GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) ANALYSIS OF ACTIVE COMPOUNDS OF *PHYLLANTHUS NIRURI* (CHANCA PIEDRA).

Musa, Y.[1*]; Madaki, M.B.[1]; Usman, J.[1] and Yerima, H.[1]
[1]Department of Chemistry, School of Sciences
Niger State College of Education, Minna.

* **E-mail**: yusufauna39@yahoo.com

Abstract

This study was carried out to analyze active compounds in *Phyllantus niruri. Phyllanthus niruri* also known as *Chanka piedra* is widely grown and used throughout the tropical and subtropical countries of the world, known by the common names gale of the wind, stonebreaker or seed-under-leaf. The plant was identified and authenticated by an expert from Niger State College of Education Minna. The plant was air dried at room temperature, pulverized in to powder, sieved into fine particles and kept in a cellophane bag in a cool dry place. The method of choice for the extraction of the crude extracts in this study was cold extraction, or maceration. The pulverized sample of Phyllantus niruri and its methanol extract were subjected to a GC-MS analysis. The results from the GC-MS analysis of the pulverized sample of *Phyllanthus niruri* revealed the presence of flavonoids, caumarine, Ligmam, carbohydrate, Tannins, Terpenoids, saponin, resins, anthraquinones, phenol and alkaloids which are useful bioactive agents that have been reported with physiological effects in humans. Furthermore, the GC-MS analysis of the crude extract of *Phyllantu niruri* revealed the presence of Bis(2-ethylhexyl) phthalate, Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, Dibutyl phthalate, Cyclohexane, 1,3,5-triphenyl- Isoxazole, 3,4-dihydroxy-10,13,dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-ly]pyran-2-one which suggest that the plant has rich medicinal properties. It was concluded that, the plant is very rich with various chemical constituents; which are the reason for its various activities against several ailments.

Key words: *Phyllantus niruri, Chanca Piedra*, methanol extract, GC-MS

1. INTRODUCTION

Nature has provided numerous sources of remedies required for the treatment of various ailments and diseases. Within the wide range of living organisms available on earth including higher plants, animals, fungi, and marine organisms, the databases of natural products have recorded more than 200,000 compounds from almost all part of the world (Füllbeck et al., 2006). Plants are by far the most widely studied source of medicinal compounds (Cragg *et al*, 1997). Dias *et al* (2012) infer that one of the ancient scripts from Egypt (2600 BC) mentioned the uses of plants as the major constituents of their traditional drugs (pills, infusions, and ointments).

Phyllanthus niruri Linnaus (Euphorbiaceae) also known as Chanka piedra is widely grown and used throughout the tropical and subtropical countries of the world, known by the common names gale of the wind, stonebreaker or seed-under-leaf. It is in the genus *Phyllantus* of the family phyllanthaceae. It grows 50–70 cm (20–28 in) tall and bears ascending herbaceous branches. The bark is smooth and light green. It bears numerous pale green flowers which are often flushed with red. The fruits are tiny, smooth capsules containing seeds. *Phyllanthus niruri* has been used in traditional medicine for treating various illnesses. Examples of usage include treating high fever, respiratory disturbance, abdominal disturbance, kidney disturbance, infection and bleeding disorders (Calixto *et al*., 1998, Syamasundar *et al*., 1985).

From a total of approximately 250.000 species of higher plant available on earth, only 15% have been investigated so far for their active compounds and only 6% have been screened for their biological properties (Füllbeck et al., 2006, Newman and Cragg, 2012). Accordingly, there are many more candidates for new active compounds are still untouched and therefore awaits discovery.

Natural compounds are typically secondary metabolites that are synthesised by an organism; many of these metabolites are found to be unique to a particular organism. They may not be essentially needed for metabolism, but may be related to the function of environmental adaptation or as a possible defense mechanism for the survival of the organism (Dias *et al*., 2012).

Over the past 30 years, there has been a significant rise in the number of publications on natural products focusing on the investigation of plant-derived lead compounds (Newman and Cragg, 2012). Fabricant and Farnsworth (2001) reviewed 122 compounds originated from 94 plants, which are widely used as important drugs in more than one country. They further added that 80% of these compounds were used for identical or related ethnomedical purpose of the active elements of the plant. However, despite a resurgence of interest in the investigation of plants as a source of natural products, the numbers of plant-derived secondary metabolites is still far from exhausted.

Phyllanthus niruri L is one of the most popular choices of natural herbal remedy to overcome many symptoms due to its wide range of therapeutic uses in traditional medicine.

This plant has been mentioned in a number of historic manuscripts, such as the ancient herbal medicine histories of Ayurveda Indian history, traditional Chinese medicine and Indonesian Jamu (Bagalkotkar *et al*., 2006). Consequently, the long history of *Phyllanthus niruri L* use in herbal medicine from all over the world has brought the interest of many scientists to explore the pharmacological properties of this plant.

Although various active compounds have been identified from this plant, there are questions that remain to be answered regarding the nature of the active compounds responsible for the observed biological activities, and the mechanisms by which they exert their medicinal effects. Therefore, this study sort to examine the GC-MS analysis of active compounds of *Phyllanthus niruri* (Chanca Piedra).

2. MATERIAL AND METHOD

2.1 Sample Collection and Preparation

The entire fresh plant of *Phyllanthus niruri* L were collected in the month of September, 2022 from Shango, Chanchaga Local Government Area of Niger State, Nigeria. The plant was identified and authenticated by a Botanist in the Department of Biology Niger State College of Education Minna, Nigeria. The fresh plants collected were washed under running tap water (to remove dust and any other foreign materials) and were allowed to drain off. The plants were spread on the laboratory bench to air-dry at room temperature for two weeks, thereafter pulverized to powder using a mortar and pestle, sieved into fine particles and kept in a cellophane bag in a cool dry place until further work.

2.2 Extraction of Plant Material

The method used for the extraction of crude extracts in this study was cold extraction, or maceration. The choice of this method of extraction was for its advantage of yielding more substances in the extract, this is because all of the thermolabile and thermostable components are preserved. Despite the large amount of solvent needed and longer time for extraction, maceration gives a benefit in extracting unknown compounds for screening their biological activities.

Exactly Thirty grams (30.0g) of the powder was weighed and dissolved in 200 mL of Methanol for 24h with periodic agitation to obtain 15% concentration (Lauk *et al*., 2003). This was then filtered using Whatman filter paper (No. 1). The extract was then concentrated using a rotatory evaporator (Model: RE200) to remove the solvent at a temperature less than 50^{o}C. The crude extract was then transferred into a sample bottle and stored for further analysis (Dada *et al.*, 2014).

2.3 Phytochemical Analysis (GC-MS Analysis)

The pulverized sample of *Phyllantus niruri* was weighed. One thousand gram (1000g) was sent to the Department of Chemistry, Usman Danfodio University Sokoto Nigeria, for phytochemical analysis using a Gas Chromatography – Mass Spectrometry Analysis (GC –MS).
GC-MS analysis was carried out using the GCMSQP2010 PLUS SHIMADZU. The column used was Perkin Elmer Elite - 5 capillary column measuring 30m × 0.25mm with a film thickness of 0.25mm composed of 95% Dimethyl polysiloxane. Helium was the carrier gas used at a flow rate of 0.5mL/min. And the sample injection volume that was utilized was 1µL, maintaining 250°C inlet temperature.
Initially, the oven temperature was programmed at 110°C for 4 min, then an increased to 240°C. And further programmed to increase to 280°C at a rate of 20°C ending within 5 minutes. The total run time was 35 minutes. At a temperature of 200°C, the MS transfer line was maintained with the source temperature maintained at 180°C. The GC-MS was analyzed using an acceleration electron impact ionization at 70eV and data were evaluated using Total Ion Count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library.

2.4 Gas Chromatography - Mass Spectrometry (GC-MS) Analysis of Active Compounds

The crude extract of the entire plant of *Phyllanthus niruri L* was subjected to GC-MS analysis using Agilent 19091S-433 equipped with column of 30 m long (0.25 µm x 250 µm). The carrier gas was helium with a flow rate of 4^{o}C/min using a split less injector mode (at 250^{o}C). About 1 µL of the sample was injected by an auto sampler, over the oven temperature programme: from 40 to 300^{o}C and a total run-time of 95 minutes. The relative percentage constituents were evaluated based on an estimate of the depicted area of the peak in the total ion chromatogram. Identification of the compounds was done by comparing the mass spectra fragmentation pattern with NIST database (NIST,2009; Adams, 1995). The GC-MS was analyzed using an acceleration electron impact ionization at 70eV and data were evaluated using Total Ion Count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library.

3. RESULTS AND DISCUSSION

Table 1 present the phytochemical constituents of *Phyllanthus niruri.* The result indicate significant presence of flavonoids, Tannins, and Phenols. Alkaloids, anthraquinones, and lignin were moderately present while carbohydrate, Terpenoids, saponin, resins and caumarine were present in low concentration. These compounds are useful bioactive agents tht have been reported to have physiological effects in humans. The result of the GC-MS analysis of *Phyllanthus niruri* are presented in figure 1 and Table 1 and 2.

Table 1: Results from the GC-MS Analysis of *Phyllanthus niruri* Methanol Extract

S/NO	COMPOUNDS	REMARK
1	Alkaloids	+ +
2	Carbohydrate	+
3	Balsan	—
4	Tannins	+ + +
5	Terpenoids	+
6	Saponin	+
7	Phlobatannins	—
8	Sterols	—
9	Resins	+
10	Anthraquinones	+ +
11	Phenols	+ + +
12	Flavonoids	+ + +
13	Lignan	+ +
14	Caumarine	+

Keys: +++ = Highly present; ++ = Moderately Present; + = Minimally present; — = Not present

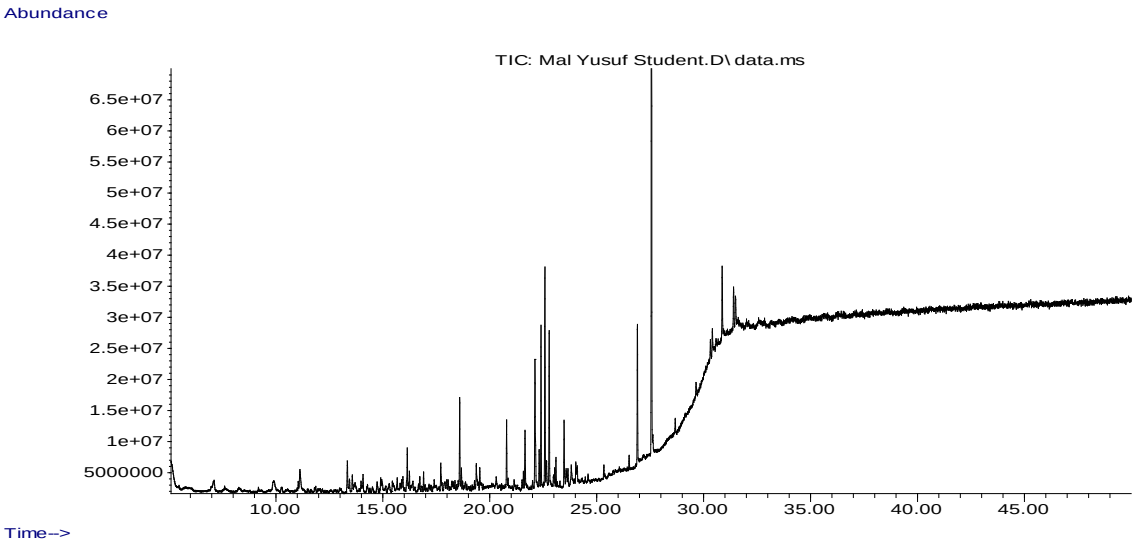

Figure 1: Chromatogram Results from the GC-MS Analysis of *Phyllanthus niruri* Methanol

Extract

Figure 1 revealed the presence of some important compounds from the ethanol extract of the whole plant of *Phyllanthus niruri* which includes: Alkaloids, Carbohydrate, Tannins, Terpenoids, Saponin, Phlobatannins, resins, anthraquinones, Phenols, Flavonoids, Lignana and Caumarine as shown by the peaks from the chromatogram. Figure 1 further revealed that Carbohydrate, Tannins, Phenols and Flavonoids are present in high amount while alkanoids,

Anthraquinones and Lignan are present in moderate amount and Terpenoids, Saponin, Phlobatannins and Caumarine are present in minimal amount.

Table 2: Results from the GC-MS Analysis of *Phyllanthus niruri* Methanol Extract

Peak No.	Retention Time (t_r)	Name of Compounds	Area Percent
1	11.124	Cyclopentasiloxane,decamethyl-3,4-Dihydroxybenzylalcohol,tris(rimethylsilyl)-Cyclopentasiloxane	1.67
2	11.33	Naphthalene. Azulene	1.46
3	16.142	1-Tetradecene Cyclotetradecane 5-Tetradecene	1.64
4	20.789	15-hydroxypentanoic acid	2.01
5	21.647	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, cholest-4-en-3-one,	2.15
6	22.110	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, Dibutyl phthalate	5.65
7	22.311	Phthalic acid, isobutyl nonyl ester,	1.23
8	22.396	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	4.43
9	22.580	3,4-dihydroxy-10,13,dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-ly]pyran-2-one	7.14
10	22.654	Phthalic acid, monohexyl ester, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1.04
11	22.780	1,2-Benzenedicarboxylic acid, buty l 2-methylpropyl ester, bis(1-methylethyl) ester	4.69
12	23.472	14-Methylcholest-8-ene-3,6-diol	2.22
13	23.810	6-Octadecenoic acid, methyl ester	1.40
14	26.905	Cyclohexane, 1,3,5-triphenyl- Isoxazole	4.67
15	27.563	Bis(2-ethylhexyl) phthalate	22.68
16	30.865	2'-Hydroxypropiophenone, 1,2-Bis(trimethylsilyl)benzene	2.21

The chemical compositions of the methanol extracts of *Phylanthus niruri* were determined by subjecting the extracts to GC-MS analyses. The compounds were identified by marching their respective mass spectrum of each compound with the library. The total ion chromatogram indicated sixteen peaks which correspond to sixteen compounds as represented in table 2.

The major compounds identified in the Methanol extract are Bis(2-ethylhexyl) phthalate, Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, 1,2-

Benzenedicarboxylic acid, bis(2-methylpropyl) ester, Dibutyl phthalate, Cyclohexane, 1,3,5-triphenyl- Isoxazole, 3,4-dihydroxy-10,13,dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-ly]pyran-2-one.

Bufalin which was the most prominent compound in the methanol extract is a steroidal triterpenoid. It is a cardiotonic steroid originally isolated from the Chinese toad venom. It is a component found in many Chinese traditional medicine. Bufalin has an antitumor effect against various malignancies such as hepatocellular and lung carcinoma (Zhang, 2014; Wu, 2014; Qiu et al., 2013).

The flavonoids identified in the extract have been detected in other plants (Foo and Wong, 1992; Foo, 1993 and 1995). Macdougallin observed in the extract have been isolated from Myrtillocactus geometrizans and tested for anti-inflammatory activity (Salazar et al., 2011).

Although, there have being no report of the isolation of steroidal compounds from the plant, most of the identified compounds are steroidal triterpenoids, which supports the phytochemical results as the analysis shows positive for both steroids and terpenes.

In Table 4.2, the presence of hydroxyl fatty acid (15-hydroxypentanoic acid), ester and reduced cholesterol (cholest-4-en-3-one,) which are implicated as physiological agents (Unander *et al* 1995) suggest that the plant has rich medicinal properties. This finding is also supported by the phytochemical constituents detected in the extract especially with the presence of steroids.

4. CONCLUSION

The importance of medicinal plants cannot be overemphasized The increasing global population have made the demand for drugs from varied sources to be continuously increasing. Various countries of the world have employed the use of medicinal plants. This is because they act as antibacterial, antifungal, antiviral, anti-parasitic and antiprotozoans activity and other hormonal inducers. Also, they had and are still playing vital roles in treating, curing and managing even life-threatening diseases. The chemical analyses results indicated that, the plant is very rich with various chemical constituents; this may be the reason for its various activities against several ailments. Further research is necessary in order to subject the plant to in-depth separation/isolation of the constituents to investigate the role of each isolate against particular disease. Therefore there is need to scientifically evaluate *Phylanthus niruri* and other plants of medicinal value to get more conclusive evidences and results with the right doses, concentrations and route of administration for the treatment of various ailments to augment the pharmaceutical drugs.

Reference

Bagalkotkar, G., Sagineedu, S. R. M., Saad, S. and Stanslas, J. (2006). **Phytochemicals from *Phyllanthus niruri* Linn. and their pharmacological properties: a review.** Journal of Pharmacy and Pharmacology · DOI: 10.1211/jpp.58.12.0001 · https://www.researchgate.net/publication/6475974

Calixto, J. B., Santos, A., Cechinel, F. V. & Yunes, R. A. 1998. A review of the plants of the genus Phyllanthus: their chemistry, pharmacology, and therapeutic potential. *Medicinal research reviews,* 18**,** 225.

Cragg, G. M., Newman, D. J. & Snader, K. M. 1997. Natural products in drug discovery and development. *Journal of natural products,* 60**,** 52-60.

Dada, E. O., Ekundayo, F. O., and Makanjuola, O. O. (2014). Antibacterial activities of Jatropha curcas (LINN) on coliforms isolated from surface waters in Akure, Nigeria. International Journal of Biomedical Science, 10(1), 25–30.

Dias, D. A., Urban, S. & Roessner, U. 2012. A historical overview of natural products in drug discovery. *Metabolites,* 2**,** 303-336.

Fabricant, D. S. & Farnsworth, N. R. 2001. The value of plants used in traditional medicine for drug discovery. *Environmental health perspectives,* 109**,** 69.

Foo LY and Wong H: Phyllanthusiin D, Unusual hydrosable tannin from *Phyllanthus amarus*. Phytochemistry 1992; 31: 711-713.

Foo LY: Amarinic acid and related ellagitannins from *Phyllanthus amarus*. Phytochemistry 1995; 39: 217-224.

Füllbeck, M., Michalsky, E., Dunkel, M. & Preissner, R. 2006. Natural products: sources and databases. *Natural product reports,* 23**,** 347-356.

Lauk, L., Lo Bue, A. M., Millazo, I., Rapisarda, A. and Blandino, G. (2003). Antibacterial Activity of Medicinal Plant Extracts Against Periodontopathic Bacteria. *Phytotherapy Research* 17 (6): 599-604.

Newman, D. J. & Cragg, G. M. 2012. Natural Products As Sources of New Drugs over the 30 Years from 1981 to 2010. *Journal of Natural Products,* 75**,** 311-335.

Qiu D.Z., Zhang Z.J., Wu W.Z., Yang Y.K. (2013). Bufalin, a component in Chansu, inhibits proliferation and invasion of hepatocellular carcinoma cells. BMC Complementary and Alternative Medicine. 13:185.

Salazar, J.R., Martinez-Vazquez, M., Cespedes, C.L., Ramirez-Apan, T., Nieto-Camacho, A., Rodriguez-Silverio, J. and Flores- Murrieta, F. (2011). Anti-inflammatory and cytotoxic activities of chichipegenin, peniocerol, and macdougallin isolated from Myrtillocactus geometrizans (Mart. ex Pfeiff.) Con. Z. Naturforsch C. 66(1-2):24-30.

Syamasundar, K. V., Singh, B., Singh, T. R., Husain, A., Yoshinobu, K. & Hiroshi, H. 1985. Antihepatotoxic principles of Phyllanthus niruri herbs. *Journal of ethnopharmacology,* 14**,** 41-44.

Unander, DW, Webster, GL and Blumberg, BS (1995). Usage and bioassays in Phyllanthus (Euphorbiaceae). IV. Lustering of antiviral uses and other effects. *Journal of Ethnopharmacology.* (45) 1: pp. 1-18.

Wu SH (2014). "Bufalin induces cell death in human lung cancer cells through disruption of DNA damage response pathways. Am. J. Chin. Med. 42 (3): 729–42.

Zhang ZJ (2014). Bufalin attenuates the stage and metastatic potential of hepatocellular carcinoma in nude mice. J. Transl. Med. 12: 57.