

1 **A Purely Green Synthesis of Silver Nanoparticles using *Carica papaya*, *Manihot esculenta*, and *Morinda***  
2 ***citrifolia*: Synthesis and Antibacterial Evaluations**

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18  
19 **Abstract**

20 Green procedure for synthesizing silver nanoparticles (AgNPs) is currently considered due to its economy and  
21 toxic-free effects. Several existing works on synthesizing AgNPs using leaves extract still involve the use of  
22 physical or mechanical treatment such as heating or stirring, which consume a lot of energy. To extend and  
23 explore the green extraction philosophy, we report here the synthesis and antibacterial evaluations of a purely  
24 green procedure to synthesize AgNPs using *Carica papaya*, *Manihot esculenta*, and *Morinda citrifolia* leaves  
25 extract without the aforementioned additional treatment. The produced AgNPs were characterized using the  
26 ultraviolet-visible spectroscopy (UV-vis), field emission scanning electron microscopy (FESEM), energy-  
27 dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR), and antibacterial  
28 investigations. For antibacterial tests, two bacteria namely *Escherichia Coli* and *Bacillus Cereus* were selected.  
29 The presently employed method has successfully produced spherical AgNPs having sizes ranging from 9 to 69  
30 nm, with plasmonic characteristics ranging from 356 to 485 nm, and energy-dispersive X-ray peak at

31 approximately 3 keV. In addition, the smallest particles can be produced when *Manihot esculenta* leaves extract  
32 was applied. Moreover, this study also confirmed that both the leaves and synthesized AgNPs exhibit the  
33 antibacterial capability, depending on their concentration and the bacteria type.

34

35 **Keywords:** *Carica papaya*, *Manihot esculenta*, *Morinda citrifolia*, green synthesis, silver nanoparticles

36

### 37 **Introduction**

38 Silver nanoparticles (AgNPs) have been the attraction for various active research subjects in recent years due to  
39 their appealing electrical [1], optical [2, 3], physical [4], and thermal behaviors [5]. Their applications are  
40 widely found in the biological products [6], photovoltaics [7], catalysts [8], and chemical sensors [9]. Of interest  
41 also are their utilization in fabrics [10], antimicrobial coatings [6, 11], biomedical devices [12, 13], and wound  
42 dressing owing to their bacterial-repelling property [14, 15]. It is well known that their properties depend  
43 strongly on their sizes, species, shapes, and procedures of the synthesis.

44 Studies on the toxicity properties of AgNPs on the bacterial growth have been well-documented in the  
45 literature. Colony forming unit (CFU) and inhibition zone are common approaches used to investigate their  
46 antibacterial capability. It is established that the nanoparticles have a large surface area compared to micro or  
47 macroparticles. Thus, nanoparticles have a higher tendency of interaction with the bacterial cells compared to  
48 the bigger particles. AgNPs with the size of 5 nm demonstrated the greatest antibacterial capability against  
49 *Escherichia coli* (*E. coli*) MTCC 443 and *Staphylococcus aureus* NCIM 5201 compared to the larger particles  
50 such as 7 nm and 10 nm sizes at similar bacterial concentrations [16]. Another study also found that smaller  
51 AgNPs with the sizes ranging from 15 to 50 nm exhibited more antibacterial activity than larger particles  
52 ranging from 25 to 70 nm, 30 to 80 nm, and 30 to 200 nm against *Pseudomonas aeruginosa* and *E. coli*. In  
53 addition, the shape of AgNPs also affected their antibacterial capability [17]. The minimum inhibitory  
54 concentration of AgNPs in other shapes such as nanocubes, nanospheres, and nanowires were found to be 37.5,  
55 75, and 100  $\mu\text{g mL}^{-1}$  when a bacterial concentration of  $10^4$  CFU  $\text{mL}^{-1}$  was employed. The high antibacterial  
56 activity of these anisotropic-shaped AgNPs was due to the basal plane with high-atom-density facets acting as  
57 the maximum reactivity sites [17].

58 Since AgNPs are proven to be toxic, their existence and applications in the environment have become a  
59 hot topic for research [18-23]. The presence of AgNPs in the environment is commonly attributed to their  
60 release from commercial products. For instance, about 5 to 95% of the total amount of AgNPs in the consumer

61 products were expelled to sewage treatment plants. In addition, they were released from six types of socks  
62 containing AgNPs with the concentrations ranging from 1.5 to 650  $\mu\text{g}$  in 500 mL of the wash water [24]. Shirts,  
63 medical masks and cloths, toothpaste, shampoo, detergent, towels, teddy bear toys, and two humidifiers were  
64 also found to release AgNPs to the wash water with the concentration of about 45  $\mu\text{g product}^{-1}$  [25].  
65 Furthermore, about 85  $\text{g d}^{-1}$  of AgNPs were discharged from the laundry into the sewer system and the  
66 municipal wastewater treatment plant [26]. It is of importance to note that AgNPs in the environment can be  
67 transformed into different forms, such as ionic Ag,  $\text{Ag}_2\text{O}$ , and  $\text{Ag}_2\text{S}$  depending on the environmental conditions  
68 via physical and chemical events [27].

69         There exist many works on synthesizing and characterizing numerous properties of AgNPs in the past  
70 several years [28-34]. Since understanding how their various properties change corresponding to different  
71 extracting techniques is vital to achieve their most desirable performance, several production approaches were  
72 continually proposed and revised. Although many extracting methods are possible, the current trend focuses on  
73 how this can be carried out in a cheaper and greener manner. Currently, synthesis of AgNPs using plant extracts  
74 is highly considered because of their simplicity, abundant availability, and potential to eliminate a complicated  
75 preparation such as the use of microorganism as presented by Mishra et al. [34]. The use of leaves, seeds, roots,  
76 and fruits in the green synthesis of AgNPs have been continually proposed and remain an active subject for  
77 research investigation. Among these, the exploration of leaves extract as the reducing and stabilizing agent to  
78 synthesize nanoparticles of AgNPs is a preferable approach due to their wide obtainability in the environment.  
79 Table 1 lists the recent pattern of AgNPs synthesis using numerous leaf extracting approaches [4, 31, 32, 35-41].  
80 Several works, for instance Salem et al. [40], Vijay Kumar et al. [41], Muthukrishnan et al. [38], and He et al.  
81 [33], on the production of AgNPs still employed physical or mechanical treatments such as heating or stirring,  
82 which consume a lot of energy.

83         Therefore, the aim of the present study is to evaluate the suitability of the use of *Carica papaya* (*C.*  
84 *papaya*), *Manihot esculenta* (*M. esculenta*), and *Morinda citrifolia* (*M. citrifolia*) leaves extract without the  
85 aforementioned additional treatment to synthesize AgNPs. It is essential to provide a purely green procedure for  
86 future exploration to minimize the use of energy, its massive consumption of which is evidenced in other  
87 alternative approaches. *C. papaya* is a climacteric tropical plant originated from the Southern Mexico. It is also  
88 generally well-populated in the tropical climate such as Brazil, Malaysia, and Indonesia. In addition, *M.*  
89 *esculenta*, generally known as Cassava, is well recognized as a carbohydrate source. In the tropical region, it is  
90 the third largest source of carbohydrates after rice and maize [42]. Furthermore, *M. citrifolia* is a medical plant

91 that is widely grown in Malaysia and Indonesia. In these countries, it is well known as Mengkudu widely  
92 explored for numerous medical applications. Leaves of these plants are commonly utilized as a vegetable source  
93 as well [43, 44]. For comprehensive details, the chemical compositions of these three types of leaf are offered in  
94 Table 2 [42, 45-48]. Many existing works have confirmed that the biomolecules contained in leaves such as  
95 proteins, enzymes, polysaccharides, amino acids, and vitamins can act as bioreductant from reducing metal ions  
96 to the formation of AgNPs in the solution [49, 50]. The use of all currently proposed leaves is due to their  
97 possession of these favorable properties as presented in Table 2. Also, since previous studies have proven that  
98 these leaves have antibacterial capability, their exploration either as extract or capping on AgNPs are interesting  
99 for further research pursuit. Such study can be highly beneficial for future medical applications.

100

## 101 **Materials and Methods**

### 102 **Materials**

103 The basic compound under study, silver nitrate ( $\text{AgNO}_3$ , QReC, Auckland, New Zealand), was used as the silver  
104 salt. *C. papaya*, *M. esculenta*, and *M. citrifolia* leaves were collected from the surrounding area of Universiti  
105 Teknologi Malaysia, Johor Bahru, Malaysia. 0.45  $\mu\text{m}$  nylon membrane (Whatman<sup>®</sup> Nylon membrane, Sigma-  
106 Aldrich, St. Louis, MO, USA) was used as the filter. For synthesizing process, solutions were prepared using the  
107 ultrapure water (resistivity 18.2  $\text{M}\Omega\text{ cm}$ ) (Arium Ultrapure Water System, Sartorius Malaysia Sdn Bhd, Kuala  
108 Lumpur, Malaysia). Bacteria, *E. coli* and *Bacillus cereus* (*B. cereus*), were obtained from the Faculty of  
109 Biosciences and Medical Engineering, Universiti Teknologi Malaysia. In addition, agar powder (OXOID  
110 CM0003, Oxoid Ltd, Cheshire, England) was used. Broth powder (Merck VM447243, Merck KGaA,  
111 Darmstadt, Germany) was also employed in this study.

112

### 113 **Preparation of leaves extract**

114 To remove impurities, fresh leaves were first washed using the tap water and followed by the ultrapure water  
115 three times each. Then, 10 g of leaf was mixed with 300 mL ultrapure water in a 500 mL Erlenmeyer flask. The  
116 mixture was heated to 250 °C for 30 min, before cooled at the room temperature. To obtain a pure leaves extract,  
117 the mixture was then filtered through a nylon membrane filter of 0.45  $\mu\text{m}$  . The solution passing the membrane  
118 was then stored in a fridge at a temperature of 7 °C for future use. The same extraction procedure was then  
119 applied for all proposed leaves.

120

### 121 **Synthesis of AgNPs**

122 Firstly, AgNO<sub>3</sub> solution (100 mL in volume) was prepared using AgNO<sub>3</sub> and the ultrapure water with a  
123 concentration of 0.15 M in a 500 mL Erlenmeyer flask. To initiate the synthesis process, 100 mL leaves extract  
124 was added slowly into the AgNO<sub>3</sub> solution. The mixtures were then left overnight to perform the reduction of  
125 metal ions to the formation of AgNPs in the solution. For control, a 100 mL AgNO<sub>3</sub> solution with the same  
126 concentration without leaves extract was also prepared. For AgNPs harvesting, the mixtures were then  
127 centrifuged at 8000×g for 45 min and the pellet obtained in this process was collected. To purify AgNPs  
128 production, the pellet was then cleansed with the ultrapure water before centrifuged at 8000×g for 45 min. The  
129 purification process was repeated for three times. Next, the pellet was air dried. AgNPs obtained using this  
130 procedure were then stored for the characterization. The same procedure was carried out for synthesizing  
131 AgNPs using other leaves extract.

132

### 133 **Plasmonic investigation**

134 Plasmonic property of AgNPs was characterized using the UV-Vis spectrometer (Perkin–Elmer, No.  
135 101N4110104) installed with the Lambda 25 software. It was operated at a resolution of 1 nm, a scan speed of  
136 960 nm min<sup>-1</sup>, and the electromagnetic wavelength in the range of 300 to 700 nm. 2 mL sample mixtures were  
137 injected into the UV-vis tube. In this inspection, the ultrapure water was used as a blank.

138

### 139 **FTIR characterization**

140 For this observation, AgNPs were mixed with potassium bromide (1:100) to produce the specimen in a pellet  
141 form. The pellet was pressed hydraulically (Specac model with a serial number of N29850) at 10 tons in  
142 pressure. The pressed pellet was then taken and placed into the FTIR holder. Biomolecules bonding of the  
143 synthesized AgNPs was identified using the FTIR spectrometer (PerkinElmer Frontier-GPOB model 96046)  
144 installed with the PerkinElmer Spectrum software. The spectrometer used OptKBr (7800 to 400 cm<sup>-1</sup>) as beam  
145 splitter and MIR TGS (15000 to 370 cm<sup>-1</sup>) as detector. This characterization was conducted using a spectrum  
146 wavelength in the range of 650 to 4000 cm<sup>-1</sup> at a resolution of 4 cm<sup>-1</sup> and accumulations of 10 scans at room  
147 temperature.

148

149

150

151 **FESEM and SEM-EDX**

152 AgNPs morphologies were also characterized by means of the field emission scanning electron microscopy  
153 (FESEM ZEISS Supra 35VP). This apparatus was supplied by Carl Zeiss Sdn Bhd. It was operated at an  
154 accelerating of 5 kV with a magnification of 50000×. AgNPs elements were then confirmed by SEM-EDX  
155 (HITACHI S-3400N) equipped with the Bruker Quantax software. It was operated at a voltage of 15 kV.

156

157 **Preparation of agar and broth nutrients**

158 To produce agar nutrient, 14 g of the nutrient agar powder was mixed with 500 mL of ultrapure water. In  
159 addition, 4 g of the broth powder was prepared with the same water mixture to produce the broth nutrient  
160 solution. In the preparation, all solutions were sterilized using the autoclave ALP (model CL-40M no.805415) at  
161 a temperature of 121 °C for 2 h.

162

163 **Colony forming test**

164 Colony forming test was prepared using the previous study by Mueller and Hinton [51] as basis. AgNO<sub>3</sub> and  
165 leaves extract were freshly prepared and mixed for this test. A 10-mL AgNO<sub>3</sub> solution (0.15 M) was prepared  
166 using the ultrapure water in a 50 mL plastic tube. A variation in the leaf quantity was made such that ratios of  
167 5:2 and 5:3 for AgNO<sub>3</sub> and leaves extract were obtained. AgNO<sub>3</sub> solution with the same concentration was also  
168 prepared as a control case. Bacterial cultures of about  $5 \times 10^{15}$  colony were taken and about 0.1 mL of inoculum  
169 was mixed with the solution and incubated at 37 °C for 24 h. For comparison purpose, the pure leaves extract  
170 was also studied. Then, the colony of the survived bacteria was counted. This test procedure was carried out for  
171 all considered leaves.

172

173 **Inhibition zone test**

174 In this investigation, antibacterial activity was determined by using the paper disk assay method as basis [52]. In  
175 this test, AgNO<sub>3</sub> and leaves extract were freshly prepared and mixed analogously to the method employed in the  
176 colony forming test. Bacterial cultures of about  $5 \times 10^{15}$  colony were taken and about 0.1 mL of inoculum was  
177 spread on each agar plate. Filter paper (2 mm in diameter) was steeped in AgNPs solution with the similar  
178 variation as the colony forming test for 1 min and then put onto the agar plate. As comparison, the filter paper  
179 was also steeped in AgNO<sub>3</sub> solution. Next, the plate was incubated at 37 °C for 24 h. An inhibition zone can be  
180 described as the clear area surrounding the filter paper containing AgNP solution deposited on the plate. The

181 diameter that defines inhibition zone was then measured using a ruler. This test procedure was similarly  
182 performed for all other test samples.

183

## 184 **Results and Discussions**

### 185 **Plasmonic property**

186 Surface plasmon resonance is well known as the collective oscillation of the electrons in the conduction band  
187 when the particles absorb the electromagnetic wave [53]. Theoretically, AgNP aggregation and dispersion  
188 phenomena can be identified with the UV-vis absorption spectra. UV-vis spectra of AgNPs synthesized using  
189 different leaves extract are depicted in Fig. 1. For comparison purpose, UV-vis spectra for AgNO<sub>3</sub> solution were  
190 also included. Figs. 2a-2c show the solution color of AgNPs synthesized using *C. papaya*, *M. esculenta*, and *M.*  
191 *citrifolia*, respectively. It is clear that the reduction of silver ion to the formation of AgNPs in the solution  
192 occurred as indicated by the change in solution color from yellow to brown or reddish yellow to deep red (see  
193 Fig. 2).

194 From UV-vis spectra, the peak absorbance can be found at 485, 356, and 471 nm for AgNPs synthesized  
195 using *C. papaya*, *M. esculenta*, and *M. citrifolia*, respectively (see Fig. 3). These findings are in agreement with  
196 those found in the previous works [4, 54]. This observation has confirmed that AgNPs reduced and stabilized  
197 using different leaves extract exhibit different characteristics in their plasmonic property. Theoretically, AgNPs  
198 have spectra in the visible region ranging from 380 to 480 nm due to the excitation of localized surface Plasmon  
199 resonance [55, 56]. It is well known that their surface plasmon oscillation is extremely affected by their size,  
200 shape, and surrounding media as well as treatment employed [3, 57]. AgNPs in spherical shape can be  
201 correlated with a single peak in the UV-vis spectrum [58]. On the other hand, AgNPs in the irregular shapes  
202 have two or more peaks depending on their symmetry. The spectra characteristics exhibited in Fig. 1 suggest  
203 that AgNPs synthesized using *C. papaya*, *M. esculenta*, and *M. citrifolia* were spherical in nature.

204 For a comprehensive overview, AgNPs synthesized using *C. papaya* shows the highest maximum peak  
205 and followed by those using *M. esculenta* and *M. citrifolia* (see Fig. 3). The different maximum peaks of their  
206 UV-vis characteristics can be associated with the size of AgNPs. For instance, increasing the size of spherical  
207 nanoparticles from 8 to 99 nm increased their maximum peak spectra from 517 to 575 nm [59]. It was measured  
208 from the current study that the absorbance at  $\lambda_{\text{max}}$  of AgNPs synthesized using *C. papaya*, *M. esculenta*, and *M.*  
209 *citrifolia* are 1.56, 1.24, and 0.82 a.u, respectively. The different absorbance of the UV-vis spectra can be

210 specifically related to the agglomeration of AgNPs. The increase in the absorbance spectra indicates a higher  
211 production of AgNPs [60].

212

### 213 **FTIR characteristics**

214 FTIR was inspected to analyze biomolecule compounds, which can act as the capping agent for stabilizing  
215 AgNPs production. This method has previously been established and proved to be reliable [4, 61]. For this  
216 purpose, the FTIR spectra of AgNPs synthesized using different leaves extract are depicted in Fig. 4. Several  
217 prominent peaks are observed around the wave numbers ranges of 1042 to 1084  $\text{cm}^{-1}$  (region I), 1384 to 1394  
218  $\text{cm}^{-1}$  (region II), 1590 to 1619  $\text{cm}^{-1}$  (region III), and 3301 to 3444  $\text{cm}^{-1}$  (region IV).

219 IR bands around 1042 to 1084  $\text{cm}^{-1}$  are characterized as the phosphorus compounds. It was obvious in  
220 Table 2 that all leaves used in this study contained phosphorus mineral. FTIR spectra around 1384 to 1394  $\text{cm}^{-1}$   
221 are associated with the nitro compounds, which are reported also in AgNPs synthesized using *Cleistanthus*  
222 *collinus* leaves extract [62]. In addition, IR bands around 1590 to 1619  $\text{cm}^{-1}$  are associated with the C=C  
223 stretching modes of vibration [63]. Finally, spectra peaks within 3301 to 3444  $\text{cm}^{-1}$  are the -NH stretching  
224 modes, which were also reported by Jeyaraj et al. [61], which used *Sesbania grandiflora* leaves extract.

225 In region I of the wave number, AgNPs synthesized using *M. esculenta* are more intense in terms of  
226 transmittance compared to the others. The similar characteristics were also observed in region II. In region III,  
227 the transmittance of AgNPs synthesized using *C. papaya* is significantly intense compared with those  
228 nanoparticles synthesized using *M. esculenta*, and *M. citrifolia*. In the region IV, AgNPs synthesized using *M.*  
229 *esculenta* did not have a prominent peak. It is apparent that there were slightly different peak intensities for all  
230 synthesized AgNPs. Such difference and obvious characteristics can be correlated with the concentration of  
231 biomolecule on the surface of AgNPs [64].

232

### 233 **Morphology and size**

234 Morphology and size of AgNPs synthesized by chemical, physical, and biological approaches are affected by  
235 the reducing agent, stabilizer, and surrounding medium. In biological synthesis particularly that using leaves  
236 extract, their properties are extremely affected by the chemical composition of the leaves. In the present work,  
237 the shape of AgNPs synthesized using *C. papaya*, *M. esculenta*, *M. citrifolia* leaves extract is found to be  
238 spherical (see Figs. 5a-5c). It is confirmed that the single peak in the UV-vis spectra (see Fig. 1) for all synthesis  
239 solution is related to the spherical AgNPs.



240 Specifically, this work found that AgNP sizes synthesized using *C. papaya* were concentrated within 13  
241 to 69 nm with an average of 40.8 nm (see Fig. 6a). In addition, AgNPs sizes synthesized using *M. esculenta*  
242 were in the range of 13 to 38 nm with an average of 23.0 nm (see Fig. 6b). For those synthesized using *M.*  
243 *citrifolia*, the range was 9 to 54 nm with an average of 26.5 nm (see Fig. 6c). By this procedure, the smallest  
244 AgNPs size was produced when *M. esculenta* leaves extraction was applied, followed by those extracted using  
245 *M. citrifolia* and *C. papaya*. These findings match with their previously discussed plasmonic properties.

246 In general, results from this study enhance the understanding of the effectiveness of the use of local  
247 leaves for synthesizing AgNPs in a purely green fashion at room temperature. It is noteworthy to see that AgNP  
248 sizes obtained using the presently employed procedure are comparable with those employing physical or  
249 mechanical treatment (see Table 1). Moreover, for certain studies such as proposed by Muthukrishnan et al. [38]  
250 and Balan et al. [35], results from this study are preferable in terms of their size. Also, the present green  
251 procedure has successfully produced AgNPs smaller than those obtained by Dipankar and Murugan [36],  
252 Prakash et al. [39], as well as the recent study by Kharat and Mendhulkar [37] and Ashraf et al. [4], who also  
253 synthesized AgNPs using leaves extract without additional treatment.

254

#### 255 **Energy dispersive characteristic**

256 Energy dispersive X-ray spectroscopy, which is sometimes abbreviated as EDS, EDX, or XEDS is a common  
257 procedure for analyzing the basic elements on the surface of sample. Their characteristics generally depend on  
258 the source of X-ray excitation and the sample. The SEM-EDX spectra of all synthesized AgNPs can be observed  
259 in Figs. 7a-7c. The spectra characteristics have confirmed the presence of AgNPs. The sharp signal peak of the  
260 spectrum exhibits that the reduction from AgNO<sub>3</sub> to AgNPs using *C. papaya*, *M. esculenta*, and *M. citrifolia*  
261 were successfully carried out.

262 Specifically, the percentages of AgNPs synthesized using *C. papaya*, *M. esculenta*, and *M. citrifolia*  
263 observed in the EDX spectra are 94.69, 92.44, and 99.22%, respectively. It is ratified that AgNPs are dominant  
264 in the samples compared to other elements. The spherical AgNPs synthesized using *C. papaya* exhibit the EDX  
265 peak at approximately 3 keV. In addition, similar characteristics were also observed for those synthesized using  
266 *M. esculenta* and *M. citrifolia*. Such peaks are typical absorptions of metallic AgNPs due to the surface plasmon  
267 resonance [65]. These findings are in line with the results from previous works, which also synthesized AgNPs  
268 using leaves extract [4, 66]. The highest peaks in Figs. 3a-3c at approximately 3 keV confirm that metal AgNPs  
269 are dominant element compared to others. In addition, other smaller elemental peaks shown in Figs. 6a-6c are

270 possibly due to the contribution from enzymes or proteins present within *C. papaya*, *M. esculenta*, and *M.*  
271 *citrifolia* leaves. In general, this study has proven that the synthesis using different leaves extract such as *C.*  
272 *papaya*, *M. esculenta*, and *M. citrifolia* can produce AgNPs with different properties in terms of plasmonic,  
273 molecule bonding, morphology, and energy dispersive. It is well-known that the synthesis of AgNPs can be  
274 divided into three categories namely physical, chemical, and biological. In the physical approach, synthesis  
275 AgNPs using laser ablation, small ceramic heater, and thermal decomposition methods has been established. For  
276 the chemical approach, this requires three main ingredients namely a silver salt, a reducing agent, and a  
277 stabilizing or capping agent. In the biological approach, the reducing and stabilizing agents are replaced using  
278 biomolecules obtained from the living organism such as plants that are explored in this work. Proteins, enzymes,  
279 and vitamins are the biomolecules contained in the employed leaves extract (see Table 2) that can act as  
280 bioreductant from reducing metal ions to the formation of AgNPs in the solution. Moreover, the proposed purely  
281 green procedure is environmentally friendly compared to chemical or physical approach since the latter uses the  
282 chemical as reducing and stabilizing agent that may dispel toxin to the environment, in addition to their  
283 employment of huge amount of energy in the production.

284

### 285 **Antibacterial investigation**

#### 286 *Antibacterial activity by all leaves*

287 Various studies had reported that *C. papaya*, *M. esculenta*, and *M. citrifolia* leaves extract can be used as  
288 antibacterial agents [67, 68]. Their studies confirmed that the antibacterial activity is strongly affected by the  
289 medium, extraction process, and type of bacteria. As summarized in Table 3, it can be seen that *C. papaya*  
290 inhibited bacteria colony growth of *E. coli* better than *M. esculenta* and *M. citrifolia*. These are supported by the  
291 inhibition zone observation also shown in Table 3. *C. papaya* leaves extract is able to inhibit *E. coli* growth to  
292 about 1 cm in zone diameter. Recently, *C. papaya* leaves extract was reported to act as an effective antibacterial  
293 component with the zones of inhibition in the range of 14 to 16 mm against *Staphylococcus aureus*, *E. coli*, *B.*  
294 *cereus*, and *Pasteurellamultocida* [67].

295 In inhibiting the *B. Cereus* growth, this study notices that *M. esculenta* is the most effective. It inhibits  
296 the growth of *B. Cereus* to about 1.2 cm in diameter. *M. esculenta* leaves extract can be used as an effective  
297 antibacterial against gram-positive bacteria such as *Corynebacterium diphtheriae*, *Listeria monocytogenes*,  
298 *Pseudomonas aeruginosa* and gram-negative bacteria such as *Vibrio cholera*, *Shigella flexneri*, and *Salmonella*  
299 *typhi* [69]. Their extract is also effective as an antibacterial against *Pseudomonas aeruginosa*, *E. coli*,

300 *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Providencia stuartii*, and *Enterobacter aerogenes* [68]. Phenolic  
301 compounds such as acubin, 1-asperuloside, alizarin, and scopoletin are the chemical composition that act  
302 towards their antimicrobial activity. On the other hand, it is worthwhile to see that *M. citrifolia* did not show any  
303 antibacterial activity, a phenomenon evidenced in both colony forming and inhibition zone studies.

304

#### 305 *Antibacterial activity by synthesized AgNPs*

306 AgNPs have been widely proven as an antibacterial and an antimicrobial agent [6, 15]. Table 4 lists the  
307 outcomes from the colony forming unit test of synthesized AgNPs against *E. coli* and *B. cereus*. It is apparent  
308 that AgNPs have completely (100%) inhibited the bacteria colonies of *E. coli* and *B. cereus* from  $10^{15}$  initial  
309 colony to 0 colony as observed in the agar plate. In comparison, about  $60 \times 10^1$  bacterial colonies were still  
310 existed when the  $\text{AgNO}_3$  solution was employed against *B. cereus* for control. In addition, the  $\text{AgNO}_3$  solution  
311 also completely inhibited the colony of *E. coli*. To the best of the authors knowledge, this is the first evidence  
312 that shows that AgNPs synthesized using local plants such as *C. papaya*, *M. esculenta*, and *M. citrifolia* can act  
313 as an antibacterial agent against *E. coli* and *B. cereus*. Further proof is shown by the inhibition zone test in Table  
314 5. It is noticed that AgNPs synthesized using *C. papaya* have inhibition zones varying from 1.8 to 2.6 cm. In  
315 addition, AgNPs synthesized using *M. esculenta* and *M. citrifolia* have inhibition zones varying from 1.7 to 2.0  
316 cm and 1.6 to 2.2 cm, respectively. As a control, the  $\text{AgNO}_3$  solution has inhibition zones about 0.7 and 1.0 cm  
317 against *E. coli* and *B. cereus*, respectively. Moreover, AgNPs synthesized using *C. papaya* show the largest  
318 average inhibition zone compared to others.

319 Although the mechanism of the bactericidal effect of AgNPs is still not well understood, several works  
320 provided some initial evidence that AgNPs can be used as an effective antibacterial agent. In terms of toxicity  
321 effect, the bacterial cell death was caused by the interaction between AgNPs and constituents of the bacteria  
322 membrane as a result of its structural change and the eventual damage [18]. AgNPs can be categorized as a  
323 hydrophobic material. Although they are hydrophobic, their bioaccumulation mechanism takes the role of their  
324 antibacterial properties [70]. AgNPs tend to accumulate at the bacterial membrane and further form aggregates  
325 once they are in contact with the organism. Consequently, the diminishment of the bacterial membrane integrity  
326 and its damage lead to bacteria cellular death [70]. There is also an implication that AgNPs can penetrate into  
327 the bacteria membrane [71]. Moreover, AgNPs can inhibit bacteria respiratory enzyme, which then facilitates  
328 the generation of reactive oxygen and consequently damages the cell [72]. Alternatively, the toxicity of AgNPs

329 is surface charge-dependent affected by the capping agent and synthesis procedure. The more negative charged  
330 AgNPs is less toxic compared with those nanoparticles having a more positive charge [64].

331

### 332 **Conclusion**

333 The aim of this investigation is to evaluate the effectiveness of the green procedure to synthesize AgNPs using  
334 *C. papaya*, *M. esculenta*, and *M. citrifolia* leaves extract without additional treatment such as physical or  
335 mechanical. This study has identified that the plasmonic property of AgNPs differs according to their size and  
336 reducing agent as well as stabilizing agent with AgNPs synthesized using *C. papaya* having the highest  
337 maximum peak and absorbance compared to the others. In addition, FTIR characteristics have confirmed the  
338 biomolecule bonding on AgNPs. FESEM investigations revealed that spherical AgNPs ranging from 9 to 69 nm  
339 were produced using this simple procedure. Specifically, the smallest nanoparticles can be produced when *M.*  
340 *esculenta* leaves were employed. Also, both leave extracts and synthesized AgNPs showed great potential as an  
341 antibacterial component against *E. coli* and *B. cereus*. In detail, *C. papaya* leaves extract was the most effective  
342 antibacterial agent against *E. coli* compared to the others. For *B. cereus*, *M. esculenta* leaves extract was the  
343 most effective to inhibit the bacteria. AgNPs synthesized using all considered leaves extract were found to  
344 completely inhibit all bacteria colonies. In addition, AgNPs synthesized using *C. papaya* showed the largest  
345 inhibition zone compared with those nanoparticles synthesized using *M. esculenta*, and *M. citrifolia*.

346 This work extends the knowledge for the exploration of natural resources particularly *C. papaya*, *M.*  
347 *esculenta*, and *M. citrifolia* as reducing and stabilizing agents for a green synthesis of AgNPs. These findings  
348 have significant implications for future medical applications particularly for providing potential antibacterial  
349 agent from natural resources and synthesized AgNPs. A further study could assess the long-term stability of  
350 AgNPs using this procedure and investigate their antibacterial performance on other bacteria.

351

### 352 **Acknowledgements**

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355

### 356 **Conflict of Interest:**

357 The authors declare that they have no conflict of interest.

358

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## Figure Captions

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564 **Fig. 1.** UV-vis spectra of AgNPs synthesized by different leaves extract

565 **Fig. 2.** The solution color of AgNPs synthesized using (a) *C. papaya*, (b) *M. esculenta*, and (c) *M. citrifolia*

566 **Fig. 3.** (a) absorbance and (b) wavelength of all solutions. Note that I, II, and III refer to the AgNO<sub>3</sub>+ *M.*  
567 *citrifolia*, AgNO<sub>3</sub>+ *M. esculenta*, and AgNO<sub>3</sub>+ *C. papaya*, respectively.

568 **Fig. 4.** FTIR spectra of AgNPs synthesized using different leaves extract

569 **Fig. 5.** FESEM of AgNPs synthesized using (a) *C. papaya*, (b) *M. esculenta*, and (c) *M. citrifolia*

570 **Fig. 6.** Size distribution histograms of all synthesized AgNPs

571 **Fig. 7.** EDX spectra of AgNPs synthesized using (a) *C. papaya*, (b) *M. esculenta*, and (c) *M. citrifolia*

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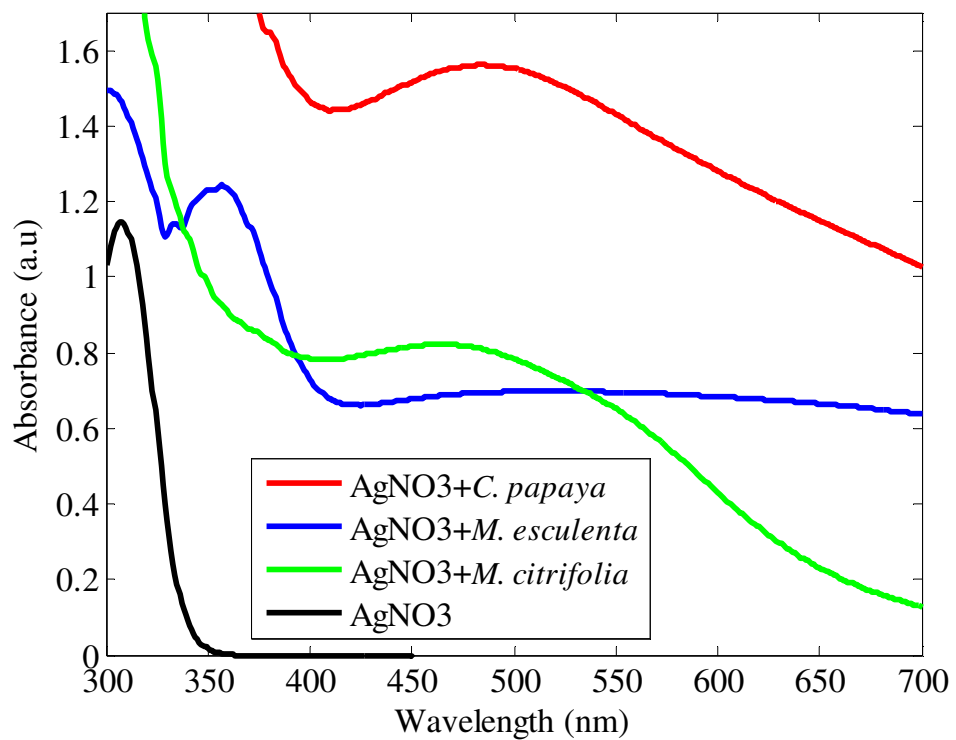
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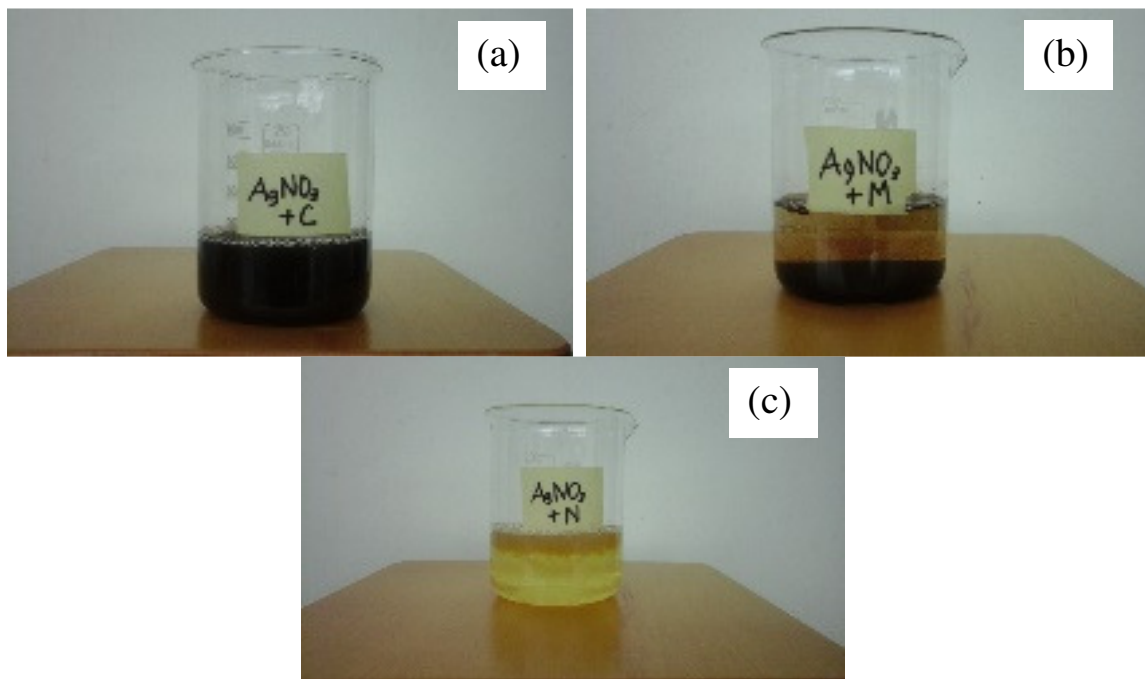
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580 **Fig. 1.** UV-vis spectra of AgNPs synthesized by different leaves extract  
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594 **Fig. 2.** The solution color of AgNPs synthesized using (a) *C. papaya*, (b) *M. esculenta*, and (c) *M. citrifolia*

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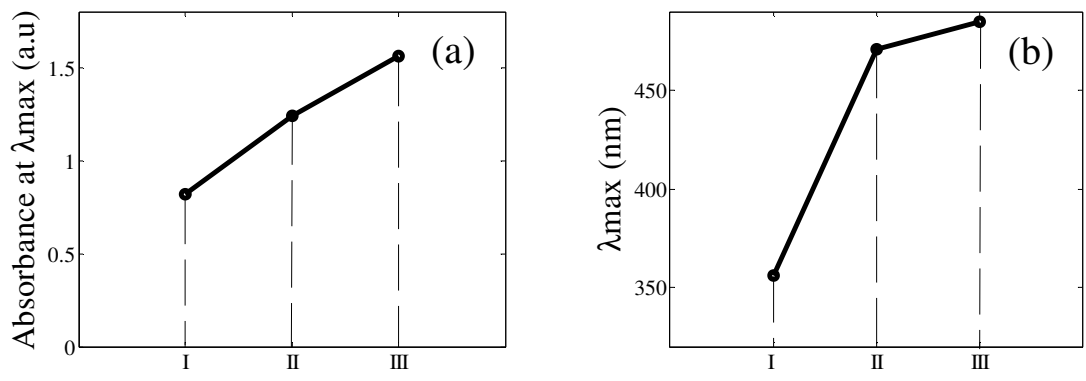
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607 **Fig. 3.** (a) Absorbance and (b) wavelength of all solutions. Note that I, II, and III refer to AgNO<sub>3</sub>+ *M. citrifolia*,  
 608 AgNO<sub>3</sub>+ *M. esculenta*, and AgNO<sub>3</sub>+ *C. papaya*, respectively.

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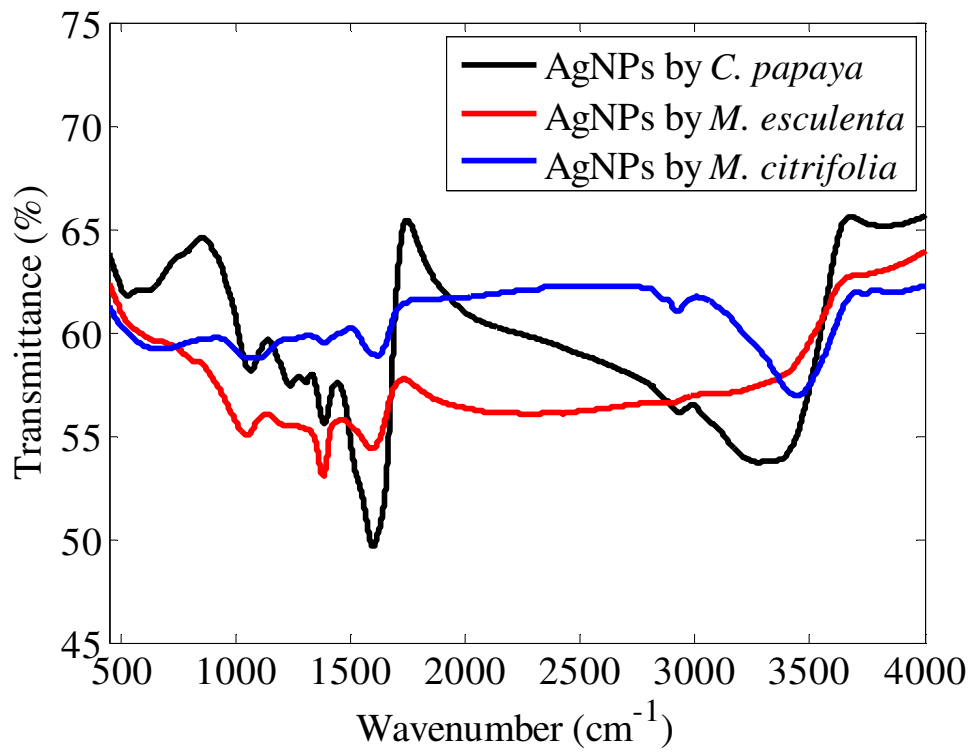
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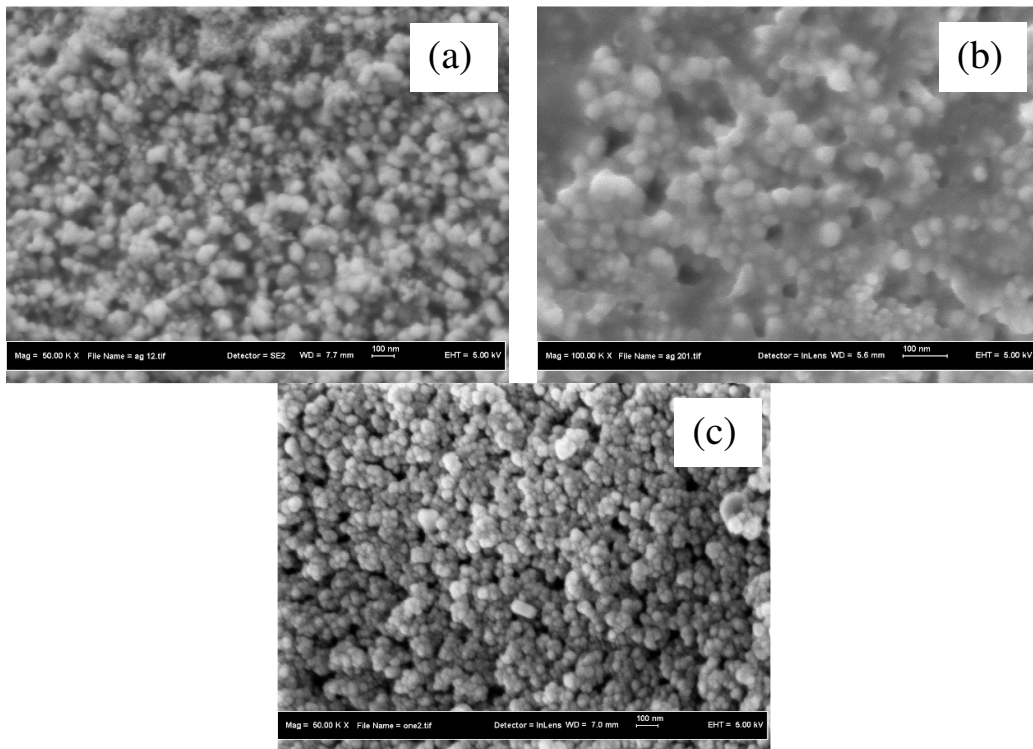
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**Fig. 4.** FTIR spectra of AgNPs synthesized using different leaves extract

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**Fig. 5.** FESEM of AgNPs synthesized using (a) *C. papaya*, (b) *M. esculenta*, and (c) *M. citrifolia*

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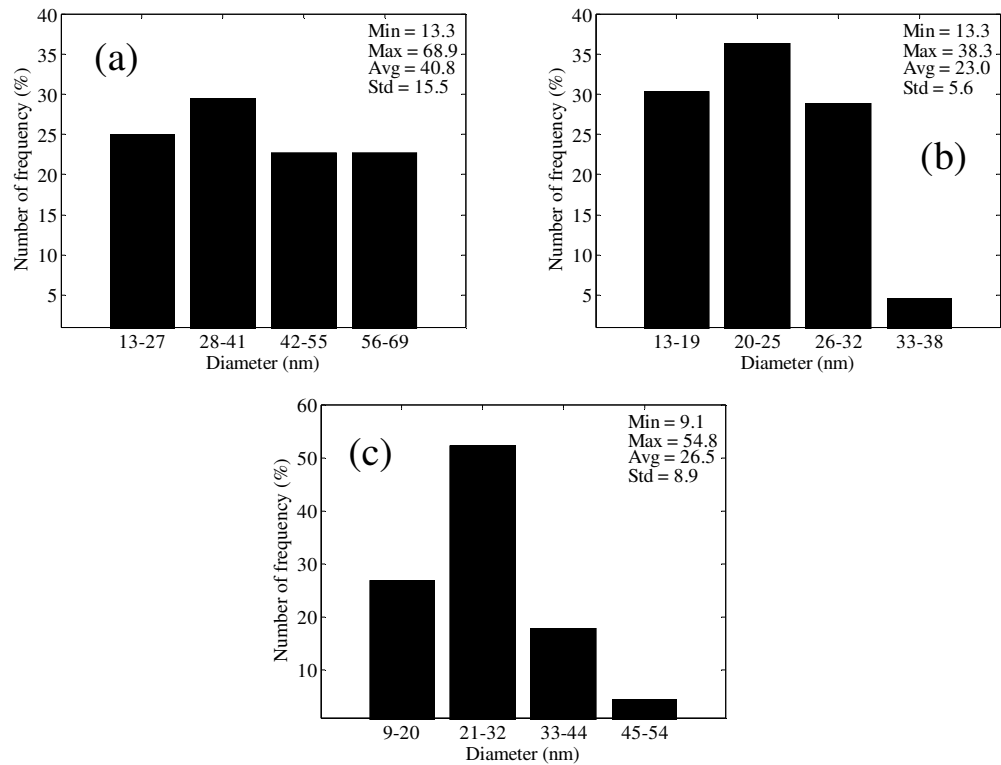
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646 **Fig. 6.** Size distribution histograms of AgNPs synthesized using (a) *C. papaya*, (b) *M. esculenta*, and (c) *M.*  
 647 *citrifolia*

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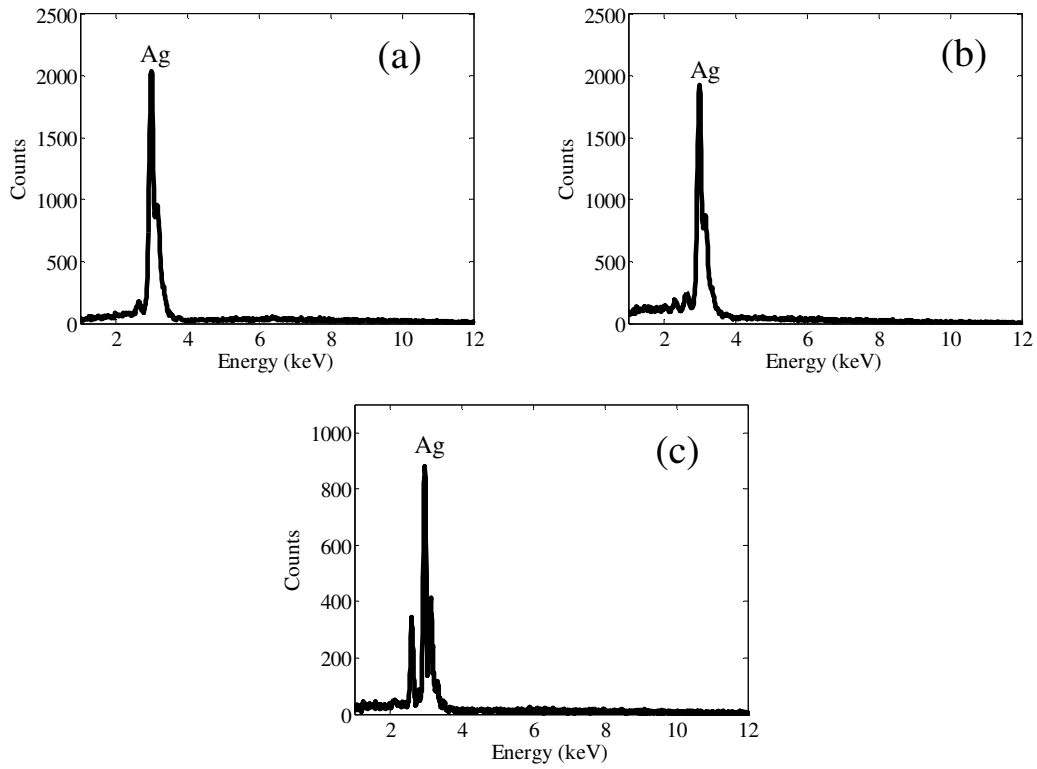
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**Fig. 7.** EDX spectra of AgNPs synthesized using (a) *C. papaya*, (b) *M. esculenta*, and (c) *M. citrifolia*

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**Table captions**

**Table 1.** Shape and size of AgNPs synthesized using various leaves extract

**Table 2.** Chemical compositions of *C. papaya*, *M. esculenta*, and *M. citrifolia* [42, 45-48]

**Table 3.** Effects of different leaves extract on the colonies and inhibition zones of *E. Coli* and *B. Cereus*

**Table 4.** Effects of AgNPs presence on the colonies of *E. Coli* and *B. Cereus*

**Table 5.** Effects of AgNPs presence on the inhibition zones of *E. Coli* and *B. Cereus*

704 **Table 1.** Shape and size of AgNPs synthesized using various leaves extract

<b>Plant origin</b>	<b>Additional treatment</b>	<b>Silver size (nm)</b>	<b>References</b>
<i>Ficus sycomorus</i>	Stirring and heating	1 to 20	Salem et al. [40]
<i>Boerhaavia diffusa</i>	Stirring and heating	25	Vijay Kumar et al. [41]
<i>Ceropegia thwaitesii</i>	Shaking	100	Muthukrishnan et al. [38]
<i>Lantana camara</i>	Stirring	14 to 34	Ajitha et al. [31]
<i>Lonicera japonica</i>	Heating	53	Balan et al. [35]
<i>Centella asiatica</i>	Stirring and heating	15	Kumar et al. [32]
<i>Iresine herbstii</i>	No	44 to 64	Dipankar and Murugan [36]
<i>Mimusops elengi</i>	No	55 to 83	Prakash et al. [39]
<i>Aloe vera</i>	No	5 to 85	Ashraf et al. [4]
<i>Elephantopus scaber</i>	No	78	Kharat and Mendhulkar [37]
<i>Carica papaya</i>	No	13 to 69	Present work
<i>Manihot esculenta</i>	No	13 to 38	Present work
<i>Morinda citrifolia</i>	No	9 to 54	Present work

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719 **Table 2.** Chemical compositions of *C. papaya*, *M. esculenta*, and *M. citrifolia* [42, 45-48]

<b>Leaves extract</b>	<b>Proximate</b>	<b>Mineral</b>	<b>Vitamin</b>
<i>C. papaya</i>	Lipid	Calcium	Vitamin A
	Protein	Magnesium	Vitamin B12
	Crude fibre	Phosphorus	Vitamin C
	Moisture	Iron	Vitamin E
	Ash		Niacin
	Carbohydrate		Thiamine
	Fat		Riboflavin (Vitamin B2)
<i>M. esculenta</i>	Caloric content	Calcium	Beta-carotene
	Water	Copper	Ascorbic acid
	Protein	Iron	Niacin
	Lipid	Magnesium	Riboflavin (Vitamin B2)
	Carbohydrates	Manganese	Thiamin
	Fiber	Phosphorous	Vitamin A
	Ash	Potassium	
<i>M. citrifolia</i>	Water	Sodium	
	Protein	Calcium	Ascorbic acid
	Fat	Phosphorous	Niacin
	Carbohydrate	Iron	Riboflavin (Vitamin B2)
	Fiber		Thiamin
	Ash		Beta-carotene

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732 **Table 3.** Effects of different leaves extract on the colonies and inhibition zones of *E. Coli* and *B. Cereus*

Leaves extract	Colony		Inhibition zone (cm)	
	<i>E. Coli</i>	<i>B. Cereus</i>	<i>E. Coli</i>	<i>B. Cereus</i>
<i>Carica papaya</i>	$2 \times 10^{11}$	$29 \times 10^9$	1	0.75
<i>Manihot esculenta</i>	$66 \times 10^{11}$	$4 \times 10^9$	None	1.2
<i>Morinda citrifolia</i>	$8 \times 10^{11}$	$4 \times 10^{15}$	None	None

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757 **Table 4.** Effects of AgNPs presence on the colonies of *E. Coli* and *B. Cereus*

Type of Leaf	<i>E. Coli</i>		<i>B. Cereus</i>	
	AgNO <sub>3</sub> + and the leaves extract (5:2)	AgNO <sub>3</sub> + and leaves extract (5:3)	AgNO <sub>3</sub> + and the leaves extract (5:2)	AgNO <sub>3</sub> + and leaves extract (5:3)
<i>C. papaya</i>	0	0	0	0
<i>M. esculenta</i>	0	0	0	0
<i>M. citrifolia</i>	0	0	0	0

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781 **Table 5.** Effects of AgNPs presence on the inhibition zones of *E. Coli* and *B. Cereus*

Type of Leaf	<i>E. Coli</i>		<i>B. Cereus</i>	
	AgNO <sub>3</sub> + and the leaves extract (5:2)	AgNO <sub>3</sub> + and leaves extract (5:3)	AgNO <sub>3</sub> + and leaves extract (5:2)	AgNO <sub>3</sub> + and leaves extract (5:3)
<i>C. papaya</i>	1.8 cm	2.6 cm	1.7 cm	1.8 cm
<i>M. esculenta</i>	1.7 cm	2.0 cm	1.7 cm	2.0 cm
<i>M. citrifolia</i>	1.6 cm	2.2 cm	1.3 cm	2.4 cm

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