CURCUMA ANGUSTIFOLIA RHIZOME EXTRACTS AS REDUCING AGENT IN SYNTHESIS OF SILVER NANOPARTICLES

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Abstract. Biological synthesis of silver nanoparticles was carried out from aqueous rhizome extracts of *Curcuma angustifolia,* by a microwave assisted approach. Dried rhizomes were solvent extracted with different solvents chosen in order of increasing polarity and were screened for the phytochemicals. For nano synthesis reaction mixture was subjected to microwave radiations at 400 W and a notable colour change from yellow to colloidal brown was observed after 30 min, which indicated the silver reduction. The nanoparticles synthesis was further confirmed by characterization using UV-VIS Spectrophotometer, FT-IR, PXRD, and SEM analysis. UV-Visible absorption spectra of the reaction medium containing silver nanoparticles showed maximum absorbance at 420 nm. FT-IR analysis confirmed reduction of Ag^+ ions to Ag ions indicating the presence of electron donor group in the aqueous extracts of the plant rhizome which would have acted as a reducing agent. The PXRD and SEM analysis revealed the particle size to be between 30–50 nm, and it was found to be spherical in structure. Antimicrobial efficacy against all the tested bacterial and fungal strains was confirmed. Cytotoxic potential of the nanoparticles was also evaluated. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages in being an ecofriendly and less time consuming protocol.

Key words: *Curcuma angustifolia*, silver nanoparticles, antimicrobial activity, cytotoxicity.

INTRODUCTION

Green nanotechnology using biological reducing and capping agents is emerging as a rapidly growing field with its application in science and technology [1, 2]. Though an array of metal ions are employed for nanosynthesis, silver nano particles are of great demand for commercial applications due to distinctive properties like stability, non-cytotoxicity, biocompatibility and anti microbial properties [8]. A wide variety of physico-chemical approaches are being used

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these days, however they often possess the threat of chemical contaminants for medical applications. Uses of plants extracts in nanoparticle synthesis have emerged as a viable alternative to chemical synthetic methods so as to eliminate use of toxic chemicals and high operational cost. The medicinally important plants like *Aloe vera* [3], *Solanum tricobatum* [9], *Acalypha indica* [7], *Erythrina indica* [12] have been reported as sources for silver nanoparticle synthesis.

The plants of genus *Curcuma* are very important for their therapeutic potentials and they have been reported to have antibacterial [5], anti-allergic, antioxidant [13], hepato-protective [6], anti cancerous [10], anti inflammatory [14] and immune stimulatory [4] properties. Rhizomes are a chief source of starch used as an excipient in medicinal tablets [11]. Considering the potentiality of the plant extracts as a source for developing environmental benign method for synthesis of nanoparticles, the project was designed to assess the effect of *Curcuma angustifolia* rhizome extract in the bioconversion of silver ions to nanoparticles.

MATERIALS AND METHODS

PLANT EXTRACT PREPARATION AND PHYTOCHEMICAL PROFILING

5 g powdered samples of *Curcuma angustifolia* rhizome were mixed with 40 mL solvents chosen in order of increasing polarity, namely methanol, ethyl acetate and chloroform for sequential extraction and kept in a shaker and centrifuged at 100 rpm for 24 hours. This process of extraction was repeated for 2 times and extracts were filtered and the filtrate was evaporated to dryness. It was then mixed well with 5 mL methanol and stored. The extracts were then screened for phytochemicals using standard test procedures.

THIN LAYER CHROMATOGRAPHY

The extracted samples were analyzed by thin layer chromatography using 7 cm × 3 cm sized silica gel 60 F254 plates. 5 μL of each sample was spotted on the plate. The spotted plates were placed in the pre-saturated TLC chamber, containing the solvent system toluene: ethyl acetate: formic acid in the ratio 5:5:1. The plates were then sprayed with 10% sulphuric acid, air dried and heated in an oven at 80 °C for 20 minutes for the development of spots. The spots were analyzed and their retention factor (*Rf*) values were calculated as follows:

$$Rf = \frac{Distance\ traveled\ by\ the\ solute}{Distance\ traveled\ by\ the\ solvent} \quad (1)$$

GREEN SYNTHESIS OF SILVER NANOPARTICLES

5 g of the rhizome derived from *Curcuma angustifolia* were boiled in 100 mL of de-ionized water and the extract was filtered through Whattmann's filter paper No.1. 2.5 mL of ammonium solution was added to 5 mL of 1 mM $AgNO_3$ solution, followed by addition of rhizome extract 1 mL and the final volume was adjusted to 50 mL by adding the appropriate amount of de-ionized water. This reaction mixture was subjected to microwaves at 400 W for different time intervals and observed for color change from yellowish to bright yellow and to dark brown.

UV-VISIBLE SPECTRA ANALYSIS

To determine the time point of maximum production of silver nanoparticles, the absorption spectra of the samples were taken 300–500 nm using a UV-visible spectrophotometer (Model-systronics AU 2701). The de-ionized water was used as the blank. The samples from the maximum time point of production of silver nanoparticles were air-dried and were subjected for further characterization.

SEM ANALYSIS

Scanning electron microscopy for the detection of morphological features of silver nanoparticles synthesized from *Curcuma angustifolia*, were done at Sophisticated Test and Instrumentation Center (STIC), CUSAT using Sophisticated Analytical Instrument Facility (SAIF). The size of nanoparticles was determined using scanning electron microscope (JEOL Model JSM – 6390LV) at an accelerating voltage of 15 kV.

FOURIER TRANSFORM INFRARED-SPECTROSCOPY ANALYSIS

FT-IR is an effective analytical instrument for detecting functional groups and determining data on covalent bonding. Synthesized nanoparticles were subjected to FT-IR spectrum analysis. Different functional group and bond stretching were identified.

ANTIMICROBIAL SCREENING

For antibacterial studies selected bacterial strains, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Bacillus subtilis,* and *Proteus mirabilis* of 24 hour culture were evenly spread onto the surface of sterile Muller-Hinton (MH) agar plates using sterile swab sticks. Fungal cultures of *Aspergillus niger, Mucar indicus* and *Rhizopus stolonifer* were used for the antifungal screening. Sterile discs saturated with plant extracts were placed on to the agar surface. DMSO served as negative control and tetracycline disc served as positive control. The plates were

incubated at 37 °C for 24 hours and observed for zones of inhibition. The zones of inhibition were then measured and antibacterial activity was expressed in terms of the mean diameter of zone inhibition in millimeters.

BRINE SHRIMP LETHALITY ASSAY

Brine shrimp nauplii (*Artemiasalina*) were obtained by hatching brine shrimp eggs in artificial sea water (3.8% non iodized sodium chloride solution) for 24 hours. Plant extracts at desired concentrations were taken as 200 µL, 100 µL, 50 µL, 25 µL, 12.5 µL and made up to 5000 µL using dimethyl sulfoxide (DMSO) with ten nauplii for each extract in vials. These vials were maintained at room temperature for 24 hours under the light and surviving larvae were counted using a magnifying lens. The mortality concentration data was calculated. LC50 values were obtained by best-fit line plotted as concentration *versus* percentage of mortality.

RESULTS

PHYTOCHEMICAL SCREENING

The phytochemical profiling showed the presence of an array phytoconstituents in the rhizome extract of *Curcuma angustifolia* species. Phytochemicals such as alkaloids, tannins, flavanoids, phenols, saponins, carbohydrates, glycosides and anthraquinones were present in the different extracts of *Curcuma angustifolia*.

Alkaloids, tannins, flavanoids, phenol, glycosides, and anthraquinone were present in chloroform, ethyl acetate, and methanol extract of *Curcuma angustifolia* variety. Saponins were only present in methanol extract. The results of the qualitative analysis of the phytochemicals present in the rhizome extract of *Curcuma angustifolia* under study are shown in Table 1.

Table 1

Qualitative analysis of phytochemicals in *Curcuma angustifolia*

Phytochemicals	C	E	M
Alkaloids	+	+	+
Tannins	+	+	+
Flavanoids	+	+	+
Phenols	+	+	+
Glycosides	+	+	+
Saponins	–	–	+
Anthraquinone	+	+	+
Carbohydrates	+	+	+

C – chloroform, E – ethyl acetate, M – methanol, + = presence, – = absence

THIN LAYER CHROMATOGRAPHY

TLC analysis of methanol, ethyl acetate and chloroform extracts from rhizome of *Curcuma angustifolia* showed the different *Rf* values indicating the presence of various compounds in the plant rhizome. 23 bands were observed in the crude rhizome extract from *Curcuma angustifolia*. Bands were observed at the *Rf* values 0.04, 0.15, 0.20, 0.25, 0.32, 0.48, 0.25, 0.70, 0.76, 0.89, 0.96 in methanol extract. For ethyl acetate extract, bands were observed at the *Rf* values 0.04, 0.11, 0.14, 0.20, 0.32, 0.42, 0.69, 0.76, 0.89, 0.97 and for chloroform extract *Rf* values were 0.07, 0.11, 0.16, 0.21, 0.32, 0.44, 0.47, 0.73 and 0.98. *Rf* value of the compounds present in the extract of plant under study are presented in Table 2. Thin layer chromatogram has been depicted in Figure 1.

Table 2

TLC analysis of rhizome extracts of *Curcuma angustifolia*

Rf values	Rhizome extracts		
	C	E	M
0.04	–	+	+
0.07	+	–	–
0.11	+	+	–
0.14	–	+	–
0.15	–	–	+
0.16	+	–	–
0.20	–	+	+
0.21	+	–	–
0.25	–	–	+
0.32	+	+	+
0.42	–	+	–
0.44	+	–	–
0.45	–	–	+
0.47	+	–	–
0.48	–	–	+
0.69	–	+	–
0.70	–	–	+
0.73	+	–	–
0.76	–	+	+
0.89	–	+	+
0.96	–	–	+
0.97	–	+	–
0.98	+	–	–

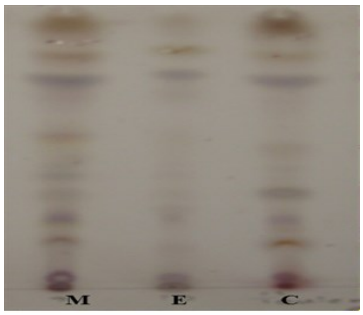

Fig. 1. Thin layer chromatogram of *Curcuma angustifolia* rhizome extracts; M – methanol, E – ethyl acetate, C – chloroform.

MICROWAVE ASSISTED NANOSYNTHESIS

Reaction mixture when subjected to microwave radiation showed a visible colour change from pale yellow to dark brown. The appearance of a colloidal brown colour clearly indicates the formation of silver nanoparticles in the reaction mixture. Figure 2 represents stages in silver nanoparticles synthesis.

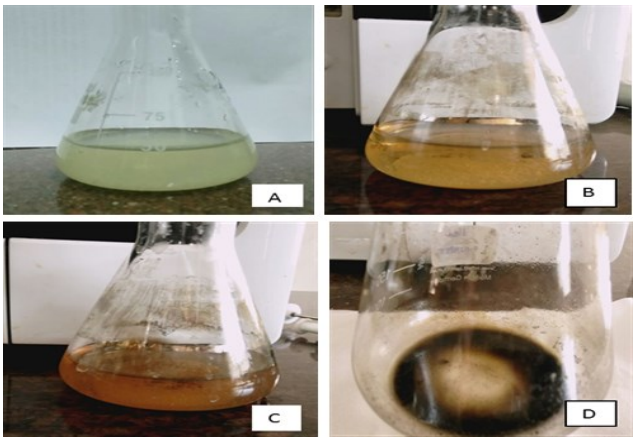

Fig. 2. Synthesis of silver nanoparticles using rhizome extracts of *Curcuma angustifolia*; A: reaction mixture, B: at 10 minutes, C: at 20 minutes, D: at 30 minutes.

UV-VISIBLE SPECTRA ANALYSIS

Reduction of silver ions into silver nanoparticles during exposure to rhizome extract was primarily confirmed by observing the color change attributed to the surface plasmon resonance phenomenon. The metal nanoparticles have free electrons, which give the absorption band, due to the combined vibration of

electrons of metal nanoparticles in resonance with light wave. Sharp bands of silver nanoparticles were observed around 420 nm in case of *Curcuma angustifolia* at 30 minutes as illustrated in Figure 3.

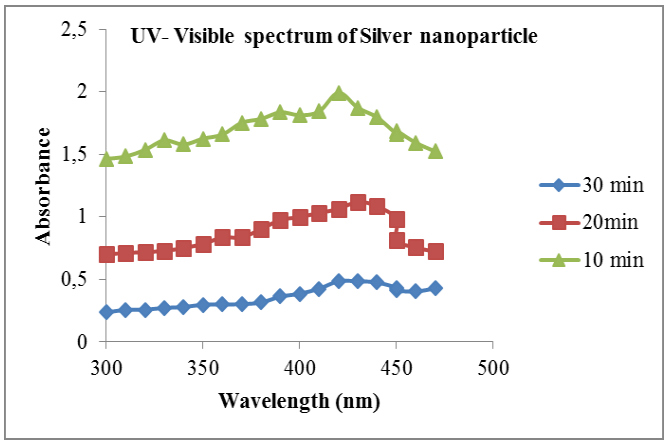

Fig. 3. UV-visible spectra of *Curcuma angustifolia* rhizome extracts at different time intervals.

SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy provided further insight into the shape and size details of the silver nanoparticles. SEM analysis revealed that the silver nanoparticles are predominantly spherical. The overall morphology of silver nanoparticle was recorded in the range of 30–55 nm. Figure 4 shows the SEM image of silver nanoparticles.

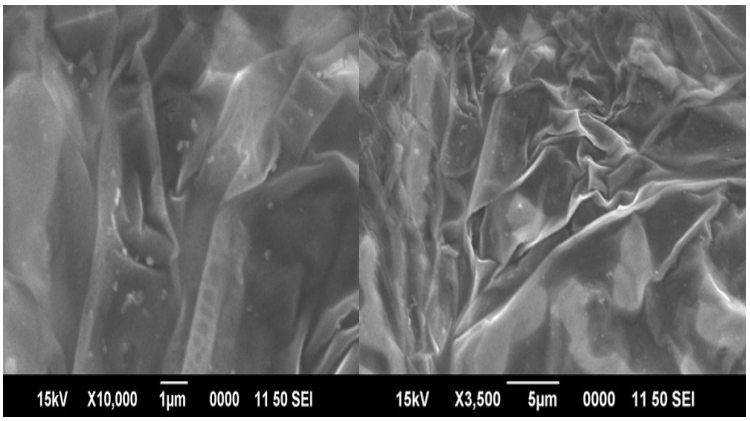

Fig. 4. SEM micrographs of silver nanoparticles.

FOURIER TRANSFORM INFRARED-SPECTROSCOPY ANALYSIS

To study the bonding nature of the resulting product the FT-IR spectrum was recorded. The absorption band of 3352.26 cm^{-1} is associated to vibration of O–H stretches. 1633.42 cm^{-1} absorption peak corresponds to the vibration of C=C stretches. The absorption peak of 1336.42 cm^{-1} is attributed by C–N stretches of amines. Peak of 1034.94 cm^{-1} is associated to the vibration of C–C stretches denoting N-alkynes. The absorption band of 666.70 cm^{-1} is linked with the vibration of C–H bending. The weak absorption band of 549.21 cm^{-1} is associated to the vibration of S–S stretches in cysteine. 563.06 cm^{-1} peak is associated to the vibration of P–O bonds in phosphate group. Minor bands at 599.56 cm^{-1} were also observed. Spectrum graph of siver nanoparticles has been depicted below in Figure 5.

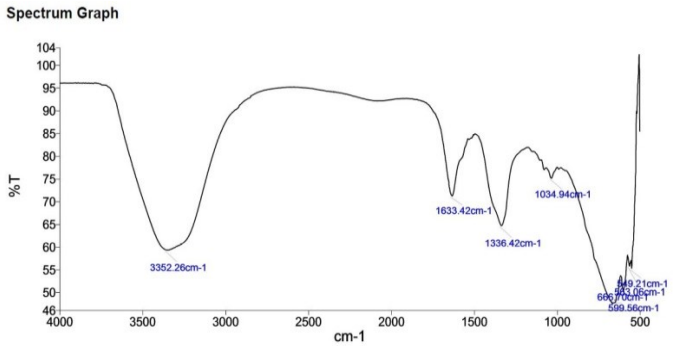

Fig. 5. FT-IR spectrum of silver nanoparticles.

ANTIMICROBIAL ACTIVITY

The tested *Curcuma angustifolia* rhizome extracts were active against all the tested bacterial organisms. Among the different extracts used the silver nanoparticles of plant extract show significantly higher activity against all bacterial organisms. The silver nanoparticles of plant extract show higher activity particularly against *Klebsiella pneumonia* and *Staphylococcus aureus* and also produced inhibition zones against *Aspergillus niger, Mucor indicus and Rhizopus stolonifer.* Methanol extract of *Curcuma angustifolia* showed similar activity against *Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Lactobacillus* and *Proteus mirabilis.* Ethyl acetate extract showed high inhibition zone against *Staphylococcus aureus* and least zone against both *Escherichia coli* and *Lactobacillus.* Chloroform extract showed more activity against *Klebsiella pneumonia* and *Lactobacillus.* All the rhizome extracts of *Curcuma angustifolia* showed maximum inhibitory zones against *Klebsiella pneumonia.* Table 3 and Figure 6 show the antibacterial activity of rhizome extracts of *Curcuma angustifolia* in mm.

The antifungal screening of different extracts of *Curcuma angustifolia* with the tested strains was less compared to the antibacterial screening. Out of all extracts silver nanoparticles of *Curcuma angustifolia* showed the maximum inhibitory zone against all fungal strains. Methanol, ethyl acetate and chloroform extracts showed the largest zones of inhibition against *Klebsiella pneumonia* and *Staphylococcus aureus*. The antimicrobial activity might be due to the presence of alkaloids, flavonoids, tannins, phenolic compounds, steroids, anthraquinones and saponins that may be attributed to the medicinal properties of plant. Table 4 and Figure 7 show the antifungal activity of rhizome extracts of *Curcuma angustifolia* in mm.

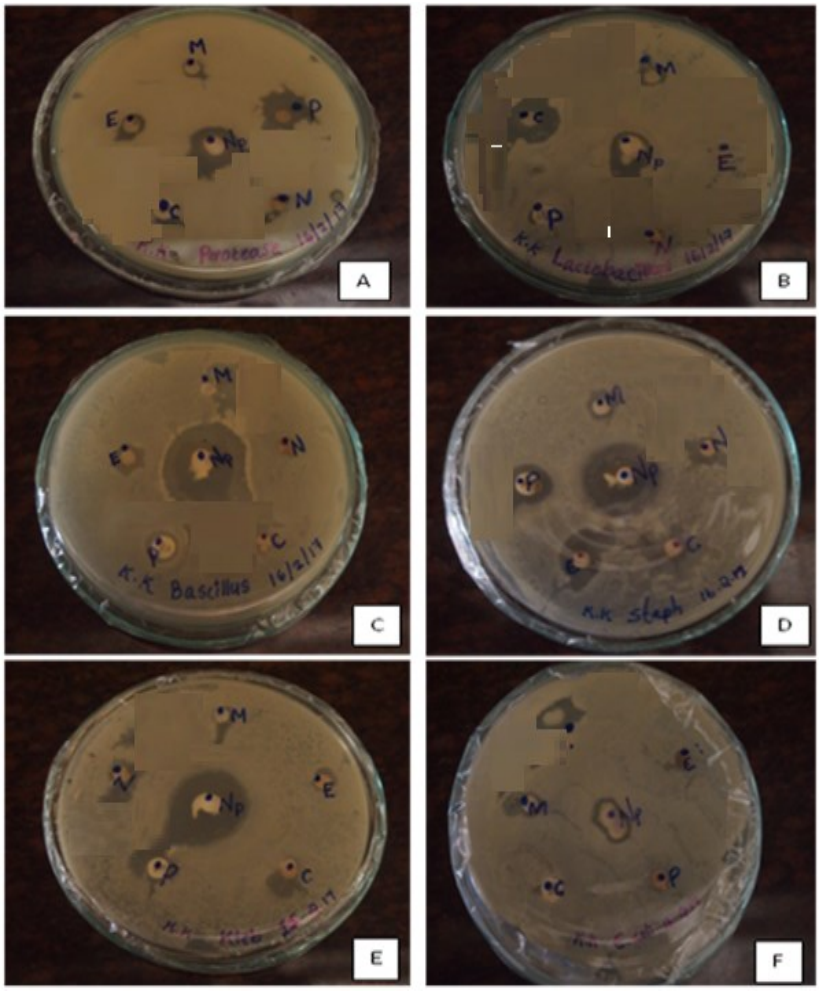

Fig. 6. Antibacterial activity of *Curcuma angustifolia*; A – *Proteus mirabilis*, B – *Lactobacillus*, C – *Bacillus subtilis*, D – *Staphylococcus aureus*, E – *Klebsiella pneumonia*, F – *Escherichia coli*.

Table 3

Antibacterial activity of rhizome extracts of *Curcuma angustifolia* (in mm)

Bacterial strains	N	C	E	M
Escherichia coli	10±0.52	8±0.5	6±0.13	6±0.25
Klebsiella pneumonia	24±0.2	10±0.2	10±0.1	6±0.2
Staphylococcus aureus	22±0.1	8±0.2	12±0.2	6±0.5
Proteus mirabilis	15±0.5	7±0.5	7±0.1	6±0.22
Bacillus subtilis	18±0.5	6±0.5	10±0.5	6±0.25
Lactobacillus	12±0.5	10±0.5	6±0.3	9±0.42

N – silver nanoparticle, M – methanol, E – ethyl acetate, C – chloroform

Table 4

Antifungal activity of rhizome extracts of *Curcuma angustifolia* (in mm)

Fungal strains	N	C	E	M
Rhizopus stolonifer	17±0.5	6±0.25	7±0.4	5±0.5
Aspergillus niger	13±0.3	8±0.25	5±0.4	5±0.22
Mucor indicus	13±0.52	7±0.2	5±0.22	5±0.2

N – silver nanoparticle, C – chloroform, E – ethyl acetate, M – methanol.

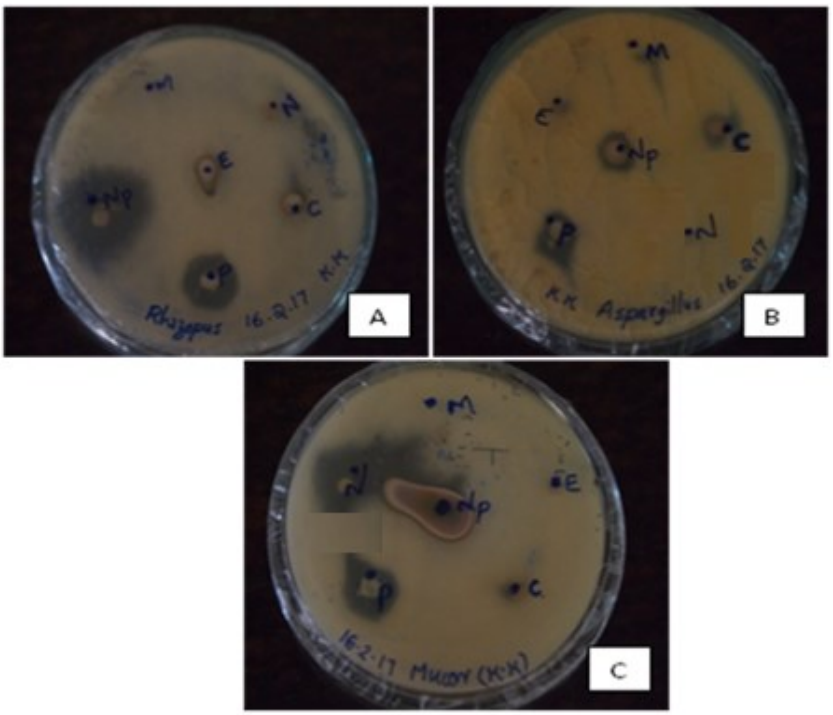

Fig. 7. Antifungal activity of *Curcuma angustifolia*; A – *Rhizopus stolonifer*, B – *Aspergillus niger*, C – *Mucor indicus*.

PXRD ANALYSIS

X-ray diffraction is a primary tool for probing structure of nano-materials. Size of the silver nanoparticles was determined to be 35 ± 1.5 nm approximately by substituting the obtained 2 theta and FWHM values in Debye-Scherer equation $D = 0.9\lambda/\text{Cos}\vartheta$. PXRD spectrum of nanoparticles is presented in Figure 8.

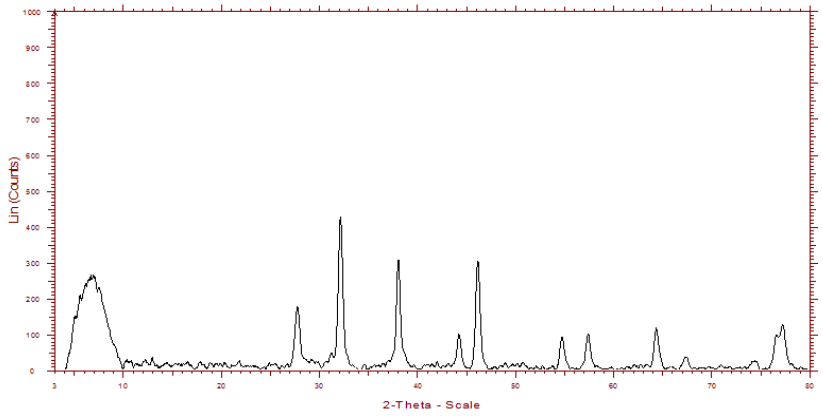

Fig. 8. PXRD spectrum of solid nanoparticles.

BRINE SHRIMP LETHALITY ASSAY

Cytotoxic potential of the plant extracts and the nanoparticles was confirmed by brine shrimp lethality assay. The percentage of mortality increased with increase in concentration of extract. The lowest value was found to be most potent. LC50 values ranged from 12.01 µg/mL to 15.51 µg/mL. The LC50 value of silver nanoparticle was 0.14 µg/mL. Silver nanoparticles showed high cytotoxic effect when compared to other extracts.

The cytotoxicity exhibited by the crude extracts was evident and this clearly indicates the presence of potent bioactive components. Table 5 represents the LC50 values of different extracts of the plant rhizome and Figure 9 plots the LC50 values of the nanoparticles.

Table 5

LC50 values of the different extracts of *Curcuma angustifolia* (in µg/mL)

Extract	Silver nanoparticles	Methanol	Ethyl acetate	Chloroform
Rhizome extract	0.14	15.51	12.01	13.56

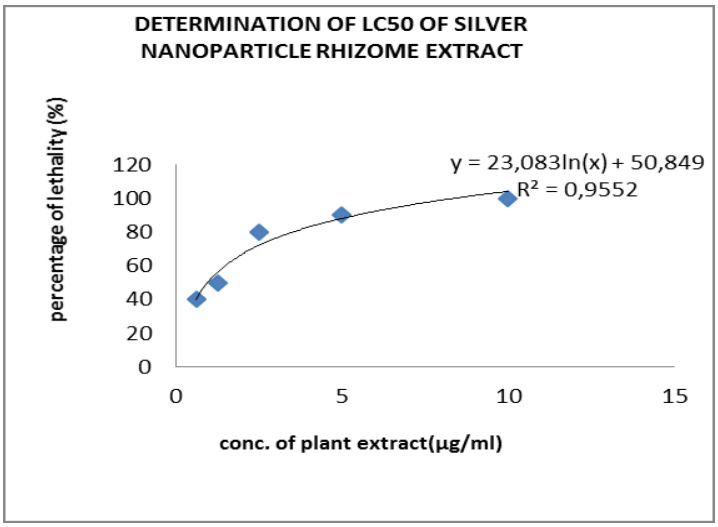

Fig. 9. Graph LC50 value of nanoparticles under study on brine shrimp lethality.

CONCLUSION

Synthesis was found to be efficient in terms of reaction time as well as stability of the synthesized nanoparticles which include *Curcuma angustifolia* rhizome extracts as reducing agents. Therefore, merging of nanotechnology and green chemistry proves to be an eco-friendly, rapid green approach providing a cost effective and an efficient way for the synthesis of silver nanoparticles. Phytochemical analysis of ethnomedicinal plants for secondary metabolites is an important area of fundamental research because of its relevance for the discovery of therapeutic agents and in providing clues for new sources of bioactive compounds. The synthesised silver nanoparticles showed efficient antimicrobial and cytotoxic activities thereby confirming the potential of plant derived nanoparticles in being used for biomedical applications.

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