Iraqi Journal of Industrial Research (IJOIR)

Journal homepage: http://ijoir.gov.iq

Phytochemical, Total Phenolic, and Antioxidant Activity of Ginger Extract and Its Use in the Synthesis of Eco-friendly Silver Nanoparticles

[1]Abdulkadir Mohammed Noori Jassim*, [2]Maryam Abdulghafor Ahmed, [1]Salam A. H. Al-Ameri, [3]Safana Ahmed Farhan, [4]Omar M. Noori

[1]Department of Chemistry, College of Science, Mustansiriyah University – Iraq

[2]Department of Energy Science, College of Energy & Environmental Sciences, Alkarkh University of Science – Iraq

[3]Polymer Research Unit, College of Science, Mustansiriyah University – Iraq

[4]Industries of Chemical and Physical Materials, Ministry of Science and Technology – Iraq

Article information

Article history:
Received: January, 05, 2023
Accepted: March, 29, 2023
Available online: October, 20, 2023

Keywords:
Ginger (Zingiber Officinale),
Phytochemicals,
GC-Mass

Corresponding Author:
Abdulkadir Mohammed Noori Jassim
kadirchem@uomustansiriyah.edu.iq

DOI:
https://doi.org/10.53523/ijoirVol10I2ID303

Abstract

The Ginger *(Zingiber Officinale)* extract contain a lot of constituents and elements. Ginger extract can effort as a good bio-reductant to produce silver nanoparticles (AgNPs). The AgNPs Green synthesis was achieved by using the Ginger plant extract with $AgNO_3$. The green synthesis of AgNPs is the best developing procedure for preparation, since this process is easier than other procedures, eco-friendly and fewer times consuming. The produced AgNPs were characterizing via UV-Vis, FTIR, AFM, SEM, Zeta potential and EDX. The optimal conditions for the synthesis AgNPs which was confirmed through UV–Vis spectrophotometer. Also, the AgNPs has been characterized by AFM with average size of 53.45 nm, SEM with average size of 21.91 to 33.79 nm. Antioxidant and free radical scavenge activity are examined through TLC and DPPH test, the results shows that the Ginger have a high activity, moreover, this activity increased with characterized AgNPs.

1. Introduction

The scientific name of Ginger (*Zingiber officinale*) is initially from southern part of China and it extent to Maluku Islands in India from Asia and West Africa. Ginger discovered first in Europe in the 1st century from Roman trade with India. It was briefly used by the Romans, decreased of use through the medieval [1]. Such of bioactive Ginger compounds had been known, such as phenolic as well as terpenes compounds. The mostly phenolic compounds are gingerols, shogaols, and paradols which explain the various Ginger bioactivities [2].

Ginger has biological activities as antioxidant, antimicrobial, anti-inflammatory also anticancer [2]. It has above 400 diverse constituents, the significant compounds are 50–70% carbohydrates, 3–8% lipids, phenolic acids and terpenes moreover phytosterols, amino acids, raw fiber, ash, protein, vitamins (vitamin A with slight quantity of vitamin B and C) and minerals are also being present [3]. This plant is an excellent source of necessary micronutrients such as potassium, magnesium, copper, manganese as well as silicon [4].

Ginger extract is a simply obtainable, safe and non-hazardous as well as have a broad metabolites range that can lead to fast reduction of metal ions than microbes, this most important advantage of using Ginger extracts for nanoparticles (NPs) preparation. The other methods such as chemical synthesis leads to some toxic chemical absorbed on the NPs surface which cause differing effect in the medical uses [5, 6]. No such effects are obtained when it moves toward synthesized NPs from green synthesis [7].

Silver Nanoparticles (AgNPs) are of importance since it has some special characteristics like size and shape which are dependent optical, electrical and magnetic feature which can be included in antimicrobial uses, biosensor equipment, composite filament, cryogenic super conducting substances, cosmetic and electronic apparatus [8]. Many of physical and chemical methods were applied for AgNPs production [8, 9].

The current study described and applied a developed biological preparation of AgNPs using Ginger extract and estimation the phytochemicals compounds in Ginger also their characterization.

2. Experimental Procedure

2.1 Materials

Ginger was taking from locally commercial markets in Baqupa / Diyala-Iraq. Silver nitrate, chemical reagents and substances are of analytical grade purity gotten from BDH, Sigma Aldrich and Merck.

2.2. Preparation of Ginger Extract

All pollutions were removed from ginger by washing using distilled water. The Ginger was parings and cut it into very small portions then placed in the mixer for a few time till it turns into juice, then it filtered with a piece of gauze to remove the fibers residues and get a clear juice then filtered throughout filter paper No. 1 to obtained the extract which set aside at 4 °C until use [10].

2.2.1. Quantitative and Qualitative Examination of Phytochemical

The extract phytochemical quantitative and qualitative examination of primary and secondary metabolites was employed for each standard method. The achieved extract was analysis for steroids, alkaloids, saponins, carbohydrate, triterpenoids, phenolic compounds, flavonoids, tannins, moisture, ash, protein and fats, following standard methods [11-14].

2.3. Determination of Total Phenolic Compounds

A standard graph for Gallic acid in the concentration range between 50-250 µg mL-1 were produced via dilution 25, 50, 75,100 and 125 µL of 5000 µg mL^{-1} stock standard solution to 2.5 mL [15]. The regression equation [Y=b x + an or Y=0.0035x+0.494, where: b is the slope=0.0035, a; is the intercept= 0.494, X is the concentration and Y is the absorbance] was achieved *via* Least Squares method [16]. The precision of this regression equation was examined by analysis of a laboratory made samples.

Also standard solutions of Ginger extract in the concentration between 50-250 µg mL-1 were formed through dilution 25, 50, 75,100 and 125 µL of 5000 µg mL-1 stock standard solution to 2.5 ml.

The total phenolic contents in the Ginger extract samples were determined through Folin–Ciocalteau reagent illustrated by Singleton & Rossi with little adjustments [17]. The absorbance was determined at 765 nm by UV–Vis spectrophotometer. The total phenolic contents amounts were characterized as Gallic acid equivalent (GAE) in milligram per 10 g initial sample with standard calibration curve [18].

2.4. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The various volatile components of methanol Ginger Extract was analysis using GC-MS using capillary column (30 mm x 0.25 mm x 0.25 µm) at temperature programming from 70 to 240 °C, Injection temperature: 240 °C, Injection Mode: Splitless at Split Ratio: 10.0. Start Time: 3.50 min, End Time: 27 min [19].

2.5. Biosynthesis of AgNPs

In a suitable test tube, 1 mL of the Ginger extract was reacted with 9 mL (1 mM) of silver nitrate, $AgNO_3$ at room temperature 25°C. The reaction color was gradually turned from light yellow to brown-yellow proposing the formation of AgNPs and the time reserved for the alterations was prominent. The AgNPs suspension gained was kept in refrigerator at 4°C [20].

2.5.1. Fixation of Biosynthetic AgNPs

2.5.1.1. Temperature

The above process was repeated for temperature optimization and then the temperature of water bath reaction was kept equally at 20, 30, 40, 50, 60, 70, 80 and 100°C with shaking. The absorbance of the final solutions was measured via UV-Vis spectrophotometer [21].

2.5.1.2 Time

The adjusted process was repeat to optimal the required reaction time achievement. The reaction was observed from 0.0 to 70.0 min at 10 min period time. The resultant absorbance was obtained via UV-Vis spectrophotometer [21].

2.5.1.3. Concentration of $AgNO_3$ Solution

The mention method (2.5.1.2) was recurrent for optimization of $AgNO_3$ concentration, where the reaction was preserved using diverse $AgNO_3$ concentrations of 0.25, 0.5, 1, 2, 3, 4 and 5 mM respectively. Other conditions were applied. The last solutions absorbance was measured via UV-Vis spectrophotometer [21].

2.5.1.4. Ratio of Extract to Ag Solution

The mentioned method (2.5.1.3) was repeated for the determination the optimal Ag and extract concentration required for the greatest produce of AgNPs. Diverse volumes 1,3,5,7,9,11 and 13 mL of $AgNO_3$ was taking with firm volume of the extract at 1 ml, then a 0.1,0.5,1,3,5,7 and 9 mL diverse volume was taken with steady 7 mL volume of $AgNO_3$ solution. The final solution absorbance was measured through UV-Vis spectrophotometer [21].

2.5.1.5. Effect of pH

The previous process (2.5.1.4) was also repeated for the pH value optimization. The reaction pH was kept at 4, 5, 6, 7, 8 and 9. Additional parameters were applied. The absorbance of the last solutions was measured with UV-Vis spectrophotometer [21].

2.5.1.6. Stability Study

The resulting solution stability was estimated at room temperature and time intervals of 1 day, 1week and 30 days [21].

2.6. Characterization of AgNPs

2.6.1. The UV-Vis Spectra Analysis

The AgNPs were established by determining the reaction mixture wave length via UV–Vis spectrum at 300 to 800 nm in 3 mL quartz cell [21].

2.6.2. FTIR Analysis

After the preparation of AgNPs, they were centrifuging for 15 minutes at 5000rpm. This method was repeated 3-5 times. Then proceeds the precipitate and dry it in oven at 40 ^{O}C for 4 hours. The powder of Ginger extract was dry in the oven. For the analysis; the dried AgNPs and Ginger powder were examined by FTIR spectra via KBr disc. The spectrum was obtained at the ranging between 500-4000 cm^{-1} [22].

2.6.3. The AFM Analysis

The AFM was used to observe the NPs size and size-distribution. The evaporation process of the dropper was applied to prepare AFM samples of fluid suspension. One drop of liquid sample was drained on a 2x6 cm^2 glass cover slide. To dehydrated the sample prior to scan, also left the sample overnight in a dust-shielded location.

2.6.4. Zeta Potential Analysis

Zeta potential measurement was used to identifying the Nano-material and the Ginger extract. Zeta potential was a probability measured by light dispersion using the Zeta Plus tool. The data were being around with 5 measurements. Size of NPs were measured by Electrophoretic light scattering (ELS) and dynamic light scattering (DLS) using Zeta Plus, while characterizing nano-materials surfaces and estimated their surface charge through the determination of the zeta potential [23].

2.6.5. Atomic Absorption Analysis

The AgNPs concentration was obtained via atomic absorption analysis. Standard Ag solutions were prepared and the samples absorption values were used for the determination of AgNPs via calibration graph.

2.6.6. The SEM Analysis

The SEM was applied for the identification the nature and size of AgNPs biosynthesized.

2.7. Antioxidant Activity

Antioxidant activity (*in vitro*) of Ginger fruit extract was achieved using DPPH, 2, 2- Diphenyl-1-picrylhydrazyl via free radical scavenge activity procedure.

2.7.1. Qualitative Determination via Free Radical Scavenging Activity; TLC Method

The antioxidant component was estimated by thin layer chromatography (TLC) using DPPH. About 100µg of Ginger extract and standard Gallic acid solutions were retained on 10 x 10 cm2 TLC plates. The plates become dry and detected at 240 and 300 nm. Varied separated spots were well-known refers to their retention factor (R_f). A 0.05% of DPPH solution in methanol was marked on the TLC plates fronts and incubate at room temperature for 30 min. The active Ginger and Gallic acid antioxidant showed as a yellow spot versus a violet background [24, 25].

2.7.2. Quantitative Determination via Free Radical Scavenging Activity; DPPH Method

Free radical scavenging activity was employed specified along with Braca *et al*, 2001 method through little changes. 0.5 mL of the extract was adding to 1 mL 0.013g/L DPPH solution in methanol. The DPPH reduction was checked at 517 nm vs. a blank later than 30 min. The free radical percentage was estimated as the sample absorbance split by that of DPPH control at similar time multiplied by 100. The quantity of sample needed to reduction the initial DPPH concentration by 50%, IC_{50}, was estimated graphically using the equation below [24, 25]:

% DPPH scavenging activity = [1 – (A test or standard sample / A control sample)] x 100

Where: A control = Absorbance of DPPH only
A sample = Absorbance of DPPH as well as diverse extract or standards concentrations

3. Results and Discussion

The analytical results of the Ginger extract proved and indicated the presence of diverse phytochemical components, Table 1.

Iraqi Journal of Industrial Research, Vol. 10, No. 2 (2023)

Table (1). Qualitative phytochemical Ginger extract tests.

Components	Reagents	Note	Results
Glycosides	Molish test Benedict test	Purple ring Bluesolution	+*ve* +*ve*
Phenolic Compounds	Ferric chloride $FeCl_3$ 3%	Green ppt	+*ve*
Alkaloids	Mayer's reagent Wagner reagent Picric acid	White ppt Brown ppt Yellowppt	+*ve* +*ve* +*ve*
Proteins	Biuret test	Purple blue	+*ve*
Steroids	Ethanol +acetic anhydride+ H_2SO_4	Blue/green	+*ve*
Saponins	Strong shaking	foam	+*ve*
Flavonoids	Ethanol + KOH	Yellow ppt	+*ve*
Terpenoids	$CHCl_3$ + H_2SO_4	Reddish brown	+*ve*

The results show that the Ginger extract contains glycosides, flavonoids, alkaloids, terpenoids, phenolic compounds, tannins, resins as well as Steroid, Tables 1. This results in agree with the study of seda *et al.* [26]. The nutritional rate per 100 gm of Ginger extract was summarized in Table 2, the results in contract with other study [27].

Table (2). Nutritional contents in Ginger extract.

Nutritional contents in Ginger extract	
Composition (g/100g)	**Percentage%**
Moisture	12.46%
Ash	3.13 %
Fiber	4.32%
C.H.O.	67.58 %
Energy	338.41Kcal
Protein	8.92 %
Fat	3.61 %

3.1. Total Phenolic Contents

Total phenolic contents of the methanolic portions of Ginger extract were estimated via folin-ciocalteu reagent and express as Gallic acid equivalents for each gram of Ginger extract using the standard Gallic acid graph, Table (3).

Table (3). Total phenolic contents for standard Gallic acid and Ginger extract.

Concentration(µg/mL)	Absorption of standard Gallic acid	Absorption of Ginger extract
250	1.345	1.337
200	1.139	1.328
150	1.125	1.321
100	0.869	1.320
50	0.606	1.315

Analysis of the total phenolic contents [26] for Ginger extract shown informed to contain these phenolic compounds as shown in Table 4.

Table (4). Total phenolic contents for Ginger extract analysis.

Sample Absorbance	Concentration (µg/mL)	The equation for standard Gallic acid	Total phenolic contents in Ginger Plant mg/mL
1.337	250	Y=0.0035x+0.494 R^2 = 0.9403	241.3
1.328	200		238.7
1.321	150		236.7
1.320	100		236.4
1.315	50		235.0

3.2. GC-MS Analysis of the Phytocomponents of Ginger

The GC-MS method shows a particular statistics of a number of components in the Ginger extract. These compounds were distinguished via MS coupled to GC. The mainly components exposed in the Ginger extract found using GC-MS are summarized in Table 5. The GC-MS chromatogram establishes 85 mixed components with varied retention times, Figure 1.

The GC-MS study of Ginger showed several phytochemicals which gives the reduction action of the plant via the presence of varied components have different groups such as OH, C-I, NH, CO, C-Br, C-S…etc, this results was in agreement with other studies [29, 30] which indicated that the Ginger contains a different antioxidant, phenolic compound, terpenes, and others.

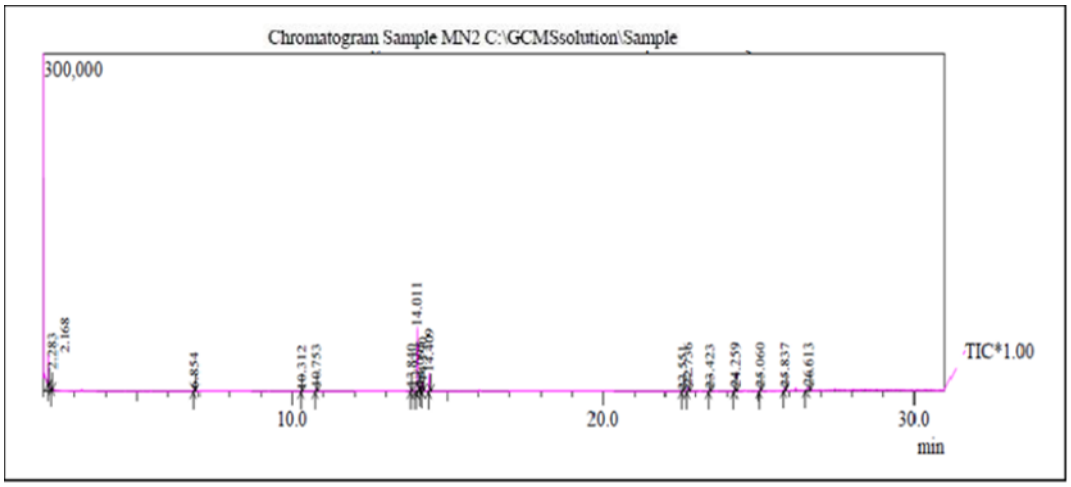

Figure (1). GC-MS chromatogram investigation of Ginger extract.

Table (5). Retention times, Molecular Formula and peak Area for some Ginger extract components by GC-MS.

Peak	R. Time	Area%	Name of the compound	Formula
1	2.168	11.76	p-Dioxane, 2,3-bis(tert-butyloxy)	C12H24O4
2	2.168	11.76	Propanoic acid, 2-methylpropyl ester	C7H14O2
3	2.283	0.83	Oxan-4-one, 2-trifluoromethyl-2-hydroxy-5,5-dimethyl	C8H11F3O3
4	2.283	0.83	1-Pentanol, 4-methyl	C6H14O
5	6.854	11.76	Bis[bicyclo[3.2.0]hept-2-en-4-yl]ether	C14H18O
6	6.854	11.76	Bicyclo[3.1.0]hexane,, 4-methyl-1-(1-methylethyl)-, didehydroderiv	C10H16
7	10.312	0.83	2-Propenoic acid, 2-methyl-, ethenyl ester	C6H8O2
8	10.312	0.83	Borane, triethyl	C6H15B
9	10.753	1.68	3,7-Nonadien-2-one	C10H16O
10	10.753	1.68	2-Propenoic acid, 2-methyl-ethenyl ester	C6H8O2
11	13.840	0.68	Benzoic acid, 4-(2 methylbenzoylamino)-, 2-methylphenyl ester	C22H19NO3
12	13.840	0.68	4-Methyl-N-(4-methyl-furazan-3-yl)-benzamide	C11H11N3O2
13	14.011	2.41	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-	C15H24
14	14.095	1.79	2-Norpinene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)	C15H24
15	14.190	44.51	1-Pentene, 5-(2,2-dimethylcyclopropyl)-2-methyl-4-methylene	C12H20
16	14.190	44.51	4-(4-Methylpent-3-enyl)-3,6-dihydro-1,2-dithiin	C10H16S2
17	14.409	2.04	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene	C15H24
18	22.551	5.14	3,4-Hexanedione, 2,2,5-trimethyl	C9H16O2
19	22.551	5.14	Pentane, 1,3-epoxy-4-methyl	C6H12O
20	22.736	12.07	Propanoic acid, 3-(4-benzyloxyphenyl)-, methyl ester	C17H18O3
21	22.736	12.07	2-Methyl-3-(3-methylenebicyclo[3.2.1]oct-6-en-8-yloxy)cyclohex-2-enone	C16H20O2
22	23.423	0.72	Decane, 1-iodo	C10H21I
23	23.423	0.72	Pentane, 1,3-epoxy-4-methyl	C6H12O
24	24.259	4.63	Sulfurous acid, hexyl pentyl ester	C11H24O3S
25	24.259	4.63	2-Bromononane	C9H19Br
26	25.060	1.83	3,4-Hexanedione, 2,2,5-trimethyl	C9H16O2
27	25.060	1.83	Ethyl propyl ketone	C6H12O
28	25.837	2.41	Borane, diethyl(decyloxy)	C14H31BO
29	25.837	2.41	Sulfurous acid, 2-ethylhexyl isohexyl ester	C14H30O3S
30	26.613	3.57	Nonane, 1-Iodononane	C9H19I

3.3. Biosynthesis of AgNPs

The extract of Ginger has several reducing phytocomponents. The color of residues Ginger extract unchanged when protected by $AgNO_3$ solution for five days. Revealing to heating up to 100ºC, the extract having $AgNO_3$ changes from light yellow to brown-yellow, specifying the AgNPs formation, Figure (2).

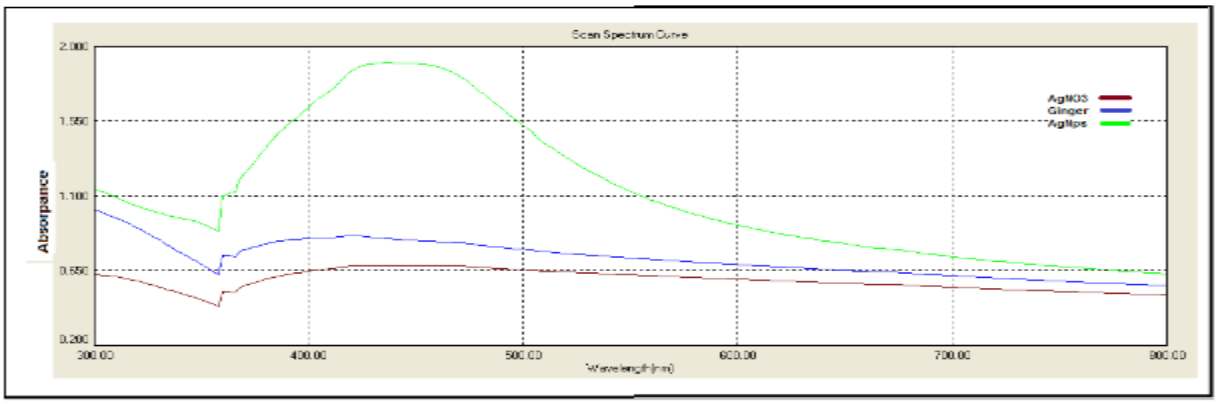

Figure (2). UV–VIS spectra and change in reaction products of $AgNO_3$, Ginger extract and AgNPs.

The maximum absorption of AgNPs was detected at 430nm, which characteristic the SPR for AgNPs, Figure (2). After spreading of $AgNO_3$ in the Ginger aqueous solution, the extract was countered with the functional groups of Ginger components to form AgNPs, Figure (٣). The AgNPs concentration via AAS, was equal to 47.0329 ppm.

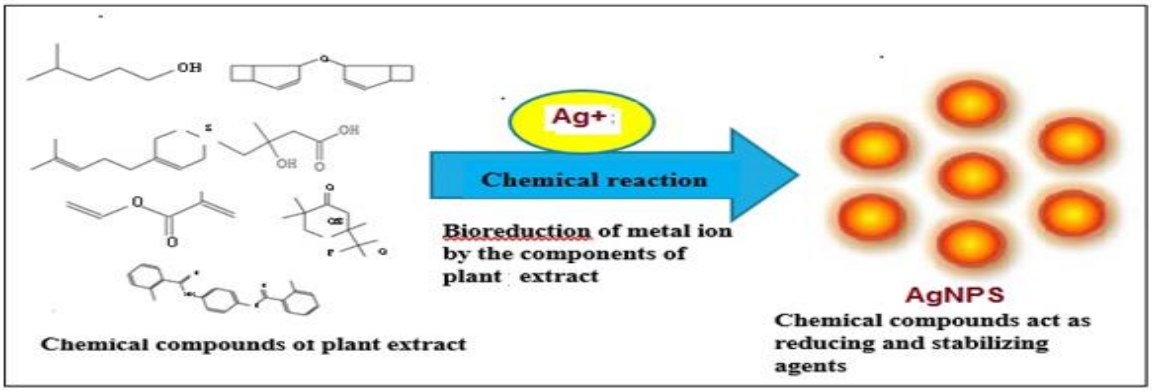

Figure (3). Probable compounds of Ginger extract responsible for the bio-reduction of silver ions.

3.3.1. Fixation of Biosynthetic AgNPs

Diverse limitations were optimized including temperature, time, silver concentration ratio and the Ginger extract concentration which have recognized as factors affect the AgNPs yields [11].

3.3.1.1. Temperature

The central factor measured was temperature, at the higher temperature, the AgNPs formation rate was distended, also the size is mainly condensed owing to the reduction in the collection of the growing NPs [11], while increasing the temperature above 100°C were assistances the crystal growth nearby the nucleus.

3.3.1.2. Time

The factor measured was the time necessary for the end of the reaction. During the reaction time resulted rising the absorbance spectra by SPR near 430 nm and the intensity of the color increasing with the incubation time. The SPR peak intensity increases as the time of reaction increase, which confirms the AgNPs concentration increasing. Owing to the variability of the formed AgNPs, an optimal duration is necessary. The optimal time needed for the ended of the reaction was 50 min.

3.3.1.3. Effect of *p*H

The size and form of bio synthesized NPs was tested by changing the *p*H of media. A chief subjective of the *p*H of the reaction is its capacity to adjust the biomolecules electrical charges which might affects their stabilizing capability and the NPs growth, the effect of *p*H on the creation of AgNPs using Ginger extract in its best

conditions was checked at *p*H range 4 to 11. The results obtained via UV-Vis spectrophotometer illustrated that the absorbance increased with *p*H increasing from 4 to 9. The maximum absorbance was in *p*H = 6 at λ max = 450 nm, so the optimal *p*H is 6 for the synthesized of AgNPs using Ginger extract [31].

3.3.1.4. Concentration of Ag Solution

Diverse concentrations of silver solution were used to develop maximum AgNPs. A maximum harvest was perceived at 0.5mM silver solution. As the Ag concentration increases, the color intensity also increasing from brown to deep brown and drizzle of silver particles on the tube walls. The AgNPs SPR peak developed separate via an increasing in Ag solution concentration. The UV-Vis spectra bands are wide with an absorption tail in the lengthy wavelengths as the Ag concentration increasing which showed an improvement in the particles size [32]. Also, the color strength increasing as it is dependent on the AgNPs size. So, as to get regular growth with lesser particle size, it was used 0.5mM Ag at reaction mention condition.

3.3.1.5. Ratio of Extract to Ag Solution

The optimization of Ag and Ginger extract concentration is necessary for the maximum fabrication of AgNPs, which observed by using a diverse ratio of Ag and Ginger extract to examine the optimal conditions in order to maximize the AgNPs yield. It was creating that the best ratio for the reaction is 7: 0.5 constructed on the number of trials and the best absorbance.

3.3.1.6. Stability Study

The stability of reaction time for realization of AgNPs by the reaction among silver ion and the reducing material in the Ginger extract were trailed for one month from synthesis. The UV-Vis spectra of synthesized AgNPs as a function of time subsequently the addition of Ginger extract indication that the increases in the reaction time results in gradual increases of absorbance with SPR at λmax between 430-450 nm. This takings that prepared AgNPs was unchanging more than one month [33] . This study showing no variation in the maximum peak at 430 nm still later one month indicated high stability of AgNPs.

3.3.2. Characterization of AgNPs:

3.3.2.1. UV-Vis Spectrophotometric Study

The features of AgNPs were known via UV-Vis spectra. The results indicated that there is no clear peak for Ginger extract, other than, after the adding of Silver nitrate, a broad peak seems at the range of 425-475 nm, Figure 4 [34]. As a further definite by other characterizations that this peak appoints the formation of mono-dispersed spherical shape of AgNPs. The reaction takes place within 50 minutes with obvious color changed. The results were in agreement with other study [35].

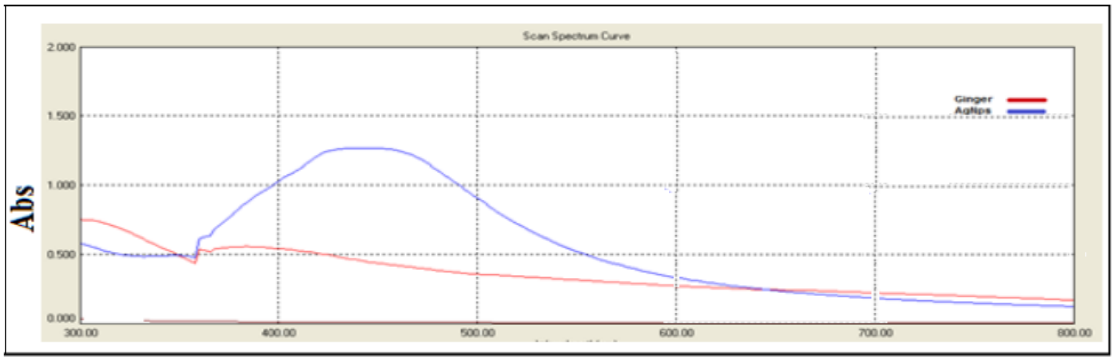

Figure (4). UV-Vis absorbance spectra for Ginger extract and prepared AgNPs.

3.3.2.2. FTIR Analysis

The FTIR spectra was using for the identifying the probable bio-reducing components in the Ginger extract and in the prepared AgNPs following reaction with AgNO3, Figures (4).

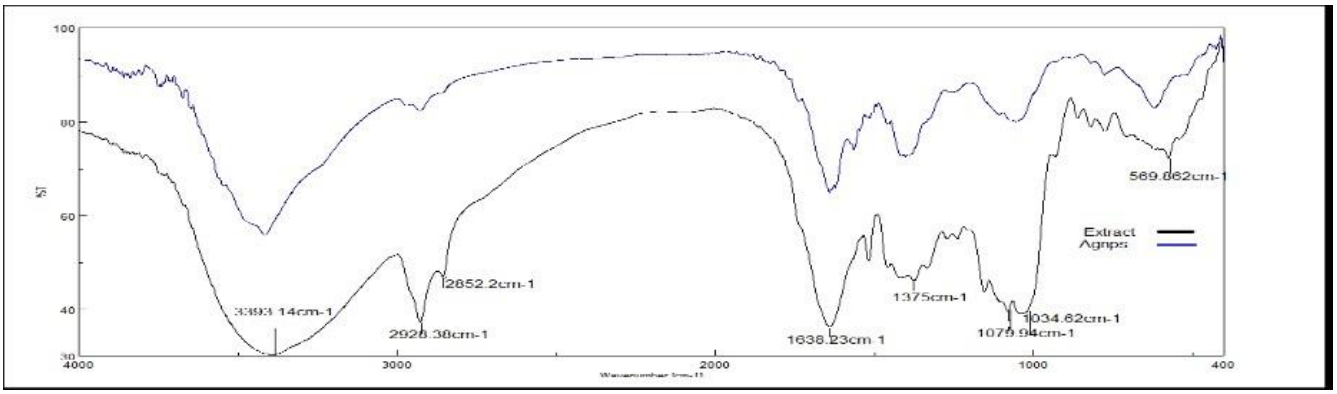

Figure (5). The FTIR for Ginger extract and synthesized AgNPs.

The Ginger extract as well as AgNPs FTIR spectra was showed bands near 569 and 540 cm^{-1} which is refer to CH bend vibrations of substituted ethylene - CH=CH-, while the peak at 1034 to 1149 cm^{-1} indicate C-O stretching vibration. The peak of 1409 to 1454 cm-1 relates to stretching C-C aromatic group, the broad band centered at 3393.14 cm^{-1} is specified to -NH and -OH stretching vibrations of the phyto-constituent obtained in the Ginger extract. The peak at 2928.38 cm−1 is assigning to stretching vibration of C-H. The bands appear to stretching C=O and bending -NH are join together as one and show a broad peak at range of 1863-1516 cm^{-1} with a focus at 1638 cm^{-1}. The peak at 1375 cm^{-1} is equivalent to the side chain vibrations bands which can clarify the Ginger extract proteins also carbohydrates, Figure (5). The AgNPs FTIR spectra indicated every one of the Ginger extract vibrations bands, which recommended that NPs were become stable by the phyto-components. Previous studies illustrate that the phyto-proteins has a tendency to create photo-induced electron transfer to reduce metal ions [36], therefore, this study advise that the biological components obtained in the Ginger extract referee the photo-induced preparation of AgNPs as well as the stability of NPs in the aqueous media.

3.3.2.3. AFM Analysis

The AFM was used to distinguish the surface morphology and to define topography which provides 2D and 3D of AFM NPs surface images at an atomic level with the conforming size distribution of AgNPs. The average particle diameter size is 53.45 nm which was calculated in nano-scale size, Figure (6). Surface analysis by AFM needs good care because of factors that affect results such as tip or contaminations [37].

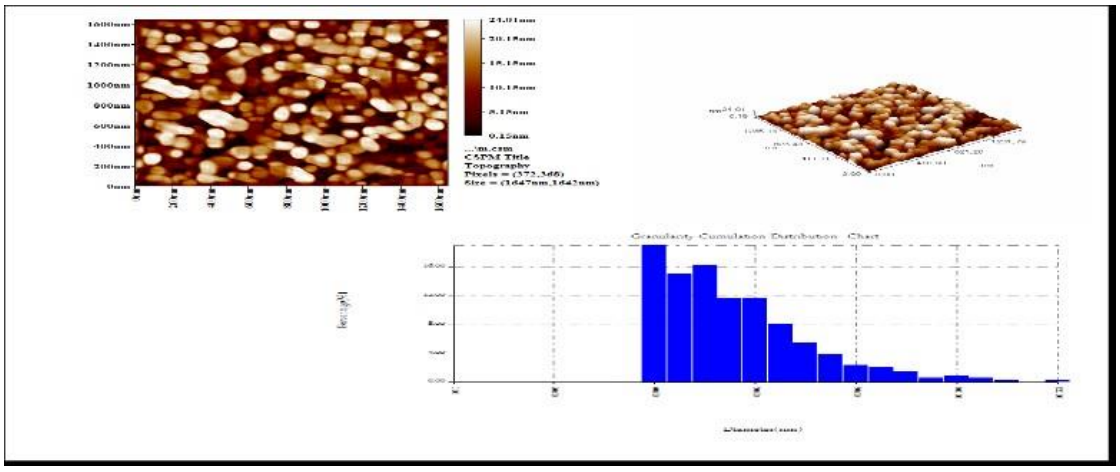

Figure (6). AFM images and size distributions of prepared AgNPs.

3.3.2.4. SEM Analysis

The SEM was applied to study the NPs structure and morphology [38], also obtained further knowledge of the AgNPs gained from the suggested biosynthesis process using Ginger extract. The SEM results explained

different shapes, such as linear and spherical, but the chief shape or the largest quantity of the NPs shape is the spherical, Figure 7.

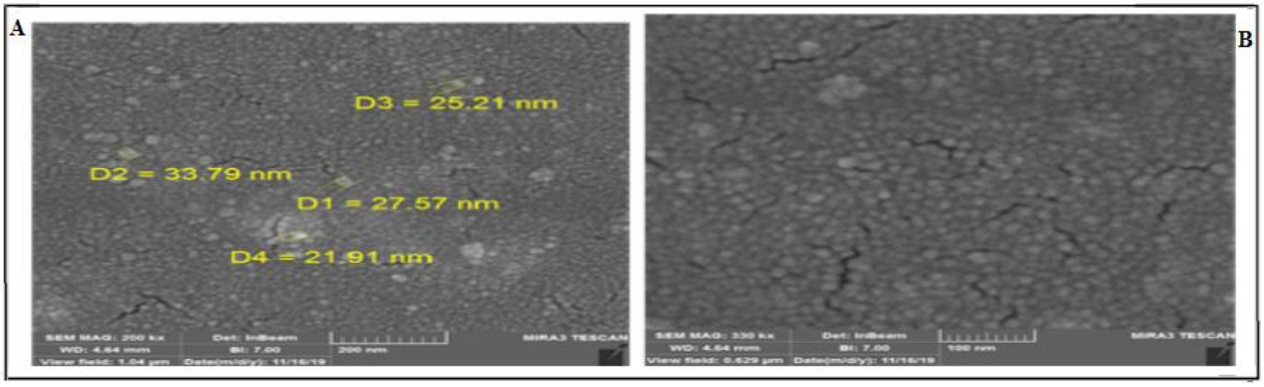

Figure (7). SEM images of AgNPs (A=200 nm, B=100 nm).

The average particles size and distribution are firm arbitrarily using SEM technique. The surface of NPs is smooth with good crystallinity, in agreement with other study [39]. The chief particles size of the AgNPs between 21 to 33 nm. Notable, this size gotten from SEM measurements is meaningfully lesser than the size gained from particle. AFM could be ascribed to existence of phyto-constituents around the NPs, for the reason that AFM measures the NPs size in the existence of hydrated capping agents to confirm that the NPs become stable by phyto-constituents.

Existence of elemental silver in formation of AgNPS was definite by EDS microanalysis, Figure 8. The spectrum shows an indication in the silver area. Metallic silver nano crystal presentation a typical absorption peak near 3 keV owing to the SPR [40, 41]. Another signs for other metals C, N, O, Na, Mg, P, S, Cl, K and Ca existing in the reaction media established that the additional cellular organic components from Ginger extract were initiate on the AgNPs surface or the nearness.

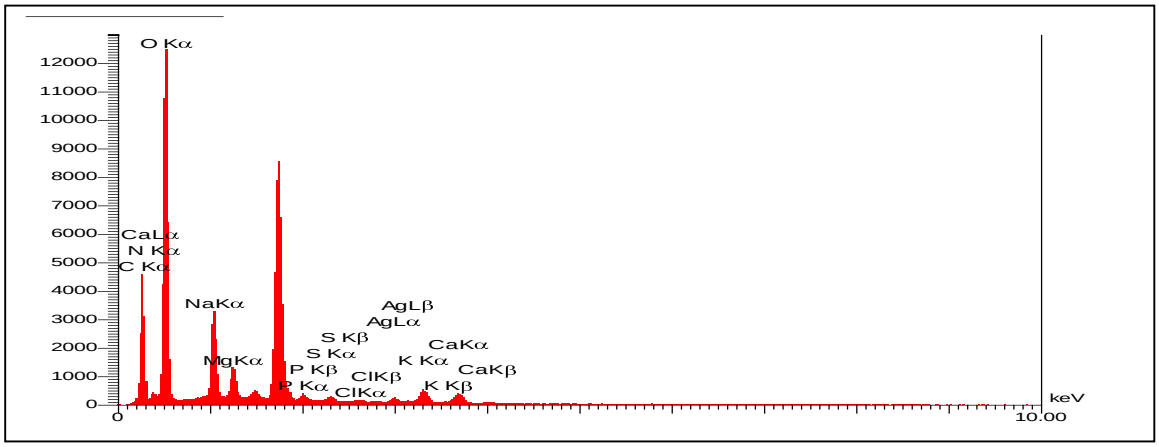

Figure (8). The EDS pattern of spherical synthesized AgNPs using Ginger extract.

3.3.2.5. Zeta Potential Study

Zeta potential measure the NPs stability or in general particles in the colloidal suspension. The zeta potential value achieved by ELS for Ginger extract was -17.88 mV with mobility value equal to -1.40 (μ/s) / (V/cm), Figure 9 A while the reading for AgNPs was -22.5 mV and -2.11, respectively, Figure 9 B. These suggest that the electrical boundary of the AgNPs is comparatively separate and reduced the NPs from more aggregation. The ELS is initially applied for characterized the charges on the colloidal particles surface or other macromolecules in an electric field for liquid media [42]. So, the obvious data showed that the particles in liquid media are fewer stable due to the zeta potential was fewer than ±30 mV and it was more stable as colloidal NPs than it was initiated in extract solution.

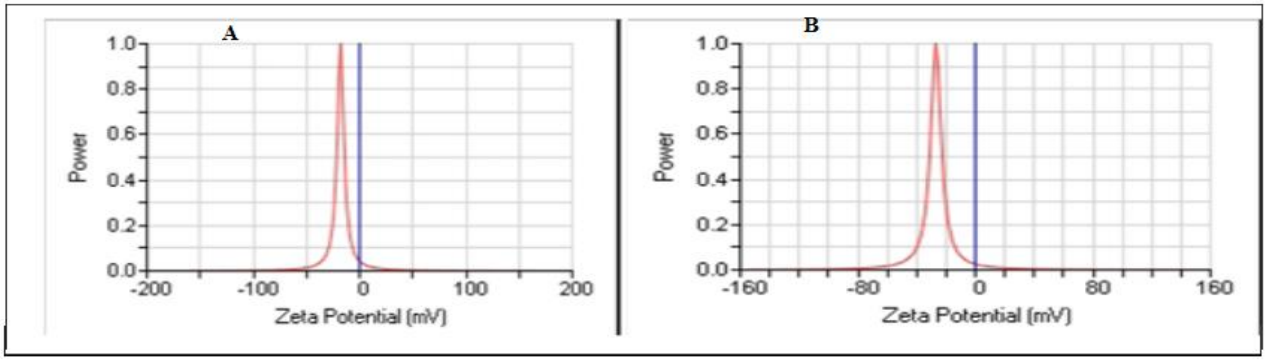

Figure (9). Zeta potential distribution of Ginger (A) and AgNPs (B).

3.7. Qualitative and Quantitative Estimation of Free Radical Scavenging Activity

The chief antioxidant action mechanism in foods is radical scavenge activity. Therefore, various processs were proceeded in which the antioxidant activity was estimated by the scavenge the developed radicals in methanol as polar organic solvents [43]. In free radical scavenge activity, DPPH is a stable and saleable nitrogen radical organic solvent which have a UV–Vis maximum absorption at 517 nm [44]. The results obtained confirm the Ginger extract antioxidant activities were determined through free radical scavenging activity which the color change from purple to yellow as the DPPH radical molar absorptivity at 517 nm reduces, leading to moving of acidic H-atom from the molecule to DPPH radical to give DPPH-H. The result decolorization is stoichiometric with admiration to an electrons captured number.

Radical-scavenging possessions of a standard Gallic acid, Ginger extract were assessed alongside the DPPH radical. Using DPPH as a TLC scatter, standard Gallic acid and Ginger extract seemed as clear yellow spots touching a purple background; mean that they have a strong scavenging activity, Figure (10). The DPPH analyze was used to study the quantitative scavenging activity for the samples, Table (6).

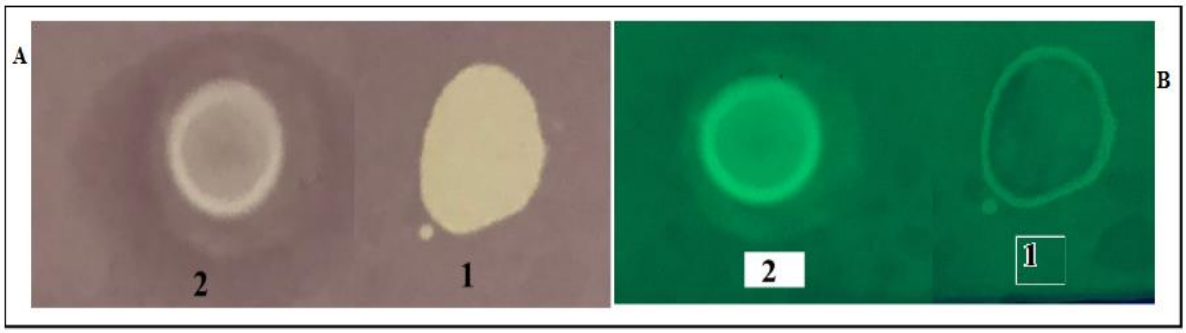

Figure (10). The TLC photo-image A and under UV-light B for (1) Gallic Acid, (2) Ginger extract.

Table (6). The inhibition percentage of standard Gallic acid, Ginger extract at different concentrations (µg/mL) by DPPH model.

Concentration (µg/mL)	% Inhibition (standard solution-Gallic acid)	% Inhibition (Ginger extract)
10	43.2	3.44
20	46.5	6.68
40	53.5	9.2
60	59.6	11.8
80	62.3	13.3
100	82.5	20.4

Current consequences expression the scavenging activity for extract associated with Gallic acid as a standard. It is understandable from the data that compounds radical scavenging activity under study increases with concentration exhibiting increasing, its dose independent nature [45]. The fall outs show that it has a potent scavenging activity with IC_{50} for standard Gallic acid was (28µg\mL), Figures (11), but it was under IC_{50} value for Ginger extract, Figure (12).

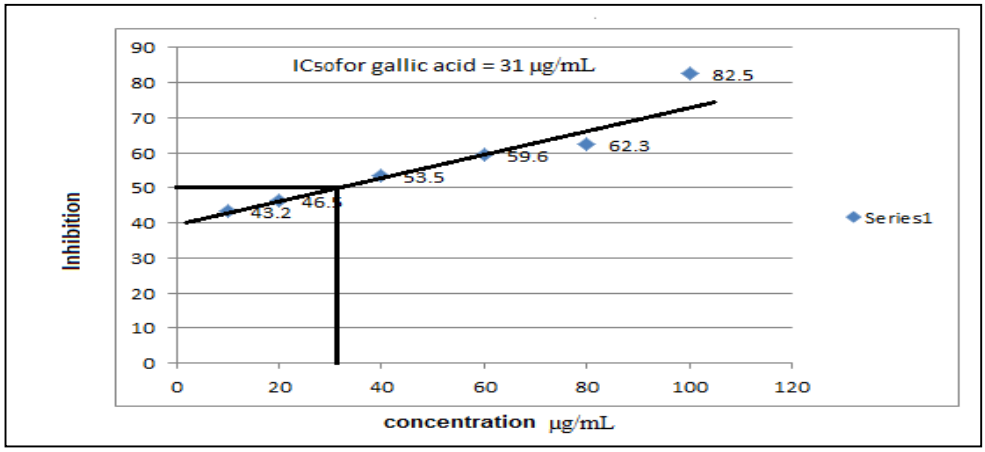

Figure (11). The DPPH free radical scavenging activity of standard Gallic acid.

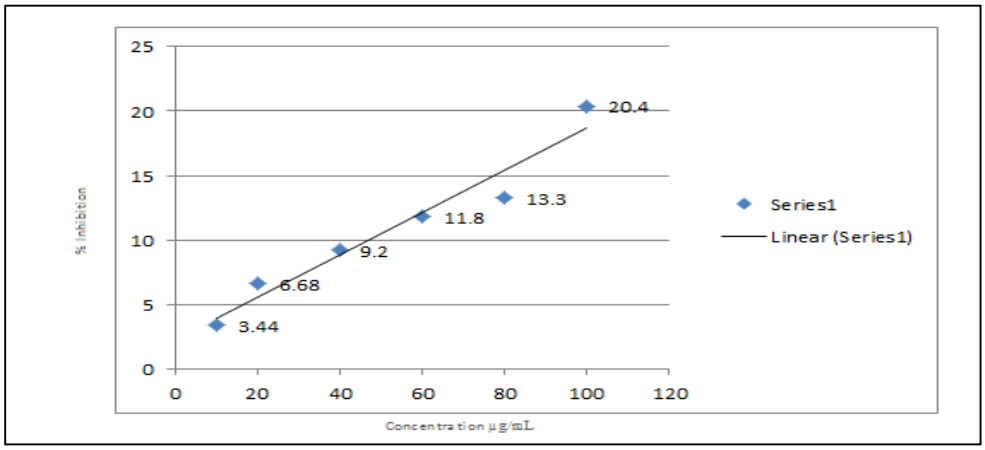

Figure (12). The DPPH free radical scavenging activity of Ginger extract.

The actual concentration required to scavenge 50% of DPPH, IC_{50}, Figure 10, for standard Gallic acid. The lesser the IC_{50}, the better it's able to scavenge radicals, mainly peroxy radicals which are propagators of the oxidation of lipid molecules and thus turn off the free radical chain reaction [46]. Besides, the consequences also showed that the increase in biosynthesized AgNPs antioxidant activity recommended that the Ginger extract itself is accountable for activity and AgNPs improves less antioxidant activity [43]. Furthermore, this extract has a good scavenging activity which could use to work as anti-bacterial and anti-inflammatory.

4. Conclusions

The present works explained the phytochemical, antioxidant activity of Ginger extract. The results showed that the Ginger was a good source for antioxidant ability observed by chemical DPPH method. The Ginger extract, was wealthy in phenolic compounds, antioxidants, unsaturated fatty acids, terpenes, many of trace elements and others. The extract biocomponents were analyzed using GC-MS. The synthesis of AgNPs using Ginger extracts has an important possibility as well as a number of essential advantages relation to traditional processes of NPs preparation. The water-soluble biocomponents from Ginger extract that act as bio-reductants of Ag^{+} to form

synthesize stable AgNPs, which have a yellowish-brown color with SPR band at 430 nm. The AgNPs have also been characterized by AFM with average size of 53.45 nm, SEM with average size of 21.91 to 33.79 nm SEM, AFM images showed that NPs had spherical morphology. Furthermore, UV-VIS, FTIR, Zetepotential analysis were carried out to identify the possible biocomponents.

Acknowledgement: The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq), Baghdad, Iraq, for helping to complete this work.

Conflict of Interest: The authors declare that there are no conflicts of interest associated with this research project. We have no financial or personal relationships that could potentially bias our work or influence the interpretation of the results.

References

[1] S. S. Braga, "Ginger Panacea or Consumer's Hype?," *Applied sciences*, vol. 9 , pp.1, 2019.
[2] Q. Mao, X. Xu, S. Cao, R. Gan, H. Corke, T. Beta, and H. Li, "Bioactive Compounds and Bioactivities of Ginger (*Zingiber offcinale Roscoe*), " *foods* , vol. 8, no. 185,pp.1-14, 2019.
[3] M. A. Mele, "Bioactive compounds and biological activity of ginger," *Journal of Multidisciplinary Sciences*, vol.1, no.1, pp.1-2, 2019
[4] N. Bhatt, M. I. Waly, M. M. Essa, A. Ali, "Ginger :A functional Herb," *Food Science and Nutrition*, pp. 5-20, 2013.
[5] S. A. Wadhwani, , M. Gorain, P. P. Banerjee, U. U. Shedbalkar, R. Singh, *et al.*,"Green synthesis of selenium nanoparticles using Acinetobacter sp. SW30: optimization, characterization and its anticancer activity in breast cancer cells,"*International Journal of Nanomedicine*, vol. 12, pp. 6841-6855, 2017.
[6] A. A. Ezhilarasi, J. J. Vijaya, K. Kaviyarasu, L. J.Kennedy, R. J. Ramalingam, H. A. Al-Lohedan," Green synthesis of NiO nanoparticles using Aegle marmelos leaf extract for the evaluation of *in-vitro* cytotoxicity antibacterial and photocatalytic properties," *Journal of Photochemistry and Photobiology B: Biology*, vol. 180, no. pp. 39-50, 2018.
[7] S. Rajeshkumar, S. Venkat Kumar, C. Malarkodi, M. Vanaja, K. Paulkumar, *et al.*, "Optimized Synthesis of Gold Nanoparticles using Green Chemical Process and its *In vitro* Anticancer Activity Against HepG2 and A549 Cell Lines, *Mechanics, Materials Science & Engineering Journal*, vol. 9, 2017.
[8] S. Verma, A. P. Srivastava, B. Singh, B. Tirumala Rao, *et al.*,"A facile synthesis of broad plasmon wavelength tunable silver nanoparticles in citrate aqueous solutions by laser ablation and light irradiation," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 527: pp. 23-33, 2017.
[9] S. M. Hosseinpour-Mashkani, M. Maddahfar, and A. Sobhani-Nasab," Novel silver-doped $NiTiO_3$: auto-combustion synthesis, characterization and photovoltaic measurements," *South African Journal of Chemistry*, vol. 70, pp. 44-48, 2017.
[10] H. G. Priyaa and K. B. Satyan,"Biological Synthesis of Silver Nanoparticles using Ginger (*Zingiber Officinale*) Extract," *J. Environ Nanotechnol*., vol. 3, no. 4, pp. 32-40, 2014.
[11] A. A. Chemists and W. Horwitz,"Official methods of analysis," vol. I,15th ed., *AOAC*, Arlington, VA, 1990.
[12] A. M. N. Jassim, G. M. Shafy, M. T. Mohammed, S. A. Farhan, and O. Mohammed Noori, "Antioxidant, Anti-Inflammatory and Wound Healing of Biosynthetic Gold Nanoparticles Using Mangosteen (G. Mangostona),"IJOIR, vol. 8, no. 2, pp. 59-74, Oct. 2021.
[13] R. Rajendran, R. Hemachander, T. Ezhilarasan, C. Keerthana, D. L. Saroja, K. V. Saichand, *et al.*, "Phytochemical Analysis and *In-Vitro* Antioxidant Activity of Mimosa pudica Lin., Leaves," *Res. J. Pharm. Technol.*, vol. 3, no. 2, pp. 551-555, 2010.
[14] M. T. Mohammed and S. I. Abbas,"Antioxidant and Anti-Inflammatory Effect of Fruit Juice of Annona muricata L (Soursop) During Ischemia Reperfusion Injury in Rats," *Iraqi Academic Scientific Journal*,. vol. 15, no.1, 118-123, 2016.
[15] M. Begum,"Phytochemical and Pharmacological Investigation of Carica papaya Leaf,", PhD, East west University, Aftabanar, Dhaka, 2014.
[16] N. James, Millar & Jane C. Millar,. "Statistic and Chemometrics for analytical chemistry", 4th ed., Prentice Hall, 2000.
[17] V. L. Singleton and J. A. J. Rossi," Colorimetric of total phenolics with phosphomolybdic- phosphotungstic acid reagents, " *American journal of Endology and Yiticulture*, vol.16, pp.144-158, 1965.

[18] R. Badmanaban, P. CN, D. J. Sen, and P. Bibhuranjan, “Studies on leaf extracts of drug Punarnava by comparing its antioxidant potential by spectrophotometric method,” Res. J. Pharm. Technol., vol. 3, no. 2, pp. 566-569, 2010.
[19] M. T. Abdo, H. A. Gad, S. H. El-Ahmady, and M. M. Al-Azizi," Quality Assessment methods for Ginger (*Zingiber officinale*): A review," *Archives of Pharmaceutical Sciences Ain Shams University*, vol. 2, no. 2, pp.87-96, 2018 .
[20] Y. Nan, L. Fuyan, J. Tiancai, L. Chongchong, S. Hushan, W. Lei, *et al.*," Biogenic synthesis of silver nanoparticles using ginger (*Zingiber officinale*) extract and their antibacterial properties against aquatic pathogens," *Acta Oceanologica Sinica*, vol. 36, no.12, pp.95-100, 2017 .
[21] A.M.N. Jassim, , S.A. Farhan and R.M. Dadoosh,"Green synthesis of silver nanoparticles using seed aqueous extract of *Abelmoschus esculentus* and study of their properties," *Advances in Environmental Biology*, vol. 10, no.4, pp. 51-67, 2016.
[22] M. A. Al-Alwani, A. B. Mohamad , A. H. Kadhum , and N. A. Ludin," Effect of solvents on the extraction of natural pigments and adsorption onto TiO_2 for dye-sensitized solar cell applications," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol.138: pp. 130-137, 2015.
[23] K. Khoshnevisan and M. Barkhi," Information about Zeta Potential," *Institute of Agricultural Biotechnology*, , pp.7-35, 2015.
[24] A. Roy and S. Saraf, “Antioxidant and antiulcer activities of an Ethnomedicine: Alternanthera sessilis,” *Res. J. Pharm. Technol.*, vol. 1, no. 2, pp. 75-79, 2008.
[25] M. C. Gunde, P. B. Suruse, N. D. Amnerkar, R. W. Lohe, M. D. Godbole, M. K. Kale, *et al.*, “Antioxidant Activity of Tubers of Arisaema Leschenaultii Blume,” Res. J. Pharm. Technol., vol. 2, no. 1, pp. 134-137, 2009.
[26] S. Sida, R. S. Samakradhamrongthai, and N. Utama-ang," Influence of Maturity and Drying Temperature on Antioxidant Activity and Chemical Compositions in Ginger," *Current Applied Science and Technology*, vol.19, no.1,pp. 30-38, 2019.
[27] V.I. Borekar, A. P. Somkuwar, R. P. Limsay, N. V. Kurkure, and S. W. Bonde," Evaluation of active components from ethanolic extract of Zingiber officinale Rhizomes," *Journal of Pharmacognosy and Phytochemistry*, vol. 7, no. 4, pp. 2781-2782, 2018.
[28] K. Barman, D. Chowdhury, and P. K. Baruah," Biosynthesized silver nanoparticles using *Zingiber officinale* rhizome extract as efficient catalyst for the degradation of environmental pollutants," *Inorganic and Nano-Metal Chemistry*, vol. 50, no. 2, pp. 57-65, 2020.
[29] S. Noori, F. Zeynali ,and H. Almasi," Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (*Zingiber officinale*) essential oil and its effect on safety and quality attributes of chicken breast fillets," *Elsevier*, vol. 84, pp.312-320, 2018.
[30] H. K. Shareef , H. J. Muhammed , H. M. Hussein, and I. H. Hameed," Antibacterial Effect of Ginger (*Zingiber officinale*)Roscoe and Bioactive Chemical Analysis using Gas Chromatography Mass Spectrum ," *Oriental Journal of Chemistry*, vol. 32, No. 2, pp.817-837, 2016.
[31] M. Balakumaran, R. Ramachandran, P. Balashanmugam, D. J. Mukeshkumar, and P.T. Kalaichelvan," Mycosynthesis of silver and gold nanoparticles: optimization, characterization and antimicrobial activity against human pathogens," *Microbiological research*, vol. 182, pp. 8-20. 2016.
[32] S. Iravani," Green synthesis of metal nanoparticles using plants," *Green Chemistry*, vol.13, no.10, pp. 2638-2650, 2011.
[33] C. Krishnaraj, R. Ramachandran, K. Mohan, and P. T. Kalaichelvan, " Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 93: pp. 95-99, 2012.
[34] B. Sadeghi and F. Gholamhoseinpoor," A study on the stability and green synthesis of silver nanoparticles using Ziziphora tenuior (Zt) extract at room temperature," *Spectrochimica Acta Part A:Molecular and Biomolecular Spectroscopy*, vol. 134, pp.310-315, 2015.
[35] G. Dinda, D. Halder, A. Mitra, N. Pal, and D. K. Chattoraj," Phytosynthesis of silver nanoparticles using(*Zingiber officinale*) extract: evaluation of Their catalytic and antibacterial activities," *Journal of Dispersion Science and Technology*, vol.41, no. 14, pp. 2128-2135, 2020.
[36] S. Megarajan, K. B. A. Ahmed, G.R.K. Reddy, P.S. Kumar, V. Anbazhagan, "Phytoproteins in green leaves as building blocks for photosynthesis of gold nanoparticles: an efficient electrocatalyst towards the

oxidation of ascorbic acid and the reduction of hydrogen peroxide," *J. Photochem. Photobiol.*, vol. 155, pp. 7-12, 2016.
[37] B. Kyeyune," Atomic Force Microscopy, Lappeenranta University Of Technology, Finland, vol. 10, 2017.
[38] J. U. Chandirika and G. Annadurai," Biosynthesis and Characterization of Silver Nanoparticles Using Leaf Extract Abutilon indicum," *Global Journal of Biotechnology & Biochemistry*, vol. 13, no. 1, pp. 7-11, 2018.
[39] A. Rohaizad, Z. A. Mohd Hir, U. A. M. Kamal, Z. Aspanut, and A. A. Pam," Biosynthesis of silver nanoparticles using Allium sativum extract assisted by solar irradiation in a composite with graphene oxide as potent adsorbents," *Results in Chemistry*, vol. 5, (100731), 2023.
[40] U. B. Jagtap and V. A. Bapat," Green synthesis of silver nanoparticles using Artocarpus heterophyllus Lam. seed extract and its antibacterial activity," *Industrial Crops and Products*, vol. 46, pp.132-137, 2012.
[41] M. A. Awad, A. A. Hendi, K. M. O. Ortashi, D. F. A. Elradi, N. E. Eisa, L. A. Al-lahieb, et al.," Silver nanoparticles biogenic synthesized using an orange peel extract and their use as an anti-bacterial agent," *Int. J. Phys. Sci.*, vol. 9, no. 3, pp. 34-40, 2014.
[42] T. Okubo and M. Suda," Absorption of Polyelectrolytes on Colloidal Surfaces as Studied by Electrophoretic and Dynamic Light-Scattering Techniques.,"*Journal of colloid and interface science*, vol. 213, no.2, pp.565-571, 1999.
[43] D. Huang, B. Ou, and R. L. Prior," The chemistry behind antioxidant capacity assays," *Journal of agricultural and food chemistry*, vol. 53, no. 6, pp. 1841-1856, 2005 .
[44] S. Rajakannu, S. Shankar, S. Perumal, S. Subramanian, and G. P. Dhakshinamoorthy," Biosynthesis of Silver Nanoparticles using Garcinia mangostana Fruit Extract and their Antibacterial, Antioxidant Activity," *Int. J. Curr. Microbiol. App. Sci*, vol. 4, pp. 944-952, 2015.
[45] M. Nurmahani, A.Osman, A. A. Hamid, F. M. Ghazali, and M. P. Dek," Antibacterial property of Hylocereus polyrhizus and Hylocereus undatus peel extracts," *International Food Research Journal*, vol. 19, no. 1, pp. 77-84, 2012.
[46] W. S. Choo and W. K. Yong," Antioxidant properties of two species of Hylocereus fruits," *Advances in Applied Science Research*, vol. 2, no. 3, pp. 418-425, 2011.