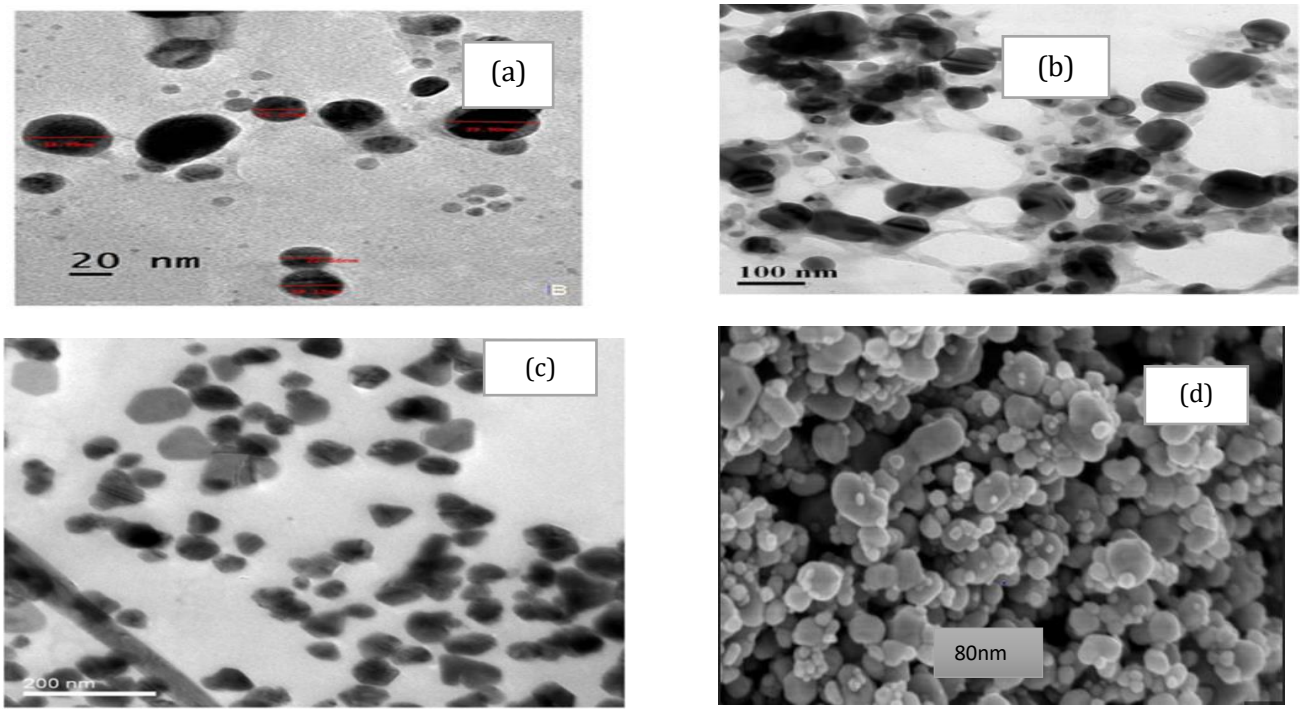


Figure- 6: SEM images of silver nanoparticles synthesised by neem (a), mango (b), lemon (c) and combination of all the leaves (d)

3.7 Antimicrobial Assay of Silver Nanoparticles Against E – Coli and S. aureus

Antimicrobial assay of biosynthesised silver nanoparticles was studied against E. coli and S. aureus bacteria using agar well diffusion method and zone of inhibition is depicted in figure- 7 and in Table- 1. Plant extract (neem, mango, lemon and combination of all the three leaves) and Silver nanoparticles was loaded into the wells with different concentrations of 20, 40, 60 and 80 micro litres respectively. The silver nanoparticles synthesised by using combination of neem, mango and lemon leaves showed the more antimicrobial activity towards both the bacteria than compare to the silver nanoparticles synthesised by using neem, mango and lemon leaves extract. On the other hand, plant extract alone did not show much antimicrobial activity this may be due to the medium extraction as well as lower concentration during experimentation (4). Thus, from the present research it is indicated that the silver nanoparticles is more effective towards both S. aeurus and E. coli bacteria because the silver nanoparticles not only interact with the surface membrane, but also penetrate inside the bacteria, and it also interact with the DNA of bacteria, preventing cell reproduction (6).

Table- 1: Antimicrobial activity of synthesised silver nanoparticles.

	COMPONENTS	ZONE OF INHIBITION (cm)							
		Gram positive (s. Aeurus)				Gram negative (E. coli)			
	Dosage (µl/100µl)	20	40	60	80	20	40	60	80
NEEM	Plant extract	NZ	NZ	NZ	0.9	NZ	NZ	NZ	NZ
	Silver nanoparticles	1	1.4	1.5	1.7	0.6	0.8	2.5	2.8
MANGO	Plant extract	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	Silver nanoparticles	1	1.1	1.2	1.4	1	1.2	1.3	1.4
LEMON	Plant extract	NZ	NZ	NZ	NZ	NZ	NZ	NZ	0.9
	Silver nanoparticles	0.6	0.6	0.8	1	0.5	0.7	0.9	1
COMBINATION ON ALL THE PLANTS	Plant extract	NZ	NZ	1.0	1.1	NZ	NZ	1.0	1.1
	Silver nanoparticles	1.2	1.4	1.8	2.0	2.4	2.7	2.8	3

4 CONCLUSION

Silver nanoparticles were successfully synthesised using neem, mango, lemon and combination of all the three leaves extract. The synthesis of silver nanoparticles using this method was achieved within 48 hours. The change of colour from yellowish to reddish brown is mainly due to the surface resonance during the reaction (6) which indicates the formation of colour and it was confirmed by UV – Visible spectrophotometer and SEM analysis. Antimicrobial study shows that silver nanoparticles has strong antimicrobial activity against E. coli and S. aeurus.

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