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Functional properties of *Averrhoa bilimbi* and green synthesis and characterization of silver nanoparticles using its fruit extracts

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Abstract
Averrhoa bilimbi is an underutilized crop possessing remarkable health benefits. The current investigation is based on anti-inflammatory, antioxidant and anti-diabetic properties of aerial parts of the crops. Additionally, silver nanoparticles synthesis using ethanolic extract of the fruit of *A. bilimbi* was performed. Characterization of synthesized nanoparticles were performed using Scanning Electron Microscopy (SEM) and the Fourier-Transform Infrared Spectroscopy (FTIR). Antibacterial properties of the synthesized nanoparticles were also evaluated. Results revealed that the leaf extract of *A. bilimbi* has shown significantly higher ($p< 0.05$) phenolics (261.58 mg GAE/ g DW), carotene (2.4357mg/g DW), antioxidant capacity (309.75±1.57mg AAE/g DW), anti-diabetic properties (α-amylase inhibition-21.41%) and anti-inflammatory properties (heat-induced hemolysis inhibition-15.29%) compared with its fruits and flower extracts. SEM images showed a size range of 400nm and the shape of nanoparticles is polyhedral. In FTIR spectrum, the major peaks were observed at 3272.37cm-1 to 2852.43cm^{-1} and 1739.23 cm^{-1} to 1034.04 cm^{-1}. In addition, the *A. bilimbi*-based silver nanoparticles showed antibacterial properties against tested *E. coli*. In conclusion, *A. bilimbi* may possess antioxidant, anti-inflammatory and antidiabetic properties and the synthesized silver-nanoparticles also show antimicrobial properties.

Keywords: *Averrhoa bilimbi*, nanoparticles, antioxidant, antibacterial, ant diabetic

1. Introduction
Averrhoa bilimbi is an evergreen plant which provides food, medicine and other commodities in its long life span. Commonly found in Asian continent, the plant grows up to a height of 15 m and a diameter of 30 cm (Alhassan and Ahmed, 2016) [3]. Fruits are fairly cylindrical and produced in clusters. The tree is often cultivated for its fruit in many areas of Sri Lanka; however, this is one of the underutilized or neglected crop species grown in Sri Lanka (Malkanthi, 2017) [23]. *A. bilimbi* is used for treating diabetes, hypertension and other disorders in traditional medicine, by people in tropical and subtropical countries (Alhassan and Ahmed, 2016) [3]. Leaf extracts are commonly taken for treating cough, cold and fever conditions, rectum inflammation, postpartum injuries, skin diseases and syphilis (Alhassan and Ahmed, 2016) [3]. Grated fruits added with salt are used on pimples in face (Ong and Nordiana, 1999) [27]. Fruit juice is recognized for treating obesity. Fruits, leaves and young stems of A. *bilimbi* plant has numerous health benefits. Fruit and leaf extracts of *A. bilimbi* have shown anti-inflammatory (Valsan and Raphael, 2016) [37], antidiabetic (Tan *et al*., 2005), antioxidant activity and antibacterial activities (Das *et al*., 2011, Rahman *et al*., 2014) [29]. These properties of *A. bilimbi* fruit have been accredited to its saponins, tannins and flavonoids (Kumar *et al*., 2013) [20].

As stated in Laurent *et al*. (2008) [22], nanoparticles are particulate materials having dimensions less than 100 nm. The size of the material can influence the physicochemical characteristics as realized with the invention of nano particles (Tiwari and Kim, 2012) [36]. Inorganic nanoparticles synthesis, characterization, and surface functionalization have come a long way and found application in therapeutic and imaging agents (Akhter *et al*., 2020). Physical and chemical methods applied for synthesis of nanoparticles are not environmental friendly. With the initiation of green nanotechnology for nanoparticles synthesis, interest has grown to synthesize metallic nanoparticles from plant extracts. Green synthesis of nanoparticles is mainly targeted for minimizing the waste generation and increasing the process sustainability. Novel technologies have been identified by the researchers in recent years to synthesize metal nanoparticles and they are successfully utilized in various applications.

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Silver is famous for its therapeutic properties as same as other noble metal nano particles identified in medicine. It also possesses antibacterial properties and antifungal properties (Zhang *et al*., 2016) [39]. The antioxidants, antibacterial properties of silver nanoparticles have been studied by many fruits such as mangoes and papaya (Samari *et al*., 2019) [32]. When compared with the chemical method, this method of green synthesis is environmental friendly, less hazardous, low cost and easily available. Even it is having a potential for large scale production. Plants produce more stable nanoparticles produced using plants are stable and therefore, very easy to scale up. There is very lower risk for contamination (Devatha and Thalla, 2018) [7]. Silver has low toxicity for animal cells when compared to the other metals when its use in low doses (Tiwari *et al*., 2011) [35]. Therefore, silver nanoparticles are applied as antimicrobial compounds for food packages as well (Emamifar *et al*., 2011) [9]. Although silver nanoparticles are listed as generally recognized as safe as mentioned by Emamifar *et al*. (2011) [9], according to Gallocchio *et al*. (2016) [10], potential health impacts caused due to ingestion of these particles migrated from packaging to food matrix should be highly concerned. In ancient times, Ayurveda and traditional medicine have used silver as an antimicrobial agent (Ashokkumar *et al*., 2014) [4]. Saravanan and colleagues (2011) [33] proved the efficiency of silver nanoparticles in acting as antintimicrobial activities against bacteria and other eukaryotic microorganisms.

Different techniques are available for the characterization of silver nanoparticles such as scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy-FT-IR. Silver nanoparticles were functionalized with biomolecules and other stabilizing functional groups as revealed by FT-IR spectroscopy. The stability of the nanoparticles is also very important to assess the properties of *A. bilimbi*. Silver nanoparticles are having unique properties such as antibacterial, and anti-inflammatory, good conductivity and chemical stability activities when compared with nanoparticles from heavy metals. As reported by Ahmad *et al*. (2003) [1], Silver nanoparticles are successfully incorporated in composite fibers, cosmetic products, cryogenic superconducting materials, electronic components and utilized in the food industry. This study evaluates the synthesizing process of silver nanoparticles from *A. bilimbi* as a naturally available reducing agent and assay its bactericidal capacity as well as other properties such as antidiabetic, antioxidant, and anti-inflammatory properties.

2. Materials and Methodology
2.1 Preparation of fruit extract
Fresh green *A. bilimbi* fruits, leaves and flower samples were collected from a home garden in Kottawa, Colombo District of Sri Lanka. Freshly collected flowers, leaves and fruits of *A. bilimbi* were washed with distilled water several times before freeze-drying. Ten grams of dried sample was mixed with 100 mL 50% ethanol solution and then heated at 60° C for 20 minutes. The crude ethanolic extract of *A. bilimbi* was filtered by filter paper (Whatman # 1) and Buchner flask. The filtered extracts prepared were refrigerated at 4°C for two weeks for further use.

2.2 Total carotene content
Total carotene content in methanolic extracts of *A. bilimbi* samples was evaluated using a method described in Gunathilake and Ranaweera (2016) [12] with slight modifications. An amount of one gram from dried powder samples was mixed with 50 mL of 96% methanol. They were vortexed for one minute. The homogenate was centrifuged (EBA 20) for 10 min at 3000 rpm. The supernatant was filtered using filter paper (Whatman # 1). The absorbance was measured Spectrophotometrically at 470, 653, and 666 nm (UV/VIS-Optima, SP-3000, Tokyo, Japan). Chlorophyll and carotene concentrations was calculated according to the following formula and reported as µg/g dry weight of the sample.

Chlorophyll a = 15.65 A_{666} - 7.340 A_{653}
Chlorophyll b = 27.05 A_{653} - 11.21 A_{666}
Carotene = 1000 A_{470} – 2.860 C_a – 129.2 C_b/245
(C_a =chlorophyll a and C_b=chlorophyll b)

2.3 Total Flavonoid Content (TFC)
Polyphenols from *A. bilimbi* samples were extracted following the method presented in Gunathilake & Ranaweera (2016) [12], with some minor alterations. One gram of dried sample was macerated for 12 h while stirring, in 20 mL methanol/water solution (70/30 v/v). After centrifuging the hydro-methanolic extract for 20 min at 6000 rpm, the supernatant was brought to 15 mL prior to storage at 4 °C for further analysis. The total flavonoid content of the extract was evaluated as described in Kumari and Gunathilake, 2020 [21]. Each 0.5 mL of methanolic extract was mixed with distilled water (2.5mL) and then 0.15mL sodium nitrite (5%) was added. After incubating the mixture at ambient temperature nearly for 6 min, 3 mL aluminum chloride solution (10%) was added before incubating for another 5 min. after the incubation, 1 mL sodium hydroxide (1M) solution was added and then the distilled water was added to made the total volume of the mixture up to 5 mL. The absorbance was obtained at 510 nm using the Spectrophotometer (UV/VIS). Catechin was used as the standard. The total flavonoids amount of the plant extract was presented as mg catechin equivalents in one gram of sample (mg/g).

2.4 Total phenolic content (TPC)
The total phenolic content (TPC) was analysed using the spectrophotometric method as described in Gunathilake *et al* (2017) [17] by using Folin-Ciocalteu's reagent. Briefly, a 0.2 mL extract/standard solution was added to ten times diluted I mL Folin-Ciocalteu reagent. Then the mixture was incubated nearly for 10 min before adding Na_2CO_3 solution (7.5%) (0.8 mL) to the mixture. The absorbance of solutions was taken at 743 nm after 30 minutes' incubation period at ambient temperature. TPC was presented as GA equivalents (mg/ g DW)

2.5 Antioxidant activities
2.5.1 Total Antioxidant Capacity
The method explained by Janarny and Gunathilake (2020) [19] was used with minor modifications as mentioned in Hettiarachchi *et al*. (2021) [17] to evaluate the total antioxidant capacity of extracts. A mixture of sample extract (0.3 mL) and 3 mL reagent solution containing 4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulphuric acid were incubated for 90 min at 95°C. Then the mixture was cooled to ambient temperature and then the absorbance of each solution was measured at a 695 nm using the spectrophotometer. The antioxidant capacity of the extract was presented as ascorbic acid equivalents (AAE).

2.5.2 DPPH Assay

DPPH radical scavenging abilities of the *A. bilimbi* extracts were done as mentioned in Hettiarachchi *et al*. (2020) [16] with minor modifications. Briefly, 100 μL extracts were mixed in 3.9 mL of freshly prepared DPPH solution (1 mM, 0.5 mL) and then vortexed for nearly 15 seconds and then kept 30 min at 30 °C in the dark. The absorbance of the prepared solution was read at 520 nm spectrophotometrically. The equation as shown below was used to calculate the percentage DPPH scavenging ability.

% radical scavenging = (A0-A1)*100/A0
(A0=the absorbance of control, A1=the absorbance of standard)

2.6. Anti-inflammatory properties

This assay was carried out as mentioned by Gunathilake *et al*. (2018). Briefly, 2.95 mL phosphate buffer (pH 7.4) were mixed 0.05 mL of blood cell suspension and 0.05 mL of hydroethanolic extracts. The mixture was subjected to incubation in a shaking water bath at 54 °C for 20 min. The mixture was centrifuged for 4 min at 2500 rpm after the incubation. The absorbance of the supernatant was measured at 540 nm spectrophotometrically. The phosphate buffer solution was used as a control for the experiment.

The level of hemolysis was computed based on the below equation

% hemolysis inhibition = $100 \times (1 - A2/A1)$,
A1 = absorption of the control, and A2 = absorption of the prepared sample mixture.

2.7 Anti-diabetic properties

The method of Chiranthika *et al*. (2021) [6] was followed with minor alterations as mentioned by Mel *et al*. (2020) [24] to assess α-Amylase inhibition. An amount of 100 μL of the samples was mixed with 200 μL α-amylase and 100 μL phosphate buffer (concentration=2 mM, pH=6.9) and then the mixture was subjected to incubation for 20 min. Then 100 μL of a 1% starch solution was added. The control samples were subjected to same procedure replacing 200 μL enzyme with the buffer solution and allowed to incubate nearly for 5 min. Then 3, 5-dinitrosalicylic acid reagent (500 μL) was mixed the control and the sample extract. Then the mixtures were kept for 5 min in a boiling water bath. The absorbances of the tested samples were measured using the UV/Visible spectrophotometer at a wave length of 540 nm. The percentage α-amylase inhibition was computed.

2.8 Synthesis of Silver Nanoparticles

The method as mentioned by Salehi *et al*. (2016) [31] was used for the silver Nanoparticles synthesis. A volume of 100 mL of aqueous silver nitrate solution (0.01 mM) was mixed with 4 mL aqueous sample extract. Continuous stirring was carried out at ambient temperature for 5 min with a magnetic stirrer before incubating the mixture. The mixtures were undisturbed during the incubation period until the colour of the mixture turned dark brown (reduction of Ag^+ to Ag^0 Nanoparticles. Then the particles were separated by 20 min centrifugation at 12,000 rpm. Then, the silver Nanoparticles were dried at 40 °C nearly for 2 hours before characterization.

2.9 Characterization of Silver Nanoparticles

2.9.1 Fourier transform infrared spectroscopic studies

As mentioned in Salehi *et al*. (2016) [31], fourier transform infrared spectroscopy (FT-IR) procedure was used with a spectrum RX 1 instrument. Then the samples were mixed with potassium bromide and pellets were formed and then the analysis was done to check the available functional groups on the *A. bilimbi* extract and the synthesized silver Nanoparticles. FT-IR spectra obtained were scanned between 4,000 cm-1-400 cm-1 at a resolution of 4 cm-1 in the transmittance mode.

2.9.2 Scanning electron microscopy

Morphological studies of the synthesized/prepared silver nanoparticles were done using a field-emission scanning electron microscope as mentioned in Salehi *et al*. (2016) [31] using a gold film for loading the dried Nanoparticles s in the instrument.

2.9.3 Stability of nanoparticles

Stability nanoparticles were evaluated based on the particle size (Salehi *et al.*, 2016) [31] during a 30 days storage at 5 °C. In addition, to evaluate the thermal stability, the nanoparticles dispersed water samples were exposed to 30 min pasteurization at 100 °C.

2.9.4 Anti-bacterial properties

The antimicrobial activity of extracts and nanoparticles against gram-negative bacteria *Escherichia coli* was done using the method explained in Rhman *et al* (2017). The test organisms were grown in a nutrient agar broth media at 35-37 °C for 24 hrs 0.5 Mcfarland standards were prepared and culture was adjusted to the same turbidity level of it. Samples were diluted several times to get the required culture density. Nutrient agar culture media was prepared by dissolving extracts and nanoparticles (100 ppm), then pour plate method was performed and incubated the samples at 37 °C for 24 hrs. Three replicates were conducted and the results were reported as the average of three sets of values.

2.10 Statistical analysis

All data gathered in the study were expressed as mean ± standard deviation of three replicates. The data will be analyzed statistically using one-way analysis of variance (ANOVA) with the SPSS/18 software.

3. Results and discussion

3.1 Total phenolic, flavonoid and carotene content

Table 1 presents the total phenolic, flavonoid and carotene amounts of the methanolic extracts of fruits, leaves and flowers of *A. bilimbi*. Total phenolic content of the aerial parts of the plant was reported as 69.13±5.47 to 261.58±30.64 mg GAE/g DW whereas the total flavonoid and carotene contents were within the range of 37.741±1.33 to 95.499±3.28 mg CE/g DW and 2.43±0.19 to 0.53±0.01 mg/g DW respectively. Leaf extracts have shown significantly higher ($p< 0.05$) phenolic, flavonoid and carotene contents compared to the extracts of flowers and fruits. Among the extracts studied, fruit extracts of *A. bilimbi* have shown significantly lower ($p< 0.05$) phenolic, flavonoid and carotene contents. Phenols, flavonoids and tannins in plants are observed with free radical scavenging ability (Hasanuzzaman *et al.*, 2013) [15]. Phenolics are a group of bioactive compounds with antioxidant properties, found in plant foods such as *A. bilimbi* and that plant parts are used in medicinal formulations to treat chronic diseases in Ayurveda medicine. Cellular stress levels affect the carotene concentration. Therefore, combined pigments assessment provides information about cellular physiological state (Zavřel, *et al.*, 2015) [38]. Previously Prasad *et al.* (2011)

[28] have done researches to identify and quantify different types of carotenoids present in underutilized fruits (Prasad *et al.*, 2011) [28].

3.2 Bioactivity of the extracts

According to Table 2, the total antioxidant capacity of the methanolic extracts of *A. bilimbi* was ranged between 148.58 ±7.68-309.75 ±1.57 mg AAE/g dry weight. Leaf extract displayed the highest antioxidant capacity (309.75 mg AAE/g dry weight), followed by flower extract (167.88 ±5.29 mg AAE/g dry weight) and fruit extract have shown the least antioxidant capacity compared with leaf and flower extracts. Various *in vitro* based methods have been used for the evaluation of antioxidant activities such as spectrophotometric method described in Prieto *et al*. (1999). This assay is relying on the reduction of MO(VI) to MO(V) by an antioxidant or a sample analyte. According to Gunathilake and Ranaweera (2016) [12], it might be more useful for measuring the total antioxidant capacity of a sample than determining the particular antioxidant molecules in a sample. The total antioxidant capacity of the methanolic extracts of leaf, flowers and fruits of *A. bilimbi* was studied and the leaf extracts have shown higher antioxidant activities than flower and fruit extracts. The total antioxidant capacity of the plant extracts may be contributed to their chemical composition including their phenolic and other antioxidants in the sample. In our study, it was found that the phenolic and carotene contents (Table 1) of *A. bilimbi* extracts correlate with the total antioxidant capacity assay (data not shown).

α-Amylase inhibition assay was performed to evaluate the anti-diabetic activity of the aerial samples *A. bilimbi*. All values are significantly different ($P < 0.05$) and the range of inhibition showed 6.48%±0.013 to 21.41%±0.031 (Table 2) where the highest inhibition was observed in leaf extract and the lowest inhibition was found in flesh extract. According to the results, the anti-diabetic properties of different Ariel parts of *A. bilimbi* show the highest inhibition percentage that leaves part compared with other flesh and flowers.

Anti-inflammatory Activity of *A. bilimbi* extracts was performed based on Heat induce hemolysis method with two different concentrations 10 μg/ml, 100 μg/ml. (Table 2). At the concentration of 10 μg/mL, the highest hemolysis inhibition was observed in leaf extract (11.57±0.08% to 15.29±0.07%) whereas the fruit extract has shown the lowest hemolysis inhibition. A similar trend was reported for 10 μg/mL extract concentration. The anti-inflammatory activity of the studied hydro methanolic extracts of different aerial parts of *A. bilimbi* were evaluated using *in vitro* heat-induced hemolysis method. Okoli and Akah (2004) [26] stated that leukocytes are responsible in the development of inflammation and therefore the effect of *in vivo* leukocyte migration should be studied. Inflammatory stimulus which influences leukocyte migration happens due to the interaction of leukocytes with chemotactic/chemoattractant and adhesion molecules (Okoli and Akah, 2004) [26]. The production of mediators, enzymes and pro-inflammatory cytokines at inflammatory sites are inhibited by these extracts.

3.3 Green synthesis of silver nanoparticles and characterization

This study targeted at the preparation of silver nanoparticles (AgNPs) using an environmental friendly, green synthetic method. The green synthesis and characterization of metal nanoparticles using aqueous extracts have been demonstrated in Ahmed *et al.* (2016) [1]. In this study, the absorption spectrum of silver nitrate (Ag_2NO_3) nanoparticles synthesized by green route using *A. bilimbi* ethanolic extract revealed the conversion of silver ions to nanoparticles. The color change of the silver nitrate solution to dark brown confirms the reduction of silver ions. This is due to the conversion of silver ions into elemental silver in the reaction medium. The Conversion process took several minutes to hours duration period. It reveals a rough prediction about the total phenolic compounds and antioxidant capacity of *A. bilimbiin.* Salehi and colleagues (2016) [31] have presented that the green synthesis of AgNPs, the reaction has taken several hours to complete.

SEM images of the synthesized AgNPs revealed that the shape of particles yielded were spherical and the average particle size was 400-450 nm (Figure 1a). EDS analysis was used to study the elemental composition of the silver particles (Figure 2) and it revealed strong signals in the silver region. Usually, due to surface plasmon resonance a typical absorption peak for Metallic AgNPs appears at the 3 keV range. According to EDS analysis the presence of pure silver was 93.7%. The other signals such as chlorine appeared in the image, confirmed the presence of organic compounds (from the plant extract), which revealed the presence of biomolecules with AgNPs (Das *et al.*, 2011). Concerning the stability of nanoparticles under heat-treated SEM images showed size range in 400nm and the shape of nanoparticles is polyhedral. Formation of different crystalline phases with different characteristics could be induced by heat. With compared to the SEM images in non-heat treated (Figure 1) nanoparticles heated (Figure 1b) sample shows unshaped nanoparticles with sharpened ends and also more aggregations. Storage stability of nanoparticles reflects more dispersed impact with lesser dense and aggregations (Figure 1c).

Figure 2a-b displays the FT-IR spectra of the sample extract taken from powdered flesh parts of *A. bilimbi* and AgNPs, respectively. Table 3 shows the types of possible bonds present. The major peaks in the spectrum (FTIR) of synthesized silver nanoparticles were measured at 3272.37cm-1 to 2852.43cm-1and 1739.23 cm-1 to 1034.04cm-1. Similarly, in the FTIR spectrum of the *A. bilimbi* fruit dried powder, the major peaks were measured at 3383.60cm-1 to 2852.78cm-1 and 1740.13cm-1 to1021.82 cm−1. The potential biomolecules in *A. bilimbi* extract responsible for the reduction of the synthesized NPs were identified using FT-IR measurements (Basavegowda *et al.*, 2014). The strong band of the spectrum at 3383.60 cm 1 in A. *bilimbi* extract was attributed to the O–H stretching band of alcohols and phenols shifted to 3272.37cm 1 in silver nanoparticles apparently due to binding of protein (Figure 2). Absorption peaks observed at 2922.20 and 2921.82 cm 1 due to alkane C–H stretching vibrational modes. It was observed that a more intense peak was present at 1740.13cm 1, signifying the presence of carbonyl (C–O) stretching vibrations. Moreover, the medium-intensity peak at 1524.77cm 1, which is absent from the AgNPs FT-IR spectrum, was assigned to the C–N stretching vibrations of aromatic molecules. After the bio-reduction, peaks at some wavelengths were totally disappeared and a decrease in the intensity of peaks could be observed (Dipankar and Murugan, 2012). It may be due to polyols and phenols which are mainly responsible for the reduction of Ag ions by oxidizing to unsaturated carbonyl groups. These are showing that the synthesized silver nanoparticles from the plant extract are surrounded by some proteins and metabolites (Dipankar and

Murugan, 2012) [8] such as terpenoids that have amine, alcohol, ketone, aldehyde and carboxylic acid functional groups. It can be concluded using the previous observations, that functional groups present in the *A. bilimbi* extract might be responsible for the bioreduction of Ag to AgNPs.

3.4 Anti-bacterial properties
Antibacterial properties of the extracts and the formulated nanoparticles were evaluated using the disk diffusion method and colony-forming unit count method. The highest antibiotic concentration of the disk is found in the disk edge and it ceases gradually as the distance from the tested disk increases to a point where it is no longer inhibition of the organism, which then grows freely. Formation of a clear zone or ring around an antibiotic disk indicates the inhibition of bacterial growth. The selected organism of *Escherichia coli* is the predominant nonpathogenic facultative flora found in the human intestine. According to Nataro and Kaper (1998) [25], some E. coli strains have developed the ability to cause disease of central nervous system and gastrointestinal, urinary

tracts in even the most robust human hosts. Most of the assays of silver nanoparticles show inhibitory zones applied in the disk diffusion method (Geoprincy *et al.*, 2011) [11]. However, this study did not observe any inhibitory zones from the *A. bilimbi* or the extracts compared with erythromycin. Several factors can be effective for not presenting inhibitory zones such as the size of nanoparticles, even shape and environmental conditions etc. Smaller nanoparticles are having a larger surface area which provides a higher interaction area and increasing intracellular penetration ability (Roy *et al.*, 2019).
A colony-forming unit count method was carried which shows comparatively better results for nanoparticles and extracts than the disk diffusion method. Silver nanoparticles showed a minimal average number of colonies compared with the control and other extracts (Table 4). The lowest colony count was appeared in the nanoparticles treatment ($70 \times 10^7 \pm 6.976$) and the highest average number of colonies appeared in the control sample (without treatment).

Table 1: Total Phenolic content and Total Flavonoid content of *A. bilimbi*.

Sample type	Total Phenolic content (mg GAE/ g DW)	Total Flavonoid content (mg CE/g DW)	Total carotene (mg/g DW)
Fruit extracts	69.13±5.47[c]	37.74±1.33[c]	0.53±0.01[c]
Leaf extracts	261.58±30.64[a]	95.49±3.28[a]	2.43±0.19[a]
Flower extracts	94.32±13.45[b]	55.46±0.89[b]	0.91±0.08[b]

Values are mean ± standard deviation (n=3)

Table 2: Antioxidant, anti-diabetic and anti-inflammatory activities of aerial parts of *A. bilimbi*

| Sample type	Total antioxidant capacity (mg AAE/g DW)	Anti-diabetic Activity (alpha amylase inhibition)	Anti-inflammatory Activity (% Heat induced Hemolysis)	
			10 µg/ml	100 µg/ml
Fruit extract	148.58 ±7.68[c]	6.48±0.013[c]	11.57±0.09[c]	23.09±0.09[c]
Leaf extract	309.75 ±1.57[a]	21.41±0.03[a]	15.29±0.07[a]	36.68±0.05[a]
Flower extract	167.88 ±5.29[b]	13.43±0.03[b]	13.81±0.09[b]	27.11±0.06[b]

Values are mean ± standard deviation (n=3)

Table 3: FTIR analysis *A. bilimbi* dry powder and Silver nanoparticles

Sample type	Type of bonds present	Peaks present at wave number (cm-1)
A. bilimbi dried powder	RNR-H, RCON-HR	3383.60
	C-H	2922.20
	R(C=O)H,R(C=O)R	1740.13
	R(CO2)H,	1437.56
	C-C- C–H	1246.76
	C-O-C	1021.82
A. bilimbi Silver nanoparticles	=CR-H,R(C=O)O-H	3272.37
	R3C-H	2921.82
	R(C=O)H,R(C=O)R	1740.13
	R2C=C(OR)R,R(CO2)H	1620.77
	RNO2	1524.77
	C-O-C	1034.04

Table 4: Antibacterial assay of colony-forming unit method

Average colony forming unit per mL			
Control	nanoparticles	Leaf extract	Fruit extract
$150 \times 10^7 \pm 5.55$	$70 \times 10^7 \pm 6.97$	$129 \times 10^7 \pm 7.36$	$114 \times 10^7 \pm 12.83$

Values are mean ± standard deviation (n=3). *E. coli* was used as the bacteria. The extract concentration used for the study was 100 ppm
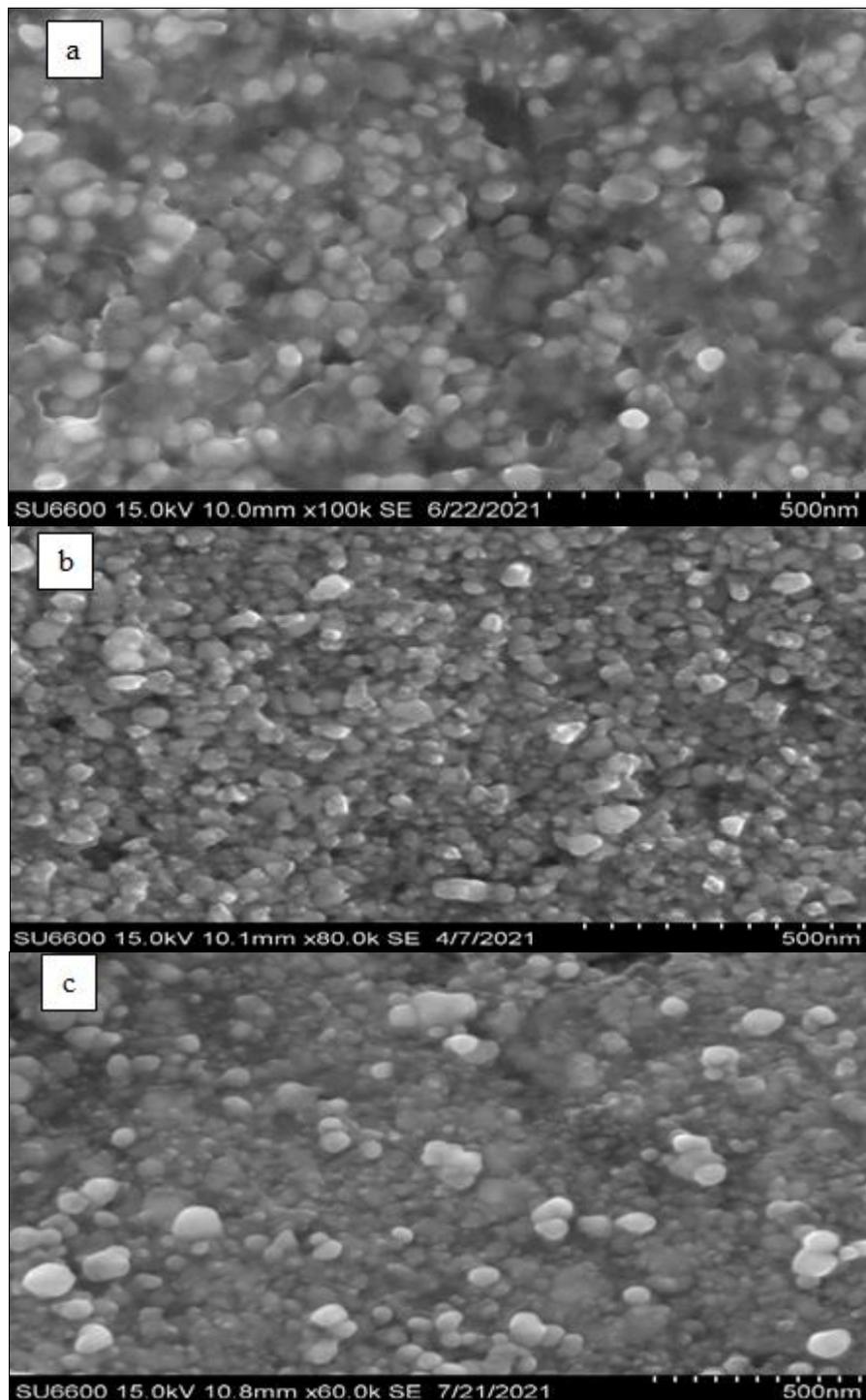
Fig 1: SEM images of silver nanoparticles (a), after the heat treatment at 100 °C (b) after 30 days storage at 5° C (c)
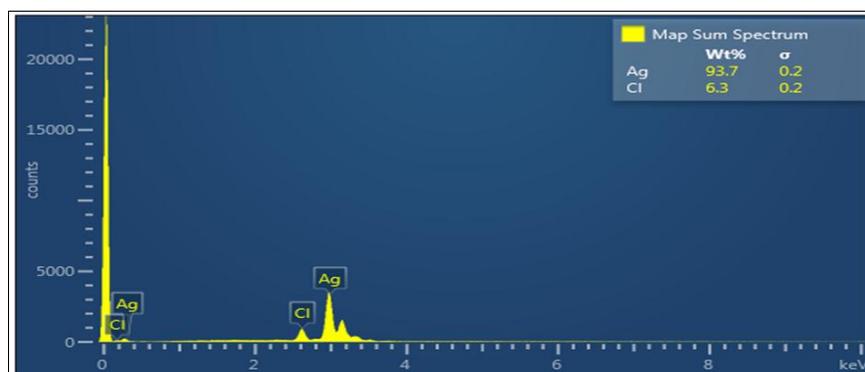
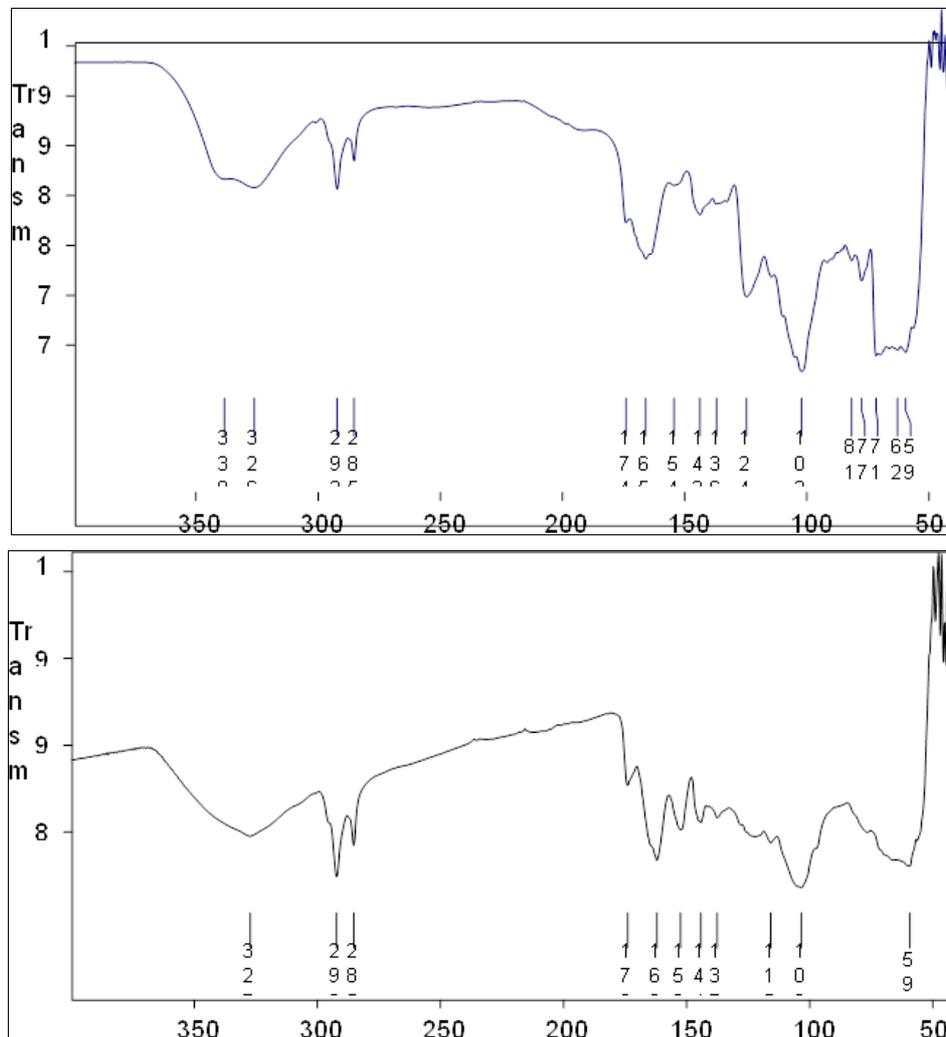**Fig 2:** EDS spectrum of the prepared AgNPs

Fig 3: FTIR spectra of *A. bilimbi* (a) flesh part dried and (b) AgNP

4. Conclusion
Leaf extracts of *A. bilimbi* showed significantly higher total phenolics, flavonoids, carotene levels compared with the extracts from fruits and flowers. Antioxidant capacity, anti-diabetic anti-inflammatory properties of the *A. bilimbi* leaf extract are higher compared with its fruits and flower extracts. Silver nanoparticles can be synthesized successfully from AgNO$_3$ through the green route procedures using the fruit of *A. bilimbi* as a biological reducing and capping agent and evaluated structural properties through SEM and FTIR. The stability of nanoparticles was evaluated under normal storage conditions and heat-treated samples through SEM analysis. Silver nanoparticles of *A. bilimbi* possess some anti-bacterial properties.

5. Acknowledgment

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