

## PHYTOCHEMICAL INVESTIGATION OF METHANOLIC LEAF EXTRACT OF *PHYLLANTHUS NIRURI* USING FOURIER-TRANSFORM INFRARED SPECTROSCOPY AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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### ABSTRACT

Medicinal plants have been used for millennia to treat a wide variety of ailments and promote health and well-being. This study analyzed the chemical composition of methanolic leaf extracts of *Phyllanthus niruri* using Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GCMS). FTIR identified functional groups including methyl ( $2922\text{ cm}^{-1}$ ), hydroxyl ( $2858\text{ cm}^{-1}$ ), carbonyl ( $1707\text{ cm}^{-1}$ ), alkyl ( $1452\text{ cm}^{-1}$ ), and ester ( $1031\text{ cm}^{-1}$ ) while GCMS detected 15 compounds with the most abundant being octadecanoic acid (23.84%) and hexadecanoic acid, methyl ester (18.25%). Other relatively abundant compounds included 12,15-octadecadienoic acid, methyl ester (5.38%) and tetradecanoic acid (3.88%). Many of the identified compounds are fatty acids and derivatives documented to have hepatoprotective, antidiabetic, antioxidant and anti-inflammatory properties aligning with *P. niruri*'s reported effects. However, further isolation, testing, and clinical evidence are still needed to fully elucidate therapeutic mechanisms and develop standardized extracts or derivatives as botanical therapies.

**Keywords:** *Phyllanthus niruri*, Phytochemicals, GC-MS analysis, FT-IR analysis

### INTRODUCTION

The use of plants for medicinal purposes has a long history spanning thousands of years across many cultures worldwide (Balick and Cox, 2020). Human civilizations have relied

on the vast diversity of natural plant compounds to treat injuries, infections, and diseases before synthetic medicines were developed (Giannenaset *al.*, 2020). Documentations of traditional plant remedies abound in many ancient texts and historical

records across different geographic regions (Van Arsdall, 2023). For instance, clay tablets from Mesopotamia dated around 5,000 years old provide evidence that Sumerians utilized medicinal plants like poppy as early as the third millennium BCE (Cartwright and Armstrong, 2020). Ancient Chinese and Egyptian papyri also describe the curative uses for herbs and plants (Alamgir and Alamgir, 2017). Hippocrates, known as the father of medicine, recorded the beneficial properties of over 200 different plant species in Greek herbal manuals circa 400 BCE (Riddle, 2013; Schiefsky, 2018).

In recent history, natural products derived from plants have contributed significantly to allopathic health systems (Kala, 2017). Experts estimate around 25% of modern pharmaceuticals contain active compounds directly or indirectly sourced from vascular plants (Napagoda and Wijesundara, 2022; Elshafieet *al.*, 2023). The World Health Organization (WHO) has emphasized the importance of traditional medicine and medicinal plants as a source of health care globally. WHO estimates indicate that 80% of the world's population relies on traditional plant-based medicine for primary healthcare needs (WHO, 2013). Additionally, the Convention on Biological Diversity has highlighted that the global market for botanical extracts and medicinal plants was estimated to be over \$100 billion in 2017 and is projected to reach nearly \$350 billion by 2027 (Arkalgud, 2016). This underscores the increasing commercial importance of medicinal plants in pharmaceutical, cosmetic, food and beverage industries worldwide.

Phytochemicals, the array of bioactive compounds produced as primary or secondary metabolites in plants, comprise the foundation upholding this tenacious human reliance on botanical treatments for assorted afflictions (Kaushik *et al.*, 2021). Major chemical classes like alkaloids, terpenoids, sterols, glycosides, flavonoids, and tannins number over 100,000 structurally diverse entities derived from an estimated 28,000 plus medicinal vascular plant

species globally (Bhatla and Lal, 2023). Well-known drug examples include quinidine obtained from *Cinchona* tree bark, morphine and codeine extracted from poppy seeds, and artemisinin isolated from *Artemisia annua*. Those drugs treat malaria, heart arrhythmia, pain relief, cough suppression, and more (Alamgir, 2017; Nahar and Sarker, 2020). Researchers anticipate that many uncharacterized plant metabolites likely hold promise as new drug leads once discovered and evaluated. However, plants remain an underutilized reservoir housing extensive chemical diversity that awaits further scientific investigation. One such plant is *Phyllanthus niruri*.

*Phyllanthus niruri*, commonly known as the gale of the wind or stonebreaker, is a widespread tropical plant belonging to the Phyllanthaceae family. It is an annual herb that grows 30–60 cm tall with numerous slender, leaf-bearing branches. Its leaves are 7–12 cm long and they are alternate, sessile oblong. It has small off-white-greenish flowers, which are solitary, auxiliary, pedicellate, apetalous and monoecious. The fruit is a smooth capsule around 1.5–2 mm across, each containing 1–2 seeds (Dahanayakeet *al.*, 2020). *P. niruri* has a long history of medicinal use in systems like Ayurveda and Traditional Chinese Medicine for problems related to the liver, kidney, urinary bladder, stomach, genitourinary system, spleen, and prostate (Bhatt *et al.*, 2018; Kamruzzaman and Hoq, 2016; Lee *et al.*, 2016; Rahman and Husen, 2021). In several cultures around the world, *Phyllanthus niruri* has traditionally been used as a medicinal herb to treat a variety of ailments.

Traditional Chinese Medicine utilizes decoctions of the whole *P. niruri* plant, called Yexiazhu, to clear heat and toxins as well as remove dampness. This helps treat issues like jaundice, enteritis, diarrhoea, and dropsy. A Traditional Chinese Medicine preparation named "yexiazhu capsule" claims to cure hepatitis B (Geethangili and Ding, 2018). In India, *P. niruri* is considered an effective diuretic. The crushed plant is also used as a

fish poison (Kaur *et al.*, 2017). In Taiwan, decoctions of young shoots or roots are traditionally employed for contagious hepatitis, acute conjunctivitis, diarrhoea, oedema, and dysentery (Geethangili and Ding, 2018). In Thailand, *P. niruri* shares the name "look tai bai" with *P. amarus* and *P. virgatus*. All three plants are utilized to address gonorrhoea, jaundice, diabetes, and liver disease (Buddhachatet *et al.*, 2015). In Malaysia, the juice stimulates appetite in children and is used to wash their tongues. An extract of *P. niruri* in Papua New Guinea functions as a febrifuge (Geethangili and Ding, 2018). In Brunei, a leaf poultice combined with coconut milk treats smallpox. Cambodia relies on it against malaria, while in Ghana a decoction treats dysentery. The Solomon Islands employ the leaves to alleviate chest pain (Geethangili and Ding, 2018).

Many pharmacological studies have aimed to scientifically evaluate these traditional uses and identify the active phytochemicals responsible. Extracts, infusion, and isolated compounds from *P. niruri* have exhibited hepatoprotective, antihepatotoxic, antilithic, antiviral, antimicrobial, antioxidant, anti-inflammatory, antispasmodic, antidiabetic, anticancer, radioprotective, and immunomodulatory effects. Okoliet *et al.* (2011) studied the antidiabetic effects of the methanol extract from *Phyllanthus niruri* aerial parts in alloxan-induced diabetic rats. Chronic oral administration of the extract lowered blood glucose, suppressed post-prandial glucose rise, reduced haemoglobin glycation, and increased liver weight and glycogen content compared to untreated diabetic rats. Treatment also prevented body weight loss caused by diabetes. In vitro, the extract strongly inhibited the carbohydrate-hydrolyzing enzymes alpha-amylase and alpha-glucosidase. These findings suggest the blood glucose lowering effects of *P. niruri* are due to blocking glucose absorption via enzyme inhibition and enhancing glucose storage in the liver.

Amin *et al.* (2012) examined the hepatoprotective effects of a *Phyllanthus niruri* extract against thioacetamide-induced

liver cirrhosis in rats. Acute toxicity testing found the extract was safe. Rats were divided into control, thioacetamide, silymarin, and low- and high-dose *P. niruri* extract groups. Significant differences between the thioacetamide group and others were seen in body and liver weights, liver biomarkers, oxidative stress markers, and histopathology. Silymarin and *P. niruri* treatments effectively restored measurements toward normal levels and comparably reduced inflammation, fibrosis, and abnormal liver architecture induced by thioacetamide. Thus, *P. niruri* extract demonstrated a protective role against thioacetamide-induced liver cirrhosis in rats similar to silymarin.

Colpoet *et al.* (2014) investigated the potential antioxidant effects of *Phyllanthus niruri* tea in a randomized crossover study of 5 healthy male volunteers aged 20-31 years. Subjects drank either *P. niruri* infusion or water, and fasting blood samples were taken before and up to 4 hours after consumption to examine levels of gallic acid and ascorbic acid as well as catalase and superoxide dismutase enzyme activities. The results showed that *P. niruri* tea significantly increased plasma levels of gallic acid at 1, 2, and 4 hours and plasma ascorbic acid at 1-hour post-ingestion, with no effects on antioxidant enzyme activities, demonstrating a slight increase in certain blood antioxidant markers from drinking *P. niruri* tea that could contribute to its pharmacological effects.

Zheng *et al.* (2016) isolated the antitumor components ethyl brevifolincarboxylate and corilagin from the ethyl acetate fraction of *Phyllanthus niruri* using bioguided fractionation and chromatographic methods followed by NMR and MS analyses. Cell cytotoxicity assays demonstrated that corilagin exhibited broad-spectrum antitumor activity against hepatocellular carcinoma cells with better potential and lower toxicity than normal cells. Moreover, corilagin was found to enhance the antitumor effects of cDDP chemotherapy. A screening of 10 corilagin-containing plants revealed *Dimocarpus longan* to have the highest corilagin content.

Therefore, corilagin was identified as the major bioactive compound from *P. niruri* responsible for its antitumor effects on hepatocellular carcinoma.

Mostofaet al. (2017) evaluated the anti-inflammatory and antiulcer activities of the methanol extract of *Phyllanthus niruri* leaves in rat models. For anti-inflammation, the extract at doses of 100-400 mg/kg demonstrated a dose-dependent reduction in carrageenan-induced paw oedema comparable to ibuprofen, as well as reduced inflammatory cell infiltration histologically. Regarding antiulcer activity against ethanol-acid-induced gastric lesions, the extract doses inhibited gastric erosion in a dose-dependent manner similar to or better than omeprazole at the highest dose, accompanied by regeneration of the mucosal layer and prevention of haemorrhage and oedema on histological examination. The findings suggest that *P. niruri* extract possesses anti-inflammatory and gastric ulcer protective effects through inhibition of inflammation and promotion of mucosal healing.

Sowjanyaet al. (2021) conducted a randomized, double-blind, placebo-controlled trial to evaluate the efficacy of *Phyllanthus niruri* compared to placebo over 4 weeks in patients with mild-moderate alcoholic hepatitis. Out of 454 screened patients, 100 eligible patients were randomly assigned to *P. niruri* or placebo groups. While liver and kidney function parameters did not significantly differ between groups, *P. niruri* demonstrated a statistically significant increase in total antioxidant levels compared to placebo at 4 weeks, in addition to an appetite stimulant effect. Thus, a 4-week administration of *P. niruri* in mild-moderate alcoholic hepatitis patients was shown to improve total antioxidant status and stimulate appetite relative to placebo based on this clinical trial analysis.

While the reviewed studies effectively demonstrated the pharmacological activities of *Phyllanthus niruri* extracts using in vitro and in vivo methods, limitations remain regarding

the identification and characterization of the specific compounds responsible. The present study aimed to address this gap by utilizing Fourier-Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GCMS) to analyze the organic compound compositions and the functional groups present in *P. niruri* extract.

## **MATERIALS AND METHOD**

### **Sample Collection**

Fresh leaves of fully grown *Phyllanthus niruri* were harvested from local farmlands in Uturu, Isuikwaoto Local Government Area of Abia State in November 2023. The plant sample was identified by a taxonomist, Mr O. Emmanuel, of the Department of Plant Science and Biotechnology, Abia State University, Uturu, Nigeria. A voucher specimen was deposited at the Herbarium in the Department of Plant Science and Biotechnology, Abia State University, Uturu for reference purposes. The samples were then transported under sterile conditions and taken to the Chemistry Laboratory of the Department of Pure and Industrial Chemistry, Abia State University, Uturu for analysis.

### **Sample Preparation**

The leaves of *Phyllanthus niruri* were cleansed by washing and they were stripped out of the stems and air-dried at room temperature for 3 weeks. The dried samples were pulverized using a Mechanical grinder and resultant powdered samples were stored in airtight sample plastic bottles for extraction.

### **Preparation of Extracts by Maceration**

The pulverized sample (95 g) was soaked in 500 ml of methanol for 72 hours. The sample was filtered using What Man No. 42 filter paper to collect the plant extract. The filtrate was concentrated by placing the sample container in a Water Bath at 40 °C. The concentrated sample was inserted into a sample container and taken for GC-MS and FT-IR analysis.

## GC-MS Analysis of Extract

The methanol leaf extract of *Phyllanthus niruri* was analyzed by GC-MS using a temperature program from 60°C to 280°C at 6°C/min with a 5-minute isothermal hold at 280°C. Helium carrier gas flow was 1 ml/min. Samples were injected in a 1:10 split ratio. Retention indices (RI) of the compounds were determined by comparing the retention times of a series and identification of each component was confirmed by comparison of its retention index with data in the literature. Interpretation of the mass spectrum was carried out by using the database of the National Institute of Standard and Technology (NIST) which has more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components which was stored in the NIST library. The molecular weight, name, chemical structure and molecular formula of the components of the test materials were ascertained.

## FT-IR Analysis of Extract

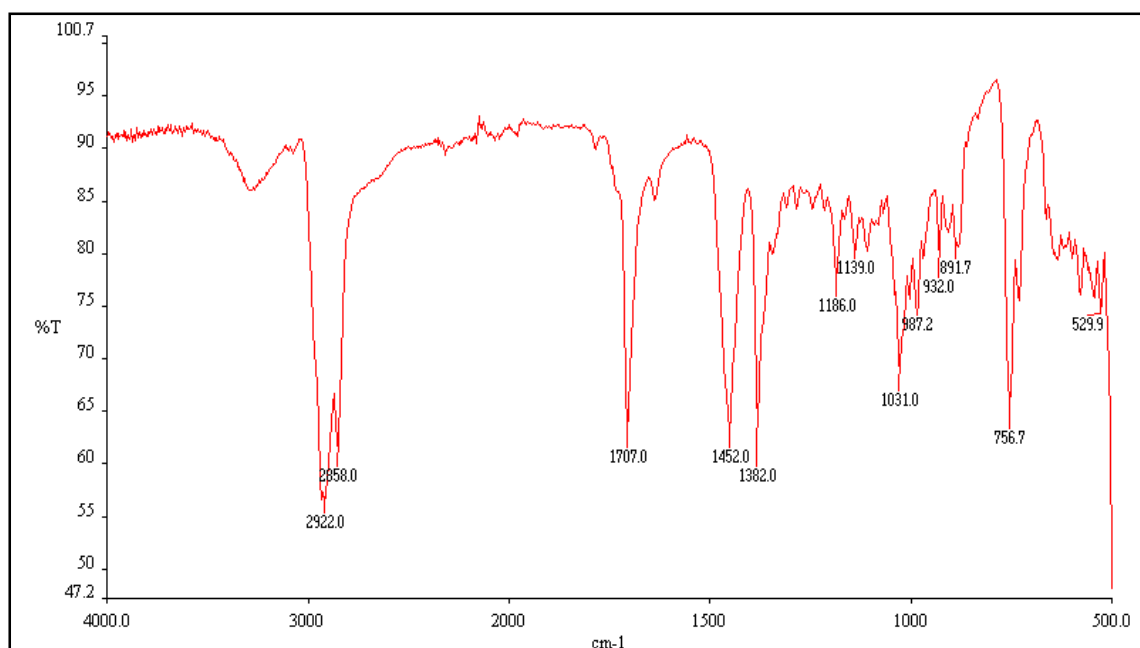
FT-IR analysis was carried out on a Lumex FT08 Fourier Transform Infrared Spectrometer using the potassium bromide pellet technique. The *Phyllanthus niruri*

methanolic leaf extract was mixed with dry KBr in a 1:100 ratio and pressed into a pellet. The background spectrum was collected using a plain KBr pellet. The sample spectrum was acquired in the mid-IR range from 400 to 4000  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$  with 16 scans. Functional groups present in the extract were identified by analyzing absorption peaks in the IR spectrum and comparing them to reference wavenumber values. Computerized matching against a standard IR library containing over 10,000 absorption spectra was also utilized.

## RESULTS AND DISCUSSION

### FT-IR Results

The FTIR spectrum of the methanolic plant extract of *Phyllanthus niruri* in the form of a KBr pellet shown in Figure 1 revealed that the peak of 2922.0  $\text{cm}^{-1}$  indicates the presence of a  $\text{CH}_3$  (methyl) group, while 2858.0  $\text{cm}^{-1}$  signifies an OH (hydroxyl) group. The wavelength of 1707.0  $\text{cm}^{-1}$  was associated with a C=O (carbonyl) group, 1452.0  $\text{cm}^{-1}$  with a C-H (alkyl) group, and 1382.0  $\text{cm}^{-1}$  with another  $\text{CH}_3$  (methyl) group—A stretching vibration. Additionally, the wavelengths of 1186.0  $\text{cm}^{-1}$ , 1139.0  $\text{cm}^{-1}$ , and 1031.0  $\text{cm}^{-1}$  correspond to C=S (thioamide), C-O-C (ether), and =C-O-C (ester) groups, respectively.



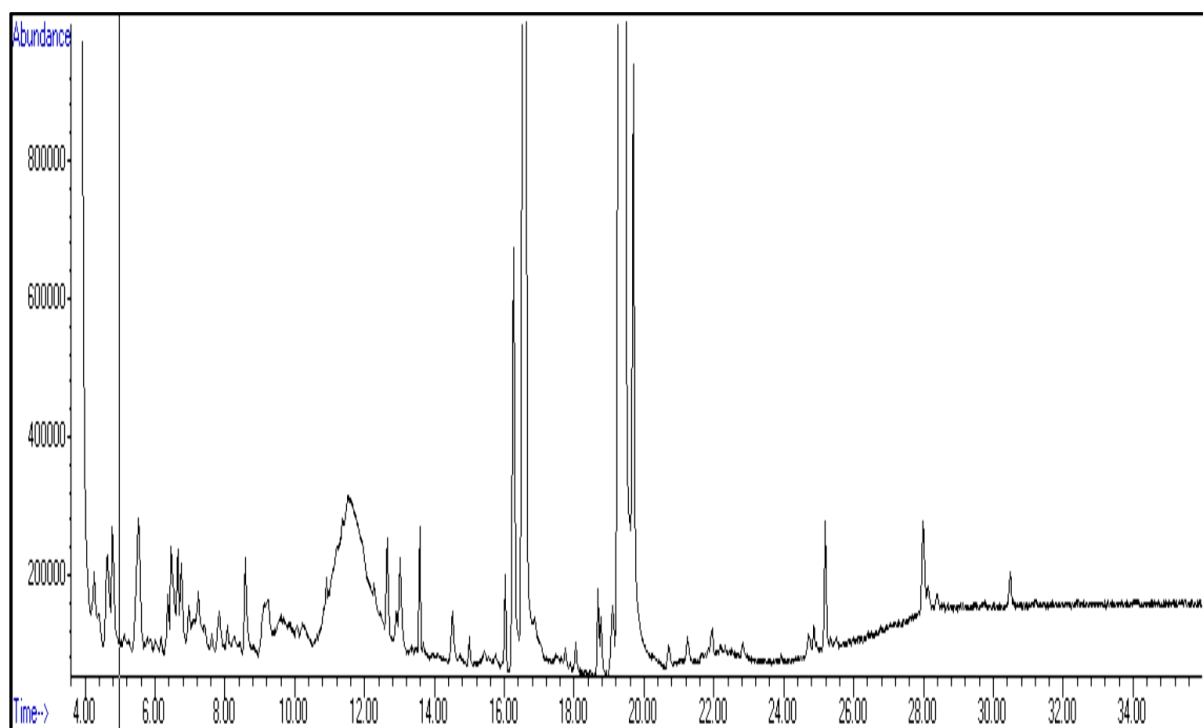
**Figure 1:** FTIR spectra of pure methanolic plant extract of *Phyllanthus niruri*

**Table 1:** FTIR Absorption bands of *Phyllanthus niruri* extract

ABSORPTION BANDS (cm <sup>-1</sup> )	ASSIGNMENTS
2922.0	CH <sub>3</sub> (Methyl group)
2858.0	OH (Hydroxyl group)
1707.0	C=O (Carbonyl group)
1452.0	C-H (Alkyl group)
1382.0	CH <sub>3</sub> (Methyl group)
1186.0	C=S (Thioamide group)
1139.0	C-O-C (Ether group)
1031.0	=C-O-C (Ester group)

### GC-MS Results

The chemical constituents identified by the GC/MS analysis of the methanolic plant extract of *Phyllanthus niruri* were enumerated along with their molecular formula, retention time, and peak area in Table 2. The results in Figure 2 captured 15 different compounds in the sample. The most abundant compounds were octadecenoic acid at 23.84% and hexadecanoic acid (methyl ester) at 18.25%. Other relatively abundant compounds included 12,15-octadecadienoic acid (methyl ester) at 5.38%, hexadecanoic acid at 4.72%, 4H-pyranone-2,3-dihydro-3,5-dihydroxy-dihydroxy-6-methyl at 4.25%, 2,4-hexadienedioic acid at 4.06%, and tetradecanoic acid at 3.88%.

**Figure 2:** GC-MS Chromatogram of the Methanolic extract of *Phyllanthus niruri*

**Table 2:** Chemical constituents present in the Methanolic extract of *Phyllanthus niruri*

Pk# <sup>1</sup>	RT <sup>2</sup> (min)	Compound name	MW <sup>3</sup> (g/mol)	Formula	Abundance (%)
1	16.52	Hexadecanoic acid, methyl ester	270	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	18.25
2	19.702	Octadecenoic acid	284	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	14.84
3	18.709	12,15-Octadecadienioc acid, methyl ester	294	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	5.38
4	16.568	n-Hexadecanoic acid	256	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	0.78
5	16.269	Hexadecenoic acid, Z-11	254	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	19.17
6	25.983	9,12,15-Octadecatrienoic acid, 2- [[trimethylsilyl]oxy]-1- [[trimethylsilyl]oxy] methyl]ethyl(9E,12E,15E)-9,12,15- octadecatriene	496	C <sub>27</sub> H <sub>52</sub> O <sub>4</sub> S i <sub>2</sub>	4.58
7	4.614	4(1H)-Pyrimidinone,2,6,-diamino	126	C <sub>4</sub> H <sub>6</sub> H <sub>4</sub> O	2.27
8	4.764	1,2,3-Propanetriol, monoacetate	134	C <sub>5</sub> H <sub>10</sub> O <sub>4</sub>	2.19
9	5.517	4H-Pyranone-2,3-dihydro-3,5- dihydroxy-dihydroxy-6-methyl	144	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	4.25
10	6.451	6-Acetyl-β-D-mannose	222	C <sub>8</sub> H <sub>14</sub> O <sub>7</sub>	3.31
11	7.588	2,4-Hexadienedioic acid	142	C <sub>6</sub> H <sub>6</sub> O <sub>4</sub>	4.06
12	13.05	1-Gala-1-ido-octose	240	C <sub>8</sub> H <sub>16</sub> O <sub>6</sub>	3.19
13	13.58	Tetradecanoic acid	223	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	3.88
14	14.52	Benzoic acid, 4-hydroxy-3,5- dimethoxy	198	C <sub>9</sub> H <sub>10</sub> O <sub>5</sub>	1.13
15	25.183	Hexadecanoic acid, 2,3- dihydroxypropyl ester	330	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	4.18

<sup>1</sup>Pk = Peak Number; <sup>2</sup>RT = Retention Time; <sup>3</sup>MW = Molecular Weight

This study analyzed the chemical composition of the methanolic leaf extract of *Phyllanthus niruri* using Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GCMS). The FTIR analysis identified key functional groups in the extract including methyl, hydroxyl, carbonyl, alkyl, and ester groups which provided insight into the types of compounds present. These functional groups belong to diverse classes of bioactive phytochemicals including alkaloids, flavonoids, terpenoids, sterols and polyphenols that contribute to the plant's

therapeutic effects (Zheng *et al.*, 2016). Specifically, free hydroxyl groups have been associated with potent antioxidant capacities while carbonyl and ester groups indicate anti-inflammatory compounds through enzyme inhibition (Mostofaet *al.*, 2017).

Meanwhile, the GCMS analysis detected and identified 15 different compounds in the extract. The two most abundant compounds were octadecanoic acid and hexadecanoic acid methyl ester. Previous pharmacological research has demonstrated hepatoprotective, antidiabetic, anti-inflammatory, and other

medicinal effects of *P. niruri* extracts. Comparing the compounds identified in this study to those activities provides clues about which specific constituents may be responsible. For example, Amin *et al.* (2012) found that a *P. niruri* extract protected against thioacetamide-induced liver cirrhosis in rats similar to silymarin. Octadecanoic acid and other fatty acids identified here such as tetradecanoic acid are known to possess hepatoprotective effects (Achi and Ohaeri, 2015; Anyasoret *et al.*, 2014). Tetradecanoic acid has exhibited antioxidant and anti-inflammatory properties that can attenuate liver damage from toxins like thioacetamide (Roshankhahet *et al.*, 2020).

Additionally, Okoliet *al.* (2011) reported the methanol extract of *P. niruri* lowered blood glucose levels and inhibited carbohydrate-hydrolyzing enzymes in diabetic rats, suggesting antidiabetic activity. The presence of compounds like hexadecanoic acid methyl ester and other fatty acid derivatives in this study's extract provides a likely source of those antidiabetic effects (Oyebodeet *al.*, 2018). Fatty acids can sensitize cells to insulin and influence lipid metabolism pathways involved in managing glucose levels (Stinkenset *al.*, 2015). Colpoet *al.* (2014) found that drinking *P. niruri* tea increased plasma antioxidant levels in human subjects. Compounds detected here such as 4H-pyranone-2,3-dihydro-3,5-dihydroxy-6-methyl and benzoic acid derivatives were known to possess antioxidant properties consistent with enhancing blood antioxidant status upon ingestion (Chen *et al.*, 2021; Van Chen *et al.*, 2022; Yuet *al.*, 2013).

The anti-inflammatory properties of *P. niruri* reported by Mostofaet *al.* (2017) also align with specific compounds characterized. For example, hexadecanoic acid and related fatty acids exhibit anti-inflammatory effects by reducing prostaglandin and leukotriene biosynthesis (Das, 2018; Kiezel-Tsuginovaet *al.*, 2018). In addition, 2,4-hexadienedioic acid identified in this study acts as a nonsteroidal anti-inflammatory drug (NSAID) via inhibition of cyclooxygenase enzymes similar

to ibuprofen (Maciel Ferreira, 2023). Furthermore, Sowjanyaet *al.* (2021) observed appetite stimulation in alcoholic hepatitis patients using *P. niruri*, and various fatty acids were known to influence appetite regulation pathways in the central nervous system and gastrointestinal tract (Byrne *et al.*, 2015; Witkamp, 2018).

Together, these correlations between the active principles elucidated in this research and prior investigations of *P. niruri* pharmacological activities provide support for the roles of specific constituents. The current study helps address knowledge gaps raised by past works regarding the identification and characterization of compounds responsible for *P. niruri*'s effects. However, limitations remain since the activities are likely mediated by multiple components in combination rather than individual isolated constituents. Further efforts should aim to isolate and test candidate compounds using different methods of extract preparation preclinically as well as determine structure-activity relationships. Additionally, standardized extracts rather than solely identifying biomarkers may better represent the complexity underlying this plant's traditional uses and documented multi-target properties.

## CONCLUSION

This study provided a valuable first step toward pinpointing some active constituents in *P. niruri*'s leaves that could underline its multi-target medicinal properties explored previously. Many of the compounds characterized correlated plausibly with reported pharmacological effects based on current understanding. However, attributing complex herbal activities to single molecules remains an oversimplification requiring further multidisciplinary research. Additional standardization efforts, mechanism elucidation, and clinical evidence are still warranted before *P. niruri* extracts or derivatives can be developed as stand-alone botanical therapies. Overall, the natural products-based approach employed here enhances the scientific rationalization of *P.*



*niruri*'s extensive traditional use and longevity as a global herbal remedy worthy of ongoing investigation.

### Conflict of Interest

The authors declare that there is no conflict of interest.

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