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Drug properties and antimicrobial evaluations of extracts from *Phyllanthus amarus*

Abstract

The development of resistance among pathogenic microorganisms against conventional or standard antimicrobial drugs has given rise to the need for alternative approaches to therapeutic measures. The methanol, ethanol, acetone, and aqueous extracts of leaves and seeds of *Phyllanthus amarus* were screened for phytochemical constituents and antimicrobial potentials. GC-MS analysis of the methanolic extract of the plant was carried out, followed by OSRIS analysis to identify compounds with drug properties. Phytochemicals detected were tannin, saponins, alkaloids, flavonoids, and phenols in the seed and leaf of the plant, depending on the extractive solvent, with exception of acetone extracts where none was detected. The GC-MS analysis identified imidazole, phytol, elemicin, methoxyeugenol, asarone, and heneicosane among others in extracted oil of *P. amarus* and most of the compounds were found to have high drug properties. The methanolic extracts of the plant produced higher antimicrobial activities than ethanolic and aqueous extracts; no antimicrobial activity was observed with acetone extracts. The minimum inhibitory concentration (MIC) of methanol extracts of both leaf and seed of *P. amarus* against test microbes ranged from 6.25 mg/mL to 50mg/mL. The seed extracts exhibited MIC consistently at 12.5-50 mg/mL. The minimum bactericidal concentration (MBC) of methanol extracts of *P. amarus* ranged from 25 mg/mL to 100 mg/mL while minimum fungicidal concentration (MFC) was observed at the range of 25mg/mL to 50 mg/mL. There was no significant difference in the antimicrobial activities between the methanolic extracts of leaves and seeds of *P. amarus* for the results of MIC, MBC and MFC. The antimicrobial efficacy of *P. amarus* may be attributable to some of its constituent biomolecules with high drug properties reported in this study.

Keywords: antimicrobial, drug-resistant microbes, *Phyllanthus amarus*

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Introduction

Medicinal plants are rich sources of antimicrobial agents, due to the metabolites they constitute, and many of the plant materials used in traditional medicine are readily available in rural areas and more accessible than orthodox drugs.[1] Medicinal plants identified in traditional medicine have a long history of consumption in nutrition and medicine in different nations. Thus, traditional medicines are a virgin resource for investigation on their efficacy and phytochemical constituents.[2,3]

Although the use of medicinal plants by man for the treatment of disease has been long in practice, forages and plants have been employed for therapeutic purposes based on the belief of medicinal values they possess; but in contemporary times researchers' interests exploring plants chemical constituents for therapeutic advancement. The chemical constituents, which is believed to be defensive molecules for plants, is appreciated by human beings because of their importance in health care.[4]

The genus *Phyllanthus* and family Euphorbiaceae are widely distributed in tropical and sub-tropical countries of the world and has been employed traditionally to treat chronic liver diseases among others.[5] The plant was first identified in Central and Southern India in the18th Century. The common names include carry me seed, stone breaker, breaker, gully leaf flower or gala of mind and in Nigeria, it is called "Oyomokeisoam-amkedem" by the Efiks, "Iyin Olobe" by the Yorubas, and "Ebebenizo" by the Binis.[6]

Phyllanthus amarus has been reported for its therapeutic potentials in several infectious and non-infectious diseases. *P. amarus* has been reported to be useful in the treatment of dropsy, jaundice, diarrhoea, dysentery, intermittent fevers, urinogenital diseases, scabies, ulcers, and wounds. *P. amarus* has been proven to possess pharmacological activities such as analgesic, antibacterial, antifungal, antihepatoxic, antihypercholesterolemic, antihyperglycemic, antihyperlipidemic, antimutagenic, antipyretic, antispasmodic, antitumor, antiviral, cardiotoxic, cytotoxic, among others.[7] Okiki and co-workers[8] reported that both seed and leaf of *P. amarus* separately possessed high levels of alkaloids and saponin; moderate in tannins and antioxidant levels; low in phytates, oxalate, protease inhibitor and phenol with no detectable cyanogenic glycosides; and they are also rich in protein, carbohydrate, fat, vitamins and minerals. The study was aimed at assessing the antimicrobial effects of extracts of the leaves and seeds of *P. amarus* against some selected microbes of medical importance.

Methods

Collection and identification of plant material

P. amarus plant was harvested from Afe Babalola University farm in March 2021 and identified at the Department of Biological Sciences, Afe Babalola University, Ado-Ekiti, Nigeria. The plant was then authenticated by a plant taxonomist and assigned Herbarium number UHA 2021412: *Phyllanthus amarus* Schum & Thorn (Euphorbiaceae) at Ekiti State University, Ado-Ekiti, Nigeria.

Preparation of *P. amarus* leaf and seed extracts

The plant was rinsed with clean water and air-dried. The seeds were separated from the leaves by sieving through a set of sieve outfits with different pores sizes. The seeds and leaves were separately ground to

smooth powder using an electric blender. The powders were kept in sterile air-tight containers before use. Four solvents namely methanol, ethanol, acetone and hot water were used for extraction. Fifty grams each of both seed and leaf were weighed and soaked for 72 hours in 250 mL of each solvent separately. The extracts were filtered using Whatman no1 filter paper and concentrated under reduced pressure at 40ºC using a rotary evaporator. The extracts collected were allowed to dry at room temperature and kept at -4ºC for further use.

Phytochemical analysis

The criteria of Trease and Evans[9] were used for qualitative analysis of phenols, tannin, saponin, alkaloids, flavonoids, cardiac glycoside, terpenoids and steroids from each extract.

GCMS analysis and identification of bioactive compounds in methanolic extract of *P. amarus*

Identification of the chemical compounds present in the oil extracted from *P. amarus* was carried out by the Gas Chromatography-Mass Spectrometry (GC-MS). Some of the GC-MS identified compounds in the crude extracts of *P. amarus* were screened computationally for their drug properties, using an online OSIRIS property explorer server. Each of the compounds was assessed for their bioavailability, hydrophobicity and membrane permeability which are linked with some molecular descriptors such as cLogP (partition coefficient) and cLogS (solubility).[10]

Source and preparation of isolates

The bacteria used in this study include *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 34089), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella Typhi* (ATCC 22648), and clinical isolates such as *Proteus mirabilis*, *Enterobacter aerogenes*, *Branchiibius cervisis*, *Staphylococcus aureus*, *Corynebacterium accolens*, *Klebsiella pneumoniae*, and *Salmonella Typhi*. Fungi used include *Candida albicans* (ATCC 10231), *Malazessia furfur* (ATCC 44349) and *Aspergillius flavus* (ATCC 204304). Before use, the bacteria species were sub-cultured in nutrient broth for 24 hours at 37ºC while the test fungal species were cultured in potato dextrose agar for 72 hours at 28±2ºC.

Antimicrobial susceptibility

The antimicrobial assay, of both leaf and seed extracts of *P. amarus*, was performed by agar well diffusion method. Mueller Hinton agar plates were inoculated separately with a suspension of each test bacterial culture by spread plate technique. The agar was carefully punched using a sterile 7 mm diameter cork-borer. The extracts were reconstituted in 5% dimethylsulfoxide (DMSO) to a final concentration of 100 mg/mL. The wells were filled with 100 mg/mL of the different solvent extracts of both seed and leaf extracts. Care was taken not to allow spillage of the solutions onto the surface of the agar. The culture plates were left at ambient temperature for 15 minutes to allow proper diffusion of these solutions before incubation at 37ºC for 24 hours. Wells in Mueller Hinton agar plates containing 5% dimethyl sulphide (DMSO) without extract served as control. The experiments were done in duplicates. After 24 hours of incubation, the plates were examined for any inhibition zone. The diameters of inhibition zones produced by each of the extracts were measured and interpreted using Clinical and laboratory standards institute (CLSI) zone diameter interpretative standards.[11]

Antifungal assay

The leaf and seed extracts of the test plant were screened for antifungal activity by agar well diffusion method using potato dextrose agar (PDA).[12] An aliquot of inoculum was introduced to molten PDA and poured into a petri dish by pour plate technique. On solidification, wells were then bored into the agar media using a sterile 7 mm cork-borer. The wells were filled with 100 mg/mL concentration of both the seed and leaf extracts in the fungi seeded cultures.

The plates were incubated at room temperature for 24-72 hours and antifungal activities were evaluated by observing the zones of inhibition. The diameters of the inhibition zones produced by each of the concentrations of the extract were measured, recorded and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) zone diameter interpretative standards.[10]

Minimum Inhibition Concentration (MIC)

Different concentrations (0.78-50) mg/mL of the extracts were prepared by serial dilution in Mueller Hinton broth medium. Each tube was then inoculated with 100µL of each of the typed bacterial strains. Two Mueller Hinton broth tubes, with and without bacterial inoculation were used as the growth and sterility controls. The experiment was incubated aerobically at 37ºC for 24 hours. After incubation, the tubes were observed for turbidity by checking the control tubes with treated tubes to access the MIC. This was followed by determining the minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) for the bacterial and fungal isolates respectively.

Statistical analysis: One-way ANOVA was used to determine significant differences between groups, using IBM SPSS Statistics 23. A value of $p \leq 0.05$ was considered significant.

Results

The qualitative phytochemical screening of *P. amarus* extracts revealed the presence of important phytoconstituents such as tannin, saponins, alkaloids, flavonoids and phenols in the seed and leaf of the plant, depending on the extractive solvent, with exception of acetone extracts where none was detected (Table 1).

Table 1 Qualitative phytochemical constituents of *Phyllanthus amarus* extracts

	Methanol		Ethanol		Acetone		Aqueous	
Phytochemicals	**Leaf**	**Seed**	**Leaf**	**Seed**	**Leaf**	**Seed**	**Leaf**	**Seed**
Phenols	+	+	+	+	-	-	+	+
Tannin	+	+	+	+	-	-	+	+
Saponin	+	+	-	+	-	-	+	+
Alkaloids	+	+	-	-	-	-	+	+
Flavonoids	+	+	+	-	-	-	+	-

Citation: Okiki PA, Egbebi A, Akharaiyi FC, et al. Drug properties and antimicrobial evaluations of extracts from *Phyllanthus amarus*. *J Microbiol Exp.* 2022;10(1):10–16. DOI: 10.15406/jmen.2022.10.00346

Table Continued...

	Methanol		Ethanol		Acetone		Aqueous	
Phytochemicals	**Leaf**	**Seed**	**Leaf**	**Seed**	**Leaf**	**Seed**	**Leaf**	**Seed**
Cardiac glycosides	+	-	-	-	-	-	+	-
Steroidal glycoside	-	-	-	-	-	-	-	-
Terpenoids	+	-	-	-	-	-	+	-
Steroids	-	-	-	-	-	-	-	-

+ present - absent

The product of GC-MS analysis of methanol extract of *P. amarus* gave spectra with 22 peaks (Figure 1). The identified compounds from the spectra include imidazole, phytol, elemicin, methoxyeugenol, asarone and heneicosane among others (Table 2). OSIRIS property explorer analysis of the identified compounds showed some of them (Elemicin, Asarone, Phytol, Imidazole and Heneicosane) having high drug properties that could be exploited for the development of new drugs (Table 3).

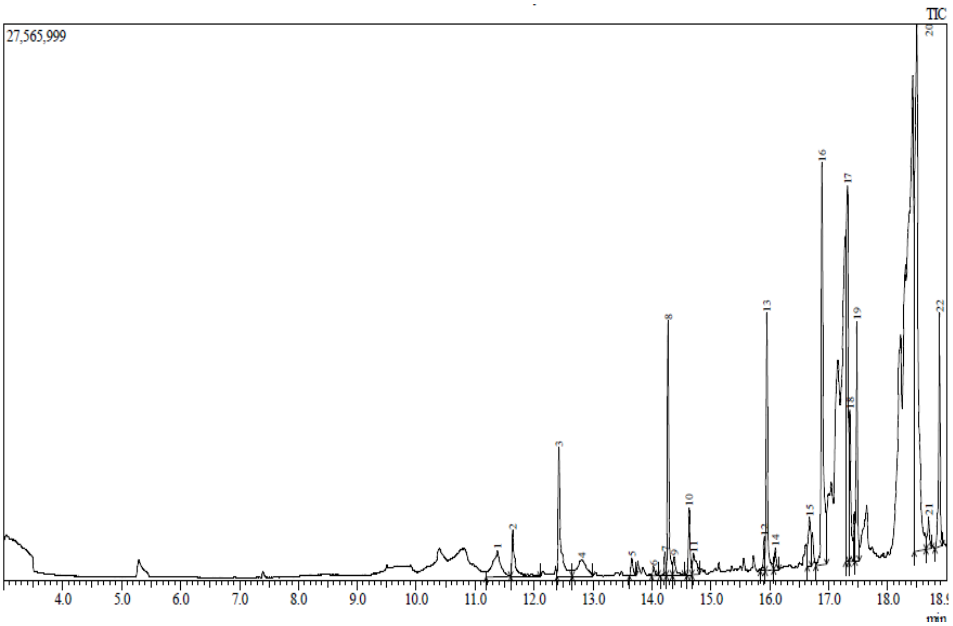

Figure 1 Chromatogram of GCMS analysis of methanol extract of *Phyllanthus amarus*.

Table 2 Biomolecules identified in GCMS analysis of *Phyllanthus amarus* methanolic extract

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	11.379	12364300	3.14	1310189	0.9	9.44	Methoxyeugenol
2	11.637	9122976	2.32	2309747	1.58	3.73	2,4-di-t-Butylphenol
3	12.422	18730853	4.76	6419684	4.39	2.92	Asarone
4	12.812	9464354	2.4	850394	0.58	11.13	Elemicin
5	13.659	2090947	0.53	896119	0.61	2.33	Dihydrophytol
6	14.027	1110353	0.28	463961	0.32	2.39	11-Butyldocosane
7	14.222	2065499	0.52	1153139	0.79	1.79	3-Eicosene
8	14.274	22700937	5.77	12605320	8.61	1.8	Octadecane
9	14.38	3032300	0.77	917441	0.63	3.31	Hexyldecanol
10	14.631	6594032	1.68	3333728	2.28	1.98	Phytol, acetate
11	14.71	4694026	1.19	1059453	0.72	4.43	Hexahydrofarnesyl acetone
12	15.905	2814991	0.72	1675620	1.14	1.68	1-Nonadecene
13	15.95	22582701	5.74	12733162	8.7	1.77	Nonadecane

Citation: Okiki PA, Egbebi A, Akharaiyi FC, et al. Drug properties and antimicrobial evaluations of extracts from *Phyllanthus amarus*. *J Microbiol Exp.* 2022;10(1):10–16. DOI: 10.15406/jmen.2022.10.00346

Table Continued...

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
14	16.095	2498189	0.63	1092200	0.75	2.29	Dodecane, 5-cyclohexyl-
15	16.676	7896573	2.01	2422032	1.65	3.26	1-Heneicosanol
16	16.887	47464819	12.06	19916792	13.61	2.38	Phytol
17	17.32	48479564	12.32	18619625	12.73	2.6	Imidazole
18	17.359	16065567	4.08	7507105	5.13	2.14	N-[2-(3,4-Dimethoxy-phenyl)-ethyl]-3-phenyl-a
19	17.479	20407383	5.19	11866597	8.11	1.72	Heneicosane
20	18.5	1.08E+08	27.33	26080579	17.82	4.12	6-Amino-9-(2-p-tolyloxy-ethyl)-9H-purine-8-th
21	18.699	4017422	1.02	1608711	1.1	2.5	2-methylhexacosane
22	18.883	21785207	5.54	11539161	7.88	1.89	Hexacosane
Total		3.94E+08	100	1.46E+08	100		

Table 3 Drug properties of some of the identified compounds in the crude extracts determined by OSIRIS property explorer

Compound	Drug likeness	Mutagenic	Tumorigenic	cLogS	Polar surface area (A°)	% Absorption	Reproductive effect	Irritability
[a]Elemicin	-4.7336	High	None	-2.382	0.16822	109	None	None
[b]Asarone	-4.0375	High	High	-2.578	0.16779	109	High	None
[c]Phytol	-3.7661	None	None	-4.633	0.046626	109	None	None
[d]Imidazole	0.44659	High	None	-0.431	0.40526	109	High	None
[e]Heneicosane	-20.398	None	None	-6.114	0	109	None	None

[a]Elemicin share the same functional group with Isoelemicin 1,2,3-Trimethoxy-5-(1-propenyl) Benzene, 5(1-propenyl)-1,2,3-trimethoxy

[b]Asarone share the same functional group with Asarone (E)-2,4,5-Trimethoxypropenylbenzene, trans-2,4,5-Trimethoxypropenylbenzene Benzene, 1,2,4-trimethoxy-5-(1-propenyl)-, (E)-

[c]Phytol share the same functional group with Phytol, acetate

[d]Imidazole shares the same functional group with Phenantro[9,10-d]imidazole, 2-(3,4-dimethoxyphenyl)- 2-(3,4-Dimethoxyphenyl)-1H-phenanthro[9,10-d] imidazole

[e]Heneicosane share the same group with Heneicosane

Note: % absorption =109-0.345(PSA)

The results of antimicrobial activities of extracts of *P. amarus* to test organisms are presented in Table 4. The methanol extracts produced higher antimicrobial activities than ethanol and aqueous extracts; no antimicrobial activity was observed with acetone extracts. All the microorganisms tested were susceptible to methanol extracts of both leaf and seed of *P. amarus* (Table 4). The MIC of methanol extracts of both leaf and seed of *P. amarus* against test microbes ranged from 6.25 mg/mL to 50mg/mL. The seed extracts exhibited MIC consistently at 12.5-50 mg/mL. The MBC of methanol extracts of *P. amarus* ranged from 25 mg/mL to 100 mg/mL while the MFC was observed at the range of 25mg/mL to 50 mg/mL (Tables 5 & 6). There was no significant difference in the antimicrobial activities between the methanol extracts of leaves and seeds of *P. amarus* for the results of MIC, MBC and MFC in this study.

Table 4 Antimicrobial activities of 100 mg/mL extracts of *Phyllanthus amarus*

	Methanol		Ethanol		Aqueous		Acetone		Amoxicillin
	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	
Bacteria									
Staphylococcus aureus (ATCC 25923)	12	12	8	10	8	10	0	0	17
Escherichia coli (ATCC 35218)	13	10	8	8	7	8	0	0	18
Klebsiella pneumoniae (ATCC 34089)	11	12	7	11	10	9	0	0	17
Pseudomonas aeruginosa (ATCC27853)	11	12	8	7	9	6	0	0	20

Table Continued...

	Methanol		Ethanol		Aqueous		Acetone		Amoxicillin
	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	
Salmonella Typhi (ATCC 22648)	9	3	9	8	9	0	0	0	15
Staphylococcus aureus	11	10	7	9	8	10	0	0	3
Klebsiella pneumoniae	13	14	8	7	11	10	0	0	10
Corynebacterium accolens	12	11	10	9	0	8	0	0	5
Proteus mirabilis	13	13	12	11	12	0	0	0	9
Enterobacter aerogenes	12	11	10	10	8	6	0	0	9
Branchiibius cervisis	13	6	8	0	0	0	0	0	7
Salmonella Typhi	14	17	15	16	10	11	0	0	9
Fungi									
Aspergillus flavus (ATCC 204304)	12	10	6	4	4	3	0	0	ND
Candida albicans (ATCC 10231)	10	7	4	5	0	4	0	0	ND
Malazessia furfur (ATCC 44349)	12	11	3	0	6	4	0	0	ND
Aspergillus flavus	9	9	5	3	6	6	0	0	ND
Candida albicans	6	7	8	6	0	2	0	0	ND

ND – Not done

Table 5 Antibacterial activities of methanolic extract of *Phyllanthus amarus*

	Minimum Inhibitory Concentration (mg/mL)		Minimum Bactericidal Concentration (mg/mL)	
Bacteria	**Leaf**	**Seed**	**Leaf**	**Seed**
Staphylococcus aureus (ATCC 25923)	25	25	25	25
Klebsiella pneumoniae (ATCC 34089)	6.25	12.5	25	25
Escherichia coli (ATCC 35218)	50	50	25	100
Salmonella Typhi (ATCC 22648)	12.5	25	50	25
Pseudomonas aeruginosa (ATCC 27853)	50	12.5	50	25
	p = 0.426		P=0.704	

ATCC – American Type Culture Collection

Table 6 Antifungal activities of methanolic extract of *Phyllanthus amarus*

	Minimum Inhibitory Concentration (mg/mL)		Minimum Fungicidal Concentration (mg/mL)	
Fungi	**Leaf**	**Seed**	**Leaf**	**Seed**
Candida albicans (ATCC 10231)	12.5	25	50	25
Malazessia furfur (ATCC 44349)	25	50	25	50
Candida albicans (Clinical isolate)	25	25	50	50
	p = 0.225		p = 1.000	

ATCC – American Type Culture Collection

Discussion

Phyllanthus amarus plant has been reported upon with therapeutic properties that include antibacterial, antioxidants and anti-inflammatory. This study showed the antimicrobial effects of this plant on bacteria and fungi of medical importance. The results showed that methanolic extracts of the leaf and seed of *P. amarus* have antibacterial activities on all the bacteria screened. This finding is in tandem with the report of Olufemi and Debiri,[13] who and could be attributed to the phytochemical constituents which include tannin, saponin, alkaloid and phenol. These phytochemicals have been reported in varieties of plant foods and beverages to prevent the initiation, progression and spread of cancer.[14]

The antimicrobial results of this study conformed to the reports of Adegoke et al.,[15] that *S. aureus*, *E. coli*, *S. Typhi* and *Klebsiella* species were susceptible at a concentration of 100 mg/mL with an increased zone of inhibition as the concentration of extract increased. However,

the ability demonstrated by the methanol, ethanol and aqueous extracts of leaf and seed to inhibit the growth of these organisms indicates that the test organisms do not exhibit a mechanism that inactivates the bioactive constituents in the extracts or other mechanisms which may include exclusion of the substance from the cell and modification of the binding site of the substance. The phytochemical constituents could contribute to the antimicrobial potency observed with the plant extracts; for example, saponins have a positive role in cholesterol metabolism as they are expectorants, cough suppressants and can be administered for haemolytic activities,[16,17] while tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them and to form irreversible complexes with proline rich protein,[18] resulting in the inhibition of cellular protein synthesis and this also confirms the report by Okwu and Okwu.[17]

P. amarus can help control infection caused by *Staphylococcus aureus* which is an important human pathogen causing infection, ranging from food poisoning or minor skin infections to severe life-threatening infections such as septicaemia and disseminated abscesses in all organs and also *Escherichia coli* which causes urinary infection (UTI), diarrhoea, sepsis and meningitis.[19] These extracts also have an antifungal property, as it showed a clear zone of inhibition against fungi tested which also conforms with the report of Sahidi et al.[20]

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) exhibited by the extracts of *P. amarus* on *Staphylococcus aureus*, *K. pneumoniae*, *E. coli*, *S. Typhi* and *P. aeruginosa* is of great significance as these organisms are of medical importance and possess threat to humans.[21]

The identified compounds present in this plant that have high drug properties are Elimicin, Asarone, Phytol, Imidazole and Heneicosane. Elemicin is a constituent of several plant species essential oils and has strong antifungal and antibacterial activities.[22] Asarone is a chemical compound of the phenylpropanoid class, it has the role of plant metabolite, it is used to kill pests and bacteria. It is clinically used as a medication for treating epilepsy, cough, bronchitis and asthma. It exhibits neuroprotective, anti-oxidative, anticonvulsive and cognitive enhancing action.[23]

Phytol is acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1, which is mainly known as the side chain of the plant pigment chlorophyll, is released during chlorophyll breakdown. Phytol is reported to have antimicrobial, anti-inflammatory, anti-mycobacterial, anti-nociceptive activities and it is also having antiviral and carminative properties.[24] It can be suggested based on the findings from this research that *P. amarus* may serve as an alternative to conventional antibiotics which may have side effects on humans or on microbes developing resistance.[25]

The imidazole is a constituent of several important natural products, including purine, histamine, histidine and nucleic acid. Imidazole drugs have broadened the scope in remedying various dispositions in clinical medicines. Medicinal properties of imidazole include anticancer, b-lactamase inhibitors, 20HETE (20-Hydroxy-5,8,11,14-eicosatetraenoic acid) synthase inhibitors, carboxypeptidase inhibitors, hemeoxygenase inhibitors, antiaging agents, anticoagulants, anti-inflammatory, antibacterial, antifungal, antiviral, antimycobacterial, antidiabetic and antimalarial.[26] Heneicosane is found, on average, in the highest concentration within a few different foods, such as black elderberries, common oregano, and lemon balms. This could make heneicosane a potential biomarker for the consumption of these foods.[27]

Conclusion

This study affirmed the antimicrobial potentials of *P. amarus* and revealed the molecules responsible for such abilities. This plant, considering its potency, availability and cost-effectiveness, may be explored for therapeutic advances against drug-resistant organisms.

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Conflicts of interest

There is no conflict of interest among the authors.

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