Isolation of Flavonoids and a Triterpene Ester with Potential Bioactivity from *Ludwigia octovalvis* (Jacq.) P.H. Raven (Onagraceae) Leaves

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ABSTRACT: Leaves of *Ludwigia octovalvis* (Jacq.) P.H. Raven (Onagraceae), a plant with a history of traditional medicinal use, were investigated to determine their bioactive constituents. Five known compounds were isolated and structurally characterized using extensive NMR spectroscopic techniques (^{1}H NMR, ^{13}C NMR, COSY, HSQC, and HMBC) and comparison with literature data. These compounds included 7,4'-dihydroxy-5,6,8-trimethoxyflavone (**1**), 6,7,4'-trihydroxy-3,5-dimethoxyflavone (**2**), 4-hydroxybenzaldehyde (**3**), palmitic acid (**4**), and 6,27-(2-ethyl)-dihydroxy-3-oxo-olean-12-enyl palmitate (**5**). Bioactivity assays were performed on various fractions derived from crude methanolic (MeOH) extract of *L. octovalvis*. The hexane (LOH) and ethyl acetate (LOEA) fractions displayed significant DPPH free radical scavenging activity, with IC_{50} values of 17.69 and 20.06 µg/mL, respectively, compared to the standard reference compound, ascorbic acid (5.96 µg/mL). The chloroform fraction (LOC) and LOH exhibited the most potent inhibition in the brine shrimp lethality bioassay (LC_{50} = 0.037 and 0.012 µg/mL, respectively) compared to the standard, vincristine sulphate (0.45 µg/mL). The LOEA fraction demonstrated promising thrombolytic activity (43.34% clot lysis) compared to the standard, streptokinase (65.15%).

Key words: *Ludwigia octovalvis*, Phytochemicals, Flavonoids, DPPH, Brine shrimp lethality assay, Thrombolytic activity.

INTRODUCTION

Investigating natural products for new therapeutic treatments continues to be essential in contemporary drug development. Plants have long been valued for their bioactive compounds, often providing the basis for modern pharmaceuticals.[1] While strides have been made in utilizing natural resources for medicine, there are still notable gaps in research. It's imperative to continue exploring these resources to better grasp and enhance their therapeutic capabilities.

Ludwigia octovalvis (Jacq.) P.H. Raven, a member of the Onagraceae family, is a flowering aquatic plant found across tropical regions globally.[2] Extracts from *L. octovalvis* have a long history of being consumed as a beneficial beverage to address a range of health issues such as edema, nephritis, hypotension and diabetes.[3] For centuries, it has been a mainstay in traditional Chinese medicine (TCM), and has been utilized for their various health benefits, including immunoregulatory, hepatoprotective, cardiovascular-protective and anti-aging properties.[4] Traditional healers in Mexico and India have also harnessed *L. octovalvis*'s potential, particularly for managing diabetes.[5] Their methods often involve consuming a boiled extract or juice prepared from the whole plant. Modern science sheds light on the potential reasons behind these age-old practices. Previous studies on the phytochemical composition of *L. octovalvis* have identified a range of bioactive compounds across its organs, including flavonoids, phenolic acids, polyphenols, saponins, sterols, tannins and triterpenoids.[6] Notably, quercetin, a

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flavonoid found in LOE, has garnered attention for its potential health benefits.[2] Overall, *L. octovalvis* extract offers a rich source of bioactive compounds with diverse therapeutic potentials, ranging from antioxidant and cardioprotective effects to potential antidiabetic properties. Further research into these compounds and their mechanisms of action could lead to the development of novel therapeutic agents derived from natural sources.

The recognition of these characteristics highlights the medicinal effectiveness of *L. octovalvis* leaves and provides a scientific basis for their longstanding traditional use in treating various health ailments. In this study, we isolated and characterized five compounds from the ethyl acetate extract of the plant. Furthermore, we evaluated the antioxidant, cytotoxic and thrombolytic properties of the plant extract. Delving deeper into the chemical composition and medicinal effects of the plant shows potential for the creation of innovative treatments for a variety of health issues.

MATERIALS AND METHODS

Sample collection and preparation. Leaves of *L. octovalvis* were gathered from the Moulvibazar district in December 2021. A taxonomist at the Jahangirnagar University Herbarium authenticated the plant and a voucher specimen was archived with the accession number 48662. Subsequently, the leaves underwent a cleaning process and were air-dried in shade for a period of two weeks. Following drying, they were pulverized into a coarse powder using a high capacity grinding machine, yielding 1 kg of powder.

Instrumentations, drugs, and chemicals. A Bruker apparatus running at 400 MHz was used to obtain nuclear magnetic resonance (NMR) spectra with $CDCl_3$ (deuterated chloroform) acting as the solvent. A German-made Buchi Rotavapor was used to evaporate the solvent. Whereas column chromatography (CC) employed the silica gel Kieselgel 60 from Sigma-Aldrich, vacuum liquid chromatography (VLC) employed Kieselgel 60H. Compound analysis was performed using Silica gel 60 F_{254} from Merck, Germany, pre-coated on thin-layer chromatography plates. The vanillin/H_2SO_4 reagent and UV light were used to visualize the spots on the TLC plates. The remaining chemicals and solvents came from reliable vendors such as Active Fine Chemicals Ltd., Bangladesh.

Extraction of plant material. One kg of coarse plant powder was soaked separately in 5 liters of ethyl acetate (EtOAc) and methanol (MeOH) for a duration of 25 days with periodic stirring. Following cold extraction, both solutions were filtered through Whatman No. 1 filter paper and subsequently evaporated using a Buchi Rotary evaporator under low pressure and temperature (39^0C). This process yielded dry crude extracts, with the EtOAc extract weighing 30 grams and the MeOH extract weighing 7 grams.

Preparation of different partitions. To partition both EtOAc and MeOH crude extract of *L. octovalvis*, we followed the procedures outlined by S. Morris Kupchan (1970) and Van Wagenen (1993)[7]. Initially, 5 grams of both crude extracts were dissolved in a 10% aqueous methanol solution (100 ml each) separately. Subsequently, based on their polarity, the extract underwent sequential extraction using n-hexane (LOH), chloroform (LOC), ethyl acetate (LOE) and aqueous (LOAQ). Each fraction was obtained by individually evaporating the solvent from these fractions using a rotary evaporator.

Isolation of compounds. The LOH fraction underwent further separation on a Sephadex LH-20 column, resulting in 170 sub-fractions using $CHCl_3$ as the eluting solvent. Compound **1** (R_f = 0.45), exhibiting a yellow color with vanillin-H_2SO_4 treatment, was isolated from Sephadex fractions 7-11. Compounds **2** (R_f = 0.52) and **3** (R_f = 0.55) were obtained from Sephadex fractions 143-148. Sephadex fractions 84-86 yielded compounds **4** (R_f = 0.65) and **5** (R_f = 0.68), which displayed purple and dark brown colors upon acid treatment and heating, respectively.

Properties of isolated compounds. 7,4'-Dihydroxy-5,6,8-trimethoxyflavone **(1)**: White crystals and soluble in ethyl acetate and chloroform; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.54 (1H, s, H-3),

8.23 (2H, d, *J* = 9 Hz, H-2', 6'), 7.03 (2H, d, *J* = 9 Hz, H-3',5'), 3.97 (3H, s, OCH_3-5), 3.89 (3H, s, OCH_3-6), 3.93 (3H, s, OCH_3-8).

6,7,4'-Trihydroxy-3,5-dimethoxyflavone (**2**): White crystals and soluble in ethyl acetate and chloroform; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.76 (1H, s, H-8), 8.15 (2H, d, *J* = 8.7 Hz, H-2',6'), 6.92 (2H, d, *J* = 8.7 Hz, H-3',5'), 3.84 (3H, s, OCH_3-3), 3.97 (3H, s, OCH_3-5).

4-Hydroxybenzaldehyde (**3**): Colorless crystals and soluble in ethyl acetate and chloroform; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.77 (1H, s, CHO-1), 6.91 (2H, d, *J* = 8.0 Hz, H-3,5), 7.77 (2H d, *J* = 8.0 Hz, H-2,6).

Palmitic acid (**4**): White crystals and soluble in ethyl acetate and chloroform; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.2-1.3 (16H, m, H-4-15), 0.88 (3H, t, H-16), 2.35 (2H, t, H-2), 1.63 (2H, q, H-3).

6,27-(2-ethyl)-dihydroxy-3-oxo-olean-12-enyl palmitate (**5**): White crystals and soluble in ethyl acetate and chloroform; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.63 (1H dd, *J*= 12.4, 5.2 Hz, H-6), 5.39 (1H t, *J*= 3.6 Hz, H-12), 1.13 (3H s, H-23), 1.08 (3H s, H-24), 1.10 (3H s, H-25), 1.14 (3H s, H-26), 0.92 (3H s, H-28), 0.92 (3H s, H-29), 0.89 (3H s, H-30), 4.08 (2H t, *J*= 12.4, CH_2OH), 2.31 (2H t, *J*= 7.6, H-2'), 1.28-1.31 (26H m, H-3'-15'), 0.91 (3H s, H-16'). ^{13}CNMR (100 MHz, $CDCl_3$): δ 39.4 (C-1), 32.8 (C-2), 217.5 (C-3), 47.3 (C-4), 54.7 (C-5), 62.6 (C-6), 35.7 (C-7), 41.9 (C-8), 46.6 (C-9), 36.9 (C-10), 23.6 (C-11), 124.2 (C-12), 145.0 (C-13), 48.1 (C-14), 25.9 (C-15), 28.7 (C-16), 34.4 (C-17), 48.2 (C-18), 46.0 (C-19), 31.0 (C-20), 34.2 (C-21), 36.4 (C-22), 26.5 (C-23), 21.5 (C-24), 15.3 (C-25), 17.3 (C-26), 40.5 (C-27), 28.3 (C-28), 33.2 (C-29), 23.5 (C-30), 64.4 (CH_2OH), 174.0 (C-1'), 34.4 (C-2'), 28.8-29.7 (C-3'-15'), 14.1 (C-16').

DPPH free radical scavenging assay. The study assessed the antioxidant capacity of plant extracts, at concentrations ranging from 500 to 0.977 µg/ml, by measuring their ability to scavenge DPPH free radicals. A 20 µg/ml methanol solution of DPPH was mixed with varying concentrations of the plant extracts and the resulting color change was compared to that of tert-butyl-1-hydroxytoluene (BHT), which served as the reference standard.[8,9]

% Inhibition of free radical DPPH = (1-Absorbance of sample / Absorbance of the control reaction) × 100

Brine shrimp lethality bioassay. The plant extract's potential cytotoxicity was evaluated using the brine shrimp lethality test. A saltwater solution (38 g NaCl in 1000 ml water, pH 8.0) was prepared for hatching brine shrimp. Vincristine sulfate (VS) served as a reference, with test concentrations from 400 to 0.78125 µg/ml. After counting the nauplii, 5 ml of saltwater was added to introduce them to the samples, and cytotoxicity was assessed based on their survival.[10]

% Morality = (Number of nauplii death / Number of nauplii taken) × 100

***In vitro* thrombolytic assay.** The process followed the methods that Ahmed *et al.* described.[11] Venous blood samples from healthy volunteers were placed in pre-weighed Eppendorf tubes, incubated at 37°C for 45 minutes to form clots. After serum separation, clot weight was calculated. Tubes containing clots were treated with 100 µl of plant extract, streptokinase (positive control), or distilled water. All tubes were incubated for 90 minutes at 37°C. The extent of clot lysis was measured as a percentage based on weight differences before and after lysis.[11]

% Clot lysis = (Weight of the clot after lysis / Weight of the clot before lysis) × 100

RESULTS AND DISCUSSION

Isolated phytochemicals from *L. octovalvis*. From the crude ethyl acetate extract of *L. octovalvis* leaves, five compounds were isolated using repeated chromatographic methods (Figure 1). These compounds were identified as 7,4'-dihydroxy-5,6,8-trimethoxyflavone (**1**), 6,7,4'-trihydroxy-3,5-dimethoxyflavone (**2**), 4-hydroxybenzaldehyde (**3**), palmitic acid (**4**), and 6,27-(2-ethyl)-dihydroxy-3-oxo-olean-12-enyl palmitate (**5**). Their structures were determined by analyzing NMR spectral data and comparing them with published data.

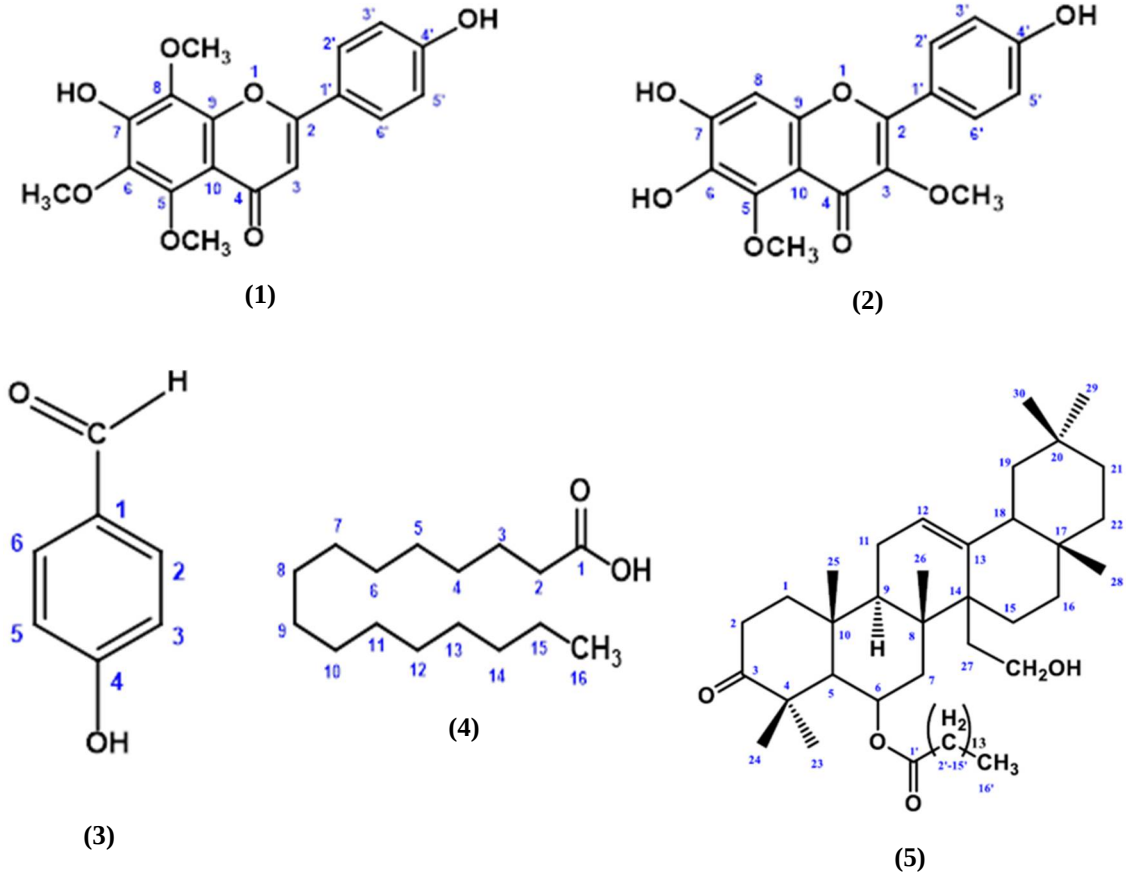

Figure 1. Structures of isolated phytochemicals from *L. octovalvis* using NMR techniques: 7,4'-Dihydroxy-5,6,8-trimethoxyflavone (**1**), 6,7,4'-Trihydroxy-3,5-dimethoxyflavone (**2**), 4-Hydroxybenzaldehyde (**3**), palmitic acid (**4**), and 6,27-(2-ethyl)-dihydroxy-3-oxo-olean-12-enyl palmitate (**5**).

The ^{1}H-NMR spectrum of compound **1** (400 MHz, $CDCl_3$) displayed one singlet at δ 6.54, which was assigned to proton at C-3. Four aromatic protons doublets at δ 7.03, d (*J*=9 Hz) and 8.23, d (*J*=9 Hz) were assignable as H-3′,5′ and H-2′,6′ respectively. These signals are typical for flavonoids. The ^{1}H-NMR spectrum displayed three singlets at δ 3.9, 3.89, 3.93 which were assigned to OCH_3-5, OCH_3-6, and OCH_3-8, respectively. Therefore, from the ^{1}H-NMR spectral data and also consecutive comparative study of its spectral value with published data[12], the identity of the compound **1** was proposed to be 7,4'-dihydroxy-5,6,8 -trimethoxyflavone.

The ^{1}H-NMR spectrum of compound **2** (400 MHz, $CDCl_3$) displayed one singlet at δ 6.76, which was assigned to proton at C-8. Four aromatic protons doublets at δ 6.92, d (*J*=8.7 Hz) and 8.15, d (*J*= 8.7 Hz) were assignable as H-3′,5′ and H-2′,6′ respectively. The spectrum also showed two singlets at δ 3.84, 3.97 which were assigned to OCH_3-3, OCH_3-5 respectively. Therefore, from ^{1}H-NMR spectral data and also consecutive comparative study of its spectral value with published data[12], compound **2** was identified as 6,7,4'-trihydroxy-3,5 -trimethoxyflavone.

The ^{1}H-NMR spectrum of compound **3** (400 MHz, $CDCl_3$) displayed two doublets at δ 6.91, d (*J*=8 Hz) and 7.77, d (*J*=8 Hz) which were assigned to protons at C-3,5 and C-2,6 respectively. One singlet at δ 9.77 proves the presence of an aldehydic

proton at C-1. Considering all the spectral data and comparing with established value[13], the structure of the compound **3** was identified as 4-hydroxybenzaldehyde.

The ^{1}H NMR spectrum of compound **4** (400 MHz, $CDCl_3$) displayed a triplet at δ 0.88 with three proton intensity corresponds to one methyl ($-CH_3$) proton. One distinct multiplet at δ 1.2- 1.3 attributed to protons of eleven methylene groups ($-CH_2$). One triplet at δ2.33 proves the presence of a methylene moiety, deshielded due to adjacent electronegative group ($-CH_2-CH_2-COOH$). One quintet at δ1.63 proves the presence of a methylene moiety, deshielded due to adjacent electronegative group ($-CH_2-CH_2-CH_2-COOH$). Considering all the spectral data and comparing with established value[14] allowed to establish the structure of compound **4** as palmitic acid.

The ^{1}H NMR spectrum of compound **5** (400 MHz, $CDCl_3$) indicated the presence of an olefinic proton at δ 5.39 t (*J*=3.6 Hz) and seven tertiary methyl singlets at δ 0.89, 0.92, 0.92, 1.14, 1.13, 1.10 and 1.08. The ^{13}C NMR spectrum indicated the presence of a carbonyl carbon at δ 217.5, an unsaturated methine carbon at δ 124.3 and an unsaturated quaternary carbon at δ 145.0, suggesting an oleanone skeleton. The DEPT-135 experiment confirmed the presence of seven methyls at δ 33.2, 28.3, 26.5, 23.5, 21.5, 17.3 and 15.3, instead of eight methyl groups of oleanane triterpenes. In the HMBC experiment, the two methyl groups at δ 1.13 and 1.08 showed ^{3}J correlations to the carbonyl carbons at δ 217.5, thus confirming the position of the carbonyl group at C-3 and that of methyls at C-23 and C-24. Another pair of methyl groups at δ 0.92 and 0.89 showed ^{2}J correlation to a quaternary carbon at δ 31.0 (C-20) and ^{3}J correlations to two methylene carbons at δ 46.0 (C-19) and 34.2 (C-21). The C-25 and C-26 methyls also showed the expected correlations to C-1,10, 9 and C-9, 8,14 respectively. In the HSQC spectrum the methyl groups showed ^{1}J correlations to δ 33.2 and 23.5, characteristic for C-29 and C-30 methyls of oleanane triterpenes. In addition to all of the above signals, the ^{1}H NMR spectrum showed two deshielded signals at δ 4.63 dd (*J*=12.4, 5.2) and 4.08 2H t (*J*=6.8) and the ^{13}C NMR spectrum displayed two carbinol carbon at δ 62.6 and 64.4. The δ_H 2.31 triplet with coupling constant 7.6 Hz was assigned to δ_C 34.4 ppm. It has HMBC correlation to δ_C 25.1, 29.2 and 174.0 ppm which were assigned to C 3', C-4' and C-1'. δH 1.28-1.31 multiplet were assigned to C-3'to C-15' with δC of 28.8-29.7 ppm. δ_H 0.91 singlet was assigned to C-16' with δ_C of 14.1ppm. All of these indicate the presence of a palmitate side chain. Value at δ_C 174.0 was for ester group at C-1'. δ_H 4.63 double doublet (*J*=12.4, 5.2 Hz) was assigned to C-6 with δ_C of 62.6 ppm. δH 4.08 triplet (*J*=6.8Hz) was correlated with δ_C of 62.6 ppm and indicates the presence of a CH_2OH group. Moreover, the ^{1}H NMR and ^{13}C NMR spectra revealed the presence of a palmitic acid moiety joined to the triterpene moiety as confirmed by the characteristic doublet of doublet at δ 4.63. The triplet at δ 4.08 indicated its attachment to a methylene group, which could be placed to the missing C-27 methyl. On the basis of above data compound **5** was identified as 6,27-(2-ethyl)-dihydroxy-3-oxo-olean-12-enyl palmitate, a tentatively new compound isolated from plant.

Effect of *L. octovalvis* extracts on DPPH free radical scavenging activity. The research aimed to assess the antioxidant potential of various extracts from *L. octovalvis* by evaluating their effectiveness in neutralizing free radicals. Findings revealed promising antioxidant activity in the plant extract. LOAQ exhibited a moderate scavenging effect with an IC_{50} value of 60.41 µg/ml. Notably, LOC and LOEA demonstrated stronger activity (IC_{50} = 17.69 and 20.06 µg/ml, respectively), approaching that of the standard ASA (5.96 µg/ml), suggesting its potential in scavenging free radical. Conversely, LOH displayed considerably weaker activity, as indicated by their substantially higher IC_{50} values (73.82 µg/ml) (Figure 2).

Effect of *L. octovalvis* extracts on brine shrimp lethality bioassay. Compared to the standard, which had an LC_{50} value of 0.451 µg/ml, LOC and LOH demonstrated substantial activity in the brine shrimp cytotoxicity test, with LC_{50} values of

0.037 and 0.012 µg/ml, respectively. In contrast, LOEA and LOAQ showed less activity, as seen by their greater LC_{50} values of 1.28 µg/ml and 0.797 µg/ml, respectively. (Figure 3).

Effect of *L. octovalvis* extracts on thrombolytic assay. The objective of the experiment was to evaluate the clot-dissolving abilities of the fractions, with the hope of identifying novel treatments for clot-related disorders. LOEA displayed notable thrombolytic activity in the experiment, achieving a clot lysis of 43.34%, whereas the standard streptokinase (SK) showed a clot lysis of 65.15% (Figure 4).

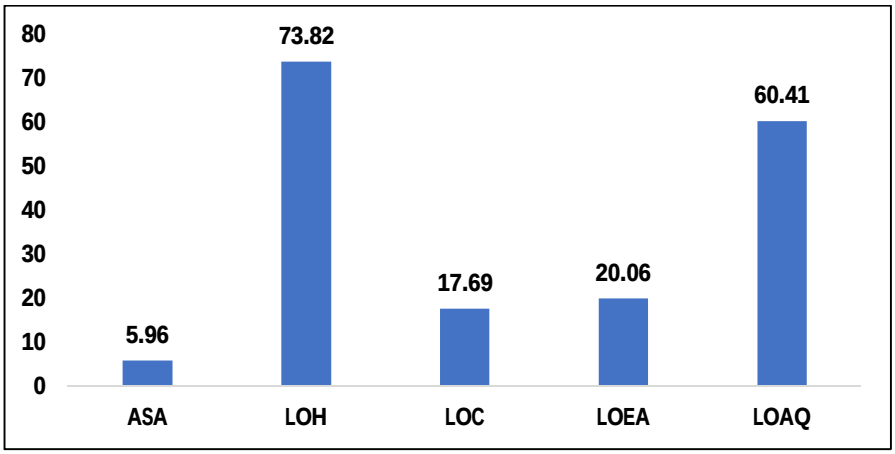

Figure 2. Comparison among IC_{50} (µg/ml) values of the different extractives of *L. octovalvis* with the standard (ASA).

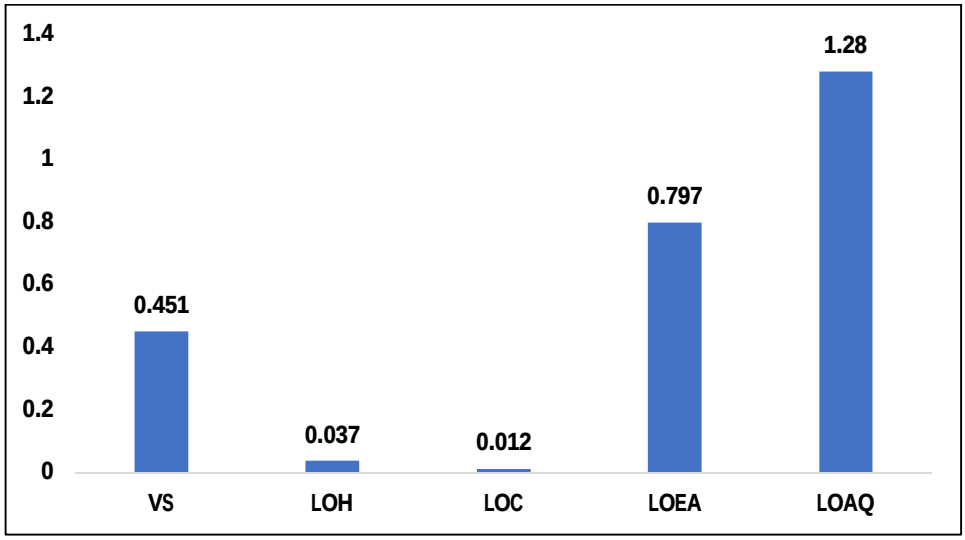

Figure 3. Comparison among LC_{50} (µg/ml) values of the different extractives of *L. octovalvis* with the standard (VS).

Exploring the medicinal properties of plants involves a complex series of stages. An essential starting point is pinpointing and understanding the bioactive molecules responsible for their potential healing effects.[15] This study offers a thorough assessment of *L. octovalvis* presenting insights into its possible therapeutic uses through a multifaceted approach. By employing chemical analysis, five familiar compounds were isolated and identified structurally using advanced nuclear magnetic resonance (NMR) spectroscopy methods, thus verifying their composition. This meticulous

characterization establishes a strong basis for future research into their biological functions.

The isolation and structural characterization of five known compounds from the leaves of *L. octovalvis* through rigorous NMR spectroscopic techniques contribute to our understanding of its chemical composition. The isolated compounds offered an interesting mix of flavonoids (**1, 2**), a simple phenolic aldehyde (**3**), a fatty acid (**4**), and a triterpene ester (**5**). The presence of flavonoids aligns with previous reports suggesting these secondary metabolites play a role in the antioxidant and potentially other biological activities in plants[16,17], the presence of fatty acids and their derivatives warrants further exploration. Palmitic acid (**4**) is a common saturated fatty acid found in many plants and animals. While palmitic acid is not traditionally recognized for potent bioactivity, recent studies suggest it may play a role in various physiological processes.[18–20] The triterpene ester, 6,27-(2-ethyl)-dihydroxy-3-oxo-olean-12-enyl palmitate (**5**), is a more intriguing isolate. Triterpene esters are a diverse group of natural products known for various biological activities, including anti-inflammatory, anticancer and antimicrobial properties.[21–24]

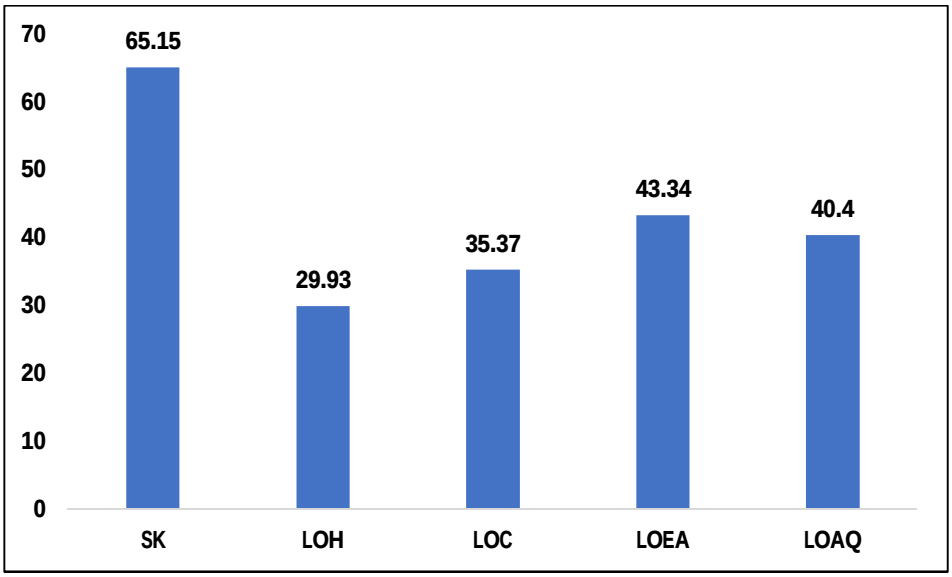

Figure 4. Thrombolytic activity of different fractions of *L. octovalvis*.

The specific bioactivity of compound **5** remains unknown and warrants further investigation. Its presence alongside the active LOEA fraction suggests a potential role, and future studies could involve isolating this compound and testing its individual bioactivity. The identification of 4-hydroxybenzaldehyde (**3**) as a simple phenolic aldehyde is another interesting finding. While seemingly less complex than the flavonoids (**1, 2**), phenolic aldehydes can also possess various bioactivities, including antioxidant and antimicrobial properties.[25,26] Further studies could explore the potential contribution of this compound to the overall bioactivity of the extract.

The bioactivity assays revealed promising results for the LOH and LOEA fractions derived from the crude methanol extract. Both fractions demonstrated significant free radical scavenging activity as measured by the DPPH assay, suggesting potential antioxidant properties. This suggests the presence of synergistic or particularly potent antioxidant compounds concentrated in these fractions. The LOC and LOH fractions exhibited impressive lethality towards brine shrimp larvae in the bioassay.

Additionally, the promising thrombolytic activity observed for the LOEA fraction highlights its potential for applications related to blood clot dissolution.

The research primarily centered on isolating and characterizing phytochemicals and assessing the bioactivities of the plant extract. Further investigation using techniques like mass spectrometry (MS) could be employed to identify any novel bioactive constituents present in the extracts. Additionally, further fractionation and bioassay-guided isolation could pinpoint the specific compounds responsible for the observed activities in the fractions. Future studies should explore the cytotoxicity of all the fractions using mammalian cell lines to assess their potential as a therapeutic agent. Similarly, *in vitro* studies using isolated blood clots could provide a more comprehensive understanding of thrombolytic activity.

CONCLUSION

In conclusion, this study successfully isolated and identified five known compounds from the leaves of *L. octovalvis*, a plant with a history of traditional medicinal use. The presence of flavonoids aligns with the family's known bioactive profile, while the identification of a triterpene ester and a phenolic aldehyde suggests further avenues for exploration. Bioactivity assays revealed promising antioxidant, cytotoxic and thrombolytic activities. While limitations exist, this study highlights the potential of *L. octovalvis* leaves as a source of bioactive compounds. Future research should focus on identifying the specific bioactive components using advanced techniques and assessing their activity. Elucidating the mechanisms of action of these compounds will be crucial for developing *L. octovalvis* as a potential therapeutic agent.

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AUTHOR CONTRIBUTION

T.S. and Z.A conceptualized and designed the research. T.S. and S.A. developed the methodology for the study. Z.A. and M.A. validated the research methods. T.S., S.D. and S.H. performed the investigation. T.S., S.A., S.D. and S.H. conducted the data analysis. Z.A. and M.A. secured the necessary resources. T.S. and S.A. drafted the original manuscript, with S.A. contributing to the review and editing process. Z.A. created the visualizations for the research, and also oversaw the project as supervisor and administrator.

REFERENCES

1. Nasri, H. 2016. Herbal drugs and new concepts on its use. *J. Prev. Epidemiol*. **1**, e01-e01.
2. Yakob, H.K., Uyub, A.M. and Sulaiman, S.F. 2012. Toxicological evaluation of 80% methanol extract of *Ludwigia octovalvis* (Jacq.) PH Raven leaves (Onagraceae) in BALB/c mice. *J. Ethnopharmacol*. **142**, 663-668.
3. Lin, W. S., Lo, J. H., Yang, J. H., Wang, H. W., Fan, S. Z., Yen, J. H., and Wang, P. Y. 2017. *Ludwigia octovalvis* extract improves glycemic control and memory performance in diabetic mice. *J. Ethnopharmacol*. **207**, 211-219.
4. Murugesan, T., Sinha, S., Pal, M. and Saha, B.P. 2002. Review on phytochemical and medicinal aspects of *Jussiaea suferuticosa* Linn. *Anc. Sci. Life*. **21**, 205-207.
5. Ramírez, G., Zavala, M., Pérez, J. and Zamilpa, A. 2012. *In vitro* screening of medicinal plants used in Mexico as antidiabetics with glucosidase and lipase inhibitory activities. *Evid. Based Complement. Alternate. Med*. **2012**, 701261.
6. Yan, J. and Yang, X.W. 2005. Studies on the chemical constituents in herb of *Ludwigia octovalvis*. *Zhongguo Zhong Yao Za Zhi* **30**, 1923-1926.
7. VanWagenen, B.C., Larsen, R., Cardellina, J.H., Randazzo, D., Lidert, Z.C. and Swithenbank, C. 1993. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*. *J. Org. Chem*. **58**, 335-337.
8. Süzen, S. 2007. Antioxidant activities of synthetic indole derivatives and possible activity mechanisms. *Bioactive Heterocycles V* 145-178.
9. Singh, T.P. and Singh, O.M. 2018. Recent progress in biological activities of indole and indole alkaloids. *Mini. Rev. Med. Chem*. **18**, 9-25.

10. Jasiewicz, B., Kozanecka-Okupnik, W., Przygodzki, M., Warżajtis, B., Rychlewska, U., Pospieszny, T. and Mrówczyńska, L. 2021. Synthesis, antioxidant and cytoprotective activity evaluation of C-3 substituted indole derivatives. *Sci. Rep*. **11**, 1-14.
11. Mounnissamy, V.M., Kavimani, S., Balu, V. and Quine, S.D. 2007. Evaluation of anti-inflammatory and membrane stabilizing properties of ethanol extract of *Cansjera rheedii* J. Gmelin (Opiliaceae). *Iran J. Pharmol. Thera*. **6**, 235-237.
12. Momeni, J., Tsopmejio, J.P., Nkouam, F.T. and Ngassoum, M.B. 2016. Antioxidant activity of the natural flavonoid 7-Hydroxy-5, 6, 4'-trimethoxyflavone isolated from the leaves of *Lippia rugosa* A. Chev. *Nat. Sci.* **8**, 70.
13. Zhang, J., Mohamad, H., Wong, J.H., Bilal, M., Ismail, A.H.B., Lloyd, A.J., Yusoff, A.A.M., Osman, H., Wong, K.T., Idris, Z. and Abdullah, J.M. 2017. The Effect of 4-hydroxybenzaldehyde on the γ-aminobutyric acid type A receptor. *Malays. J. Med. Sci*. **24**, 94.
14. Ruksilp, T. 2020. Fatty acids and an ester from the leaves of *Millettia utilis* Dunn. *Naresuan Uni. J. Sci. Techno*. **28**, 63-68.
15. Süntar, I. 2020. Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. *Phytochem. Rev.***19**, 1199-1209.
16. Agati, G., Brunetti, C., Fini, A., Gori, A., Guidi, L., Landi, M., Sebastiani, F. and Tattini, M. 2020. Are flavonoids effective antioxidants in plants? Twenty years of our investigation. *Antioxidants* **9**, 1098.
17. Shen, N., Wang, T., Gan, Q., Liu, S., Wang, L. and Jin, B. 2022. Plant flavonoids: classification, distribution, biosynthesis and antioxidant activity. *Food. Chem*. **383**, 132531.
18. Carta, G., Murru, E., Banni, S. and Manca, C. 2017. Palmitic acid: physiological role, metabolism and nutritional implications. *Front. Physiol*. **8**, 306122.
19. Mancini, A., Imperlini, E., Nigro, E., Montagnese, C., Daniele, A., Orrù, S. and Buono, P. 2015. Biological and nutritional properties of palm oil and palmitic acid: effects on health. *Molecules* **20**, 17339-17361.
20. Korbecki, J. and Bajdak-Rusinek, K. 2019. The effect of palmitic acid on inflammatory response in macrophages: an overview of molecular mechanisms. *Inflam. Res*. **68**, 915-932.
21. Sultana, N., Qazi, M.S. and Kamal, M. 2020. New anti-inflammatory triterpene esters and glycosides from *Alstonia scholaris*. *Antiinflamm. Antiallergy Agents Med. Chem.* **19**, 370-386.
22. Poirier, B.C., Buchanan, D.A., Rudell, D.R. and Mattheis, J.P. 2018. Differential partitioning of triterpenes and triterpene esters in apple peel. *J. Agric. Food Chem*. **66**, 1800-1806.
23. Novakovic, M., Nikodinovic-Runic, J., Veselinovic, J., Ilic-Tomic, T., Vidakovic, V., Tesevic, V. and Milosavljevic, S. 2017. Bioactive pentacyclic triterpene ester derivatives from *Alnus viridis* ssp. *viridis* Bark. *J. Nat. Prod*. **80**, 1255-1263.
24. Viet Ho, D., Thi Hoang, H.N., Quoc Vo, H., Minh Nguyen, H., Raal, A. and Thi Nguyen, H. 2018. A new triterpene ester and other chemical constituents from the aerial parts of *Anodendron paniculatum* and their cytotoxic activity. *J. Asian Nat. Prod. Res*. **20**, 188-194.
25. Figueiredo, A.R., Campos, F., de Freitas, V., Hogg, T. and Couto, J.A. 2018. Effect of phenolic aldehydes and flavonoids on growth and inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*. *Food Microbiol*. **25**, 105-112.
26. Mulabagal, V., Alexander-Lindo, R.L., DeWitt, D.L. and Nair, M.G. 2011. Health-beneficial phenolic aldehyde in *Antigonon leptopus* tea. *Evid. Based Complement. Alternate. Med*. **2011**, 601249.