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Evaluation of Phytochemical, Total phenolic and Antioxidant Activity of *Carica Papaya* Seed for Its Use in Biosynthesis of Gold Nanoparticles

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Abstract

Synthesis of nanoparticles using green methods with study of their propertie is one of great researchers' concerns in fields of pharmaceutical and biomedical products. The present paper aiming to use of an eco-friendly route is used for biosynthesis of gold nanoparticles (AuNPs) using *C. Papaya* seeds as both reducing and stabilizing agent. Study of *C. Papaya* seed contents showed many compounds which analyzed via qualitative, quantitative and GC-MS analysis. The influence of *C. Papaya* concentration, contact time and temperature on the reaction rate and shape of AuNPs are confirmed

The biosynthesized NPs are characterize via UV-Vis, AFM and SEM. The AFM and SEM analysis exhibit a size between 40-105nm with average diameter 75.68nm, and spherical in structure. The antioxidant activity and free radical scavenge activity are studied by using TLC and DPPH analysis. The results show that the *C. Papaya* seeds and AuNPs have potent antioxidant activity; moreover, the total phenolic contents are characterized. The data confirmed that the *C. Papaya* seed extract was a good bio-reductant way for AuNPs that can be applied as a promising field in different bio-applications.

Keywords: *C. Papaya* seed, GC-Mass, Total phenolic contents, Antioxidant, Gold nanoparticles ;

1. Introduction

The term "Nanotechnology" includes the manufacture, characterization and/or manipulation of components that have approximately 1-100 nm in length in one of its dimension. When particle size is decreased lower than this dimension, the resulting materials appear chemical and physical properties differ greatly from macro scale components [1]. In recent years, Nanoparticles (NPs) are investigated in various fields which consist of healthcare, environment, chemical production, makeup, electronics, chemical manufacturing, water management, catalyst, mechanics, optics, sensors [2]. Presently, the request for NPs was assembly by combination during biological, physical and chemical techniques [3]. These NPs in colloidal forms are extra appropriate for biological uses since the formula do not include any hazard chemicals. While, careful selection is essential in this situation to find out the plant whose extracts contain excellent reducing with stabilize influence [4].

One of such plant is *C. Papaya*, a tropical fruit, member of the family Caricaceae, frequently shown as yellow-green, orange-red and yellow-orange shapes with a wealthy orange pulp. Entire parts of *C. Papaya* , bark , roots, fruits, seeds, peel and pulp defined as containing a medicinal characteristic [5, 6]. It is a tropical plant with large number of dietary antioxidants (tocopherols, total phenols, vitamin C, and β-carotene), also, a potent phyto-components with antioxidant activity (benzyl isothiocyanate) [7, 8]. Panzarini *et al.* indicated that *C. Papaya* seed water extract is beneficial for protection towered oxidative stress. Also, *C. Papaya* seed contains some functional properties that may act as antimicrobial and antiparasitic factors, and immuno-modulatory and anti-inflammatory agents. In addition, *C. Papaya* seed has been used for decades in parts of Asia and

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South America as a vermifugal agent, and in folk medicine because of its abortive features, also used as menstrual flow. Furthermore, because of spicy flavor in *C. Papaya* seed, it can be used to adulterate black pepper [8].

However, many workers deals and characterized the composition of *C. Papaya* seed. These seeds are abundant in lipids (28.2 to 30.7%), in proteins (27.3 to 28.3%), and in crude fibers (19.1 to 22.6%). Moreover, considerable amounts of phosphorus and calcium in *C. Papaya* seeds; Also, existence of toxicants, like glucosinolates, was also distinguish [6, 9]. Masson *et al.* [10] identified many of bioactive components and fatty acid in the oil extracted from *C. Papaya* seed. Fartheremore, Kadiri *et al*. [11] indicated that *C. Papaya* seed contain phenolic compounds like *p*-coumaric acid, caffeic acid, ferulic acid, quercetin-3-galactoside, *p*-hydroxybenzoic acid, and kaempferol-3-glucoside. Also, Salla *et al*. [12] indicated that *C. Papaya* seed is abundant in saponins, flavonoids, alkaloids, tannins and polyphenolic compounds. These phenolic compounds are contributed in the defense toward oxidative species damage and in protection against reactive oxygen species (ROS) [13]. The main scavenging potential of phenolic compounds is due to the existence of OH groups [14]. Also, a rapid, biosynthesized method deals with extraction of plan has progresses an interesting manner in AuNPs synthesis due to green chemistry approach. Furthermore, it is simple, cost-effective, eco-friendly, easily scaled up for large-scale synthesis.

Thus, our study was conducted to estimated the physicochemical properties, assessing the antioxidant potential, total phenolic components of the *C. Papaya* seed and synthesis, characterize the AuNPs using these seeds without external addition of capping agent, surfactant or reducing agent, also, aiming to investigate the influence of various conditions to producing AuNPs.

2. Experimental

Preparation of *C. Papaya* Seed

The *C. Papaya* seed was obtained from fresh fruit. In round bottom flask with condenser, 2 g of seeds were mixed with 100 mL distilled water .The solution was stirred at magnetic stirrer for 2 hours. The extract was filtered with a piece of gauze to remove any residues and Whatman No.1 filter paper to obtained clear extract and filtrate solution was centrifuged for 10 min. at 2500 rpm. Later, seed extract was kept frozen at -20ºC until the time of use [7].

Quantitative and Qualitative Estimation of Phytochemical

The extract of seed was phytochemical quantitative and qualitative estimated for primary and secondary metabolites. The extract was detected for glycosides, alkaloids, saponins, phenolic compounds, tannins, resins, flavonoids, proteins, ash, protein, fats, employed to each standard method. The *C. Papaya* seed was sent to Nutrition Research Institute to determining the seeds components [7, 15].

GC-Mass Analysis

Two milliliter of the methanolic seed extract prepared by mixing 0.2 g of seed with 10 mL methanol in round bottom flask with condenser. The solution was stirred at magnetic stirrer for 2 hours. Later, the extract was filtered by Whatman No.1 filter paper and the filtrated solution was centrifuged for 10 min. at 2500 rpm. The obtained methanol portion was collected, and then concentrated by evaporation through vacuum (at 55 ºC). The seed extract was analyzed using GC-MS (QP2010Ultra, Shimadzu Co., Kyoto, Japan) [15].

Total Phenolic Contents

A standard graph for Gallic acid between 50-250 $\mu g.mL^{-1}$ in methanol were prepared via dilution 25, 50, 75, 100 and 125µL of 5000 $\mu g.mL^{-1}$ stock standard solution to 2.5 mL [16]. The regression of equation [Y=0.0035x+0.494, where slope=0.0035, intercept= 0.494, X= concentration and Y=absorbance] was achieved using Least Squares Method [17]. Also standard solutions of *C. Papaya* seed extract between 50-250 $\mu g.mL^{-1}$ in methanol were prepared through dilution 25, 50, 75,100 and 125 µL of 5000 $\mu g.mL^{-1}$ stock standard solution to 2.5 mL. The total phenolic contents in the *C. Papaya* seed extract were determined through Folin–Ciocalteau reagent illustrated by Singleton & Rossi with little adjustments [18]. The absorbance was determined at 765 nm by UV–Vis spectrophotometer. Also, the contents of total phenolic compounds were identified as Gallic acid which equivalent (GAE) in milligram per gram initial of sample with standard calibration curve.

Biosynthesis of AuNPs

Five milliliter of seed extract has been added to 5mL of 1mM aqueous $HAuCl_4$ solution in a test Tube. The solution was heating (80 ºC) for reduction of Au^{+3} to Au^0. Bio-reduction of gold ions was periodically detected using the UV–Vis spectrophotometer. Reduction of Tetrachloroaurate into AuNPs was established by gradually color exchange from yellowish ($HAuCl_4$ + Seeds extract Solution) to purple, then pink.

Characterization of AuNPs

UV-Vis Analysis

The UV–Vis analysis was done via spectrophotometer to monitoring the reduction of pure Au^{+3} ions as a spectrum of the reaction medium at room temperature with 1nm resolution (spectrum between 190 and 900 nm) and used of deionized water as the blank..

AFM Analysis

Atomic Force Microscope (AFM) has been employed to measure the AuNPs size and size distributions. To preparing AFM samples from suspension solution, Droplet-evaporation procedure was used. One drop was deposited on glass cover slide (2x2 cm^2) from AuNPs. Then, the sample was dried before the measurement [7, 15].

SEM Analysis

Scanning electron microscopy (SEM) was used for identify the biosynthesized AuNPs shape, morphologies and topography.

Antioxidant Activity

A-Qualitative Assay of Free Radical via TLC Method

Antioxidant component was analyzed via thin layer chromatography (TLC) followed by DPPH. A100 µg of a *C. papaya* Seed extract, AuNPs and Gallic acid as standard solution were placed on TLC plates. The active antioxidant ingredients showed yellow spots versus a violet background [4, 19].

B-Quantitative Assay of Free Radical via DPPH Method

The *C. Papaya* seed extract scavenging activity via DPPH method was tested *in vitro* [4, 20]. The Gallic acid was use as a standard. The quantities of sample needed to reduce DPPH concentration by 50% signify to IC_{50}. The scavenging activity was estimated graphically via:

% DPPH scavenging activity=[1- (Absorbance of test or standard /Absorbance of control)]x100

Antimicrobial Activity:

The biosynthesized AuNPs from *C. Papaya* seed was tested for their antimicrobial activity by well diffusion method toward pathogenic organisms including: *Escherichia coli* (*E. coli*), *Pseudomonas aurius* (*P. aureus*) *Klebsiella pneumonia* (*k. pneumoniae*) and *Staphylococcus aureus* (*S. aureus*). Using micropipette, the samples [No.1 = AuNPs (measured by AAS) equal to 3.8085 ppm, No.2 =dilution of AuNPs 1:1, No.3 =*C. papaya* Seed extract], respectively, were made using serial dilution of stock solution, then transferred into plate wells, and incubated. After that, zone of inhibition were measured in millimeter [7, 15].

3. Results and Discussions

Phytochemical Constituents of *C. Papaya* Seed:

The qualitative chemical analysis of *C. Papaya* seed was shown in Table 1, which indicated that seed components are (carbohydrate, proteins, phenolic compounds, tannins, resins, flavonoids and alkaloids).

Other studies showed that seed of papaya which indicated the presence of carbohydrate, glycosides, proteins, phenolic compounds, alkaloids, terpenoids, flavonoids, saponins and steroids in *C. Papaya* seed [8, 21]. Nevertheless, the *C. Papaya* seeds were used in Asia and South America, for decades as a vermifugal agent and its preparations have been used in folk medicine due to its abortive properties and to favor a good menstrual flow [9]. The phytochemical quantitative contents of *C. Papaya* seed are shown in Table 2.

The data in agree with other study indicated that the plant rich in many substances, including crude proteins, fats, tocopherol, carotenoid, fiber, carbohydrates and ash [21, 22]. Furthermore, Ocloo *et al* [23] indicated the existence of phenols, flavonoids, tannins, alkaloids, saponins, reducing sugars, and terpenoids in aqueous and organic extract of dried *C. Papaya* seed. These materials can be used as potent components to biosynthesized AuNPs

Composition (g/100g)	Percentage %
Moisture	6.37 %
Protein	30.58 %
Fat	23.79 %
Ash	8.44 %
Fiber	10.06 %
Carbohydrate C.H.O	20.78 %

Table 1: Chemical components (qualitative analysis) of *C. Papaya* seed

Table 2: Chemical components (quantitative analysis) of *C. Papaya* seed

Components	Reagents	Result	Note
Carbohydrate	Molish test Iodine test Benedict test	+Ve -Ve +Ve	-Purple ring separate between two Layer -Green ppt with blue sol.
Phenolic compounds	Ferric chloride $FeCl_3$	+Ve	Brawn ppt
Tannins	Lead acetate 0.1%	+Ve	Yellow ppt
Resins	EtOH	-Ve	-
Flavonoids	EtOH + KOH	-Ve	-
Alkaloids	Picric acid	+Ve	Yellow ppt
Protein	Biurat	+Ve	Turbidity Sol.
Fatty Acid	Cupper Acetate	-Ve	-
Saponins	Fasting Sterring	+Ve	Low foam for Few Sec.
Amino acids	Ninhydrin	+Ve	Dark Blue Sol.

Table 3: Name, molecular formula, and retention time of major components in methanol fraction of *C. Papaya* seed via GC-MS

Name of the compound	Formula	Peak	R. Time
Formamide	$C_2H_5NO_2$	1	3.283
Ethoxyacetaldehyde	$C_8H_{18}O_3$		
Methoxyacetaldehyde	$C_7H_{16}O_3$		
Ethyl 2-oxopropyl sulfide	$C_5H_{10}OS$		
Tetradecanoic acid	$C_{19}H_{36}O_2$	2	19.375
11-Dodecen-1-ol trifluoroacetate	$C_{14}H_{23}F_3O_2$	3	21.208
Octadecanoic acid	$C_{18}H_{36}O_2$	4	21.367
Palmitin, 1,2-di-, 2-aminoethyl hydrogen	$C_{19}H_{38}O4$	5	22.117
Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{37}H_{74}NO_8P$	6	22.542
2-Hydroxy-1-(hydroxymethyl)ethyl pentadecanoate	$C_{23}H_{46}O_4$		
2-Hydroxy-1-(hydroxymethyl)ethyl icosanoate	$C_{18}H_{36}O_4$		
15-Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$		
7-Tetradecenal	$C_{14}H_{26}O$	7	24.108
9-Octadecenoic acid, 1,2,3-propanetriyl ester	$C_{57}H_{104}O_6$	8	25.867
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$		
Thunbergol	$C_{20}H_{34}O$	9	26.842
9,19-Cyclo-9.beta.-lanost-24-en-3.beta.-ol, acetate	$C_{32}H_{52}O_2$		
9,19-Cyclo-5.alpha.,9.beta.-ergost-24(28)-en-3.beta.-ol	$C_{30}H_{50}O$		
Cyclododecene epoxide	$C_{12}H_{22}O$	12	29.417

GC-MS Analysis

The analysis of GC-MS for methanol fraction indicated presence of many components in *C. Papaya* seed. The main components identified in *C. Papaya* seed, summarized in Table 3.

The GC-MS analysis of *C. Papaya* seed showed several components which provide the potential reducing action of this plant via existence of these components, which have different functional groups such as OH, NH, CO, COOH, C-S…etc, working as reducing and capping of AuNPs biosynthesis; it can be responsible for long time stability (Figure 1). The data obviously showed that the seed extract of *C. Papaya* contain abundant of phenolic compounds, antioxidants, saturated fatty acid , unsaturated fatty acid, terpenes, and others, which in agreement with other studies [8, 9, 21], documented that the *C. Papaya* contains a different antioxidant, phenolic compound, terpenes, tocopherol, carotenoid and others. Other peaks remained unidentified of the GC-MS, since that absence of authentic specimen and library document in GC-MS device for other compounds.

Total Phenolic Contents

The total phenolic contents of *C. Papaya* seed in the methanolic portions was measured via folin-ciocalteu reagent and express as Gallic acid equivalents for each gram of *C. Papaya* seed extract using standard Gallic acid graph, Table 4.

In the Table 4, the total phenolic content for *C. Papaya* seed extract was shown. It is clear from the data that the total phenolic content of seed extract found in high concentration and it may be involved closely operative antioxidant activity and in the reduction of AuNPs. Our data indicated that the plant possesses antioxidant activities which can counteract the oxidative damage. The total phenolic analysis provides information on the reactivity of plant extract with a stable free radical.

The *C. Papaya* seed extract contain different phenolic compounds. The amounts of phenolic compounds in various organs of *C. Papaya* seed extract varied depending on solvent type, extraction technique, maturity of plant and the drying process. The presence of phenolic compounds constitutes a major group of compounds that act as primary antioxidants which are mainly responsible for the reducing property of the extract [8, 24]. In accordance with our research, Kadiri *et al*. [25] indicated that the seed of Papaya contains many components including phenolic compounds such as *p*-coumaric acid, ferulic acid, quercetin-3-galactoside, kaempferol-3-glucoside, *p*-hydroxybenzoic acid, and caffeic acid.

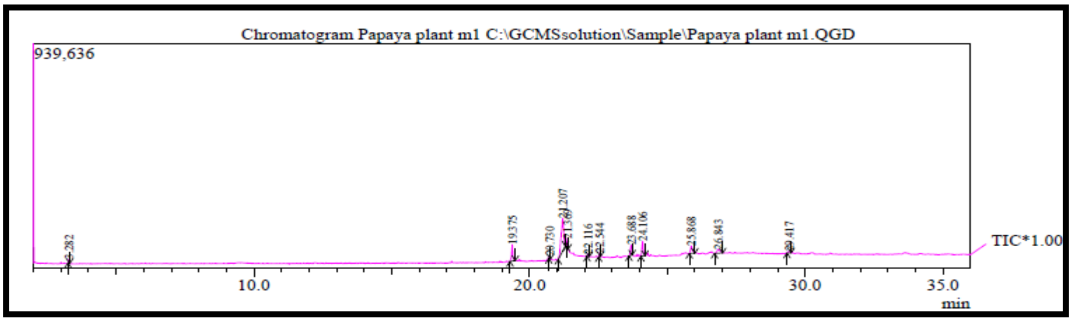

Figure 1: Chromatogram of *C. Papaya* seed of methanolic fraction via GC-MS

Table 4: Total phenolic contents for *C. Papaya* seed extract and standard Gallic acid

Absorption of *C. Papaya* seed extract	Absorption of standard Gallic acid	Concentration (µg/mL)	The equation for standard Gallic acid	Total phenolic contents of *C. Papaya* seed extract in (mg/g)
1.327	1.345	250	Y=0.0035x+0.494 R^2 = 0.9403	238.45
1.320	1.139	200		236.45
1.317	1.125	150		235.60
1.312	0.869	100		234.17
1.310	0.606	50		233.60

Biosynthesis of AgNPs

Biosynthesis supply a preferment over other methods (physical and chemical) like it is environmentally eco-friendly, cost effective, clearly scaled up with wide-ranging of synthesis. Indeed, in our procedure, it's not requiring to use high temperature, pressure, toxic chemicals and energy [26]. Plants are mainly valuable metabolites that are capable to reducing metal ions to produce the NPs. The *C. Papaya* seed components are added in order to react with aqueous ($HAuCl_4$) solution to produce Au^o as NPs.

Characterization of AuNPs

Monitoring of bio-reduction process of gold ions in the solution to form AuNPs was done through scanning the UV-Vis spectroscopy of the solutions as documented in Figure 2.

Different parameters were optimized (Results not shown) including temperature (80^{o}C), time (20min), concentration of $HAuCl_4$ (1mM), concentration ratio of *C. Papaya* seeds extract (0.1mL) to $HAuCl_4$ (1mL) , which had been characterized as a main factor affecting on quantity of AuNPs. Reduction of gold ions to AuNPs was established by the color exchange of the colorless $HAuCl_4$ to form purple, mat purple, then dark purple AuNPs. The biosynthesizes AuNPs have

maximum absorbance at near 538 nm. The solutions were then stored at 4C° in dark glass bottles to other use.
The AFM technique was used to identify the AuNPs size, size distributions and morphology. Evaporation method of a drop was used to prepare AFM samples in liquid suspension. The surface morphology for the particles sizes with irregularly shaped as well as the size distribution of biosynthesized AuNPs using *C. Papaya* seed extract are elucidate in Figure 3, which indicate that average size equal to 24.66 nm.

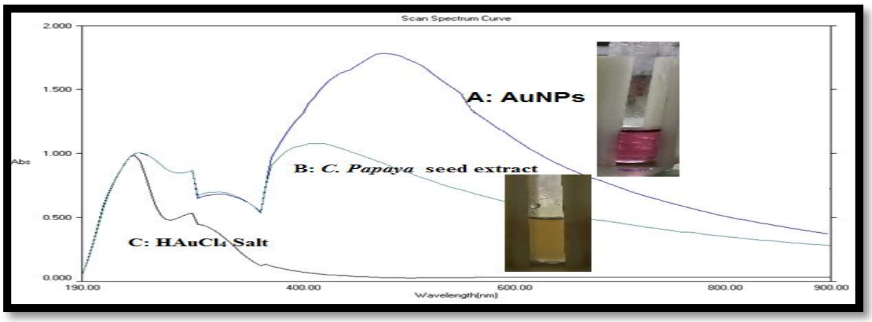

Figure 2: UV–Vis absorption spectra of A- AuNPs, B- *C. Papaya* seed extract, C- $HAuCl_4$ Salt Solution.

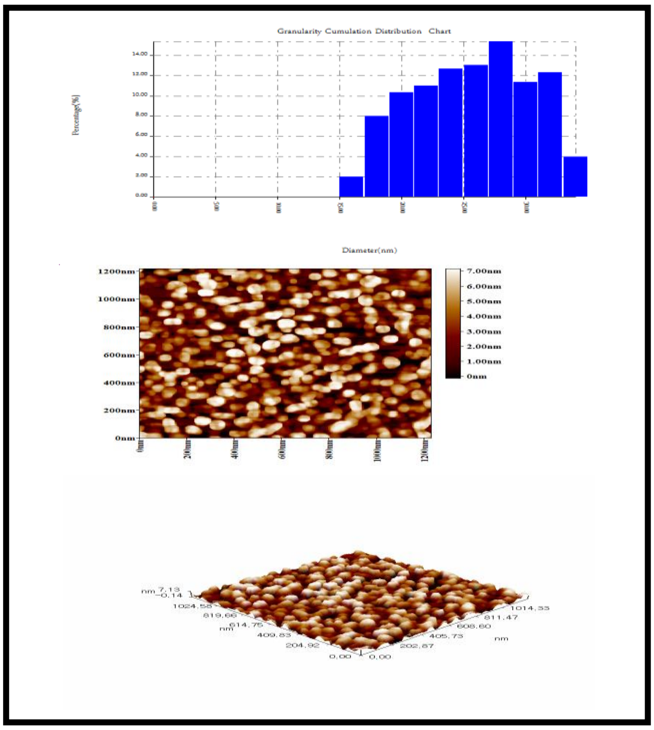

Figure 3: AFM images (2D & 3D), size distributions of biosynthesized AuNPs

The AuNPs concentration was featured by atomic absorption spectrometry. Calibration curve for gold standard solution was made, and then the absorption values of the above corresponding samples were estimated. The Concentration of AuNPs calculated was 3.8085ppm.

The SEM is exhibit in Figure 4, A-L is used to characterize the structure as well as morphology of AuNPs to present the characteristic of biosynthesized AuNPs obtained from *C. Papaya* seed extract, the image indicated relatively spherical in shape for these NPs with the coating materials from *C. Papaya* seed, and a diameter ranging from 71-92 nm (as shown in Figure 4, L).
The relatively spherical shaped AuNPs with a different diameter range using SEM which synthesized were also indicated in previous study using *Boswellia* [27]; *Shorea tumbuggaia* [28]; *Aloe vera* extract [29]; *C. Papaya* peel extract [30] and orange peel extract [31].

Antioxidant Activity (Qualitative and Quantitative estimation)

The main potential antioxidant mechanism in foods is radical scavenge activity. Therefore, many process was proceed in which the antioxidant activity was estimated by scavenging of radicals in a methanol as a polar organic solvents [32].

The antioxidant activities of *C. Papaya* seed extract and AuNPs were determined using free radical scavenging activity method which the color alter from purple to yellow as the DPPH radical reduces, because of translate the acidic H-atom from the molecules to DPPH radical to produce the DPPH-H. In this study, DPPH reagent was used as a TLC spray. So, *C. Papaya* seed extract, AuNPs and standard Gallic Acid; seems as clear yellow spots against a purple background; indicate that they have a good scavenging activity, as shown in Figure 5. Furthermore, the DPPH method was used to study the quantitative scavenging activity for these samples, as illustrated in Table 5.

Table 5: The inhibition percent of (A) *C. Papaya* seed extract (B) AuNPs (C) standard Gallic Acid at different concentrations (µg/mL) by DPPH model.

Concentration (µg/ml)	% Inhibition (C-Papaya seed extract)	% Inhibition (AuNPs)	% Inhibition (standard solution-Gallic acid)
10	14.40	14.90	43.2
20	14.70	15.80	46.5
40	17.90	18.51	53.5
60	24.01	26.02	59.6
80	30.34	32.01	62.3
100	31.60	35.81	82.5

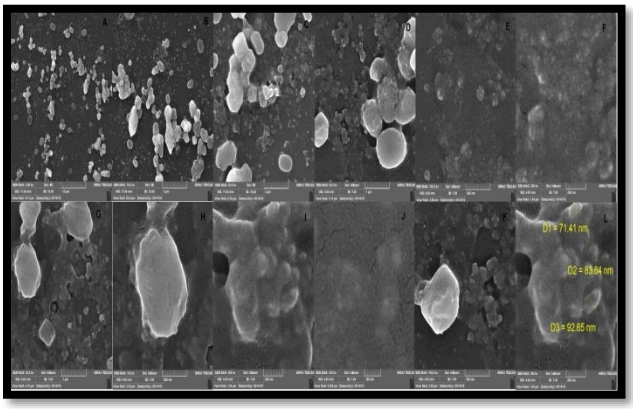

Figure 4: SEM images of biosynthesized AuNPs (A=10µm, B=5µm, C=2µm, D=1µm, E=500nm, F=200nm, G=1µm, H=500nm, I=200nm, J=100nm, K=500nm, L=200nm)

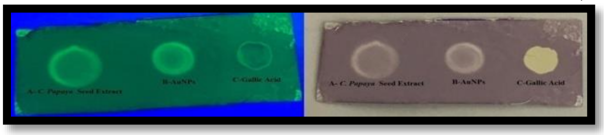

Figure 5: The TLC photo-image (Right) and under UV-light (Left) for (A) *C. Papaya* seed extract (B) AuNPs (C) standard Gallic Acid

The data in Figure 6, indicated that it has a potent scavenging activity for standard Gallic acid, with IC_{50} equal (28 µg\mL), while they were under IC_{50} for both *C. Papaya* seed extract and AuNPs as showed in Figures 7 and Figure 8, respectively.

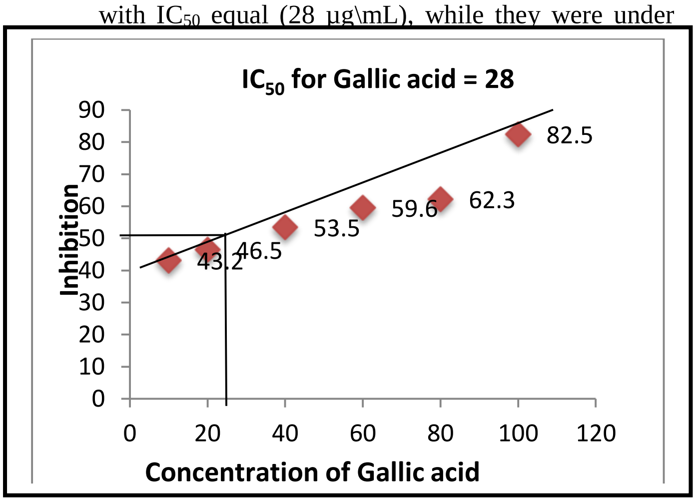

Figure 6: The DPPH free radical scavenging activity of standard Gallic acid

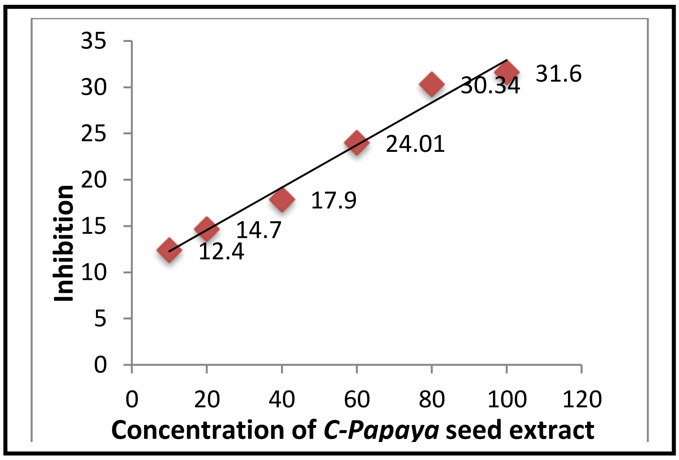

Figure 7: The DPPH free radical scavenging activity of *C-Papaya* seed extract

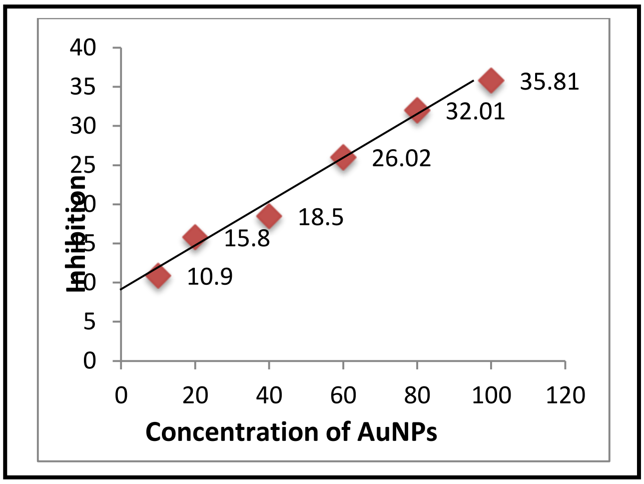

Figure 8: The DPPH free radical scavenging activity of AuNPs.

It's clear that the lesser the IC_{50}, the better it's able to scavenge radicals. In addition to these, AuNPs has more antioxidant activity than seed extract used to biosynthesized. In accordance, with earlier research by Kumar and Devi [33] showed that seed of Papaya plant have lycopene, made it more potent against free radicals. Also, *C. Papaya* seed have a quantity of β-carotene, Vitamin E, and Vitamin C, which have potent antioxidant activity [34]. In agreement with this, a study by Salla *et al*. [12] indicated that *C. Papaya* seed has a potent anti-oxidative activity. Furthermore, Maisarah *et al* [35] compare the antioxidant activity, phenolic content, and flavanoid content of various fraction of Papaya plant including leaves, seed and fruit. It is clear that increasing of total phenolic compounds and total flavanoids provide their antioxidant potential. So, we can conclude that biosynthesized AuNPs and seed extract have a good scavenging activity which can be using as antibacterial, anti-inflammatory and Antioxidant.

Antimicrobial Activity by Well Diffusion Method

Antimicrobial activity was shown in Figure 9. Inhibition zone in plates contain nutrient agar toward *E. coli*, *P. aureus*, *k. pneumoniae* and *S. aureus* as a function of amount of AuNPs and *C. papaya* seed extract. The results demonstrated that gram positive, gram negative bacteria and fungi had low effected by different solution with different extents.

Figure 9: Antimicrobial activity of AuNPs (1), AuNPs diluted (1:1) (2) and *C. Papaya* seed extract (3) against *E. coli, P. aureus, k. pneumoniae* , *S. aureus* and *Candida*

Many other studies have been showed antimicrobial activities of *C. Papaya* seed. In accordance with this, Ocloo *et al* [23] estimated the activity of antibacterial against *S. aureus*, *E. coli* and *S. flexneri* using the disc diffusion method. Indeed, Muhamad *et al*. [36] indicated the antibacterial properties of *C. Papaya* seed extract toward *B. cereus*, *V. vulnificus*, *P. mirabilis*, and *S. enteritidis*. Also, Okoye [37] has been estimated antibacterial and antifungal activity of ethanolic *C. Papaya* extract toward four various bacteria and fungi. The four estimated bacteria are *P. aeruginosa*, *S. aureus*, *S. typhi* and *E. coli*. The four estimated fungi are *Aspergillus Niger*, *Penicillium notatum*, *fusarium solani* and *candida albicans*. In accordance with our work, Peter *et al*. [38] illustrated the efficacy of *C. Papaya* seed against *S. aureus*, *P.aeruginosa*, *E. coli*, and *S. typhi*. Furthermore, Hidayati *et al*. [39] showed the antibacterial activity of *C. Papaya* seed against *S. typhi* and *E. coli*. In accordance with this, Masfufatun *et al*. [40] illustrated the activities of *C. Papaya* seed toward *Vibrio cholerae* and opportunistic pathogenic yeast *Candida albicans*. The earlier study by Singh and Ali [41], indicated the antifungal activity of *C. Papaya* seed against *A. flavus, C. albicans,* and *P. citrinum*.

4. Conclusion

The *C. Papaya* seed was abundant in antioxidants, total phenolic contents, terpenes, unsaturated fatty acid and other components which identified by qualitative, quantitative and GC-MS analysis. This study established that the *C. Papaya* seed was a useful bio-reductant for the biosynthesized of AuNPs because this seed contains many of these components. The biosynthesized AuNPs were confirmed by UV–Vis, AFM and SEM. the results confirm that biosynthesized AuNPs has a higher antioxidant activity compared to *C. Papaya* seeds extract alone, which can be effective use as promising agents in different bio-applications

5. Acknowledgements:

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6. Conflicts of interest

There are no conflicts to declare

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