ORIGINAL ARTICLE

Isolation and characterization of flavonoid glycoside from *Cordia obliqua* Willd. leaf

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Abstract

Aim: *Cordia obliqua* Willd. (Clammy cherry) tree of family Boraginaceae and genus *Cordia* is a medium-sized deciduous tree and very well distributed all over India as well as other warmer regions of the world. **Objective:** The objective of the study was to isolate phytoconstituent from *C. obliqua* leaf methanol extract by column chromatography and characterization by various spectroscopic techniques such as ultraviolet-visible, infrared, nuclear magnetic resonance, ,and mass. **Materials and Methods:** The *C. obliqua* leaf methanol extract was prepared by successive solvent extraction using Soxhlet apparatus. The methanol extract was found rich in phytoconstituents with the help of chemical tests, and also it was found effective against pain, inflammation, and pyrexia in experimental animal studies. Hence, methanol extract was selected for isolation of important plant constituents by column chromatography. The column was carried out with the different solvent system used in particular ratios. A total of 50 fractions were collected and studied by thin-layer chromatography for pooling. The pooled F2 fraction was further studied by spectroscopic techniques to characterize the compound. **Result:** On isolation by column chromatography, a flavonol glycoside molecule of quercetin aglycone "3'-*O*-Methyl Quercetin-3-glucose-6-gallic acid" was characterized by spectroscopic techniques. It justified the effect of leaf extract in the treatment of pain, swelling, and inflammation and also as an antioxidant because flavonoids are a group of therapeutic active compounds due to their supreme antioxidant action. **Conclusion:** It was concluded that *C. obliqua* leaf methanol extract was rich in plant constituents and also has a number of therapeutic active constituents which suggest the plant used for other activities.

Key words: Boraginaceae, clammy cherry, column chromatography, quercetin glycoside, spectroscopy

INTRODUCTION

The natural products, either in the form of pure compounds or as standardized plant extracts, provide infinite opportunities for drug discoveries. However, only 10% of the world's biodiversity has only been explored, and it represents wide scope for the discovery of new molecules from plant sources.[1] With this view, we have selected *Cordia obliqua* Willd. plant for our research. It is a very well distributed tree all over India and in other warmer regions of the world. It belongs to Boraginaceae or Forget-me-not family and *Cordia* genus. Commonly it is named as clammy cherry and lasora. It is a vigorous, medium height, and deciduous tree. It is of two types on the basis of its fruit size. The plant having smaller fruit is more common.[2,3] The various biological activities performed on different parts of this plant are anti-inflammatory activity of seed constituents[4] and stem bark,[5] hypotensive and respiratory stimulant activity of fruit mucilage,[6] antimicrobial activity of seed and leaf extract,[7] diuretic activity of fruit[8] and analgesic, anti-inflammatory, and antipyretic activity of leaf.[9] The chemical examination of *C. obliqua* seeds has resulted in isolation and characterization of alpha-amyrin, octacosanol, beta-sitosterol, betulin, lupeol-3-rhamnoside, beta-sitosterol-3-glucoside, hentriacontane, taxifolin-3,5-di rhamnoside, hentriacontane, and hesperetin-7-rhamnoside.[10]

Other isolated chemical compounds from *C. oblique* plant are Hesperetin-7-rhamnoside from roots,[11] Lupa-20,29-ene-3-o-β-D-maltoside from roots,[12] Lupa-20(29)-ene-3-O-alpha-L-rhamnopyranoside from roots,[13] natural gums, and mucilage from fruits.[14] Constituents such as Allantoin-β-sitosterol and some flavonoids were also isolated from the stem bark of *C. obliqua*.[15]

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Received: 04-02-2018
Revised: 24-03-2018
Accepted: 08-04-2018

However, no any isolation work has been performed on leaf part of the plant. Hence, this work was aimed to isolate and characterize important phytoconstituents from *C. oblique* plant leaf.

MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of *C. oblique* Willd. were procured from Jammu. These were authenticated and identified by Dr. (Mrs) Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum (RHMD), Council of Scientific and Industrial Research-National Institute of Science and Information Resources (CSIR-NISCAIR), New Delhi, with the reference no. NISCAIR/RHMD/Consult/-2014/2383-163.

Requirements

Chemicals and solvents

All solvents, reagents, and chemical used were of AR and high-performance liquid chromatography (HPLC) grade from Loba Chemie Pvt. Ltd. Mumbai, HiMedia Mumbai, and Qualigens fine chemicals Mumbai. Distilled water and pre-coated silica gel G plates (6 × 6, 0.2 mm thickness, aluminum base, and E Merck) were used.

Instruments

Major instruments used were ultraviolet (UV)-visible spectrophotometer (Pharma Spec UV-1700) from Shimadzu, Rotary vacuum evaporator (Heidolph), Bruker's Fourier-transform infrared (FT-IR) spectrophotometer, Avance II Bruker nuclear magnetic resonance (NMR) spectrophotometer, Waters Q-Tof Premier Micromass spectrometer, and UV cabinet for thin-layer chromatography (TLC) study by Camag.

Preparation of extract

The plant material was dried in the shade and powdered. About 1.0 kg of powdered plant material was extracted successively in Soxhlet apparatus using solvents in order of increasing polarity, namely hexane, chloroform, methanol, and water. After each extraction, the solvent was recovered using rotary vacuum evaporator, and residue plant material is dried, then extracted with next solvent. The methanol extract was of dark green color and non-sticky. Its yield was 1.2%w/w. The dried extracts were stored in vacuum desiccators for further study.[16]

ISOLATION OF CHEMICAL CONSTITUENT BY COLUMN CHROMATOGRAPHY

To isolate phytoconstituent from the methanol extract of *C. obliqua* leaf, column chromatography method was selected. It is a convenient laboratory scale method for isolation. The selected column was of 100 cm length and 4.5 cm diameter with sintered glass disc for support to stationary phase. Silica Gel (60–120 mesh) was used as an adsorbent. For mobile phase, various solvents in order of increasing polarity (hexane, chloroform, ethyl acetate, and methanol) were used ratio wise. The solvent ratios were used such as hexane (100), hexane:chloroform (50:50), chloroform (100), chloroform:ethyl acetate (75:25), chloroform:ethyl acetate (50:50), chloroform:ethyl acetate (25:75), ethyl acetate (100), ethyl acetate:methanol (80:20), ethyl acetate:methanol (60:40), ethyl acetate:methanol (40:60), and ethyl acetate:methanol (20:80).

Column packing method

Wet filling method about 200 g of Silica was used for stationary bed formation. The column was tapped during filling to remove air bubbles. Once the stationary bed was formed, it was not allowed to dry; otherwise, the column bed will show cracks and could not be used for separation.

Sample amount

10 g of leaf methanol extract was used for separation and it was adsorbed on about 80 g of silica. The sample was properly dried and passed through a mesh to make it free-flowing. Then, it was filled in column in dry form.

Column development

After placing sample in column, mobile phase run was started. All the solvents were used in order of increasing polarity and ratio wise. The flow of mobile phase was continuous (2–3 ml/min) and fractions were collected volume wise (150 ml/fraction). A total of 50 fractions were collected in about. These all fractions were concentrated to 1/10th of their original volume by rotary vacuum evaporator; solvent was recovered and studied by TLC technique.

TLC study of column fractions

The column fractions were analyzed by TLC method on pre-coated silica gel plates using solvent system "ethyl acetate:methanol:water in ratio 77:15:8," detection by UV light at 254 nm and 366 nm before spray and by spraying reagent anisaldehyde-sulfuric acid with heating at 100°C in hot air oven for 5–10 min.

Pooling of column fractions

On the basis of TLC study result, the 50 column fractions were pooled in five parts and termed as F1 from 1 to 13, F2 from 14 to 22, F3 from 25 to 28, F4 from 29 to 40, and F5 from 41 to 50. All these pooled fractions were dried and weighed. The weighed amount of these fractions was as F1 = 0.754 g, F2 = 0.503 g, F3 = 0.342 g, F4 = 1.51 g, and F5 = 2.31 g.

Characterization of pooled fraction

Among this F2 was studied by the spectroscopic method to identify and characterize the phytoconstituents. The spectroscopic techniques used were UV-visible spectroscopy, FT-IR spectroscopy, 1H-NMR, 13C-NMR, and Mass Spectrometry. Bruker FT-IR was used for analysis of functional groups. It has high resolution (1/cm) and broad scan range from 4000/cm to 250/cm. For NMR spectra Avance-II Bruker NMR spectrophotometer was used with a frequency of 400 MHz for 1H, and 100 MHz for 13 C. DMSO-d_6 was used as a solvent. Mass spectrum was recorded on Waters Q-Tof Premier Micromass mass spectrometer.

RESULTS

Column chromatography images

The following images [Figures 1-3] are showing process of isolation by column, various column fractions (concentrated), TLC study to pool the similar fractions and detection in UV light and by spraying reagent.

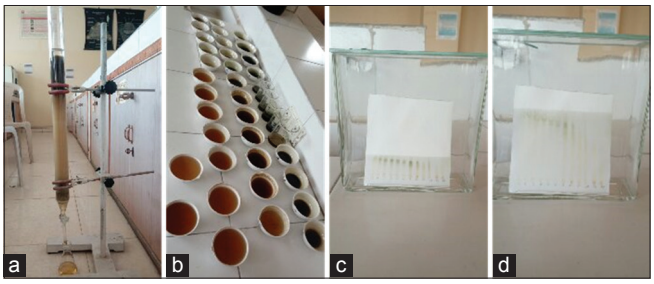


Figure 1: Column chromatography images. (a) Running column; (b) collected fractions; (c and d); thin-layer chromatography study of column fractions

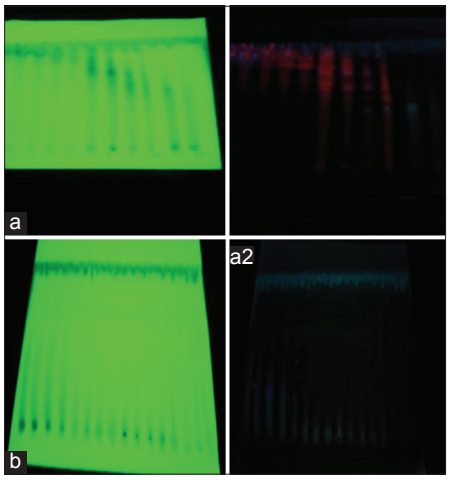


Figure 2: Thin-layer chromatography (TLC) study. (a) Detection of column fractions at 254 nm before spraying. (a2) Detection of column fractions at 366 nm before spraying. (b) Detection of column fractions at 254 nm after spraying. (b2) Detection of column fractions at 366 nm after spraying

Characterization of F2

General properties

Weight was 0.503 g (5.03%), Color - Greenish-Yellow, and Form - Solid mass.

UV λ_{max} (methanol)

UV λ_{max} was 260 nm and 354 nm

Infrared peaks (in cm^{-1}) [Figure 4]

3744.85 (-OCH_3 or free -OH), 3385.69 (Bonded -OH), 2926.40 and 2856.90 (sp^3 C-H stretch), 1734.91 (For C=O), 1656.22 (C=C stretch), 1605.79, 1458.07, 1239.90 (C-H bending), 1086.15 (C-O str), 816.24 and 754.26.

1H-NMR spectrum (DMSO-d_6) [Figures 5 and 6]

6.26 (s, 1H), 6.81 (s, 1H), 6.89 (d, 1H), 7.12 (d, 1H), 7.78 (s, 1H), 12.665 (s, 1H), 5.38 (d, H-1 glucose), 3.11-3.68 (m, sugar protons), and 6.91 (1H, s, h-2, 6 of gallic acid).

13C-NMR spectrum (DMSO-d_6) [Figure 7]

170.48 (C=O of ring C), 130.18 (-C of ring C), 114.68 (C-O of aromatic ring A), 104.15 (C-O of aromatic ring A), 98.01 (–CH of aromatic ring A), 101.84 (-CH of aromatic ring A),

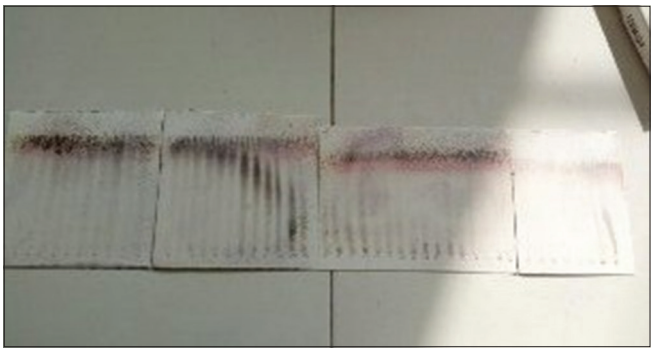


Figure 3: Thin-layer chromatography study; detection of column fractions after detecting reagent

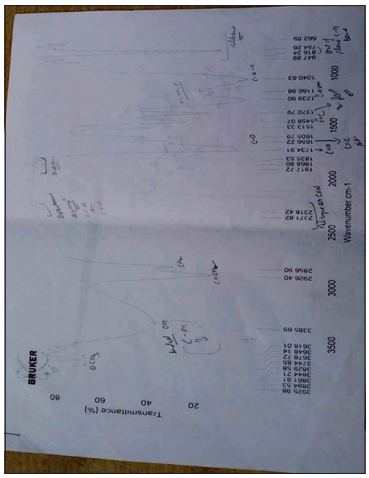


Figure 4: Fourier-transform infrared spectrum of F2

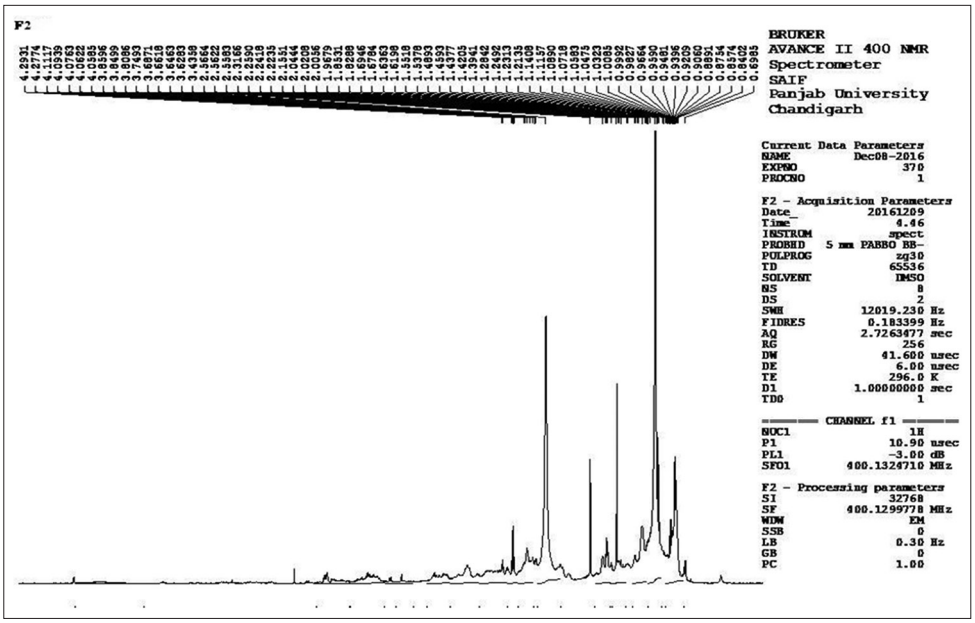


Figure 5: 1H-nuclear magnetic resonance spectrum of F2

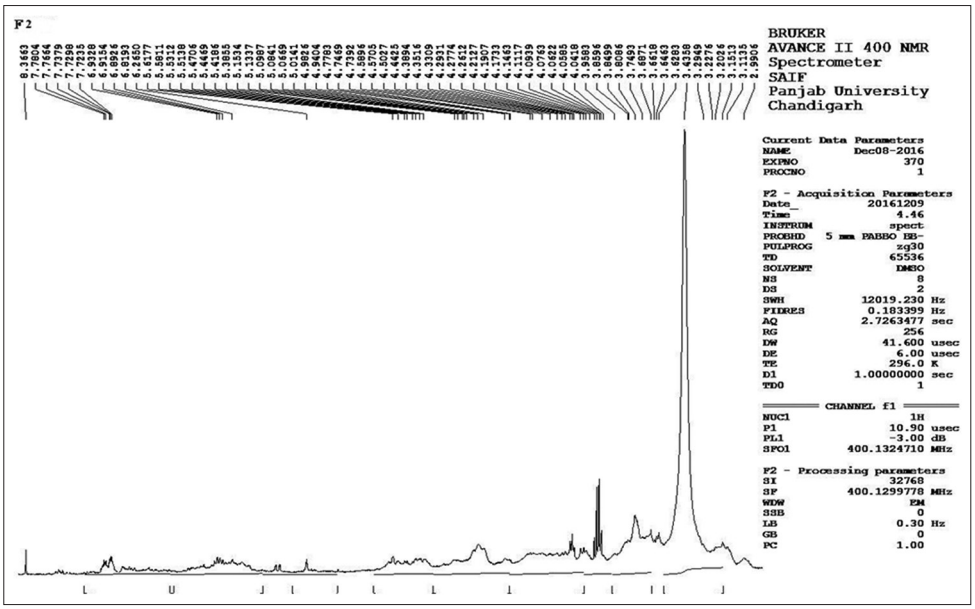


Figure 6: 1H-nuclear magnetic resonance spectrum of F2

80.93 (-CH of aromatic ring B), 81.68 (-CH of aromatic ring B), 81.43 (C-1 glucose), 75.09 (C-2 glucose), 75.84 (C-3 glucose), 69.76 (C-4 glucose), 76.54 (C-5 glucose), and 61.03 (C-6 glucose).

Mass spectrum [Figure 8]

Mol. Wt. - 631, RT - 13 min

Main fragment peaks are - 631.2 (M), 477 (M-H-gallic acid), 315 (M-H-gallic acid-hexose), 301 (M-H-gallic acid-hexose-CH_2), 169 (for gallic acid), and 180 (for hexose).

Characteristic peaks for quercetin (301) (Z+H)- 274 (Z+H-CO), 191 (Z+H-$C_6H_5O_2$ or B ring), 179 (Z+H-$C_7H_6O_2$), 151 (Z+H-$C_7H_6O_2$-CO), and 107 (Z+H-$C_7H_6O_2$-CO-CO_2).

Characteristic peaks for hexose sugar (Y) - 202.1 $(Y+H+Na)^+$, !80.1 $(Y+H)^+$,162 $(Y+H-H_2O)^+$, 145 (loss of H_2O), 129 (loss of H_2O), 104.1 and 85.2.

Chemical name: 3'-*O*-Methyl Quercetin-3-glucose-6-gallic acid [Figure 9]

Molecular formula: $C_{29}H_{27}O_{16}$

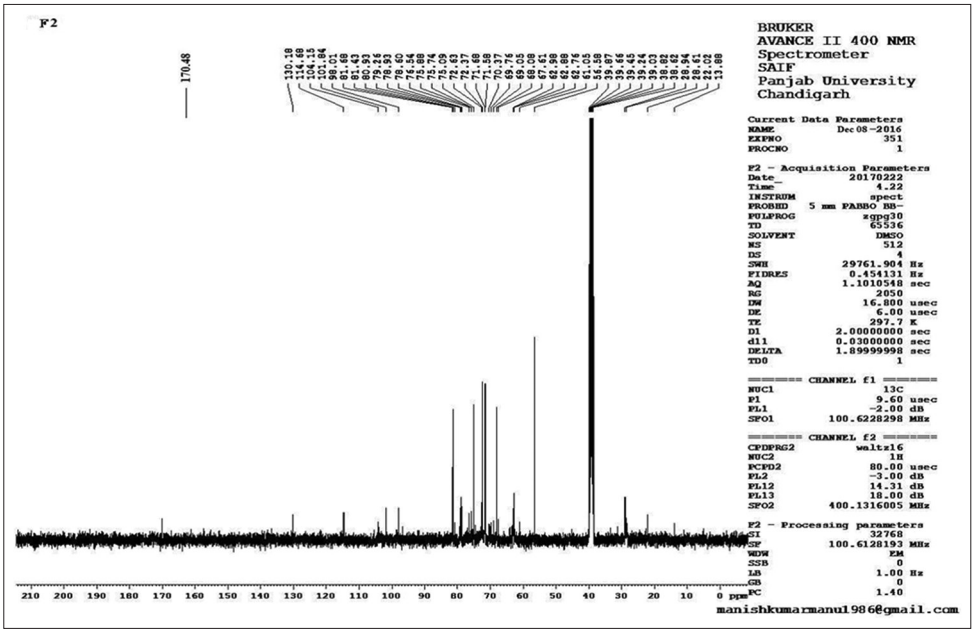


Figure 7: 13C-nuclear magnetic resonance spectrum of F2

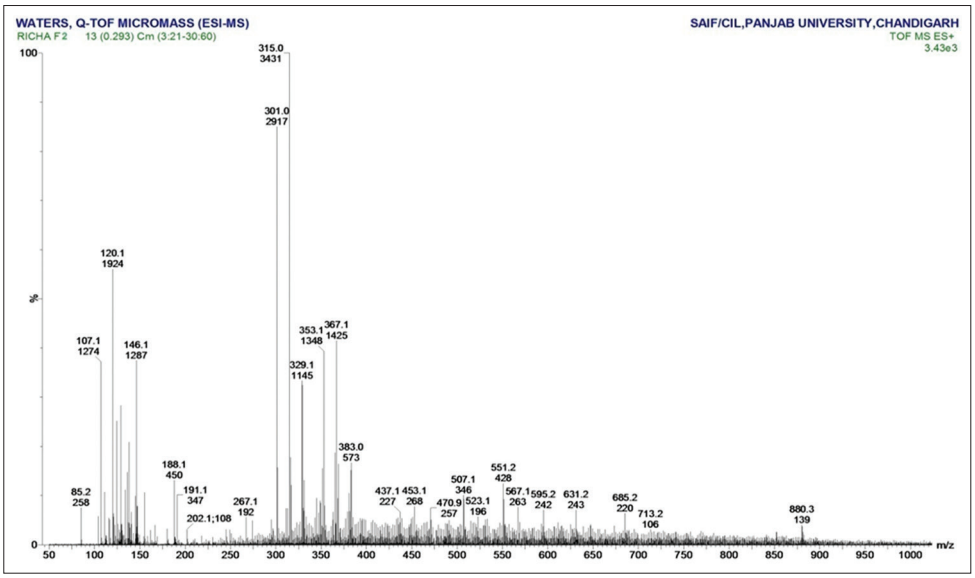
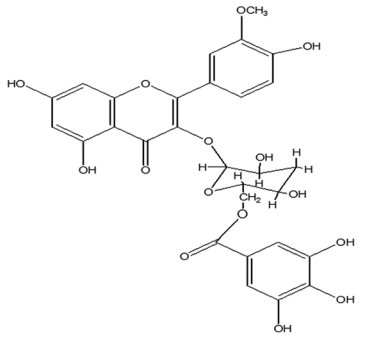


Figure 8: Mass spectra of F2

Chemical structure:



(6-(5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4-oxo-4*H*-chromen-3-yloxy)-3,5-dihydroxy-tetrahydro-2*H*-pyran-2-yl)methyl 3,4,5-trihydroxybenzoate

DISCUSSION

Column chromatography is a laboratory scale method which can be used for a variety of constituents, solvents, adsorbents and a big range of sample quantity. For isolation of phytoconstituent from *C. obliqua* Willd. leaf methanol extract, silica gel (60–120 mesh) was selected as the stationary phase, and various solvents in order of increasing polarity (hexane, chloroform, ethyl acetate, methanol, and water) were used ratio wise as the mobile phase. 50 Fractions were collected according to fix volume (150 ml). Fractions were concentrated, and the solvent was recovered. Then, these were pooled in five parts on the basis of TLC study. From the pooled fraction, F2 was studied by a spectroscopic technique.

The fraction F2 (0.503 g) was of green-yellow color solid mass. It has shown two absorption peaks in UV-visible spectroscopy study, namely 260 nm and 354 nm, which indicates the presence of any phenol or flavonoid compound.[17] The characteristic absorption peaks of FT-IR spectrum indicated the presence of bonded hydroxyl group (3385.69/cm), C-H stretch (2926.40 and 2856.90/cm), carbonyl group (1734.91/cm), C=C stretch (1656.22/cm), and C-O stretch (1086.15). The NMR spectrum of F2 in DMSO-d_6 has shown a complex spectrum, and it was studied with reference to available literature and research papers. On the basis of this study, the F2 was thought to be a flavonoid glycoside molecule because 1H-NMR spectrum has shown presence of various aromatic hydrogen environments between 6 and 7.5 δ values in ppm, a singlet for one H atom at 12.665 ppm showing the presence of hydrogen bonding in flavonoid,[18] multiplet with δ value between 3 and 4 ppm for hexose sugar hydrogens, and singlet at 6.91 ppm for hydrogen of gallic acid. Likewise, 13C-NMR spectrum has indicated various carbon signals for a flavonoid molecule and sugar moiety, for example, 170.48 (C=O of ring C), 130.18 (-C of ring C), 114.68 (C-O of aromatic ring A), 81.43 (C-1 glucose), 75.09 (C-2 glucose), 75.84 (C-3 glucose), 69.76 (C-4 glucose), 76.54 (C-5 glucose), and 61.03 (C-6 glucose).[19-22] This supposition for a flavonoid glycoside was confirmed by a study of the mass spectrum of F2. It was clearly indicating the characteristic m/z values for the molecular ion at 631 (M^+), 477 (after loss of gallic acid moiety), 315 (after loss of hexose sugar), 301 (for Quercetin after loss of CH_2 group), and 180 for hexose sugar and 169 for Gallic acid.[23-25]

CONCLUSION

Finally, on the basis of above observations, it was concluded that F2 was "3'-*O*-methyl quercetin-3-glucose-6-gallic acid" a flavonoid glycoside. We have confirmed the basic flavonoid glycoside nucleus but not confirmed for the exact position of substituent groups, as our compound was not so pure and spectra obtained were complex. It is the first reported work on isolation of phytoconstituent from *C. obliqua* Willd. leaf and the work may be carried out further to get more authentic results. The presence of flavonoid glycoside in methanol extract also supports the therapeutic potential of the plant.

ACKNOWLEDGMENTS

The authors are thankful to IK Gujral Punjab Technical University, Jalandhar, and management committee members of ASBASJSM College of Pharmacy, Bela (Ropar), for all necessary assistance and facilities provided to carry out this research work. We also thank Sophisticated Analytical Instrument Facility, Punjab University, Chandigarh, for spectral analysis.

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Source of Support: Nil. **Conflict of Interest:** None declared.