Isolation and Characterization of Polyprenols from *Litsea monopetala* (Roxb.) Pers.

Mir Shahriar Kamal, Sania Ashrafi, Sara Sultana, Mohammad A. Rashid, Monira Ahsan, Mohammad Mehedi Masud and Mohammad Rashedul Haque

Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy
University of Dhaka, Dhaka-1000, Bangladesh

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ABSTRACT: *Litsea monopetala* (Roxb.) Pers. (Family: Lauraceae) is a plant that holds substantial ethnobotanical significance due to its traditional and cultural importance. In this study, we conducted an extensive chemical investigation on *L. monopetala*, a plant native to the regions including Nepal, India, and Bangladesh. Two unique secondary metabolites were isolated and characterized from ethyl acetate (EtOAc) extract of the leaves of *L. monopetala* which were identified as tri-*trans* poly-*cis* prenol-15 (**1**), and its acetylated derivative tri-*trans* poly-*cis* prenol-15 (**2**)**.** The structures of the isolated compounds were determined through in-depth analysis of their high-resolution ^{1}H NMR, ^{13}C NMR, COSY, HSQC, and HMBC spectroscopic data, along with comparison to existing published data. Both the compounds are isolated from the plant for the first time. Further studies are recommended to analyze the potential bioactivity associated with the molecules.

Key words: *Litsea monopetala*, NMR, phytochemicals, polyprenols, acetylated polyprenols.

INTRODUCTION

The utilization of plant-derived metabolites dates as far back as 2600 BC. Over the next 4000 years, secondary metabolites from plants were primarily employed for medicinal and toxic purposes, as well as in food. In 1806, morphine became the first natural substance to be isolated from the opium poppy (*Papaver somniferum*), marking the beginning of a ground breaking era in secondary metabolite research.[1]

Litsea monopetala (Roxb.) Pers., belonging to the Lauraceae family, is a petite tree with the potential to grow up to 18 meters tall. This species is predominantly native to Nepal but is also distributed in various other Asian nations, including India and Bangladesh. The plant is extensively distributed in various regions of Bangladesh, including the Chittagong Hill Tracts, Sylhet, the Sal (*Shorea robusta*, family Dipterocarpaceae) forests of Gazipur, Madhupur, and Dinajpur.[2] In Bangladesh, the plant is commonly referred to as "Kharajora." It plays a significant role in Ayurvedic medicine, where it is known as "Maidaa-lakdi" in Sanskrit. Within Ayurveda, the bark of this plant is renowned for its stimulating, astringent, spasmolytic, and antidiarrheal properties. Additionally, the roots are used externally to alleviate pain, treat bruises, and manage contusions.[3] The flower oil of *L. monopetala* contains compounds such as α-caryophyllene alcohol, pentacosane, caryophyllene oxide, humulene oxide, and tricosane, while the fruit oil is characterized by the presence of decanal, nonanol, and capric acid. Its bark extracts have revealed the presence of eugenol, chalcone, and their derivatives, β-sitosterol, and actinodaphnine. Additionally, the bark oil of this plant contains tetradecanal, tridecanol, myristic acid, and tridecanal. Furthermore, an arabinoxylan has been documented in the mucilage of *L. monopetala* leaves, contributing to the comprehensive understanding of its phytochemical composition.[4,5] In our study, we focused our research on *L. monopetala* and we, here in, report the presence of two

Correspondence to: Mohammad Rashedul Haque
Email: haquemr@du.ac.bd

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polyprenols, from an ethyl acetate extract of the leaf of the plant.

MATERIALS AND METHODS

Sample collection and preparation. The whole plant of *L. monopetala* was collected from Gazipur, Bangladesh, and a voucher specimen DACB-38437 was carefully preserved for authentication. Expert validation of the plant's identity was conducted at the Bangladesh National Herbarium (BNH). The leaves were carefully collected, cleansed to eliminate any debris, and subjected to a two-week drying process in shaded areas with adequate ventilation, followed by additional exposure to sunlight. Subsequent to this drying period, the leaves were ground by a high-capacity grinding machine, resulting in approximately 1 kg of coarse powder.

Instrumentations, drugs and chemicals. In this study, Nuclear Magnetic Resonance (NMR) spectra were recorded using a Bruker instrument operating at 400 MHz, and deuterated chloroform ($CDCl_3$) was used as the solvent. Solvent evaporation was carried out using a Buchi Rotavapor, sourced from Germany. Vacuum liquid chromatography (VLC) procedures were performed using Kieselgel 60H, while column chromatography (CC) involved the use of Kieselgel 60 (70-230 mesh ASTM) from Sigma-Aldrich in the USA. For compound analysis, we utilized pre-coated thin-layer chromatography (TLC) plates with Silica gel 60 F_{254}, procured from Merck, Germany. To visualize spots on the TLC plates, UV light and the vanillin/H_2SO_4 reagent were used. All other chemicals and solvents were of analytical grade and were obtained from reputable suppliers, including Active Fine Chemicals Ltd. in Bangladesh, Merck, Germany, and DaeJung, Korea.

Extraction of plant material. The coarse plant powder was carefully transferred into a clean 3-liter amber bottle. Ethyl acetate was added to completely submerge the powder, and this mixture was allowed to soak for a period of 18 days with regular agitation. Following this soaking period, the mixture was subjected to filtration using a cotton plug and Whatman number 1 filter paper. The resulting product was the crude extract, which was subsequently concentrated through a low-temperature evaporation process, with temperature maintained below 40°C and appropriate pressure. The final concentrated crude extract weighed 25.5 g.

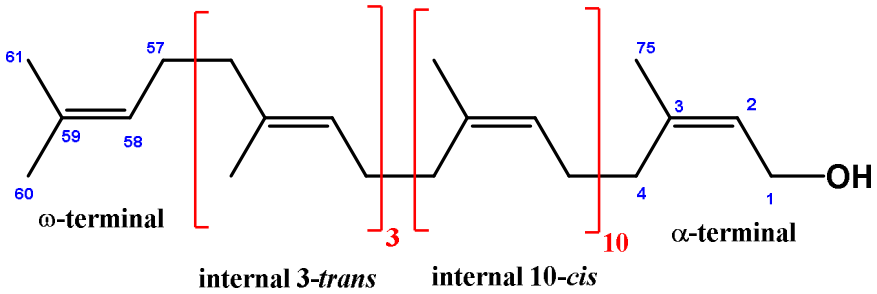
Isolation of compounds. An aliquot (25.5 g) of the crude extract was processed through vacuum liquid chromatography (VLC), employing hexane and EtOAc as the solvents.[6] This separation procedure yielded a total of 34 VLC fractions, each 150 ml. Based on the TLC features, VLC fractions 10-13, 14-17, and 18-19 underwent separation using size exclusion chromatography over Sephadex® LH-20, employing a gradient solvent system to separate molecules based on their sizes and polarities. The eluents were collected in 1ml aliquots in test tubes. For the ultimate purification, preparative TLC was employed. Compound **1** was obtained from Sephadex fractions 28-31 of VLC fraction 18-19 (PTLC at 50% hexane in toluene), and compounds **2** was isolated from Sephadex fractions 27-31 of VLC fraction 10-13 (PTLC at 75% hexane in toluene).

Properties of isolated compounds. Tri-*trans* poly-*ci s*prenol-15 (**1**). Colorless mass and soluble in chloroform; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.10 (2H, d, *J* = 7.2 Hz, H-1), 5.46 (1H, t, *J* = 7.2 Hz, H-2), 1.75 (3H, s, H-75, CH_3-3), 2.05 (56H, m, H-4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 56, 57), 5.12 (14H, br. s, H-6, 10, 14, 18, 22, 26, 30, 34, 38, 42,46, 50, 54, 58), 1.60 (12H, s, C$\underline{H}_3$-*trans* 62, 63, 64 and ω- C$\underline{H}_3$-*trans* 61), 1.68 (33H, s, C$\underline{H}_3$-*cis* 65, 66, 67, 68, 69, 70, 71, 72, 73, 74 and ω-C$\underline{H}_3$-*cis* 60).

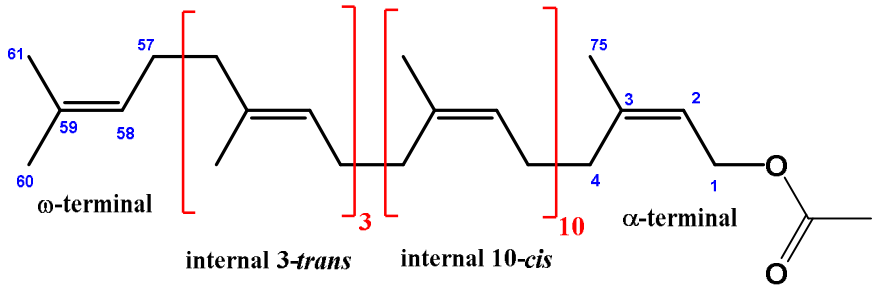
Acetylated tri-*trans* poly-*cis* prenol-15 (**2**): Colorless mass and soluble in chloroform; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.56 (2H, d, *J* = 7.2 Hz, H-1), 5.39 (1H, t, *J* = 7.2 Hz, H-2), 1.76 (3H, s, H-75, CH_3-3), 2.05 (56H, m, H-4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 56, 57), 5.12 (14H, br. s, H-6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, 58), 1.60 (12H, s, C$\underline{H}_3$-*trans* 62, 63, 64 and ω-C$\underline{H}_3$- *trans* 61), 1.68 (33H, s, C$\underline{H}_3$-*cis* 65, 66, 67, 68, 69, 70, 71, 72, 73, 74 and ω-C$\underline{H}_3$-*cis* 60), 2.17 (3H s, C$\underline{H}_3$CO).

RESULTS AND DISCUSSION

Two compounds (as shown in Figure 1) were successfully isolated from the crude EtOAc extract of the leaves of *L. monopetala* through a series of repeated chromatographic separations. The structures of the isolated compounds were subsequently elucidated and characterized as tri-*trans* poly-*cis* prenol-15 (**1**), and acetylated derivative of tri-*trans* poly-*cis* prenol-15 (**2**). Both the compounds have been isolated for the first time from this plant.



(1)



(2)

Figure 1. Structures of the isolated phytochemicals from *L. monopetala*: tri-*trans* poly-*cis* prenol-15 (**1**) and acetylated derivative of tri-*trans* poly-*cis* prenol-15 (**2**).

The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **1** indicated the presence of olefinic proton of the α-terminal isoprene unit at δ 5.46 (1H, t, J = 7.2 Hz) and the methylene protons at δ 4.10 (2H, d, J = 7.2 Hz). A signal at δ 1.75 (3H, s) could be assigned to the *cis*-methyl of the α-terminal isoprene unit.[8] The signal at δ 1.60 integrated for 12 protons were assigned to three internal *trans*-methyl and a ω-terminal *trans*-methyl group. The spectrum further confirmed the presence of eleven cis-methyl groups resonating at δ 1.68 (33H, s) and among them ten were internal *cis*-methyl and the rest was for ω-terminal *cis*-methyl. Additionally, the spectrum showed twenty-eight methylene groups at δ 2.05 (56H, m) of the fourteen isoprene units.

The ^{13}C-NMR spectrum further supported the characterization of compound **1** including the peak at δ 59.19 which can be assigned to the C-1 at the α-terminal and at δ 125.20 assigned to the methine group of the internal isoprene units. The spectrum also revealed the position of C-2 at δ 135.42 which showed ^{3}J correlation to H-75 (δ 1.75) in HMBC spectrum. The COSY spectra showed all the expected coupling correlations including H-1 at δ 4.10 showing a cross peak with H-2 at δ 5.46. Based on the above analysis, compound **1** was identified as tri-*trans* poly-*cis* prenol-15 which is isolated for the first time from this plant (Table 1).

The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **2** indicated the presence of olefinic proton of the α-terminal isoprene unit at δ 5.39 (1H t, J = 7.2

Hz) and a deshielded methylene protons at δ 4.56 (2H d, *J* = 7.2 Hz) due to acetylation. A signal at δ 1.76 (3H, s) could be assigned to the *cis*-methyl of the α-terminal isoprene unit.[7] The signal at δ 1.60 integrated for 12 protons were assigned to three internal *trans*-methyl and a ω-terminal *trans*-methyl groups. The spectrum further confirmed the presence of eleven cis-methyl groups resonating at δ 1.68 (33H, s) and among them ten were internal *cis*-methyl and the rest is one ω-terminal *cis*-methyl. Additionally, the spectrum showed twenty-eight methylene groups at δ 2.05 (56H, m) of the fourteen isoprene units. Finally, a peak at δ 2.17, integrated for 3 protons, indicated the presence of an acetyl group. The deshielded oxymethylene proton at δ 4.56 (2H) instead of δ 4.10 (2H) in case of tri-*trans* poly-*cis* prenol-15 further supported the acetylation of the carbinol group.

Table 1. ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data for compounds 1 & 2.

Position	Compound 1	Compound 2	Tri-*trans* poly-*cis* prenol- 12[7]
	δ_H	δ_H	δ_H
H-1	4.1, 2H, d (*J* = 7.2 Hz)	4.56, 2H, d (*J* = 7.2 Hz)	4.09
H-2	5.46, 1H, t (*J* =7.2 Hz)	5.39, 1H, t (*J* =7.2 Hz)	5.44
H-75 (CH_3-3)	1.75, 3H, s	1.76, 3H, s	1.74
H-4,5,8,9,12,13, 16,17,20, 21,24, 25, 28,29,32,33,36, 37, 40,41,44,45,48,49.52,53,56,57	2.05, 56H, m	2.05, 56H, m	2.03, m[8]
H-6,10,14,18,22, 26,30,34,38,42,46,50,54,58	5.12, 14H, br. s	5.12, 14H, br. s	5.12, br. s
H-62,63,64 (CH_3-trans) and H-61 (ω- CH_3- trans)	1.60, 12H, s	1.60, 12H, s	1.60
H-65,66,67,68,69,70,71, 72,73,74 (CH_3-cis) and H-60 (ω- CH3-cis)	1.68, 33H, s	1.68, 33H, s	1.68
$C\underline{H}_3CO$-	-	2.17, 3H, s	2.1-2.5

Based on the above analysis, compound **2** was identified as the acetylated derivative of tri-*trans* poly-*cis* prenol-15 which is isolated from this plant for the first time (Table 1).

Polyprenols (polyisoprenoid alcohols) are lipid linear polymers comprised of multiple isoprenoid units. They are widely distributed in nature, found in various organisms ranging from bacteria to plants, including mammals, yeast, bacteria, and fungi.[9] Polyprenols exhibit a range of favorable characteristics in humans, as they are non-toxic, non-mutagenic, non-teratogenic, and non-carcinogenic.[10] Moreover, they possess notable biological activities, including significant anti-tumor, anti-hepatitis C virus, and anti-HIV effects, making them valuable adjuvants in chemotherapy for leukemia and radiotherapy.[11,12] Furthermore, polyprenols have shown promise in addressing conditions such as hypertension, high cholesterol, diabetes, gout, lupus, and other immune function disorders, as noted in.[10,13] As a result, there has been a growing emphasis on research and development efforts aimed at harnessing the potential of polyprenols for various applications. In recent years, there has been a notable surge in research activities related to polyprenols and their derivatives. These investigations have encompassed various aspects such as extraction and purification, synthesis, elucidation of structure and function, exploration of biological activity, and investigation into the pharmacology of polyprenols and their derivatives.[13]

In the course of our research, we have successfully isolated and purified two distinct polyprenol and its acetylated derivatives. The research involved a rigorous process of extraction, isolation, followed by meticulous purification and ultimately characterizing these valuable polyprenol derivatives through extensive NMR spectroscopic analysis. NMR spectroscopy has emerged as a powerful and adaptable instrument for organic

chemists, facilitating structure elucidation. It has also become indispensable for structural biologists engaged in determining the structures and dynamics of macromolecules.[15]

The term polyprenol is employed to describe linear polymers consisting of multiple isoprene units, ranging from a few to well over a hundred. While the fundamental polymeric structure of polyprenol may seem straightforward, there are nevertheless recognized structural variations within this class of compounds.[16] Two primary categories of polyisoprenoid alcohols have been identified, distinguished by the hydrogenation status of their OH-terminal isoprene unit: polyprenols (α-unsaturated at the terminal position) and dolichols (α-saturated at the terminal position). Polyisoprenoids are typically stored in organisms either as free alcohols or as esters in conjunction with carboxylic acids, with a relatively small fraction also existing in the phosphorylated form.[17] Notably, our study has identified the presence of acetylated polyprenols, a less common occurrence in this context, which adds to the interest and significance of our findings.

For the future aspects of this study, there are several promising directions to consider. Firstly, conducting comprehensive bioactivity testing is essential to further evaluate the therapeutic potential of the isolated polyprenols from *L. monopetala*. Secondly, elucidating the precise mechanisms of action underlying the observed bioactivity of the plant is crucial. Additionally, exploring potential synergistic effects with other known drugs or compounds can enhance their therapeutic applications. Lastly, investigating the stability, formulation, and pharmacokinetics of these polyprenols is essential for their development into potential drug candidates.

CONCLUSION

In conclusion, our study on *L. monopetala* has unveiled the intriguing presence of two unique polyprenols with promising bioactivity. This plant, native to regions such as Nepal, India, and Bangladesh, showcases a rich diversity of phytochemicals, making it an enticing subject for further exploration. The isolation and identification of these polyprenols underscore the potential pharmacological importance of *L. monopetala* and its underexplored compounds. Subsequent research endeavors should focus on the broader chemical profile of *L. monopetala* and explore novel methods for extracting and purifying its valuable constituents.

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