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PHYTOCHEMICAL SCREENING OF *URENA LOBATA* L. GROWING IN HUE CITY

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SUMMARY:

Medicinal plants contain bioactive compounds that are used in the treatment of various human diseases and play a crucial role in the healing process. The primary objective of this study was to investigate the presence of phytochemical constituents in the roots and in the seeds of Urena lobata *L., collected from Hue city, through phytochemical screening and Gas Chromatography–Mass Spectrometry (GC-MS) analysis. The results revealed the presence of saponins, reducing sugars, flavonoids, terpenoids and phenolic compounds in the roots of* Urena lobata *L.. Analysis of essential oil from seeds of this specices identified the main components as esters, including: 2-ethylhexyl undecyl terephtalate, bis(2-ethylhexyl) 1,2-benzenedicarboxylate, methyl palmitate and methyl linoleate.*

Keywords: *Urena lobata L., phytochemical screening, GC-MS.*

I. INTRODUCTION

Urena lobata L. (U. lobata), a species belonging to the Malvaceae family, is an evergreen plant widely distributed across tropical regions of South America, Africa, and Asia. In Vietnam, it is predominantly found in the northern and central regions[1 - 3].

In traditional medicine, *U. lobata* has been utilized in several countries, including Bangladesh, India, China, and Vietnam, for the treatment of various health conditions. Previous research has suggested that *U. lobata* may serve as a promising source of phytotherapeutic compounds. It has been recognized for its diverse pharmacological activities, such as: antioxidant, anti-inflammatory, antimicrobial, antidiarrheal, antidiabetic, and antihyperlipidemic effects[4-7].

This study reports the screening of phytochemical constituents and the identification of chemical compounds present in the essential oil from seeds of *U. lobata*.

II. MATERIALS AND METHODS

2.1. Plant materials

Fresh roots and seeds of *U. lobata* were collected from some of the plants in Hue city, in August, 2024. Its scientific name was identified by Dr. Hoang Xuan Thao. A voucher specimen (UL-T0824) was deposited at the Department of Chemistry, Hue University of Education, Hue University, Vietnam.

2.2. Extraction and distillation procedure

Extraction

The dried sample was sequentially extracted with *n*-hexane, dichloromethane, ethyl acetate, and methanol at room temperature. The extracts were then concentrated under reduced pressure using a rotary evaporator to obtain the corresponding residues, including *n*-hexane (ULH), dichloromethane (ULD), ethyl acetate (ULE), and methanol (ULM) extracts.

Distillation procedure

The fresh ground seeds of the plant (150g) were transferred to a Clevenger-type apparatus consisting of a 1,000ml round-bottomed flask. 750ml of deionized water was added into the flask and heated by a heating mantle with a temperature of 120°C. The hydrodistillation was generated for 4h under reflux, at normal pressure to separate the essential oil from the leaves. The essential oil was dried over anhydrous sodium sulfate Na_2SO_4, then filtered and stored in a vial at 4°C until further analysis.

2.3. General experimental procedures

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS was used to identify the constituents in the essential oil from seeds of U. lobata. The GC-MS analysis was performed on a GCMS-QP2010 Plus system (Shimadzu, Japan) fitted with Equity-5 capillary column (30m × 0.25mm, 0.25μm film thickness) and a mass spectrometer (MSD QP2010 Plus). The ULH was diluted with n-hexane at a ratio of 1:100, and 1ml was used for analysis. The analytical conditions were as follows: carrier gas helium (1.5ml/min), injector temperature of 280°C, interface temperature of 280°C, and column temperature ranging from 60°C (held for 2 min) to 240°C at 3°C/min (held for 10 min) and then increased to 280°C at 5°C/min (held for 40 min). The sample was injected by using a splitless mode. MS conditions were as follows: ionization energy of 70eV; mass scan range of 45-500 (m/z) at a sampling rate of 1.0 scan/s. The identification of the compositions was carried out by comparing of mass spectra with those of NIST 11 and WILEY 7 stored in the GC/MS database. The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

Phytochemical analysis of extracts

Test for Flavonoids

Shinoda test: The extract was dissolved in methanol. One piece of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta color demonstrated the presence of flavonoids[8-10].

Test for Steroids

Salkowaski test: 10mg of extracts was dissolved in 2ml of chloroform and 2ml of concentrated sulphuric acid was added from the side of the test tube. Test tube was shaken for few minutes. The development of red color in chloroform layer indicated the presence of sterols[8-10].

Test for Alkaloids: 5mg of extract was taken in 5ml of 1.5% v/v hydrochloric acid and filtered. These filtrates were then used for testing alkaloids[8-10].

Dragendorff's test: Dragendorff's reagent was added in 2ml of filtrate. Formation of orange-brown precipitate indicated the presence of alkaloids.

Mayer's reagent: To a 1ml of test filtrate in a watch glass, a few drops of Mayers reagent were added. If the formation of cream colored precipitate it shows the presence of alkaloids[8-10].

Test for Saponins

Foam test: 1ml solution of extract was diluted with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes. Development of stable foam suggests the presence of saponins[8-10].

Test for reducing sugars

Fehling's Test: Equal volumes of Fehling's A and Fehling's B solutions (1 ml each) were mixed and boiled for 1 minute. An equal volume of the test solution was then added. The mixture was heated in a boiling water bath for 5-10 minutes. The formation of a brick-red precipitate confirmed the presence of reducing sugars[8-10].

Test for phenolic compounds

Ferric chloride test: To 2ml of the extract, 2ml of distilled water followed by 10 % $FeCl_3$ solution was added. Bluish black colour indicates the presence of phenolic compounds[8-10].

Test for phenolic terpenoids

Liebermann-Burchard test: 1ml of concentrated sulphuric acid was added to 10mg of extract in 1ml of chloroform. A reddish - blue color exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of terpenoids[8-10].

III. RESULTS AND DISCUSSION

3.1. Phytochemical analysis of extracts

Table 3.1: Phytochemical analysis of the root extracts of *U. lobata*

No	Qualitative test	Positive reaction observation	Observation			
			ULH	ULD	ULE	ULM
Flavonoids	Shinoda test	Appearance of magenta color	-	-	-	++
Steroids	Salkowski test	The development of red color in chloroform layer	-	-	-	-
Alkaloids	Mayer's reagent	Formation of cream colored precipitate	-	-	-	-
	Dragendroff's test	Formation of orange-brown precipitate	-	-	-	-
Reducing sugars	Fehling's test	brick-red precipitate	+	++	+++	++++

No	Qualitative test	Positive reaction observation	Observation			
			ULH	ULD	ULE	ULM
Saponins	Foam test	Development of stable foam	-	-	-	+
Phenolic compounds	Ferric chloride test	Bluish black colour	-	-	-	+
Terpenoids	Liebermann - Burchard test	reddish - blue color exhibited by chloroform layer and green fluorescence by the acid layer	+	+	-	-

Note: **+: Compound detected (**Positive reaction, clearly visible)**.**

-: No compound detected (Negative reaction).

+ / + / +++: Increasing intensity of positive reaction

The results of the qualitative phytochemical analysis (Table 3.1) indicated that the presence of several classes of secondary metabolites in the root extracts of *U. lobata* using different solvents. Specifically, **reducing sugars** were detected in all extracts, with the intensity of the reaction increasing from ULH to ULM. **Flavonoids** were detected only in the methanol extract (ULM). **Terpenoids** were present in the ULH and ULD extracts. Meanwhile, **saponins** and **phenolic compounds** were detected exclusively in the ULM extract. These findings are fully consistent with the "Rule of thumb" regarding the solubility of compounds in solvents with different polarities. Notably, **steroids and alkaloids were not detected** in any of the extracts.

Comparison with previous studies (Table 3.2) reveals that the chemical composition of extracts from the roots and other plant parts exhibit similarities in terms of secondary metabolite classes. However, a notable distinction is observed: earlier research on other parts such as leaves and stems reported the presence of steroids and alkaloids[11-13], whereas in the current study focused on root extracts, steroids and alkaloids were not detected. This discrepancy can be attributed to the biosynthetic pathways and differential accumulation of these compounds in various plant tissues.

Table 3.2: Comparison of chemical compositions of extracts from the roots and other plant parts

No	Chemical compositions of extracts from the roots	Chemical compositions of extracts from the leaves[11, 12]	Chemical compositions of extracts from the stem[13]
Flavonoids	+	+	+
Steroids	-	+	+
Alkaloids	-	+	+
Reducing sugars	+	+	+
Saponins	+	+	+
Phenolic compounds	+	+	+
Terpenoids	+	+	+

Note: **+:** indicates the compound was **detected**

-: indicates the compound was no detected

3.2. Chemical constituents of the essential oil from seeds of U. lobata

Gas chromatography-mass spectrometry (GC-MS) was employed to identify the components of the essential oil from seeds of *U. lobata*. The resulting essential oil was analyzed by GC-MS and 06 chemical constituents were identified based on their retention indices (Table 3.3), accounting for 100% of the total constituents.

Table 3.3: Chemical constituents of the essential oil from seeds of *U. lobata*

No	RT	Compound	RI_E	RI_L	Content (%)	
1	4.50	4-hydroxy-4-methylpentan-2-one	830	831		1.43
2	4.58	3-methylpentanol	833	833		5.30
3	46.44	Methyl palmitate	1928	1921		36.94
4	51.74	Methyl linoleate	2096	2095		55.90
5	64.67	Bis(2-ethylhexyl) phthalate	2551	2550		0.22
6	72.50	Bis(2-ethylhexyl) isophthalate	2753	2752		0.21
Total						100.00

RT: Retention time, RI_E: Experimental retention indices, RI_L: Literature retention indices.

The GC-MS analysis revealed that chemical constituents of the essential oil from seeds of *U. lobata* consisted of six identified compounds, accounting for 100% of the total detected constituents. Among them, the most abundant were methyl linoleate (55,90%) and methyl palmitate (36,94%), both belonging to the class of fatty acid esters. These methyl esters of fatty acids are well-known bioactive compounds with anti-inflammatory, antibacterial, and antioxidant effects[14]. Methyl palmitate, in particular, has been reported to inhibit bacterial growth and protect cells from oxidative stress. Methyl linoleate plays a crucial role in maintaining cell membrane integrity and regulating biological processes[15].

IV. CONCLUSION

This study is the first to analyze the chemical constituents of the roots and seed essential oil of *Urena lobata* collected from Hue City, Vietnam. The results reveal a diverse array

of secondary metabolites present in the root extract. The chemical profile is predominantly composed of saponins, reducing sugars, flavonoids, terpenoids, phenolic compounds, and fatty acid methyl esters. These classes of compounds are likely contributors to the extract's observed antimicrobial, antioxidant, and anti-inflammatory activities. The findings underscore the potential of *U. lobata* as a source of bioactive compounds with applications in phytotherapy and agricultural protection. Further studies are recommended to elucidate the specific pharmacological mechanisms of these constituents and to explore their therapeutic and agronomic potential.

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