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Phytochemical Analysis and Chemical Characterization of Carica Papaya and Carica Papaya-Synthesized Magnesium Oxide Nanoparticles

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Abstract
Plants act as natural medicine from time immemorial. Natural medicines act as good substitutes and sometimes more potent substitutes to drugs especially antibiotics. The phytochemicals present in these plants are active compounds which help both in preventive and curative medicine. *Carica papaya* is a known fruit with health benefits. The leaves are no exception to its potency as a result of various phytochemicals present in it. Nanoparticles also show good promise in the fight against infectious diseases as they aid in drug delivery. However, biosynthesized nanoparticles show more promise as they are more effective, less-expensive and ecofriendly. This study shows the use of the leaves of *Carica papaya* in the synthesis of nanoparticles. The leaves of *Carica papaya* were extracted using methanol as a solvent. There was a percentage yield of 6.245%. The phytochemicals present in the leaves were evaluated both quantitatively and qualitatively. Alkaloids, tannins, flavonoids, steroids, terpenoids, saponnins and cardiac glycoside were present, while reducing sugar was absent. The quantitative evaluation of saponins, alkaloids, flavonoids, tannins show values of 7.8%, 3.6%, 3.4% and 4.3% respectively. The *Carica papaya*-synthesized magnesium oxide nanoparticles (MgONPs) were chemically evaluated with values of pH, absorption maxima, X-ray Flourescence Spectrometry (XRF), Atomic Absorption Spectroscopy (AAS), Fourier Transform Infrared (FTIR) and Scanning Electron Microscopy (SEM) put into consideration.

Keywords: *Carica Papaya*, Phytochemicals, Magnesium Oxide Nanoparticles, FTIR, SEM

Introduction

Medicinal herbs are age old sources of drugs (Alabi *et al.*, 2012) [1]. Various active compounds mainly secondary metabolites are synthesized by medicinal herbs. Carica papaya is a herbaceous plant whose fruits, leaves, seeds and latex are used medicinally. Traditionally, the leaf extract was used as a tonic for the heart, analgesia and treatment for stomach ache (Giove Nakazawa, (1996) [13]. Leaves extract contains folic acid, vitamins B12, A, and C, alkaloids, saponins, glycosides, tannins, and flavonoids. C. papaya plants have medicinal value due to the presence of natural metabolites found in leaf, bark, and twigs that possesses both anti-tumor and pesticidal properties (Basalingappa *et al.*, 2018) [6].

Nanotechnology refers to any technology that is implemented at the nanoscale and has actual applications. (Mostafa, 2020) [20]. A nanoparticle or ultrafine particle is usually defined as a particle of matter that is between 1 and 100 nanometres (nm) in diameter (Vert, *et al*, 2012). The advancements made in fields such as nanomedicine and nanotechnology have led to significant progress in the development of nanomaterials that can be used in medical-related applications such as therapeutic, diagnosis, and imaging (Teleanu *et al*., 2019) [25]. Nanomaterials bring unique properties that can overcome the disadvantages of conventional therapies, such as lack of specificity and high drug concentrations (Bae *et al*., 2011) [5]. As compared to microparticles, nanomaterials display superior intracellular uptake, which makes them suitable as vehicles for drug delivery (Faisal and Kumar, 2017, Teleanu *et al*., 2019 [25]). Moreover, due to their small sizes, nanomaterials have the potential to overcome the biological barriers.

Several methodologies are available for the synthesis of NPs namely, chemical methods, physical methods and biological methods. Biological synthesis of NPs from herbal extract and/or microorganisms has appeared as an alternative approach as these routes have several advantages over the chemical and physical methods of synthesis. It is also a well-established fact that these routes are simple, cost-effective, eco-friendly, and easily scaled up for high yields and or production (Siddiqi and Husen, 2016) [23]. Plants and their parts contain carbohydrates, fats, proteins, nucleic acids, pigments and several types of secondary

metabolites which act as reducing agents to produce nanoparticles from metal salts without producing any toxic by-product. (Durán *et al*., 2005) [7] Magnesium oxide is used for relief of heartburn and dyspepsia, as an antacid, magnesium supplement, and as a short-term laxative. It is also used to improve symptoms of indigestion. (Tan *et al*., 2013) [24]. Characterization of nanoparticles is vital for determination of the phase purity, shape, size, morphology, electronic transition plasmonic character, atomic environment and surface charge, etc. Number of techniques are used for this purpose, including UV-visible spectroscopy, Scanning Electron Microscopy (SEM), Fourier Transmission Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), and Dynamic Light Scattering (DLS). In this study, phytochemical investigation of C. papaya leaf is carried out, alongside biosynthesis of Magnesium oxide nanoparticle from the leaf and chemical characterization of the MgONPs.

Materials and Method

Sample Collection and Processing

The leaves of *Carica papaya* were obtained, proper identification and deposition of plants was conducted at the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Agulu, Anambra State. A voucher specimen was allocated in the herbarium of the Department of Pharmacognosy. The leaf of *C. papaya* was issued voucher number – PCG/474/C/023. The leaves of *C. papaya* was thoroughly rinsed, cut into small pieces, dried completely in the hot air oven at 40°C and pulverized using industrial blender (Folorunso *et al*., 2019) [12].

Preparation of *Carica papaya* Methanol Plant Extract

200g of the pulverized *Carica papaya* leaves was put into a plastic bucket and 1000ml of methanol was poured into it. It was stirred and the bucket was covered with a lid and left for 48 hours. Afterwards, the extract was filtered using Whatman filter paper. The extract was concentrated by placing it in a water bath with a temperature of 40ºC.

Phytochemical Analysis

Qualitative Test

The preliminary qualitative screening of the phytochemical components was carried out using standard methods for analysis of tannins, saponins, alkaloids, phenolic compounds and phytosterols (Prabhu *et al*., 2017) [21].

Tannin

1ml of the extract was added to a reaction tube; afterward, 4 drops of 0.1% ferric chloride ($FeCl_3$) at 10 % was added. The appearance of a brownish green precipitate indicated the presence of non-hydrolysable tannins.

Flavonoid

1ml of the extract was added to a reaction tube; then, 4 drops of 1% Aluminium chloride was added, the presence of the yellow colour indicated the presence of flavonoids.

Steroid

1ml of extract was placed in a tube; 1 ml of acetic anhydride and few drops of concentrated H_2SO_4 in a slanting manner were added. The generation of blue at the interface indicated the presence of steroids.

Terpenoid

1 ml of extract was placed in a tube; 1 ml of chloroform and few drops of concentrated H_2SO_4 were added. The generation of reddish brown at the interface indicated the presence of terpenoids.

Alkaloid

1 ml of extract was placed in a reaction tube; 300 ml of 2 N hydrochloric acid (HCl) and 300 µL of Wagner's reagent were added. The presence of a reddish brown precipitate indicated the presence of the alkaloids.

Saponin

1 ml of extract was added to a reaction tube; 2 ml of distilled water was added. The mix water-extract was vigorously stirred for 10 seconds. The presence of persistent and stable foam indicated the presence of saponin (Rubio-Melgarejo *et al.*, 2020).

Cardiac Glycoside

Glacial acetic acid was mixed with 2 drops of 0.1% ferric chloride solution. This was added to 1 ml of the extract. Concentrated H_2SO_4 was added at the side of the reaction tube. The reddish brown colour at the interface indicated the presence of cardiac glycoside.

Reducing Sugar

Benedict's solution was added to 1 ml of the plant extract and warmed in a water bath. Absence of colour change indicated the absence of reducing sugar.

Quantitative Test

The content of total alkaloids, flavonoids, tannin and saponins were determined according to the procedures described next.

Total Flavonoids

Method of Harbone, 1973 was used. 5g of sample was boiled in 100ml of 2M HCl solution for 40 minutes. It was allowed to cool to room temperature before being filtered through Whatman filter paper. Flavonoid in the extract was precipitated by drop wise addition of concentrated ethyl acetate until in excess, following filtration. The flavonoid precipitate recovered was oven dried and the weight of flavonoid obtained by difference and expressed as a percentage of the sample analyzed.

$$\% \text{ flavonoid} = \text{Sample weight} / \text{Precipitate weight} \times 100$$

Total Tannin

The total tannin was determined by slightly modified folin and Gocalter method. Briefly 0.5ml of sample extract was added with 3.75mls of distilled water and 0.25ml of folin phenol reagent and 0.5ml of 35% sodium carbonate added. The absorbance was measured at 725nm. Tannic acid dilution (0 to 0.5 mg/ml) were used as standard solution. The result of tannin was expressed in terms of tannic acid in mg/ml of extract.

$$\text{Tannic acid (mg/100g)} = \frac{\text{C x extract vol. x 100}}{\text{Aliquot vol. x wgt. of sample}}$$

Where C = Concentration of tannic acid

Total Saponins

Exactly 100cm^3 of 20% aqueous methanol was added to 20g of the powdered sample in a 250 cm^3 conical flask. The mixture was treated over a hot water bath for 4 hours with continuous stirring at a temperature of 55ºC. The residue of the mixture was reextracted with another 100cm^{3}of 20% aqueous ethanol after filtration and heated for 4 hours at a constant temperature of 55ºC with constant stirring. The combiner extract was evaporated to 40 cm^3 over water bath at 90ºC. 20cm^3 of diethyl ether was added to the concentrate in a 250cm^3 separation funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. The purification process was repeated twice. 60cm^3 of n-butanol was added and extracted twice with 10cm^3 of 5% sodium chloride. After discarding the sodium chloride layer, the remaining solution was heated on a water bath for 30 minutes after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated as a percentage. (Rubio-Melgarejo *et al.*, 2020) [22]

% saponin = weight of saponin / weight of sample x 100

Total Alkaloids

Quantitative determination of Alkaloid was according to the methodology of Harbone, 1973. Exactly 200cm^3 of 10% acetic acid in ethanol was added to 5g of the plant sample in a 250cm^3 beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by the addition of 15 drops of concentrated ammonium hydroxide dropwise to the extract until the precipitation was completed immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitates are washed with 20cm^3 of 0.1 m of ammonium hydroxide and then filtered using gem filter paper. The residue was dried in an oven and the percentage of alkaloid was expressed mathematically as;

% alkaloid = weight of alkaloid / weight of sample x 100

Magnesium Oxide Nanoparticle Biosynthesis

5g of $Mg(NO_3)_2.6H_2O$ was calcinated in an electric furnance at 500 °C for 2 hours and 20 minutes. Approximately 1.025 g of $Mg(NO_3)_2.6H_2O$ was dissolved in 500 ml deionized H_2O contained in conical flask and placed on a magnetic stirrer then mixed drop wise with 50 ml of the plant extracts contained in burette onto magnetic stirrer. 5g of the plant extract was weighed and dissolved in 500ml of deionized water. It was added to the calcinated mixture and placed in the mechanical shaker at 25°C and left for 24 hours to produce a final concentration with colour change. At the end of the incubation period, the solution was filtered, centrifuged and rinsed severally with deionized H_2O to remove any impurities before being oven-dried at 40°C for 3 hours. The controls including plant extracts and $MgNO_3.6H_2O$ solution ran alongside the experiment under the same conditions (Hassan *et al*., 2021; Saied *et al.* 2021) [16].

Physical and Chemical Characterization of Magnesium Oxide Nanoparticle (MgONPs)

pH

The pH of the produced MgONPs was determined using multi meter analytical instrument (Model Ph-2603, China). The samples were dispensed in beakers and duplicate readings were taken after calibrations of the parameters as instructed by the manufacturer (APHA, 2012) [3].

UV-Vis spectroscopy

The colloidal nanoparticle solution was analyzed to monitor the bioreduction of Magnesium ($Mg^{2+} \rightarrow Mg^0$) using a UV-Visible spectrophotometer in the wavelength range of 200 – 800 nm at a resolution of 1nm. 0.01g of the extract was put in 10ml of distilled water and 1ml was poured into a cuvette. Deionized water was used as a blank. Self-test was conducted prior to the commencement of the analysis and the blank was run afterwards. Wavescan method was used alongside the cuvette mode with its path-length at 10mm. The start wavelength was 200nm and the end wavelength was 900nm. Smoothing was 1 and the dilution factor used was 1.000nm

X-Ray Fluorescence Spectrometry

The structural characterization of the MgONPs was carried out using an X-ray Fluorescence Spectrometer to determine the chemical composition, major elements and in traces. (Moustafa, 2017; Hassan *et al*., 2021 [16]).

Atomic Absorption Spectroscopy

The characterization of the total concentration of elemental magnesium stock content in MgONPs was carried out using Atomic Absorption Spectroscopy to determine the chemical element. 0.03g of MgONPs was dissolved in 10ml of deionized water, which was digested before the sample was run.

FTIR Spectroscopy

The Fourier transform infrared (FTIR) spectroscopy was used to determine the functional groups of produced NPs. FTIR measurements were carried out in the wavenumber range of 4,000 – 500 cm^{-1} with a resolution of 4 cm^{-1} at an average of 32 scans per sample. (Moustafa, 2017; Hassan *et al*., 2021 [16]).

Scanning Electron Microscopy

The size and morphologies of the formed NPs was determined by using scanning electron microscopy (SEM). Samples were mounted on 12 mm aluminium specimen stubs with double-sided carbon tape, coated with gold palladium, and examined with a FEI Quanta 250 FEG SEM operating at 10kV. Micrographs were taken with a Gatan bottom mount camera using Digital Micrograph software (Kgatshe *et al.*, 2019) [19].

Results and Discussion

Table 1: Percentage yield of *Carica papaya*

Parameter	Weight/Yield (g/%)
Starting Weight	200
Extracted Weight	12.49
Percentage Yield	6.25

Table 2: Qualitative and Quantitative Phytochemical Analysis of *Carica papaya*

Parameter	Qualitative	Quantitative (%)
Alkaloid	++	3.6
Saponin	+	7.8
Tannin	++	4.3
Flavonoid	+	3.4
Steroid	++	ND
Terpenoid	++	ND
Cardiac Glycoside	+	ND
Reducing Sugar	-	ND

Key:
++ indicates highly present
+ indicates moderately present
- indicates absent
ND indicates Not Determined

Table 3: pH values of *Carica papaya*

Parameter	Value
pH (1st Reading)	6.97
pH (2nd Reading)	6.98
pH (Average)	6.98

Table 4: Absorption maxima of *Carica papaya*-synthesized magnesium oxide nanoparticles

Wavelength (nm)	Absorbance (mm) *Carica papaya*	Absorbance (mm) Blank
215	2.690	0.000
227	2.700	0.000
285	2.550	0.000
327	0.280	0.000
652	0.017	0.000
900	0.030	0.000

Table 5: XRF analysis of *Carica papaya*-synthesized magnesium oxide nanoparticle

S. No	Mineral	Standard Error (%)	Real Value (%)
1	Iron	1.58	-
2	Gold	0.514	< LOD
3	Silver	0.847	6.357
4	Platinum	0.863	< LOD
5	Palladium	0.618	5.404
6	Rhodium	1.039	13.693
7	Ruthenium	0.245	2.376
8	Iridium	0.883	< LOD
9	Cadmium	1.112	8.43
10	Gallium	1.057	< LOD
11	Germanium	0.499	< LOD
12	Nickel	2.343	< LOD
13	Cobalt	1.505	< LOD
14	Chromium	6.205	< LOD
15	Zinc	0.342	0.911
16	Indium	1.39	9.115
17	Tin	1.602	7.762
18	Tungsten	2.385	< LOD
19	Copper	2.045	< LOD
20	Manganese	3.211	< LOD
21	Titanium	4.502	35.238
22	Lead	0.589	< LOD

Key: LOD: Limit of Detection

Table 6: Atomic absorption spectroscopy of magnesium in the synthesized magnesium oxide nanoparticle from *Carica papaya*

Sample	*Carica papaya* (mg/L)	*Carica papaya* (%)
Absorbance at 540nm (1st Reading)	962.9817	32.0994
Absorbance at 540nm (2nd Reading)	959.9391	31.9980
Average Absorbance at 540nm	961.4604	32.0487

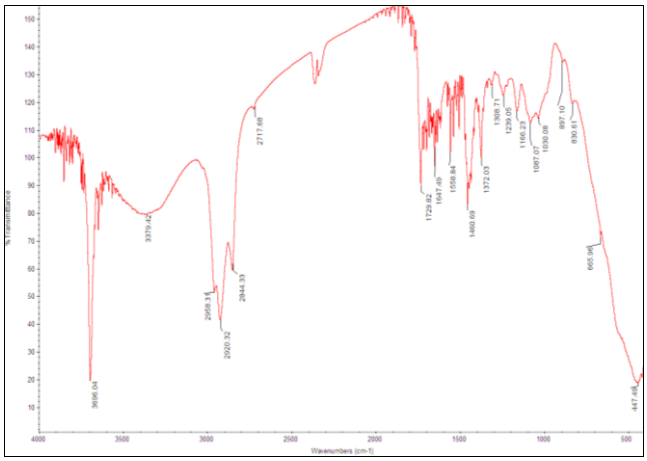

Fig 1: FTIR profile of *Carica papaya*-synthesized magnesium oxide nanoparticles

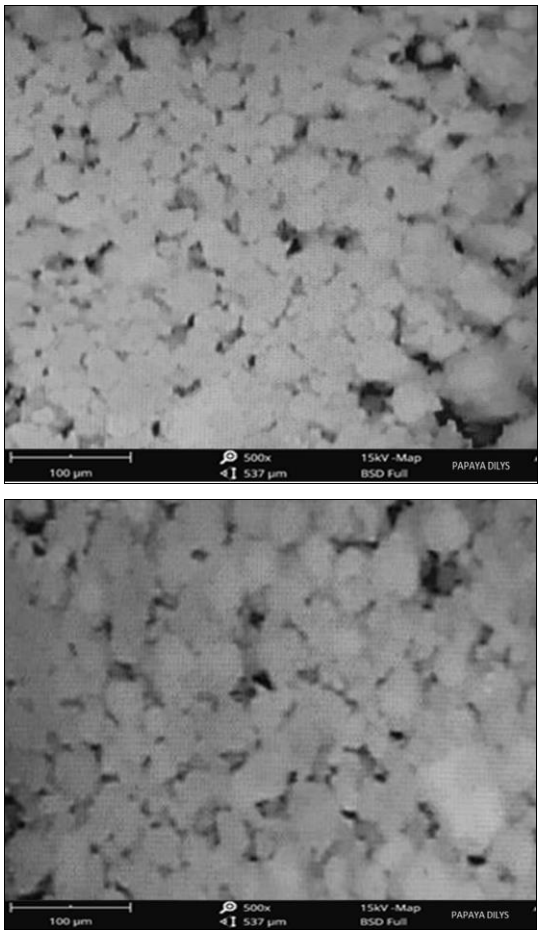

Fig 2: Scanning electron microscopic profile of *Carica papaya*-synthesized magnesium oxide nanoparticles

The yield of the leaf extract at the end of the extraction gave 12.49g of *Carica papaya* and a percentage yield of 6.25%. The results of the phytochemical qualitative evaluation

showed that the methanol extract of *Carica papaya* positively contained several types of phytochemicals such as alkaloids, tannins, flavonoids, steroids, terpenoids, cardiac glycoside, saponins (Table 2). This result is slightly consistent with the work of Jaji *et al.*, 2020 whose methanol extract of *Carica papaya* revealed the presence of flavonoids, alkaloids, phenols, tannins, steroid, saponins, and terpenoids while cardiac glycoside was absent in the extract. Iwu *et al.*, 2016 also confirms the presence of flavonoids, alkaloids, phenols, tannins, and saponins in the leaves of *Carica papaya.* The phytochemical quantitative results showed evaluation of saponin, alkaloids, flavonoids and tannin. Saponin had the highest value of 7.8% with alkaloid, flavonoids and tannin having 3.6%, 3.4% and 4.3% respectively. Saponin is known to have good antimicrobial effect. Flavonoids are a large group of polyphenolic compounds that contain several classes: isofavonoids, favonols, chalcones, favones, and favanones, which can actively chelate and reduce metal ions into nanoparticles. Flavonoids contain various functional groups capable of nanoparticle formation "as a reducing agent". Transformations of favonoids from the enol-form to the keto-form may release a reactive hydrogen atom that can reduce metal ions to form nanoparticles (Mostafa, 2020) [20].

pH measures how acidic or basic a substance is; the pH scale ranges from 0 to 14. A pH of 7 is neutral. A pH less than 7 is acidic and a pH greater than 7 is alkaline. The first and second pH values of Magnesium oxide nanoparticles from *Carica papaya* were 6.97 and 6.98. This indicated that the nanoparticle was slightly acidic or almost neutral. This may want to suggest low toxicity even when consumed by humans. Determination of the absorption maxima for *Carica papya*-synthesized nanoparticles revealed the maximum absorption wavelength for each nanoparticle. It was done in the range of 200-900 nm and the maximum absorbance was observed at 227 nm for *Carica papaya* magnesium oxide nanoparticle formation as seen in table 4. These results are quite similar to those reported for El-Rafie *et al.*, 2016. XRF analysis was done to affirm the presence and quantity of minerals and metals present in the synthesized *Carica papaya* magnesium oxide nanoparticle. It tested for the presence of Iron (Fe), Gold (Au), Silver (Ag), Platinum (Pt), Palladium (Pd), Rhodium (Rh), Ruthenium (Ru), Iridium (Ir), Cadmium (Cd), Gallium (Ga), Germanium (Ge), Nickel (Ni), Cobalt (Co), Chromium (Cr), Zinc (Zn), Indium (In), Tin (Sn), Tungsten (W), Copper (Cu), Manganese (Mn), Titanium (Ti) and Lead (Pb). The *Carica papaya*-synthesized magnesium oxide nanoparticle contained Silver (Ag), Palladium (Pd), Rhodium (Rh), Ruthenium (Ru), Cadmium (Cd), Zinc (Zn), Indium (In), Tin (Sn) and Titanium (Ti) while Gold (Au), Platinum (Pt), Iridium (Ir), Gallium (Ga), Germanium (Ge), Nickel (Ni), Cobalt (Co), Chromium (Cr), Tungsten (W), Copper (Cu), Manganese (Mn) and Lead (Pb) were below limits of detection. The presence of these minerals may detect impurity, however some of these minerals are known to beneficial to humans.

Table 6 shows the result of the Atomic Absorption Spectroscopy (AAS) analysis of *Carica papaya*-synthesized magnesium oxide nanoparticles. This was done to evaluate the presence of magnesium in the *Annona muricata*-synthesized magnesium oxide nanoparticles. This served as an alternative to the XRF spectroscopy. The magnesium content was calculated in mg/ml but was converted to percentage with the dilution factor put in consideration. The analysis showed that magnesium was present in the *Carica papaya* -synthesized magnesium oxide nanoparticles in 962.9817mg/ml and 959.9391mg/ml which is equivalent to 32.0994% and 31.9980% respectively after the analysis was run twice. Functional group's determination and their role in the synthesis of MgONPs in methanol extract of *Carica papaya* was performed using FT-IR analysis. The functional group is considered to be responsible for the capping and stabilization of the nanoparticle. FTIR analysis was carried out to investigate absorbed molecules or functional groups on the surface of MgO nanoparticles synthesized using *Carica papaya* leaf extract. Fig 1 shows the FTIR spectra of MgO nanoparticle samples synthesized, displaying absorption bands between wavenumbers of 3696 cm^{-1} and 447 cm^{-1}, which shows the presence of Mg-O interactions. It also indicated peaks around 3696, 3379, 2958, 2920, 2844, 2717, 1729, 1647, 1558, 1460, 1372, 1308, 1239, 1166, 1087, 1030, 897, 830, 665 and 447 cm^{-1}. A band in the region of 3500 to 3800 cm^{-1}, is related to the stretching of the N-H group stretches of amines and amides. The peaks at 3379cm^{-1} was assigned to the O-H alcohols, N-H amines and amides. Peaks at 2958, 2920, 2844, 2717, indicate C-H alkane stretches and aldehydes. Peaks at 1729cm and 1647cm indicate C=O stretches of ketones, esters and amides. The C=O, C-N, C-O, C-H, and C=C stretching vibrations of aldehydes, carboxylic acids, amines, alkyl halides, ethers, aromatic compounds, and alkenes were assigned to the peaks at 1729, 1647, 1558, 1460, 1372, 1308, 1239, 1166, 1087 and 1030cm^{-1}. Wave numbers between 897, 830, 665cm^{-1} indicate =C-H and C-H bend with stretches of alkynes and aromatic compounds. The peak at 447cm^{-1} indicates alkyl halides. FTIR spectra revealed the presence of different functional groups like Alcohols, phenols (O-H stretch, free hydroxyl), Alcohols, phenols (O-H stretch, H-bonded), α, β- unsaturated esters (C=O stretch), Alcohol, carboxylic acids, esters, ethers (C=O stretch). These results are consistent with the works of Jaji *et al.*, 2020, El-Rafie *et al.*, 2016 and Asafa *et al.*, 2021 which had varying bands both in the functional and fingerprint region. Morphological analysis of MgO nanoparticles synthesized was carried out using SEM. Fig 2 shows a scanning electron microscopy (SEM) image of *Carica papaya* MgO nanoparticles, which shows that the resulting MgO nanoparticles depicts a cluster of relatively spherical and uniformly distributed MgO NPs with a degree of aggregation. It has a range of diameter that measures 100µm which is equivalent to 0.1nm. Magnification measures 5.37µm. Fatiqin *et al.*, 2021 and Essien *et al.*, 2020 synthesized MgO nanoparticles using the aqueous extract of *Chromolaena odorata* leaf.

Conclusion

Methanol extract of *Carica papaya* leaf contains secondary metabolites which are steroids, alkaloids, flavonoids, saponins, phenols etc. and have various medicinal potentials. They also act as reducing and capping agents in the synthesis of nanoparticles. Nanoparticles have various activities especially when synthesized biologically. The chemical characteristics of the nanoparticles such as shape, functional group, mineral constituents etc play various roles to aid in drug delivery especially biologically synthesized nanoparticles.

Competing Interests
The authors declare no conflict of interest.

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