Phenolic profile and antimicrobial activity of green synthesized *Acalypha wilkesiana* seed’s silver nanoparticles against some food borne pathogens

Diaa A. Marrez¹, Mohamed A. El Raey², Ali M. El-Hagrassi², Mohamed M. Seif¹, Tamer I.M. Ragab³, Sabry I. El Negoumy², Mahmoud Emam²

¹Food Toxicology and Contaminants Department, National Research Centre, El-Behoos St. 33, Dokki-Cairo 12622, Egypt  
²Phytochemistry and Plant Systematic Department, National Research Centre, El-Behoos St. 33, Dokki-Cairo 12622, Egypt  
³Natural and Microbial Product Chemistry, National Research Centre, El-Behoos St. 33, Dokki-Cairo 12622, Egypt

*Correspondence: elraiy@gmail.com  Accepted: 08 Aug. 2017 Published online: 27 Nov. 2017*

Food safety is one of the major concerns due to threatening human health by various foodborne pathogens. The main objective of our study is to investigate the effect of green synthesis of silver nanoparticles (AgNPs) using *Acalypha wilkesiana* seeds ethyl acetate extract on antimicrobial activity against some pathogenic bacteria and mycotoxigenic fungi. HPLC-MS/MS analysis of phenolic profile of *A. Wilkesiana* extract showed presence of high content of phenolic metabolites including tannins and phenolic acids. The AgNPs were characterized by UV-visible spectrum and transmission electron microscope (TEM). AgNPs showed strong antibacterial activity against (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*) and antifungal activity against (*Fusarium proliferatum*, *Aspergillus ochraceus* and *Candida albicans*). The phenolic structures of *A. Wilkesiana* could be used for simple, non-hazardous, eco-friendly, cost-effective and efficient synthesis of AgNPs that can be used for large-scale production in the field of food and pharmaceutical industries.

**Keywords:** *Acalypha wilkesiana*, Silver nanoparticles, plant phenolics, antimicrobial activity, HPLC-MS/MS.

**INTRODUCTION**

Food industries lose about 25% of their food productions every year due to food contamination by pathogens (Sarhan and Azzazy, 2015). The major foodborne pathogens including *Salmonella typhii*, *Clostridium perfringens*, *Staphylococcus aureus*, *Campylobacter* spp. *Escherichia coli* and *Listeriamono cytogenes* (Scallan et al. 2011). Food borne also associated with mycotoxigenic fungi such as *Aspergillus* spp., *Fusarium* spp. and *Penicilium* spp. (Wood et al. 2003). Various natural and chemical food preservatives were used to control these foodborne pathogens (Juneja et al. 2012). However, chemical preservatives are not preferable due to their known side effects. Therefore, the use of bio-preservatives agents from different natural sources is necessary (Pawlowska et al. 2012).

Gold and silver nanoparticles are considered as strategies which have applicable uses in different fields like biology, chemistry, medicine and material science (Alaqad and Saleh, 2016). Silver nanoparticles have received greater attention due to their antimicrobial properties. Green synthesis of silver nanoparticles using plant extracts, microbial cell biomass and biopolymers drawn attention, because of its rapid, eco-friendly, non-pathogenic, economical and providing as a
single step technique for biosynthetic processes (Kharissova et al. 2013 and Ahmed et al. 2016). Silver nanoparticles were prepared by reduction of silver ions by medicinal plant extracts which contains biomolecules such as proteins, amino acid enzymes, polysaccharides, alkaloids, phenolics, saponins, terpinoids and vitamins (Kulkarni and Mudnapur, 2014).

_Acalypha_ is the fourth largest genus in family Euphorbiaceae with about 450 species (Webster, 1994). Several species of _Acalypha_ genus have been reported that they had wound healing, detoxification, antioxidant, antibacterial, antifungal and anticancer activities (Perez Gutierrez and Vargas, 2006; Marwah et al. 2007). _Acalypha wilkesiana_ is used traditionally, for the management of gastrointestinal disorders, hypertension, fungal skin infections, and diabetes (Akinyemi et al. 2005). _A. wilkesiana_ has been reported to possess anticancer, antibacterial and antifungal, anti-diabetic and anti-hypertensive properties (Al-Attar, 2010; Anokwuru et al. 2015). These activities due to the presence of sesquiterpense, monoterpenes, tri terpenoids, polyphenols,saponins, and anthraquinones in phytochemical constituents (Adesina et al. 2000 and Oladunmoye, 2006).

Several studies have focused on the antimicrobial activity of _A. wilkesiana_ leaves and seeds extract (Majekodunni and Nubani, 2014 and El-raey et al. 2016). The present study was aimed to green synthesis of silver nanoparticles (AgNPs) using _A. wilkesiana_ seeds ethyl acetate extract, evaluate its activity against pathogenic bacteria and mycotoxigenic fungi comparing with the activity of untreated ethyl acetate extract in addition to study of phenolic profile of _A. wilkesiana_ seeds ethyl acetate extract by HPLC/MS/MS.

**MATERIALS AND METHODS**

**Plant material**

The seeds of _Acalypha wilkesiana_ f. tricolor were collected from the garden of National Research Centre, Cairo, Egypt, in June 2016, and identified by Dr. Mohamed Al-Gebaly, former researcher of Botany, National Research Centre. A voucher specimen was deposited at the herbarium of National Research Center (CAIRAC) at number M109.

**Preparation of _A. wilkesiana_ extract**

The ethyl acetate fraction of _A. wilkesiana_ forma tricolor was prepared according to (El raey et al. 2016).

**HPLC-PDA-MS/MS analysis**

The phytochemical analysis of _A. wilkesiana_ ethyl acetate extract was performed using high performance liquid chromatography–mass spectrometry (HPLC-PDA-MS/MS). The LC system was Thermo finigan (Thermo electron Corporation, USA) coupled with an LCQ-Duo ion trap mass spectrometer with an ESI source (Thermo Quest). A C18 reversed-phase column (Zorbax Eclipse XDB-C18, Rapid resolution, 4.6 × 150 mm, 3.5 µm, Agilent, USA) was used for separation. A gradient of water and acetonitrile (ACN) (0.1 % formic acid each) was applied in which ACN was increased from 5% to 30% over 60 min in flow rate of 1 ml min⁻¹ and a 1:1 split before the ESI source. The auto sampler surveyor Thermo Quest was used for automated injection of samples. The instrument was under control by Xcalibur software (Xcalibur™ 2.0.7, Thermo Scientific). The MS operated in the negative mode with a capillary voltage of - 10 V, a source temperature of 200 °C, and high purity nitrogen as both sheath and auxiliary gas with flow rate of 80 and 40 (arbitrary units), respectively. MS/MS fragmentation was operated with Collision energy of 35%. The ions were detected within a mass range of 50-2000 m/z in a full scan mode.

**Synthesis of silver nanoparticles (AgNPs)**

Silver nanoparticles were prepared by the reduction of 10 ml of aqueous AgNO₃ (1 mM) solution with 0.4 ml of _A. wilkesiana_ seeds ethyl acetate extract at room temperature. The mixture was hand shaken and allowed to stand in the dark (to minimize the photo activation of silver nitrate) at room temperature. To study the effect of extract quantity on AgNPs synthesis, the quantity was varied from 100 µl to 500 µl per 10 ml of silver nitrate solution (1 mM). The obtained nanoparticle solution was purified by repeated centrifugation at 12,000 rpm for 20 min followed by redispersion of the pellet in deionized water. This process was repeated twice to isolate the pure AgNPs and exclude the presence of any unbound plant extract residue (Zayed et al. 2015).
Characterization of AgNPs

UV-Visible spectral analysis

Synthesis of AgNPs was monitored using a Schimadzu UV-1601 scanning spectrophotometer UV–visible (Schimadzu Corporation, Japan). The UV-Vis spectra were recorded between 200-500 nm.

Transmission electron microscopy (TEM)

The morphology and size of the reductive AgNPs were investigated by TEM (JEOL-JEM-1011, Japan). The samples were prepared by placing few drops of the nanoparticles suspension on carbon coated copper gride, followed by allowing the solvent to evaporate slowly before recording the TEM image.

Antimicrobial assay

Tested microorganisms

The inhibitory effects of Acalypha wilkesiana ethyl acetate extract and AgNPs were assayed against six species of pathogenic bacteria, two Gram-positive bacteria: Bacillus cereus EMCC 1080, Staphylococcus aureus ATCC 13565 and four Gram-negative bacteria: Salmonella typhi ATCC 25566, Escherichia coli 0157 H7 ATCC 51659, Pseudomonas aeruginosa NRRL B-272 and Klebsiella pneumoniae LMD 7726. Eight fungal and one yeast species were used for antifungal assay, Aspergillus flavus NRRL 3357, A. parasiticus SSWT 2999, A. westerdijikia CCT 6795, A. steynii IBT KLN 23096, A. ochraceus ITAL 14, A. carbonarius ITAL 204, Fusarium verticillioides ITEM 10027, F. proliferatum MPVP 328, Penicillium verrucosum BFE 500 and Candida albicans ATCC 10231.

Disc diffusion method

Antibacterial assay

From the 24 h incubated nutrient agar slant of each bacterial species a full loop of the microorganism was inoculated in a tube containing 5 ml of tryptic soy broth. The broth culture was incubated at 35°C for 2-6 h until it achieves the turbidity of 0.5 McFarland BaSO₄ standard. The bioactivity of A. wilkesiana ethyl acetate extract and AgNPs were examined against all the tested bacterial species using disc diffusion method of Kirby-Bauer technique (Bauer et al., 1966). Using cotton swabs, nutrient agar plates were uniformly inoculated with tryptic soy broth of bacterial cultures. A concentration of 1 mg ml⁻¹ for each ethyl acetate extract and AgNPs was prepared by dissolving in 1 ml of dimethyl sulfoxide (DMSO). Sterilized discs (6 mm) from Whatman No. 1 filter paper were loaded by either extracts or fractions and dried completely under sterile conditions. The discs were placed on the seeded plates by using a sterile forceps. DMSO and tetracycline (1mg ml⁻¹) represented the negative control and positive control, respectively. Inoculated plates were incubated at 37°C for 24 h, and then the inhibition zones were measured and expressed as the diameter of clear zone including the diameter of the paper disc.

Antifungal assay

The fungal strains were plated onto potato dextrose agar (PDA) and incubated for 5 days at 25°C. The spore suspension (2x10⁸ cfu ml⁻¹) of each fungus was prepared in 0.01% Tween 80 solution by comparing with the 0.5 McFarland standard. Petri dishes containing yeast extract sucrose (YES) medium were inoculated with 50 µl of each fungal culture and uniformly spread using sterile L-glass rod. Sterilized discs (6 mm) were loaded by either ethyl acetate extractor AgNPs (1 mg ml⁻¹) and dried completely under sterile conditions, then placed on the seeded plates by using a sterile forceps. DMSO and commercial fungicide Nystatin (1000 Unit ml⁻¹) were considered as a negative and positive control, respectively. The inoculated plates were incubated at 25°C for 48 h, and then the antifungal activity was assessed by measuring the zone of inhibition (mm), (Medeiros et al., 2011). The average of the results was calculated from at least three replicates for each assay.

Estimation of minimum inhibitory concentration (MIC)

The determination of MIC was conducted using tube dilution method (Irith et al., 2008). A 24 h culture of the tested bacterial species was diluted in 10 ml of tryptic soy broth (TSB) with reference to the 0.5 McFarland standard to achieve inocula of 10⁵ cfu ml⁻¹. In culture tube containing nine different concentrations of each A. wilkesiana ethyl acetate extract and AgNPs (4.0, 2.0, 1.75, 1.5, 1.0, 0.75, 0.50, 0.25, 0.1 mg ml⁻¹ in DMSO) were prepared. Each tube was inoculated with 100µl of bacterial cell suspension and incubated at 37°C for 24 h. The growth of the inoculum in broth is indicated by turbidity of the broth and the lowest concentration which inhibited the growth of the test organism was taken as the minimum inhibitory concentration (MIC).
MIC against fungi was performed by using the technique of Perrucci et al. (2004). The ethyl acetate extract and AgNPs at different concentrations were separately dissolved in 0.5 ml of 0.1% Tween 80 (Merck, Darmstadt, Germany), then mixed with 9.5 ml of melting, 45°C, PDA and poured into Petri dish (6 cm). The prepared plates were centrally inoculated with 3µl of fungal suspension (10^6 cfu ml^-1; 0.5 McFarland standard). The plates were incubated at 25°C for 48h. At the end of the incubation period, mycelial growth was monitored and MIC was determined.

### Determination of total carbohydrates by acid hydrolysis

Complete acid hydrolysis of the sample polysaccharides were carried out according to the modified method by (Fischer and Dorfel 1955) as follows: 0.5 mg of the sample was carefully stirred with 0.5 ml ice cold 80% H2SO4 to give a paste and was kept at room temperature for 15 h, then diluted with a mixture of ice and distilled water (up to 13 ml) until the strength of sulfuric acid reached 2N. The solution was further hydrolyzed by heating in a sealed tube in a boiled water bath for 20 min. Thereafter, the color density was measured at 490 nm Spectrophotometer UNICO 7200. The quantities of sugars were determined by comparison to appropriate standard curves constructed under the same conditions.

### RESULTS AND DISCUSSION

#### Phenolic profile of A. wilkesiana seeds ethyl acetate extract

HPLC-PDA-MS/MS has been used to identify the polyphenolic content of the ethyl acetate extract of the A. wilkesiana seeds. The twenty phenolic compounds have been identified based on their molecular weights, fragmentation pattern and the spectral data from the PDA detector. All compounds fragmentations were compared with those published data as shown in (Table 1).

Strong acid hydrolysis was applied and showed that total carbohydrates are 4.3%, using the phenol-sulfuric acid. Monosaccharide constituents of acid hydrolysis for A. wilkesiana seeds (% w/w) was mainly 96.5% D-glucose.

Phenolic constituents were investigated and showed that: Compound 1 exhibited [M-H]^- ion at m/z 169 and showed fragments at m/z 125 due to loss of one CO2 molecule suggesting compound 1 to be gallic acid (Song et al. 2010). Compound 2, showed [M-H]^- ion at m/z 481and showed fragments at m/z 463 due to loss of one H2O molecule and fragment at m/z 301 typical for ellagic acid. By comparing these fragments data with those published (Fischer et al. 2011) the structure of compound 2 suggested to be HHDP-glucose which was isolated from this plant as 3,6-HHDP-\(^1\)C\(_4\)β-D-glucopyranoside.
<table>
<thead>
<tr>
<th>No</th>
<th>R&lt;sub&gt;t&lt;/sub&gt; (min.)</th>
<th>[M-H]</th>
<th>MS/MS</th>
<th>Identified compounds</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.44</td>
<td>169</td>
<td>125</td>
<td>Gallic acid</td>
<td>Song et al. 2010</td>
</tr>
<tr>
<td>2</td>
<td>2.33</td>
<td>481</td>
<td>301</td>
<td>Hexahydroxydiphenoyl hexoside (HHDP hexoside)</td>
<td>Fischer et al. 2011</td>
</tr>
<tr>
<td>3</td>
<td>3.32</td>
<td>331</td>
<td>169</td>
<td>Galloyl-hexoside</td>
<td>Meyers et al. 2006</td>
</tr>
<tr>
<td>4</td>
<td>5.87</td>
<td>321</td>
<td>163, 233, 277</td>
<td>Digallic acid I</td>
<td>Abu-Reidah et al. 2015</td>
</tr>
<tr>
<td>5</td>
<td>8.31</td>
<td>321</td>
<td>233, 277</td>
<td>Digallic acid II</td>
<td>Abu-Reidah et al. 2015</td>
</tr>
<tr>
<td>6</td>
<td>10.14</td>
<td>291</td>
<td>247, 203</td>
<td>Brevifolin carboxylic acid</td>
<td>Zhu et al. 2015</td>
</tr>
<tr>
<td>7</td>
<td>11.33</td>
<td>483</td>
<td>169, 331</td>
<td>Digalloyl-hexoside I</td>
<td>Meyers et al. 2006</td>
</tr>
<tr>
<td>8</td>
<td>13.06</td>
<td>483</td>
<td>169, 331</td>
<td>Digalloyl-hexoside II</td>
<td>Meyers et al. 2006</td>
</tr>
<tr>
<td>9</td>
<td>14.34</td>
<td>183</td>
<td>125, 169</td>
<td>Methyl gallic acid</td>
<td>Ramirez et al. 2013</td>
</tr>
<tr>
<td>10</td>
<td>14.98</td>
<td>633</td>
<td>301, 463</td>
<td>Galloyl-HHDP-glucose I</td>
<td>Zhu et al. 2015</td>
</tr>
<tr>
<td>11</td>
<td>15.58</td>
<td>301</td>
<td>229, 257, 301</td>
<td>Ellagic acid</td>
<td>Fischer et al. 2011</td>
</tr>
<tr>
<td>12</td>
<td>17.37</td>
<td>633</td>
<td>257, 301, 463, 615</td>
<td>Galloyl-HHDP-glucose II</td>
<td>Zhu et al. 2015</td>
</tr>
<tr>
<td>13</td>
<td>18.34</td>
<td>951</td>
<td>301, 463, 613, 915, 933</td>
<td>Galloyl-HHDP-DHDP-hexoside</td>
<td>Fischer et al. 2011</td>
</tr>
<tr>
<td>14</td>
<td>18.34</td>
<td>951</td>
<td>301, 463, 613, 915, 933</td>
<td>Galloyl-HHDP-DHDP-hexoside II</td>
<td>Fischer et al. 2011</td>
</tr>
<tr>
<td>15</td>
<td>20.74</td>
<td>305</td>
<td>245, 273</td>
<td>Methyl brevifolin carboxylate</td>
<td>Zhu et al. 2015</td>
</tr>
<tr>
<td>16</td>
<td>21.97</td>
<td>951</td>
<td>301, 463, 613, 915, 933</td>
<td>Galloyl-HHDP-DHDP-hexoside III</td>
<td>Fischer et al. 2011</td>
</tr>
<tr>
<td>17</td>
<td>22.16</td>
<td>633</td>
<td>249, 301, 331, 463, 615</td>
<td>Galloyl-HHDP-hexoside III</td>
<td>Zhu et al. 2015</td>
</tr>
<tr>
<td>18</td>
<td>23.14</td>
<td>951</td>
<td>301, 613, 765, 933</td>
<td>Galloyl-HHDP-DHDP-hexoside IV</td>
<td>Fischer et al. 2011</td>
</tr>
<tr>
<td>19</td>
<td>23.33</td>
<td>379</td>
<td>159, 271, 303, 347, 361</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>24.42</td>
<td>379</td>
<td>159, 271, 303, 347, 361</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Phenolic constituents of *A. Wilkesiana seeds* ethyl acetate extract:

Figure. 1. Base beak chromatogram of *A. wilkesiana* seeds ethyl acetate extract.
Compound 3, showed [M-H]⁻ ion at m/z 331 and showed fragments at m/z 169 due to loss of one glucose moiety, suggesting compound 3 to be mono galloyl glucose (Meyers et al. 2006). Compounds 4 and 5 showed [M-H]⁻ ion at m/z 321 and showed fragments at m/z 277 due to loss of CO₂ molecule and fragment at m/z 233 due to loss of another CO₂ molecule prove that these compounds contain two carboxylic groups. By comparing these fragments data with published data (Abu-Reidah et al. 2015), compounds 4 and 5 are positional isomers and identified to be digallic acid. Compound 6 showed [M-H]⁻ ion at m/z 291 and showed fragments at m/z 247 due to loss of CO₂ molecule and fragment at m/z 203 due to loss of another CO₂ suggesting this compound to be brefiolin carboxylic acid (Santo et al. 2015 & Zhu et al. 2015). Compounds 7 and 8 showed [M-H]⁻ ion at m/z 483 and showed fragments at m/z 331 due to loss of galloyl moiety and fragment at m/z 169 characteristic peak for gallic acid, suggesting compounds 7 and 8 to be digalloyl glucose. Compound 9, exhibited [M-H]⁻ ion at m/z 183 and showed fragments at m/z 169 characteristic for gallic acid suggesting compound 9 to be methyl gallate (Ramirez et al. 2013). Compounds 10, 12 and 17 showed [M-H]⁻ ion at m/z 633 and showed fragments at m/z 615 due to loss of one H₂O molecule followed by loss of galloyl moiety to show molecular ion peak at m/z 463 and followed by loss of hexose moiety to show molecular ion at m/z 301 which are typical for ellagic acid. By comparing these fragments data with those published (Zhu et al. 2015) the structure of compounds 10, 12 and 17 suggested to be galloyl HHDP-glucose which was isolated from this plant as 1-O-galloyl-3,6- HHDP.⁻C₆-β-D-glucopyranoside (El raey et al. 2016). Compound 11, exhibited [M-H]⁻ ion at m/z 301 and showed fragments at m/z 229 and 257, suggested that compound 11 to be ellagic acid (Fischer et al. 2011). Compounds 13, 14, 16 and 18 showed [M-H]⁻ ion at m/z 931 and showed fragments at m/z 933 due to loss of one H₂O molecule and 915 due to loss of two water molecules followed by m/z 613 due to loss of ellagic acid after two water molecules then it loss gallic acid to show m/z 445 and showed also characteristic fragment at m/z 301 of ellagic acid. Based on these fragments and comparing of these results with those published data (Fischer et al., 2011). Compounds 13, 14, 16 and 18 are positional isomers and identified to be (galloyl-HHDP-DHHDP-hexoside). Among these compounds, geraniin was isolated before (El raey et al. 2016) from the seeds of this plant.

Compound 15 showed [M-H]⁻ ion at m/z 305 and showed fragments at m/z 245 and 273 suggesting this compound to be Methyl brevifolin carboxylate (Zhu et al. 2015). The structures of the identified compounds were illustrated in (Fig. 2)

Formation of nanoparticles:

Green synthesis of silver nanoparticles was achieved through the reduction of silver by phenolic constituents of A. wilkesiana seed ethyl acetate extract as shown in Table 1.

UV-visible and TEM studies:

Formation of Ag nanoparticles (Zayed et al., 2015) was recognized by observing the colour change of the reaction medium from yellow, red and finally brown (Emam et al., 2017). UV-vis spectrophotometer showed distinct bathochromic shift of the A. wilkesiana extract from 433 to 458, 468 nm with increasing concentrations (0.4 mM) of A. wilkesiana extract (100 μL to 500 μL) added to ml of AgNO₃ (1 mM) (Fig. 3). The colloidal AgNPs were examined by TEM micrographs revealed the formation of AgNPs with an external spherical shape ranging from 4 to 30 nm (Fig. 4).

UV-vis spectroscopic studies

Ag nanoparticle (AgNPs) were characterized by their brilliant yellowish brown color in the reaction mixture, this is due to the surface plasmon resonance (SPR). The UV-vis spectra of Ag nanoparticles prepared by adding different amounts (100, 200, 300, 400 and 500 μL) of A. wilkesiana to 10 ml of 10⁻³ AgNO₃ solution illustrated an absorption in the visible region at 430-470 nm. This symmetrical absorption band reflects the monodispersity of the silver nanoparticles. Also, the absorbance SPR band grows with increasing addition of the A. wilkesiana from 100 to 500 μl that increasing at intensity indicates that more Ag⁺ ions are reduced to Ag nanoparticles.

TEM studies

Shape and size of prepared Ag nanoparticles are characterized by using the TEM technique. (Fig. 4) images of the AgNPs prepared by addition of 300 μL showed mainly spherical in shape with particle size varies between 4-30 nm with separated particles from each other which reflect the capping action of the plant extract in the preparation process.
Marrez et al. LC/MS and Antimicrobial activity of *A. wilkesiana* Silver nanoparticles

Galllic Acid Derivatives

- Gallic acid
- Methyl gallate
- Mono galloyl glucose
- Di-gallic acid I
- Di-gallic acid II

Ellagitannins

- Galloyl HHDP-glucose
- Galloyl DHHDP-HHDP-glucose

Brevifolin carboxylic acids derivatives

- Brevifolin carboxylic acid
- Brevifolin carboxylic acid methyl ester

Figure 2. Isolated phenolic metabolites
Fig. 3. UV–Vis spectra of Ag NPs formed by the reaction of different concentration of *A. wilkesiana* seeds ethyl acetate extract with 1 mM AgNO$_3$ concentrations.

Figure. 4. TEM image of Ag nanoparticle prepared with 0.3 mL of *A. wilkesiana seeds ethyl acetate* extract at 1 mM AgNO$_3$. 
Antimicrobial activity

Antibacterial activity

Table 2 revealed that *A. wilkesiana* seeds ethyl acetate extract and its AgNPs inhibited the growth of all tested bacteria in varying degree of inhibition zones. Moreover, the AgNPs showed highest activity against all pathogenic bacteria when compared with normal form of extract untreated. The highest antibacterial activity was noticed against *S. aureus* followed by *E. coli* with inhibition zones of 16.0 and 14.8 mm, respectively. Also, AgNPs appeared lower MIC value against all tested bacteria than normal form of extract with MIC value ranged between 0.2 to 0.37 mg ml\(^{-1}\) (Fig. 5). The lowest MIC was observed with *E. coli* and *S. typhi* at a concentration of 0.2 mg ml\(^{-1}\).

Many studies have been reported that natural phenolics and their AgNPs can be effective as bactericidal agents (Al Jaouni S and Selim S, 2017, Sondi and Salopek, 2004, Rai et al. 2012), but it should be noted that the antimicrobial activity of AgNPs depends on particle size (Panácek et al. 2006). There are conflicting statements on the effect of AgNPs on gram positive and gram negative bacteria. Where, Kim et al. (2007) had reported that gram negative bacteria were more sensitive against AgNPs than gram positive bacteria; Shrivastava et al. (2007) found that gram negative bacteria were more resistant to the effect of AgNPs than gram positive bacteria. On the other hand, Peticae et al. (2008) revealed that AgNPs had equal inhibitory effect against both gram positive and gram negative strains. In the present study AgNPs showed equal inhibitory effect against both tested gram positive and gram negative bacteria. Krishnaraj et al. (2010) found that synthesized silver nanoparticles using *A. indica* leaf extract showed antibacterial activity against water borne pathogens *E. coli* and *Vibrio cholerae*.

El-raey et al. (2016) reported that the ethyl acetate extract of *A. wilkesiana* F. tricolor seeds had antibacterial activity against *B. cereus*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhi* and *K. pneumonia* with MIC value ranged between 0.27 and 0.55 mg ml\(^{-1}\).Haruna et al. (2013) reported that leaves of *A. wilkesiana* ethyl acetate fraction showed antibacterial activity against *S. aureus*, *P. aeruginosa*, *P. vulgaris*, *S. pyogenes* and *E. coli*. Kanyanga et al. (2014) indicated that leaf of *A. wilkesiana* aqueous and methanolic extracts had antibacterial activity against *E. coli*, Enterobacter cloacae, *K. pneumonia*, *K. oxytoca*, *Proteus mirabilis*, *Schigella flexneri*, *S. aureus* and *S. thyhimurium*. Saranraj et al. (2010) evaluated the antibacterial activity of the ethanol and ethyl acetate extracts of mature leaves of *A. indica* against nine pathogenic bacteria and maximum inhibition zone, 30 mm, was showed against *P. aeruginosa*, *E. coli* and *B. subtilis*.

Table 2: Antibacterial activity of *A. wilkesiana* ethyl acetate extract and its AgNPs.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Inhibition zone mm (Mean±*S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>negative control</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>0</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>0</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>0</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>0</td>
</tr>
</tbody>
</table>

n=3, *S.E*: standard error, negative control: DMSO, ACE: *A. wilkesiana* ethyl acetate extract
Table 3: Antifungal activity of *A. wilkesiana* ethyl acetate extract and its AgNPs.

<table>
<thead>
<tr>
<th>fungi</th>
<th>Inhibition zone mm (Mean±S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative control</td>
</tr>
<tr>
<td><em>A. parasiticus</em></td>
<td>0</td>
</tr>
<tr>
<td><em>A. ochraceus</em></td>
<td>0</td>
</tr>
<tr>
<td><em>A. westerdijikia</em></td>
<td>0</td>
</tr>
<tr>
<td><em>A. carbonarius</em></td>
<td>0</td>
</tr>
<tr>
<td><em>F. proliferatum</em></td>
<td>0</td>
</tr>
<tr>
<td><em>P. verrucosum</em></td>
<td>0</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>0</td>
</tr>
</tbody>
</table>

n=3, *S.E: standard error, negative control: DMSO.

Figure 5. Minimum inhibitory concentration of *A. wilkesiana* seeds ethyl acetate extract and its AgNPs.

**Antifungal activity**

The antifungal activities of *A. wilkesiana* ethyl acetate extract and its AgNPs against different strains of mycotoxigenic fungi are illustrated in Table 3. Generally, the AgNPs appeared higher antifungal activity than ethyl acetate extract. The highest activity was recorded against *F. proliferatum* followed by *C. albicans* with inhibition zones 20.3 and 17.0 mm, respectively. Interestingly, the synthesized AgNPs observed higher antifungal activity against all tested toxigenic fungi than the antibiotic standard, Nystatin. The results of MIC value of AgNPs were lowest when compared with ethyl acetate extract (Fig. 6). MIC for silver nanoparticles are ranged from 0.33 to 1.06 mg ml\(^{-1}\), while MIC for ethyl acetate extract are ranged between 0.58 to 1.27 mg ml\(^{-1}\). The lowest MIC was recorded against *C. albicans* at a concentration of 0.33 mg ml\(^{-1}\).

El-raey et al. (2016) reported that the ethyl acetate extract of *A. wilkesiana* seeds had antifungal activity against wide range of mycotoxigenic fungi include *A. flavus, A. ochraceus, A. parasiticus, A. westerdijikia, A. carbonarius, F. verticillioides and F. proliferatum.*
Figure 6. Minimum inhibitory concentration of *A. wilkesiana* seeds ethyl acetate extract and its AgNPs.

*Acalypha wilkesiana* leaves ethyl acetate fraction, methanol and aqueous extracts showed antifungal activity against *A. niger* and *C. albicans* (Onocha and Olusanya, 2010; Haruna et al. 2013 and Kanyanga et al. 2014). Somchit et al. (2010) reported that the chloroform extract of *A. indica* leaves had antifungal activity against *Candida albicans*, *C. tropicalis*, *Microsporum canis Aspergillus fumigatus*.

No available studies examined the antimicrobial activity of *A. wilkesiana* seeds ethyl acetate extract AgNPs against foodborne bacteria or mycotoxigenic fungi. Krishnaraj et al. (2012) revealed that silver nanoparticles synthesized by using *A. indica* leaf extract as reducing agents had antifungal activity against plant pathogens, *Alternaria alternata, Sclerotinia sclerotiorum, Macrophomina phaseolina, Rhizoctonia solani, Botrytis cinerea* and *Curvularia lunata*. Rout et al. (2012) revealed that *Ocimum sanctum* leaves methanolic extract AgNPs showed higher antifungal activity against *C. albicans*, *C. kefyr* and *A. niger* whereas intermediated activity were showed against *C. tropicalis*, *C. krusei*, *A. flavus* and *A. fumigates*. Liga Rao et al. (2013) found that AgNPs synthesized by *Allamanda cathartica* leaves aqueous extract had antifungal activity against *Fusarium oxysporum, Curvularia lunata, Rhizopus arrhizus, Aspergillus niger* and *A. flavus*.

**CONCLUSION**

Silver nanoparticles which are formed by the reducing power of phenolic content of *A. wilkesiana* seeds extract showed considerable antimicrobial activity compared to the untreated extract. This biosynthesized AgNPs prove to be potentially antimicrobial agents to be used in active food packaging film, coating and in pharmaceutical applications.

**CONFLICT OF INTEREST**

The authors have declared no conflict of interest.

Compliance with Ethics Requirements
This article does not contain any studies with human or animal subjects

ACKNOWLEDGEMENT
The authors are grateful for Dr. M. Sobeh and Dr. B. Wetterauer (IPMB), Universität Heidelberg, for collecting HPLC-MS/MS data.

AUTHOR CONTRIBUTIONS
All authors contributed equally in all parts of this study.

Copyrights: © 2017 @ author(s).
This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

REFERENCES
Identification of condensed and hydrolyzable tannins in tanoak acorns (Lithocarpus densiflorus). Journal of agricultural and food chemistry, 54(20), 7686-7691.


and Products, 47, 262-269.