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Phytochemical and Antimicrobial of Latex Serum of *Calotropis Procera* and its Silver Nanoparticles Against Some Reference Pathogenic Strains

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Abstract: The present study was carried out to evaluate the potential antimicrobial activity of latex serum extracts (*ethanol*, methanol, chloroform and aqueous) of *Calotropis Procera*, against some reference pathogenic strains of bacteria and fungi, Results revealed that the latex extracts showed a varying degree of inhibition against all tested microorganisms. The aqueous and ethanolic extracts were more effective antimicrobials against all tested strains, while chloroform extract showed only antibacterial activity. In addition, Phytochemical analysis were also carried out to assess the presence of bioactive compounds in the plant latex serum. The analysis revealed the presence of Cardiac glycosides, Saponins, Phenolic compound, Terpenoids, Alkaloids, Flavonoids, Tannins, and Resins in some of the extracts. The concentration of the various classes of secondary metabolites varies amongst the extracts evaluated. The ethanolic and the aqueous extract were rich in Saponins, Terpenoids and flavonoids than in methanolic extract, while the chloroform extract was poor in their phytochemical contents where it contains only Cardiac glycosides and Terpenoids. The present investigation was concerned also with the synthesis of latex silver nanoparticles (LAg-NPs) using the latex of *Calotropis procera* as reducing and capping agent, the synthesized LAg-NPs were characterized using UV-VS spectrophotometer analysis, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD) and Transmission Electron Microscopy (TEM). Further, the antimicrobial effect of the synthesized silver nanoparticles was determined against the reference pathogenic strains and compared with the effect of crude latex and latex solvent by agar well diffusion methods. The synthesized nanoparticles exhibited a variable growth inhibition against the tested strains. A significant increase in the effect of nanoparticles compared with crude latex and latex serum.

Keywords: Phytochemical; Antimicrobial; *Calotropis procera*; Pathogens; silver nanoparticles

1 Introduction

Nowadays many research efforts have been directed towards the development of drugs from medicinal plant for the treatment of microbial disease [1]. Plants are an important source of drugs; especially in traditional medicine, It is a common practice in many parts of the world to use plant in the form of crude extracts or tincture to treat common and chronic infection[2, 3]. According to WHO, about 80% of individuals in developing countries still use plants as remedies from many diseases, using their own personal recipes which have been passed through generations [4]. The medicinal value attributed to plants is a function of the bioactive phytochemical constituents [5]. The most important of these plants bioactive chemical constituents are flavonoids, alkaloids, tannins and phenolic compounds [6, 7]. *Calotropis procera*, also known as "**Ushar**" is a species of flowering plant which belongs to the family *Asclepiadaceae*; xerophytic, erect shrub, growing widely throughout many regions of Africa and Asia [8]. This plant is popularly known due to the abundance of latex in its green parts which is easily collected when the plant is wounded and it is used for the treatment of various diseases in different parts of the world [9]. Several reports in the literature indicate many therapeutic activities of *C. procera* including analgesic [10], anti-inflammatory [11], cytotoxic [12], anticancerous and hepatoprotective effects [13], and as antimicrobial agents [14].

Silver nanoparticles (AgNPs) have been widely used in

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many biological and pharmaceutical applications because of its antimicrobial properties [15]. Green methods used for synthesis of nanoparticles usually involve using of medicinal plants and microorganisms for pharmaceutical and biological applications [16, 17]. Many literatures have beenreported the syntheses of silver nanoparticlesusing microorganisms including bacteria, fungi andplants; because of their reducing propertiestypically responsible for the reduction of metal compoundsin their respective nanoparticles. The use of plant extracts ispotentially advantageous over microorganisms because of the easeof improvement, the less biohazard and elaborate process ofmaintaining cell cultures [18]. It is environmentally friendly, cost effective as compared to the other physical and chemical methods. It was also interesting that silver nanoparticles were able to exert inhibitory effect at a concentration that is below their cytotoxic limits. So they were regarded as safe to be used as antimicrobials [19]. Many recent reports were published on biosynthesis of AgNPs using plant latex [20, 21], natural rubber latex [22] or by the whole plant showing promising biological activities such as cytotoxic and antimicrobial activities [17, 19].

The present investigation was designed to study the phytochemical and antimicrobial of latex serum of *Calotropis procera* and its silver nanoparticles against some reference pathogenic microorganisms compared with the reference antimicrobial agents.

2 Material And Methods

2.1 Plant Latex Collection

Latex of *Calotropis procera* was collected from Industrial Zone, Arab El-Awamer, Abnoub, about 20 km north Assiut city, Egypt. It was collected in the early morning by scratch the leaves near the stem or by incision the trunk of the plant, the white milky latex was receiving into a sterile wide necked screw-capped bottles, with slightly shaken during collection to avoid coagulation, the bottles were closed and stored at 4 °C until used.

2.2 Fractionation of plant latex

Plant latex was centrifuged at 17000 rpm for 20 min at 4 C° by using centrifuge model (a Hermle Z 200A, Germany). It was separated into three layers: rubber, serum and lipids. The serum layer was lyophilized and powdered. Ten grams from the lyophilized latex serum of *C. procera* dissolved in 100 ml of different organic solvents (methanol, ethanol, chloroform) and aqueous solvent (water). With the Soxhlet extractor, each of the four solvents were allowed to remaining in contact with the latex powder for 24 h, then the filtrate was evaporated using vacuum oven (model Gallenkamp) at 45°C in order to remove the excess solvent .The obtained residue was kept in sterile bottle at (4°C) until used. [8, 23].

2.3 Phytochemical Analysis

Dry latex extracts were subjected to the phytochemical screening for alkaloids, phenolic compounds, flavonoids, terpenoids, tannins, saponins, resins and cardiac glycosides. by the methods described as the following[24,26].

2.3.1 Test For Alkaloids

Few drops of dilute HCL and 0.5 ml Wagner's reagent (1 g iodine and 2g KI in 300 ml H_2O) were added to a portion of latex extracts. A brown flocculent precipitate indicates the presence of alkaloids.

2.3.2 Test For Phenolic Compounds

Phenolic compounds were detected by mixing a portion of the latex fraction with few drops of diluted FolinCiocalteu reagent (mixture of phosphomolybdate and phosphotungstate) and aqueous sodium carbonate solution. The mixture was allowed to stand for 10 min and formation of gray color indicates the presence of phenolic groups.

2.3.3 Test For Flavonoids

The qualitative detection of flavonoids was assessed bytwo methods. (1) A portion of latex extract was dissolved in 10% HCl, and then a small amount of Zinc powder was added. Appearance of effervescences with pink color indicates the presence of flavonoids. (2) Latex extract was dissolved in concentrated H_2SO_4, and the formation of intense color indicates the presence of flavonoids.

2.3.4 Test For Terpenoids

A red to purple color formation indicates the presence of Terpenoids, when a portion of latex extraction was treated with an equal volume of concentrated H_2So_4.

2.3.5 Test For Tannins

A portion of latex extraction was mixed with few drops of 0.1% ferric chloride. The development of brownish green coloration indicates the presence of tannins.

2.3.6 Test For Saponins

One ml of the latex extraction was dissolved in 5 ml of distilled water in a test tube. The solution was vigorously shaken and observed for a stable persistent foamas evidence for the presence of Saponins.

2.3.7 Test For Resin

One ml of extraction was treated with few drops of acetic anhydride solution followed by one ml of conc. H_2So_4. Resins give coloration ranging from orange to yellow.

2.3.8 Test For Cardiac Glycosides (Keller Kelliani's test)

Five ml of latex extraction was treated with 2 ml of glacial acetic acid in and a drop of ferric chloride solution. This was carefully underlay with 1 ml concentrated sulfuric acid. A brown ring at the interface indicated the presence of deoxy sugar characteristic of cardenolides.

2.4 Synthesis Of Latex Silver Nanoparticles (LAg-NPs)

About 20 ml of plant latex serum was mixed with 80 ml of silver nitrate (0.1 M) in 250 ml Erlenmeyer conical flask, with continuous stirring at 40 C° within 60 min a yellow coloration appeared, indicating the onset of LAg-NPs formation. the mixture was further incubated in water bath until the color of solution became stable. At the same condition, latex serum without $AgNo_3$ solution saved as control and run along with the experiment.

2.5 Characterization Of LAg-NPs

Formation of LAg-NPs noticed visibly through gradual change in the color of the reaction mixture then was subjected to optical measurements using an UV-Vis spectrophotometer scanning using Perkin-Elmer Lamda-45 spectrophotometer, in a 1cm path quartz cell at a resolution of 1 nm in range of 200 nm to 800 nm. Transmission electron microscopy (TEM) analysis using JEOL-JEM-100 CXII (Electron Microscope Unit, Assiut University, Egypt) with secondary electron detectors at an operating voltage of 20 kV was used to record the size and the morphology of the of synthesized LAg-NPs (dry powder). The dried powder of latex nanoparticles was used for X-ray diffraction (XRD) analysis using X-ray diffractometer (Model PW 1710 control unit Philips Anode Material Cu, 40 KV, 30 M.A, optics: Automatic divergence slit) with Cu Kα radiation λ=1.540562 °A. FTIR spectrum of the synthesis mixture was recorded on FTIR Nicolet Avatar 660 FTIR spectrometer in the range of 4000-400 cm^{-1}[27].

2.6 Antimicrobial Activity

The crude latex, latex solvent extracts and synthesized latex nanoparticles were individually screened for their antibacterial and antifungal activity.

2.6.1 Tested Microorganisms

Cultures of six pathogenic reference strains used in this study were related to Gram-negative bacteria (*Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027), Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538P and *Bacillus subtilis* ATCC 6633) and fungal strains of (*Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404). All strains were obtained from Assiut University Mycological Centre (AUMC). Bacterial and fungal strains were sub-cultured on nutrient agar and Sabouraud dextrose agar (SDA) respectively, stored at 4 °C until use.

2.6.2 Culture Media And Inoculum Preparation

Mueller-Hinton broth medium was inoculated with bacterial strains and incubated at 37°C for 24 h and Sabouraud dextrose broth for fungal strains and incubated at 28°C for 48 h. after incubation period each culture was diluted with sterile normal saline NaCl 0.9 % to bring optical density (OD) value 0.260 nm that is equivalent to turbidity of 0.5 McFarland units (10^8 CFU/ml)[28, 29].

2.6.3 Working Concentration

The antimicrobial activity of crude latex, latex serum, latex solvent extract and latex nanoparticles against the tested microorganisms was determined using the agar well diffusion method.A stock solution was prepared by weighing (10 mg) from each one of dry extract, crude latex and latex serum, dissolved in 1ml of Dimethyl Sulfoxide (DMSO) in sterile Eppendorf separately, which results in concentration of 10,000 µg/ml. From crude latex and latex serum, five different concentrations (50, 100, 200, 250 and 300 µg/ml) were prepared, as the same as from latex fraction and latex nanoparticles five different concentration (25, 50, 75, 100 and 125 µg/ml) were prepared and used for testing their antimicrobial activities [30, 31].

2.6.4 Antimicrobial Activity Bioassay

Under aseptic conditions, the prepared inoculums were spreaded by glass spreader until totally absorbed in Muller Hinton agar (for bacteria) and SDA (for fungi). Wells were made by punching into the agar surface with a sterile cork borer. Into each of these wells, 100 µL from each concentration were added separately by using a micropipette. The plates were incubated at 37°C for 24 h and 28°C for 48 hr., respectively for the bacterial and fungal cultures. The antimicrobial activity was assessed by measuring the diameter of the inhibition zone formed around the wells. Streptomycin and Clotrimazole reference antibiotics were used as a positive control [32]. Finally, the minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibit the growth of each strain, All tests were carried out in triplicates [33].

2.6.5 Determining The Growth Curve Of Crude Latex, Latex Serum And Latex Nanoparticles Against Three Selected Standard Pathogenic Strains

An overnight cultures form Gram Negative bacteria *E. coli* ATCC 8739 and Gram-Positive bacteria *S. aureus* ATCC 6538P were inoculated in Mueller-Hinton broth and *C. albicans* ATCC 10231 in Sabouraud dextrose broth and adjusted to equivalent turbidity of 0.5 McFarland standard. Each tested strain supplemented individually with minimum inhibitor concentration from each of crude latex,

latex serum and latex nanoparticles (LAg-NPs), control culture was treated in a similar fashion but without any supplements, Then the cultures were incubated at its specific temperature with rapid shaking at 150 rpm/min. The microbial growth was determined by measuring the optical density using UV-vis spectrophotometer each hourat λ=600 nm. The growth curve was plotted between optical density and time.

3 Results

3.1 Fractionation

Results revealed that latex of *C. procera* when centrifuged at 17000 rpm for 20 min at 4°C was separated into three layers; Sticky top layer containing natural rubber; clear middle fraction of serum, and small amount of heavy fraction at the bottom (lipids). The obtained serum when treated with the four-different solvent gave various percentage of fraction yield. Highest yield (8.5 g) was from aqueous extract, (5.2 g) and (4.6 g) in chloroform and methanol extract respectively, and the lowest yield from ethanol (1.2 g) % as showed in Table1.

Table 1. Percentage yield of *C. procera* latex serum extract in different solvent.

Solvent	Latex serum dry weight (g)	Solvent volume (ml)	Final extract weight (g)	Percentage %
Aqueous	10	100	8.5	85
Chloro form	10	100	5.2	52
Methanol	10	100	4.6	46
Ethanol	10	100	1.2	12

3.2 Phytochemical analysis of bioactive compound in latex extracts of C. Procera

The latex extracts were screened for the presence of various bioactive compounds. The screening revealed the presence of cardiac glycosides, saponins, phenolic compound, terpenoids, alkaloids, flavonoids, tannins, and resins in some of the extracts, with a note that the concentration of the various classes of secondary metabolite varies amongst the extracts evaluated. The ethanolic and the aqueous extract were rich in saponins, terpenoids and flavonoids more than in methanolic extract. While the chloroform extract was poor in their phytochemical contents where it contains only cardiac glycosides and terpenoids as show in Table 2.

Table 2. Phytochemical composition of bioactive compound in latex extracts.

Bioactive compounds	Latex extraction of *C. procera*			
	Methanol	Ethanol	Chloroform	Aqueous
Cardiac glycosides	+	+	++	+
Saponins	+	++	-	++
Phenolic compound	+	+	-	-
Terpenoids	-	++	+	++
Alkaloids	+	+	-	+
Flavonoids	+	++	-	++
Tannins	-	-	-	+
Resins	+	-	-	+

3.3 Synthesis Of Silver Nanoparticles From Latex Of *C. Procera*

3.3.1 Visual Observation

It has been observed that, addition of latex serum to silver nitrate solution ($AgNO_3$ 0.1 M) changes the color of solution to yellowish brown after 50 minutes and turned to brown after 3 hours and the color become stable after 5 hours (Figure 1)

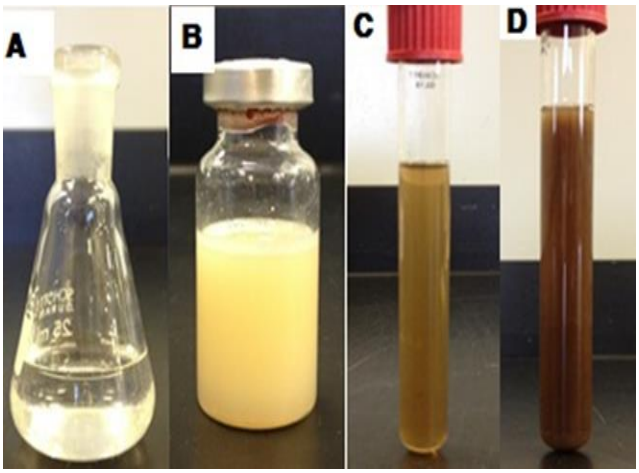


Fig 1. A) Silver nitrate (0.1 M), B) Plant latex, C) latex supplemented with silver nitrate After 50 min, D) After 5 hours

3.3.2 Characterization Of LAg-NPs

*3.3.2.1 UV-Vis Spectral Analysis*UV-VS spectrophotometer analysis showed a sharp peak at 280 nm corresponding to the Plasmon absorbance of silver nanoparticles (Fig 2)

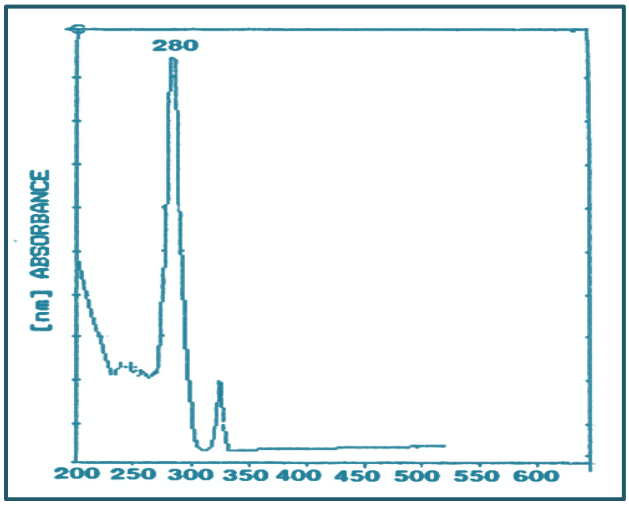


Fig 2. UV-VIS spectral analysis of LAg-NPs absorption band around 280 nm.

3.3.2.2 FTIR Analysis Of LAg- NPs

The nature of the biomolecules involved in the reduction and formation of silver nanoparticle was studied by FTIR (Fig 3). The FTIR signals of LAg-NPs were observed at 3492, 2955, 2880, 2310, 1682, 1392, 1475, 1100, 988, and 812 cm^{-1}.

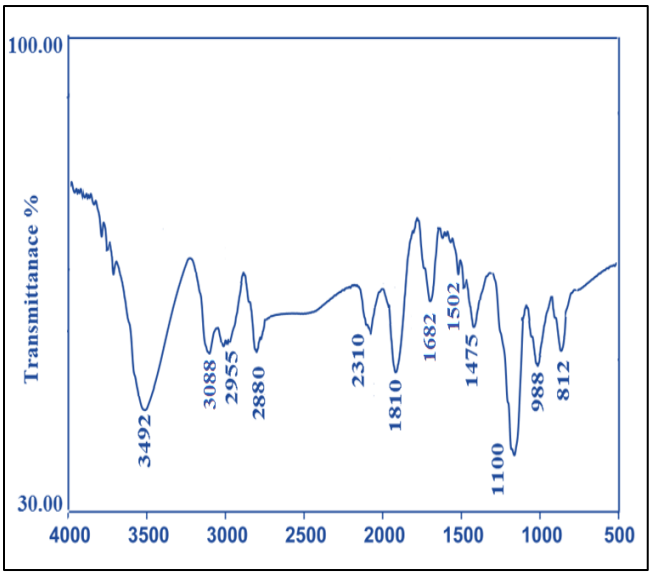


Fig 3. FTIR analysis of LAg- NPs

3.3.2.3 XRD Analysis of LAg- NPs

The structural properties of silver nanoparticles were confirmed using XRD technique, the typical XRD pattern (Fig. 4) showed diffraction peaks at 2θ = 28.2°, 32.1°, 46.8° and 58.5° indexed to (111), (200), (220) and (311) planes of silver that confirmed the main composition of the nanoparticles was silver. It is evident that silver nanoparticles were crystalline in nature.

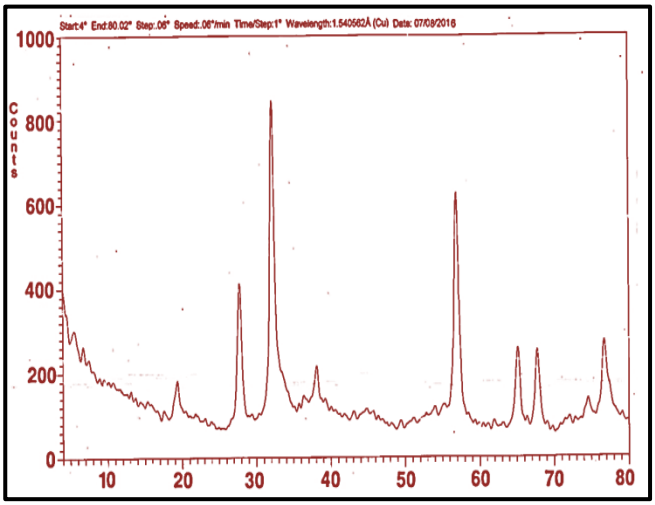


Fig. 4. XRD pattern of synthesized LAg-NPs.

3.3.2.4 TEM Analysis of LAg- NPs

TEM micrographs revealed that the latex silver nanoparticles are dispersed and aggregated, and mostly having spherical shape within the size range from 2.26 nm up to 30 nm.

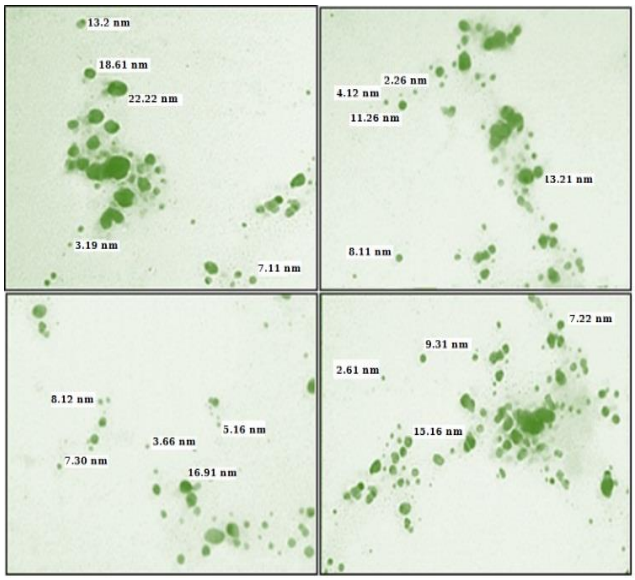


Fig 5. Transmission electron microscopy (TEM) of LAg-NPs

3.4 Determination Of Antimicrobial Activity

3.4.1 Crude latex and latex serum.

Results revealed that crude latex exhibited weak antibacterial activity against the Gram-positive bacteria only *S.aureus* and *B. subtilis*, with the minimum inhibitory concentration (MIC) at 250 µg / ml. on the other hand latex serum showed more activity against all tested

microorganisms than crude latex with MIC at 200 µg / ml as show in Table 3.

The latex extracts of C. *procera* were screened for their antimicrobial activity and results showed considerable antibacterial and antifungal activities against the tested microorganisms. Activities of different latex extracts varied with the tested microorganisms and concentration used. Ethanol and aqueous extracts showed the highest antimicrobial activity against all tested bacterial and fungal strains with minimum inhibitor concentration at MIC 50 µg / ml and Gram-Positive bacteria *S. aureus* and *B. subtilis* were more susceptible than gram negative bacteria and fungal isolates. chloroform extraction showed good antibacterial activity (except with *P. aeruginosa*) but has no antifungal activity. Methanol extract showed activity against gram positive bacteria and *C. albicans* only (Table 4)

3.4.2 Latex Nanoparticles

fungi). compared with crude latex, Latex serum and standard antibacterial (Streptomycin) and antifungal

The biosynthesized LAg-NPs exhibited good antimicrobial activity against all tested strains (bacteria and (Clotrimazole) agents. LAg-NPs exhibited higher antimicrobial activity and the minimum inhibitory concentration (25 µg / ml) Table 5.

3.5 Growth curves of bacterial cells treated with different treatments

The results showed that, growth curve of each strain tested decreased in the presence of either latex or LAg-NPs compared to control medium. Microbial growth of the cells treated with crude latex were inhibited after 10 h, 9 h and 18 h for *S. aureus, E. coli* and *C. albicans* respectively. on the other hand, microbial cells treated with LAg- NPs were inhibited after 3 h as shown in table (6) and Figure 6.

Table 3. MIC value of crude latex and Latex serum against some standard pathogenic microorganisms by using agar well diffusion method. (SD)

Tested Strains	Diameter of inhibition zone mm (mean of 3 replicates)									
	Crude latex					Latex Serum				
	Concentration (µg / ml)									
	50	100	200	250	300	50	100	200	250	300
S. aureus	0	0	0	6.2±1.1	7.6±2.1	0	0	8.3±1.1	16.3±0.5	20.3±1.5
B. subtilis	0	0	0	8.3±1.0	8.5±1.3	0	0	10.6±0.8	13.5±0.6	18.6±0.7
E. coli	0	0	0	0	0	0	0	10.1±1.6	12.8±0.2	16.4±0.8
P. aeruginosa	0	0	0	0	0	0	0	6.2±0.9	6.4±1.2	13.3±1.9
C. albicans	0	0	0	0	0	0	0	11.3±0.9	13.3±1.1	12.3±0.7
A. niger	0	0	0	0	0	0	0	9.3±1.0	10.4±0.7	10.5±0.7

Table 4. MIC value of Four different latex extractions against some standard pathogenic microorganisms by using agar well diffusion method.

Latex extraction	Conc. (µg / ml)	Diameter of inhibition zone (mm) mean of 3 replicates					
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
Ethanol	Control*	0	0	0	0	0	0
	25	0	0	0	0	0	0
	50	8.3±0.9	9.3±0.8	6.3±0.6	0	8.5±0.1	8.2±0.2
	75	10.2±1.1	11.3±1.3	8.3±1.2	8.9±1.5	10.5±0.5	10.2±1.2
	100	13.4±1.0	13.6±1.1	12.3±0.6	11.2±1.2	10.6±2.1	11.3±0.5
	125	15.4±0.9	14.6±0.3	19.6±0.5	13.2±0.6	16.3±0.7	13.3±1.3
Methanol	Control	0	0	0	0	0	0
	25	0	0	0	0	0	0
	50	0	0	0	0	0	0
	75	6.3±0.2	7.5±2.2	0	0	6.3±0.2	0
	100	10.3±0.5	11.5±2.1	0	0	11.3±1.2	0
	125	13.4±1.2	11.6±0.5	0	0	12.2±1.5	0
Chloroform	Control	0	0	0	0	0	0
	25	0	0	0	0	0	0
	50	0	0	0	0	0	0
	75	0	0	0	0	0	0
	100	6.3±0.5	7.5±0.5	6.2±1.2	0	0	0
	125	11.5±0.6	10.3±2.2	9.2±0.3	0	0	0
Aqueous	Control	0	0	0	0	0	0
	25	0	0	0	0	0	0
	50	6.3±0.3	7.5±0.2	6.2±1.3	0	6.3±0.3	0
	75	10.3±1.2	11.5±1.2	9.2±1.1	9.6±0.5	11.3±0.6	8.9±2.0
	100	11.3±1.0	11.5±0.6	8.2±2.1	8.6±1.2	13.3±0.1	9.9±1.5
	125	15.6±1.1	13.6±0.8	14.3±0.2	13.2±2.2	12.2±1.1	15.3±0.2

Table 5. MIC conc. of latex nanoparticles (0.1 M), standard antibacterial (Streptomycin) and antifungal (Clotrimazole) against some standard pathogenic microorganisms by using agar well diffusion method.

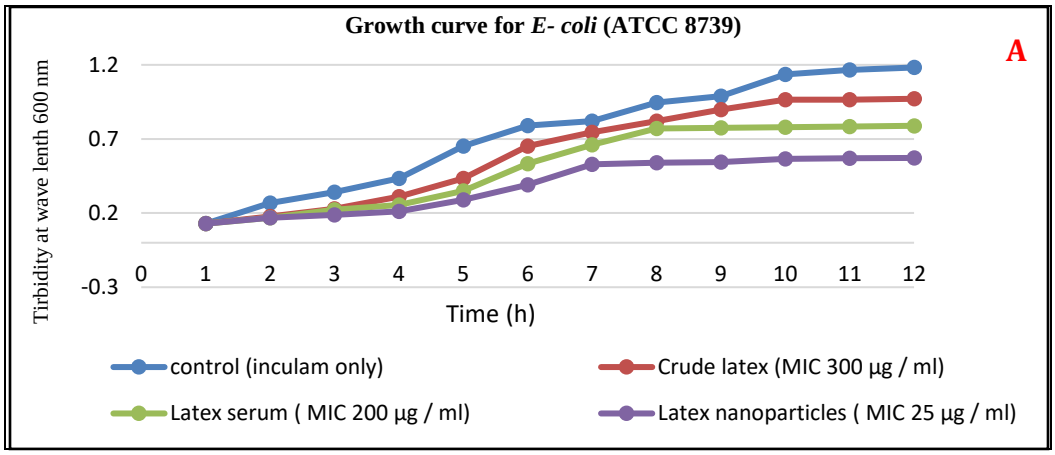
Antimicrobial agent	Conc. (µg / ml)	Diameter of inhibition zone (mm)					
		Volume (100 µL)					
		S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. niger***
LAg-NPs	**25**	6.3±0.5	7.4±0.2	6.5±1.1	7.1±0.2	7.9±2.1	8.1±0.2
	50	7.3±0.2	9.2±0.3	8.5±1.2	7.5±0.2	8.5±0.2	10.2±0.6
	75	10.6±1.3	12.5±1.2	13.5±2.1	13.9±1.2	15.6±0.6	17.5±1.5
	100	13.1±2.1	15.2±1.1	16.1±1.3	16.6±2.2	17.7±0.5	17.2±1.2
	125	16.2±0.5	18.1±0.1	21.2±0.2	17.7±1.2	22.2±1.2	20.7±2.1
Streptomycin	**25**	0	0	0	0	-	-
	50	0	0	0	0	-	-
	75	0	0	0	0	-	-
	100	8.2±0.2	5.2±0.5	0	0	-	-
	125	10.2±1.2	12.6±0.3	11.5±0.4	15.2±1.2	-	-
Clotrimazole	**25**	-	-	-	-	0	0
	50	-	-	-	-	0	0
	75	-	-	-	-	6.5±1.2	9.1±0.2
	100	-	-	-	-	8.9±2.2	13.5±0.6
	125	-	-	-	-	12.1±1.1	19.2±0.5

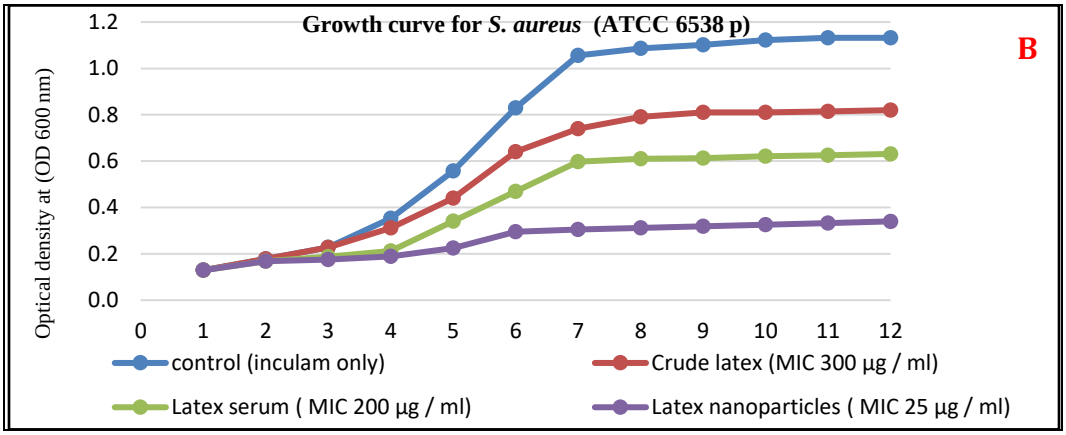
Table 6. Killing time of some standard pathogenic microorganisms by using agar well diffusion method.

Strains	**control**	**Crude latex**	**Latex serum**	**LAg -NPS**
	Inhibition times per hour			
E. coli	12	10	8	7
S. aureus	10	9	7	6
C. albicans	20	18	16	15

4 Disscusion

The phytochemical analysis of latex extracts of *C. procera*revealed the presence of cardiac glycosides, saponins, phenolic compound, terpenoids, alkaloids, flavonoids, tannins, and/or resins in some of the extracts, and the concentration of the various classes of secondary metabolite varies amongst the extracts evaluated. Ethanolic and aqueous extracts were rich in their phytochemical contents, while chloroform extract was poor where it contains only cardiac glycosides and terpenoids. these results match with those found by other researchers[34,37]. However Ahmed *et al*.,[38]reported that there is a significant variation in chemicalcompositions of plant extract of the same species collectedfrom different parts of world and analyzed in different laboratories. These active compounds of plant extract may possess the antimicrobial property. In the present study, the aqueous (water) and organic solvents (ethanol, Methanol and chloroform) extracts of the *C. procera* showed considerable antibacterial and antifungal activities against some of the tested microorganisms. Ethanol and aqueous extract provided maximum inhibitor effect against Gram positive, Gram negative and fungal strains. Methanol extraction didn't show any activity against gram negative as well as *A. niger*, Chloroform extract showed high antibacterial activity but no antifungal activity has been observed. These results indicate that the active ingredients in plant latex could be extracted much better using ethanol and aqueous solvent than other solvent used, and the highest antibacterial activity of *C. procera* was observed due to the presence of secondary metabolites such as alkaloids, flavonoids and terpenoids against tested microorganisms. The antimicrobial activity of *C. procera* extracts against bacteria and fungi is well documented [1, 9, 14]. Nenaah*et al*.,[39] reported that Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* were more susceptible to *C. procera* solvent extract than the Gram negative bacteria such as *Pseudomonas aeruginosa* and *Salmonella enteritidis*and that yeast species were more susceptible than the filamentous fungi. The ability of *Calotropis procera* extracts to reduce the total viable count of bacteria was also reported by Shittu*et al*.[40]. Renisheya*et al.,* [41]tested the antimicrobial property of aqueous and alcoholic extract of *Calotropis procera* prepared by decoction and hot percolation process against the pathogens *Staphylococcus aureus, Escherichia coli* and *Aspergillus* species and found antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.* Mako *et al*.,[42] studied the antimicrobial activity of aqueous and





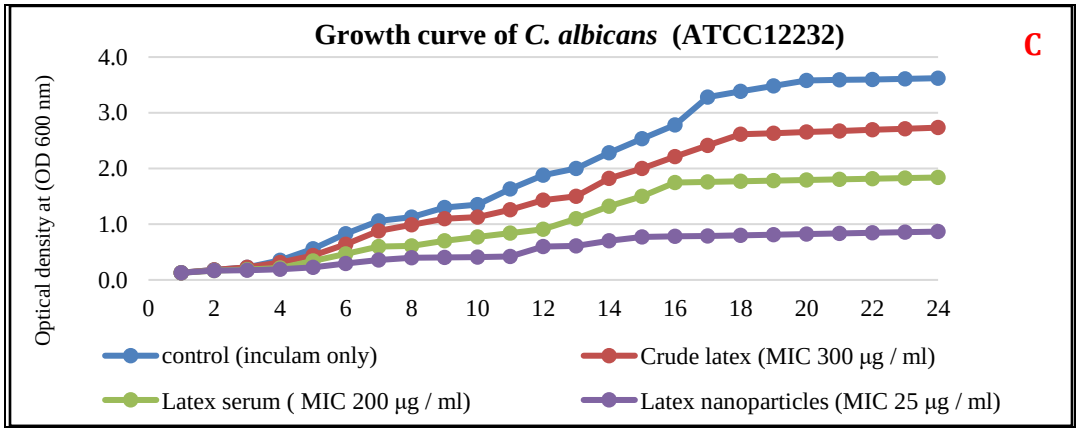


Fig 6. Growth curve of crude latex, Latex serum and latex nanoparticles against *E. coli* (A), *S. aureus* (B), and *C. albicans* (C).

ethanol extract of leaves of *Calotropis procera* against *Staphylococcus aureus*, *Streptococcus pyogen*, *Escherichia coli* and *Pseudomonas aeruginosa*, by disc method and results provide a support for the use of *Calotropis procera*, in traditional medicine .

In the present study, biosynthesis of LAg-NPs was successfully obtained by the green method which involves treatment of latex serum of *C. procera* with silver nitrate ($AgNO_3$). This method displayed that the plant latex can be used as an effective stabilizing reducing agent for the synthesis of LAg-NPs. The advancement of green synthesis of nanoparticles over physical and chemical methodswhich are eco-friendly, cost effective, no need to use high energy and toxic chemicals, provide healthier work placesand communities, protecting human health and environmentleading to lesser waste and safer products. more effective in avariety of applications especially in antimicrobial activities [43]. The preliminary visual observation showed that the color of the reaction mixture (plant latex and 0.1 M $AgNO_3$) changed from pale-yellow to light brown, then to dark brown due to excitation of surface Plasmon vibration confirming the formation of silver nanoparticles, at the same time, control (latex serum without $AgNO_3$ solution) showed no color change [44]. UV-vis spectroscopic study of the colored solution confirmed the synthesis of LAg-NPs as distinct surface Plasmon resonance band with a peak centered at 280 nm this similar to was obtained by Mallikarjuna *et.al*,[45]. TEM image of LAg-NPs showed that the particles are spherical in shape, well dispersed with a diameter range from 2.26 nm up to 30 nm [46]. XRD was used to confirm the crystalline nature of the particles. A number of Bragg reflections at 2θ values of 28.2°, 32.1°, 46.8°, 58. 5°, are shown corresponding to 111,200, 220, and 311 plans respectively [47]. FTIR an important tool in understanding the participationof functional groups in relation between metal particles and biomolecules which is used to search the chemical composition of the surface of the silver nanoparticles and identify the biomolecules for capping and efficient stabilization of the metal nanoparticles [48].There were many functional groups present which may have been responsible for the bio-reduction of Ag+ ions. The band intensities in different regions of the spectrum for plant latex and silver nanoparticles were analyzed**.** FTIR spectrum shows different major peak positions at 3492, 2955, 2880, 2310, 1682, 1392, 1475, 1100, 988 and 812 cm^{-1}. The peak located at 1639 cm^{-1} could be assigned to C=O stretching or amide bending [49]. The broad and intense peak at 3464 cm-1 corresponds to OH stretching vibrations of phenol/carboxylic group present in plant latex. A peak observed at 2922 and 2886 cm-1 is due to C-H stretching of alkanes [47]. The peak at 1384 cm-1 assigned to nitro N-O bending [50] and a peak at 1109 cm-1 to C-O-C stretching aromatic ring. It showed peak in the range of 628 cm-1 relating to the alkyl halides band especially the C-Cl bond [51]. In the same context, AgNPs become an important application in the field of microbiology as antibacterial and antifungal agents. The exact mechanisms of antimicrobial activitiesby silver nanoparticles are still in investigation. The positive charge on the Ag ions is avital for antimicrobial activities. In itsionized form, silver is inert but when itcontacts withmoisture it releases silver ions, slowly withtime, or the silver ions can comefrom ionizing the surface of a solid piece of silver as with silvernanoparticles. Some literature demonstrated theelectrostatic attraction between positive charge of nanoparticlesand negative charge of microbial cells and they aresuggested to be most suitable bactericidal agent, these nanoparticles have been shown to accumulate insidethe membrane and can subsequently penetrate into the cellscausing damage to cell wall or cell membranes. It was also suggestedthat Ag ion enters the cell anddenaturing the DNA molecule byintercalates the purine and pyrimidine base pairs leading to disruptingthe hydrogen bonding between the two anti-parallelstrands.[52, 53].

From the current results, it can be concluded that the antimicrobial activity of various extracts of *Calotropis procera* was proven on some common pathogenic microorganisms, which may result into the development of potent natural remedy for many infections after advance studies in future. At the same time, latex serum of *Calotropis procera* was found to display highly activity for the synthesis of AgNPs and as antimicrobial agents. which were found to be higher than crude latex and latex serum.

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