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Evaluation of the phytochemical, antioxidant and silver nanoparticles synthesized from the leaf extracts of *Melia dubia* and *Carica papaya* L. leaves

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Abstract

Melia dubia and *Carica papaya* leaves contain a number of important medicinal compounds. In the current study, an effort was made to study the phytochemical analysis, antioxidant, anti-inflammatory, synthesized silver nanoparticles and antimicrobial activity of synthesized Ag-NPs from *M. dubia* and *C. papaya* leaf extract. The preliminary screening test is executed for the presence of secondary metabolites and was reported in order to understand the phytochemicals of leaf extract. Both qualitative and quantitative phytochemical constituents of alkaloids, aminoacids, carbohydrate, coumarins, flavonoids, phenol, protein, quinones, saponins, steroids, tannins, terpenoids and triterpenoids was analysed. Each extracted solvents is found to be recorded with carbohydrates and flavonoid contents. Studies on the antioxidant and anti-inflammatory activities of recognized phytochemicals present in plant leaves are also determined. The synthesized silver nanoparticles using the plant leaf extract of *M. dubia* and *C. papaya* are characterized by using UV-visible and their antimicrobial activities were performed. It was concluded that, Phytochemicals content presented in the leaf extracts are capable of rendering health benefits and thereby demonstrating that *M. dubia* and *C. papaya* is a potential medicinal plant and essentially replaced as a complement to synthetic anti-microbial agents. Especially, silver nanoparticles synthesized by the plant extracts lead a significant role in biological activities such as antimicrobial and antioxidant. The presence of significant plant leaf secondary metabolites contributes to its antioxidant and antimicrobial activity in future endeavor.

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Introduction

Plants are considered to be good source for the exploration and discovery of new pharmaceutical compounds as well as medicines which can be the potential drug for humans as they act as intermediate for synthesis of useful drug (Makkar *et al.*, 2009). Among these, *M. dubia* (earlier known as *M. composita*) commonly known as Malabar Neem because its wood is very popular in southern states of India for its fast growth and wide adaptability in diverse edaphic and climatic conditions. Plant leaf is a good source of essential oil with a volatile monoterpene camphene and shown good anti-microbial activity inhibited 88% of skin pathogens. The leaf and bark extracts exhibited anti-feed ant, insecticidal, anti-bacterial, anti-viral and anti-fungal property (Dinesh *et al.*, 2020). Plants possess various phytochemicals with several bioactivities such as anti-inflammatory, antioxidant and anticancer and one among them such as *Carica papaya* is widely used in the treatment of many ailments in Ayurvedic as well as herbal and folk medicine (Samatha *et al.*, 2012). Several parts of *M. dubia* have been studied for their medicinal properties as they contain triterpenoids, alkaloids, flavonoids, anthraquinones, glycosides, tannins and various essential oils (Trung *et al.*, 2023). Several studies have shown that plants' secondary and essential metabolites preserve health in many different ways such as anti-inflammatory, antioxidant, antiallergy, antimicrobials, antivirus, antibacterial, anticancer and others (Candra *et al.*, 2024).

Plants are a major source of raw materials for medicines which are used to treat a wide range of human ailments, because of their compatibility with our biological system; natural-source medications have captivated the interest of modern civilization (Nikkitha *et al.*, 2021). Medicinal plants are the most used antimicrobial agents for treating fungi and have been used traditionally. Papaya (*Carica papaya* Linn), a fruit that belongs to the Caricaceae family, is recognized worldwide for its nutritional and beneficial properties. The various parts of the papaya plant have been used for medicinal uses since ancient times (Aljuhani *et al.*, 2024). Moreover, there are also reports found on various other biomolecules like carbohydrates, sugars, alkanoids, proteins, fats, terpenoids, flavonoids, phenols, enzymes and tannins for the efficient synthesis of AgNPs, which promotes the binding of NPs with the applied substrates (Faridul *et al.*, 2022). As nature is a big source of sustainable materials available throughout the globe, green synthesis of AgNPs using various plant extracts is showing new opportunities and routes to develop sustainable and bio-based green products through minimizing the consumption of inorganic chemicals (Maria *et al.*, 2023).

Currently there are different types emerging diseases and other drug resistance diseases. It is very difficult to cure human and animal with the currently known drugs, therefore there is a need of other alternative drugs. Antibacterial effects against certain resistant infectious pathogens are profound and effective if combinations of different plant extracts are used. Various studies have established that herbal medicines can be developed as safe, effective and less costly alternatives to the current medicines to the treat certain bacterial infections (Dagne *et al.*, 2021). A study was conducted to investigate the phytochemical analysis, antioxidant properties, anti-inflammatory effects, synthesized silver nanoparticles and antimicrobial activity of silver nanoparticles derived from the leaf extracts of *M. dubia* and *C. papaya*.

Materials and methods

Collection of plants

Fresh leaves of *M. dubia* and *C. papaya* leaves were collected from the Srinivasapuram, Thanjavur district. The collected plant leaves were washed with tap water and rinsed with distilled water to remove dust particles and then the leaves were shade dried and made into a fine powder with an electric grinder. The powdered sample was sealed in polythene bags and stored in desiccators.

The dried 20 g leaves were powdered and soaked separately in organic and inorganic components from

aqueous, methanol, chloroform and benzene extract were detected using polar and non-polar solvents as per the solubility (Yadav and Agarwala, 2011).

Phytochemical screening

M. dubia and *C. papaya* leaf samples were screened qualitative and quantitative phytochemicals using the following standard laboratory techniques (Harborne, 1992).

Qualitative phytochemical analysis

Test for alkaloids (Dragendorff's reagent test): A mixture of 1 ml of the extract and 1 ml of potassium bismuth iodide reagent resulted in the formation of an orange-red precipitate.

Test for aminoacids

Measure 1 mL of the amino acid solution into a test tube, then introduce several drops of ninhydrin reagent and thoroughly mix the contents. Subsequently, immerse the test tube in a boiling water bath for duration of 5 minutes. After this period, remove the test tube and allow it to cool to room temperature. The development of a purple to blue hue will indicate the reaction.

Test for carbohydrate

1 ml of extract was combined with 5 ml of Benedict's reagent, and the resulting mixture was subjected to heating for duration of 2 minutes. The appearance of a greenish hue suggested the presence of carbohydrates.

Test for coumarins

1 ml of extract was combined with 10% sodium hydroxide, followed by the addition of several drops of chloroform, and the mixture was thoroughly agitated. A yellow precipitate was observed as a result.

Test for flavanoids (Shinoda test)

1 ml of the extract was combined with four magnesium ribbons and two drops of concentrated hydrochloric acid, resulting in the formation of a reddish hue.

Test for phenol

1 ml of the extract solution was combined with several drops of a 5% ferric chloride solution, resulting in the formation of a dark green to bluish-black coloration.

Test for protein

A few drops of Ninhydrin reagent were added to 1 ml of the extract, which was then subjected to heating in a boiling water bath. The development of a purple-blue color signifies the presence of proteins.

Test for quinones

1 milliliter of plant extract was combined with several drops of alcoholic potassium hydroxide, resulting in the formation of a precipitate that transitioned from red to blue.

Test for saponins (foam test)

A total of 2 ml of distilled water was incorporated into 1 ml of the extract, followed by vigorous shaking. The development of a stable white foam served as an indicator for the presence of saponins.

Test for steroids

Approximately 1 mL of the crude extract was mixed with 10 mL of chloroform and 10 mL of sulfuric acid. The resulting bilayer, characterized by a red upper layer and a greenish lower layer, indicates the presence of steroids.

Test for tannins

A few drops (2-3) of $FeCl_3$ solution were introduced to 1 ml of the extract, resulting in the formation of a greenish-black precipitate, which serves as an indicator of tannin presence.

Test for terpenoids

A mixture was prepared by combining 5 ml of the extract with 2 ml of chloroform, followed by the addition of concentrated sulfuric acid to create a distinct layer. The appearance of a reddish-brown hue serves as an indicator of terpenoid presence.

Test for triterpenoids

1 ml of plant extract was subjected to filtration, followed by the addition of several drops of concentrated sulfuric acid (H_2SO_4). The mixture was thoroughly shaken and subsequently allowed to rest, resulting in the formation of a golden yellow layer at the bottom.

Anti-inflammatory test

Effect of *M. dubia* and *C. papaya* leaf extracts on bovine serum albumin (BSA) denaturation assay was carried out using a method described by (Anyasor *et al.*, 2019) with minor modifications. The reaction mixtures consist of varying concentrations (100, 200, 300, 400 and 500 µg/mL) of extracts or reference drug diclofenac sodium (2-[(2,6 dichlorophenyl)amino] benzene acetic acid sodium salt) (an NSAID), 1% w/v BSA and phosphate buffered saline (PBS, pH 6.4) separately while PBS was used as control. The reaction mixtures were incubated at 37 °C for 20 min and the temperature was increased to keep the samples at 70 °C for 5 min. After cooling, turbidity was measured at 660 nm using UV-visible spectrophotometer (Schimadzu Double Beam UV-2600, Japan). The control represents 100% protein denaturation. The percentage inhibition of BSA denaturation was calculated as stated below:

% inhibition of BSA denaturation = 100 × {1 – (A2/A1)}

Where A1 = absorbance of the control, and A2 = absorbance of the test sample.

Antioxidant activity

Scavenging of hydrogen peroxide

Hydrogen peroxide (H_2O_2) is characterized by its relatively low reactivity and is classified as a weak oxidizing agent. However, it can exhibit cytotoxic effects due to its potential to generate hydroxyl radicals within cells (Banik *et al.*, 2017). The compound is capable of rapidly traversing cell membranes, and once intracellular, H_2O_2 may interact with ferrous (Fe^{2+}) and cupric (Cu^{2+}) ions, leading to the formation of hydroxyl radicals, which are likely responsible for many of its deleterious effects. A 40 mM solution of hydrogen peroxide was prepared in a phosphate buffer solution at pH 7.4 (0.6 ml, 40 mM). The absorbance of the hydrogen peroxide solution was measured 10 minutes later at a wavelength of 230 nm, using a blank solution of phosphate buffer devoid of hydrogen peroxide for comparison. The percentage of hydrogen peroxide scavenging by both plant extracts and standard compounds was subsequently calculated.

Scavenging % = AC – AS/AC × 100

Where,

AC = the absorbance of control

AS = the absorbance in the presence of the sample`s extracts and standards.

DPPH scavenging method

The antioxidant activity of plant extract and standard antioxidant were measured in-vitro in terms of hydrogen donating or free radical scavenging ability by using the 1,1 diphenyl-2-picryl hydrazyl (DPPH). DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extract. The purple DPPH becomes yellow diphenyl picryl hydrazine while reduced (Banik *et al.*, 2017). Extract solution was prepared by dissolving 1mg of dry plant extract in 1ml methanol. An aliquot of 2.5ml of 75 µM DPPH solution in methanol and 0.5ml of plant extract in methanol at various concentrations were mixed and incubated at room temperature for 90 minutes and absorbance of the test mixture was read at 517 nm using a UV-VIS spectrophotometer (Systronic 119) against a DPPH control containing 1ml of methanol were measured. The experiment was done in triplicated. Gallic acid was used as standard. Percentage scavenging activity was calculated as follows:

Scavenging % = Absorbance of control – Absorbance of test / Absorbance of control × 100

Reducing power

Ferric reducing activity of plant extracts was performed according to previous study (Mandal *et al.*,

2015). The plant extract of different concentration was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min and the reaction was terminated by adding 2.5 ml trichloroacetic acid (10%) followed by centrifugation at 5000 rpm. 2.5 mL of the upper layer was pipette out in a new test tube and 0.5 ml of $FeCl_3$ (1%) was added and allowed to stand for 30 min before measuring the absorbance at 700 nm. Increased absorbance of the reaction mixture indicated with increased reducing power.

Thiobarbituric acid (TBA) assay

The standard solution of 4.0 mM of TBA with glacial acetic acid was prepared. Consequently, in 100 mL of glacial acetic acid, 57.66 mg of TBA was dissolved. The *M. dubia* and *C. papaya* leaf extract sample were added with 100% glacial acetic acid (AA) and 50% glacial acetic acid with water (AW). With 1 mL of TBA reagent 1ml of leaf extract was mixed and the above method was repeated for five times (n = 5) (Zeb and Ullah, 2016). The TBARS was evaluated using the formula as μμM/g of the sample:

TBARS (μμM/g) = (Ac × VV)/WW

Where Ac is referred as the amount determined from the calibration curve and WW is weight of the sample taken while VV is the volume in mL or dilution factor of the total leaf extract prepared.

Synthesized silver nanoparticles (Ag-NPs)

Fresh *M. dubia* and *C. papaya* leaves were washed with distilled water and removed the dust. The 25 gram of fine-cut fruits was boiled with 50 ml of water at 80°C for 15 minutes (Firdaus *et al.*, 2017). The plant leaves extract was separated from the remaining small solids by filtration through Whatman no. 1 filter paper. Silver nitrate with concentration 1 mM was prepared in Erlenmeyer flasks and the appropriate volume of fruit extract was added to the mixture under sunlight irradiation for 15 minutes. In order to optimize the AgNPs biosynthesis condition, a variation of different concentration of fruit extracts added into $AgNO_3$ solution were also conducted. The volume ratios of leaves extract to $AgNO_3$ were 0.5, 1.0, 1.5, 2.0 and 2.5mM. An aliquot of the mixture was taken periodically to monitor the progress of AgNPs biosynthesis by using UV–vis spectrophotometer. UV–vis spectra were recorded at a resolution of 1 nm. The pH of the reaction was kept at 4.5.

Antimicrobial assay (Anibijuwon and Udeze, 2009)

Using pathogenic microorganism

In the experiment, four bacterial and four fungal strains such as *E. coli, K. pneumoniae, Enterococcus* sp., *Staphylococcus aureus* and *Aspergillus niger, A. terreus, Penicillium* sp., *P. chrysogenum* were used for antimicrobial activity. The preserved strains were obtained from the Indian Biotrack Research Institute, Thanjavur.

Agar well – diffusion method

The agar well diffusion technique was used for determine the antimicrobial activity of the synthesized silver nanoparticles from leaf extracts. One millilitre of the different standardized organisms were introduced separately and thoroughly mixed with 30 milliliters of molten nutrient agar each in a sterile Petri dish and allowed to set then labelled. A sterile 8mm cork borer was then used to punch holes (i.e. 5wells) in the inoculated agar and the agar was then removed. Four wells that were formed and filled with different concentrations of the extract which were labelled accordingly, the different concentration of 100 to 500mg/ml while the 5th well contained the extractant i.e. the solvent used for the extraction to serve as control. These were then left on the bench for 1hour for adequate diffusion of the extracts and incubated at 37°c for 48hours. After incubation, the diameter of the zones of inhibition around each well were measured to the nearest millimetres along two axis i.e. 90° to each other and the mean of the two readings were then calculated.

Statistical analysis

Experiments were carried out in triplicate and the results are expressed as mean values with standard deviation.

Results and discussion

Qualitative estimation of the phytochemicals conducted on *M. dubia* leaf extracts revealed the presence of alkaloids, phenolics, flavonoids, and tannins, whereas *M. dubia* leaf extract revealed the presence of alkaloids, tannins, terpenoids, flavanoids and phenolics. The active principles of many drugs found in plants are known for secondary metabolites (Dinesh *et al.*, 2020). Medicinal and healing properties of herbs are closely related to their chemical constituents which are classified into some major groups like alkaloids, terpenoids, phenols, tannins, sugar, proteins, quinones, saponins and these chemicals out into the herbal remedy depends upon the solubility of these components in various solvents. The alkaloids have been reported and derived from medicinal plants shows that biological activities like anti-inflammatory, anti-malarial, anti-microbial, cytotoxicity and pharmacological effects (Singh *et al.*, 2018).

Table 1. Qualitative phytochemical analysis of leaf extracts in *Melia dubia* and *Carica papaya* from different solvents

Phytochemical compounds	Inference							
	Melia dubia				*Carica papaya*			
	Aqueous	Methanol	Chloroform	Benzene	Aqueous	Methanol	Chloroform	Benzene
Alkaloids	+	+	-	-	+	+	-	-
Amino acids	+	+	+	+	+	+	+	+
Carbohydrate	+	+	+	+	+	+	+	+
Coumarins	+	+	-	-	+	+	-	-
Flavonoids	++	+	+	+	+	++	+	+
Phenol	+	+	+	+	+	+	+	+
Protein	+	+	+	-	+	+	+	+
Quinone	+	+	+	-	+	+	-	+
Saponins	++	+	-	+	+	+	+	-
Steroid	+	+	+	+	+	+	-	+
Tannins	++	+	-	-	+	++	-	-
Terpenoids	-	+	+	-	+	+	+	+
Triterpenoids	+	+	-	-	+	+	-	-

(++) – Strongly present, (+) – Present, (-) – Absent

Table 2. Quantitative phytochemical analysis of leaf extract in *Melia dubia* and *Carica papaya* from different solvents

Phytochemical compounds	Quantity (mg/g)							
	Melia dubia				*Carica papaya*			
	Aqueous	Methanol	Chloroform	Benzene	Aqueous	Methanol	Chloroform	Benzene
Alkaloids	12.26±0.08	12.21±0.07	-	-	11.27±0.05	11.29±0.12	-	-
Amino acids	13.06±0.12	13.59±0.23	12.05±0.01	13.85±0.01	12.61±0.14	12.94±0.09	13.18±0.06	12.48±0.01
Carbohydrate	15.59±0.19	15.53±0.16	15.32±0.20	15.22±0.21	14.32±0.20	14.35±0.08	14.24±0.10	14.27±0.14
Coumarins	13.05±0.01	16.17±0.00	-	-	12.41±0.16	13.08±0.02	-	-
Flavonoids	17.79±0.26	12.72±0.07	12.26±0.17	12.20±0.17	11.62±0.09	16.76±0.16	11.45±0.10	11.39±0.13
Phenol	11.02±0.05	11.65±0.00	10.07±0.04	10.36±0.18	11.04±0.03	10.57±0.00	11.44±0.06	09.00±0.11
Protein	14.34±0.07	14.23±0.12	14.25±0.24	-	13.56±0.12	13.63±0.04	13.32±0.21	13.32±0.22
Quinone	06.08±0.00	08.97±0.34	06.18±0.05	-	05.19±0.17	09.16±0.23	-	08.05±0.16
Saponins	17.81±0.27	12.75±0.23	-	12.24±0.10	11.61±0.07	11.72±0.14	11.34±0.18	-
Steroid	11.94±0.34	11.87±0.09	11.27±0.15	11.24±0.14	11.58±0.17	11.46±0.04	-	11.66±0.16
Tannins	18.86±0.28	12.84±0.05	-	-	11.77±0.17	18.81±0.11	-	-
Terpenoids	-	11.64±0.12	11.32±0.23	-	11.69±0.09	11.76±0.09	11.65±0.24	11.31±0.18
Triterpenoids	10.30±0.12	09.22±0.14	-	-	14.09±0.12	10.07±0.15	-	-

The values are expressed in terms of (Mean ± Standard deviation)

Preliminary phytochemical studies revealed the acidic nature of all present extracts. Methanolic extract was found to contain alkaloids, aminoacids, carbohydrate, coumarins, flavonoids, phenol, protein, quinone, saponins, steroids, tannins, terpenoids and triterpenoids from *M. dubia* leaf extracts while aqueous extract gave tests for the absence of terpenoids, chloroform extract tests for the alkaloids,

coumarins, saponins, tannins and triterpenoids were absent and benzene extract tests for the absence of alkaloids, coumarins, protein, quinones, tannins, terpenoids and triterpenoids. The aqueous and methanolic extract of *C. papaya* leaves was found to contain a variety of compounds including alkaloids, amino acids, carbohydrates, coumarins, flavonoids, phenols, proteins, quinones, saponins, steroids, tannins, terpenoids, and triterpenoids. In contrast, the chloroform extract showed no presence of alkaloids, coumarins, quinones, steroids, tannins and triterpenoids. Additionally, the benzene extract did not contain alkaloids, coumarins, saponins, tannins and triterpenoids (Table 1 & 2).

Table 3. Anti-inflammatory activity of leaf extract in *Melia dubia* and *Carica papaya*

Different concentration (µl)	Percentage of inhibition (%)				
	Different solvents				
	Standard (Diclofenac sodium)	*Melia dubia*		*Carica papaya*	
		Aqueous	Methanol	Aqueous	Methanol
100	34.88±0.16	25.52±0.06	17.37±0.06	27.82±0.11	19.48±0.53
200	37.00±0.25	32.03±0.17	26.61±0.17	31.15±0.06	26.52±0.67
300	45.83±0.72	40.81±0.35	31.11±0.87	45.21±0.87	37.67±0.08
400	39.12±0.63	33.21±0.41	24.46±0.91	39.56±0.17	25.17±0.89
500	28.33±0.17	24.01±0.63	15.16±0.77	22.11±0.68	18.65±0.46

The values are expressed in terms of (Mean ± Standard deviation)

Table 4. Antioxidant activity of hydrogen peroxide assay from leaf extracts

Different concentration (µl)	% of activity				
	Hydrogen peroxide assay (H_2O_2)				
	Standard (Ascorbic acid)	*Melia dubia*		*Carica papaya*	
		Aqueous	Methanol	Aqueous	Methanol
100	28.08±0.02	24.38±0.09	24.07±0.07	27.99±0.12	24.31±0.07
200	31.25±0.00	27.82±0.23	27.20±0.74	29.35±0.33	26.50±0.18
300	35.48±0.06	31.43±0.02	28.46±0.23	30.76±0.78	27.49±0.23
400	36.19±0.03	33.62±0.37	30.53±0.77	32.85±0.14	28.67±0.56
500	39.74±0.15	36.75±0.47	32.42±0.64	35.16±0.78	29.65±0.71

The values are expressed in terms of (Mean ± Standard deviation)

Table 5. Antioxidant activity of reducing power assay from leaf extracts

Different concentration(µl)	% of activity				
	Reducing power assay				
	Standard (Ascorbic acid)	*Melia dubia*		*Carica papaya*	
		Aqueous	Methanol	Aqueous	Methanol
100	63.23±0.16	54.47±0.67	52.69±0.56	58.04±0.65	51.85±0.11
200	68.05±0.05	59.93±0.94	54.19±0.13	66.64±0.37	56.12±0.04
300	73.00±0.28	60.78±0.35	67.08±0.56	70.02±0.20	62.66±0.10
400	80.34±0.61	72.33±0.22	78.28±0.04	76.91±0.06	66.10±0.57
500	94.17±0.47	80.90±0.11	83.06±0.34	81.15±0.51	72.22±0.49

The values are expressed in terms of (Mean ± Standard deviation)

Table 6. Antioxidant activity of thiobarbituric acid assay from leaf extracts

Different concentration (µl)	% of activity				
	Thiobarbituric acid assay				
	Standard (Ferric cyanide)	*Melia dubia*		*Carica papaya*	
		Aqueous	Methanol	Aqueous	Methanol
100	32.11±0.04	29.69±0.85	31.80±0.33	30.10±0.08	27.85±0.16
200	37.11±0.10	31.10±0.07	34.83±0.05	35.63±0.56	31.97±0.52
300	41.27±0.34	35.47±0.36	35.59±0.41	38.32±0.81	42.27±0.71
400	47.05±0.11	42.89±0.12	38.05±0.67	41.53±0.18	46.24±0.67
500	50.41±0.37	47.66±0.19	42.54±0.21	48.70±0.07	49.05±0.60

The values are expressed in terms of (Mean ± Standard deviation)

This assay is based on the stabilization of human red blood cell (HRBC) membrane. It is a well-known fact that hydrolytic enzymes released during inflammation generate different disorders. The extra cellular activity of these enzymes is said to acute or chronic inflammation (Krishna and Jyoti, 2019). Collectively, the *in-vitro* studies reported here indicated that papaya extracts (leaf and seed) possess an ability to modulate inflammatory markers in various cell types exposed to a variety of stressors. The role of tissue-resident macrophages in inflammation conditions is evident (Pandey *et al.*, 2016). In the present study, maximum percentage protection of the aqueous and methanol extract observed at 300µg/ml from both plant leaves. The extract assessed plant in the present investigation showed high membrane stabilization property in the concentration dependent manner (Table 3).

Free radical scavenging potential of the extract was determined by DPPH assay. Since the DPPH assay is sensitive enough to detect natural compound at low concentration, it is extensively used for evaluating the free radical scavenging potential of natural antioxidants. Hydroxy radicals are an extremely reactive free radical formed in biological system and has enhanced as highly damaged to almost every molecule found in living cells. These radicals have the capacity to join nucleotides in DNA and cause strand breakage which contributes to carcinogenesis, mutation and cytotoxicity and considered to be one of the quick initiators of lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids (Dinesh *et al.*, 2020).

In the present study, all the concentrations tested showed a dose dependent increase in the percentage antioxidant activity. In each concentration, the tests were performed in triplicate and the mean ± SD of the three tests were taken. The percentage inhibition of methanolic leaf extracts of *Melia dubia* and *Carica papaya* at various concentrations (µg/ml) in antioxidant activity. Similarly, the percentage inhibition at various concentrations of the standard ascorbic acid also showed a linear increase in antioxidant activity. The maximum percentage inhibition was observed at 500µg/ml concentration in all assays from antioxidant activity for aqueous and methanol solvents, respectively (Table 4-7).

Table 7. Antioxidant activity of DPPH assay from leaf extracts

Different concentration (µl)	% of activity				
	DPPH assay				
	Standard (Vitamin C)	*Melia dubia*		*Carica papaya*	
		Aqueous	Methanol	Aqueous	Methanol
100	40.36±0.11	38.84±0.56	30.81±0.03	23.60±0.82	23.29±0.09
200	45.08±0.08	43.05±0.48	32.21±0.45	29.81±0.23	26.42±0.34
300	47.15±0.04	45.09±0.19	33.90±0.66	42.27±0.44	31.24±0.78
400	50.38±0.37	49.64±0.15	35.78±0.87	46.24±0.13	36.15±0.18
500	53.06±0.22	51.19±0.23	37.51±0.04	51.05±0.05	40.66±0.71

The values are expressed in terms of (Mean ± Standard deviation)

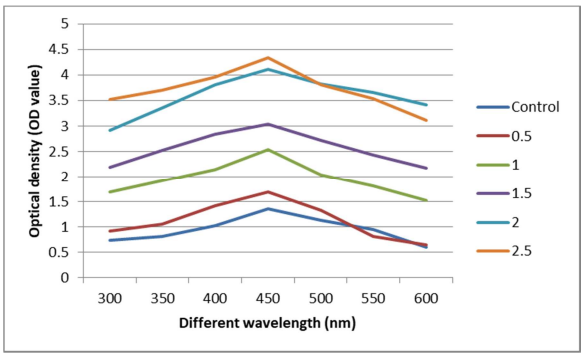

Fig. 1. Bio synthesis of silver nanoparticles from *Melia dubia* leaf extract

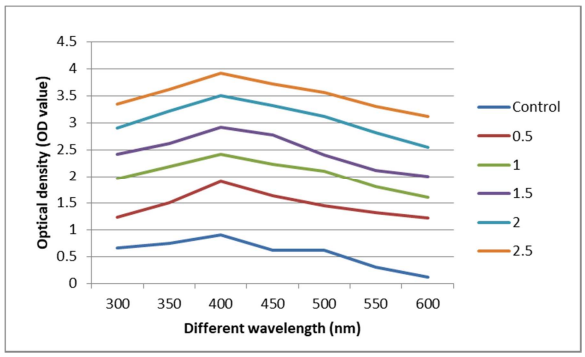

Fig. 2. Bio synthesis of silver nanoparticles from *Carica papaya* leaf extract

Biosynthetic methods can employed plant extracts for nanoparticles production. In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. Based on the tradition knowledge the *M. dubia* and *C. papaya* leaf extract was synthesized of silver nanoparticles. There was visible colour changed after addition of silver nitrate solution with leaves extract. The colour of the aqueous silver nitrate solution was turned into yellowish brown within 15 minutes with the addition of leaves extract. Intensity of brown color became deep with direct proportion to the incubation period. The colour change from watery to brown due to the excitation of surface Plasmon resonance with the silver NPs, which indicated the formation of silver nanoparticles in the mixed solution. The reduction of silver ions and the formation of stable nanoparticles occurred rapidly within 2 hours of reaction making it one of the fastest bioreducing methods to produced Ag nanoparticles, but in the case of present study the silver nanoparticles were settled down with in 30 minute after mixing (Gunasekaran *et al.*, 2017). Even though the silver nanoparticle settled down in the bottom of the conical flask after 10 min of mixing with the *M. dubia* and *C. papaya* leaves extract. The presence of silver nanoparticles was confirmed by UV-spectrophotometer analysis at 450nm and 400nm (Fig. 1 & 2).

Table 8. Antibacterial activity of *Melia dubia* leaf with extracts against clinical bacteria

Name of the bacteria	Zone of inhibition (mm)							
	Melia dubia leaf							
	Aqueous				Methanol			
	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl
E. coli	09.7±0.09	10.2±0.64	12.7±0.37	13.8±0.02	07.9±0.65	08.4±0.68	09.4±0.06	10.9±0.97
K. pneumoniae	10.5±0.12	12.6±0.96	13.0±0.61	14.1±0.57	10.9±0.85	11.8±0.07	13.6±0.47	14.9±0.08
Enterococcus sp.	08.6±0.55	10.9±0.75	11.4±0.81	13.8±0.45	07.5±0.34	08.6±0.82	09.4±0.64	10.7±0.17
S. aureus	10.6±0.27	11.9±0.06	13.8±0.89	15.8±0.16	10.6±0.51	11.5±0.06	13.5±0.11	14.8±0.64

The values are expressed in terms of (Mean ± Standard deviation)

Table 9. Antifungal activity of *Melia dubia* leaf with extracts against clinical fungi

Name of the fungi	Zone of inhibition (mm)							
	Melia dubia leaf							
	Aqueous				Methanol			
	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl
Aspergillus niger	05.2±0.55	06.3±0.96	08.3±0.44	07.3±0.65	09.6±0.61	07.4±0.09	08.6±0.14	07.5±0.04
A. terreus	06.3±0.88	05.4±0.31	09.6±0.57	12.7±0.48	09.8±0.11	10.5±0.07	12.4±0.15	10.6±0.17
Penicillium sp.	07.2±0.31	09.3±0.78	11.8±0.12	08.7±0.22	09.4±0.45	10.7±0.59	09.4±0.31	10.5±0.44
P. chrysogenum	05.6±0.13	06.1±0.20	08.6±0.44	06.9±0.51	08.7±0.18	07.6±0.46	08.3±0.01	09.4±0.07

The values are expressed in terms of (Mean ± Standard deviation)

Table 10. Antibacterial activity of *Carica papaya* leaf with extracts against clinical bacteria

Name of the bacteria	Zone of inhibition (mm)							
	Carica papaya leaf							
	Aqueous				Methanol			
	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl
E. coli	11.4±0.23	14.09±0.15	12.6±0.74	10.6±0.57	09.6±0.12	08.9±0.08	09.4±0.23	07.5±0.08
K. pneumoniae	12.7±0.61	11.3±0.08	14.5±0.09	10.7±0.22	10.2±0.67	09.8±0.11	08.4±0.66	07.4±0.17
Enterococcus sp.	12.6±0.07	13.9±0.49	11.2±0.31	13.8±0.06	09.8±0.57	10.2±0.89	09.5±0.46	08.4±0.75
S. aureus	13.7±0.06	11.7±0.56	12.1±0.11	11.3±0.46	11.3±0.75	10.6±0.08	09.3±0.27	08.6±0.17

The values are expressed in terms of (Mean ± Standard deviation)

The zone of inhibition (mm) for both *Candida albicans* and *Streptococcus mutans* as antibacterial and antifungal activities of three varying combinations of nanocomposites were measured and noted (Dhevishri *et al.*, 2023). Microbial activity testing Distilled water was used to dilute the AgNPs to obtain a stock solution concentration of 1024 µg/ mL. All stock solutions underwent sterilization of

microfiltration (0.22 µm), and aliquots were diluted to create the various lower-concentration solutions required for the antimicrobial testing procedure. The antimicrobial activity of the AgNPs was assessed against the same Gram-negative bacteria used in a study (Arsene *et al*., 2023).

In the present study, eight microorganisms were cultured and studied for the zone of inhibition around these synthesized nanocomposites against *E. coli, K. pneumoniae, Enterococcus* sp., *Staphylococcus aureus* and *Aspergillus niger, A. terreus, Pencillium* sp., *Pencillium chrysogenum.* Antibacterial and antifungal activities of Ag nanoparticles were observed by well-diffusion method. Agar agar served as the medium for microbial activation which was taken in culture plates. Four wells were prepared and nano-silver with three varying ratios of nanocomposites. These well loaded agar plates were incubated at 36˚C for 24 hours. After incubation, the zone of inhibition around the well was observed; this denotes the antimicrobial activities of Ag nanoparticles individually and in combination. The antibacterial and antifungal activities of Ag nanocomposites in varying concentrations compared with nano-silver of 1µL in concentration. The zone of inhibition (ZOI) of Ag nanocomposites is demonstrated against bacterial and fungal cultures. The highest ZOI against the *S. aureus* for *M. dubia* and *K. pneumoniae* for *C. papaya* was displayed when nano silver are in equal proportions in bacteria. However, the highest ZOI were observed at the *A. terreus* for *M. dubia* and *C. papaya* against fungi. Ag exhibits their antimicrobial potential through their mechanism of adhesion penetration into the cell membrane of these microbial cells. These nanoparticles possess the ability of free radical production against these microbes, thereby inhibiting their growth in these matrices (Table 8-11).

Table 11. Antifungal activity of *Carica papaya* leaf with extracts against clinical fungi

Name of the fungi	Zone of inhibition (mm)							
	Carica papaya leaf							
	Aqueous				Methanol			
	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl
Aspergillus niger	05.1±0.13	05.9±0.56	06.7±0.08	05.7±0.11	05.1±0.86	05.4±0.37	07.4±0.41	06.9±0.24
A. terreus	06.8±0.25	07.9±0.05	09.7±0.67	08.6±0.04	09.3±0.57	10.1±0.07	10.7±0.34	09.3±0.75
Penicillium sp.	05.7±0.08	05.9±0.16	07.8±0.13	06.9±0.59	06.9±0.12	07.5±0.17	11.6±0.58	09.3±0.74
P. chrysogenum	09.6±0.33	10.9±0.27	13.8±0.55	14.7±0.04	06.1±0.28	05.7±0.03	07.4±0.11	05.8±0.56

The values are expressed in terms of (Mean ± Standard deviation)

Conclusion

This study has shown the phytochemical screening, anti-inflammatory and antioxidant activity, synthesized silver nanoparticles and its antimicrobial activity of *Melia dubia* and *Carica papaya* leaf extracts. Phytochemicals such as alkaloids, aminoacids, carbohydrate, coumarins, flavonoids, phenol, protein, quinone, saponins, steroids, tannins, terpenoids and triterpenoids were present in both extracts. Only one in *vitro* study has examined the potential anti-inflammatory and antioxidant activities of polar and non-polar extracts of plant leaves from *M. dubia* and *C. papaya*. Leaf extract of *M. dubia* and *C. papaya* catalyzed the formation of the nanoparticles because of presence of various proteins that involved in the reduction of silver ions. Nanoparticles also showed remarkable inhibition against sewage pathogens through its ability to bind to the nanoparticles. For the synthesis of nanoparticles employing plants can be advantageous over other biological entities which can overcome the time consuming process of employing microbes and maintaining their culture which can lose their potential towards synthesis of nanoparticles. Hence, use of plant extract for synthesis can form an immense impact in coming decades. This was evidenced from the zone of inhibition in the antimicrobial activity against tested organisms used for the study. The zone of inhibition for the synthesized leaf extracts suggests the significant degree of efficacy on test organisms. This research has

confirmed the antimicrobial properties of the leaves extracts of *M. dubia* and *C. papaya* and can be suggested that *M. dubia* and *C. papaya* may be recommended as useful source to prepare natural bioactive products from which we can develop new antimicrobial drugs which will be cost effective. Therefore, the medicinal properties of *M. dubia* and *C. papaya* render them appropriate for utilization in the fourth generation.

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