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-propenitrile, 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.
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 dysmenorrhea 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° 45' to 6° 15' east longitude and 5° 151 to 6° 45' 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°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 °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 ºC for 0.4 minutes, then it was raised to 200 ºC at a rate of 25 ºC/min, 280 ºC at a rate of 8 ºC/min, and finally to 300 ºC at a rate of 25 ºC/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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Agilent Lichrospher 100-5RP8 (250 x 4.6 mm) (C18)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.00 ml/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>10 µL</td>
</tr>
<tr>
<td>Column temperature</td>
<td>35 °C</td>
</tr>
<tr>
<td>Mobile phase A</td>
<td>0.1% phosphoric acid</td>
</tr>
<tr>
<td>Mobile phase B</td>
<td>Methanol</td>
</tr>
<tr>
<td>Run time</td>
<td>25 minute</td>
</tr>
<tr>
<td>Gradient</td>
<td>% B Time</td>
</tr>
<tr>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>

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*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>+ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>+ve</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+ve</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>+ve</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+ve</td>
</tr>
</tbody>
</table>

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)-imidazolidione, bromophos-ethyl, semicarbazide were detected in ascending order of percentage area.
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Table 3 GC-MS analysis of the methanolic extract of *Urena lobata* powdered leaves

<table>
<thead>
<tr>
<th>S/N</th>
<th>Retention Time(min)</th>
<th>Compound</th>
<th>Area %</th>
<th>Molecular weight (g/mol)</th>
<th>Molecular formula</th>
<th>Base Peak (m/z)</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5.668</td>
<td>9-octadecenamide (Z)</td>
<td>1.98</td>
<td>281.5</td>
<td>C₁₈H₃₅NO</td>
<td>59</td>
<td>Fatty amide.</td>
</tr>
<tr>
<td>2.</td>
<td>25.666</td>
<td>4-(3-dimethylaminopropoxy) benzaldehyde</td>
<td>1.16</td>
<td>207.27</td>
<td>C₁₂H₁₇NO₂</td>
<td>161</td>
<td>Benzaldehyde</td>
</tr>
<tr>
<td>3.</td>
<td>25.741</td>
<td>Pyridine</td>
<td>1.25</td>
<td>79.1</td>
<td>C₃H₈N</td>
<td>79</td>
<td>Pyridines</td>
</tr>
<tr>
<td>4.</td>
<td>26.324</td>
<td>2,1-benzisoxazole-4-carboxylic acid</td>
<td>3.37</td>
<td>179.13</td>
<td>C₄H₅NO₄</td>
<td>106</td>
<td>Benzisoxazoles</td>
</tr>
<tr>
<td>5.</td>
<td>26.456</td>
<td>2-propenenitrile</td>
<td>3.29</td>
<td>53.06</td>
<td>C₃H₅N</td>
<td>41</td>
<td>Nitriles.</td>
</tr>
<tr>
<td>7.</td>
<td>26.977</td>
<td>Pyrazoline</td>
<td>5.96</td>
<td>70.09</td>
<td>C₃H₄N</td>
<td>68</td>
<td>Pyrazolines</td>
</tr>
<tr>
<td>8.</td>
<td>27.114</td>
<td>2-amino-5-chlorophenyl phosphonic acid</td>
<td>3.15</td>
<td>207.55</td>
<td>C₆H₇ClNO₃P</td>
<td>128</td>
<td>Organophosphonic acids</td>
</tr>
<tr>
<td>9.</td>
<td>27.331</td>
<td>3-nitrophthalhydrazide</td>
<td>8.34</td>
<td>207.14</td>
<td>C₆H₈N₂O₄</td>
<td>147</td>
<td>Hydrazides</td>
</tr>
<tr>
<td>10.</td>
<td>27.726</td>
<td>2,4-(1H, 5H)-imidazoledione</td>
<td>8.62</td>
<td>114.09</td>
<td>C₃H₈N₂O₂</td>
<td>84</td>
<td>Imidazolediones</td>
</tr>
<tr>
<td>11.</td>
<td>28.058</td>
<td>1,2,4-Triazin-5 (2H)-one</td>
<td>3.57</td>
<td>97.08</td>
<td>C₃H₅N₂O</td>
<td>73</td>
<td>Triazinones</td>
</tr>
<tr>
<td>12.</td>
<td>28.499</td>
<td>Semicarbazide</td>
<td>36.01</td>
<td>75.07</td>
<td>C₂H₇NO₂</td>
<td>44</td>
<td>Semicarbazides</td>
</tr>
<tr>
<td>13.</td>
<td>28.762</td>
<td>Bromophos-ethyl</td>
<td>11.46</td>
<td>394.04</td>
<td>C₁₂H₁₂BrCl₂O₃PS</td>
<td>186</td>
<td>Organophosphate</td>
</tr>
<tr>
<td>14.</td>
<td>28.974</td>
<td>4-aminobenzoic acid</td>
<td>6.96</td>
<td>137.14</td>
<td>C₇H₇NO₂</td>
<td>92</td>
<td>Aminobenzoic acid</td>
</tr>
</tbody>
</table>

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 anti-inflammatory potentials (Singh et al., 2021). 3-nitrophthalhydrazide 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.

![HPLC chromatogram of methanolic extract of Urena lobata powdered leaves](image)

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.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST**

None

**REFERENCES**


