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CONSTITUENTS AND ANTIBACTERIAL ABILITY OF WATER BUG (*Lethocerus indicus* Lep.) ESSENTIAL OILS

Nguyen Van Loi[1,*], Do Thi Hanh[2] and Pham Hoang Nam[3]
[1]*Faculty of Environmental Sciences, University of Science, Vietnam National University, Hanoi city, Vietnam*
[2]*Faculty of Chemical Technology, Hanoi University of Industry, Hanoi city, Vietnam*
[3]*Department of Life Sciences, University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology, Hanoi city, Vietnam*
[*]Corresponding author: Nguyen Van Loi, e-mail: nguyenvanloi@hus.edu.vn

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Abstract. The water bug, *Lethocerus indicus* Lep., is an aquatic insect commonly found in deep rice fields, lakes, ponds, and rivers in Vietnam. In this study, male specimens were collected from fields, ditches, ponds, and lakes in Dai Cuong commune, Ung Hoa district, Hanoi, Vietnam. The objective of this study is to determine the constituents and antibacterial properties of the essential oil extracted from *L. indicus*. Gas chromatography–mass spectrometry (GC-MS) analysis identified fifteen major components, primarily hydrocarbons and oxygenated hydrocarbons. In which, specifically, the essential oil comprised 12.41% monoterpenes, 25.12% sesquiterpenes, 21.98% alcohols, 1.79% aldehydes, and 36.63% esters. In particular, (*E*)-2-hexenol-acetate was the predominant component, accounting for 36.63%, and is believed to contribute to the characteristic aroma of the essential oil. The antibacterial activity of the essential oil was evaluated against six microorganisms: *Staphylococcus aureus*, *Clostridium botulinum*, *Clostridium perfringens*, *Escherichia coli*, *Campylobacter jejuni*, and *Vibrio parahaemolyticus*.

***Keywords**:* antibacterial ability, constituents, essential oil, water bug.

1. Introduction

The volatile compounds found in *Lethocerus indicus* Lepare are employed in the manufacture of lubricants, emulsifiers, solvents, rubber surfactants, paints, coatings, household detergents, food products, cosmetics, and pharmaceutical industries [1]. *L. indicus*, commonly known as the water bug, is an aquatic insect widely distributed in Vietnam, particularly in deep rice fields, lakes, ponds, and rivers. These insects possess an elongated, elliptical body measuring approximately 64 - 80 mm in length and 23.5 - 29.7 mm in width. Water bugs are edible and considered a rich source of nutrition. In recent years, the abundance of water bugs has declined due to extensive pesticide use in the fields and environmental pollution, despite growing consumer demand. Water bug has a variety of roles in maintaining ecological balance, human health, and food security [2]. They are rich in essential nutrients, including sodium, calcium, and magnesium, as well as proteins, lipids, vitamins, amino acids, and fatty acids. Moreover, water bugs contain notable amounts of essential oils [3], [4]. In Vietnam, water bugs are water bugs are traditionally used in culinary applications, including in specialty dishes and in the preparation of fish sauce. The essential oil of water bug has a characteristic, attractive aroma and is widely used in food. Traditionally, the male giant water bug has been used as an essential 'flavour' component of chili paste and as an ingredient in some types of fish sauce. Hence, the added odour or scent of male bugs is important for consumer acceptance in those food products. Its popularity stems from the unique and desirable odour it imparts to food and food products [5]. Only the male giant water bugs have this special odour, which is contained in their abdominal reservoir or scent gland. There are very few research projects on the processing of water bugs' essential oils. Therefore, the aim of this study is to determine the chemical constituents and antibacterial properties of the essential oil of the water bug, thereby providing a scientific foundation for its potential application in the food industry.

2. Content

2.1. Materials and methods

2.1.1. Materials

- *Water bugs*: Using male water bugs caught in fields, ditches, ponds, and lakes in Dai Cuong commune, Ung Hoa district, Hanoi, Vietnam, from April to August 2024.

Morphological characteristics: Male water bugs measure approximately 7 - 9 cm in length, while females are larger, ranging from 9 - 12 cm. On average, male body dimensions were 70.3 ± 0.25 mm in length and 26.3 ± 0.3 mm in width, whereas females measured 78.64 ± 0.33 mm in length and 28.63 ± 0.06 mm in width. Thus, females are significantly larger than males.

Biological characteristics: Male water bugs possess two essential oil sacs located at the base of the third pair of legs (counting from the head). These scent glands are well-developed in males but absent in females. Additionally, males can be distinguished by the presence of two black spots at the sides of the terminal abdominal segment, which are not present in females. The male genital capsule, comprising the phallobase, aedeagus, diverticulum, and parameres, and the female genital capsule, consisting of the gonapophyses, valves, and anal cone, were anatomically described as distinguishing features between the sexes. One of the most definitive characteristics for sex identification is the ratio of length to width of the last abdominal segment, which is 2.66 in males and 2.12 in females, respectively [6]. After collection, the insects were placed in a Styrofoam box layered with crushed ice to maintain the temperature below 5 °C. The box was then sealed and transported in an air-conditioned vehicle to the laboratory for essential oil extraction and analysis.

- *Laboratory tools and equipment*: Laboratory tools and equipment used include stainless steel razor blades, stainless steel tweezers, medical syringes, dark glass bottles, refrigerators, and styrofoam boxes.

- *The tested microorganisms*: Experimental microorganisms were provided by VNTEST Institute for Quality testing and Inspection, including *Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Escherichia coli, Campylobacter jejuni,* and *Vibrio parahaemolyticus.*

2.1.2. Methods

- *Exploitation and recovery of water bug essential oil*

Each water bug was placed ventral side down with the dorsal side facing up. Using a razor blade, an incision was made transversely across the back at the junction with the thorax, near the base of the third pair of legs. The specimen was then gently bent ventrally to expose the essential oil sacs, which protrude upward from the abdominal cavity. The sacs were carefully extracted using stainless steel tweezers and transferred to a ceramic bowl. Essential oil was then withdrawn from the sacs using a sterile syringe and transferred into a dark glass vial. The vial was tightly sealed and stored in a freezer at −5 °C for preservation [7].

- *Determining the constituents of the water bug essential oil*

The 0.5 µl of the sample prepared was injected into the GC-MS. The GC-MS employed has Trace1300 as Gas chromatography and TSQ DUO as Mass Spectrophotometry. The GC has the silicacapillary column (30 m × 0.25 mm; 0.25 µm film thickness), TG-5 MS interfaced with the MS. The carrier gas used in the instrument was helium with a flow rate of 1 ml/min for 60 minutes. The ionization energy was set at 70 eV, and the mass transfer line temperature at 250 °C, and the ion source temperature at 280 °C. The column temperature was programmed from 40°C for 1 min to 280 °C at the rate of 5 °C/min by a heating ramp for 20 min and held at 250 °C. The inlet injector temperature was set at 240 °C with a split mode of 1:20 maintained. The mass spectra were filtered from the mass range of 35 to 450 Mw. The identification of the volatile compound was determined by comparing the spectra obtained from the sample with the reference to the mass spectrum given by the National Institute of Standards and Technology (NIST) GC/MS Libraries 2017 [8], [9].

- *Determining the antibacterial ability of water bug essential oil*

The agar diffusion method was used to determine antibacterial activity. A 50 µl water bug essential oil was placed in wells on plates containing the tested bacterial strains. The activity was estimated by the diameter of the antibacterial round (mm), which was calculated using the formula D- d (mm), where D was the diameter of the antibacterial round (mm) and d was the hole diameter (mm) [10], [11]. The experiment was repeated three times and processed using biostatistical methods.

2.2. Results and discussion

2.2.1. Exploitation and recovery of water bug essential oils

Using the method of extraction and recovery of essential oil of water bugs in section 2.1.2, the results showed that, on average, one water bug gave 0.02 mL of essential oil. The extracted essential oil was clear, light yellow, and possessed a characteristic aroma.

2.2.2. The constituents of water bug essential oils

The chemical constituents of water bug essential oil were identified using gas chromatography-mass spectrometry (GC–MS). The analytical results are presented in Table 1 and illustrated in Figure 1.

Table 1. The chemical constituents of water bug essential oils

No.	Constituents	Retention time (min)	Proportion (%)
	Monoterpenes		**12.41**
1	*β*-pinene	13.73	0.87
2	*α*-phellandrene	15.10	11.54
	Sesquiterpenes		**25.12**
3	longifolene	18.94	23.08
4	valencene	19.37	2.04
	Alcohols		**21.98**
5	*p*-cresol	13.84	0.94
6	undecanol	14.80	0.97
7	2-methylhexan-1-ol	15.47	1.02
8	hexadecanol	16.06	1.17
9	allyl-cyclohexane-1,2-diol	16.34	1.05
10	2-hexyloctanol	16.68	1.13
11	Indole	18.60	13.56
12	2-methylundecanol	19.05	2.14
	Aldehydes		**1.79**
13	(*E*)-nonen-2-al	17.58	0.97
14	heptadecen-2-al	17.66	0.82
	Esters		**36.63**
15	(*E*)-2-hexenol acetate	14.15	36.63
	Total		**97.93**

Noted: (%) was calculated by the percentage of chromatographic peak area

Fifteen constituents were predicted in the water bug essential oils, Table 1. Four constituents were hydrocarbons (monoterpenes 12.41%, and sesquiterpenes 25.12%), and the rest were oxygenated hydrocarbons (alcohols 21.98%, aldehydes, 1.79%, and esters 36.63%). Including some high percentages of constituents as (*E*)-2-hexenol acetate 36.63%, longifolene 23.08%, indole 13.56%, *α*-phellandrene 11.54%, 2-methylundecanol 2.14%, and valencene 2.04%. The results of this study are also consistent with the published results by Mutum R.D *et al*., [5]. Author Mutum R.D et al suggested that constituent (*E*)-2-hexenol acetate was the most common volatile constituent detected in male water bugs [5]. The comparative analysis of abundant volatile compounds illustrates that the male water bug has a higher composition of 2-hexenol acetate and volatile substances, which have been used as ingredients in the food and cosmetic industry due to their fruity, apple, and waxy oily aroma [5]. Among the unique volatile compounds of male insects, indole has been used as a promising agent for the treatment of antimicrobial activity, malaria, diabetes, cancer, migraines, convulsions, and hypertension [12], [13].

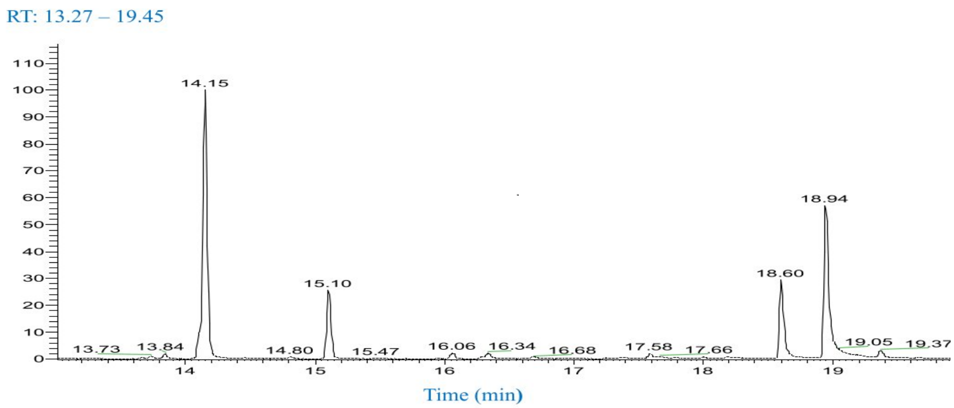

Figure 1. Gas chromatogram of the water bug essential oil

2.2.3. The antibacterial activity of water bug essential oils

The agar diffusion method was used to evaluate the antibacterial potential of the water bug essential oils. The tested microorganisms used in this experiment were *Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Escherichia coli, Campylobacter jejuni,* and *Vibrio parahaemolyticus.* The research results of the antibacterial activity of the water bug essential oils are shown in Table 2.

Table 2. The antibacterial activity diameters of water bug essential oils

No.	Tested microorganisms	Diameter of an antibacterial round (mm)
1	*Staphylococcus aureus*	21.16 ± 0.24
2	*Clostridium botulinum*	19.84 ± 0.17
3	*Clostridium perfringens*	19.32 ± 0.26
4	*Escherichia coli*	16.47 ± 0.31
5	*Campylobacter jejuni*	14.28 ± 0.19
6	*Vibrio parahaemolyticus*	13.75 ± 0.37

The results showed that the water bug essential oils possessed antibacterial activity against all six strains of selected microorganisms. Among them, the antibacterial activity against *Staphylococcus aureus, Clostridium botulinum* and *Clostridium perfringens* was the highest, then strains of microorganisms *Escherichia coli, Campylobacter jejuni,* and *Vibrio parahaemolyticus*. The zones of inhibition of the water bug essential oils for *Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Escherichia coli, Campylobacter jejuni,* and *Vibrio parahaemolyticus* were 21.16 ± 0.24 mm, 19.84 ± 0.17 mm, 19.32 ± 0.26 mm, 16.47 ± 0.31 mm, 16.47 ± 0.31 mm, and 13.75 ± 0.37 mm respectively. These results indicate that Gram-negative bacteria were generally less susceptible to the essential oil than Gram-positive bacteria. This difference in susceptibility may be attributed to the structural characteristics of Gram-negative bacterial cell walls, which are more complex and contain higher levels of lipopolysaccharides, lipids, and lipoproteins, providing greater resistance to antimicrobial agents. These findings suggest that water bug essential oils are a potential natural antibacterial agent.

3. Conclusions

The study on water bug (*Lethocerus indicus* Lep.) essential oils from Dai Cuong commune, Ung Hoa district, Hanoi city, reveals significant insights into its chemical composition and antibacterial properties. The essential oil was analyzed using gas chromatography-mass spectrometry, identifying fifteen key components, predominantly hydrocarbons and oxygenated hydrocarbons. In which, the monoterpenes group accounted for 12.41%, the sesquiterpenes group accounted for 25.12%, the alcohols group accounted for 21.98%, the aldehydes group accounted for 1.79%, and the esters group accounted for 36.63%. This composition underscores the potential of water bug essential oil as a rich source of bioactive compounds. The antibacterial efficacy of water bug essential oil was tested against six microorganisms: *Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Escherichia coli, Campylobacter jejuni* and *Vibrio parahaemolyticus.* The results demonstrated notable antibacterial activity, particularly against *Staphylococcus aureus* and *Clostridium botulinum*, with inhibition zones measuring 21.16 ± 0.24 mm and 19.84 ± 0.17, respectively. These findings suggest that water bug essential oil could serve as a natural antimicrobial agent, supporting its use not only in culinary applications but also in food preservation and safety.

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