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Der Pharmacia Lettre, 2016, 8 (10):150-160
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USA CODEN: DPLEB4

Synthesis of silver nanoparticles and its antibacterial activity from *Moringa oleifera, Murraya koingii* and *Ocimum sanctum* against *E.coli* and *S.aureus*

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ABSTRACT

Silver nanoparticles (AgNPs) due to biocompatibility, antibacterial action and its various applications in the field of electronics, optical, magnetic have attracted considerable attention. In this study silver nanoparticles were successfully produced from silver nitrate using leaves extracts of Murrayakoenigii, Moringaoleifera and Ocimum sanctum. The AgNPs were characterized using UV-visible spectroscopy at the wavelength of 300-600nm. The antibacterial activities against different pathogens (Escherichia coli, Staphylococcus aureus) were reported. The zone of inhibition was observed both in gram positive and gram negative bacterial strains. They were found to have considerable inhibitory action against the mentioned microorganisms.

Keyword*:* *Murrayakoenigii* extract, *Moringaoleifera* extract, *Ocimum sanctum* extract, AgNPs, antibacterial.

INTRODUCTION

Nanotechnology has induced great scientific advancement in the field of research and technology. Nanotechnology is the study and application of small objects which can be used across all fields such as chemistry, biology, physics and engineering. Nanoparticle is a core particle which performs as a whole unit in terms of transport and property. As the name indicates Nano means a billionth or 10-9 unit. Its size ranges from 1-100nm[1] due to small size it occupies a position in various fields of Nano science and nanotechnology.Nanocrystalline silver particles have found tremendous applications in the field ofhigh sensitivity bio molecular detection and diagnostics, antimicrobials and therapeutics; catalysis and microelectronics.Currently there are several methods for the production of nanoparticles like chemical and physicalmethods. But there are evidences regarding the harmfulness of these methods to environment[2].Development of plant mediated reliable green, nontoxic and environmentally friendly process for the synthesis of silvernanoparticles are best suited to the environment. In this experiment, the rapid synthesis of stable silver nanoparticles has been demonstrated using the extracts *Moringaoleifera*, *Murrayakoingii* and *Ocimumsanctum*.

Moringaoleifera(*M. oleifera*) (Family: Moringaceae, English name: drumstick tree) has been reported to be essentially used as an ingredient of the Indian diet since ages The plant leaves have also been reported for its antitumor, cardioprotective, hypotensive, wound and eye healing properties[3].

Murrayakoenigii (common name- kadipatta or curry leaves).It is a small strong smelling perennial shrub. Leaves are bitter, acrid, astringent, cooling, demulcent, depurative, anthelmintic, febrifuge, stomachic, appetizing, carminative, anti-inflammatory and antiseptic.

Ocimum sanctum(local name *Tulasi*) is a traditional medicinal plant of India has source of bio-reductant and stabilizers. It has been reported to contain alkaloids, glycosides, tannins, saponins and aromatic compounds [4]. It is used in the treatment of headaches, coughs, diarrhea, constipation, worts, worms and kidney malfunctions [5].In the present study, synthesis of AgNPs in cold water extract and methanol extract have been reported, reducing the silver ions present in the silver nitrate solution by the aqueous extract of *M. oleifera*, *Murayakoingii*and*Ocimum sanctum*leaves.

MATERIALS AND METHODS

2.1Plant material

The fresh leaves of *Moringaoleifera, Murrayakoingii* and *Ocimum sanctum* were collected from Elispettai, Erode, Tamil Nadu state, India. Fresh plant material were washed under running tap water, air dried and homogenized into fine powder and stored in air tight polythene papers.

2.2Bacterial strain isolation

A total of 6 diarrhea bacterial strains were used in the study, among these were three Gram negative ,*Escherichiacoli* species and three Gram-positive, *Staphylococcus aureus* species .All the tested strains are reference strains, and were collected from Perundurai Medical College and Hospital Erode.

2.3 Isolation of Pathogens:

An aseptically collected diarrhea samples was inoculated on the EMB, Mannitolsalt agar and Mac Conkey Agar plates and was incubated aerobically at 37°C for 24 hours. Isolates obtained were maintained on nutrient agar slants at 4°C until required.

2.4 Identification and biochemical characterization of bacterial isolates:

The isolated pathogens were identified on the basis of Gram's reaction and biochemical characteristics [6].and the results were identified with the help of Bergey's Manual of systemic Bacteriology [7].Biochemical tests such as indole test, methyl red test, VP test, citrate utilization test, Nitrate reduction test, Triple sugar iron agar test and catalase test.

2.5 Preparation of Plant extracts

Preparation of Cold aqueous extract:

The well air dried fresh leaves of *Moringaoleifera, Murrayakoingii*and *Ocimum sanctum* were crushed directly by grinder into fine powder, and 10 grams sample was dipped into 50 ml Cold distilled water in a BOD bottle and left for 24 hours. Filtered off using sterile filter paper (Whattman no. 1) into a clean conical flask and subjected to water bath evaporation where the aqueous solvent was evaporated at its boiling temperature of 100°C. The standard extracts obtained were then stored in a refrigerator at 4°C

Preparation of Methanol extract:

The well air dried fresh leaves of *Moringaoleifera, Murrayakoingii*and*Ocimum sanctum* were crushed directly by grinder into fine powder, and 10 grams sample was dipped into 50 ml methanol in a BOD bottle and left for 24 hours. Filtered off using sterile filter paper (Whattman no. 1) into a clean conical flask and subjected to water bath evaporation where the aqueous solvent was evaporated at its boiling temperature of 100°C. The standard extracts obtained were then stored in a refrigerator at 4°C

Antibacterial sensitivity testing

Antibacterial susceptibility testing of antibiotics was performed by disc diffusion method [8]. Antibiotic discs included kanamycin (30mcg), erythromycin (15mcg), rifampicin (5mcg), oflaxacin (5mcg) and oxacillin (5 mcg). For susceptibility testing, a sterile cotton swab was dipped into the standardized inoculum and rotated firmly against the upper inside wall of the test tube to remove excess inoculum from swab. Entire sterile and dried Mueller Hinton agar surface of the plate was streaked with the cotton swab. The plates were incubated at 37° C for 24 hrs. The assessment of antibacterial activity was done by measuring the diameter of the growth inhibition zone formed around disc.

Antibacterial assay

Antibacterial activity of the two different samples; of Cold water extract and Methanol extracts of *Moringaoleifera, Murrayakoingii*and*Ocimum sanctum* were individually tested against studied bacteria. In vitro antibacterial test was then carried out by agar well diffusion method [9].The bacteria suspensions were swabbed on nutrient agar plates. The discs (6 mm in diameter) were added with 100 µl of each extract solution, separately to each well and allowed

to diffuse at room temperature for 15-20 minutes. The plates were incubated at 37°C for 24h. Antibacterial activity was evaluated by measuring the zones of inhibition against the tested bacteria.

Synthesis of Sliver Nanoparticiles

Stock solution was prepared by dissolving 1mM sliver nitrate (AgNO3; Merck, Mumbai, India) and volume made up to 150 ml with distilled water. 5 ml Cold water extractof*Moringaoleifera, Murrayakoingii*and*Ocimum sanctum*were added to 10 ml of 1mM AgNO3 solution and allowed to react at room temperature.

Characterization of Silver Nanoparticles UV –Vis Spectroscopy

The periodic scans of the optical absorbance between 300 and 600nm with a UV- Vis spectrophotometer (ELICO SL159) at a resolution of 1 nm were performed to investigate the reduction rate of silver ions by leaf extract of *Moringaoleifera, Murrayakoingiiand Ocimumsanctum*.

Antibacterial Activity of Silver Nanoparticles

The potential of silver nanoparticles as effective antimicrobial agents is well known. The antibacterial activities of silver nanoparticles were carried out against pathogenic strains of *Escherichia coli* and *Staphylococcus aureus*, by disc diffusion method6 nutrientagar medium plates were prepared, sterilized and solidified. After solidification the formed discs (6 mm in diameter) were added with 100 µl of each extract solution, separately to each well. The plates were incubated at 37°C for 24h. Antibacterial activity was evaluated by measuring the zones of inhibition of silver nanoparticle against the tested bacteria.

Minimum inhibitory concentration (MIC)

MIC was also determined using the initial bacterial inoculums with different concentrations of the AgNPs (0.048,0.097,0.19,0.39,0.78,1.56,3.12,6.25,12.5,25,50 and 100 µl) in 2 Microtiter plates. Microtitre plate wells from each column in row 1 were marked and 100 µ1 (500mg/ml) of stock (Methanol, Cold water) was added. 50µ1 of sterile distilled water was added to rows 2-12. Two fold serial dilutions were performed by transferring 50 µ1 of solution from row 1 to row 2, using a multichannel pipette. This was repeated down the row 2 to row12. 14µ1 of double strength nutrient broth and 10µ1 of different bacterial solution were added to all the wells in separate column, so the final concentrations of the inoculum in all the wells .To prevent dehydration, the plates were covered with a plastic cover and then incubated at 37°C overnight. The bacterial growth was determined after addition of 40µ1 of Triphenyltetrazodiumchloride red (0.02mg/m1). The minimum inhibitory concentrations (MIC) of the isolates were taken as the lowest concentration of the antibiotic of which the bacterial tested did not show visible growth.

Phytochemical Analysis

The freshly prepared extracts were subjected to standard preliminary phytochemical analysis for the presence of alkaloids, flavonoids, tannin, saponins, terpenoids, cardiac glycosides, coumerin, pytosterols, phylobotannins, cholesterol, carbohydrates and cartenoidsas described by various authors [10].

RESULTS AND DISCUSSION

Antibacterial activity of standard commercial antibiotics

Antibacterial activity of standard commercial antibiotics is summarized in **(Table 1**).The results indicate that *S.aureus* and *E.coli* species pathogens were inhibited by Kanamycin with inhibition zone 17.2-20.3mm, Erythromycin with 15.35mm, Rifampicin with 9.4-15.15 mm, Oflaxacinwith 11.25-27.15 mm. All the strains produced sensitive result except Oxacillin as shown in **(Figure 2 and 3)**

Antibacterial activity

Cold water extract and Methanol extracts of *Moringaoleifera, Murrayakoingii* and *Ocimum sanctum* leaves were subjected to preliminary screening for their antibacterial activity was determined on the basis of zone of inhibition against *S.aureus* and *E.coli***(Table2) (Figure 4).**The results revealed that *Moringaoleifera* cold water showed highest zone of inhibition of 14.25mm against *S.aureus 2* while Methanol extract *Moringaoleifera* showed a maximum zone of inhibition at 14.15mm against *S.aureus* 1.This an observation in agreement with reported by [1} *Murraya koingii*, cold water showed a zone of inhibition at 10.1mm against*S.aureus 2* and the Methanol leaf extract showed antibacterial activity at 15.15mm against *E.coli 1* with a diameter of 15.15mm.*Ocimum sanctum* Cold water extract had a zone of inhibition at 9.45mm against *S.aureus2* while Methanol leaf extract showed antibacterial potential of 14.35mm against*S.aureus 3*.In the present studies Methanol extract was found to be more effective than aqueous extracts to the tested microbial strains. Most sensitive bacteria were *S.aureus* than *E.coli*.It was also evident that Methanol extracts of leaves have a broad spectrum against both Gram positive as well as Gram negative while aqueous extract has no antibacterial activity **.(Figure 5,6,7)**

Synthesis of Silver Nanoparticles

Addition of Silver nitrate to the *Moringaoleifera, Murrayakoingii* and *Ocimum sanctum*leaf extracts resulted in the change of the plant extracts from colourless to brown **(Fig. 1).** That brown colour indicated that surface plasmon vibrations, typical of silver nanoparticles. As the of *Moringaoleifera, Murrayakoingii*and *Ocimum sanctum*aqueous leaf extract was added to the silver nitrate solution and incubated the mixture color was changed rapidly from the transparent color to brown yellowish color due to the formation of AgNP's. The appearance of yellowish brown color was due to the excitation of surface plasmon vibrations of silver nanoparticles.

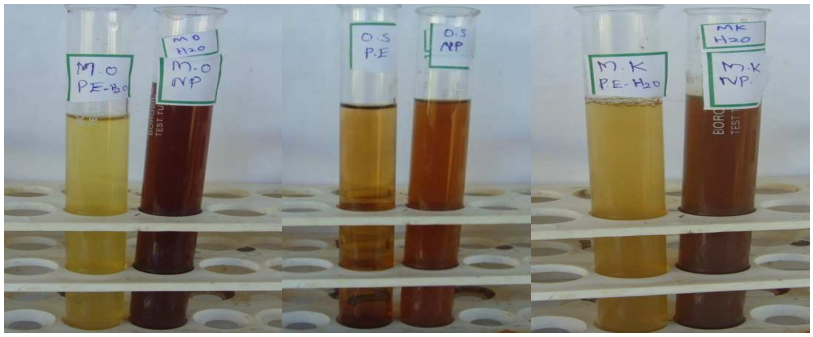

Figure 1: Photograph of *Moringaoleifera, Murrayakoingii*and *Ocimum sanctum* aqueous Bio-nanocomposites

Charaterization of Sliver Nanoparticles UV Spectrometry

The formation of silver nanoparticles was confirmed through measurement of UV-Visible spectrum of the reaction mixture. The UV-Visible spectrophotometric analysis of silver nanoparticles using *Moringaoleifera*cold water leaf extract showed peak at 480. (Stepanov1997) reported absorbance peak at 400- 420 nm. The maximum peak was observed at 480 nm in the case of *Murrayakoingii* cold water leaf extract nanoparticle. Laura *et al*. reported maximum peak at 435 nm for the nanoparticle synthesized using*Murrayakoingii* leaf extract. The UV-Spectra showed maximum peak at 440 nm for *Ocimum sanctum* cold water leaf extract nanoparticle and similar results were reported by Bindhani *et al*., 2015 whereby the UV spectra showed maximum peak at 440 nm for *Ocimum sanctum* Nanoparticle. The maximum peak observed for *Moringaoleifera* Methanol leaf extract was at 440nm whereas Elumalai *et al*., 2011 reported absorbance bands between 430-440 nm for*Moringaoleifera* Nanoparticle .(**Figure 8,9,10,11**)

Antibacterial activity

The antibacterial activity of the synthesized nanoparticles was analyzed using well diffusion method (**Table 4).** Synthesized nanoparticles showed antibacterial activity against the bacteria (*Escherichia coli, Staphylococcus aureus*). The highest zone of inhibition was found to be 15.1 mm in *Moringaoleifera* Cold water extract against *S. aureus1* and in *Moringaoleifera* Methanol extract was 16.25 mm against *S. aureus1*. The zone of inhibition of *Murrayakoingii* Cold water extract nanoparticles was found to be 15.35mm against *S. aureus2. Ocimum sanctum* Cold water extract had the highest zone of inhibition at 15.3mm against *S. aureus2* while *Ocimum sanctum* Methanol extracthad highest zone at 10.45 against *S. aureus2*. (**Figure 12, 13, 14)**Results revealed that leaf that leaf extract (Methanol) *Moringaoleifera* was more effective than those of other extract.

Minimum Inhibition Concentration (MIC)

Various concentrations of Cold water extract and Methanol extracts (0.048-100mg/ml) was used to inhibit the test organism. The Minimum Inhibition Concentration (25µl/ml) was found against the *S. aureus*3 in *Moringaoleifera* Methanol extract. This suggests that Gram positive is more sensitive for test extract.

Phytochemical analysis

Phytochemical analysis of *Moringaoleifera* revealed the presence of alkaloids, saponins, terpernoids and phytosterols.*Murrayakoingii* had alkaloids, tannins, cholesterol, phytobotannins and phytosterols. *Osmium sanctum* had alkaloids, tannins, cholesterol, terpernoids and phytosterols.This is in analogy with other workers [12] (Doughier *et al.*, 2007; Krishnaiahh *et al.*, 2009) (**Table 5**).Qualitative analysis of *Moringa oleifera, Murraya koingii* and *Ocimum sanctum* flavonoid content (**Table 6)** was found to be 1.28mg, 1.02mg, 1.06mgrespectively while the ash content was found to be 0.23, mg 0.19 mg, 0.30mgand crude fiber were found to be 0.04 mg, 0.05mg and 0.04mg respectively as shown in (**Fig 16)**

Table 1: Antibacterial activity of Commercial Antibiotics against *S.aureus* and *E.coli*

Isolates	Zone of Inhibition(mm)				
	Kanamycin (30mcg)	Erythromycin (15mcg)	Rifampicin (5mcg)	Oflaxacin (5mcg)	Oxacillin (5 mcg)
S.aureus 1	17.25±0.35	R	R	11.25±0.35	R
S.aureus 2	20.3±0.42	R	15.15±0.21	27.15±0.21	R
S.aureus 3	R	R	18.2±0.28	13.35±0.49	R
E.coli 1	R	15.35±0.49	10.25±0.35	20.1±0.14	R
E.coli 2	R	R	10.3±0.42	14.45±0.63	R
E.coli 3	17.2±0.28	R	9.4±0.56	20.3±0.42	R

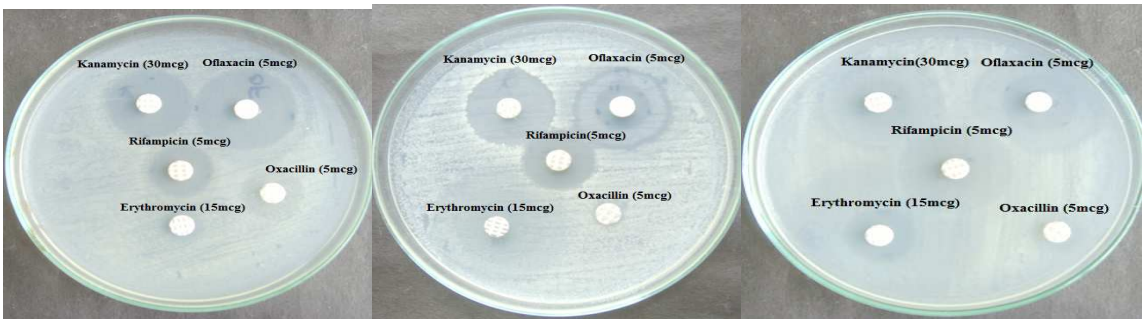

Figure 2: Antibacterial activity of Commercial Antibiotics against *S.aureus* and *E.coli*

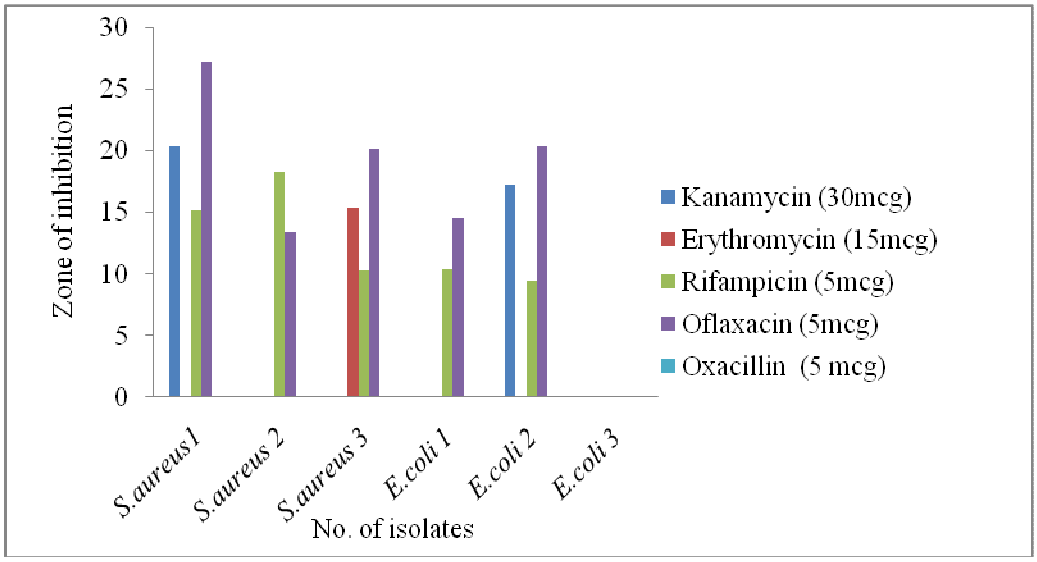

Figure 3: Antibacterial activity of Commercial Antibiotics against *S.aureus* and *E.coli*

Table 2: Antibacterial activity of *Moringaoleifera, Murrayakoingii* and *Ocimum sanctum* against *S.aureus* and *E.coli*

Isolates	*Moringaoleifera*		*Murrayakoingii*		*Ocimum sanctum*	
	Cold water	Methanol	Cold water	Methanol	Cold water	Methanol
S.aureus 1	8.1±0.14	14.15±0.35	7.25±0.35	12.35±0.49	7.25±0.35	13.1±0.14
S.aureus 2	14.25±0.35	9.1±0.14	10.1±0.14	7.35±0.49	9.45±0.63	14.25±0.35
S.aureus 3	9.15±0.21	13.4±0.56	8.3±0.42	12.25±0.35	6.2±0.28	14.35±0.49
E.coli 1	10.35±0.49	7.45±0.63	6.2±0.28	15.15±0.21	7.1±0.14	10.1±0.14
E.coli 2	7.15±0.21	10.25±0.35	8.4±0.56	9.1±0.14	8.2±0.28	13.4±0.56
E.coli 3	9.45±0.63	8.2±0.28	9.25±0.35	10.35±0.49	9.4±0.56	12.3±0.42

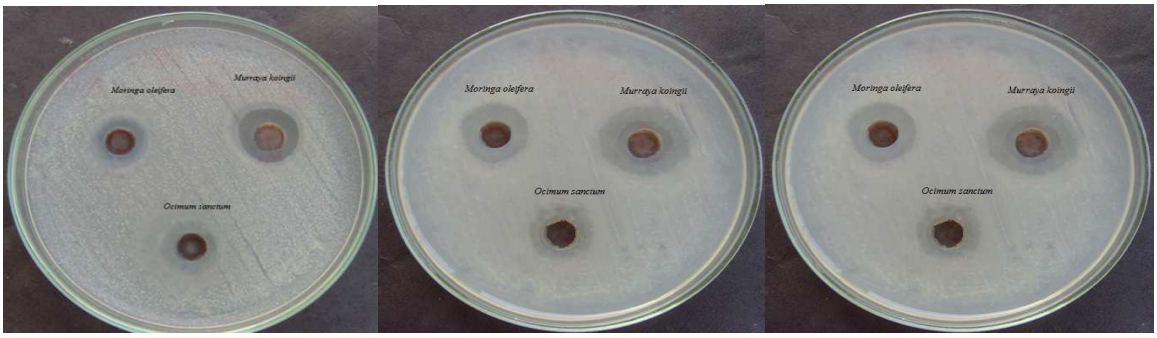

Figure 4: Antibacterial activity of *Moringa oleifera, Murrayakoingii* and *Ocimum sanctum*

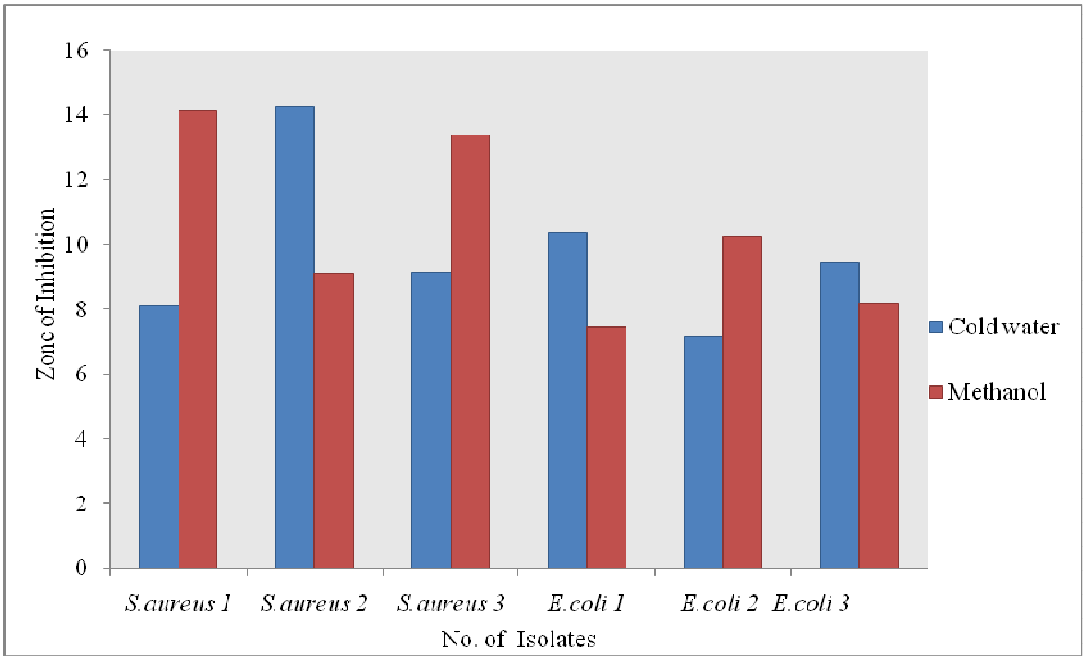

Figure 5: Antibacterial activity of *Moringaoleifera*

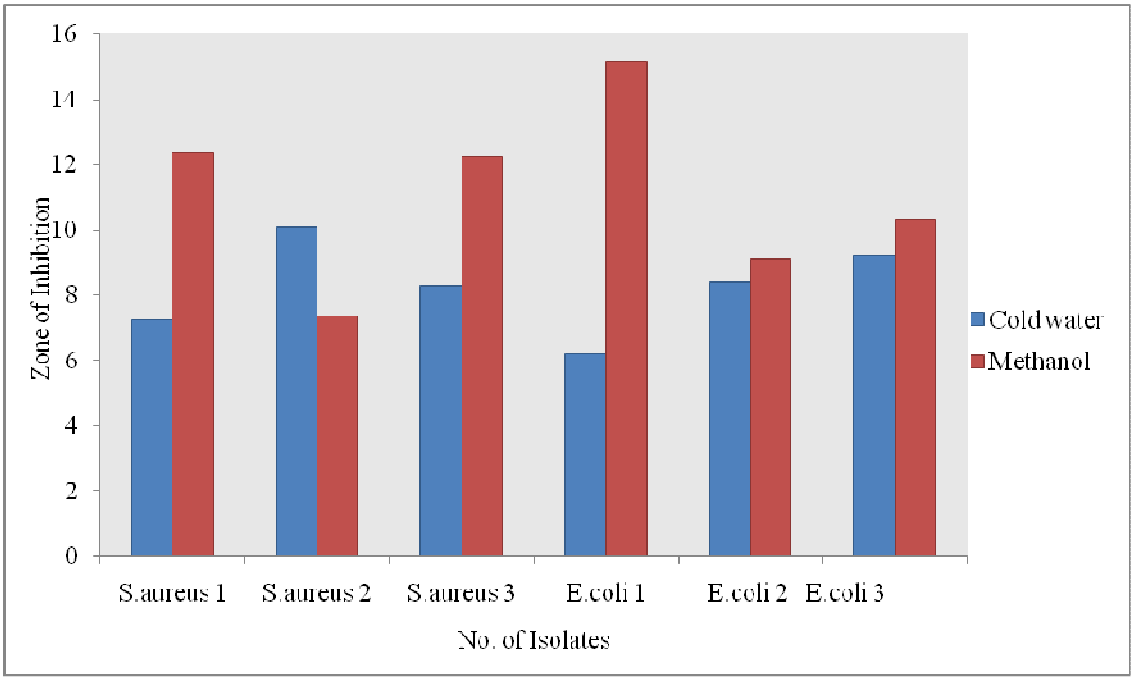

Figure 6: Antibacterial activity of *Murrayakoingii*

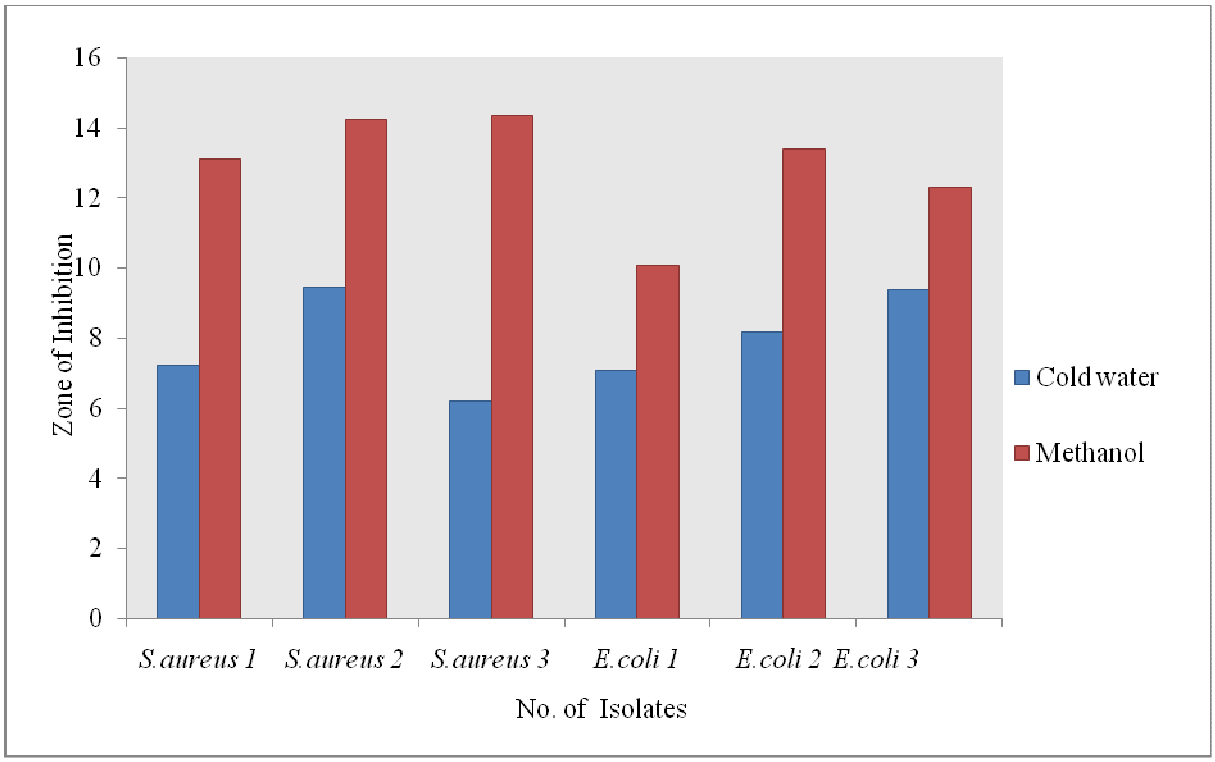

Figure 7: Antibacterial activity of *Ocimum sanctum*

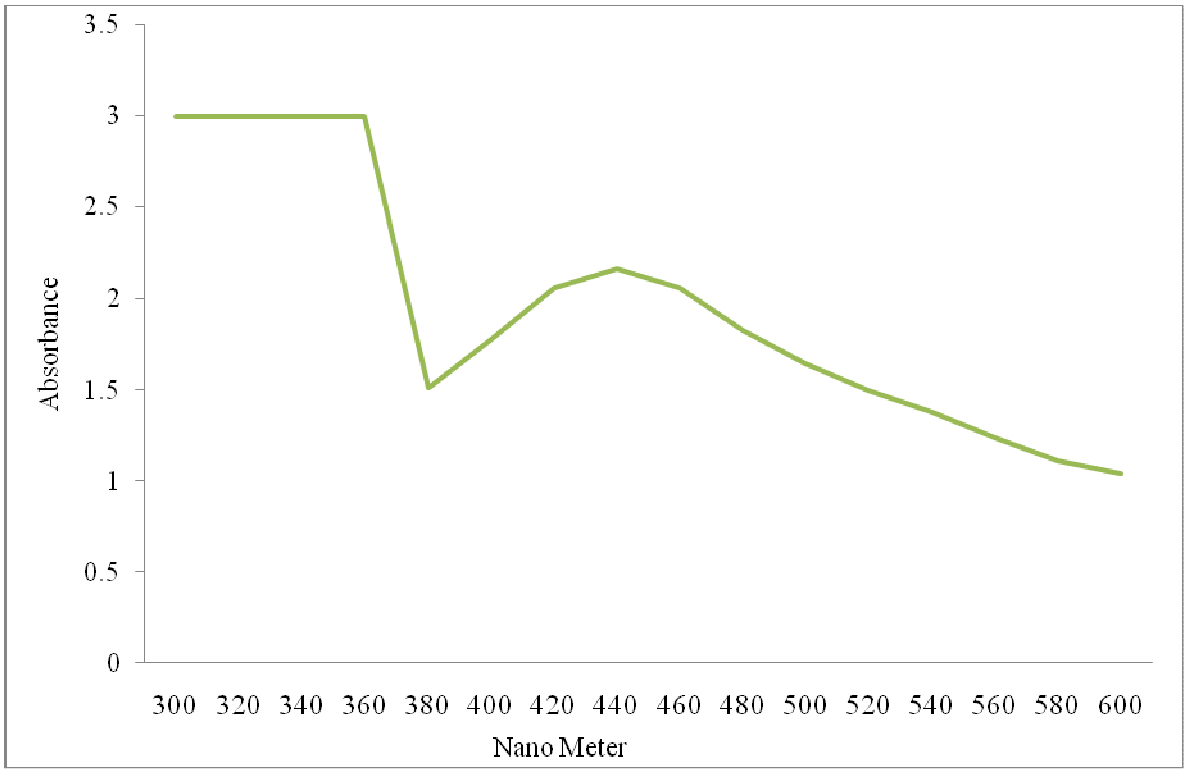

Figure 8: Synthesis of Silver Nanoparticles using *Moringaoleifera* Cold Aqueous Extract

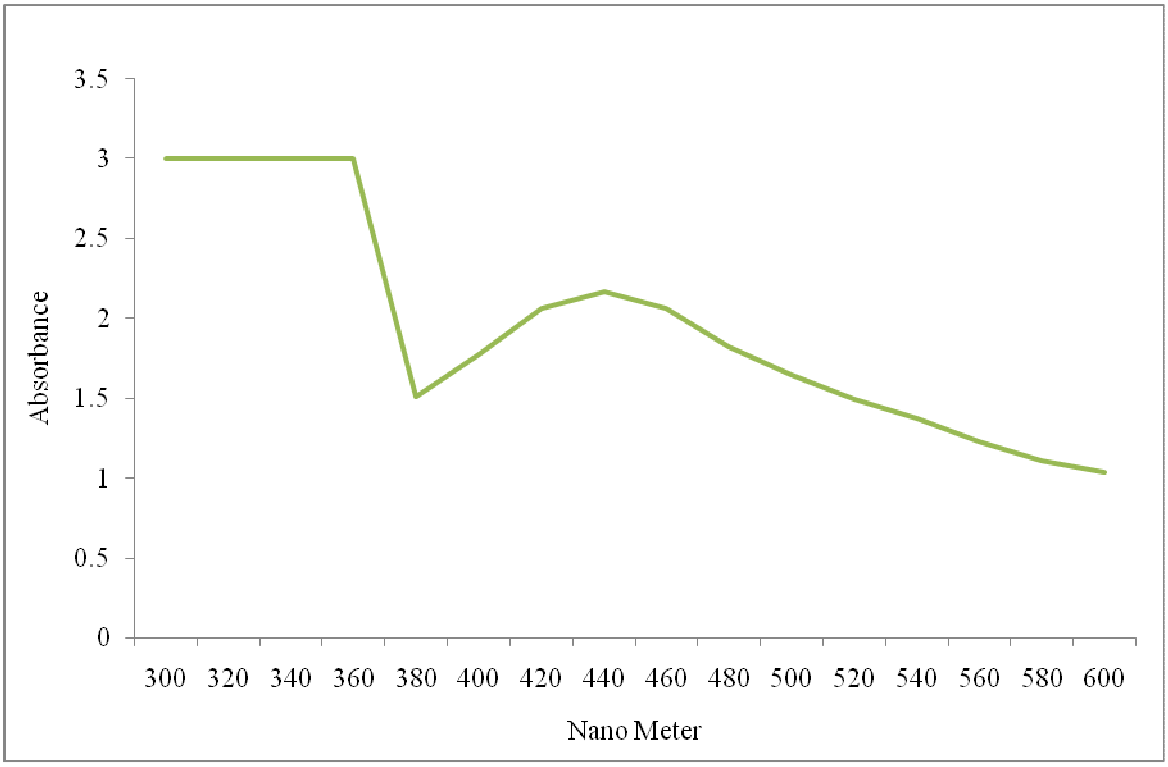

Figure 9: Synthesis of Silver Nanoparticles using *Murrayakoingii* Cold Aqueous Extract

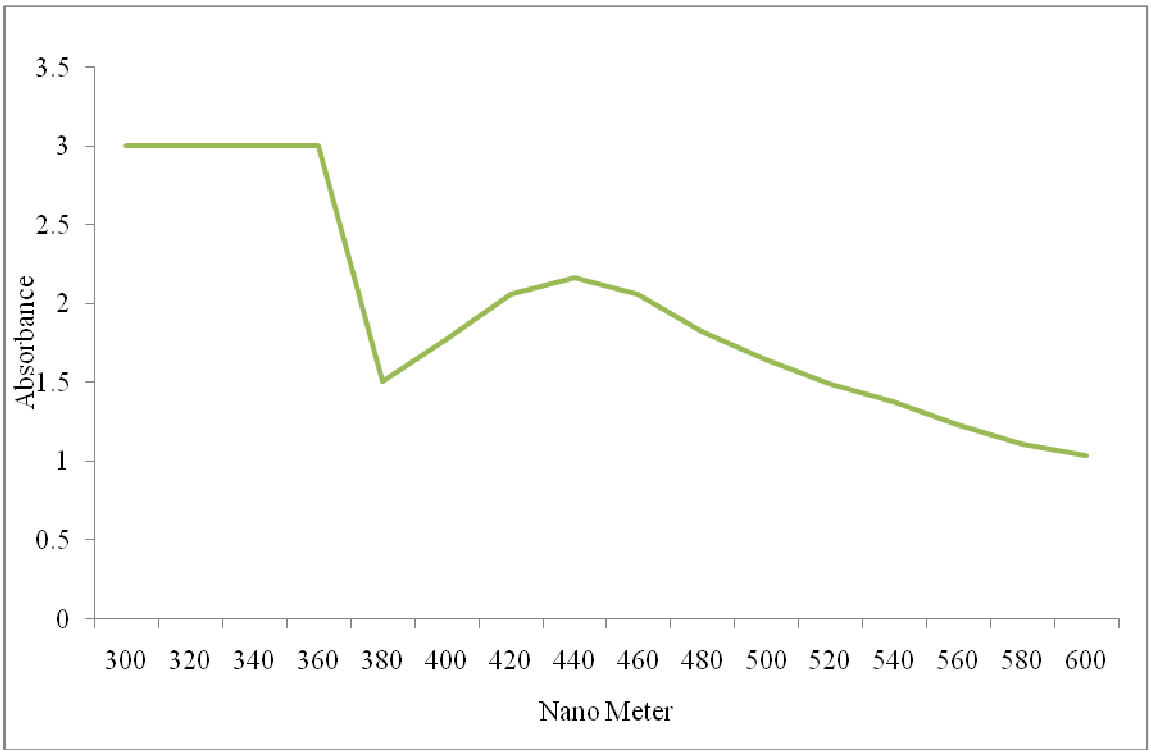

Figure 10: Synthesis of Silver Nanoparticles using *Ocimum sanctum* Cold Aqueous Extract

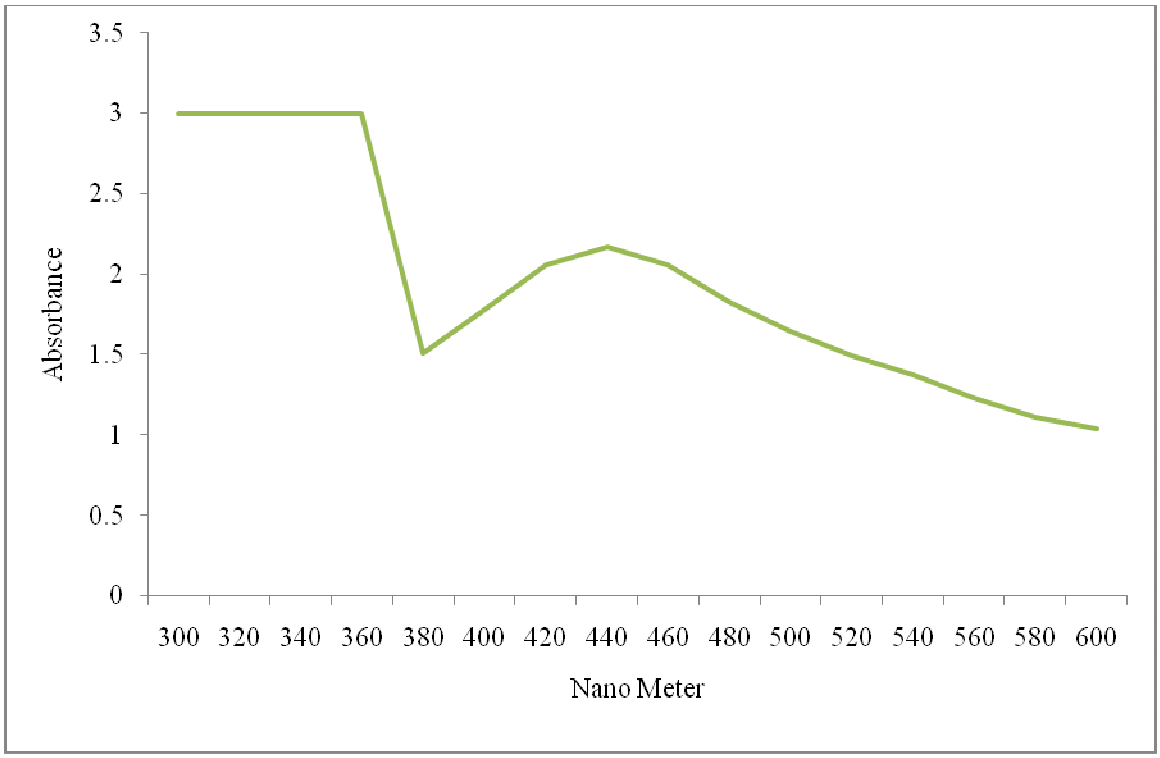

Figure 11: Synthesis of Silver Nanoparticles using *Moringaoleifera* Methanol Aqueous Extract

Table 4: Antibacterial activity of Silver Nanoparticles from *Moringaoleifera, Murrayakoingii* and *Ocimum sanctum* against *S.aureus* and *E.coli*

Isolates	*Moringaoleifera*		*Murrayakoingii*		*Ocimum sanctum*	
	Cold water	Methanol	Cold water	Methanol	Cold water	Methanol
S.aureus 1	15.1±0.14	16.25±0.35	13.1±0.14	7.25±0.35	14.1±0.14	6.1±0.14
S.aureus 2	14.2±0.28	16.2±0.28	15.35±0.49	10.1±0.14	15.3±0.42	10.45±0.63
S.aureus 3	9.1±0.14	7.35±0.49	10.1±0.14	8.3±0.42	9.1±0.14	9.15±0.21
E.coli 1	7.15±0.21	8.25±0.35	7.25±0.35	9.45±0.63	8.15±0.21	7.1±0.14
E.coli 2	8.25±0.35	10.1±0.14	9.2±0.28	6.2±0.28	7.45±0.63	8.2±0.28
E.coli 3	10.3±0.42	9.45±0.63	8.3±0.42	8.4±0.56	10.2±0.28	9.4±0.56

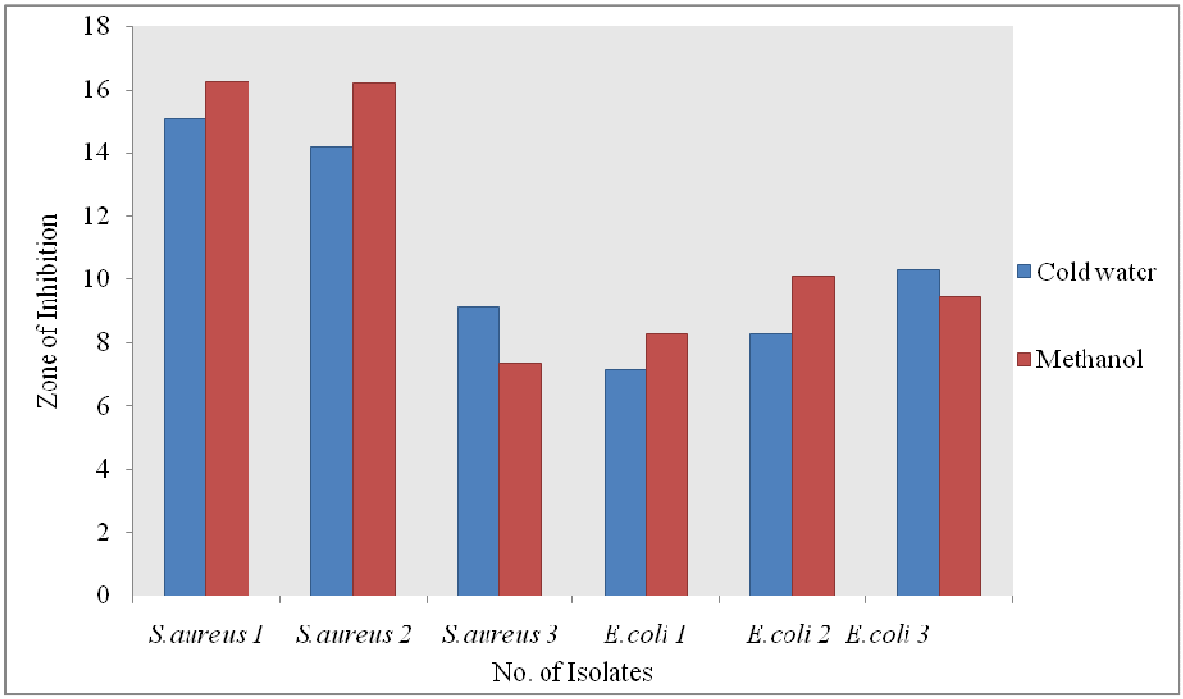

Figure 12: Antibacterial activity of Silver Nanoparticles using *Moringaoleifera*

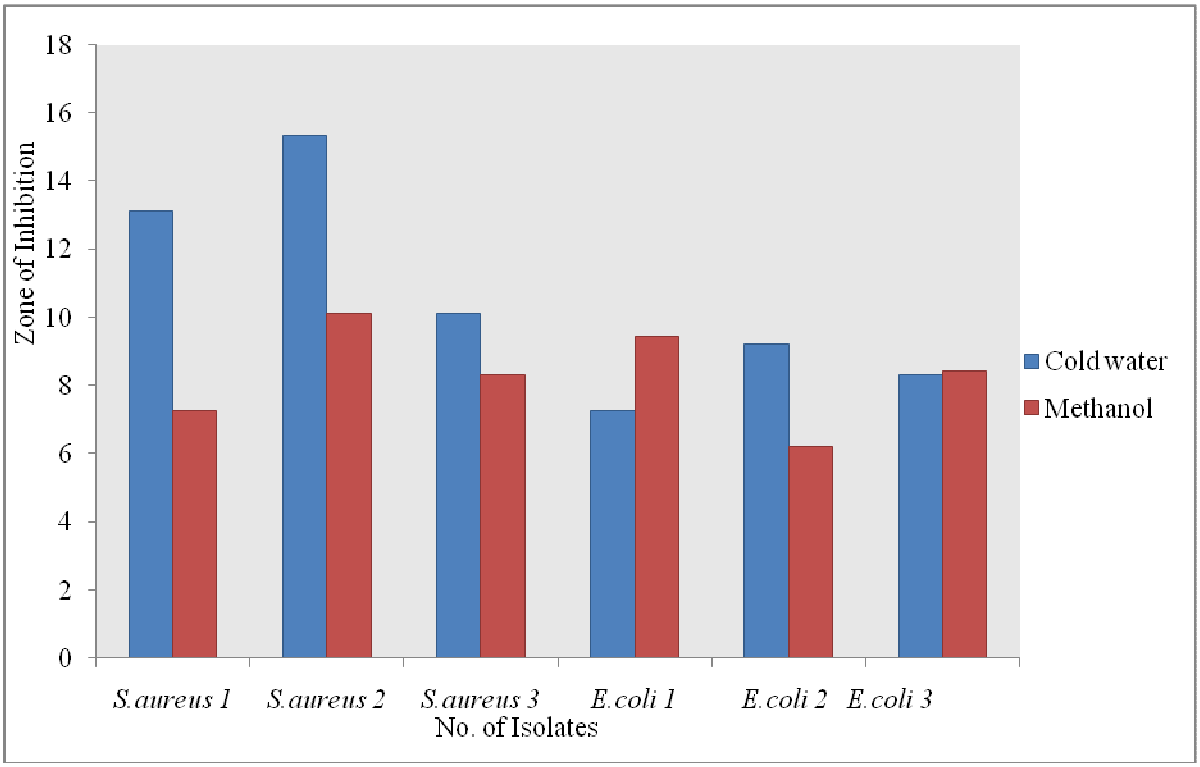

Figure 13: Antibacterial activity of Silver Nanoparticles using *Murrayakoingii*

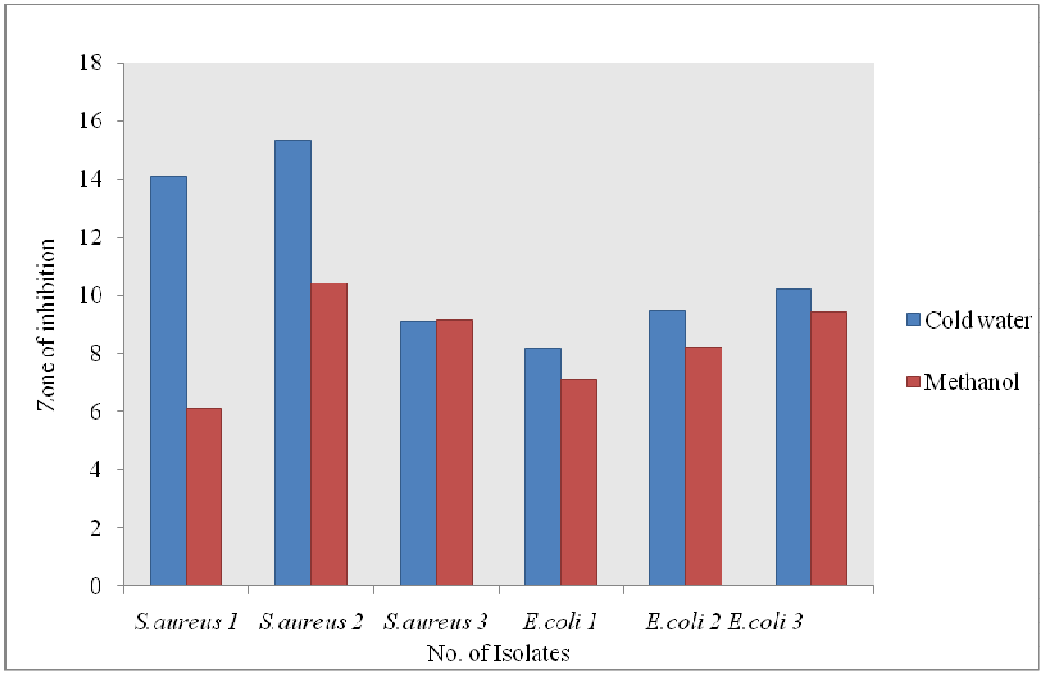

Figure 14: Antibacterial activity of Silver Nanoparticles using *Ocimum sanctum*

Figure 15:Antibacterial activity of Silver Nanoparticles

Table 5: Preliminary Phytochemical screening of *Moringaoleifera, Murrayakoingii*and *Ocimum sanctum*

S.No	Chemical compound	Name of the plant		
		Moringaoleifera	***Murrayakoingii***	***Ocimum sanctum***
1	Alkaloids	+	+	+
2	Flavanoids	-	-	-
3	Saponins	+	-	-
4	Tannins	+	+	+
5	Terpenoids	+	-	+
6	Coumerin	-	-	-
7	Cholesterol	-	+	+
8	Cardiac Glycosides	-	-	-
9	Phytotannins	-	+	-
10	Carotenoids	-	-	-
11	Carbohydrates	-	-	-
12	Phytosterols	+	+	+

CONCLUSION

In the present observation, green synthesis shows that the environmentally benign and renewable source of *Moringaoleifera*, *Murrayakoingii*and*Ocimumsanctum*are used as an effective reducing agent for the synthesis of AgNPs. This biological reduction of silvernanoparticles would be boon for the development of clean, nontoxic, and environmentally acceptable green approach to produce AgNPs, involving organisms even ranging to higher plants. The formed AgNPs are highly stable and have significant activity against diarrhea bacterial strains (*Escherichia coli*, *Staphylococcus aureus*).

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