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 properties 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 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. Farthermore, 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-glucose. 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 seed extract were determined through Folin–Ciocalteau reagent illustrated by Singleton & Rossi Method [17]. Also standard solutions of C. Papaya seed extract between 50–250 μg.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. 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.

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].

Total Phenolic Contents

A standard graph for Gallic acid between 50-250 μg.mL⁻¹ in methanol were prepared via dilution 25, 50, 75, 100 and 125μL of 5000 μg.mL⁻¹ stock 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. Also standard solutions of C. Papaya seed extract between 50-250 μg.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 HAuCl₄ solution in a test Tube. The solution was heating (80 °C) for reduction of Au³⁺ to Au⁰. Bio-reduction of gold ions was

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 -20°C until the time of use [7].
periodically detected using the UV–Vis spectrophotometer. Reduction of Tetrachloroaurate into AuNPs was established by gradually color exchange from yellowish (HAuCl₄ + 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 cm²) 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 showned 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 IC₅₀. 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

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

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

<table>
<thead>
<tr>
<th>Components</th>
<th>Reagents</th>
<th>Result</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>Molish test, Iodine test</td>
<td>+Ve</td>
<td>-Purple ring separate between two Layer</td>
</tr>
<tr>
<td></td>
<td>Benedict test</td>
<td>-Ve</td>
<td>-Green ppt with blue sol.</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>FeCl₃</td>
<td>+Ve</td>
<td>Brawn ppt</td>
</tr>
<tr>
<td>Tamins</td>
<td>Lead acetate 0.1%</td>
<td>+Ve</td>
<td>Yellow ppt</td>
</tr>
<tr>
<td>Resins</td>
<td>EtOH</td>
<td>-Ve</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>EtOH + KOH</td>
<td>-Ve</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Picric acid</td>
<td>+Ve</td>
<td>Yellow ppt</td>
</tr>
<tr>
<td>Protein</td>
<td>Biurat</td>
<td>+Ve</td>
<td>Turbidity Sol.</td>
</tr>
<tr>
<td>Fatty Acid</td>
<td>Cupper Acetate</td>
<td>-Ve</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>Fasting Sterring</td>
<td>+Ve</td>
<td>Low foam for few Sec.</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Ninhydrin</td>
<td>+Ve</td>
<td>Dark Blue Sol.</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Name of the compound</th>
<th>Formula</th>
<th>Peak</th>
<th>R. Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formamide</td>
<td>C₂H₅NO₂</td>
<td>1</td>
<td>3.283</td>
</tr>
<tr>
<td>Ethoxyacetaldehyde</td>
<td>C₆H₁₂O₁</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methoxyacetaldehyde</td>
<td>C₆H₁₀O₁</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl 2-oxopropyl sulfide</td>
<td>C₃H₇OS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetradecanoic acid</td>
<td>C₁₀H₂₀O₂</td>
<td>2</td>
<td>19.375</td>
</tr>
<tr>
<td>11-Dodecen-1-ol trifluoroacetate</td>
<td>C₁₁H₂₁F₂O₂</td>
<td>3</td>
<td>21.208</td>
</tr>
<tr>
<td>Octadecanoic acid</td>
<td>C₁₈H₃₈O₂</td>
<td>4</td>
<td>21.367</td>
</tr>
<tr>
<td>Palmitin, 1,2-di- 2-arnoimethyl hydrogen</td>
<td>C₁₅H₂₉O₄</td>
<td>5</td>
<td>22.117</td>
</tr>
<tr>
<td>Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester</td>
<td>C₁₅H₂₀NO₄P</td>
<td>6</td>
<td>22.542</td>
</tr>
<tr>
<td>2-Hydroxy-1-(hydroxymethyl)ethyl pentadecanoate</td>
<td>C₂₀H₂₃O₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Hydroxy-1-(hydroxymethyl)ethyl isosanoate</td>
<td>C₂₀H₂₃O₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-Hydroxypentadecanoic acid</td>
<td>C₁₅H₂₃O₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Tetradecenal</td>
<td>C₁₆H₃₂O</td>
<td>7</td>
<td>24.108</td>
</tr>
<tr>
<td>9-Octadecenoic acid, 1,2,3-propanetriyl ester</td>
<td>C₁₆H₃₀O₆</td>
<td>8</td>
<td>25.867</td>
</tr>
<tr>
<td>9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester</td>
<td>C₁₆H₃₀O₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thunbergol</td>
<td>C₂₀H₂₃O₇</td>
<td>9</td>
<td>26.842</td>
</tr>
<tr>
<td>9,19-Cyclo-9.beta.-lanost-24-en-3.beta.-ol, acetate</td>
<td>C₂₀H₃₀O₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclododecene epoxide</td>
<td>C₂₀H₃₂O</td>
<td>12</td>
<td>29.417</td>
</tr>
</tbody>
</table>

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**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.

**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**

<table>
<thead>
<tr>
<th>Absorption of <em>C. Papaya</em> seed extract</th>
<th>Absorption of standard Gallic acid</th>
<th>Concentration (µg/mL)</th>
<th>The equation for standard Gallic acid</th>
<th>Total phenolic contents of <em>C. Papaya</em> seed extract in (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.327</td>
<td>1.345</td>
<td>250</td>
<td>Y=0.0035x+0.494</td>
<td>238.45</td>
</tr>
<tr>
<td>1.320</td>
<td>1.139</td>
<td>200</td>
<td>R² = 0.9403</td>
<td>236.45</td>
</tr>
<tr>
<td>1.317</td>
<td>1.125</td>
<td>150</td>
<td></td>
<td>235.60</td>
</tr>
<tr>
<td>1.312</td>
<td>0.869</td>
<td>100</td>
<td></td>
<td>234.17</td>
</tr>
<tr>
<td>1.310</td>
<td>0.606</td>
<td>50</td>
<td></td>
<td>233.60</td>
</tr>
</tbody>
</table>

**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 (HAuCl₄) solution to produce Au⁺ as NPs.

**Characterization of AuNPs**

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.

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°C), time (20min), concentration of HAuCl₄ (1mM), concentration ratio of *C. Papaya* seeds extract (0.1mL) to HAuCl₄ (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 HAuCl₄ 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 4°C 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- HAuCl₄ Salt Solution.](image)

The concentration of AuNPs calculated was 3.8085 ppm.

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).

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

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 relatively spherical shaped AuNPs with a different diameter range using SEM which synthesized were also indicated in previous study using Boswellia [27]; Shorea tumbugaga [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.

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

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 IC₅₀ equal (28 µg/mL), while they were under IC₅₀ 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
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](image)

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 Carica papaya seed against S. typhi and E. coli. In accordance with this, Masufatun et al. [40] illustrated the activities of Carica papaya seed toward Vibrio cholerae and opportunistic pathogenic yeast Candida albicans. The earlier study by Singh and Ali [41], indicated the antifungal activity of Carica papaya seed against A. flavus, C. albicans, and P. citrinum.

4. Conclusion

The Carica 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 Carica 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 Carica papaya seeds extract alone, which can be effective use as promising agents in different bio-applications

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6. Conflicts of interest

There are no conflicts to declare

References


