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Evaluation of antimicrobial potential of silver nanoparticles synthesized by curry *(Murraya koenigii)* against pathogenic bacteria

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Abstract
This study was aimed to synthesizing AgNP from Curry *(Murrayakoenigii)*, plant leaf and investigates its antibacterial activity against different bacteria. AgNP was synthesized by Herbal method. The characterization of nanoparticles was done by XRD, FTIR and Uv-spectroscopy method. Antibacterial activity was determined by disc diffusion, well diffusion method and MIC.
The size of biosynthesized silver nanoparticles was found to be within the range of 20-60 nm. To identify the compounds for the reaction of silver ions, the functional groups present in plant extract were investigated by FTIR. XRD analysis confirms the nanocrystalline phase of silver with FCC crystal structure. The formation of silver nanoparticles in dispersion was monitored through the analysis of absorbance spectra by UV-Visible spectrophotometer at different stage during the process of synthesis. The result showed that all three leaves play an importance role in the reduction and stabilization of silver to silver nanoparticles. This green synthesis method is alternative to chemical methods, since it is cheap, pollutant free and eco-friendly. Thus the obtained AgNPs, demonstrated remarkable antibacterial activity against three human pathogenic bacteris when used in combination with commercially available antibiotics. AgNPs have become an important approach for applications in nanobiotechnology in the development of antibiotic treatment of different bacterial infections.

Keywords: AgNPs nanoparticles, antibacterial activity *S. typhi, E. coli, and S. aureus*

Introduction
In ayurveda medicinal plants have played special role from century to till date. Medicinal plant and plant products are the main inexpensive source of Indian ayurveda. Different plants and their products are the most dapper regimentations of research and contemporary materials as nanoparticles in nanobiotechnology (Den, 2005). The nanoparticles are generally less than 100nm in size and it is also called Nps. Nanoparticles are glorious mineral materials of silver, platinum and gold. Gold, silver and platinum are well accredited as NPs and have significant application in optoelectronic, electronics, magnetic and information storage (Gratzal, 2001; Dai and Murray *et al.*, 2001; Bruening, 2002; Okuda *et al.*, 2005).
In Roman and Greece silver particles are used to fight infections due to its antimicrobial effect from the immemorial time. They are considerable substrates for surface enhancement probe single molecules in Raman scattering (SERS) (Shiraishi and Toshima, 1991; Tao *et al.*, 2003). Saha *et al.* (2017) observed many methods for the preparation of silver nano-particles which can be biological, physical and chemical. Synthesis of silver nano-particles by chemical methods was toxic and various hazardous chemicals were used for their synthesis. Green synthesis is preferred over conventional synthesis because it is cost-effective, ecofriendly, single-step method that can be easily scaled up for large scale synthesis and does not require high energy, temperature, pressure and toxic chemicals. Thus the use of eco-friendly methods, for the synthesis of silver Nps is known as ‘‘Green synthesis’’

Material and Methods
Bacterial genera
The following three bacterial genera were used for present investigation:-
- *Escherichia Coli.*
- *Staphylococcus aureus.*
- *Salmonellae typhi* etc.

Plants
The following plant was used for synthesis of nanoparticels:-
1. **Curry *(Murrayakoenigii)***

Synthesis of Silver nanoparticles
Syntheses of nanoparticls from plants were done by method described by (Chandran *et al.*, 2006) [4].

Antibacterial test

Antibacterial test was done by disc diffusion and well diffusion (Nastasiji *et al*., 2002) [17].
Antibacterial test was done by minimum inhibitory concentration (MIC) Vasundriyan *et al.*, 2002)

Media

The following media were used for present research work:-

- Nutrient agar for *E. coli.*
- Manittol salt agar for *S. aureas* and *S. typhi.*

Characterization of nanoparticles

Characterization of nanoparticles was done by XRD, FTIR and UV spectroscopy method of (Markova, 2010; Usman *et al.*, 2012 and Krithiga *et al.*, 2013) [14, 26, 9].

Results and Discussion

Characterizations of silver nanoparticles synthesis from Curry leaf (*Murraya koenigii*)

***A.* XRD X-ray diffraction**

Characterizations of silver nanoparticles by X-ray diffraction were done to confirm the crystalline nature of the Ag Nps synthesis from Curry leaf (*Murraya koenigii*). The silver nanoparticles were used as dry powers for XRD analysis. Diffracted intensitie was observed from 20º to 80º at 2θ angles. Compared the XRD spectrum with the standard spectrum for confirm that the formed Ag Nps were in the form of nanocrystals. Various diffraction lines were observed at 2θ angle 28.2, 38.40, 44.47, 46.2, 67. 62 (Fig1).
Calculations using Scherrer's equation showed that the average particle size was in the range of 6 to 8 nm.

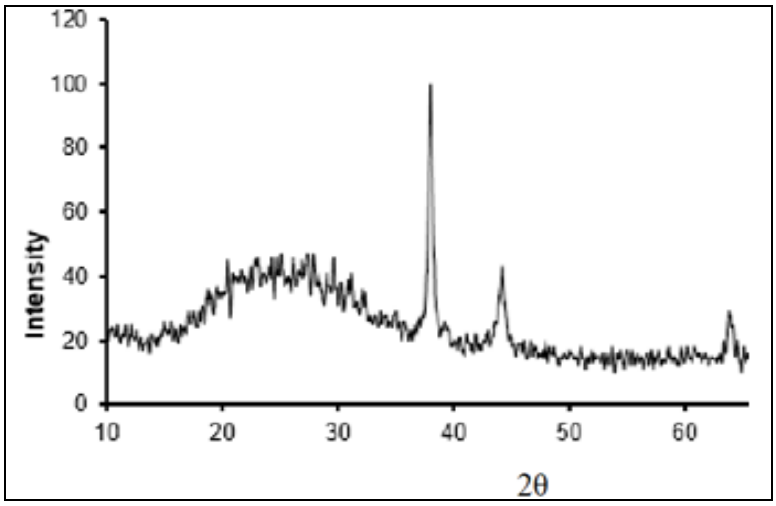

Fig 1: XRD analysis of silver nanoparticles from curry leaf *Murraya koenigii* extract

B. FTIR (Fouries Transform Infrared Spectroscopy)

FTIR observation was done for identification of the biomolecules which responsible for capping and stabilization of the Ag nanoparticles synthesized from *M. koenigii* leaf extract. The FTIR spectrum of *M. koenigii* leaf extract mediated Ag nanoparticles showed the different peaks at 1039, 1267, 1377, 1379, 1445, 1577, 1633 and 1740 cm1 (Fig2).

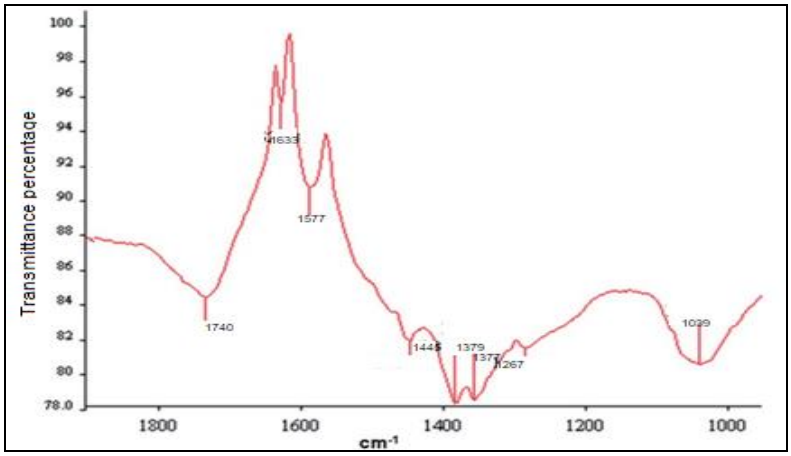

Fig 2: FTIR spectrum of *M. koenigii* leaf extract mediated Ag nanoparticles

C. UV- Visible Spectroscopy

The UV- Vis spectroscopy used to examine the size and shape controlled nanoparticles in aqueous suspensions. The absorption spectrum of aqueous solution *M. koenigii* leaf extract mediated Ag nanoparticles was observed in the range of 300-800 nm and maximum absorbance was recorded at 430 nm. The result reveal that the reduction of Ag+ into Ag NPs. The sharing of peak indicates the particle shape (Fig 3).

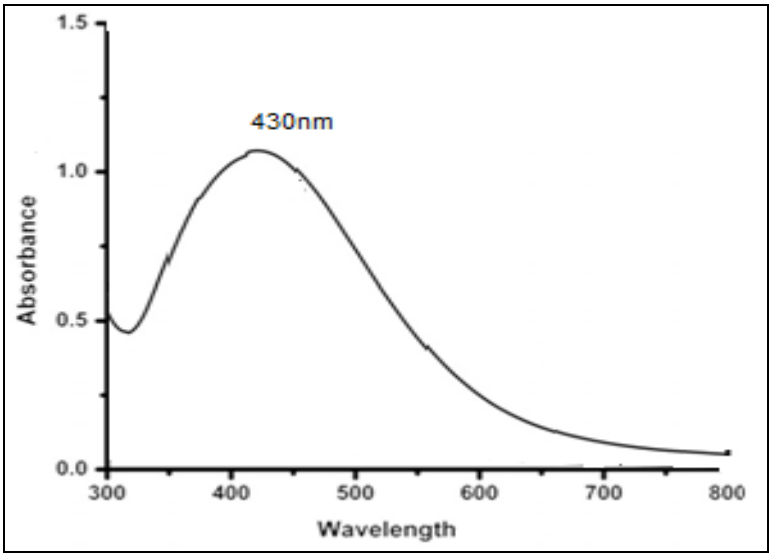

Fig 3: UV- Vis spectroscopy of *M. koenigii* leaf extract mediated Ag nanoparticles

1. Antibacterial activity of silver nanoparticles.

Disc diffusion method

In negative control water was applied in disc of cultured petriplates. There was no inhibition zone against *E. coli*, *S. typhi* and *S. aureas* bacteria.

In positive control (experiment) the *Murraya koenigii* silver nanoparticles used with solvent (such as water) and different concentration (0.1gm 0.2gm 0.3gm 0.4gm and 0.5gm/ml) applied over on bacterial culture plate. Solvent with nanoparticles showed the zone of inhibition. The results are summarized in table 1and figures 4 and 5. In disk diffusion *Murraya koenigii* silver nanoprticles + water showed the zone of inhibition against different bacteria. The zones on inhibition were 1.3cm, 13cm, 1.5cm,1.6cm and1.7cm, against *E. coli* and 1.0cm, 1.1cm, 1.1cm, 1.1 and 1.2cm against *S. aureus* and 0.7cm, 0.9cm, 1.0cm, 1.0 and 1.1cm against *S. typhi* with different concentration (0.1gm 0.2gm 0.3gm 0.4 and 0.5gm/ ml) respectively.

Table 1: Antibacterial activity of water + silver nanoparticles of *Murraya koenigii* different bacteria by disc diffusion method.

S. No	Organism (pathogens)	Water (-) ve Control	Inhibition zone (Radius) in cm				
			0.1gm/ml	0.2gm/ml	0.3gm/ml	0.4gm/ml	0.5gm/ml
1	*E. coli*	-	1.3 ± 0.26	1.3 ± 0.19	1.5 ± 0.21	1.6 ± 0.18	1.7 ± 0.28
2	*S. aureus*	-	1.0 ± 0.16	1.1 ± 0.35	1.1 ± 0.31	1.1 ± 0.28	1.2 ± 0.30
3	*S. typhi*	-	0.7 ± 0.14	0.9 ± 0.38	1.0 ± 0.26	1.0 ± 0.19	1.1 ± 0.19

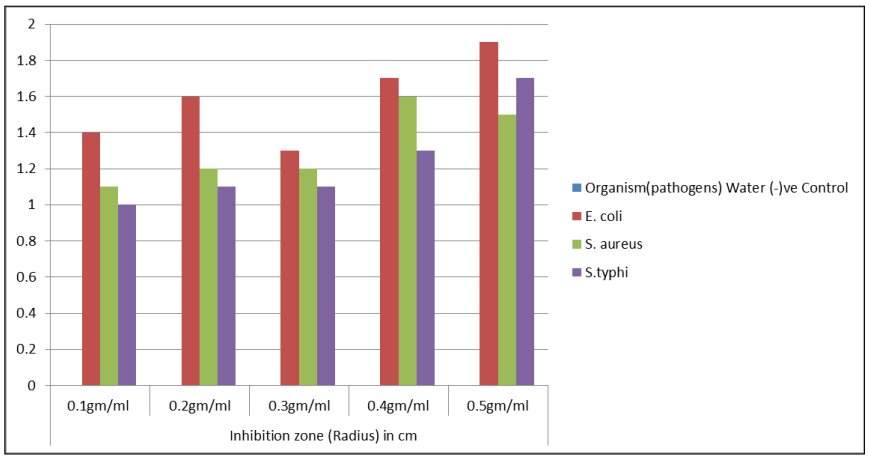

Fig 4: Antibacterial activity of water + silver nanoparticles of *Murraya koenigii* different bacteria by disc diffusion method.

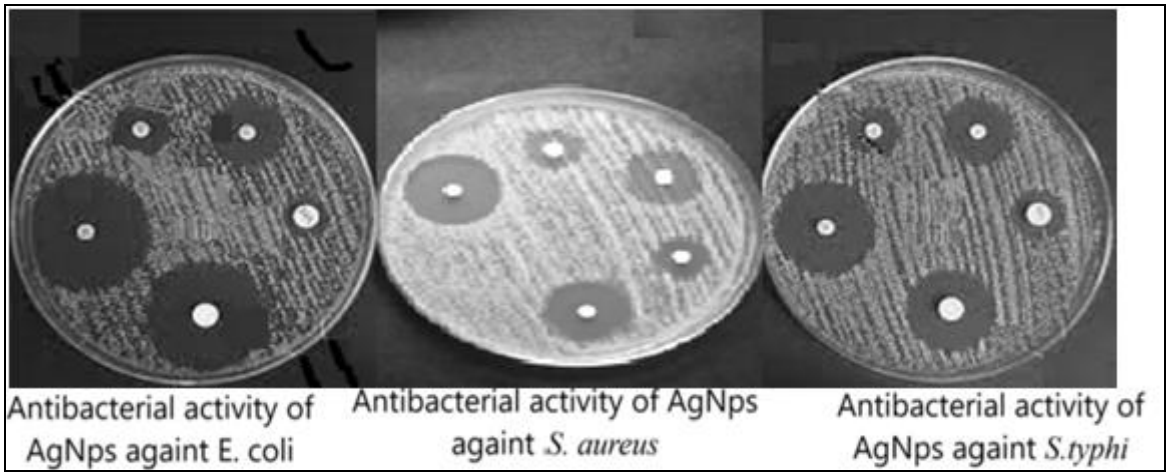

Fig 5: Antibacterial activity of water + silver nanoparticles of *Murraya koenigii* different bacteria by disc diffusion method.

Well diffusion method

In negative control water was applied in disc of cultured petriplates. There was no inhibition zone against *E. coli, S. typhi* and *S. aureas* bacteria.

In positive control (experiment) the *Murraya koenigii* silver nanoparticles used with different solvent and different concentration (0.1gm 0.2gm 0.3gm 0.4gm and 0.5gm/ ml)) applied over on bacterial culture plate. Solvent with nanoparticles showed the zone of inhibition. The results are summarized in table 2and figures 6 and 7.

In well diffusion *Murraya koenigii* silver nanoprticles + water showed the zone of inhibition against different bacteria. The zones on inhibition were 1.4cm, 1.6cm, 1.3cm,1.7cm and1.9cm, against *E. coli* and 1.1cm, 1.2cm, 1.2cm, 1.6 and 1.5cm against *S. aureus* and 1.0cm, 1.1cm, 1.1cm, 1.3 and 1.7cm against *S. typhi* .with different concentration (0.1gm 0.2gm 0.3gm 0.4 and 0.5gm/ ml)) respectively.

Table 2: Antibacterial activity of water + silver nanoparticles of *Murraya koenigii* different bacteria by well diffusion method.

S. No	Organism(pathogens)	Water (-)ve Control	Inhibition zone (Radius) in cm				
			0.1gm/ml	0.2gm/ml	0.3gm/ml	0.4gm/ml	0.5gm/ml
1	*E. coli*	-	1.4 ± 0.17	1.6 ± 0.23	1.3 ± 0.26	1.7 ± 0.16	1.9 ± 0.21
2	*S. aureus*	-	1.1 ± 0.25	1.2 ± 0.33	1.2 ± 0.19	1.6 ± 0.44	1.5 ± 0.28
3	*S.typhi*	-	1.0 ± 0.34	1.1 ± 0.15	1.1 ± 0.45	1.3 ± 0.52	1.7 ± 0.16

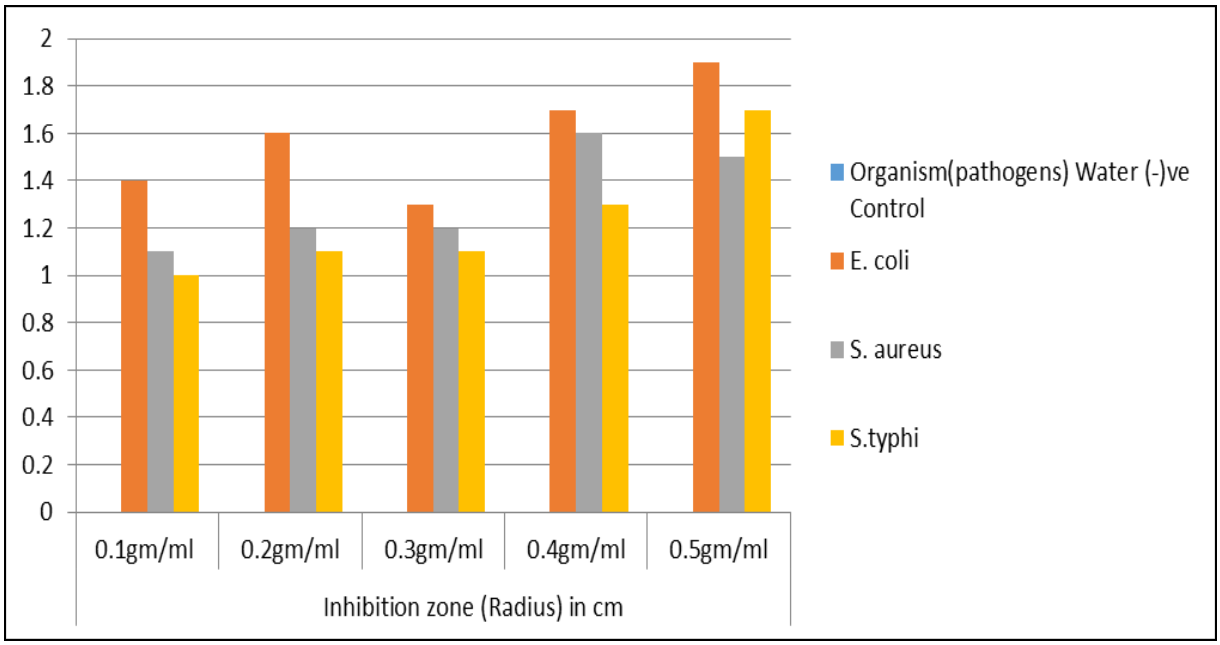

Fig 6: Antibacterial activity of water + silver nanoparticles of *Murraya koenigii* different bacteria by well diffusion method.

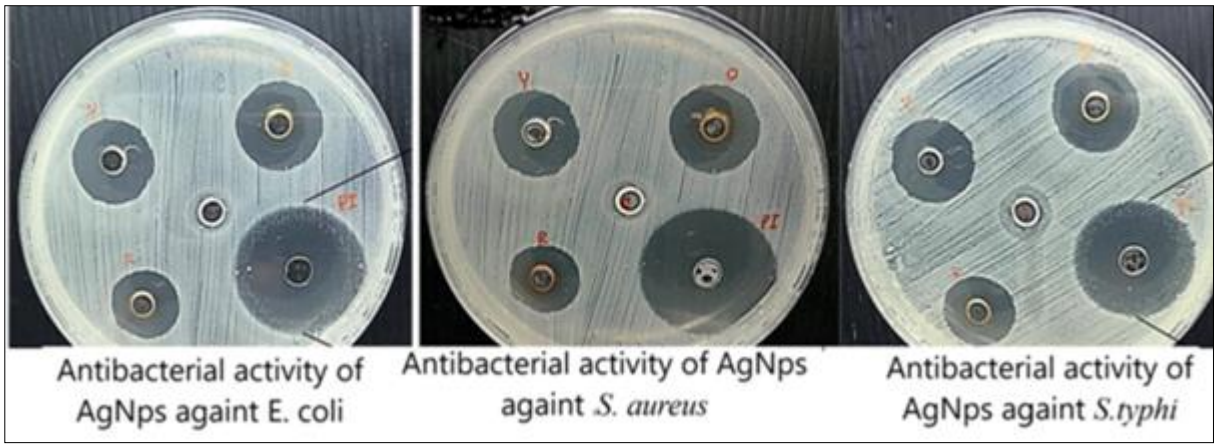

Fig 7: Antibacterial activity of water + silver nanoparticles of *Murraya koenigii* different bacteria by well diffusion method.

MIC effect of Ag Nanoparticles on different bectaria

The efficiency of Ag Nanoparticles synthesis form *Murraya koenigii* was assessed on the basis on O.D at different time interval against *E. coli, S. typhi* and *S. aureas* at 1gm/100ml concentration and at different time interval i.e. 24, 48, 72 and 96hours. The results were summarized in table 3and graph 6.

After 24 hours O.D was found decreased. The Nanoparticles Inhibit the growth of *E. coli, S. typhi* and *S. aureas* after 48, 72 and 96 hours. The O.D of silver Nanoparticles was more effective against *E. coli* followed by *S. aureus* than *S. typhi*.

Table 3: MIC effect different Nanoparticles of *Murraya koenigii* on *E.coli, S. aureus* and *S. typhi* bacteria at different time interval.

S. No.	Sample	Con. of Nps	OD at 630 nm at different time intervals			
			24hrs.	48 hrs.	72hrs.	96 hrs.
1	Blank	Plan Media	00	00	00	00
2	AgNps against *E. coli*	1gm/100ml	0.28 ±0.10	0.12 ±0.12	0.11 ±0.14	0.08 ±0.16
3	AgNps against *S. aureus*	1gm/100ml	0.24 ±0.10	0.11 ±0.12	0.10 ±0.14	0.07 ±0.16
4	AgNps against *S. typhi*	1gm/100ml	0.23 ±0.10	0.09 ±0.12	0.08 ±0.14	0.05 ±0.18

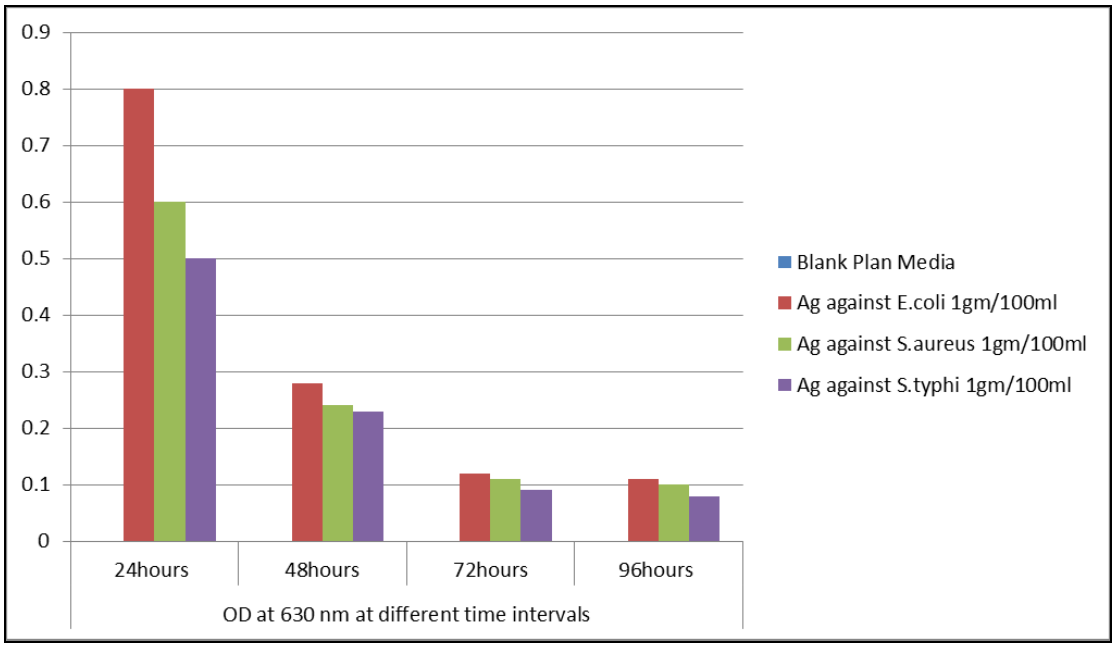

Fig 8: MIC effect different Nanoparticles of *Murraya koenigii* on *E. coli, S. aureus* and *S. typhi* bacteria at different time interval.

Nanoparticles prepared for the present experiments showed effective antibacterial activity. It was observed that nanoparticles have penetrated inside the bacteria and have caused damage by interacting with phosphorus and sulfur containing compound such as DNA (Shetty *et al.*, 2006) [22] may have lose its replication power and cellular proteins and become inactive after treatment with nanoparticles. In addition, nanoparticles may be

preventing the growth and cell division. The nanoparticles have an additional contribution to the bactericidal efficacy. Heavy metals are toxic and react with proteins, therefore they bind protein molecules and as a result cellular metabolism is inhibited causing death of microorganism (Moghaddam *et al.,* 2009) [15]. Bactericidal efficacy of silver nanoparticles was investigated by many researchers and their effective potential against broad range of microb was proved, including antibiotic-resistant bacteria. Thus silver nanoparticles are also termed as new-generation of antimicrobials. The group of researchers actively proved the bactericidal potential of silver nanoparticles (Lansdown *et al.,* 1997) [12]. In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. These biosynthesis of gold nanoparticles by plants such as *alfalfa* (Shetty *et al.,* 2006; Ahmed *et al.,* 2002) [22, 2] *Aloe Vera* (Gupta *et al.,* 2006) *Cinnamomum camphora* (Singh *et al.,* 1996) [23] *Azadirachta indica* (Samjon *et al.,* 2007) [19] *Embica officinal's* (Sood *et al.,* 2006) [24] *lemongrass* (Sharma *et al.,* 2002) [21]. *Tamarinds indica L/n* (Kantak *et al.,* 1992) [7] have also been reported. Synthesis of plants extract mediated copper nanoparticles and their impact on pathogenic becteria was studied by (Ijjatdar *et al.,* 2018) [20] they reported that synthesis of CuNP Garlic (*Allium sativum*) plants and investigate its antibacterial activity against different bacteria. Among the different types of metallic nanoparticles, silver nanoparticles can be highlighted for their broad-spectrum antimicrobial potential (Gupta *et al.,* 2017; Loo *et al.,* 2018) [6, 13]. In the present investigation *Murraya koenigii* AgNPs showed antibacterial activity against *E. coli, S.typhi* and *S. aureus.* Thus corroborate with finding of previous authors that plant mediated nanoparticles may be good antibacterial agent.

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