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New Flavonoid Glycoside from the Medicinal Plant *Vitex negundo*

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Abstract: A new polyphenolic flavonoid glycoside has been isolated from the leaf extract of the medicinal plant *Vitex negundo* Linn. Structure of the isolated compound-1 has been elucidated as methyl 6-(5-(5,7-dihydroxy-4-oxo-4*H*-chromen-2-yl)-2-hydroxyphenoxy)-3,4,5-trihydroxy tetra hydro-2*H*-pyran-2-carboxylate based on the FT-IR and GC-Mass spectroscopic data analyses.

Keywords: *Vitex negundo*, FT-IR, GC-MS, Medicinal plant.

INTRODUCTION

Vitex negundo (*VN*) Linn. (Locally known as Ban Nishinda) belonging to the family verbenaceae, is a large shrub growing in Bangladesh, India, and Nepal, specially, in the warmer region of the respective countries. It is one of the common plants used in traditional medicine that have a variety of biological activities. All parts of the *VN* are used as medicine, however, the leaves are specially considered to be the most potent for the isolation of medicinal constituents. It has been used for the treatment of eye-disease, inflammation, leucoderma, and toothache, skin-ulcers, in catarrhal fever, rheumatoid arthritis, gonorrhea, sinuses and bronchitis[1]. It should also be used as tonics, vermifuge, lactagogue, emmenagogue, antibacterial, antifeedant, antifilarial, antifungal, anti-larval, anti-viral, insecticidal, larvicidal, and mosquito repellant agents[2–8]. Its extract showed anticancer activity against ehrlich ascites tumour cells[9]. The aqueous extract and oil of seeds of *VN* possessed anti-oxidant and anti-inflammatory properties[10]. Among the chemical constituents, the major classes of compounds such as flavonoids, iridoids,

terpenoids, steroids, alkaloids have been isolated from the plant *VN* [11–13]. The anti-inflammatory and free radical scavenging property of the constituents extracted from the seeds and leaves of the *VN* have also been reported[14–16]. In spite of being widely used in traditional systems of medicines, only few numbers of structures of the compounds has been elucidated. In this present study, we report a new polyphenolic flavonoid glycoside component extracted from a typical fractionate of the dia-ion resin adsorbed fraction of the methanolic extract of the plant *VN* leaves.

MATERIALS AND METHODS

Plant collection and preparation of extracts: Fresh *Vitex negundo* (*VN*) plant was collected during the period of April from the adjacent area of the Rajshahi University Campus, Bangladesh. The leaves were parted from the plant and were dried in laboratory in absence of sunlight. The dried leaves were pulverized to make powder by an electric grinder. The ground power was placed in a tank previously filled in methanol (Mark, analytical grade) for the extraction process. The methanolic extracts were cumulatively collected in conical flask for about 24h. The juicy extract "called crude extract" was filtered through a Whatmann filter paper and was concentrated under reduced pressure at 54°C by using Rotavapor (Buchi Rotavapor R-200). The crude extract was suspended in water in order to separate the water soluble components. The water soluble components were passed through a column which was well packed with dia-ion resin. After evaporation, the fraction was designated as dia-ion resin adsorbed fraction. Since the dia-ion resin adsorbed fraction showed promising antioxidant property, therefore, we have been considered it for further isolation of bioactive compound.

Isolation of the pure compound: Typical 10.21 g dia-ion resin adsorbed fraction mixed with 12.0 g silica gel (mesh 60-120 nm) "called slurry" was used for column packing. The column about 3 feet length was cleaned by flowing double distilled water about three times and then it was dried at room temperature. The slurry was placed on the top of silica gel layer of the column and a piece of cotton was placed on top of the slurry so that it should not scattered during pouring of the solvent. First, the column was eluted with pure chloroform and then amount of methanol was increased. Elutes (30~70 mL) were collected in a series of conical flask and was monitored by TLC. Elutes of similar TLC behavior were combined together and were designated. A pure compound was isolated and crystallized out by evaporating a typical fractionate with the solvent ratio of chloroform t methanol = 5 t 2. The crystal was successively washed with n-hexane, petroleum ether and diethyl ether and designated as pure compound-1. A photograph of the pure compound-1 is shown in **Figure 1**.

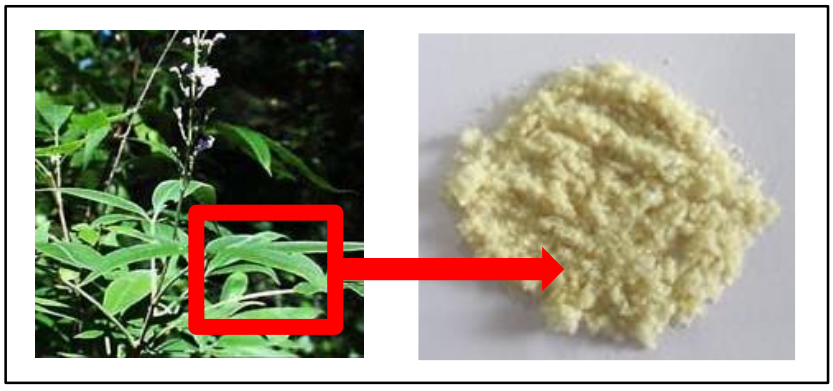

Figure-1: Photograph of the plant *Vitex negundo* and the isolated compound-1.

RESULTS AND DISCUSSION

The compound-1 was obtained as white colored crystalline solid. The melting point was recorded to be 156~158 °C (Buchi melting point, B-545) and the R_f value was calculated to be 0.657 in the solvent system of chloroform t methanole (5 t 2). The nitrogen, halogen and sulphur elements were absent confirmed by the elementary analyses. Simultaneously, the functional groups such as phenolic –OH, carbonyl >C=O, ester –$COOCH_3$, and carboxyl group –COOH has been confirmed[17].

Figure 2 shows the FT-IR spectrum (PERKIN ELMER) of the compound-1. The spectrum was recorded by the background subtraction of KBr. The main band observed at ~3324 cm^{-1} which was due to the –OH vibration. The –OH group was also confirmed by the qualitative functional group analyses. Another strong band observed at ~1715 cm^{-1} owing to the >C=O stretching vibrations which was also confirmed by the functional group analyses. The band at 3005 and 2974 cm^{-1} were due to the aromatic C–H bond. The other bands observed at 1509, 1430 and 1377 cm^{-1} was due to the aromatic ring system. The bands at 1310 ~1333 cm^{-1} were reported to be the bending vibrations of C–O–H bonds.

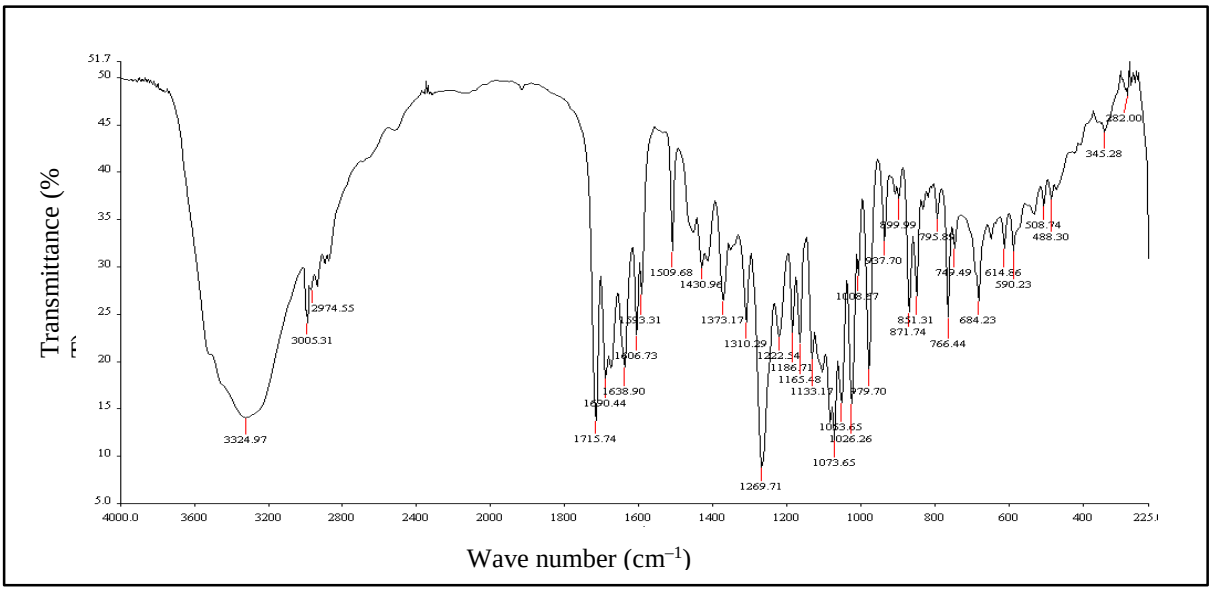

Figure-2: FT-IR spectrum of the unknown compound-1 extracted from the medicinal plant *Vitex negundo.*

Recently, the GC-MS spectroscopic analysis has been considered as a tool for the conclusive proof of identity of an unknown compound. In this study, the structure of the unknown compound-1 isolated from the *VN* leaf extract was elucidated by the GC-MS spectroscopic analyses. Typical 2.0 µl sample was dissolve in methanol and then it was run for taking GC-MS spectra. The GC–MS was conducted on a Gas Chromatograph Mass Spectrometer (GCMS-TQ8040, SHIMADZU). Helium (99.99%) was used as carrier gas at a flow rate of 1 mL/min. The pressure was 45 kPa and the inlet temperature was maintained at 200˚C. The column oven temperature was programed initially at 30˚C and increased to 275°C. The GC–MS spectra was taken using electron impact ionization at 70 eV and the data were evaluated using total ion count for compound identification and quantification. The spectrum of the compound-1 was compared with the database of spectra of known components stored in the GC–MS library at Bangladesh

Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. The name, molecular weights and structure of the compound-1 was hereby ascertained.

The GC–MS spectrum of the compound-1 is shown in **Figure 3**. A sharp peak has been observed in spectrum. The molecular weight was found to be 476.393 emu. The trivially name of the compound was as vitegnoside has been ascertained. The possible structure of the unknown compound-1 is shown in **Figure 4**.

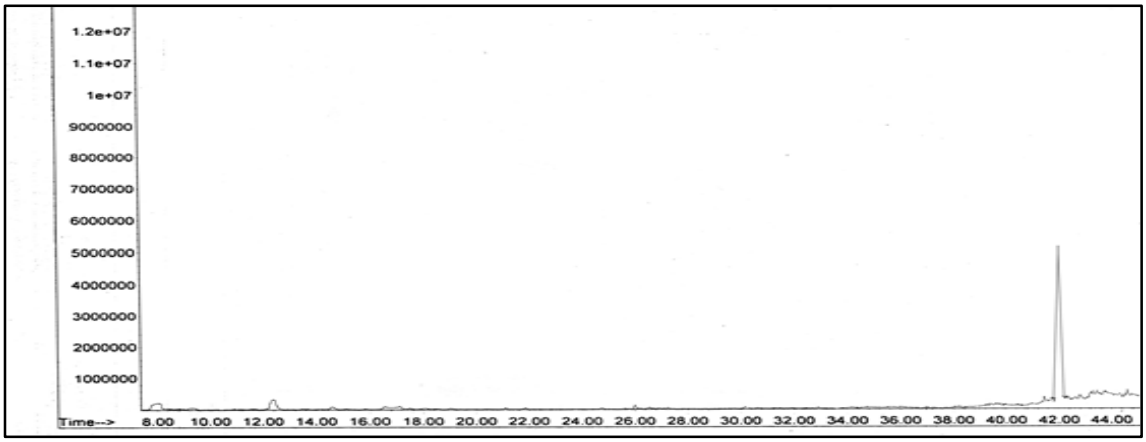

Figure-3: GC–MS spectrum of the unknown compound-1 extracted from the medicinal plant *Vitex negundo.*

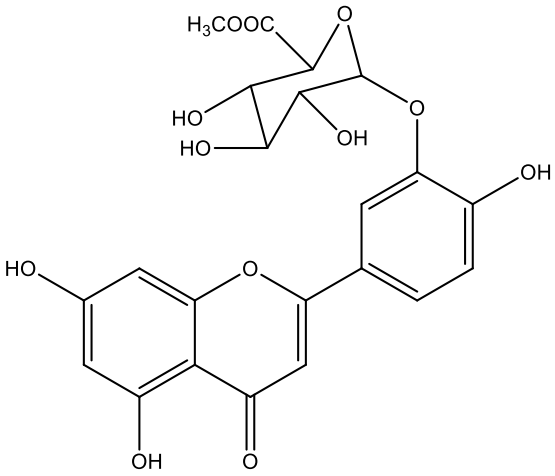

Figure-4: Proposed chemical structure of the unknown compound-1 extracted from the medicinal plant *Vitex negundo.*

CONCLUSION

In this study, one new vitegnoside [methyl 6-(5-(5,7-dihydroxy-4-oxo-4*H*-chromen-2-yl)-2-hydroxyphenoxy)-3,4,5-trihydroxy tetra hydro-2*H*-pyran-2-carboxylate)] has been isolated from the medicinal plant *VN* through systematic guided fractionation. The structure was assigned on the basis of the FT-IR and GC-MS spectroscopic analyses. However, more spectroscopic analyses like NMR, single

X-ray crystallography etc. should be performed to ensure the structure but due to some limitations we could not provide other data. Further research has been taken to explore the detail biological profile of the newly isolated compound-1.

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