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Screening and Chromatographic identification of the Phytochemicals in the Powdered leaves of Urena *lobata L.* (Malvaceae)

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Abstract

Urena lobata is a widely distributed tropical and subtropical weed with varying pharmacological activities, ascribed to the presence of bioactive principles in the plant. This study aimed to identify phytochemicals in the powdered leaves of Urena lobata using two chromatographic techniques. Phytochemical screening was evaluated for the powdered leaves using standard methods, Gas Chromatography-Mass Spectrometry (GC-MS) and High Pressure Liquid Chromatography (HPLC) were utilized to determined the phytochemicals in the methanolic extract of the leaves. Results obtained indicate that alkaloids, cyanogenic glycosides, flavonoids, saponins, steroids, tannins and terpenoids were detected in the methanolic extract of the leaves. The GC-MS analysis showed the presence of 4-(3-dimethylaminopropoxy)benzaldehyde, pyridine, 9-octadecenamide, 2-amino-5-chlorophenyl phosphonic acid, 2-propenenitrile, 2,1-benzisoxazole-4-carboxlic acid, 1,2,4-triazin-5(2H)-one, pyridine-3-carboxamide, 2-pyrazoline, 4-aminobenzoic acid, 3-nitrophthalhydrazide, 2,4 (1H, 5H)-imidazoldione and bromophos-ethyl, semicarbazide. While the HPLC analysis for selected alkaloids detected imidazole and pyrazoline alkaloidal moieties from the extract. Owing to the results obtained in this study, it can be concluded that phytochemicals of important pharmacological potentials were detected in the methanolic extract of Urena lobata leaves.

Keywords: Methanolic extract, Gas chromatography-mass spectrometry, High pressure liquid chromatography, phytochemicals.

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INTRODUCTION

The Malvaceae family, sometimes known as the mallows, belongs to dicotyledonous flowering plant, that is distributed in the tropic and temperate regions of the world (Babu *et al*., 2016). It is made up of about two hundred and fifty (250) genera and over four thousand (4000) species (Xu & Deng, 2017). Numerous ethno-botanical uses of different parts of the plants belonging to this family have been documented (Sheema *et al*., 2023). They are used as diuretic and demulcent, equally employed in the treatment of fevers, chest infections, gonorrhea, urethritis and bleeding piles. Also they are use as an astringent, anthelmintic; lessens perspiration; good in strangury and urinary complaints. Other reported uses include aphrodisiac, laxative and emollient agents. They are good for the treatment of bronchitis, cough, gleet and chronic cystitis. They are used as nervine tonic while infusions from plants in this family are useful for managing leprosy, leucoderma, haematuria and stones in bladder. While some plants in this family may be used as an abortifacient to induce menstrual flow, others cause dysmenorrhoea and suppression of menstruation. Others stimulates uterine contractions and hastens difficult labour (Gasparreto *et al*., 2012; Haq & Singh, 2020; Mousavi *et al*., 2021; Sheem *et al*., 2023). It promotes abortion or onset of menstruation and reduces menstrual flow (Rahman & Gondha, 2014).

Urena lobata L. is a member of the malvaceae family and is commonly called Caesar weed or Congo jute (Kumar *et al*., 2020). It is an annual, erect and a rising under-shrub that can grow to a height of 0.5–2.5 m (MPB, 2017). The stems are frequently tinted purple and coated with tiny star-shaped hairs (Islam & Uddim, 2017). It is widely distributed as a weed in the tropical and subtropical regions of the World, including Nigeria, Ghana, and Senegal (Njoku *et al*., 2021). In one of the tribes in Edo State, the plant is called Oronhon.

Traditionally, *Urena lobata* root infusion is used in the treatment of swelling, ointment of the powdered preparation is used in the treatment of lumbago and rheumatism. Decoction of the root and stem are used in the treatment of watery stool with blood. Infusion and extract from the flowers are used as gargle for treatment of sore throat and as expectorant in the management of dry cough (Rahman & Gondha, 2014). The leaves infusion is used in the treatment of abscess, while the leaves can also be used in the preparation of delicacy for breast feeding mothers so as to enhance lactation (Shaba *et al*., 2017).

Several reports in the literature have indicated the pharmacological uses of the plant, including analgesic and anti-inflammatory (Purnomo and Tilaqza, 2022), antitumor (Mathappan *et al*., 2019), antifungal (Fokou *et al*., 2023), ant-helmintic (Rajagopal *et al*., 2019) and antiarthritic effects (Rajagopal *et al*., 2019). The above mentioned effects are due to the bioactive principles expressed by the physiological machinery of the plant (Chikezie *et al*., 2015). An insight into the type and nature of the phytoconstituents present in the plants could further strengthen the reported biological effects. Keke *et al*., (2023) reported different fatty acids as the most abundant compounds in the methanolic and ethanolic extracts of *Urena lobata* leaves. This study was therefore conducted to identify other phytochemicals in the methanolic extract of *Urena lobata* powdered leaves using Gas Chromatography-Mass Spectrometry (GC-MS) and High Pressure Liquid Chromatography (HPLC).

MATERIALS AND METHODS

Collection of *Urena lobata* Leaves and Extraction

The plant was obtained in January 2023 from the Ekosodin community, which is located behind the University of Benin, with the following coordinates of 5^{o} 451 to 6^{o} 151 east longitude and 5^{o} 151 to 6^{o} 451 north latitude, within the central province of Edo State. Prof. H. A. Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life

Science, University of Benin, authenticated the plant. The sample was deposited in the Departmental herbarium with the specimen sample number UBH-U614. The leaves were dried under shade for two weeks and pulverized to fine powder. The fine powdered (58 g) was macerated using methanol (95%) for five days. Whatman (no. 1) filter paper was used to decant and filter the extract. A rotary evaporator operating at 40°C was used to evaporate the filtrate. After that, the extract was stored at 4^{o}C in the refrigerator until it was needed (Odion *et al*., 2022).

Phytochemical Screening

The fine powder from the dried leaves was screened using conventional procedures outlined by Sofowora (1993); Trease & Evans (1989) for alkaloids, tannins, glycosides, terpenoids, flavonoids, steroids, and saponins.

Preparation of methanolic extract of *Urena lobata* leaves for GC-MS analysis

A 50 mg methanolic extract was diluted in 5 mL of a 1:1 n-hexane: dichloromethane solvent mixture. Silica gel of mesh size 100–200 mm was triturated with the mixture using a pestle and mortar. A well-packed column containing silica was carefully loaded with the dried adsorbed mixture, and 3 g of anhydrous sodium sulphate was added to the adsorbed silica. The column was isocratically eluted with 3 x 10 mL of n-hexane. The resulting eluate was bulked up and evaporated to 2 mL in a rotary evaporator operating at 40 ^{o}C. GC-MS analysis was then performed using this extract (Odion *et al*., 2020).

Gas Chromatography-Mass Spectrometric Analysis

The Agilent mass spectrometric detector was linked to an autosampler equipped with Agilent 6890N gas chromatography. The concentrated eluent was then injected into the GC-MS via the port in the pulsed spitless mode, and 1 µL was applied to a 30 m x 0.25 mm ID DB 5MS coated fused silica column with a 0.15 µm film thickness. The carrier gas utilized in this experiment was helium, and a constant flow rate of 1 mL/min was achieved by maintaining the column head pressure at 20 psi. There were predefined operational conditions. The temperature of the column was first maintained at 55 ^{o}C for 0.4 minutes, then it was raised to 200 ^{o}C at a rate of 25 ^{o}C/min, 280 ^{o}C at a rate of 8 ^{o}C/min, and finally to 300 ^{o}C at a rate of 25 ^{o}C/min, held for 2 minutes. Standards from the National Institute of Standard and Technology was utilized in comparing data obtained (Odion *et al*., 2020)

Preparation of Stock and Working Standards for HPLC Analysis

The stock solution of all standards; Quinolinamine, Benzenesulfonamide, Allylamine, Benzamide, Indolizine, Pyrazoline, Imidazole, Propargylamine, Ethylenimine, Difluoramine, Isoxazolidine, Simulansamide, Colchicine, Norethindrone, Androstane, Methanamine, Isoxazolidine, Isobutylamine, and Amphetamine (1,000 µg/mL each), were prepared in methanol and stored at 4 °C when not in use.

Sample Preparation and Separation of Compounds using HPLC Analysis

Two hundred milligram (200 mg) of methanolic extract of *Urena lobata* leaves was homogenized, followed by the addition of 200 mL of deionized water. The mixture was refluxed for an hour while stirring constantly, this was then allowed to cool at room temperature. The resultant extract was then diluted to a ratio of 1:3 (v/v) using a 2 % ammonia solution after being filtered through Whatman filter paper (No. 1) with a diameter of 125 mm. Hydrochloric acid (0.01 M) was then used to adjust the pH to 7. HPLC analysis was carried out following the procedure reported by Odion *et al*. (2023). Five (5) µL of a diluted stock solution containing 80 µg/mL was injected into the HPLC and the peak separation was

optimized at 242 nm. All stock standards were carefully observed. Table 1 provides a summary of the HPLC conditions used to analyze the methanol extract of *Urena lobata* leaves.

Table 1: Conditions utilized in the HPLC analysis of the methanolic extract of *Urena lobata* leaves

Parameter	Condition			
Column	Agilent Lichrospher 100-5RP8 (250 x 4.6 mm) (C18)			
Flow rate	1.00 ml/min			
Injection volume	10 µL			
Column temperature	35 ^{o}C			
Mobile phase A	0.1% phosphoric acid			
Mobile phase B	Methanol			
Run time	25 minute			
Gradient	Time	0	2.5	6
	% B	25	25	50

RESULTS AND DISCUSSION

Table 2 depicts the phytochemicals in the powdered leaves of *Urena lobata.* These phytochemicals include flavonoids, saponin, tannins, cyanogenic glycosides, alkaloids and terpenoids.

Table 2: Phytochemical screening of the powdered leaves of *Urena lobata*

Phytochemicals	Results
Flavonoids	+ve
Saponins	+ve
Tannins	+ve
Carbohydrates	+ve
Steroids	+ve
Alkaloids	+ve
Cyanogenic glycosides	+ve
Terpenoids	+ve

Key: +ve =Present

The presence of these phytochemicals in the powdered leaves of *Urena lobata* have been implicated in many of the pharmacologically expressed activities (keke *et al*., 2023). Take for example, oral administration of the ethanolic extract of *Urena lobata* leaves in experimental animals resulted in significant reduction in pain (Islam *et al*., 2012). Also, the aqueous extract of *Urena lobata* leaves has improve the structure and function of islets beta cells in male sprague Dawley rats by increasing the glucose like peptide-1 (GLP-1) bioavailability (Purnomo *et al*., 2017).

Table 3 shows the bioactive compounds identified via GC-MS analysis in methanolic extract of *Urena lobata* leaves. From the chromatogram, fourteen (14) peaks were seen having several compounds . In ascending order of percentage area, they are; 4-(3-dimethylaminopropoxy)benzaldehyde, pyridine, 9-octadecenamide, 2-amino-5-chlorophenyl phosphonic acid, 2-propenenitrile, 2,1-benzisoxazole-4-carboxlic acid, 1,2,4-triazin-5(2H)-one, pyridine-3-carboxamide, 2-pyrazoline, 4-aminobenzoic acid, 3-nitrophthalhydrazide, 2,4 (1H, 5H)-imidazoldione, bromophos-ethyl, semicarbazide were detected in ascending order of percentage area.

Table 3 GC-MS analysis of the methanolic extract of *Urena lobata* powdered leaves

S/N	Retention Time(min)	Compound	Area %	Molecular weight (g/mol)	Molecular formula	Base Peak (m/z)	Class
1.	5.668	9-octadecenamide (Z)	1.98	281.5	$C_{18}H_{35}NO$	59	Fatty amide.
2.	25.666	4-(3-dimethylaminopropoxy) benzaldehyde	1.16	207.27	$C_{12}H_{17}NO_2$	161	Benzaldehyde
3.	25.741	Pyridine	1.25	79.1	C_5H_5N	79	Pyridines
4.	26.324	2,1-benzisoxazole-4-carboxylic acid	3.37	179.13	$C_8H_5NO_4$	106	Benzisoxazoles
5.	26.456	2-propenenitrile	3.29	53.06	C_3H_3N	41	Nitriles.
6.	26.719	Pyridine-3-carboxamide	4.89	122.12	$C_6H_6N_2O$	106	Nicotinamides.
7.	26.977	2-Pyrazoline	5.96	70.09	$C_3H_6N_2$	68	Pyrazolines
8.	27.114	2-amino-5-chlorophenyl phosphonic acid	3.15	207.55	$C_6H_7ClNO_3P$	128	Organophosphonic acids
9.	27.331	3-nitrophthalhydrazide	8.34	207.14	$C_8H_5N_3O_4$	147	Hydrazides
10.	27.726	2,4 (1H, 5H)-Imidazoledione	8.62	114.09	$C_3H_4N_2O_2$	84	Imidazolediones
11.	28.058	1,2,4-Triazin-5 (2H)-one	3.57	97.08	$C_3H_3N_3O$	73	Triazinones
12.	28.499	Semicarbazide	36.01	75.07	CH_5N_3O	44	Semicarbazides
13.	28.762	Bromophos-ethyl	11.46	394.04	$C_{10}H_{12}BrCl_2O_3PS$	186	Organophosphate
14.	28.974	4-aminobenzoic acid	6.96	137.14	$C_7H_7NO_2$	92	Aminobenzoic acid

2-propenenitrile possesses antifungal potentials with low hemolytic action on blood type, this compound has been detected in *Byrsonima gardneriana* (Sousa-Melo *et al*., 2021). 9(Z)-octadecenamide has been identified from essential oil in mountain celery seeds and possess the ability of lowering serum TG, TC, LDL-c, LDL-c/HDL-C and hepatic TG (Cheng *et al*., 2010). Benzisoxazole moiety has been implicated as antiglycation, anticancer, anti-inflammatory and antibacterial agents (Kabi *et al*., 2022). pyridine-3-carboxamide has been identified as a novel CB2 agonist, thus having analgesia property (Mitchell *et al*., 2009). 1,2,4-triazin-4-one possesses wide variety of chemotherapeutic activities which range from fungicidal, antimalarial, antibacterial, anticancer, antiviral and ant-inflammatory potentials (Singh *et al*., 2021). 3-nitronaphthalhydrazide is considered to be a nitro analogue of phenytoin and known to possess anti-inflammatory activity by inhibiting COX-2 enzymes responsible for releasing inflammatory mediators (Li *et al*., 2019). 4-Aminobenzoic acid has shown activity against fungal infection in pear (Laborda *et al*., 2019), cytotoxic activity when the pharmacophore was combined with other moieties such as aromatic aldehyde (Kratky *et al*., 2019) and management of Alzheimer disease due to the ability of its derivatives to inhibit acetylcholinesterase (Correa-Basurto *et al*., 2005).

Figure 1 shows the class of alkaloids that were identified from the powdered leaves of *Urena lobata*. The class of alkaloids identified are imidazoles and pyrazolines.

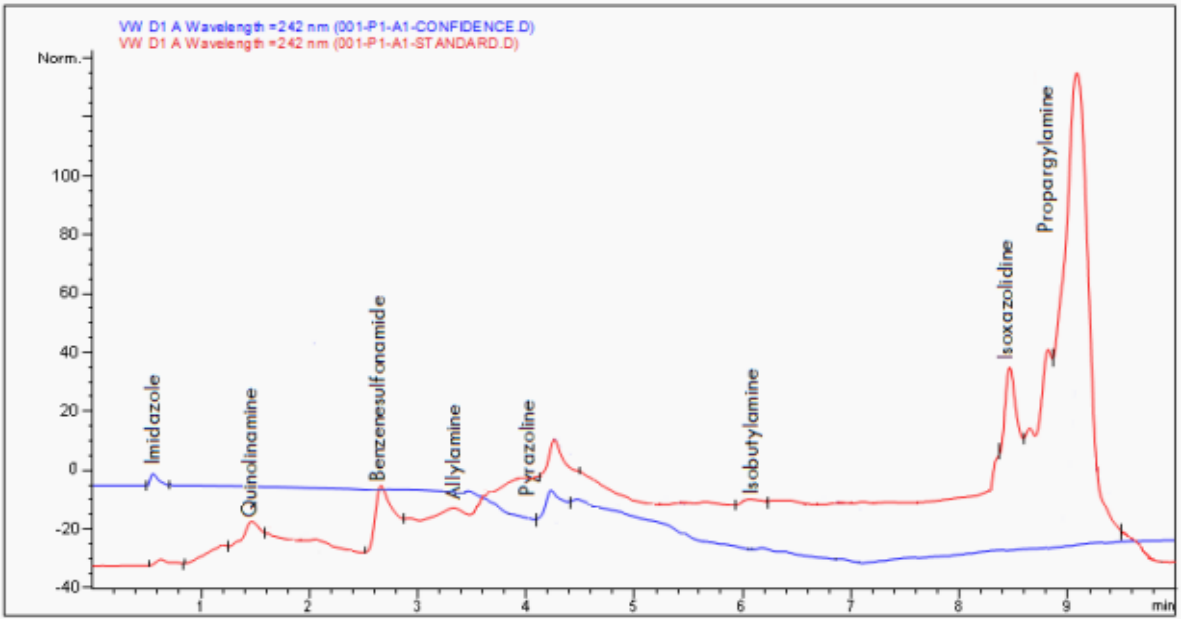

Figure 1: HPLC chromatogram of methanolic extract of *Urena lobata* powdered leaves

The chromatogram showed a red plot representing the standard alkaloidal compounds while the blue plot represent the methanol extract of *Urena lobata*. Imidazole (6.58 ng/µL) and pyrazoline (8.50 ng/µL) were identified are the common moieties in this analysis with pyrazoline as the most abundant. These moieties can be seen in 2-pyrazoline and 2,4(1H,5H)-imidazoledione previously identified by the GC-MS analysis. The presence of these moieties in an extract has ascribed anti-inflammatory and anticancer properties to such (Ali *et al*., 2017; Lai *et al*., 2022).

CONCLUSION

Results obtained in this study have revealed the presence of different classes of phytochemicals. Phytochemicals; flavonoids, saponin, tannins, cyanogenic glycosides, alkaloids and terpenoids were identified and were linked with specific pharmacological activities. Some of the phytochemicals detected in this study have already been documented while many others are yet to be evaluated. Our study have shown that *Urena lobata* powdered leaves posses anti-inflammatory and anticancer potential due to the presence of compounds with such properties therein. Particularly, the moieties (pyrazoline and imidazole) identified in this study are both alkaloidal compounds.

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CONFLICT OF INTEREST

None

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