Malaysian Journal of Chemistry, 2019, Vol. 21(1), 96-103

GC-MS Analysis and Antiasthmatic Activity of Hexane Extract of *Phyllanthus amarus* (Chanca piedra) L. in Guinea Pig

Osaro Iyekowa[1*], Oladele Oyelakin[1] and Oghenekohwiroro Edjere[2]
[1]Department of Chemistry, University of Benin, Benin City, Nigeria/Division of Physical and Natural Sciences, School of Arts and Sciences, University of The Gambia
[1]Division of Physical and Natural Sciences, School of Arts and Sciences, University of The Gambia
[2]Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria
[*]Corresponding author (e-mail: osaro.iyekowa@uniben.edu)

Phyllanthus amarus (chanca piedra), plant family—*Euphorbiaceae*, have been used in the treatment of several ailments among traditional medicine practitioners. The plant after treatment and extraction with hexane solvent was screened for phytochemical constituents. Isolation of oil from the extract was done using vacuum liquid chromatography and characterized using gas chromatography-mass spectrometry. The acute toxicity test was determined to estimate the lethal dose (LD_{50}) value of the extract in Balb/c albino mice. Bronchial asthma was induced into the guinea pigs in the test groups with 10% histamine dihydrochloride aerosols. Chloro-pheniranmine (8 mg/kg), saline (5 ml/kg) and the plant extract (10 mg/kg, 20 mg/kg and 30 mg/kg) were administered orally for 30 minutes prior to the exposure of histamine aerosols for 6 minutes. Phenolics, flavonoids, and alkaloids were present in the extract. The major components detected from the isolated yellow oil of *P. amarus* among others were 1, 14-tetradecandiol [retention time (Rt): 17.47, 14.86%], a dihydric alcohol and cholest-4-en-3-one (Rt:19.49, 15.01%), a reduced cholesterol. LD_{50} value of 28.18 mg of extract/kg body weight of the mice was derived from the regression graph. Upon exposure of the treated guinea pigs to the histamine aerosols, those which developed typical histamine asthma (characterised by dyspnoea: convulsions and asphyxia) within 6 minutes of exposure were removed from chamber for possible recovery while those which did not develop typical asthma after the 6th minute were taken as protected. The results of the study generally indicated that the plant extract exhibited significant bronchodilatory activity against histamine.

Key words: *Phyllanthus amarus*; antiasthmatic activity; toxicity; phytochemicals

Received: July 2018; Accepted: May 2019

Phyllanthus amarus (chanca piedra), plant family—*Euphorbiaceae,* locally called 'ebe iyekezukpe' and 'iyin-olobe' among the Bini and Yoruba tribes in southern Nigeria respectively, is a medicinal plant used as vermifuge among the 'Binis' in Edo State of Nigeria. The decoction of the plant is used as purgative. The plant is made into a paste, and this paste is leaked twice daily for seven days for seven days [1].

The plant is employed for numerous other conditions by the indigenous peoples of Edo State, Nigeria, in the treatment of diabetes, malaria, dysentery, fever, flu, tumors, jaundice, vaginitis, gonorrhea [1]. In India, *Phyllanthus niruri*, a specie of *Phyllanthus* has been used in traditional medicines used for the treatment of jaundice, asthma, hepatitis and urolithic disease [2]. Over the years, the *P. amarus* has been used to manage cough, itchiness, arthritis, otitis, swelling, skin ulcer irregular menstruation, tachycardia, dysentery, spasmodic and weakness of male organ [3]. *P. niruri* is used to treat renal calculi in Brazil [4]. In South Africa, it is used in folk medicine to treat hyperuricemia [5]. While in Nigeria, aqueous extract of *P. amarus* has application in Nigerian homes for the elimination of waste from the body. It is also used to restore liver activity, blood tonic, and enhance body defence system [6]. In another research, the hydro-alcoholic extract of leaves of *P. amarus* was also found to have anti- hyperlipidemic potential in hyperlipidemic rats [7]. The nephroprotective effect of *P. amarus* has also been reported. Here, the aqueous extract of *P. amarus* at doses of 200 mg and 400 mg/kg/day for 14 days, were found to protect against the nephrotoxic effect of paracetamol and gentamicin in rat, through the maintenance of the level of blood urea nitrogen and serum creatinine within the normal range compared to control group [8]. Two compounds

isolated from *P. amarus*, 8-(3-methyl-but- 2–enyl)-2-phenyl chroman-4-one and 2-(4-hydroxyphenyl)-8-(3-methyl-but-2–enyl)-chroman-4-one were also reported to have antinematodal activity against *Meloidegyne incognita* and *Rotelenchulus reniformis* [9]. The antibacterial activity of *P. amarus* indicated that the extract showed the lowest minimum inhibitory concentration in some selected pathogens which includes, *Bacillus stearothermophilus, Staphylococcus aureus, Bacillus subtilis, Micrococcus leuteus, Salmonella typhi, Enterobacter aerogens, Proteus mirabilis,* and *Proteus vulgaris* [10]. *P. amarus* has been demonstrated to inhibit the DNA polymerase in Hepatitis B virus and Woodchuck hepatitis virus [11], blocked enzymes that play an important role in the reproduction of hepatitis B virus [12] while methanol extracts of the leaves of *P. amarus* led to a decrease in sperm motility and count of male guinea pigs in a dose- dependent manner and this effect was comparable to the observed effects of Vitamin E on sperm parameters [13]. On reproductive health using experimental animals, the methanol extract of *P. amarus* leaves caused significant increase in the level of testosterone of male Guinea pigs in a dose- and time- dependent manner with changes in the levels of leutenizing hormones (LH) and follicle- stimulating hormones (FSH). These changes caused by the plant were comparable to the changes caused by vitamin E on LH and FSH hormones [14]. It is reported that *P. amarus* improved antioxidant activity in liver and blood of irradiated mice [15] and prevented the genotoxic effect of radiation on mice chromosome, prevented the intestine from radiation-induced damages as evident by decreased peroxidation level of intestinal membrane and elevated antioxidant system [16]. More so, the plant offers protection against chemical carcinogenesis. It was reported that the aqueous extract of *P. amarus* significantly inhibited hepatocarcino-genesis induced by N-nitrosodiethylamine in a dose-dependent manner in male Wistar rats [17].

Since the mid-1960s, *P. amarus* has been the subject of much phytochemical research to determine the active constituents and their pharmacological activities. It is a rich source of plant chemicals, including many which have been found only in the Phyllanthus genus. Many of the "active" constituent in the plants include lignans, glycosides, flavonoids, alkaloids, ellagitannins, and phenyl propanoids found in the leaf, stem, and root of the methanol extract. Common lipids, sterols, and flavonols also occur in the nonpolar fraction of the plant. The main plant chemicals in chanca piedra include alkaloids, nirurin, brevifolin, carboxylic acids, methyl salicylate, nirurin, niruriside, phyllanthin, phyllanthine [18]. The plant is highly abortive (at high dosages), so should be considered contraindicated during pregnancy. It may increase the effect of diabetes and high blood pressure [18].

Asthma may be regarded as a diffuse, obstructive lung disease with hyper-reactivity of the airways to a variety of stimuli and a high degree of reversibility of the obstructive process, which may occur either spontaneously or as a result of chemical induction [19]. It is a chronic inflammatory disease, characterized by both bronchoconstriction and airway inflammation. Anti-asthmatic drugs like cortico-steroids, theophylline, salbutamol are widely used in the treatment of asthma but these drugs produce some adverse effects like immune suppression, cardiac problems [20]. Recently, Wu [21], revealed that *P. amarus* alleviated Th2 response in OVA-induced AHR via modulation of endogenous markers in a murine model of asthma. The approach on herbal medicine to reduce the adverse effects of asthma among other diseases has been increased and *P. amarus* is one of the traditional herbal medicine which claims to have many therapeutically beneficial effects. Thus, the present study aims to scientifically evaluate the antiasthmatic activity of *P. amarus*.

EXPERIMENTAL

Sample Collection and Treatment

The fresh succulent and herbaceous plant of *P. amarus* were collected from the University of Benin Teaching Hospital environment. The plant was identified and authenticated by a taxonomist Prof. J. F. Bamidele, with herbarium voucher number (UBHm 0204) deposited in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. It was air-dried in the laboratory for five weeks and then powdered using a milling machine (Christy laboratory mill, England) and stored in an airtight container.

580 g of powdered plant was extracted using n-hexane solvent in the soxhlet extractor for 8 h. 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 (122 g, yield: 21.03%) was then transferred into a sample bottle and stored for phytochemical analysis and antiasthmatic activity.

Phytochemical Screening of the Plant Extract

The plant extract was subjected to phytochemical screening using standard procedures by Sofowara, Trease and Evans, and Odebeyi and Sofowara [22-24]

to determine the presence of active components in the plant.

Acute Toxicity of the Hexane Extract of *P. Amarus*

The acute toxicity assay was performed according to the procedure described by [25] to estimate the lethal dose (LD_{50}) values of the hexane extract of *P. amarus* using BALB/c albino mice.

Induction of Bronchial Asthma in Guinea Pigs

Bronchial asthma was induced in guinea pigs using histamine dihydrochloride, according to the method described by Parmar, Gangwal, and Sheth [26]. Guinea pigs fasted for 24 h after which they were exposed to an atomized fine mist of 10% histamine dihydrochloride aerosols (using a nebulizer at a pressure of 300 mmHg in a chamber) to induce bronchial asthma. The guinea pigs were divided into 5 groups of 5 animals each. Chlorpheniramine (8 mg/kg), Saline (5 ml/kg) and the hexane extract of *P. amarus* (10, 20 and 30 mg/kg) were administered orally (via the use of a gastric cannular) 30 min prior to the exposure to histamine aerosols. Upon exposure of the treated guinea pigs to the histamine aerosols, animals which developed typical histamine asthma (characterized by dysponea — a condition that refers to difficulty in breathing which may lead to convulsions, asphyxia, and death) within 6 min of exposure were quickly removed from the chamber to fresh air for possible recovery. Those animals which did not develop typical asthma after the 6th min of exposure to histamine aerosols and the varying extract doses were taken as protected.

Isolation of Oil

Seventy grammes of the crude extract was partitioned with 100 ml of hexane: methanol mixture (ratio: 8: 2) and shaken vigorously in a separatory funnel. The upper hexane fraction was separated, concentrated and then subjected to vacuum liquid chromatography, using silca gel (particle size: 200-425 mesh) as the solid phase and hexane: methanol mixture (4:1) as the mobile phase. A yellow oily phase obtained was dried over Na_2SO_4 and concentrated to recover the pure oil (12 g, yield: 17.14%).

GC-MS Analysis

The analysis was carried out on a GC-Mass spectrometer filled with an HP-5 MS (5% phenysiloxane) column at a temperature programme of 70^{o}C (2 minutes) increase at 10^{o}C/min to 280^{o}C and held for 7 min. The carrier gas was nitrogen and flow rate, 1.80 ml/min.

RESULTS AND DISCUSSION

The phytochemical screening of *P. amarus* n-hexane extract revealed the presence of flavonoids, phenolic, terpenoids, and alkaloids which are useful bioactive agents that have been reported with physiological effects in humans [22].

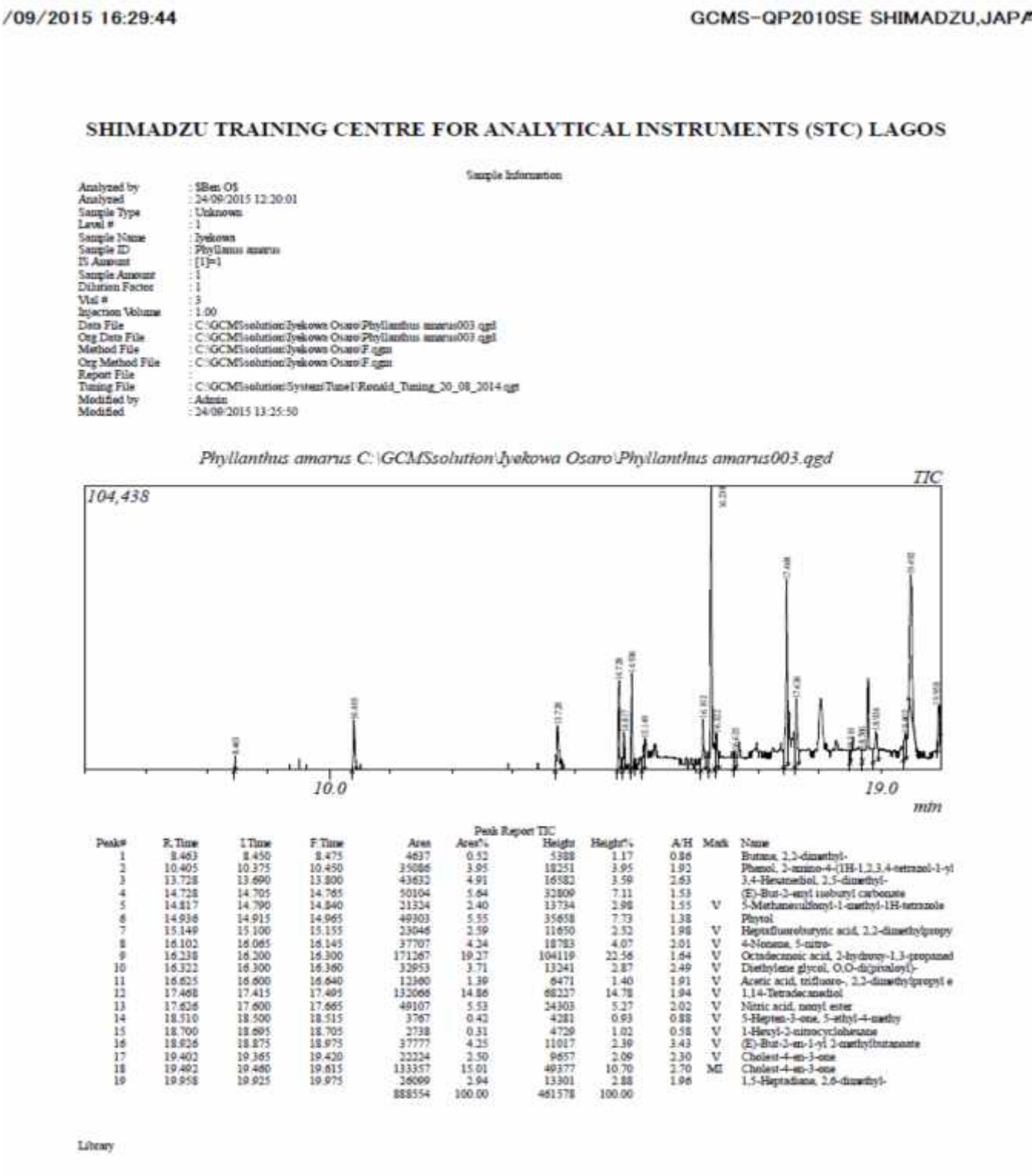
Table 1. Phytochemical screening of *P. amarus* hexane extract.

S/N	Phytochemical constituents	Name of the test	Hexane extract
1	Saponin	Foam Test	-
2	Flavonoid	Lead acetate test	+
3	Phenolics	Ferric chloride	+
4	Tannin	Ferric chloride	-
5	Eugenol	KOH/HCl	+
5	Steroids	Acetic acid/H_2SO_4	+
6	Terpenoid	Salkowski Test	+
7	Alkaloid	Picric acid Test	+

- = absent; + = present

GC-MS Analysis

The GC-MS chromatogram of the isolated yellow oil given in Figure 1 showed 19 peaks indicating from the search list of the chemical abstract service nineteen compounds. The chemical compounds identified in the oil fraction is presented in Table 2.



/09/2015 16:29:44 GCMS-QP2010SE SHIMADZU,JAPA

SHIMADZU TRAINING CENTRE FOR ANALYTICAL INSTRUMENTS (STC) LAGOS

Sample Information

Analyzed by	: \$Ben O\$
Analyzed	: 24/09/2015 12:20:01
Sample Type	: Unknown
Level #	: 1
Sample Name	: Iyekowa
Sample ID	: Phyllanus amarus
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 3
Injection Volume	: 1.00
Data File	: C:\GCMSsolution\Iyekowa Osaro\Phyllanthus amarus003.qgd
Org Data File	: C:\GCMSsolution\Iyekowa Osaro\Phyllanthus amarus003.qgd
Method File	: C:\GCMSsolution\Iyekowa Osaro\F.qgm
Org Method File	: C:\GCMSsolution\Iyekowa Osaro\F.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune\Ronald_Tuning_20_08_2014.qgt
Modified by	: Admin
Modified	: 24/09/2015 13:25:50

Phyllanthus amarus C:\GCMSsolution\Iyekowa Osaro\Phyllanthus amarus003.qgd

TIC

104,438

10.0 19.0 min

Peak Report TIC

Peak#	R. Time	I. Time	F. Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	8.463	8.450	8.475	4637	0.52	5388	1.17	0.86		Butane, 2,2-dimethyl-
2	10.405	10.375	10.450	35086	3.95	18251	3.95	1.92		Phenol, 2-amino-4-(1H-1,2,3,4-tetrazol-1-yl
3	13.728	13.690	13.800	43632	4.91	16582	3.59	2.63		3,4-Hexanediol, 2,5-dimethyl-
4	14.728	14.705	14.765	50104	5.64	32809	7.11	1.53		(E)-But-2-enyl isobutyl carbonate
5	14.817	14.790	14.840	21334	2.40	13734	2.98	1.55	V	5-Methanesulfonyl-1-methyl-1H-tetrazole
6	14.938	14.915	14.965	49303	5.55	35658	7.73	1.38		Phytol
7	15.149	15.100	15.155	23046	2.59	11650	2.52	1.98	V	Heptafluorobutyric acid, 2,2-dimethylpropy
8	16.102	16.065	16.145	37707	4.24	18783	4.07	2.01	V	4-Nonene, 5-nitro-
9	16.238	16.200	16.300	171267	19.27	104119	22.56	1.64	V	Octadecanoic acid, 2-hydroxy-1,3-propanedi
10	16.322	16.300	16.360	32953	3.71	13241	2.87	2.49	V	Diethylene glycol, O,O-di(pivaloyl)-
11	16.625	16.600	16.640	12360	1.39	6471	1.40	1.91	V	Acetic acid, trifluoro-, 2,2-dimethylpropyl e
12	17.468	17.415	17.495	132066	14.86	68227	14.78	1.94	V	1,14-Tetradecanediol
13	17.626	17.600	17.665	49107	5.53	24303	5.27	2.02	V	Nitric acid, nonyl ester
14	18.510	18.500	18.515	3767	0.42	4281	0.93	0.88	V	5-Hepten-3-one, 5-ethyl-4-methy
15	18.700	18.695	18.705	2738	0.31	4729	1.02	0.58	V	1-Hexyl-2-nitrocyclohexane
16	18.926	18.875	18.975	37777	4.25	11017	2.39	3.43	V	(E)-But-3-en-1-yl 2-methylbutanoate
17	19.402	19.365	19.420	22224	2.50	9657	2.09	2.30	V	Cholest-4-en-3-one
18	19.492	19.460	19.515	133357	15.01	49377	10.70	2.70	MI	Cholest-4-en-3-one
19	19.958	19.925	19.975	26099	2.94	13301	2.88	1.96		1,5-Heptadiene, 2,6-dimethyl-
				888554	100.00	461578	100.00			

Library

Figure 1. GC-MS Analysis of isolated yellow oil of *P. amarus.*

Table 2. GC-MS Analysis of isolated yellow oil of *P. amarus* .

Peak no.	Retention time (Rt)	Name of compound	Area percent (%)	Moecular formula	Mol. weigh (g/mol)
1	8.46	2,2-dimethyl butane	0.52	C_6H_{14}	86
2	10.41	2-amino-4 (1H-1,2,3,4-tetrazol-1-yl) phenol	3.95	$C_7H_7N_5O$	177
3	13.73	2,5-dimethyl-3,4-hexandiol	4.91	$C_8H_{18}O_2$	146
4	14.73	3-methyl-2-octanol	5.64	$C_9H_{20}O$	144
5	14.82	3-methyl-4-penten-1-ol	2.40	$C_6H_{12}O$	100
6	14.94	3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol)	5.55	$C_{20}H_{40}O$	296
7	15.15	4-butoxybutanol	2.59	$C_8H_{18}O_2$	146
8	16.10	1,6-heptadiene	4.24	C_7H_{12}	96
9	16.24	15-hydroxypentanoic acid	19.27	$C_{15}H_{30}O_3$	258
10	16.32	Diethylene glycol	3.71	$C_{14}H_{26}O_5$	274
11	16.63	3,3-dimethyl butanoic acid	1.39	$C_6H_{12}O_2$	116
12	17.47	1,14-tetradecandiol	14.86	$C_{14}H_{30}O_2$	230
13	17.63	3-methyl-1,2-cyclopentandiol	5.53	$C_6H_{12}O_2$	116
14	18.51	5-ethyl,4-methyl,5-hepten-3-one	0.42	$C_{10}H_{18}O$	154
15	18.70	Tridecyn-4-ol	0.31	$C_{13}H_{24}O$	196
16	18.93	But-2-en-1-yl-2-methylbutanoate	4.25	$C_9H_{16}O_2$	156
17	19.40	Cholest-4-en-3-one	2.50	$C_{27}H_{44}O$	384
18	19.49	Cholest-4-en-3-one	15.01	$C_{27}H_{44}O$	384
19	19.96	2,6-dimethyl 1,5-heptadiene	2.94	C_9H_{16}	124
Total			100.00		

In Table 2, the presence of hydroxyl fatty acid (15-hydroxypentanoic acid, Rt: 16.24, 19.27%), ester and reduced cholesterol (cholest-4-en-3-one, Rt: 19.40:2.50%) which are implicated as physiological agents [27] 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.

Antiasthmatic Activity (Dysponea observed during exposure of guinea pigs to histamine aerosols).

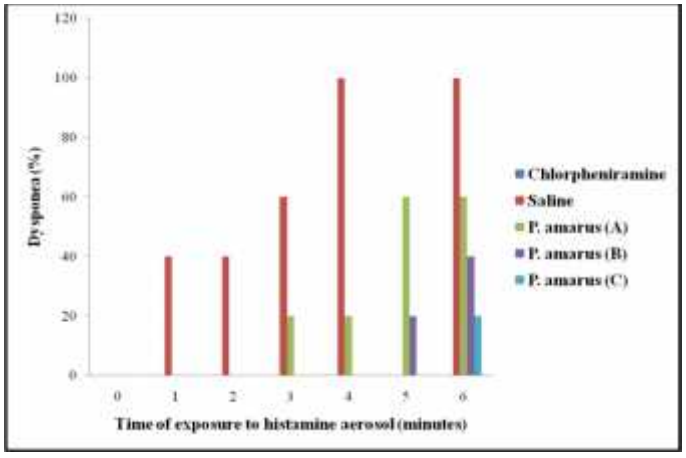


Figure 2. Profile of dysponea observed during exposure of guinea pigs to histamine aerosols.

Dysponea seen in saline- , chlorpheniramine- , and *P. amarus*-treated guinea pigs were compared. Dysponea was not seen in all the guinea pigs treated with chlorpheniramine, but was observed in 40% of the guinea pigs treated with saline within the first minute of administration of histamine aerosols. The remaining 60% of guinea pigs treated with saline however exhibited the symptoms of dysponea within the 4th minute of administration of histamine aerosols. 60% of the guinea pigs that were administered with 10 mg *P. amarus* extract (A) exhibited dysponea within the 5th minute of administration of histamine aerosols, while within the 6th minute of administration of histamine aerosols, dysponea was respectively exhibited in 40% of the guinea pigs that were administered with 20 mg *P. amarus* extract (B) and 20% of the guinea pigs that were administered with 30 mg *P. amarus* extract (C) (Figure 2).

(A) represents guinea pigs that received 10 milligrams hexane extract of *P. amarus* per kilogram body weight; (B) represents guinea pigs that received 20 milligrams hexane extract of *P. amarus* per kilogram body weight; (C) represents guinea pigs that received 30 milligrams hexane extract of *P. amarus* per kilogram body weight; while the remaining guinea pigs received chlorpheniramine (8mg/kg body weight), and saline (5ml/kg body weight), respectively. All doses of the *P. amarus* were administered based on the LD_{50} value of 28.18 milligrams /kg body weight of the animal.

Figure 3 represents profile of protection against symptoms of bronchial asthma observed after exposure of guinea pigs to histamine aerosols. Protection exhibited by the varying concentrations of *Phyllantus amarus* against bronchial asthma induced by histamine aerosols was dose-dependent in the guinea pigs examined. The protective effect (80%) was highest in the guinea pigs administered with 30 milligrams *P. amarus* extract / kg body weight and lowest (40%) in the guinea pigs administered with 10 milligrams *P. amarus* extract / kg body weight. There was no significant difference ($P < 0.05$) when the average protection exhibited by the varying concentrations of *P. amarus* extract was compared with the protection (100%) exhibited by chlorpheniramine (positive control).

Generally, the bronchodilatary effect of the *P. amarus* extract was found to exhibit significant protection ($P < 0.05$) against bronchial asthma induced by histamine aerosols, when compared to the protection provided by the reference standard (chlorpheniramine) thus, confirming that *P. amarus* had valuable antihistaminic activity.

CONCLUSION

In conclusion, the results of the present investigation suggested that *P. amarus* exhibited significant bronchodilatory activity against histamine-induced asthma.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

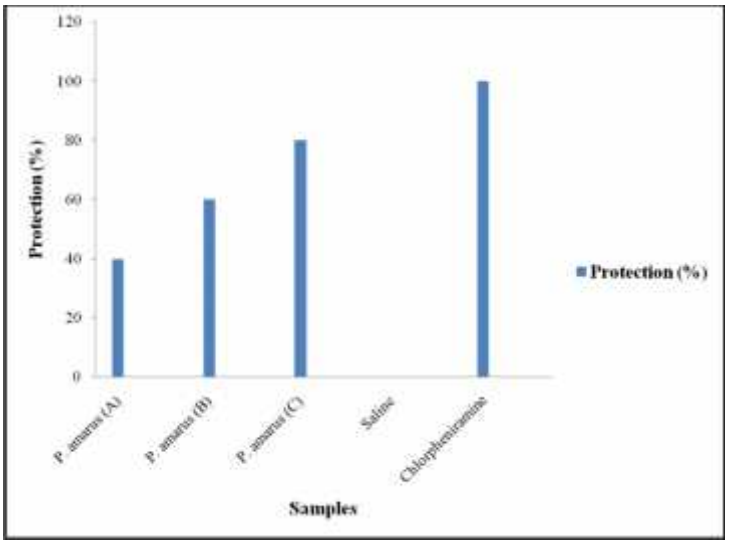


Figure 3. Profile of protection against symptoms of bronchial asthma observed after exposure of guinea pigs to histamine aerosols.

ACKNOWLEDGEMENT

The authors are grateful to Mr Odaro Stanley Imade of the Department of Biological Sciences, Igbinedion University, Okada (IUO), Edo State, Nigeria for his technical assistance.

REFERENCES

1. Gills, L. S. (1992) *Ethomedical uses of plants in Nigeria*, Uniben Press, University of Benin, Benin City, Nigeria.

2. Ishimaru, K., Yoshimatsu, K., Yamakawa, T., Kamada, H. and Shimomura, K. (1992) Phenolic constituents in tissue cultures of Phyllanthus niruri, *Phytochemistry*, **31**(**6**), 2015-2018.

3. Yeap, L. F., Wong, H. and Phyllanthusiin, D. (1992) An unusual hydrolysable tannin from Phyllanthus amarus, *Phytochemistry*, **31(2)**, 711-713.

4. Nishiura, J. L., Campos, A. H., Boim, M. A., Heilberg, I. P. and Schor, N. (2004) Phyllanthus niruri normalizes elevated urinary calcium levels in calcium stone forming (CSF) patients, *Urol. Res*., **32**, 362-366.

5. Murugaiya, V., and Chan, K.-L. (2009) Mechanism of antihyperuricemic effect of Phyllanthus niruri and its lignin constituents, *Journal of Ethnopharmacology*, **124**, 233-239.

6. Igwe, C.U., Nwaogu, L. A. and Ujuwondu, C. O. (2007) Assessment of the hepatic effects, phytochemical and proximate compositions of Phyllanthus amarus, *African Journal of Biotechnology*, **6(6)**, 728-731.

7. Khanna, A. K., Rizvi, F. and Chander, R. (2002) Lipid lowering activity of *Phyllanthus niruri* in hyperlipemic rats, *Journal of Ethnopharmacology*, **82**, 19- 22.

8. Adeneye, A. A. and Senebo, A. B. (2008) Protective effect of aqueous leaf and seed extract of P. amarus on gentamicin and acetaminophen induced nephrotoxic rats, *Journal of Ethnopharmacology*, **111**, 318-323.

9. Shakil, N. A., Pankaj Kumar, J., Pandey, R. K. and Saxena, D. B. (2008) Nematicidal prevylated flavonones from Phyllanthus niruri, *Phytochemistry*, **69**, 759-764.

10. Komuraiah, A., Bolla, K., Rao, K. N., Ragan, A., Raju, V. S. and Charya, M. A. S. (2009) Antibacterial studies and phytochemical constituents of South Indian Phyllanthus species, *African Journal of Biotechnology*, **8(19)**, 4991-4995.

11. Blumberg, B. S., Miilman, I., Venkateswaran, P. S. and Thyagarajan, S. P. (1990) Hepatitis B virus and primary hepatocellular carcinoma: treatment of HBV carriers with Phyllanthus amarus, Vaccine, *8 Supplement*, S86-S92.

12. Naik, A. D., Juvekar, A. R. (2003) Effect of alkaloidal extract of Phyllanthus niruri on HIV replication, *Indian Journal of Medical Science*, **57(9)**, 387- 393.

13. Obianime, A. W. and Uche, F. I. (2009) Comparative effects of methanol extract of *Phyllanthus amarus* leaves and Vitamin E on the sperm parameters of male guinea pigs, *J. Appl. Sci. Environ. Manage*, **13(1)**, 37- 41.

14. Obianime, A. W. and Uche, F. I. (2009) The Phytochemical constituents and the effects of methanol extracts of Phyllanthus amarus leaves (kidney stone plant) on the hormonal parameters of male guinea pigs, *J. Appl. Sci. Environ. Manage*, **13(1)**, 5-9.

15. Kumar, K. B. H. and Kuttan, R. (2004) Protective effect of an extract of *Phyllanthus amarus* against radiation induce damage in mice, *J. Radiat. Res*., **45**, 133-139.

16. Harikumar, K. B. N. and Kuttan, R. (2007) An extract of *Phyllanthus amarus* protects mouse chromosome and intestine from radiation induced damages, *J. Radiat. Res*., **48**, 469-476.

17. Jeena, K. J., and Joy, K. L. Kuttan, R. (1999) Effect of Emblica officinalis, Phyllanthus amarus and Picrorrhiza kurroa on N-nitrosodiethylamine induced hepatocarcinogenesis, *Cancer Letters*, **136**, 11-16.

18. Patel., M. Ali., T. E. Javad., H. and Amir, A. (2011) A systematic review about effect of aerial portion of Urtica dioica (Nettle) on some cardiovascular risk factors in diabetes mellitus, *Int. Res. J. Pharm.*, **2(12)**, 4-11.

19. Nelson, H. S. (2003) Prospects for antihistamines in the treatment of asthma, *J. Allergy Clin. Immunol*, **112**, 96–100.

20. Horwitz, R. J. and Busse, W. W. (1995) Inflammation and asthma, *Clin. Chest Med*., **16**, 583-620.

21. Wu, W., Li, Y., Jiao, Z., Zhang, L., Wang, X.

and Qin, R. (2018) Phyllanthin and hypophyllanthin from Phyllantus amarus ameliorates immune-infllammatory response in ovalbumin-induced asthma: role of IgE, Nrf2, iNOs, TNF-a and ILS, *Immunopharmacol and Immunotoxicol.,* **12**, 1-13.

22. Sofowora, A. (1993) *Medicinal plants and traditional medicine in Africa,* Spectrum Books Limited, Ibadan.

23. Trease, G. E. and W.C. Evans (1989) *Pharmacognosy*, 15th edn., W. B. Saunders, Edinburgh. [*Indian Journal of Pharmaceutical Sciences* (1997), **59,** 142–144].

24. Odebiyi, O. O. and Sofowara, E. A. (1978) Phytochemical screening of Nigeria medicinal plants, *Lloydia,* **41(3),** 234-246.

25. Lorke, D. (1983) A new Approach to practical acute toxicity Testing, *Arch. Toxicol.,* **54**, 275-287.

26. Parmar, S., Gangwal, A. and Sheth, N. (2010) Evaluation of antiasthmatic activity of a polyherbal formulation containing four plant extracts, *J. Clin. Pharmaceut. Res*., **2(1)**, 40 – 44.

27. Doughari, J. H. (2012) Phytochemicals: extraction methods, basic structures and modes of action as potential chemotherapeutic agents, phytochemicals — a global perspective of their role in nutrition and health, *InTech*., 1-5.