

## Phytochemical Screening and Larvicidal Evaluation of Phyto-synthesized Silver Nanoparticles using Palmyra Palm Sprout Extract

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**ABSTRACT.** One of the objectives of nanotechnology is to synthesize effective nano-insecticides in a bid to reduce the prevalence of the numerous diseases caused by mosquitoes. This synthesis is even more vital in cases where phytochemicals from plant extracts are used as reducing agents. This study aims to determine the phytochemicals present in Palmyra (*Borassus aethiopum*) sprout extract, perform green synthesis of silver NPs with the sprout extract, and spectroscopic investigation using FT-IR and UV-Visible spectrophotometers, in addition to the main aim of evaluating the AgNPs' applications as a nano-larvicide. For this study, a total of ten phytochemical analyses was performed. Subsequently, the sprout extract was used as a reducing agent in the synthesis of silver nanoparticles (AgNPs). Characterization with Fourier transform infrared and Ultraviolet-visible spectrometry was then carried out to confirm the synthesis. In addition, the AgNPs were further analyzed for larvicidal potency against 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instars *Culex quinquefasciatus* mosquito larvae, at interval concentrations of 5, 10, 20, 25, and 50 ppm. The results confirmed the presence of alkaloids, flavonoids, saponins, coumarins, glycosides, tannins, phenols as well as quinines, however, sterols, steroids, and terpenoids were not detected. The LC<sub>50</sub> and LC<sub>90</sub> values discovered to be 9.103 ppm and 134.463 ppm with a correlation of 0.815, as well as 10.316 ppm and 118.810 ppm with a correlation of 0.801, for the 1<sup>st</sup> and 2<sup>nd</sup> instars, respectively. Meanwhile, the 3<sup>rd</sup> and 4<sup>th</sup> instars had LC<sub>50</sub> and LC<sub>90</sub> values of 17.585 ppm and 179.052 ppm, respectively, with a correlation of 0.807. This study provides a basis for extracting and analyzing the reduction potential of the phytochemicals present in the sprout extract, as well as the application of AgNPs, in controlling the mosquito larvae population.

**Keywords:** AgNPs; *Culex quinquefasciatus*; larvicidal activity; Palmyra palm; phytochemical screening

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### INTRODUCTION

Phytochemical screening is a crucial step in the discovery of bioactive compounds (Altemimi *et al.*, 2017), while a report by Kwaji *et al.* (2018) showed some phytochemicals have been known to exhibit biological properties. Phytochemicals are natural, usually bioactive compounds, often found in plants as primary or secondary components (Chikara *et al.*, 2018). The primary compounds are utilized exclusively used by plants, hence, the secondary compounds are more fascinating for studies. Prior to modern science, plants were consumed without any knowledge of the health benefits or side effects of the component compounds (Ma & Zhang, 2017).

*Borassus* is a genus comprising of six species of fan palm, found in tropical Africa (Salako *et al.*, 2015; Siebert & Struwig, 2019),

Asia (Eagleton, 2016; Pipatchartlearnwong *et al.*, 2017; Sakulsathaporn *et al.*, 2017) and New Guinea (Sudhakara *et al.*, 2013; Pangau-Adam & Muehlenberg; 2014; Jerry, 2018). *Borassus aethiopum*, an edible crop with highly nutritious fruits and sprouts, commonly called Palmyra or Palmyra palm, is one of these species (Ali *et al.*, 2010; Srinivasababu *et al.*, 2014). However, previous studies provide better insights on *Borassus flabellifer*. This species has a higher carbohydrate, fiber, amino acids, and protein content, contains essential phytochemicals, also exhibits significant antibacterial activity at the different parts of the plant such as root (Sahni *et al.*, 2014; Aziz *et al.*, 2016), leaves (Jamkhande *et al.*, 2016; Sarmini & Premaratne, 2018), fruit (Vijayakumari *et al.*, 2014; Singchai *et al.*,

2015), and seeds (Arunachalam *et al.*, 2011; Rahman *et al.*, 2020).

Meanwhile, *Culex quinquefasciatus* is a mosquito vector responsible for various diseases, including bancroftian filarial parasite, the major cause of human lymphatic filariasis (Sutthanont *et al.*, 2019). Velayutham *et al.* (2016) and Morejón *et al.* (2018) reported silver nanoparticles to have larvicidal properties, while Awwad & Salem (2012) the metal has been widely recognized due to the numerous applications in nanotechnology. Furthermore, AgNPs are one of the earliest synthesized nanoparticles and have long been used for medical purposes. However, the conventional methods of synthesis involve chemicals with harmful effects on humans and the environment (Chikkanna & Neelagund, 2018). Recently, alternative, environmentally friendly and cost effective techniques were developed to replace chemical and physical reduction methods (Kalpana & Rajeswari, 2018). This study therefore, aims to determine the phytochemicals present in Palmyra sprout extract, perform green synthesis of silver NPs with the sprout extract, and spectroscopic investigation using FT-IR and UV-Visible spectrophotometers, in addition to the main aim of evaluating the AgNPs' applications as a nano-larvicide. Palmyra palm sprout extract exhibits significant larvicidal potency, while its nanoparticles can be a better alternative to available chemicals to control and against mosquitos.

## MATERIALS AND METHODS

### Sample collection and preparation.

Palmyra sprouts were collected from Gombe metropolis, packaged in polyethylene bags, transported to the General Chemistry Laboratory II in Gombe State University, and then identified in the Laboratory of Botany, Department of Biological Science. Subsequently, the sprouts were washed with clean tap water, followed by distilled water, cut into tiny pieces, crushed using mortar and pestle, and then weighed. About 20 g of the sample was then mixed with 250 ml of distilled water and heated with a hot plate at 60°C, for 30 min. This was followed by cooling and

filtering, using Whatman No.1 filter paper. The filtrate was subjected to phytochemical screening, and stored at 4°C, prior to further analyses (Sivakumar, 2019).

**Phytochemical analyses.** Test for phenols and tannins (Lead acetate test) was performed by adding 0.5 ml of 1% lead acetate solution to 10 mg of the sprout extract. The formation of a white precipitate indicated the presence of tannins and phenolic compounds (Gibbs, 1974; Bhatt & Dhyani, 2012). Test for saponins was carried out by diluting 0.5 mg of extract in 20 ml distilled water, and shaking in a graduated cylinder for 15 min. The formation of emulsion up to 1 cm long, confirmed the presence of saponins (Odebisi & Sofowora, 1978; Bhatt & Dhyani, 2012). Test for coumarins was performed by adding 10% sodium hydroxide to 2 ml of the extract. The formation of yellow precipitate indicated the presence of coumarins (Schönberg & Aziz, 1955; Ryu & Yook; 1967). Test for quinones was carried out by treating 2 ml of the plant extract with 5 ml of HCl. The formation of a yellow precipitate indicated the presence of quinone (Loganathan *et al.*, 2017). For flavonoids test, 1 ml of the extract was treated with 1 ml of sulphuric acid. The formation of orange coloration confirmed the presence of a flavonoid (Tyagi, 2017). Test for glycosides was performed by dissolving 1 ml of the extract in distilled water and then adding aqueous NaOH solution. The formation of yellow coloration is indicative of glycoside presence (Jagessar, 2017). Test for steroids and sterols (Salkowski's test) was used 2 ml of extract dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turned red and lower layer turned yellow with green fluorescence, indicating the presence of the steroids and sterols in the extract (Singh & Kumar, 2017). Test for terpenoids was performed by treating 2 ml of the extract with 2 ml of acetic acid, followed by adding 2 ml of sulphuric acid. The formation of deep red coloration confirmed the presence of terpenoids (Harborne, 1978; Trease & Evans, 1989). Test for alkaloid was carried out by adding 3 ml of 1% HCl to 3 ml of the extract stirred on water bath for 20 min. The mixture

was then cooled and used to perform the Wagner and Mayer's test. A total of 1 ml of Wagner reagent was added drop-wise to the mixture and a reddish brown precipitate indicated alkaloid presence, while Mayer test was performed by adding Mayer reagent drop-wise to 1 ml of the mixture in a test tube and a greenish coloration or cream precipitate confirmed the presence of alkaloid (Harborne, 1978; Trease & Evans, 1989).

**Synthesis of silver nanoparticles.** A total of 100 ml of aqueous Palmyra sprout extract was mixed with 500 ml of 0.01 M silver nitrate ( $\text{AgNO}_3$ ) in a 1 L erlenmeyer flask. The mixture was then boiled at  $600^\circ\text{C}$  until the color changed to dark brown and a precipitate was formed. Subsequently, the mixture was left to stand for 24 h at  $27^\circ\text{C}$ , to enable the silver nanoparticles settle at the bottom. This was followed by filtration using Whatman No. 1 filter paper. The residue was then dried at  $105^\circ\text{C}$  in a hot air oven for 6 hours to obtain fine AgNPs (Banne *et al.*, 2017; Razali *et al.*, 2017).

**Ultraviolet-visible spectroscopic analysis.** For this characterization, two clean cuvettes, one filled with distilled water (blank solvent), and the other with the filtrate obtained from the AgNPs, were placed in the spectrometer. Subsequently, the absorbance values were recorded at intervals of 100 nm, from 200 nm to 800 nm, and a graph of absorbance against wavelength was plotted (Razali *et al.*, 2017 with modification).

**Fourier transform infrared spectroscopic analysis (FT-IR).** This characterization technique was performed to study the phytochemicals involved in the phyto-synthesis of AgNPs. A mixture comprising about 99% KBr and 1% NPs was crushed with a mortar and pestle and pelletized with a pelletizer and hydraulic press, then placed into FTIR sample holder and introduced into the infrared spectrophotometer (Sytu & Camacho, 2018).

**Larvicidal bioassay.** The AgNPs' larvicidal activity against *Culex quinquefasciatus* larvae was evaluated, as described by Lamayi *et al.* (2020). *Culex quinquefasciatus* larvae were obtained from stagnant water in Gombe metropolis and

divided into three groups based on growth stage. The third and the fourth instars were group in one because of the difficulty to obtain homogenous population. Meanwhile, a 100 ppm AgNPs stock solution was prepared, and used to produce other required subsequent concentrations through serial dilution. A paper cup was then filled with 100 ml of distilled water and used as the control sample. Subsequently, 100 ml of 5, 10, 20, 25, and 50 ppm AgNP solutions were prepared and evaluated for larvicidal activity on all the instars, and the larval mortality was recorded after 24 hours of exposure.

## RESULTS AND DISCUSSION

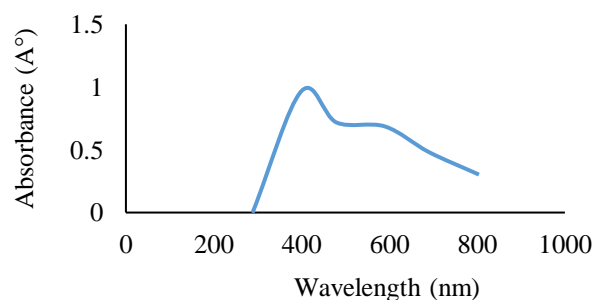
**Phytochemical screening.** Based on the results of phytochemical screening, alkaloids, saponins, flavonoids, and coumarins, were present in considerable quantity, glycosides and quinones were present in minute quantity, while terpenoids and steroids were absent (Table 1).

**Table 1.** Phytochemical screening of Palmyra palm.

Metabolites	Result
Alkaloids	++
Saponins	++
Flavonoids	++
Glycosides	+
Coumarins	++
Quinones	+
Steroids and Sterols	-
Terpenoids	-
Phenols and tannins	+

Notes: -not detected; +present; ++much present

A similar study by Alamelumangai *et al.* (2014) on *Borassus flabellifer* Linn, reported the presence of flavonoids, saponins, glycosides, tannins and terpenoids.

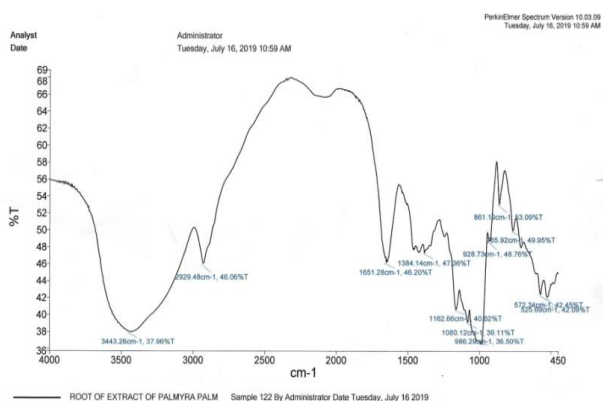


**Fig. 1.** UV-Visible spectrum for silver nanoparticles (AgNPs).

**Ultraviolet-visible spectroscopic analysis.** Fig. 1 shows the UV-Vis spectrum of AgNPs obtained by plotting absorbance against wavelength. The maximum absorbance, 0.968 was recorded at a wavelength of 390 nm, near the wavelength for violet color. This corresponds to the color change of the solution from pale yellow to dark brown, due to the excitation and vibration of bio-reducing and stabilizing agents. Elamawi *et al.* (2018) also

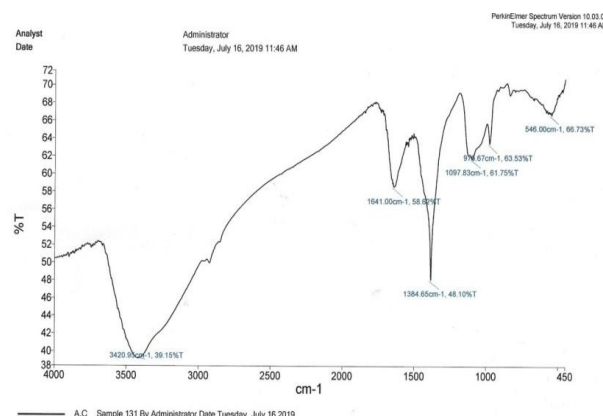
reported a similar maximum absorption value of 385 nm, for AgNPs.

**Fourier transform infrared spectroscopic analysis (FT-IR).** This was performed to study the interactions between the Palmyra sprout extract and aqueous silver nitrate salt. Fig. 2 and Fig. 3 show the FT-IR spectra of the extract and the AgNPs, respectively.



**Fig. 2.** FT-IR spectrum for the sprout extract of Palmyra palm.

Meanwhile, the major absorption bands in the raw extract's spectrum were attributed to various functional groups. The peak at 3443.26  $\text{cm}^{-1}$ , 37.96% T is attributed to O-H, -NH group of terpenoids, alkaloids other alcohols and phenols, the peak at 2929.48  $\text{cm}^{-1}$ , 46.06% T is probably due to  $\text{sp}^3$  C-H bond stretching, the



**Fig. 3.** FT-IR spectrum for AgNPs.

peak at 1651.28  $\text{cm}^{-1}$ , 48.20% T is possibly due to C=O or C=N groups in alkaloids and terpenoids, while the peaks at 1384.14  $\text{cm}^{-1}$ , 47.36% T and 1162.66  $\text{cm}^{-1}$ , 40.62% T are probably due to C-H rock bending vibration and C-C stretching in terpenoids, respectively.

**Table 2.** Larvicidal activity of synthesized silver nanoparticles.

Larvae	Conc.(ppm)	% mortality	LC <sub>50</sub>	LC <sub>90</sub>	95% Confidence		X <sup>2</sup>	R
					LC <sub>50</sub>	LC <sub>90</sub>		
1 <sup>st</sup> instar	5	45	6.042	118.144	3.354-	66.672-	0.654	0.863
	10	60			8.472	362.001		
	20	70						
	25	75						
	50	80						
2 <sup>nd</sup> instar	5	30	10.490	86.565	8.165-	59.112-	1.941	0.855
	10	50			12.781	158.833		
	20	70						
	25	70						
	50	80						
3 <sup>rd</sup> / 4 <sup>th</sup> instar	5	25	20.263	326.146	15.886-	120.155-	0.184	0.923
	10	38			26.925	1325.843		
	20	50						
	25	55						
	50	65						

The numerous peaks formed after the synthesis of the AgNPs (Fig. 3). The major absorption bands occurred at 3420.95  $\text{cm}^{-1}$ , 39.155% T, 1641.00  $\text{cm}^{-1}$ , 58.62% T, 1384.64  $\text{cm}^{-1}$ , 48.10% T. Furthermore, the disappearance of peaks at 2929.48  $\text{cm}^{-1}$ , 46.06% T, 986.29  $\text{cm}^{-1}$ , 36.50% T, as well as the other bands between 450  $\text{cm}^{-1}$  to 1500  $\text{cm}^{-1}$ , confirms the formation of silver nanoparticles as previously explained by Danbature *et al.* (2020a).

**Larvicidal activity.** Table 2 is the result of the larvicidal activity assay of silver nanoparticles at various concentrations (5, 10, 20, 25, 50) against the first, second and third/fourth instars of mosquito larvae. These evaluations were repeated twice and the average values were computed. For the first instar, the  $\text{LC}_{50}$  (lethal concentration at 50%) was discovered to be 6.042 ppm, while the  $\text{LC}_{90}$  (lethal concentration at 90%) was found to be 118.144 ppm, with a correlation of 0.863. Meanwhile, for the second instar, the  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values were discovered to be 10.490 ppm and 86.565 ppm, respectively, with a correlation of 0.855. In addition, the  $\text{LC}_{50}$  and  $\text{LC}_{90}$  for the third/fourth instar, were found to be 20.263 ppm and 326.146 ppm, respectively, with a correlation of 0.923. The nanoparticles obtained from this synthesis were discovered to be less active compared to the report by Danbature *et al.* (2020b), and to be more active compared to the study by Shehu *et al.* (2020), at all concentrations. It is reasonable to state that the mortality rates were positively correlated with the concentration of AgNPs. Bioactive constituents of Palmyra are proving valuable compounds as an ideal eco-friendly approach in controlling the mosquito larvae population.

## CONCLUSION

The phytochemical screening of *Borassus aethiopum* sprout extract confirmed the presence of seven out of the nine secondary compounds studied. From this evaluation, alkaloids, saponins, glycosides, quinones, phenols, tannings, coumarins and flavonoids were discovered to be present within the extract, while terpenoids and sterols/steroids,

were not detected. The detected phytochemicals were responsible for the reduction of Ag ion and the formation of silver nanoparticles. Furthermore, the FT-IR and UV-Visible spectrophotometric characterizations confirmed the formation of AgNPs. The synthesized nanoparticles were also discovered to exhibit significant larvicidal potency, hence, there is a need to fully characterize the AgNPs and carry out further studies regarding the potential applications against the organism.

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