



Research Article

Phytochemical Components and *In Vitro* Antioxidant Activity of Methanol Leaves Extract of *Phyllanthus niruri* Linn. (*Chanca piedra*)

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ABSTRACT

Oxidative stress has been recognized as a co-occurring pathogenic process that initiates and advances liver injury. Various risk factors, such as alcohol, drugs, environmental toxins, and irradiation, can cause the liver to experience oxidative stress, which in turn can lead to serious liver conditions such as alcoholic liver disease. This study was carried out to evaluate the antioxidant potentials of methanol leaf extract of *Phyllanthus niruri* Linn. (*Chanca piedra*). The sample was obtained from Maiduguri, Borno State, Nigeria. The methanol leaves extract was subjected to qualitative phytochemical analysis and *in vitro* radical scavenging activity, using standard laboratory procedures. The methanol leaves extract of *Phyllanthus niruri* Linn. (*Chanca piedra*) exhibited a significant antioxidant activity on both hydrogen peroxide (H₂O₂) and di-phenyl-picryl hydrazyl (DPPH) radical scavenging activity assay with IC₅₀ values of 3.70±0.06 and 6.78±0.01 µg/ml respectively, as compared to the standard (Ascorbic acid) with IC₅₀ values of 2.39±0.01 and 1.25±0.01 µg/ml, respectively. This study therefore suggests that the methanol leaves extract of *Phyllanthus niruri* Linn. (*Chanca piedra*) possessed antioxidant activity.

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INTRODUCTION

Antioxidants are chemical substances that stop or delay the oxidation of macromolecules. They play a crucial role in reducing or stopping these chain reactions (Duarte and Lunec, 2005). Scientific research revealed that antioxidants lower the risk for chronic diseases including cancer and heart disease. Whole grains, fruits, and vegetables are the main sources of naturally occurring antioxidants. Antioxidants from plants, such as vitamin C, vitamin E, carotenes, phenolic acids, etc., have been shown to have the ability to lower the risk of disease (Annonymous, 2002). The majority of antioxidant substances in a regular diet come from plant sources and falls under different classes of substances with a wide range of physical and chemical characteristics (Sravani and Paarakh, 2012).

Cellular macromolecules such as nucleic acids, proteins, and lipids can chemically react with reactive species produced in the cell during normal biological metabolism, resulting to their oxidative modification, which can change their composition and potentially harm their cellular functions (Bapat *et al.*, 2013).

Phyllanthus niruri Linn. (also known as *Chanca piedra*, gale of the wind, stonebreaker or seed-under-leaf in English and Geron tsuntsaye in Hausa) is a plant possessing several pharmacological properties. It grows 50–70 cm 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 (Patel *et al.*, 2011). *Phyllanthus niruri* Linn. (*Chanca piedra*) possesses a wide range of therapeutic characteristics. It is well-known in conventional healthcare systems. It is taken for diuretic, hypoglycemia, and hypertensive situations in addition to treating kidney stones, gallbladder stones, ailments such cancer and jaundice (Bagalkotkar *et al.*, 2006).

Phyllanthus niruri Linn. (also known as *Chanca piedra*, gale of the wind, stonebreaker or seed-under-leaf in English and Geron tsuntsaye in Hausa) is a plant possessing several pharmacological properties. It grows 50–70 cm tall and bears ascending herbaceous branches. The bark is smooth and light green.

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MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in the study are of analytical grade and were purchased from BDH Chemicals Ltd. Poole, England.

Plant Collection and Identification

The fresh leaves of *Phyllanthus niruri* Linn. (*Chanca piedra*) was collected from a garden, behind Federal Government College Maiduguri, Borno State, Nigeria, on 23rd December, 2021. The sample was identified and authenticated by Professor S.S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Nigeria.

Preparation of Plant Material

The fresh leaves of *Phyllanthus niruri* Linn. (*Chanca piedra*) was washed and shade dried. It was ground into fine powder using mortar and pestle. Then sieved to remove debris and coarse plant materials. A powdered form of the leaves was stored under laboratory condition prior to extraction. **Methanol Extract Preparation**

Five Hundred grams (500 g) powder of *Phyllanthus niruri* Linn. (*Chanca piedra*) leaves was extracted with one liter of 70 % methanol using cold maceration method, the product was concentrated to dryness at low temperature on a rotary evaporator and then stored at 4^o.

Qualitative Phytochemical Screening of Methanol Leaves Extract of *Phyllanthus niruri* Linn. (*Chanca piedra*)

The qualitative phytochemical analysis of the methanol leaf extract of *Phyllanthus niruri* Linn. (*Chanca piedra*) leaf was determined by standard methods as described by Trease and Evans, (2002) and Harbourne, (1984).

In vitro Antioxidant Studies

Hydrogen Peroxide Scavenging (H₂O₂) Activity Assay

The hydrogen peroxide (H₂O₂) scavenging activity of the methanol leaves extract of

Phyllanthus niruri Linn. (*Chanca piedra*) was determined as described by Ruch *et al.* (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM pH 7.4). The concentration of hydrogen peroxide was determined by absorbance at 230 nm using a spectrophotometer. Extract (20-60 µg/mL) in distilled water was added to hydrogen peroxide and absorbance at 230 nm was determined after 10 minutes against a blank solution containing buffer without hydrogen peroxide (Ruch *et al.*, 1989). The percentage of hydrogen peroxide scavenging was calculated using the formula below:

$$\% \text{ Scavenged (H}_2\text{O}_2) = \frac{(A_i - A_t)}{A_i} \times 100$$

Where A_i – Absorbance of the control and A_t = absorbance of the test.

Di-Phenyl-Picryl-Hydrazyl (DPPH) Scavenging Activity Assay

The di-phenyl-picryl-hydrazyl (DPPH) scavenging activity of the methanol leaves extract of *Phyllanthus niruri* Linn. (*Chanca piedra*) was determined according to the method described by (Vaidyaratnam, 2002; Patel and Patel, 2011 and Koleva and Beek, 2002).

Solution of DPPH (0.1 mM) in methanol was prepared. About 1 mL of the solution was added to 3 ml. of different concentrations of

$$\% \text{ Inhibition of DPPH radical} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A₀ was the absorbance of control reaction and A₁ was the absorbance in presence of test or standard sample.

Data Analysis

Results were expressed as mean ± Standard Error of Mean (SEM). Differences among mean of the groups was determined by student t-test using SPSS software version 23.0. P < 0.05 was considered statistically significant (Mead and Curnow, 1982). The IC₅₀ values of the sample (*Phyllanthus niruri*) and the standard (ascorbic acid), which is the concentration of sample required to inhibit

the extract (*Phyllanthus niruri*) in methanol (15, 30, 45, 60, 75, 90 µg/ml) (Vaidyaratnam, 2002). After that, the mixture was shaken vigorously and kept in complete darkness for 30 min. then, absorbance was measured at 517 nm. by using spectrophotometer (PEC Medical USA). Reference standard compound being used was ascorbic acid. (Patel and Patel, 2011). The Lower absorbance of the reaction mixture indicated higher free radical activity (Koleva and Beek, 2002). The percentage DPPH scavenging effect was calculated by using following equation:

50% of the H₂O₂ and DPPH free radicals, was calculated using MS Excel (2013 version).

RESULTS

Qualitative Phytochemical Constituents of Methanol Leaf Extract of *Phyllanthus niruri* Linn. (*Chanca piedra*)

The results of the phytochemical screening (Table 1) of the methanol leaves extract of *Phyllanthus niruri* Linn. (*Chanca piedra*) revealed the presence of alkaloids, tannins, flavonoids, anthocyanins, cardiac glycosides, beta cyanin, and phenols however, saponins,

steroids, quinones and terpenoids were not detected.

Table 1: Phytochemical Constituents of Methanol Leaves Extract of *Phyllanthus niruri* Linn. (*Chanca piedra*)

Constituents	Results
Alkaloids	+
Tannins	+
Flavonoids	+
Saponins	-
Anthocyanin	+
Steroids	-
Quinones	-
Terpenoids	-
Cyanogenic glycosides	+
Beta cyanin	+
Phenols	+

Key: + = Detected - = Not Detected

Hydrogen Peroxide (H₂O₂) Scavenging Activity

Hydrogen peroxide (H₂O₂) scavenging activity of the methanol extract and standard (Ascorbic acid) caused a good inhibition of hydrogen peroxide (Fig. 1). The methanol leaves extract of *Phyllanthus niruri* Linn. (*Chanca piedra*) revealed an excellent scavenging activity as compared to the standard (Ascorbic acid). The IC₅₀ value was found to be 3.70±0.06 µg/ml compared to the standard ascorbic acid 2.39±0.01 µg/ml. (Table 2).

Di-Phenyl-Picryl-Hydrazyl (DPPH) Scavenging Activity

Di-Phenyl-Picryl-Hydrazine (DPPH) scavenging activity of the methanol extract and standard (Ascorbic acid) caused a strong inhibition of DPPH. (Fig. 2). The methanol leaves extract of *Phyllanthus niruri* Linn. (*Chanca piedra*) revealed an excellent scavenging activity as compared to the standard (Ascorbic acid). The IC₅₀ value was found to be 6.78±0.01 µg/ml compared to the standard ascorbic acid 1.25±0.01 µg/ml (Table 2).

Table 2: IC₅₀ (µg/ml) Values for Methanol Leaves Extract of *Phyllanthus niruri* Linn. (*Chanca piedra*) and Standard (Ascorbic Acid)

Samples	IC ₅₀ (H ₂ O ₂)	IC ₅₀ (DPPH)
<i>Chanca piedra</i> (µg/ml)	3.70±0.06	6.78±0.01*
Standard (Ascorbic acid (µg/ml)	2.39±0.01	1.25±0.01

Values are presented as mean ± SEM, of three determinations. Values with different asteric (*) along the column are significantly different (P < 0.05).

Key:

H₂O₂ = Hydrogen Peroxide
 DPPH = Diphenyl Picryl Hydrazyl
 IC = Inhibitory Concentration

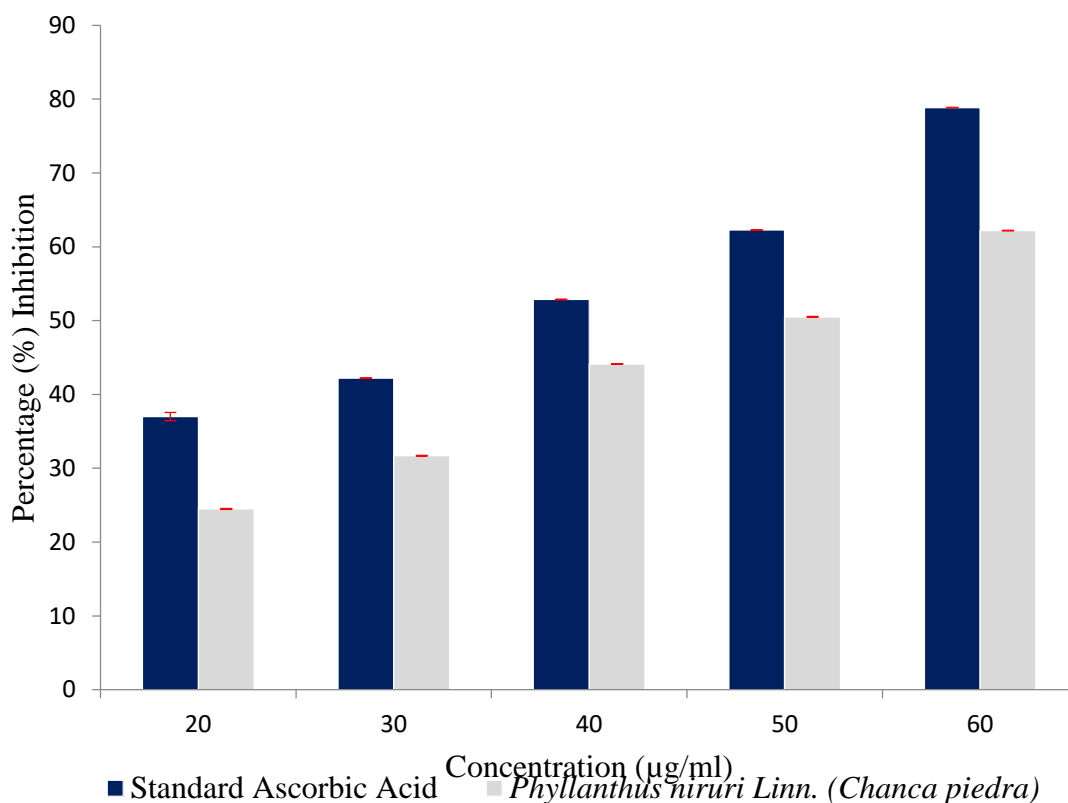


Figure 1: Percentage inhibition (%) of Hydrogen Peroxide (H₂O₂) Radical Scavenging Activity of Methanol Leaves Extract of *Phyllanthus niruri* Linn. (*Chanca piedra*) and Standard Ascorbic acid.

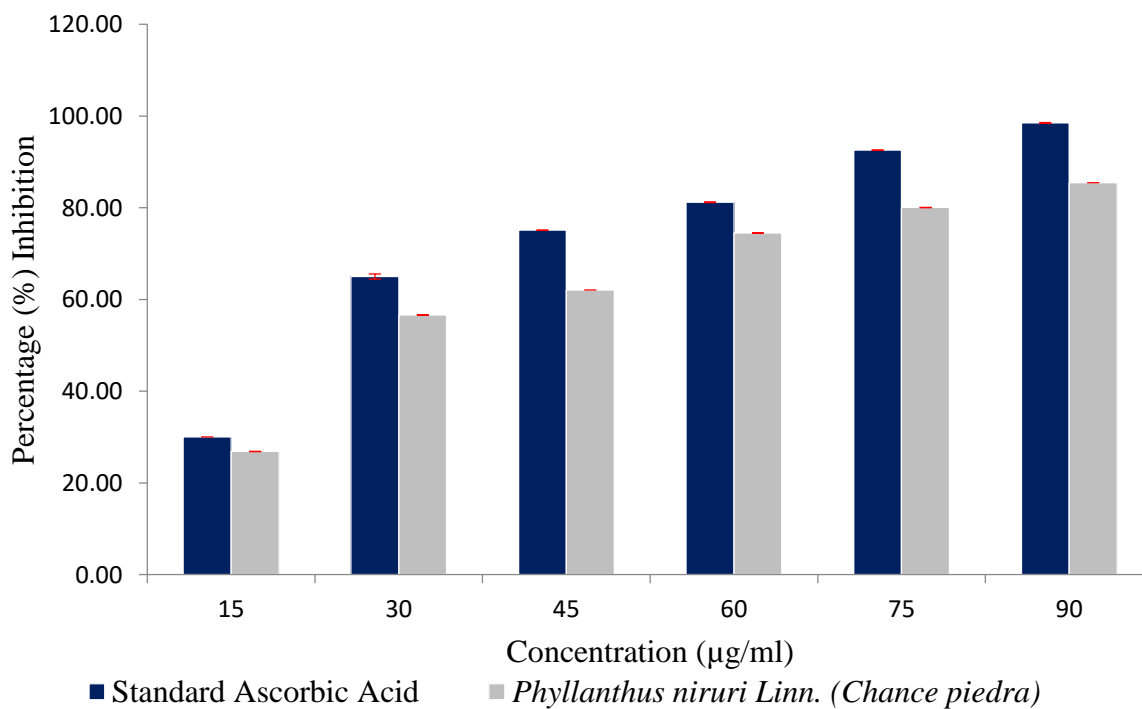


Figure 2: Percentage Inhibition (%) of Di-Phenyl-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity of Methanol Leaf Extract of *Phyllanthus niruri* Linn. (*Chanca piedra*) and Standard (Ascorbic acid)

DISCUSSION

Phytochemicals are physiologically active, naturally occurring bioactive compounds found in plants, which provide a wide variety of health benefits to humans more than those associated with micronutrients and macronutrients (Hasler and Blumberg, 1999).

The preliminary phytochemical screening conducted in this research revealed that methanol leaf extract of *Phyllanthus niruri* Linn. (*Chanca piedra*) leaf extract contained alkaloids, tannins, flavonoids, anthocyanins, cyanogenic glycosides, cardiac glycosides, beta cyanin, and phenols however, saponins, steroids, quinones and terpenoids were not detected (Table 1). This result is in conformity with the findings of Calixto *et al.* (1998) which stated that *Phyllanthus niruri* (*Chanca piedra*) is a good source of lignans, tannins, polyphenols, alkaloids, flavonoids, terpenoids and steroid.

The protective effects of these phytochemicals have received more attention against free radical induced liver toxicities (Frei and Higdon, 2003). Flavonoids played an important role in the protection against oxidative stress, (Okada *et al.*, 2001) especially in the case of cancer (Babich *et al.*, 2005). Also known as natural antioxidants, phytochemicals strengthen the endogenous antioxidants defenses from reactive oxygen species (ROS) and restore the optimal balance by neutralizing reactive species (Al-Mamary *et al.*, 2002); (Fetouh and Azab, 2014).

The *in vitro* antioxidant activity assay as presented in Table 2 showed that the methanol leaf extract of *Phyllanthus niruri* Linn. (*Chanca piedra*) exhibited a significant ($P < 0.05$) antioxidant activity in both hydrogen

peroxide (H_2O_2) and di-phenyl-picryl hydrazine (DPPH) scavenging activity assays, as compared to the standard (Ascorbic acid).

Phenolics and flavonoids are radical scavengers, reducing agents, metal chelators, and hydrogen donors (Khadri *et al.*, 2010; Saeed and Farzaneh, 2010). Therefore, it is worthwhile to measure the total phenolics and flavonoids content of a plant extract in order to determine its antioxidant capacity. The *in vitro* antioxidant activity of the methanol leaves extract of *Phyllanthus niruri* Linn. might be due to the presence of phenolics, flavonoids and other phytocomponents as demonstrated by hydrogen peroxide (H_2O_2) and di-phenyl-picryl-hydrazyl (DPPH) scavenging activity (Khadri *et al.*, 2010; Saeed and Farzaneh, 2010).

CONCLUSION

In conclusion, the findings of the research revealed that the methanol leaves extract of *Phyllanthus niruri* (*Chanca piedra*) Linn. contain phytochemicals possessing several pharmacological activities. This study, therefore, suggests that the leaf extract of *Phyllanthus niruri* Linn. (*Chanca piedra*) is a good source of antioxidant, and exhibited a significant antioxidant activity which might be helpful in the prevention and treatment of various liver diseases and ailments associated with oxidative stress.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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