JOURNAL OF PURE AND APPLIED MICROBIOLOGY, FEBRUARY 2014. Vol. **8**(1), p. 251-262

Biosynthesis, Characterization and Biological Activates of Silver Nanoparticles by *Streptomyces bikiniensis* Strain Ess_amA-1

Maged S. Ahmad[1], Manal M. Yasser[1] and Essam N. Sholkamy[1,2]*

[1]Department of Botany, Faculty of Science, University of Beni-Suef, Beni-Suef - 62511, Egypt.
[2]Department of Botany and Microbiology, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia.

(Received: 11 October 2013; accepted: 26 December 2013)

In the current study, *Streptomyces bikiniensis* was isolated from insect (*Tapinoma simrothi*), Riyadh, Saudi Arabia, and identified by classical and molecular methods. The biosynthesized silver nanoparticles (AgNPs) were characterized by determining Fourier transform infrared spectroscopy (FTIR), Energy Dispersive Spectroscopy (EDS) and transmission electron microscopy (TEM). TEM photos of nanoparticles showed the spherical shapes of nanoparticles in the size range of 3–70 nm. Moreover, the minimum inhibitory concentration of biosynthesized silver nanoparticles against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Salmonella typhi* were at 8 μg per paper disc (6 mm), and the inhibition zones were 7 to 9 mm while was at 16μg per paper disc in case of *Candida albicans*, the inhibition zone was 8 mm. The lethal inhibitory concentration (IC_{50}) of AgNPs on Hep2 and MCF-7 cells was obtained at 253.6 and 119.6 μg/ml, respectively. Thus, bioconversion of silver nanoparticles by *Streptomyces bikiniensis* strain Ess_amA-1 can be employed as a potential Nano-drug to kill pathogenic microbes.

Key word: Silver nanoparticles, Streptomyces, SEM, IC_{50}.

Insects show a vast array of symbiotic relationships with a wide diversity of microorganisms, whichsome of them could benefits to the host as part of nutrition and protection from natural enemies[1].The variety of partnerships between eukaryotes and prokaryotes was astounding in the arthropods, where bacteria were living within body cavities, somatic cells, and germ line cells, and perform a vast array of functions[2]. Fungiculture in the insect world was practiced by ants, termites, beetles and gall midges[3]. Fungal associates of ants werebelong to the tribe Attini (Hymenoptera, Formicidae), and a monophyletic group of more than 210 species of fungus-growing ants, distributed in 12 genera[4]. One of the primary mechanisms used by fungus-growing ants to defend their fungus garden involved a mutualistic association with bacteria[5].*Streptomyces* spp. were alsofrequently found in pollen, provisions and alimentarycanals of alfalfa leafcutter bees andthese bacteria were considered as part of resident microfloraof the bee[6].Barke *et al*.,[7] had concluded that a combination of co-evolution and environmental sampling results in the diversity of actinomycetesymbionts and antibiotics associated with attine ants**,** using a bioassay-guided approach, they isolated an antifungal compound produced by a *Streptomyces* spp. associated with leaf-cutting ants.

Nanotechnology was been defined as an investigation for the design, synthesis, and manipulation of structure of particles with dimension smaller than 100 nm, andhad a variety of applications in fields such as optics, electronics,

* To whom all correspondence should be addressed.
Mob.: +966599342324; Fax: +966114682742
E-mail: Essam_92003@yahoo.com, elisi@ksu.edu.sa

biomedicine[8,9], magnetic, mechanics, catalysis, energy science[10]. The development of environmentally friendly, kindly and green process technologies for the production of nanoparticles with a range of chemical and physical properties is one of the challenges in the newly emerging field of Nano-biotechnology. The extensive use of toxic solvents and hazardous reducing agents in chemical procedures to synthesize nanoparticles hadimproved the necessity in view of eco-friendly and green chemistry approach. Hence, biological synthesis of nanoparticles was being the best important one of non-toxic and eco-friendly potent methods[11].

Over the last few years, the biosynthesis of nanoparticles by bacteria, fungi, and plant had gained much of interest work of good conductivity, chemical, stability, catalytic and antimicrobial activity[12, 13]. Sadhasivam *et al*.,[14] used the extracellular components from a *Streptomyces hygroscopicus* culture medium to synthesize silver nanoparticles, and characterized their antimicrobial activity. *Streptomyces* sp.was well known for its unique potential ability to produce a wide variety of secondary metabolites, such as antibiotics, immunosuppressors and many other biologically active compounds[15]. Exploitation of *Streptomyces* in nanotechnology had recently received considerable attention[14].

Silver nitrate was used for the treatment of venereal diseases, fistulae from salivary glands, and bone and perianal abscesses[16].Silver had been used for the treatment of burns and chronic wounds, and also to make water potable[17]. Silver nitrate was used in its solid form and was known by different terms like, "Lunar caustic" in English, "Lapis infernale" in Latin and "Pierre infernale" in French[18]. In the 1940s, after penicillin was introduced, the use of silver for the treatment of bacterial infections minimized[19]. Previous studyhad been reported that silver does not interfere with epidermal proliferation and possess antibacterial property against *S. aureus*, *P. aeruginosa* and *E. coli* [20]. In 1968, silver nitrate was combined with sulfonamide to form silver sulfadazine cream, which served as a broad-spectrum antibacterial agent and was used for the treatment of burns against bacteria like *E. coli*, *S. aureus*, *Klebsiella* sp., *Pseudomonas* sp. It also possesses some antifungal and antiviral activities[21]. Recently, the clinicians had returned to silver wound dressings containing varying level of silver due to the emergence of antibiotic-resistant bacteria and limitations of the use of antibiotics[19].The present study aimed to biosynthesize silver nanoparticles by *Streptomyces bikiniensis* strain Ess_amA-1, and to determine their characterization and potential role as antimicrobial activity and their cytotoxicity against cell line.

MATERIALS AND METHODS

Isolation of *Streptomyces* from insect

Tapinoma simrothi was collected in sterile airlock polyethylene bags and stored at 4°C from Eldrieh, Riyadh, Saudi Arabia. Suspension of *Tapinoma simrothi* was inoculated on starch casein agar medium[22,23] supplemented with antibiotics such as cycloheximide (40 g/ l), nystatin (30 g/ l) and nalidixic acid (10 g/ l). The plates were incubated at 30°C until the appearance of colonies with a tough leathery texture, dry or folded appearance, and branching filaments with aerial mycelia[24]. Pure colonies were isolated and subcultures were carried out by streaking the particular isolate directly on ISP-2 agar medium.

Morphological and physiological characterization of *Streptomyces* isolate

Color of aerial mycelium was determined from mature, sporulating aerial mycelia of the actinomycete colonies on different media such as ISP-2, ISP-4, ISP-6, ISP-7, Czapex dox, starch casein agar. The color was determined using the color names lists [25]. Color of the soluble pigments was determined visually by observing the color changes in the medium due to the diffusing pigments produced by actinomycete isolate[26, 27] and Bergey's manual[28,29].Utilization of carbohydrates was investigated with a basal carbon nutrient medium[30,31]. Methods and media used for physiological test were as described by Luedemann and Brodsky[32] and Luedemann[33]. All the cultures were incubated at 30 p C for 7 days. The assayfor enzymatic activity was performed according to Hopwood[34]

Extraction of DNA

Total genomic DNA of actinomycete isolate was extracted by a modiûcation of a method of Smoker and Barnum[35]. Actinomycete isolate was grown in 10 ml International Streptomyces Project

Medium 2 (ISP-2) [26] with agitation at 30 °C for 96 h. Cells (4 ml) were harvested by centrifugation (12000 rpm for 2 min), washed once with 500 ml of 50 mM Tris-HCl/1 mM EDTA (TE) buffer (pH 8.0),the pellet wasresuspended in 500 ml of 50 mM Tris-HCl (pH 8.0) –5 mM EDTA (pH 8.0) –50 mMNaCl, add 20 µl lysozyme (1 mg/ml), and the solution was incubated at 55°C for 30 min. After the addition of 10 µl of proteinase K (10 mg/ml) and 20 µl of 10% sodium dodecyl sulfate, the mixture is incubated at 55°C for 10 min or until the solution cleared (complete cell lysis). The solution was chilled on ice and extracted with an equal volume of phenol-chloroform-isoamylalcohol (25:24:1). The organic extraction was repeated. The supernatant is added to an equal volume of 4 M ammonium acetate. Total genomic DNA is precipitated by the addition of 2 volumes of isopropanol followed by centrifugation (13000 rpm) for 10 min at room temperature**.** Supernatant was removed after centrifugation (13000 rpm) and pellet was washed with 70% ethanol. The pellet was dissolved in 100 µl of TE buffer or double distilled water.

Amplification of 16S rDNA and its sequencing

The 16S ribosomal DNA gene was amplified by PCR using the universal primer pair Star-F ^{5}-GAGTTTGATCMTGGCTCAG and 1387-R CGGGCGGTGTGTACAAGG^{-3}. The amplified products were analyzed by GATC Biotech, European Custom Sequencing Centre, D-51105 Cologne, Germany. DNA sequence analysis was then performed by BLAST network services at the NCBI. The 16S rRNA gene sequence of the isolate was aligned with reference sequences obtained from gene Bank using Crustal W[36]. Phylogenetic tree was generated using the maximum likelihood method with MEGA 5 package[37,38]. The evolutionary distance matrix was derived with Jukes and Cantor model[39]. Topology of phylogenetic tree was evaluated by bootstrap analysis based on 500 replicates[40].

Biosynthesis and characterization of Silver nanoparticles (AgNPs)

This method was used by Li G. *et al.*, [41] in biosynthesis of nanoparticles. The isolate was further cultured in ISP-2 medium (pH 7.2) and grown for 72 h at 30 °C in an orbital shaker at 220 rpm. Cell filtrate (CF) was obtained by centrifugation at 4 p C, 10,000 rpm for 10 min.Bio-reduction process to occur, $AgNO_3$ (Qualigens 99.8%) was added to 100 ml CF at molarity 1 mM and incubated at 30 °C in dark for 48 h. Followed by initial observation of color change in the bioreduction process, UV–visible spectrometric measurements were performed on Hitachi double beam equipment (Model Lambda 35) at range 210 to 800 nm. The bio-transformed products present in cell-free filtrate after 72 h of incubation were freeze-dried and diluted with potassium bromide in the ratio of 1: 100. FTIR spectrum of samples was recorded on FTIR instrument mode Nicolet 6700 spectrometer at a resolution of 4 cm^{-1}in the range of 400– 4000 cm^{-1}. For energy dispersive spectroscopy (EDS), sample was prepared on a copper substrate by drop coating of silver nanoparticles, and was carried out using JEOL (JSM-6380 LA) equipped with scanning electron microscopy. Transmission electron microscopy was performed on JEOL (JEM-1010) instrument, with an accelerating voltage of 80 kV after drying of a drop of aqueous AgNPs on the carbon-coated copper TEM grids Samples were dried and kept under vacuum in desiccators before loading them onto a specimen holder. The particle size distribution of silver nanoparticles was evaluated using ImageJ 1.45s software.

Test pathogens used

The antibacterial activity of biosynthesized AgNPs was assessed against bacterial species: Gram positive (*Streptococcus pyogenes* ATCC 19615, *Staphylococcus aureus* ATCC 25923) and Gram negative (*Salmonella typhi* ATCC 6539, *pseudomonas aeruginosa* ATTC 27853 and *Klebsiella pneumonia* ATCC 700603), and *Candida albicans* strain ATCC 90028 for antifungal activity which was growing sabouraud agar. The test pathogen samples were procured from Khalid hospital, Riyadh. All the test pathogen samples were maintained in Brain HeartInfusion medium (BHI) at – 20 °C. 300 µL of each stock-culture were added to 3 mL of BHI broth. Overnight cultures were kept for 24 h at 37°C ± 1°C and the purity of cultures was checked after 8 h of incubation.

Antimicrobial activity of AgNPs

The antimicrobial effects of the microbiologically synthesized AgNPs were evaluated using method of disk inhibition zone(agar diffusion method). The tested

concentrations were 3, 8, 16, 30 ug. In disk inhibition zone method, the nutrient agar medium was inoculated with freshly prepared cells of each bacteria, and *Candida albicans* on Sabouraud agar. After solidification of the agar, a number of sterilized disks were dipped into CF as positive control and biosynthesized AgNPs, and placed on the plates. After incubation at 37°C for 24 h, the antimicrobial activity was measured as diameter of the inhibition zone formed around the disk.

Scanning Electron photomicrograph of affected bacteria by AgNPs

The bacteria were been inhibited by biosynthesized silver nanoparticles were prepared for scanning electron microscope (SEM). The paper disc containing bacteria were fixed in 3 % (v/ v) glutaraldehyde buffered with 0.1 M sodium phosphate buffer (pH 7.2) for an hour at room temperature and then washed four times in sodium phosphate buffer. It was then post-fixed in 1 % (w/ v) osmium tetroxide for an hour and then washed four times in the buffer. They were dehydrated in a graded alcohol series. The last stages of dehydration were performed with propylene oxide. The specimens were dried and were mounted onto stubs using double-sided carbon tape, and then were coated with a thin layer of gold by a Polaron SC 502 sputter coater, and were examined in a Joel JSM 6060 LV scanning electron microscope[42].

Cytotoxicity assay

Cytotoxicity tests using specialized cells have proved most useful when the invivotoxicity of a silver nanoparticles is already well established and where in vitro investigations using specialized cell cultures have been used to clarify the mechanisms oftoxic action on the target tissue. The human breast adenocarcinoma cells (MCF-7) and Human larynx carcinoma cell line (Hep-2)procured from ATCC (Rockville, MD, USA) were used in this study. The cells were cultured in humid environment at 37°C and 5% CO_2 in a cell culture minimum essential medium (MEM, Invitrogen, USA) supplemented with 15% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Invitrogen, USA). At 85-90% confluence, cells were harvested using 0.25% trypsin/ EDTA solution and sub-cultured into 96-well plate. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay developed by Mosmann[43] with modification was used to screen the cytotoxic activity of silver nanoparticles. Briefly, the MCF-7 and Hep- 2cells (1×10^4 cells/ well) were grown for overnight in 96-well flat bottom culture plates, and then exposed to six different concentrations (i.e. 1.00 mg/ ml, 0.5 mg/ ml, 0.25 mg/ ml, 0.12 mg/ ml, 0.6 mg/ ml and 0.3 mg/ ml) of the biosynthesized silver nanoparicles for 24 hrs. In addition, negative/ vehicle control, and positive control (Doxorubicin) were also used for comparison. After the completion of desired treatment, 10 µl of MTT reagent (Invtrogen, USA) was added to each well and further incubated for 3 hrs at 37°C. Finally, medium with MTT solution was removed, and 100 µl of DMSO (Sigma Aldrich, St. Louis, MO, USA) was added to each well and further incubated for 20 min. The optical density (OD) of each well was measured at 570 nm using a microplate reader (Synergy, BioTek, USA). The percentage of cytotoxicity compared to the untreated cells was determined.Triplicates were maintained for each treatment. Lethal concentration (LC_{50}) was determined by calculating the % of viability:

% of viability = Mean Test OD × 100/ Mean OD of Control

RESULTS AND DISCUSSION

Actinomycete isolate was isolated from *Tapinoma simrothi* which were collected from Eldrieh, Riyadh, Saudi Arabia through October at 2012 After 7 days incubation at 30°C, pure colonies were isolated on SCA agar, and sub cultured on ISP-2 agar medium. Aerial and substrate mycelia of the isolate were examined under bright field microscopy. Results of the biochemical characterization and carbon source utilization tests were as summarized in Table 1, 2 [26,44]. SEM images indicated that the isolate possessed substrate mycelia and extensively aerial hyphae that further differentiated into smooth surfaced spores (Rectus)(Fig. 1). The strain exhibited superior growth on ISP-2, 4, 6, 7, moderate growth on ISP-5 and Czapex dox. Melanin was noticedon ISP-6. By morphological and physiological characterizations of actinomycete isolate, the isolated identified according to Bergey's manual[28-29] as *S. bikiniensis.*

The total length of the 16 S rRNA gene sequenced in the present study possess 1206 base pair, and showed 99% similarity index with *S.*

Table 1. Physiological and biochemical properties of *S. bikiniensis*strain Ess_amA-1

Name of the test	*Streptomyces bikiniensis*
Carbon sources	
Rhaffinose	+
Fructose	-
Sucrose	+
Arabinose	-
lactose	+++
D-galactose	+
D-xylose	+
Citrate	-
Nitrogen sources	
L-Cysteine	-
L-Valine	-
L-Phenylalanine	+
L-Histidine	+
Enzyme activity	
amylase	+
Protease	+
Chitinase	+
catalase	+
DNase	+
Hydrolysis of esculin	+
Lecithin hydrolysis	+
H_2S production	-
Nitrate reduction	-
Urea hydrolysis	+
Lipid hydrolysis	+
Growth at different conc. NaCl	
1 %	+++
4 %	+
7 %	-
10 %	-
14 %	-
Growth at different pH	
5	-
7	+
10	-
Growth at different temperatures	
4	-
26	++
30	+++
37	+
45	-
Antibiotic resistance	
E Erythromycin (15 µg)	-
GM Gentamamicin (10 µg)	+
PG Pencillin G (10 µg)	+
RP Rifampicin (30 µg)	-
K Kanamycin (1000 µg)	-
VA Vancomycin (5 µg)	-
CO Colisttinsulphate (10 µg)	+
AK Amikacin (30 µg)	-
ATM Aztreonam (30 µg)	+
C Chloramphenicol (30 µg)	-
CAZ Ceftazidime (30 µg)	+
IMI Imipenem (10 µg)	-
CIP Ciprofloxacin (1 µg)	+
PRL Piperacillin (100 µg)	+
T Tetracycline (30 µg)	-

bikiniensis clone ZD Hu (Gene Bank, Accession No. AY946043.1). The result of cladistics analysis of generated sequence together with the related sequences accession retained from gene Bank was consistent with the result of Blast search (Fig. 2); and thus, the isolate was named as *Streptomyces bikiniensis* strain Ess_amA-1 (Accession No. KF588366).

The result in Fig.3showed the change in color of a mixture of the cell filtrate occurred after mixing with silver nitrate and this indicatedprocess of biosynthesis of silver nanoparticles by cell filtrate of *S. bikiniensis*.Mulvaney[45] also reported that the dark brown color was the result of excitation of surface Plasmon vibration in the metal nanoparticles and formation of the silver nanoparticles. The role of NADH dependent nitrate reductase from fungi in the biosynthesis of silver nanoparticles was recently reported[46, 47]; however, different NADH-dependent reductases may be produced also by *S. bikiniensis*.Previous study was reported that the color development was result as different reductase enzymes in filtrate of *Streptomyces* sp.[14].

Results of energy dispersive spectroscopy (EDS) of the silver nanoparticles confirmed the presence of elemental silver signal shown in Fig. 4 which was reported that the spectrum of nanoparticles sample showed mainly Ag (94.01%) and only minor amounts of other elements (5.99 %), the signals for N and O indicated the presence of proteins as a capping material on the surface of silver nanoparticles. Magudapathy *et al.*,[48] also reported that the spectrum of nanoparticles sample showed mainly Ag (94.1%) and only minor amounts of other elements (5.9 %),

Table 2. Cultural characterization of *S. bikiniensis*strain Ess_amA-1on different culture media

Parameter Medium	Color of aerial mycelium	Color of substrate mycelium	AM/ SM	pigmentation	Melanin production	growth	Form of spore chain
ISP-2	Gray with green	Dark pink	A M	Brown	- —	good	Rectus
ISP-4	gray	red	A M	red	-	good	Rectus
ISP-5	black	Light grey	A M	- -	- -	moder ate	Rectus
ISP-6	gray with White	Light white	Am	Brownish black	+ ve	good	Rectus
ISP-7	gray with White	Violet	Am	voilet	-	good	Rectus
Czapex Dox	Gray	Light white	Am	-	-	Moder ate	Rectus
Starch casein agar	Gray	Light white	Am	red	-	good	Rectus

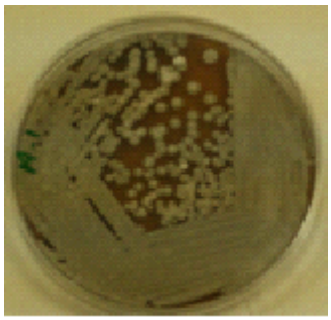
Table 3. Minimum inhibition concentration of AgNPs against pathogenic microbes

Pathogens Conc. of AgNPs	*K. pneumonia*	*S. aureus*	*S. typhi*	*S. pyogenes*	*P.aeruginosa*	*C.albicans*
3µg	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
8 µg	7±0.2	8±0.1	9±0.7	7±0.1	9±2	0
16 µg	11 ±0.4	9 ±1	12 ±1	9 ±0.5	12 ±0.9	8±0.5
30 µg	13 ±0.8	13 ±2	14 ±0.9	12 ±2	14 ±1	11 ±1

the signals for N and O indicated the presence of proteins as a capping material on the surface of silver nanoparticles.

Results in Fig. 5 showed FTIR spectraat two bands at 1627.6 cm^{-1} that corresponds to the bending vibrations of the amide I and amide II bands of the proteins while their corresponding stretching vibrations of primary amines were seen at 3433.04 cm^{-1}.Similarity, Gole *et al.*,[49] and Jain *et al.*,[50] also reported the presence and binding of protein with silver nanoparticles which could lead to their possible stabilization.

Result in Fig.6 showed different sizes of nanoparticles, mostly spherical in shape but aggregated to a lesser extent. In Fig.7, there were four distinct sizes of Ag nanoparticles was synthesized by *S. bikiniensis*. The size of Ag nanoparticles was approximately in range 3 to 70 nm with standard deviation 2.3nm.This result was similar to previous study[51] for biosynthesis of silver nanoparticles from *Bacillus subtilis* using microwave radiation. Sadhasivam *et al.*,[14] also reported nanoparticle sizes of 20–30 nm when produced by the extracellular from a *Streptomyces*



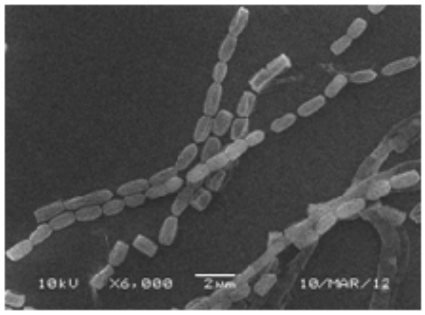


Fig. 1(a). *S. bikiniensis* strain Ess_amA-1on ISP-2 medium, b) Under SEM microscope. Bar scale = 2µm

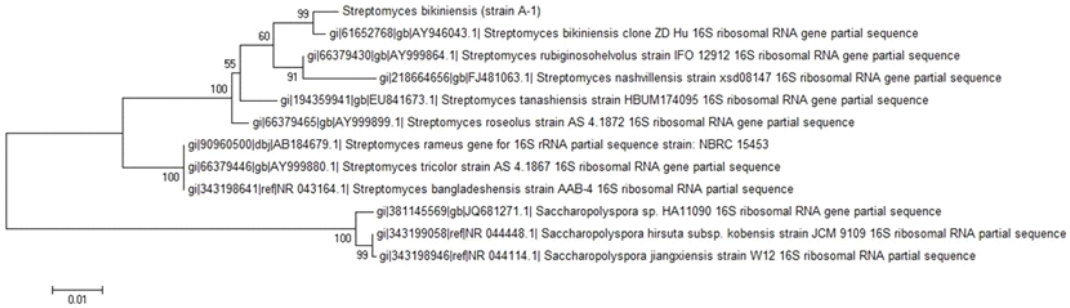


Fig. 2. The phylogenetic tree of *S. bikiniensis* (Accession No. KF588366) was constructed using the neighbor-joining method with aid of MEGA 5.0 program. The Bootstrap values above 50%, presented as percentages of 500 replications, are shown at the branch points. Bar 0.5 substitutions per nucleotide position

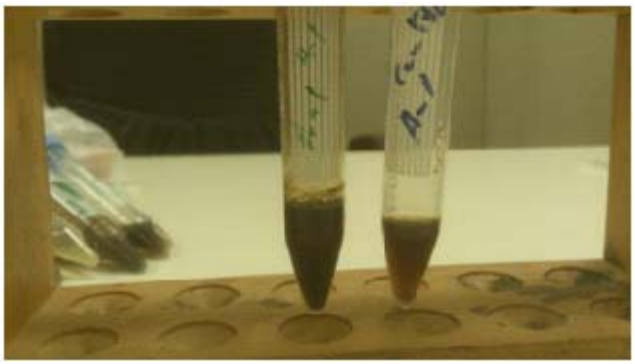


Fig. 3. Test tubes containing the filtrate of the *S. bikiniensis* strain Ess_amA-1at: (1) before addition 1 mM $AgNO_3$ (right), (2) after 72 h incubation with 1 mM AgNO3 (left)

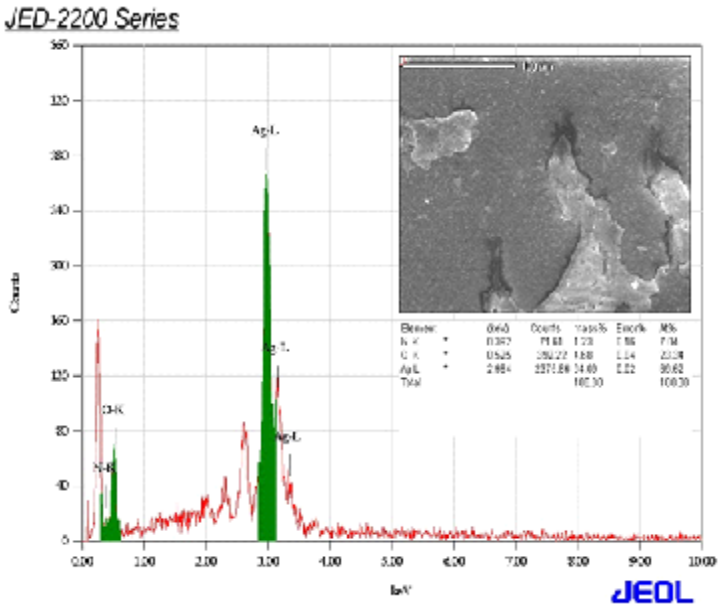


Fig. 4. EDS spectra of silver nanoparticles

culture. The different size distribution of nanoparticles may be due to differences in reductases produced by *S. bikiniensis*, or the effects of other proteins coating the nanoparticles. The size distribution of nanoparticles was very important for application purposes. For example, smaller nanoparticles were more effective as an antimicrobial against pathogens[52].

Figs.8clearly, showed biosynthesized nanoparticles had activity against Gram positive and Gram negative bacteria.In case of bacterial strains, the minimum inhibitory concentrations of silver nanoparticles were at 8 µg per paper disc (6 mm), and the inhibition zones were 7 to 9 mm while in case of *C. albicans*, the MIC was at 16µg per paper disc, the inhibition zone was 8 mm. Shrinking of bacterial cell and degradation of bacterial cell was observed under SEM (Fig. 9).Stoimenov *et al.*,[53]also confirmed that metal nanoparticles possessed excellent bactericidal effects.The mechanism of inhibitory action of silver ions on microorganisms revealed that upon Ag^+treatment, DNA loses its replication ability[54] and expression of ribosomal subunit proteins as well as some other cellular proteins and enzymes essential to ATP production becomes inactivated[55]. It had also been hypothesized that Ag^+ primarily affects the function of membrane-bound enzymes, such as those in the respiratory chain[56, 57]. In a previous report[58] on the bactericidal activity of silver nanoparticles, it was shown that the interaction between silver nanoparticles and constituents of the bacterial membrane caused structural changes in or damage to membranes, finally leading to cell death.

The cytotoxic activity of AgNPs synthesized by using *S.bikiniensis* was determined by MTT assay.In the present study, the inhibitory concentration(IC_{50}) of AgNPs on Hep2 and MCF-7 cells was obtained at 253.6 and 119.6 µg/ml respectively as in figure 10. Exposure to highest concentrations of AgNPs showed cytotoxic activity on Hep2 and MCF-7 cells. Ourstudy correlates with the results of an earlier study[59,60] where *Sapium* leaves showed the highest cytotoxic activity againstHeLa cell line. Cannonball leaves had also been reported tohave antioxidant activity, and this might have a role to play inthe observed activity in the cancer cell lines as antioxidants play a complex role in cancer prevention[61].

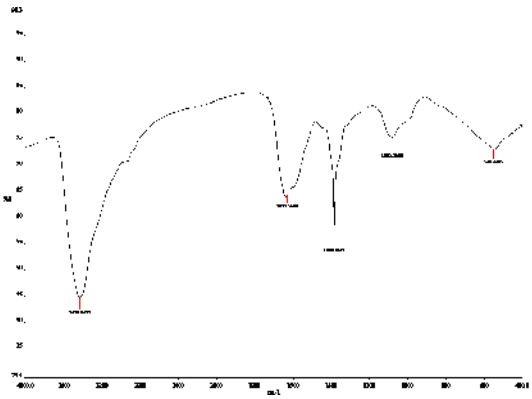


Fig. 5. FTIR spectrum of biosynthesized AgNPs by filtrate of *S. bikiniensis* strain Ess_amA-1

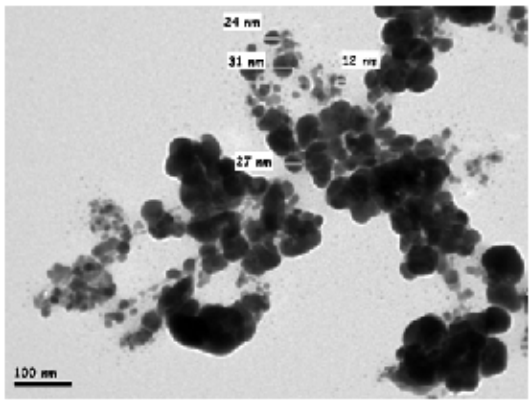


Fig. 6. Images of biosynthesized silver nanoparticles by Transmission Electron Microscopy (TEM)

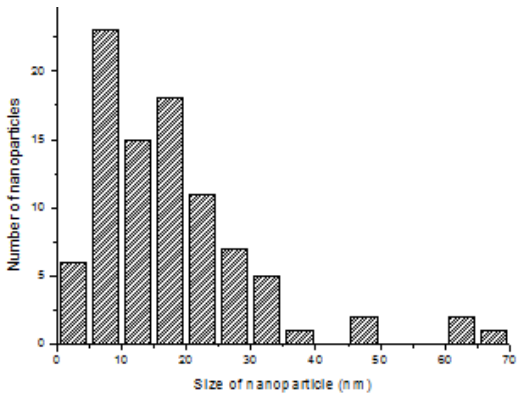


Fig. 7. Histogram for different sizes of biosynthesized silver nanoparticles from TEM images

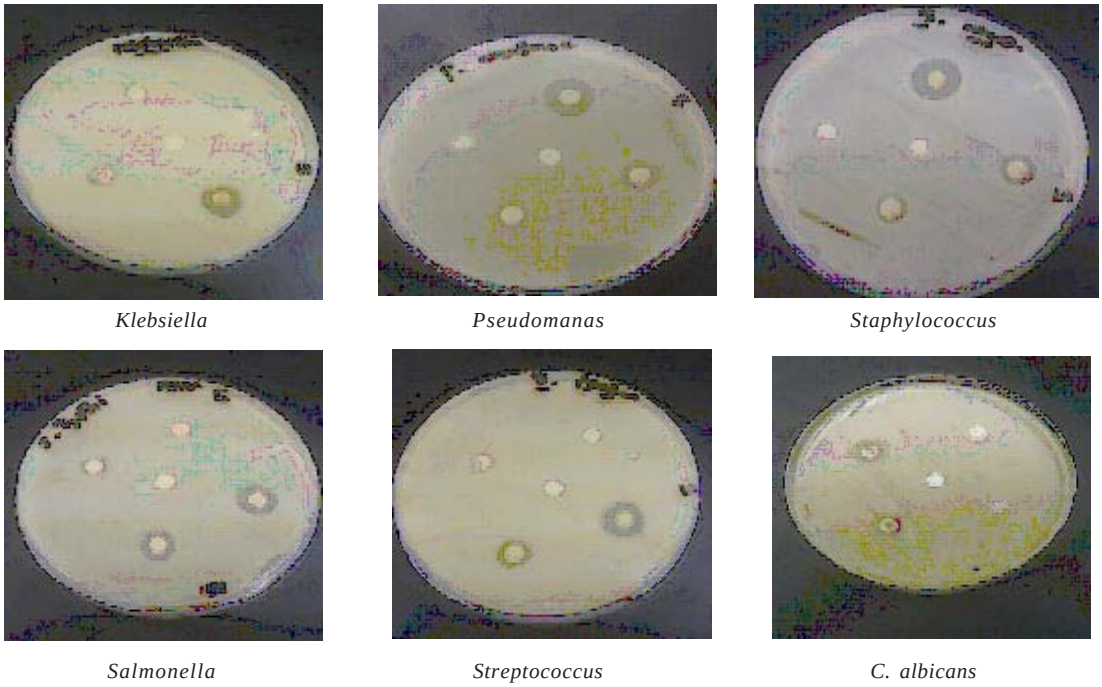


Fig. 8. Antimicrobial activity of AgNPs (3, 8, 16 and 30 µg) against pathogenic microbes

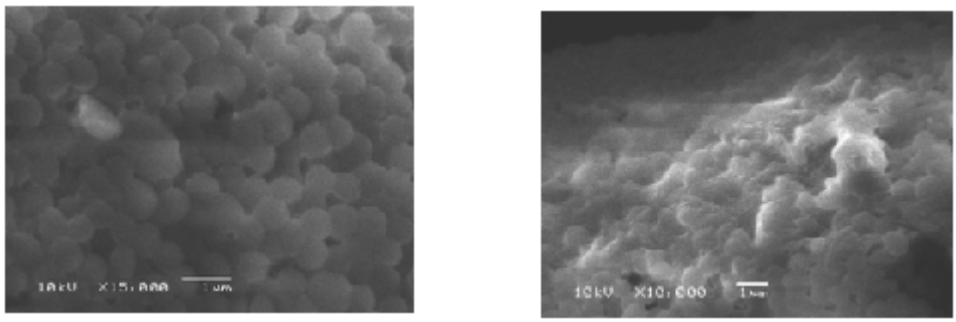


Fig. 9. Effect of AgNPs on growth of bacterial cell under SEM. Untreated cells (left) and treated cells (right)

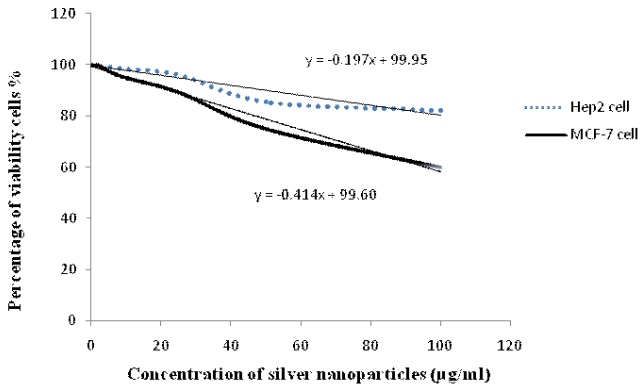


Fig. 10. Cytotoxicity of silver nanoparticles against Hepatocellular carcinoma cells (HepG-2) and breast cell line at 5 different concentrations. Cell viability was determined using the MTT bromide colorimetric assay

CONCLUSION

In conclusion, , it had been established that *S. bikiniensis* strain Ess_amA-1 was capable of producing silver nanoparticles extracellular and the silver nanoparticles was quite stable, and had biomedicine applications. Biological methods could establish to be an excellent alternative for synthesis of AgNPs. *S. bikiniensis* can be a good candidate for the synthesis of the AgNPs using silver nitrate in the size range3- 70 nmnm. In summary, it was concluded that biosynthesized AgNPs have effective inhibition action against pathogenic microbes and then its potential role as antimicrobial s against pathogenic microbes; and anticancer agents against HepG2 and CMF-7 cell line.

ACKNOWLEDGMENTS

This work was supported by King Saud University, Deanship of Scientific Research, College of Science,Research Center.

REFERENCES

1. Klepzig, K.D. Adams, A.S., Handelsman, J., Raffa, K.F., Symbioses: a key driver of insect physiological processes, ecological interactions, evolutionary diversification, and impacts on humans. *Environ. Entomol.*, 2009; **38**; 67-77.
2. Baumann, P., Moran, M.A., Baumann, L., Bacteriocyte-associated endosymbionts of insects. In: Dworkin, M. (Ed.), The Prokaryotes. Sprin, New York, http://link.springer.de/link/service/books/10125, 2001.
3. Kaltenpoth, M., Actinobacteria as mutualists: general healthcare for insects.*Trends Microbiol*., 2009; **17**: 529-535.
4. Mueller, U.G., Schultz, T.R., Currie, C.R., Adams, R.M.M., Malloch, D., The origin of the attine ant–fungus mutualism. *Q. Rev. Biol*., 2001; **76**: 169–197.
5. Currie, C.R., Poulsen, M., Mendenhall, J., Boomsma, J.J., Billen, J., Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. *Sci.*, 2006; **311**: 81-88.
6. Inglis, G.D., Sigler, L., Goette, M.S., Aerobic microorganisms associated with alfalfa leafcutter bees Megachilerotundata. *Microb. Ecol*., 1993; **26**: 125-143.
7. Barke, J., Seipke, R.F., Grüschow, S., Heavens, *et al.*, A mixed community of actinomycetes produce multiple antibiotics for the fungus farming ant Acromyrmex octospinosus. *BMC Biology*, 2010; **8**: 109.
8. Jain, P.K., Huang, X.H., El-Sayed, I.H., El-Sayed, M.A., Noble metals on the nanoscale: optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. *Acc. Chem. Res*., 2007; **41**: 1578-1586.
9. Klaus-Jeorger, T., Jeorger, R., Olsson, E., Granqvist, C., Bacteria as workers in the living factory: metal accumulating bacteria and their potential for material science. *Trends Biotechnol*., 2001; **19**: 15–20.
10. Dura´n, N., Marcato, P., Alves, O., De Souza, G., Esposito, E., Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. J. Nanobiotechnol., 2005; **15**: 3-8.
11. Mukherjee, P., Roy, M., Mandal, B.P., Dey, G.K., *et al.*, Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*. Nanotechnol., 2008; **19**: 075103.1–075103.7
12. Sivalingama, P., Antony, J., Sivab, D., Achiramanb, S., Anbarasua, K., Mangrove Streptomyces sp. BDUKAS10 as nanofactory for fabrication of bactericidal silver nanoparticles. Colloids and Sur. B. *Biointerfac.*, 2012; **98**: 12-17.
13. Antony, J.J., Sivalingam, P., Siva, D., Kamalakkannan, S., *et al.*, Comparative evaluation of antibacterial activity of silver nanoparticles synthesized using Rhizophora apiculata and glucose. *Colloids Surf*. B. 2011; **88**: 134-140.
14. Sadhasivam, S., Shanmugam, P., Yun, K.S., Biosynthesis of silver nanoparticles by *Streptomyces hygroscopicus* and antimicrobial activity against medically important pathogenic microorganisms. *Colloids Surf. B.*, 2010; **81**: 358-362.
15. Chater, K.F., Genetic differentiation of *Streptomyces*, *Annu. Rev. Microbiol*., 1993; **47**: 685–713.
16. Landsdown, A.B.G., Silver I: its antibacterial properties and mechanism of action. *J. Wound Care*, 2002; **11**: 125–38.
17. Castellano, J.J., Shafii, S.M., Ko, F., Donate, G., *et al.*, Comparative evaluation of silver-containing antimicrobial dressings and drugs. *Int. Wound J*., 2007; **4**(2): 114-22.
18. Klasen, H.J., A historical review of the use of silver in the treatment of burns, Part I early uses. *Burns*, 2000; **30**: 1-9.
19. Chopra, I., 2007. The increasing use of silver-

based products as antimicrobial agents: a useful development or a cause for concern. *J. Antimicrob. Chemother*., 2000; **59**: 587–90.

20. Moyer, C.A., Brentano, L., Gravens, D.L., Margraf, H.W., Monafo, W.W., Treatment of large human burns with 0.5% silver nitrate solution. *Arch. Surg*., 1965; **90**: 812-67.
21. Fox, C.L., Modak, S.M., Mechanism of silver sulfadiazine action on burn wound infections. Antimicrob. *Agents Chemother*., 1974; **5**(6): 582–8.
22. Pisano, A.M., Sommer, J.M., Lopez, M.M., Application of pretreatment for the isolation of bioactive actinomycetes from marine sediments. *Appl. Microbiol. Biotechnol*., 1986; **25**: 285-288.
23. Hayakawa, M., Ohara, Y., Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J. Ferment.Technol.*, 1987; **65**: 501-509.
24. Mince, T.J., Jensen, P.R., Kauffman, C.A., Fenical, W., Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. *Appl. Environ. Microbiol.*, 2002; **68**: 5005–5011.
25. Pridham, T.G., Colour of Streptomyces: report of an international workshop on determination of colour of *Streptomyces. Appl. Microbiol.*, 1964; **13**: 43-61.
26. Shirling, E.B., Gottlieb, D., Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.*, 1966; **16**: 313-340.
27. Williams, S.T., Goodfellow, M., Alderson, G., Wellingtone, *et al.*, Numerical classification of Streptomyces and related genera. *J. Gen. Mocrobiol.*, 1983; **192**: 1743-1813.
28. Williams, Wilkins, Bergey's manual of determinative bacteriology. Holt (ed.). Co., Baltimore, 1994.
29. William, B., Whitman, Wilkins, *et al.*, Bergey's manual of systematic bacteriology. 2012; **5**(2th ed.), Co., Baltimore.
30. Pridham, T.G., Gottlieb, D., The utilization of carbon compounds by some Actinomycetes as an aid for species determination. *J. Bacteriol.*, 1948; **56**: 107-114.
31. Waksman, S.A., The Actinomycetes classification, identification and determination of genera and species. The Williams and Wilkins Co., Baltimore, 1961; **2**: 1-363.
32. Luedemann, G.M., Brodsky, B.G., Taxonomy of gentamicin producing *Micromonospora*. Antimicrob. *Agents Chemother*., 1964; **63**: 116-124.
33. Luedemann, G.M., Species concepts and criteria in the genus *Micromonospora*. Trans. N.V. *Acad. Sci*., 1971; **33**: 207-218.
34. Hopwood, D.A., Genetic recombination in *Streptomyces coelicolor*. *J. Gen. Microbial*., 1957; **16**: 2-3.
35. Smoker, J.A., Barnum, S.R., Rapid small-scale DNA isolation from filamentous cyanobacteria. *FEMS. microbiol. Let*., 1988; **56**(1): 119-122.
36. Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res*., 1997; **25**: 4876–4882.
37. Saitou, N., Nei, M., The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*., 1987; **4**: 406–425.
38. Tamura, K., Peterson, D., Peterson, N., Stecher, G., *et al.*, MEGA5, Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Biol. Evol.*, 2011; **28**: 2731–2739.
39. Jukes, T.H., Cantor, C.R., Evolution of protein molecules, in: Mammalian Protein metabolism III. Academic Press, London, 1969; 21–132.
40. Felsenstein, J., Confidence limits on phylogenies: an approach using the bootstrap. *Evolut.*, 1985; **39**: 783-791.
41. Li, G., He, D., Qian, Y., Guan, B., *et al.*, Fungus-mediated green synthesis of silver nanoparticles using *Aspergillus terreus*. *Int. J. Mol. Sci*., 2012; **13**: 466-476.
42. Hayat, M.A., Principles and techniques of electron microscopy. Vol. 1. London, Edward Arlond Lt., 1981; 522 p.
43. Mosmann, T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. of Immunol. Methods*, 1983; **65**: 55-63
44. Miller, J.H., A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for *E. coli* and Related Bacteria. Cold Spring Harbor Lab., Cold Spring Harbor, New York, 1992; 456 p.
45. Mulvaney, P., Surface plasmon spectroscopy of nanosized metal particles. *Langmuir*, 1996; **12**: 788–800.
46. Kumar, S., Abyaneh, M., Gosavi, S., Kulkarni, S., *et al*., Nitrate reductase-mediated synthesis of silver nanoparticles from $AgNO_3$. *Biotechnol. Lett*., 2007; **29**: 439–445.
47. Bai, H.J., Yang, B.S., Chai, G.J., Yang, G.E., *et al*., Green synthesis of silver nanoparticles using *Rhodobacter Sphaeroides*. *World J. Microbiol. Biotechnol*. 2011; **27**: 2723–2728.
48. Magudapathy, P., Gangopadhyay, P., Panigrahi, B. K., Nair, K.G.M., Dhara, S., Electrical transport studies of Ag nanoclusters embedded

in glass matrix. *Phys. B.*, 2001; **299**: 142.
49. Gole, A., Dash, C., Ramakrishnan, V., Sainkar, S.R., *et al.*, Pepsin gold colloid conjugates, Preparation, characterization, and enzymatic activity. *Langmuir*, 2001; **17**: 1674-1679.
50. Jain, N., Bhargava, A., Majumdar, S., Tarafdarb, J.C., Panwar, J., Extracellular biosynthesis and characterization of silver nanoparticles using *Aspergillus flavus* NJP08 A mechanism perspective. *Nanoscal*. 2011; **3**: 635.
51. Saifuddin, N., Wong, C., Yasumira, A., Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. *E. J. Chem.*, 2009; **6**(1): 61-70.
52. Elechiguerra, J., Burt, J., Morones, J., Camacho-Bragado, A., *et al.*, Interaction of silver nanoparticles with HIV-1. *J. Nanobiotechnol.*, 2005; **23**: 3-6.
53. Stoimenov, P.K., Klinger, R.L., Marchin, G.L., Klabunde, K.J., Metal oxide nanoparticles as bactericidal agents. *Langmuir*. 2002; **18**: 6679-6686.
54. Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., *et al.*, A mechanistic study of the antibacterial effect of silver ions on *E. coli* and *S. aureus*, *Biomed. Mater Res*., 2000; **52**(4): 662-668.
55. Yamanaka, M., Hara, K., Kudo, J., Bactericidal actions of a silver ion solution on E. coli, studied by energy-filtering transmission electron microscopy and proteomic analysis. *Appl. Environ. Microbiol*., 2005; **71**(11): 7589-93.
56. Bragg, P.D., Rainnie, D.J., Can, J., The effect of silver ions on the respiratory chain of *E. coli. Microbiol.*, 1974; **20**(6): 883-9.
57. McDonnell, G., Russell, A.D., Review Antiseptics and disinfectants: activity, action, and resistance. *Clin. Microbiol. Rev.*, 1999; **1**: 147-79.
58. Sondi, I., Salopek-Sondi, B., Silver nanoparticles as antimicrobial agent: a case study on *Escherichia coli* as a model for Gram-negative bacteria. *J. Colloid Interface Sci*., 2004; **275**(1): 177-82.
59. Devaraj, P., Kumari, P., Aartiand, C., Renganathan, A., Synthesis and characterization of silver nanoparticles using cannonball leaves and their cytotoxic activity against mcf-7 cell line. *J. of Nanotechnol*., 2013; 1-5.
60. Sowemimo, A., van de Venter, M., Baatjies, L., Koekemoer, T., Cytotoxic activity of selected Nigerian plants. *Afri. J. of Trad. Complementary and Alternative Medicines*, 2009; **6**(4): 526–528.
61. Martýnez, A., Conde, E., Moure, A., Domnguez, H., Estevez, R.J., Protective effect against oxygen reactive species and skin fibroblast stimulation of Couroupitaguianensis leaf extracts. *Nat. Product Research*, 2012; **26**: 314–322.