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Bioinspired synthesis of silver nanoparticles and their mechanistic approach on antimicrobials using *C. dactylon*

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ABSTRACT

In recent years' nanoparticle have attracted interest because of their wide application in biomedicine. Nanotechnology has acquired interest because of their eco-friendly approach for the synthesis of the silver nanoparticle. This work demonstrates the efficacy of biologically synthesised Ag nanoparticle by means of *Cynodon dactylon*. Phytochemical analysis of *C. dactylon* suggests the presence of active constituents in polar solvents. Agnps were optimized using different parameters, and in general, the effect for temperature was 75°C within 1 hr, in a neutral condition, the concentration was found to be 1mM. Uv- vis analysis of synthesized silver nanoparticle indicates that the absorption peak was observed at 240nm. Absorbance peak at 2917^{cm-1}, 1648^{cm-1} indicate the presence of capping agent responsible for synthesis. A broad spectrum of antimicrobial activity was reported in 400µl of synthesised silver nanoparticle (23.3 mm) against P. *aeuroginosa*, followed by *Bacillus sp.* (22.8 mm) and *F. oxysporum* (22.1 mm). HPLC analysis indicates the presence of active constituents in the sample. This study reveals that *C. dactylon* acts as a potential source for nanoparticle synthesis.

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INTRODUCTION

Nanoparticles are often referred to as particles with a maximum size of 100 nm. In the midst of the fine metals (e.g., Ag, Pt, Au and Pd), silver (Ag) is preferred for prospective usage in the field of biological systems, organisms and medicine (Jain *et al.*, 2009). Silver nanoparticles are important materials that have been studied extensively. Methods opted for synthesis includes physical, chemical and biological methods (Annadhasan *et al.*, 2012; Abbasi *et al.*, 2012; Vijayaraghavan *et al.*, 2012). Chemical and physical methods have productively involved in nanoparticle synthesis, these processes are usually costly and involve the use of toxic chemicals. Moreover, these toxins are being adsorbed onto the surface of nanoparticles and cause undesirable effects. Therefore, the necessity for the bio-inspired synthesis of silver nanoparticles has become noteworthy. Persons infected with multidrug-resistant (MDR) bacteria are not easily treated and left hospitalized for extensive periods (Webb *et al.*, 2005). As a result, attempts to find a substitute for antibiotics has been developed in order to evade the further development of antibiotic resistance. Silver and its derivatives have been used as antimicrobial agents against a wide array of microbes (Pugazhenthiran *et al.*, 2008; Fayaz *et al.*, 2009; Xie *et al.*, 2007). Biological methods prove to be cost-effective, nontoxic and eco-friendly to generate Ag-NPs (Gerricke *et al.*, 2006, Harris *et al.*, 2008). Applications in diverse fields such as drug delivery (Keun *et al.*, 2008), biosensors (Amanda *et al.*, 2005), bioimaging (Mohammed *et al.*, 2009), antimicrobial activity (Mohammed *et al.*, 2010), food preservation (Mohammed *et al.*, 2009) have been reported.

However, an extensive literature survey revealed that there are minor reports (Rajendran *et al*., 2012) on nano synthesis using agricultural wastes. Silver ion is highly lethal to most microorganisms (Jung *et al*., 2008) and antimicrobial action of nanoparticles is through a deliberate release of silver ions via oxidation within or outside the cell.

Medicinal plants are prosperous in potential drugs, and it holds healthier and simple exchange for synthetic drugs (Rai *et al*., 2007). The English name of *Cynodon* is Bermuda grass (Harlan, 1970) and belongs to the family of Poaceae. It is native to East Africa, Asia, Australia and southern Europe. It is a weed and has been found to possess various potential medicinal properties (Singh *et al*., 2009). The plant is traditionally used as an agent to control diabetes in India (Kirtikar and Basu, 1996). Phytochemical constituents of *C. dactylon* indicates the presence of alkaloids, flavonoids, terpenoids, glycosides, steroids, saponins, tannins (Paranjpe 2011; Kumar *et al*., 2011; Annapurna *et al*., 2013; Abhishek and Thakur, 2012; Dhande and Khan, 2012). The plant was used for the treatment of diarrhoea, dysentery, wounds, haemorrhages and hyperdypsia. Fresh extract of the plant was used as a demulcent, astringent and in the treatment of dropsy, catarrhal ophthalmia, secondary syphilis, chronic diarrhoea and dysentery. Plant extract was used in hemuturesis, vomiting, to treat catarrhal ophthalmia, applied in wounds, chronic diarrhoea and dysentery (Auddy *et al*., 2003; Warrier *et al*., 1994; Nadkarnai A.K and K.M, 1995; Ferdinand 1986; Jolly and Narayanan 2000].

In the present study, silver nanoparticle was synthesized using *C. dactylon*, and the antimicrobial assay was performed. Plants were selected for the reduction process on the basis of their extraction method. Synthesized Ag-NPs were analysed by ultraviolet (UV)–visible spectrophotometer, Fourier transform infrared spectroscopy and scanning electron microscopy (SEM). In addition, the bactericidal activity of Ag-NPs was tested against bacteria and fungi, the biosynthesis of Ag-NPs was achieved by the reduction of silver nitrate using plant extracts as bio-reducing agents.

MATERIALS AND METHODS

Preparation of extract: Powdered plants were successively extracted with acetone, chloroform, petroleum ether, ethanol, methanol for 2 days. The solvent was allowed to evaporate using rotary evaporator under reduced pressure at 37º C. Condensed extract was refrigerated at 4ºC for further applications.

Qualitative phytochemical analysis: *C. dactylon* extract was subjected to phytochemical analysis by the method described by Harborne (1973). The extract was tested for the presence of bioactive compounds like alkaloid, flavonoid, glycosides, phenol, saponin, steroid, tannin and terpenoids.

Biosynthesis of silver nanoparticles: 90 ml of 1 mM $Agno_3$ solution was mixed with 10 ml of *C. dactylon*. The flask was kept in the dark condition at room temperature. The colour change was monitored for 4 to 6 days — the silver nitrate solution act as a control. As an outcome, a brown coloured solution indicates the development of AgNPs and the aqueous silver ions can be condensed by water extract of *C. dactylon* to produce even nanoparticles (Kumar *et al*., 2010).

Antimicrobial assay of Silver Nanoparticles: Biosynthesis of Agnp by means of *C. dactylon* were screened for antimicrobial activity through well-diffusion technique against pathogenic microbes. The pure cultures of organisms were subcultured on Nutrient broth at 35°C. Wells of 6 mm diameter were made on Nutrient agar plates using gel puncture. Using sterile cotton swabs, each strain was swabbed uniformly onto the Petri plates of the sample of water as a control, liquid culture filtrate, silver nitrate and silver nanoparticle were loaded onto the well using a micropipette. After incubation at 37°C for 48 h, the different levels of the zone of inhibition were calculated (Kirby Bauer *et al*., 1996).

Characterisation of silver nanoparticles

Synthesized silver nanoparticles were sampled at regular intervals, and the absorption maxima were scanned by UV–Vis spectra, at the wavelength of 200–700 nm in UV-3600 Shimadzu spectrophotometer at 1-nm resolution. Further, the reaction mixture was subjected to centrifugation at 15,000 rpm for 20 min, and the resulting pellet was dissolved in deionised water and filtered through a Millipore filter (0.45 μm). An aliquot of this filtrate containing silver nanoparticles was used for scanning electron microscopy (SEM), and Fourier transforms infrared (FTIR) studies.

UV-Vis spectroscopy: Leaves extract were subjected to 100 ppm $AgNO_3$ solution. The mixture was experimented and illustrated according to colour change, and one ml of the reaction mixture were withdrawn from time to time for investigation of surface plasmon resonance of silver nanoparticles using a UV-Vis spectrophotometer at the resolution of 1 nm in the range of 200 to 800 nm.

FT-IR analysis: The absorbance spectrum of nanoparticles was qualitatively confirmed by using FTIR spectroscopy (Stuart 2002). Experimentation of FTIR was done using Shimadzu FT-IR

Table 1: Phytochemical analysis of C. dactylon

Phyto constituents	Acetone	Chloroform	Diethyl ether	Methanol	Ethyl acetate	Ethanol	Petroleum ether	Water
Alkaloid	+	+	-	+	+	+	+	+
Flavanoid	-	-	-	+	-	-	-	+
Saponin	+	+	+	-	-	-	+	+
Tannin	+	-	-	-	-	+	-	+
Phenol	-	-	+	+	-	+	-	+
Glycosides	+	+	+	+	+	+	-	+
Terpenoid	-	+	-	-	+	+	-	-
Steroid	-	+	-	+	+	+	-	-

Table 2: Percentage of phytochemical constituents

Phytochemicals	*C. dactylon*(mg/10 gm)	
	Chloroform	Ethanol
Alkaloid	0.08±0.1	0.03±0.0
Flavanoid	0.24±0.04	1.3±0.1
Saponin	0.6±0.03	1.7±0.02
Tannin	0.8±0	1.2±0.1
Phenol	0.94±0.2	1.43±0.1

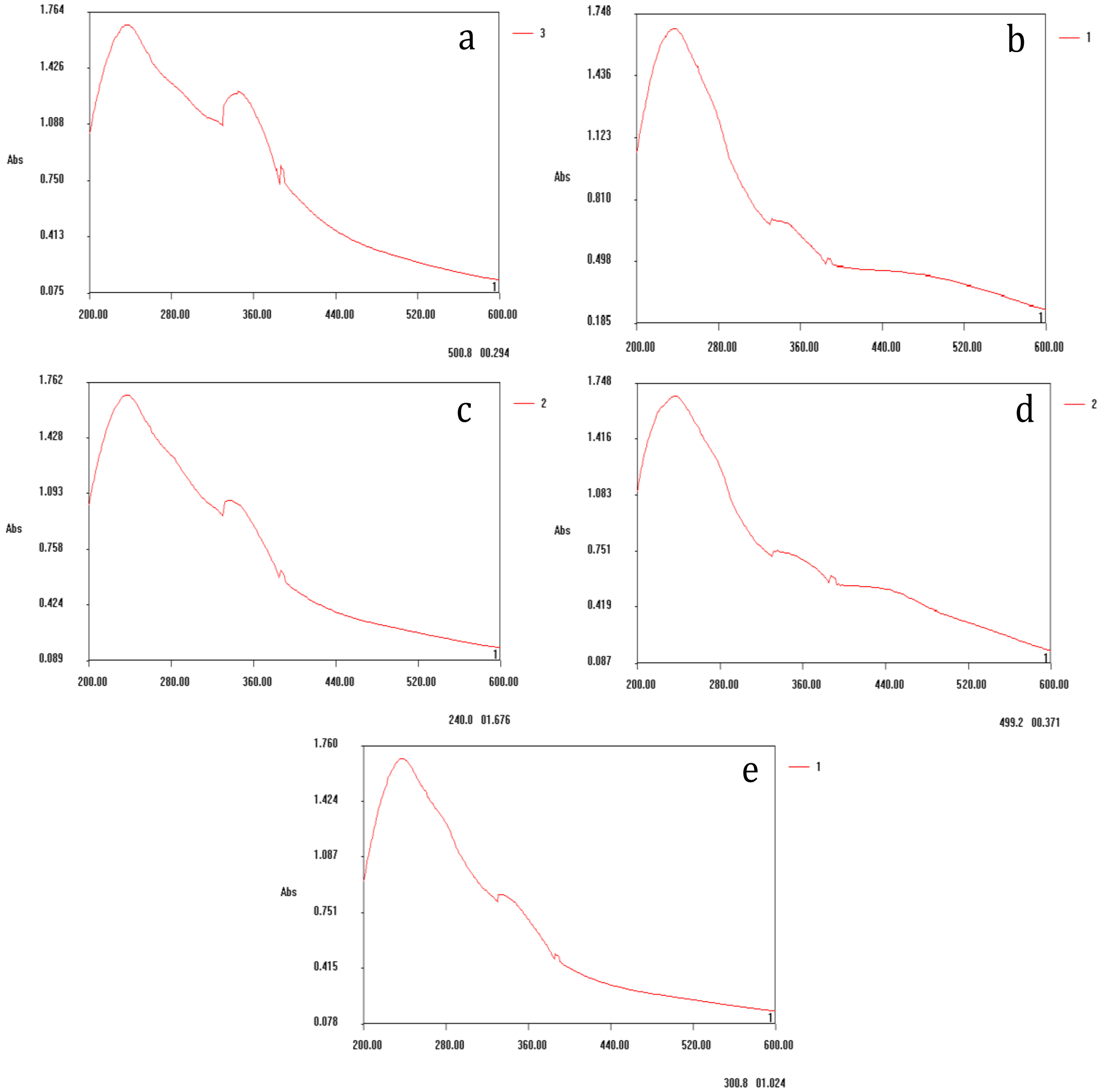


Figure 1: UV-vis spectrum of synthesized silver nanoparticle a) 75°C; b) 60 min; c) pH-4; d) pH-8; e) 1mM

Table 3: Antimicrobial activity of plant extract

		Cynodon dactylon			
S.no	Microorganism	Acetone	Ethanol	Ethyl acetate	Petroleum ether
1.	*Bacillus cereus*	16.3±0.2	15.5±0	17.1±0.2	14±0
2.	*Klebsiella pneumonia*	18.5±0.5	20.6±0.5	16±0.8	12.3±0.2
3.	*Pseudomonas aeuroginosa*	14.8±0.2	13.6±0.5	14.1±0.2	12.5±0.5
4.	*Staphylococcus aureus*	15.1±0.2	18.6±0.2	14.8±0.7	15±0
5.	*Escherichia coli*	15.5±0.5	18.8±0.5	14.6±0.2	18.8±0.2
6.	*Mycobacterium mucliaginosus*	23±0	18.1±0.2	20±0	19.5±0
7.	*Klebsiellaterrigena*	15.1±0.2	22.5±0	18.6±0.2	19±0
8.	*Fusarium oxysporum*	22.1±0.2	16.3±0.2	17.6±0.2	13.8±0.2
9.	*Penicillium*	19.5±0.5	14.5±0	16.6±0.2	15.5±0
10.	*Aspergillus niger*	17±0	15.5±0	16.1±0.2	15±0

*values are mean of ± S.D n=3

Table 4: Antimicrobial activity of synthesized silver nanoparticle

Microorganism	Plant samples used in the study Zone of inhibition in mm			
	Cynodon dactylon			
	100	200	300	400
Bacillus sp.	16.6±0.76	17.5±0	21.1±0.28	22.8±0.76
Escherichia coli	14.3±0.28	16.5±0	19.5±0.5	21±0.5
Mycobacterium mucilaginosus	13.5±0	15.5±0.5	17.6±0.76	20.3±0.28
Klebsiella terrigena	15.5±0	16.8±0.28	18.5±0.5	19.5±0
Pseudomonas aeruoginosa	18.5±0	18.5±0.5	22±0.5	23.3±0.28
Shigella	12±0.5	13.5±0	15.1±0.28	16±0
Staphylococcus epidermis	15.3±0.28	17.1±0.28	18.5±0	21.3±0.28
Fusarium oxysporum	13.6±0.5	16±0.8	17.6±0.2	22.1±0.2
Penicillium	14±0	15.5±0	16.3±0.2	19.5±0.5
Aspergillus niger	15±0	15.1±0.2	16.1±0.2	17±0

*values are mean of ± S.D n=3

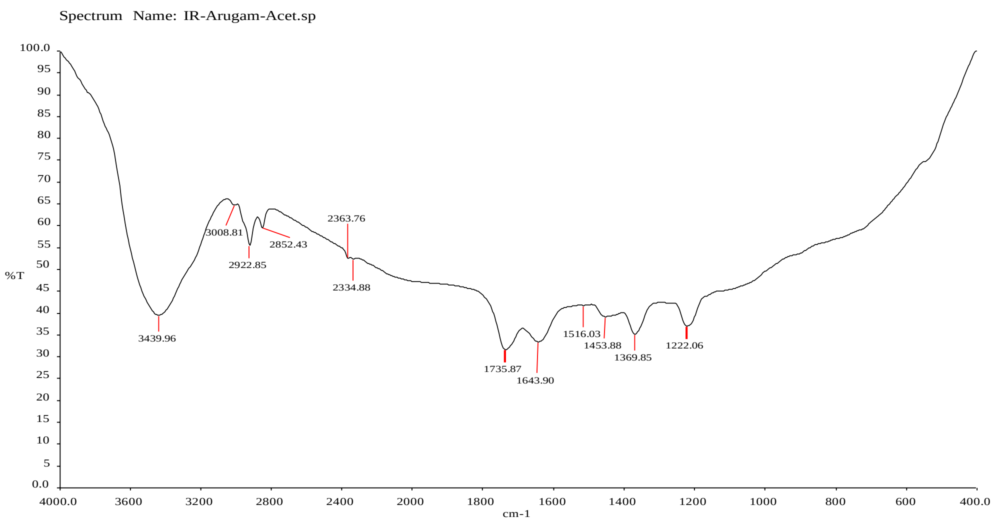


Figure 2: FTIR spectrum of biosynthesized Agnps using Cynodon dactylon

model number 8400. 3 mg of powdered leaves along with 300 mg of KBr was mixed well in mortar and pestle, and then pellets were prepared. Scans per sample were performed in a range of 400-4000cm^{-1}.

Scanning electron microscopy (SEM): Scanning electron microscope was done for assessing the configuration and composition of purified silver particles using a 10-kV ultra-high resolution. 1 ml of a solution containing purified Ag nanomaterials obtained after repetitive centrifugation was sputter coated on carbon-coated copper grids and the imagery descriptions of nanoparticles were studied using SEM.

RESULTS AND DISCUSSION

Phytochemical analysis of *C. dactylon:* Phytoconstituents in plants were identified by means of qualitative phytochemical screening is represented in Table: 1. Plants produce biologically active compounds like alkaloid, flavonoid, glycoside, saponin, tannin, steroid, phenols and terpenoids, which acts as a protective mechanism (Leyinson 1976). Extraction of phytochemicals was found to be effective in polar solvents such as ethanol, methanol and water. Ethanol extract of *C. dactylon* leaf extract possesses active constituents except for flavonoid and saponin, whereas in aqueous extract steroid and terpenoids were absent. Glycosides were found to be present in all of the extracts, except in petroleum ether. Terpenoids and steroid were found to be present in chloroform and ethanol extract. Methanol and aqueous extract displayed positive result towards flavanoid, whereas phenols displayed positive result towards diethyl ether, methanol and ethanol.

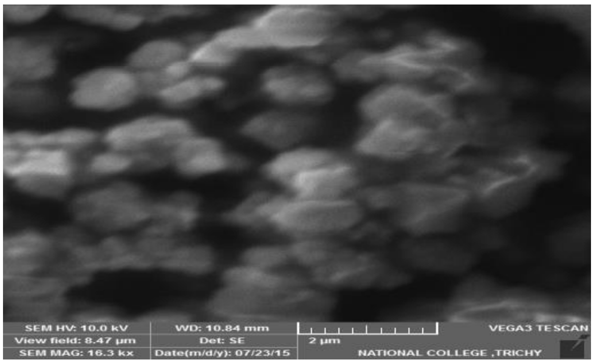


Figure 3: SEM analysis of synthesized Agnp using Cynodon dactylon

Percentage of phytochemical constituents in *C. dactylon*

Percentage of phytochemical constituent were represented in Table: 2. *C. dactylon* ethanol extract possess maximum amount of saponin (1.7 mg) followed by phenol (1.4 mg) and flavanoid(1.3 mg), whereas chloroform extract possess higher amount of phenol(0.94 mg), followed by tannin(0.8 mg) and saponin(0.6 mg).

Green synthesis of silver nanoparticle

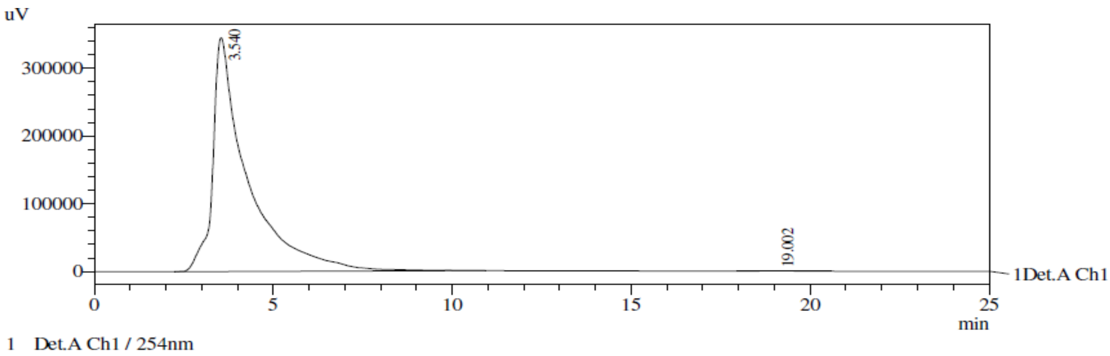
Photosynthesis of nanoparticles was confirmed by the formation of brownish colour. Plant along with silver nitrate acts as a catalyst which aids in the biosynthesis of nanoparticles. After the addition of plant extract of *C. dactylon*, the colour changed to light brown. Uv-visible spectroscopy helps to monitor the bioreduction mechanism of silver ions, and the readings were recorded periodically. Visualisation of brown colour in solution is owing to the excitation of surface plasmon vibrations in Ag nanoparticles (Krishnaraj *et al.*, 2010).

Optimisation studies for the synthesis of silver nanoparticles

Uv- vis analysis: Uv- vis analysis of synthesized silver nanoparticle was represented in Figure: 1(a-e). $Agno_3$ was mixed along with *C. dactylon* leaf extract, the development of Ag nanoparticles was recorded in Uv- vis spectra in the absorbance peak at 240 nm and broadening of peak confirms that the particles were found to be polydispersed. Different factors such as pH, temperature, time and concentration of silver nitrate were optimized for the production of Ag nanoparticles. The first aspect considered was temperature, the rate of silver nanoparticles formation increase by means of increasing the temperature at 75° C. Secondly, pH of the reaction medium plays a vital role in synthesis. Basic pH was found to enhance the rate of synthesis of Agnp. At low pH, the aggregation of nanoparticles forming superior particles was supposed to be favoured over the nucleation. At elevated pH level, functional assignments for binding of metals enhance the synthesis process. The time required for the completion of reaction contributes a significant role. Ag nanoparticles are formed at maximum duration. In the present study, synthesis has occurred at 60 min. The next factor was the concentration of Ag nitrate, which acts as a major source in the rate of synthesis. Therefore, maximum yield was obtained with 1 mM Ag nitrate solution. Besides that, the ratio of silver nitrate solution (1 mM) and the leaves extract was changed to examine the optimum composition to make the best use of the yield of silver nanoparticles. Therefore, in the present study rate of synthesis have occurred at 75°C in 60 min at the concentration of 1 mM and neutral pH plays a vital role in the synthesis of Agnp.

FTIR analysis of synthesized silver nanoparticle using *Cynodon dactylon*

FTIR analysis of synthesized silver nanoparticle was represented in Figure: 6. The functional assignments in *C. dactylon* leaf extract indicate the formation and stabilization of nanoparticles. Prominent IR bands were observed at 2848^{cm-1}, $1648\ ^{cm-1}$, $1457\ ^{cm-1}$. The peak at 3273 indicates C-H stretching due to alkynes. Alkanes, phenols and hydrogen-bonded carboxylic acid peak were depicted in $2917\ ^{cm-1}$corresponding to the OH stretch vibrations. The peak at $1648\ ^{cm-1}$ indicates C=C stretching vibrations of alkanes, NO_2 asymmetric stretching vibrations to nitro compounds and NH bending vibrations due to amines (Supraja *et al.*, 2013). The functional assignments at $1457\ ^{cm-1}$, $1383\ ^{cm-1}$ indicates deformation of a methyl group and NO_2 symmetric stretching vibrations to nitro compounds. Wavenumber obtained at $1238\ ^{cm-1}$ indicates C-O stretching due to alcohols, ethers, carboxylic acids, ethers and esters, NO_2 symmetric stretching vibrations to nitro



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.540	23349091	344229	99.831	99.812
2	19.002	39568	647	0.169	0.188
Total		23388659	344876	100.000	100.000

Figure 4: HPLC analysis of Cynodon dactylon

compounds and C-N stretching due to amines. Absorbance bands were observed at 1522 $^{cm-1}$, 1340 $^{cm-1}$, these bands are known to be associated with the stretching vibrations for –C–C– [(in-ring) aromatic], C–O–C

(ethers) And C–O (–C–OH). FTIR analysis confirmed that the bioreduction of Ag+ ions to silver nanoparticles is due to the reduction by capping material of plant extract. Reduction of Ag ions has occurred because of the polyol groups, where they get oxidised to unsaturated carbonyl groups leading to a broad peak at 1660 $^{cm-1}$ (for reduction of Ag).

SEM analysis of synthesized silver nanoparticle using *Cynodon dactylon*

SEM image of the manufactured silver nanoparticle using *C. dactylon* was depicted in the Figure: 7. The image depicts the visualisation of dimension, nature and range of Ag nanoparticles. The nanoparticles were polydispersed and roughly spherical.

HPLC analysis of *Cynodon dactylon*

The highest peak was seen at the retention time at 3.54 min. After comparing with HPLC chromatogram of standard, shows the presence of phytochemicals like phenols and flavonoids.

Antimicrobial activity of plant extract

Preliminary screening of antibacterial assay of *C. dactylon* extract experimented against a variety of pathogens such as *Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumonia, Klebsiella terrigena, Pseudomonas aeruoginosa* and *Staphylococcus aureus* using well diffusion technique were represented in Table: 3. Higher resolving strength of ethanol yields maximum percentage, comparatively more bioactive compounds to produce considerable antimicrobial activity. Therefore, the results suggest that ethanol has a superior activity for extraction of bioactive constituents when compared to ethyl acetate (Madigan *et al*., 2009). The maximum inhibitory effect was recorded in acetone (23 mm), and ethyl acetate (20 mm) extract against *Mycobacterium mucilaginous*. *E. coli* remained sensitive towards ethanol and petroleum ether extract possessing an inhibition of 18.8 mm, followed by *S. aureus* (18.6 mm) and *K. pneumonia* (18.5 mm). Ethanolic extracts possess active bioconstituents like phenolic, saponins and terpenoids which generates a wide spectrum of antimicrobial action (Kafaru 1994, Singh and Gupta, 2008). Moderate zone of inhibition was observed in ethyl acetate (17.1 mm), and acetone (16.3 mm) extract against *Bacillus cereus*. The minimum inhibitory effect was recorded in petroleum ether extract against *B. cereus* (14 mm) and *P. aeuroginosa* (13.6 mm).

Fungicidal assay of plant extract experimented through various fungi such as *Fusarium oxysporum*, *Penicillium* and *Aspergillus niger*. Inhibition range was found to be superior in acetone extract against *F. oxysporum* (22.1 mm) and *Penicillium* (19.5 mm), whereas the zone of inhibition was found to be moderate in ethyl acetate (17.6 mm) against *F. oxysporum* and acetone (17 mm) against *A. niger*. Minimum zone of inhibition was observed in petroleum ether (13.8 mm), and ethanol (14.5 mm) extract.

Microbicidal screening of photosynthesised Agnp nanoparticle using *C. dactylon*

Microbicidal assay of synthesized Ag nanoparticle using plant samples was represented in Table: 4. Experimentation was done in the activity of metal nanoparticles in *E. coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Salmonella enteric, typhi*(all species of Gram-negative bacteria) was reported by Morones *et al*. Previous reports suggest that phytomediated synthesis of metal nanoparticles using plant extract have been recorded. (Gardea-

Torresdey *et al*., 2003; Park *et al*., 2011). Invading mechanism of nanoparticles through the bacteria was detected by means of their interaction with cells and membrane. In the present study broad spectrum of antimicrobial activity was reported at 400µl of the synthesised silver nanoparticle using *C. dactylon* (23.3 mm) leaf extract against *P. aeuroginosa*, followed by *Bacillus sp*. (22.8 mm) and *F. oxysporum* (22.1 mm). Metal synthesis of Ag nanoparticle was performed by fungi isolated from soil was reported by 300 µl of synthesised silver nanoparticle possess maximum inhibition against *P. aeuroginosa* (22 mm) followed by *Bacillus sp*. (21 mm) and *E. coli* (19.5 mm). When Agnps is incubated along with bacteria such as *S. aureus*, it spoils the cell mechanism by invading through the cells and leads to death. Therefore pits have been formed in bacterial cells (Tarad *et al*., 2017). The zone of inhibition was found to be moderate at *P. aeuroginosa* (18.5 mm), *Bacillus* (17.5 mm) and *S. epidermis* (17.1 mm). The minimum inhibitory effect was recorded against *M. mucilaginous* (13.5 mm) and *Shigella* (12 mm).

CONCLUSION

Therefore, in this study bioinspired synthesis of Ag nanoparticle was achieved using *C. dactylon*. Different techniques used to characterize AgNPs showed the successful capping of phytoorganic components on silver, to form well – dispersed, spherical nanoparticles. The bioactive potential is confirmed by means of functional assignments on their surface group. Therefore, the facile approach is done by means of plant extract based metal nanoparticles and shows an alternative promise in biomedical applications.

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