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PHYTOCHEMICAL COMPOSITION AND FUNCTIONAL GROUP PROFILE OF *Millettia aboensis* EXTRACTS TO ELUCIDATE THEIR BIOCHEMICAL BASIS FOR LARVICIDAL ACTIVITY AGAINST *Anopheles* AND *Culex* MOSQUITOES

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ABSTRACT
Mosquitoes are major vectors of life-threatening diseases and their control is increasingly challenged by the emergence of insecticide resistance. *Millettia aboensis* has demonstrated promising larvicidal activity against mosquito species. This study evaluated the phytochemical composition and functional group profile of *M. aboensis* extracts to elucidate the biochemical basis of their larvicidal activity against *Anopheles* and *Culex* mosquitoes. Plant materials collected from Lafia metropolis were extracted by maceration and subjected to qualitative and quantitative phytochemical analyses, alongside Fourier Transform Infrared (FTIR) spectroscopy. Qualitative screening revealed the presence of alkaloids, flavonoids, saponins, steroids, terpenoids, cardiac glycosides, phenols, and anthraquinones, with the leaf extracts showing greater phytochemical diversity and abundance of active constituents. Quantitative analysis indicated that saponins (18.78 mg/100 g) and steroids (16.65 mg/100 g) were predominantin the leaf extract. The FTIR spectroscopy further validated these findings by identifying characteristic absorption bands corresponding to hydroxyl (–OH), carbonyl (C=O), amine (–NH), ether (C–O–C), and chloro (C–Cl) groups, which are associated with alcohols, phenols, carboxylic acids, and alkaloids. The presence of these functional groups suggests multiple larvicidal mechanisms, including oxidative stress induction, neurotoxic interference, enzymatic inhibition, and cuticular disruption. The integrated phytochemical and FTIR analyses provide molecular evidence supporting the larvicidal potency of *M. aboensis*, highlighting its potential as a sustainable botanical alternative to synthetic larvicides. The findings establish *M. aboensis* as a promising bio-resource for the development of eco-friendly vector control agents and contribute to the growing body of knowledge on plant-based mosquito management strategies.

Keywords: FTIR, *Millettia aboensis*, Functional group, Phytochemical, Plant extracts

INTRODUCTION
Mosquitoes (Order Diptera, Family Culicidae) are small, slender, holometabolous insects that typically measure between 3 and 6 mm in length. Their evolutionary success and global persistence are largely attributed to ecological plasticity, physiological adaptability, and specialized suctorial mouthparts that enable hematophagy. To date, over 3,500 mosquito species across approximately 40 genera have been documented worldwide (Livinus *et al*., 2022; Singh *et al*., 2024; Joshua, 2025). Except for a few extreme polar and isolated oceanic regions, mosquitoes inhabit nearly every terrestrial environment on Earth (Singh *et al*., 2024; Walter Reed Biosystematics Unit [WRBU], 2024). Although only a fraction of species feed on humans, those that do constitute the most important disease vectors known to science. Globally, illnesses like as malaria, filariasis and dengue infections continue to impose a disproportionate health and socioeconomic burden, particularly in sub-Saharan Africa (George *et al*., 2023).

Nigeria remains one of the most heavily affected nations: the World Malaria Report (2024) reported that Nigeria accounts for approximately 27 % of global cases of malaria infection, with about 31 % deaths, and children below five years as well as pregnant women the most prone. The principal mosquito genera of medical importance in Nigeria include *Anopheles*, the primary malaria vector, and *Culex*, which transmits lymphatic filariasis and several arboviruses. Their extensive distribution, breeding diversity, and behavioral plasticity contribute to the persistence of these diseases in both rural and urban habitats (Bhuvaneswari *et al*., 2023; World Health Organization [WHO], 2024; Usman, 2025).

Historically, vector-control programs have relied heavily on synthetic insecticides such as pyrethroids, organophosphates, and carbamates applied by indoor residual spraying (IRS), insecticide-treated nets (ITNs), and larvicidal applications. The consistent and indiscriminate use of these has however led to widespread insecticide resistance, driven by mechanisms including metabolic detoxification, target-

site mutations, and behavioral avoidance (Siddiqui *et al*., 2023; WHO, 2023). The escalation of resistance now undermines the long-term efficacy of conventional chemical interventions, posing a major obstacle to malaria elimination goals.

Consequently, scientific attention has increasingly shifted toward discovering eco-friendly and sustainable alternatives to synthetic insecticides. Botanically derived insecticides are particularly attractive because they are biodegradable, locally available, cost-effective, and pose minimal environmental risk (Rattan, 2019; Ngegba *et al*., 2022; Ullah *et al*., 2022). One promising species in this regard is *Millettia aboensis* (Fabaceae), locally known as *Odudu* in Nigeria. The genus *Millettia* plays a pivotal role in African ethnomedicine and local livelihoods. Approximately 59 % of known *Millettia* species have documented medicinal applications, reflecting the genus's high therapeutic potential and cultural significance (Chen & Lou, 2025). Traditionally, species within this genus are employed to treat diverse ailments, including diabetes mellitus, hypertension, inflammatory disorders, and microbial infections (Olaniyi *et al*., 202).

In southern Nigeria, *M. aboensis* - an endemic but under-studied species - occupies a vital place in indigenous healthcare systems. Decoctions, infusions, and poultices prepared from its leaves, bark, seeds, and roots are used for both preventive and curative purposes. Phytochemical analyses have identified flavonoids, alkaloids, tannins, terpenoids, and phenolic compounds as the major constituents, many of which demonstrate antioxidant, antimicrobial, hematopoietic, and anti-inflammatory activities (Bello *et al*., 2024; Joshua *et al*., 2025). Traditionally used in ethnomedicine for treating fever, malaria, and parasitic infections, *M. aboensis* contains diverse phytochemicals - such as alkaloids, flavonoids, and terpenoids, with demonstrated insecticidal and antimicrobial potential (Igoli *et al*., 2005; Joshua *et al*., 2025). Despite these promising attributes, limited empirical evidence exists regarding the entomological efficacy and resistance dynamics of *M. aboensis* extracts against local mosquito populations. Furthermore, the comparative potency of its various plant parts, leaves, seeds, and bark remains poorly understood, even though preliminary findings suggest differing phytochemical compositions (Igoli et al., 2005).

Accordingly, this study investigates the phytochemical composition and functional group profile of crude methanolic extracts from the leaf, seed, and bark of *M. aboensis*, and evaluates their relevance to larvicidal activity against *Anopheles* and *Culex* mosquitoes in Lafia, Nasarawa State, a region characterized by high malaria endemicity and ecological diversity. The findings are expected to support the development of locally sourced, plant-based vector control strategies aligned with sustainable public health interventions.

Figure 1: Leaves and flowers of *Millettia aboensis* (Field Photo, 2025)

MATERIALS AND METHODS

Study Area

The study was conducted at the Federal University of Lafia, Nasarawa State, north-central Nigeria (8°29′N, 8°51′E). Lafia has an estimated population of 330,712 based on the 2006 National Population Census. The area is characterized by agrarian activities, including the cultivation of yam (*Dioscorea* spp.), cassava (*Manihot esculenta*), rice (*Oryza sativa*), maize (*Zea mays*), and legumes. The presence of irrigation practices and stagnant water bodies provides favorable breeding conditions for mosquitoes, contributing to the transmission of malaria and lymphatic filariasis.

Plant Material Collection and Authentication

Fresh leaves of *Millettia aboensis* were collected from farmlands within Lafia metropolis, Nasarawa State, Nigeria. The plant was identified and authenticated at the Herbarium Unit, Federal College of Forestry, Jos, Plateau State, and a voucher specimen (No. 235) was deposited. The leaves were washed, shade-dried at ambient temperature (29 ± 1 °C) for three weeks, and pulverized into fine powder using a mechanical grinder. The powdered samples were stored in airtight containers at room temperature until extraction (Handa *et al*., 2008; Joshua *et al*., 2025).

Preparation of Plant Extracts

Extraction was performed using both methanol and aqueous solvents following the maceration method described by Handa *et al*. (2008) with slight modifications.

Methanol Extraction

Approximately 400 g of *M. aboensis* leaf powder was weighed and divided into two equal portions (200 g each). Each portion was soaked in 2 L of methanol in separate amber bottles, maintaining a 1:10 w/v ratio (100 g of plant material per 1 L solvent). The mixtures

were intermittently agitated and left to stand for 72 hours at room temperature to facilitate solvent penetration and dissolution of bioactive compounds. After maceration, the mixtures were filtered using non-absorbent cotton wool followed by Whatman No. 1 filter paper. The combined filtrates were concentrated to dryness in clean, grease-free aluminum trays at ambient temperature (31 °C). The resulting crude extract was carefully scraped, transferred into sterile labeled containers, and stored in a refrigerator (4 °C) until required for analysis.

Phytochemical Screening and Analytical Procedures
Phytochemical screening was carried out to identify the major classes of secondary metabolites present in both the aqueous and methanol leaf extracts of *M. aboensis*. These bioactive compounds are responsible for the plant's therapeutic, insecticidal, and antimicrobial properties (Ajima *et al*., 2021; Desta & Abd El-Aty, 2023; Onwudiwe *et al*., 2023; Joshua *et al*., 2025). The analysis employed standardized qualitative and quantitative protocols as described by Sofowora (1993), Trease and Evans (2002), and subsequently refined by Harborne (1998), Edeoga *et al*. (2005), Usman *et al*. (2007, 2009) with modifications to align with current phytochemical research standards (WHO, 2023).

Qualitative Phytochemical Screening
The qualitative analysis involved testing for the presence of key secondary metabolites including alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, glycosides, and phenolic compounds. The following standard procedures were adopted:

Test for Alkaloids (Dragendorff's and Mayer's Tests)
A portion of the extract was acidified with 1 % hydrochloric acid and filtered. Filtrates were treated separately with Dragendorff's and Mayer's reagents. The appearance of an orange-brown precipitate (Dragendorff's) or creamy-white precipitate (Mayer's) indicated the presence of alkaloids (Harborne, 1998).

Test for Flavonoids (Alkaline Reagent and Lead Acetate Tests)
Small portions of the extract were treated with 10 % sodium hydroxide, producing an intense yellow coloration that disappeared upon addition of dilute hydrochloric acid. Similarly, the addition of 10 % lead acetate produced a yellow precipitate, confirming the presence of flavonoids (Edeoga *et al*., 2005).

Test for Saponins (Frothing Test)
About 2 mL of the extract was vigorously shaken with 5 mL of distilled water and allowed to stand for 15 min. Persistent frothing indicated the presence of saponins (Sofowora, 1993).

Test for Tannins (Ferric Chloride Test)
A few drops of 5 % ferric chloride solution were added to 2 mL of the extract. The formation of a dark green or blue-black coloration confirmed the presence of tannins (Trease & Evans, 2002).

Test for Terpenoids (Salkowski Test)
Five milliliters of the extract were mixed with 2 mL of chloroform and 3 mL of concentrated sulfuric acid. The appearance of a reddish-brown interface indicated terpenoids (Harborne, 1998).

Test for Steroids (Liebermann–Burchard Test)
The extract was mixed with acetic anhydride and a few drops of concentrated sulfuric acid. A bluish-green coloration indicated the presence of steroids (Edeoga *et al*., 2005).

Test for Glycosides (Keller–Killiani Test)
Two milliliters of the extract were treated with glacial acetic acid containing ferric chloride, followed by the addition of concentrated sulfuric acid. A brown ring at the interface indicated the presence of cardiac glycosides (Trease & Evans, 2002).

Test for Phenolic Compounds
A small quantity of extract was treated with 5 % ferric chloride solution. The development of a bluish-green color confirmed the presence of phenolic compounds (Sofowora, 1993).

Quantitative Phytochemical Analysis
Quantitative estimation of the major bioactive constituents was performed to determine their relative concentrations in the methanol and aqueous extracts. Analytical determinations followed procedures described by Obadoni and Ochuko (2001) and Edeoga *et al*. (2005), with the following parameters measured:

Alkaloid Content
Determined by acid-base titration using 10% acetic acid in ethanol and precipitation with ammonium hydroxide.

Flavonoid Content
Quantified by the aluminum chloride colorimetric method using quercetin as standard.

Saponin Content
Determined by gravimetric method following aqueous ethanol extraction and n-butanol partitioning.

Tannin Content
Measured using the Folin–Denis spectrophotometric method at 760 nm.

Phenolic Content
Estimated using the Folin–Ciocalteu reagent with gallic acid as the standard reference compound.

Terpenoid and Steroid Content
Quantified spectrophotometrically at 538 nm following ethanol extraction and sulfuric acid treatment. All spectrophotometric measurements were carried out using a UV–Visible Spectrophotometer (Shimadzu

Model UV-1800). Each assay was conducted in duplicate and presented as mean values.

Data Analysis

All data were analyzed using R Statistical Software (Version 2.14.0). Mean values of replicate Quantitative analysis were obtained using Standard deviation.

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening of *M. aboensis* Plant Extracts

Qualitative Phytochemical screening of *M. Aboensis* parts revealed eight (8) active ingredients present (Table 1). The Methanol extract showed high presence of saponins, steroids, terpenoids and alkaloids in the leaf; cardiac glycosides were present in high concentration in the Seed and Bark. Moderately high concentrations of flavonoids and anthraquinones were found in all the extracts while phenols were either not detected, or present in low concentration in the extracts.

Table 1: Qualitative phytochemical screening of *M. aboensis* plant extracts

Phytochemicals	Leaf	Seed	Bark
Saponins	+++	++	++
Flavonoids	++	++	++
Steroids	+++	++	++
Cardiac glycosides	++	+++	+++
Phenols	-	++	+
Terpenoids	+++	++	++
Alkaloids	+++	++	++
Anthraquinones	++	++	++

+++ = Present in very high concentration; ++ = Present in moderately high concentration; + = Present in low concentration; – = Not detected

Table 2: Quantitative phytochemical screening of *M. aboensis* plant extracts

Phytochemical	Leaf	Seed	Bark
Alkaloids	15.95 ± 0.08	7.47 ± 0.12	10.45 ± 0.11
Steroids	16.65 ± 0.09	4.94 ± 0.10	2.89 ± 0.15
Terpenoids	15.70 ± 0.28	7.48 ± 0.05	10.89 ± 0.25
Flavonoids	5.83 ± 0.20	6.83 ± 0.18	7.38 ± 0.08
Anthraquinones	7.52 ± 0.13	7.49 ± 0.17	7.49 ± 0.04
Saponins	18.78 ± 0.17	7.65 ± 0.09	7.02 ± 0.06

*Values represent mean ± standard deviation of two determinations

Quantitative Phytochemical Screening of *M. aboensis* Plant Extracts

The quantitative analysis of *M. aboensis* extracts (Table 2) revealed variations in phytochemical concentrations among the leaf, seed, and bark. The leaf extract recorded the highest levels of saponins (18.78 mg/100 g) and steroids (16.65 mg/100 g), while the seed extract exhibited the lowest concentrations of most phytochemicals. Flavonoids were moderately higher in the bark (7.38 mg/100 g) compared to the leaf (5.83 mg/100 g). Anthraquinones were relatively uniform across all extracts (7.49–7.52 mg/100 g). These results suggest that the leaf part of *M. aboensis* may be a richer source of bioactive compounds than the seed or bark.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

Results obtained from the FTIR Analysis are presented in Tables 3–5 as well as Figs 2 – 4, and reveal the presence of several prominent absorption bands indicative of diverse phytochemical constituents. In all three extracts, major peaks corresponding to hydroxyl (–OH), amine (–NH), and carbonyl (C=O) groups were observed within the ranges of 3200–3500 cm^{-1} and 1600–1750 cm^{-1}. These functional groups indicate the presence of alcohols, phenols, amides, carboxylic acids, and carbonyl compounds commonly found in flavonoids, tannins, and saponins.

FTIR Spectrum of *Millettia aboensis* Leaf Extract

The FTIR analysis of the *M. aboensis* leaf extract revealed major absorption peaks at 3285, 2918, 1604, 1377, and 831 cm^{-1}, which correspond to hydroxyl (O–H), carbonyl (C=O), amine (N–H), and chloro (C–Cl) functional groups. The strong band at 3285 cm^{-1} suggests hydrogen-bonded O–H stretching vibrations, indicating the presence of alcohols, phenols, and possibly carboxylic acids. The peaks between 1707 and 1604 cm^{-1} represent carbonyl stretching typical of aldehydes, ketones, and esters, while those around 1377–1244 cm^{-1} confirm C–O stretching of phenolic compounds. The presence of absorption bands between 831 and 766 cm^{-1} indicates C–Cl stretching, signifying halogenated organic compounds (Table 3, Fig. 2).

These results imply that the leaf extract contains a complex mixture of organic compounds, including alcohols, phenols, aldehydes, esters, and chloro-organics, which support the earlier quantitative findings on the presence of flavonoids, tannins, alkaloids, and other phenolic constituents. The abundance of O–H and C=O functional groups may account for the plant's reported antioxidant and antimicrobial activities.

FTIR Spectrum of *Millettia aboensis* Seed Extract

The FTIR spectrum of the seed extract displayed characteristic absorption peaks at 3237, 3009, 2109, 1986, and 1032 cm^{-1}, corresponding to hydroxyl (O–H), amine (N–H), carbonyl (C=O), and ether (C–O–C) groups. The strong O–H stretching vibration at 3237 cm^{-1} reflects the presence of alcohols and phenolic compounds, while the peaks between 2900 and 3009 cm^{-1} denote aliphatic C–H stretching vibrations. The absorption bands at 2109 and 1986 cm^{-1} are attributed to C=O stretching of aldehydes, ketones, and esters. Furthermore, the peak observed at 1032 cm^{-1} corresponds to C–O stretching vibrations, indicative of ethers or glycosidic linkages (Table 4, Fig. 3). The presence of these functional groups suggests that the seed extract contains alcohols, phenols, amines, aldehydes, and esters, consistent with the phytochemical presence of saponins, glycosides, and polyphenols. These compounds are known to contribute to the antioxidant and antimicrobial potential of plant-

derived products. The FTIR results, therefore, reinforce the role of *M. aboensis* seeds as a potential source of bioactive compounds.

FTIR Spectrum of *Millettia aboensis* Bark Extract

The FTIR analysis of the bark extract exhibited prominent absorption peaks at 3256, 2920, 2851, 1604, 1157, and 766 cm^{-1}, corresponding to hydroxyl (O–H), aliphatic C–H, carbonyl (C=O), amine (C–N), and C–Cl stretching vibrations. The strong O–H stretching band at 3256 cm^{-1} confirms the presence of alcohols and carboxylic acids, while the carbonyl peaks around 1604–1709 cm^{-1} indicate aldehydes and ketones. The absorptions at 1157 and 1030 cm^{-1} are due to C–N and C–O stretching, suggesting the presence of amines and nitro compounds, whereas the low-frequency bands between 878 and 766 cm^{-1} are characteristic of C–Cl bonds (Table 5, Fig. 4). These observations imply that the bark extract of *M. aboensis* contains hydroxyl, carbonyl, amine, and chloro groups, indicative of tannins, alkaloids, and phenolic acids. The presence of these bioactive compounds supports the plant's traditional use for medicinal and antimicrobial applications.

Table 3: FTIR – functional group analysis for *M. aboensis* leaf extract

Group Freq. (cm^{-1})	Functional Group	Observed Freq. (cm^{-1})	Vibration Assignment
3750-3500	O-H	3285,2918 2849	Hydroxyl group H-bonded OH stretch. Monomeric alcohols, phenols, Carboxylic acids
3570-3200	N-H, O-H	3285,2918	Amine hydroxyl overlap
3300-2900	N-H	2189	Amine stretch
3000-2700	C-H	2849, 2374	Aromatic ring C-H, Alkenes
675-870	C-H	669	Aromatic ring C-H
3200-3600	C-H	3000,3100	Hydroxyl group H-bonded, Alcohol, Phenols
1900-1650	C=O	1604, 1707	Aldehyde, Alkenes, Ketone, Carboxylic acid stretch, Esters
1300-1200	C-O	1377,1244	Phenol CO stretch
1090-1020	C-N, C-O	1034	Primary Amine CN Carbonyl
1300-1500	NO_2, C=	1377,1375	Nitro Compounds
900-860	C - Cl	881,818,	Chloro compound C-Cl stretch
800-700	C-Cl	831, 766	Chloro compound C-Cl stretch

Table 4: FTIR – functional group analysis for *M. aboensis* seed extract

Group Freq. (cm^{-1})	Functional Group	Observed Freq. (cm^{-1})	Vibration Assignment
3570-3200	N-H, O-H,	3237,	Amine hydroxyl overlap
3300-2900	N-H, C-H	2900, 3009, 2885	Amine stretch
3000-2700	C-H	2853, 2374	Aliphatic C-H, Alkenes
3200-3600	C-H	3334,3477	Hydroxyl group H-bonded, Alcohol, Phenols
1900-1650	C=O	2109, 1986	Aldehyde, Ketone, Carboxylic acid stretch, Esters
2500-3000			
1300-1200	C-O	1032	Phenol CO stretch
1090-1020	C-N, C-O	1038	Primary Amine CN Carbonyl
1300-1500	NO_2	1407,1407	Nitro Compounds
900-860	C-Cl	862	Chloro compound C-Cl stretch
800-700	C-Cl	831, 719	Chloro compound C-Cl stretch

Table 5: FTIR – functional group analysis for *M. aboensis* bark extract

Group Freq. (cm^{-1})	Functional Group	Observed Freq. (cm^{-1})	Vibration Assignment
3750-3500	O-H	3256, 2920, 2851	Hydroxyl group H-bonded OH stretch, Carboxylic acid, Amine stretch
3000-2700	C-H, N-H	2851, 2920	Aliphatic C-H, Alkenes Hydroxyl group H-bonded, Alcohol, Phenols
1900-1650	C=O	1604, 1709	Aldehyde, Ketone, Carboxylic acid stretch, Esters
1500-1600	C=O	1575,	Phenol CO stretch, Alcohols, ethers, Carboxylic acids.
1090-1020	C-N, C-O	1157,1030	Primary Amine CN Carbonyl
1300-1500	NO_2	1438,	Nitro Compounds
900-860	C-Cl	878, 8219	Chloro compound C-Cl stretch
800-700	C-Cl	766	Chloro compound C-Cl stretch

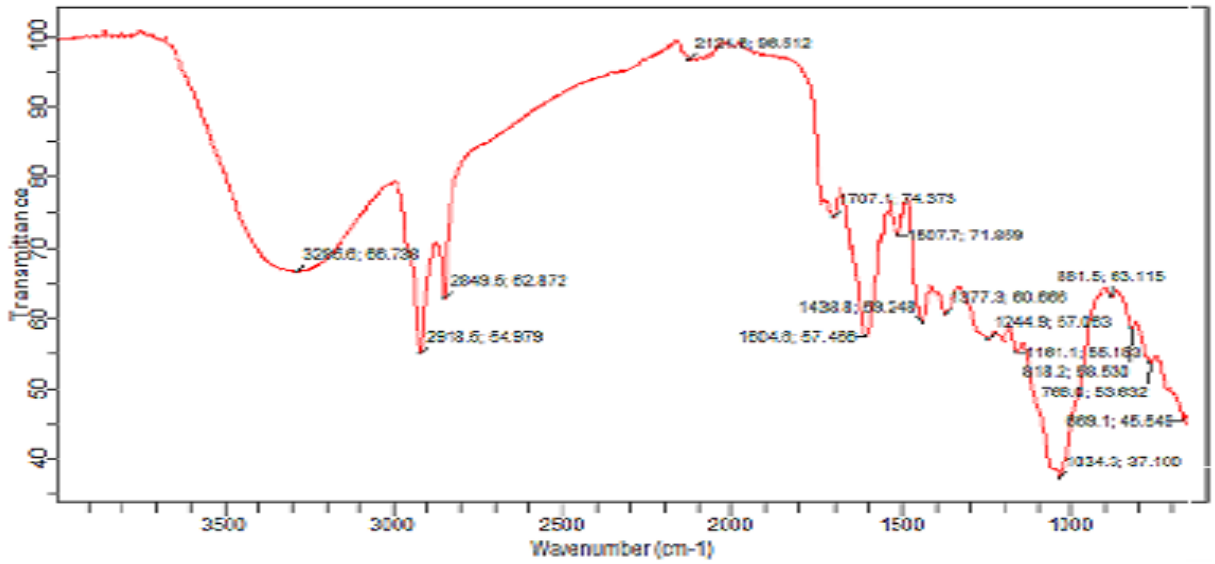

Figure 2: FTIR spectrum of *Millettia aboensis* leaf extract

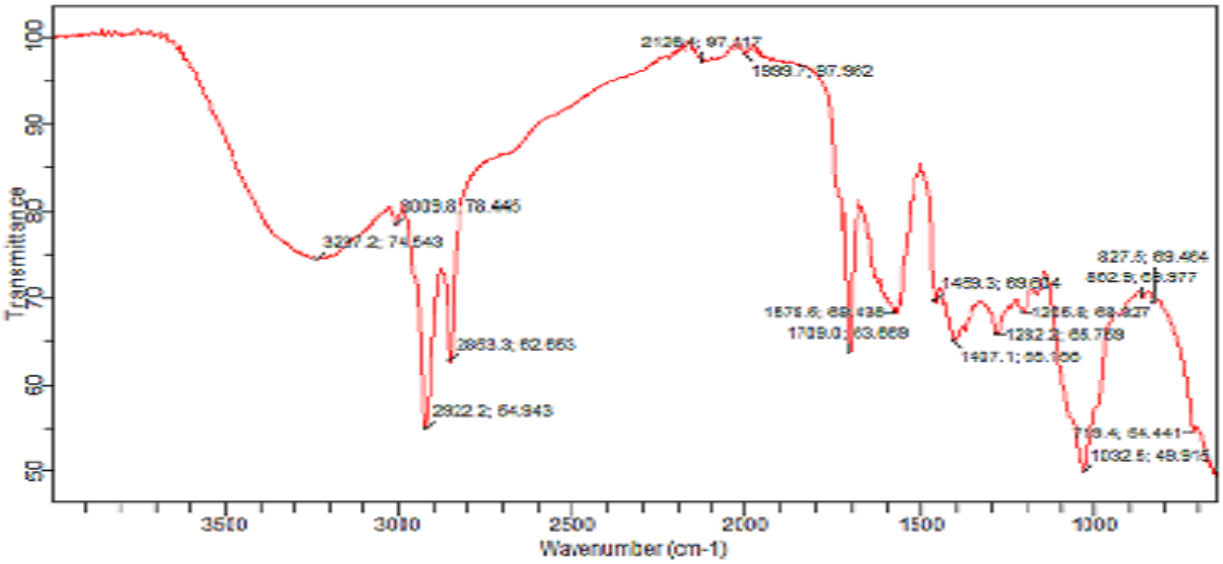

Figure 3: FTIR spectrum of *Millettia aboensis* seed extract

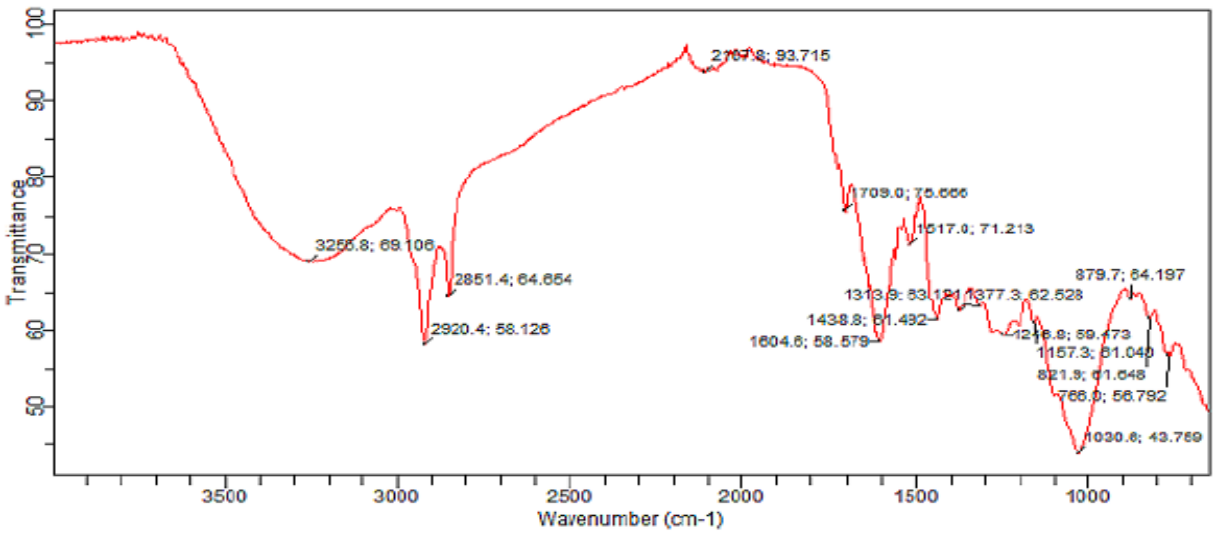

Figure 4: FTIR spectrum of *Millettia aboensis* bark extract

Table 6: Correlation of FTIR findings and documented larvicidal potency

Functional Group	Possible Compound Type	Biological Effect on Larvae	Reference
O–H (Hydroxyl)	Phenols, flavonoids	Induces oxidative stress; disrupts membranes	Mane & Khilare (2021)
C=O (Carbonyl)	Aldehydes, ketones	Denatures larval proteins and enzymes	Singh *et al*. (2022)
–NH / C–N	Alkaloids, amines	Neurotoxicity via acetylcholinesterase inhibition	Shaheen *et al*. (2022)
C–O–C (Ether)	Glycosides, esters	Respiratory interference and cuticular blockage	Shaheen *et al*. (2022)
C–H (Aliphatic)	Terpenoids, hydrocarbons	Cuticle disruption and dehydration	Govindarajan *et al*. (2021)

Correlation of FTIR Findings and Documented Larvicidal Potency

Table 6 summarizes the correlation between functional groups identified via FTIR and their biological significance in larvicidal mechanisms in Literature. The strong presence of these bioactive functional groups provides molecular justification for the high mortality rates recorded in bioassays. Similar associations have been reported in *Cassia fistula*, *Azadirachta indica*, and *Ocimum gratissimum* extracts exhibiting comparable FTIR profiles.

The qualitative phytochemical analysis of *Millettia aboensis* revealed the presence of several secondary metabolites known for their biological and larvicidal activities. The major phytochemical constituents detected across the leaf, stem bark, and seed extracts included alkaloids, flavonoids, saponins, phenols, glycosides, steroids, anthraquinones and terpenoids, though their concentrations varied among plant parts. The leaf extract was generally richer in alkaloids, steroids, terpenoids and saponins. This slightly agrees with the findings of Nandagopal and Raju (2025) who also reported similar metabolite, but with the absence of glycoside content. The seed and bark showed high concentration of glycosides. The distribution pattern suggests a compartmentalization of bioactive molecules within the plant tissues, likely reflecting their adaptive defense functions. The result therefore, is somewhat consistent with research by Senthilkumar *et al*. (2018), Edward *et al*. (2023), Usman and Abdulkarim (2023) and Joshua *et al*. (2025b) which found that *Sida acuta* contained alkaloids, saponin, steroids, phenols, tannins, flavonoids, terpenoids, and hydrogen cyanide

Flavonoids have been widely reported to exert larvicidal effects through oxidative stress induction, disruption of cellular metabolism, and inhibition of essential enzymatic systems in insect larvae (Shaalan *et al*., 2005; Şengül-Demirak & Canpolat, 2022). The abundance of flavonoids across the extracts of *M. aboensis* therefore points to a possible link between these compounds and possible larval mortality. Alkaloids present in many plant extracts can exert larvicidal and neurophysiological effects on mosquito larvae by inhibiting key neuronal enzymes such as acetylcholinesterase and interfering with ion channels and neurotransmission pathways, leading to impaired nerve function, paralysis, and increased larval mortality (Ganesan *et al*., 2023). Alkaloids are known to act on acetylcholinesterase enzymes, leading to paralysis and eventual death of insect larvae. Saponins, which are plant-derived glycosides with surfactant properties that can disrupt membrane permeability and cause cell lysis, have been associated with larvicidal activity in mosquito larvae. For example, saponin isolated from *Achyranthes aspera* exhibited potent larvicidal effects against *Aedes aegypti* and *Culex quinquefasciatus* larvae in laboratory bioassays (Bagavan *et al*., 2008). The presence of phenolic compounds and terpenoids further enhances the insecticidal spectrum of *M. aboensis*. Phenols are reactive compounds that interfere with respiratory enzymes, while terpenoids disrupt hormonal regulation of growth and molting in mosquitoes. The combined action of these metabolites suggests that *M. aboensis* operates through multiple physiological and biochemical pathways, resulting in an efficient and multifaceted larvicidal response (Isman, 2020).

The leaf extract recorded the highest levels of saponins and steroids, while the seed extract exhibited the lowest concentrations of most phytochemicals. Flavonoids were moderately higher in the bark compared to the leaf, while anthraquinones were relatively uniform across all extracts. These results suggest that the leaf part of *M. aboensis* may be a richer source of bioactive compounds than the seed or bark. These quantitative differences suggest that the biosynthetic pathways for phenolic and nitrogenous metabolites vary among plant organs. Leaves, being the primary site of photosynthesis and secondary metabolite synthesis, often accumulate higher amounts of antioxidant compounds such as flavonoids and phenols (Singh *et al*., 2022). In contrast, bark tissues, which serve a protective role, tend to be richer in alkaloids and tannins that deter herbivory and microbial invasion (Mane & Khilare, 2021).

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the characteristic functional groups present in the leaf, seed, and bark extracts of *M. aboensis*. The FTIR spectra provide qualitative evidence of the diverse phytochemical constituents that correspond to bioactive compounds known to contribute to larvicidal activity. The analysis complements the quantitative phytochemical data by confirming the presence of key metabolites such as alkaloids, flavonoids, tannins, phenols, and saponins. The correlation of these functional groups with larvicidal activity is vital for understanding the biochemical mechanisms underlying the insecticidal efficacy of *M. aboensis* extracts.

The functional groups detected through FTIR are well-known for their larvicidal and insecticidal properties. Hydroxyl and carbonyl-containing compounds (phenols and flavonoids) act as strong antioxidants capable of generating reactive oxygen species (ROS) that damage

larval tissues and disrupt metabolic enzymes (Mane & Khilare, 2021). Additionally, alkaloids and amines interfere with the acetylcholinesterase enzyme, leading to neuromuscular paralysis and eventual larval death (Shaheen *et al*., 2023). The seed extract's ester and saponin-related bands indicate surface-active compounds that reduce water surface tension, impairing larval respiration by blocking the spiracular openings. Terpenoids identified in the bark extract contribute to cuticle disruption and desiccation (Govindarajan *et al*., 2021; Shaheen et al., 2022). In line with previous findings by Rajkumar and Jebanesan (2019) and Govindarajan *et al*. (2021), phytochemicals containing these functional groups have demonstrated strong larvicidal efficacy against *Anopheles gambiae* and *Culex quinquefasciatus*.

The integration of the phytochemical and FTIR data provides a biochemical basis for understanding the larvicidal potency of *M. aboensis* against *Anopheles gambiae* and *Culex quinquefasciatus*. The diversity of metabolites and functional groups suggests that larval mortality results from a combination of toxic, oxidative, and physiological disruptions rather than a single mode of action. For instance, the presence of flavonoids and phenolics may induce oxidative stress by generating reactive oxygen species within larval cells, damaging vital biomolecules such as lipids, proteins, and DNA (Elumalai et al., 2021). Alkaloids could further interfere with neuromuscular coordination, leading to paralysis and death. Saponins' surfactant action may destabilize larval membranes, increase permeability and causing electrolyte imbalance, while terpenoids and tannins may inhibit chitin synthesis and hinder normal molting cycles (Pavela & Benelli, 2019).

CONCLUSION

This study demonstrated that *Millettia aboensis* possesses a rich array of phytochemicals with possible larvicidal potential against *Anopheles gambiae* and *Culex quinquefasciatus*. The qualitative and quantitative analyses revealed that alkaloids, flavonoids, saponins, terpenoids, steroids, phenols, and anthraquinones occur abundantly in different plant parts, particularly the leaves. The Fourier Transform Infrared (FTIR) analysis further confirmed the presence of key functional groups - hydroxyl, carbonyl, and amine groups - associated with larvicidal bioactivity. The study established that possible larval mortality in exposed mosquito populations likely results from synergistic biochemical mechanisms involving oxidative stress induction, neuro-inhibition, membrane destabilization, and interference with molting and respiratory pathways. These multiple modes of action, arising from the interplay of secondary metabolites, suggest that *M. aboensis* is capable of exerting potent, broad-spectrum larvicidal effects with a low risk of resistance development. By demonstrating the biochemical basis for its larvicidal efficacy and validating its traditional ethnobotanical relevance, this research provides strong scientific evidence supporting the potential use of *M. aboensis* as an eco-friendly biolarvicide for integrated vector management.

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