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Antioxidant, chelating and HPLC quantification of phenols in extract of *Phyllanthus niruri*

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Abstract

*Phyllanthus niruri*is a known medicinal plant. The leaves of *Phyllanthus niruri* were evaluated for antioxidant activity utilizing different methods and the quantification of phenols and flavonoid using HPLC (High Performance Liquid Chromatography). The leaves of *Phyllanthus niruri* were harvested, shade dried, grounded and extracted with methanol. The extract was subjected to total antioxidant capacity, DPPH radical scavenging, nitric oxide (NO) scavenging, ferrous chelation and HPLC quantification of phenolics and flavonoids. The result showed total antioxidant capacity of 13.04 ±0.08 mgAAE/g. The % scavenging of DPPH radical of gallic acid a standard antioxidant was slightly higher than *Phyllanthus niruri* at concentrations of 0.1 – 1 mg/ml. In the case of nitric oxide (NȮ), the scavenging ability of *Phyllanthus niruri* was higher than the standard antioxidant quercetin at 0.1 – 1 mg/ml. the chelating ability of *Phyllanthus niruri* and EDTA at the same concentrations of 0.1 – 1 mg/ml shown in table 3 shows that *Phyllanthus niruri* has about 70 % of the chelating ability of EDTA. Some important phenols and flavonoids detected in *Phyllanthus niruri* are P-coumaric acid, gallic acid, caffeic acid, kaempferol, ferulic, luteolin, quercetin amongst others. This investigation shows that extract of *Phyllanthus niruri* acts as an antioxidant due to the abundance of phenolics and flavonoids.

Keywords: *Phyllanthus niruri*; Phenols; Flavonoids; Antioxidant; Chelating

1. Introduction

Phyllanthus niruri has broad medicinal properties and has been known in the health care system of tropical countries in the globe [1]. *Phyllanthus niruri*is used as a folk medicine for treating kidney stones [2], gallbladder stones, liver related diseases such as liver cancer & jaundice, apart from these it is also administered for diuretic, hypoglycemic and hypertension cases and it also shows anti-inflammatory, anti-tumor [3], anti-nociceptive and anti-oxidant [4] properties [5]. The aim of this study is to determine reactive oxidative species scavenging and HPLC quantification in extract of *Phyllanthus niruri*.

2. Material and methods

2.1. Reagents

DPPH (1, 1-diphenyl–2 Picrylhydrazyl), sodium nitroprusside, Griess reagent, copper sulfate ($CuSO_4$),pyrocatechol, thiourea, acetic acid, ethanol , ammonia solution, Quercetin, sodium hydroxide (NaOH), ,L-ascorbic acid, sodium phosphate, ammonium molybdate, EDTA were all of analytical grade.

2.2. Plant collection

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The plant *Phyllanthus niruri* (fresh leaves) were collected from Amassoma Community, Southern Ijaw L.G.A. Bayelsa State. The plant was identified and authenticated in the Department of Botany, Niger Delta University, Bayelsa State. November 2021.

2.3. Preparation of methanolic extract of *Phyllanthus niruri*

Phyllanthus niruri plant was harvested and was shade dried for 19 days (two weeks and 5 days). It was then grounded into fine powder. A total of 35.2 g of the pulverized plant was soaked in 800ml of methanol and kept for 72 hours (3 days). It was consecutively filtered using cheese cloth and filter paper. The extract collected was evaporated to dryness in a constant temperature hot water bath at 60°C. The paste formed (30.89 g) was then stored in the refrigerator for further use.

2.4. NO scavenging activity

The scavenging effect of plant extract on NO was measured according to the method of [6].

2.5. Fe2+chelation assay

The Fe2+chelating ability of the extracts were determined using a modified method of [7] with a slight modification by [8].

2.6. 1, 1-Diphenyl–2 Picrylhydrazyl Radical Scavenging

The free radical scavenging ability of the extracts against DPPH (1, 1-diphenyl–2 picrylhydrazyl) free radical was evaluated as described by [9].

2.7. Total Antioxidant by the phosphomolybdenum method

The antioxidant capacity of plant extract was evaluated by the method of [10].

2.8. High Performance Liquid Chromatography Quantification

Phenols and flavonoids were further extracted from the crude methanolic extract of *Phyllanthus niruri* and detected by high performance liquid chromatography.

3. Results

3.1. Percentage yield of plants

Percentage yield (%) = weight of extract/weight of dry plant × 100

Weight of Extract = 30.89 g

Weight of dry plant = 35.2 g

Percentage yield (%) = 30.89 g/35.2 × 100

=87.75

Table 1 Results of total antioxidant in Extract of *Phyllanthus niruri*

Sample	Total antioxidant
P. niruri	13.04 ±0.08 mgAAE/g

Values are mean ± S.D n = 3, AAE= Ascorbic Acid Equivalent

Table 2 Results of DPPH and Nitric oxide scavenging in *Phyllanthus niruri*

	DPPH Scavenging		**NO scavenging**	
Conc. mg/ml	***Phyllanthus niruri***	**Gallic acid**	***Phyllanthus niruri***	**Quercetin**
0.1	16.19 ±2.14	21.69±1.70	14.67 ± 0.06	18.09 ±1.73
0.2	34.25±2.04	36.04±1.15	28.05 ±2.93	29.75 ±2.18
0.4	55.69 ±0.92	61.17±0.78	48.35 ±1.22	48.62 ±1.54
0.6	61.63 ±0.97	73.77±0.25	61.41 ±1.95	57.58 ±1.37
0.8	78.62 ±1.15	84.58±1.04	79.52 ±2.5	66.97 ±1.94
1.0	81.20 ±1.69	94.19±3.04	83.15 ±1.39	76.61 ±1.87

Values are mean ± S.D n = 3

The % scavenging of DPPH radical of gallic acid a standard antioxidant was higher than *Phyllanthus niruri* at concentrations of 0.1 – 1 mg/ml. In the case of NO,˙The scavenging ability of *Phyllanthus niruri* was higher than the standard antioxidant quercetin at 0.1 – 1 mg/ml.

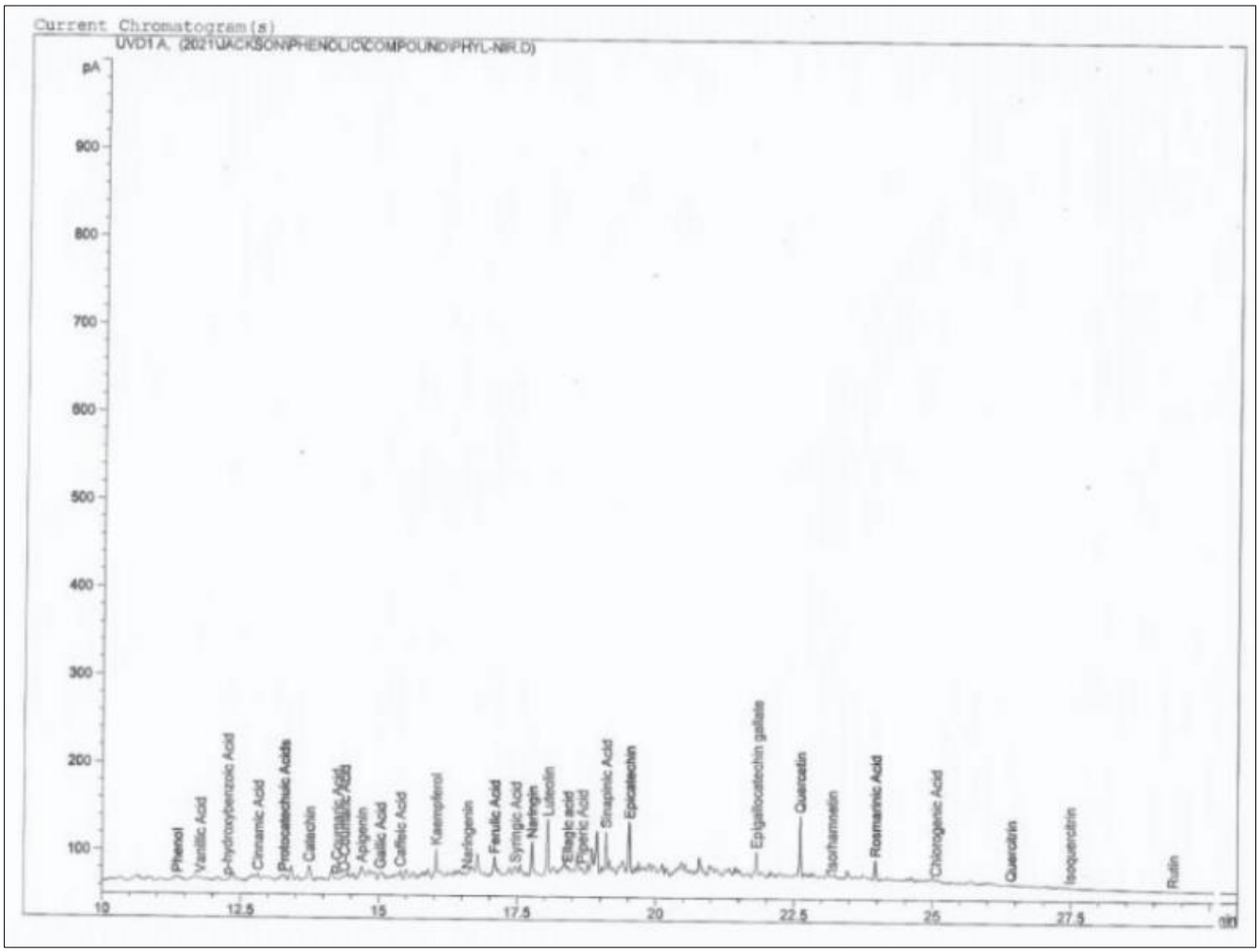

Figure 1 Chromatogram of phenolics in methanolic extract of *Phyllanthus niruri*

Comparing the chelating capacities of *Phyllanthus niruri* and EDTA at the same concentrations of 0.1 – 1 mg/ml table 3 shows that EDTA has chelating ability than *Phyllanthus niruri.*

Table 3 Results of Fe^{2+}Chelation in Extract of *Phyllanthus niruri*

Concentration	*P. niruri*%	EDTA%$FeCl_2$
0.1 mg/ml	15.97±1.04	22.88 ±1.25
0.2 mg/ml	23.92 ±1.55	34.67 ±1.77
0.4 mg/ml	58.89 ±1.52	62.64 ±2.17
0.6 mg/ml	73.91 ±2.55	80.49 ±1.34
0.8 mg/ml	85.01 ±2.37	88.72 ±2.91
1.0 mg/ml	91.45 ±1.64	96.54 ±3.04

Values are mean ± S.D n = 3

Table 4 Phenolic and flavonoids in extract of *Phyllanthus niruri*

RT (min)	Area	Amount (mg/100g)	Name of compound
11.35	123.34	8.86×10^{-3}	Phenol
11.77	69.02	1.64	Vanillic acid
12.26	24.79	4.92×10^{-2}	p-hydroxybenzoic acid
12.82	53.53	4.22×10^{-2}	Cinnamic acid
13.28	110.97	1.32	Protocatechuic acid
13.74	113.46	1.78×10^{-2}	Catechin
14.24	34.30	41.23	p-coumaric acid
14.37	60.34	1.82×10^{-3}	o-coumaric acid
14.69	153.12	1.78×10^{-2}	Apigenin
15.02	106.54	71.99	Gallic acid
15.39	72.42	44.16	Caffeic acid
16.04	142.27	83.10	Kaempferol
16.60	44.26	5.32×10^{-4}	Naringenin
17.09	144.73	87.82	Ferulic acid
17.46	124.45	2.27×10^{-2}	Syringic acid
17.77	212.28	1.22	Naringin
18.05	274.87	56.51	Luteolin
18.41	78.98	24.08	Ellagic acid
18.66	100.34	9.84×10^{-5}	Piperic acid
19.10	174.50	6.16	Sinapinic acid
19.52	263.15	1.85×10^{-5}	Epicatechin
21.82	203.56	1.66×10^{-2}	Epigallocatechin gallate
22.60	257.25	56.02	Quercetin
23.18	46.12	5.41×10^{-4}	Isorhamnetin
23.96	123.22	2.49×10^{-1}	Rosmarinic acid
25.06	29.00	3.29×10^{-1}	Chlorogenic acid
26.41	30.57	3.67×10^{-4}	Quercitrin
27.48	20.91	9.68×10^{-1}	Isoquercetrin
29.34	7.90	4.84×10^{-1}	Rutin

RT = Retention Time

4. Discussion

Phyllanthus niruri is a medicinal plant in Africa and other parts of the world. The percentage yield of *Phyllanthus niruri* was 87.75 %. The total antioxidant capacity of *Phyllanthus niruri* determined by the phosphomolybdate method showed 13.04±0.08 mgAAE/g [11].

Table 2 shows the scavenging effect of *Phyllanthus niruri* against DPPH and nitric oxide (NO). For DPPH radical gallic acid was a better scavenger than *Phyllanthus niruri*, but for NO *Phyllanthus niruri* was a better scavenger than the standard quercetin. This potential in *Phyllanthus niruri* may be due to phenols, flavonoids and other phytocompounds detected in *Phyllanthus niruri*. The potential to chelate ferrous was also evaluated and results showed in table 3 indicated that EDTA acts as a better chelator than *Phyllanthus niruri*.

The antioxidant, chelating and scavenging potentials of *Phyllanthus niruri* are due to secondary metabolites (phenols and flavonoids). These were detected by HPLC and displayed in table 4. P-coumaric acid, gallic acid, caffeic acid, kaempferol, ferulic, luteolin and quercetin were found in great quantities in the extract of *Phyllanthus niruri*. Other phenolics and flavonoids detected in lesser quantities also contributed to the medicinal value of *Phyllanthus niruri* are rutin, quercetrin, chlorogenic acid and many others [12].

5. Conclusion

The findings of this investigation indicated that methanolic extract of *Phyllanthus niruri* plant contains a good number of phytochemicals (phenolics and flavonoids) which are present in different quantities and are responsible for the antioxidant, chelating, scavenging and medicinal properties of *Phyllanthus niruri*. This methanolic fraction may however represent just a portion of the plant's medicinal value. Therefore further research is needed for complete extraction using other solvents and subsequent fractionation, identification and quantification.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest.

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