OPEN ACCESS Freely available online

Journal of Chromatography and Separation Techniques

Research Article

Development of HPTLC Method for the Analysis of Flavonoids and Tri-terpenoid Glycoside Isolated from *Carya Illinoinensis* Bark and their Biological Activity: A Brief Report

Md. Sarfaraj Hussain[1*], Mohd. Zaheen Hassan[2]

[1]Department of Pharmacognosy and Phytochemistry, Lord Buddha Koshi Pharmacy College, Baijanathpur, Bihar, India; [2]Department of Pharmaceutical Chemistry, University of King Khalid, Abha, Saudi Arabia

ABSTRACT

Medicinal uses of *Carya illinoinensis* have drawn the attention of researchers towards identifying and establishing the active principles responsible for different biological activities. Application of advanced tools and techniques has been in isolating and studying a new active principle CI-01 from the bark of *Carya illinoinensis* plant besides developing validated HPTLC method for its ethanolic extract from bark. The active principle CI-01 after its identification as Farnesoic acid α-L-glucoside has been investigated for its anti-inflammatory and analgesic activity at a dose of 10 mg/kg body weight. Besides, *in silico* studies like molecular docking followed by simulation has enabled to predict the probable mechanism of it studied biological actions.

Keywords: *Carya Illinoinensis*; HPTLC; Anti-inflammatory; Molecular docking; Molecular simulation

INTRODUCTION

With the advancement in the field of modern analytical tools, development of new and efficient methods for the isolation and identification of phytoconstituents from a plant has become quiet easier, less time consuming, affordable as well as eco-friendly. Isolation of a particular constituent, its identification, characterization and structure determination followed by their biological activity have been thrust areas of research over the decades [1]. *Carya illinoinensis*, well known as sweet pecan or pecan is a deciduous tree. Though native to the southern parts of United States and northern parts of Mexico, the tree is now cultivated in various parts of the world. Significant work on this plant has been reported over the decades and suggests its medicinal importance [2]. Extracts from different parts of this plant like leaves, barks, nuts, nutshells are used medicinally owing to their various biological activities such as anti-oxidant, antimicrobial, anti-proliferative, anti-diabetic etc.

The plant is a rich source of vitamin B complex, gamma tocopherol, and minerals besides proteins and lipids [3]. These obtained methanolic extract of nut shell and husk, lyophilized it, determined the total phenolic and flavonoid contents and carried out anti-oxidant, anti-proliferative and antibacterial activities [4,5]. The prepared nut shell extrusion of *Carya illinoinensis* and carried out anti-oxidant activity [6]. As reported the presence of phenolic contents in leave extract if *Carya illinoinensis* and demonstrated their anti-oxidant and hepato-protectrive activities [7]. As reported two new flavonol methyl ethers- caryatin-3' sulfate and caryatin-3' methyl ether-7-O-β-d-glucoside from the butanol extract of *Carya illinoinensis* bark [8]. These compounds besides other known isolated phenolic compounds were investigated for their anti-diabetic activity. Novel compound caryatin-3' methyl ether-7-O-β-d-glucoside appeared to have potential aldose reductase inhibitory activity [9]. The determined antimicrobial activity of aqueous and ethanolic extract of *Carya illinoinensis* leaves which contained phenolics, flavonoids and tannins [3].

Recently, reported that development of a simple and efficient method for the analysis of flavonoids and tri-terpenoid glycosides based on HPTLC as well as its validation [10]. This article has been written to analyse their efforts critically along with contemporary works in this area that may be fruitful for further research on *Carya illinoinensis*.

Correspondence to: Md. Sarfaraj Hussain, Department of Pharmacognosy and Phytochemistry, Lord Buddha Koshi Pharmacy College, Baijanathpur, Bihar, India, E-mail: sarfarajpharma@gmail.com

Received: 28-Oct-2022, Manuscript No. JCGST-22-19869; **Editor assigned:** 31-Oct-2022, PreQC No. JCGST-22-19869 (PQ); **Reviewed:** 14-Nov-2022, QC No. JCGST-22-19869; **Revised:** 22-Nov-2022, Manuscript No. JCGST-22-19869 (R); **Published:** 30-Nov-2022, DOI: 10.35248/2157-7064.22.S1.003.

Citation: Hussain MDS, Hassan MZ (2022) Development of HPTLC Method for the Analysis of Flavonoids and Tri-terpenoid Glycoside Isolated from Carya Illinoinensis Bark and their Biological Activity: A Brief Report, J Chromatogr Sep Tech. S1:003.

MATERIALS AND METHODS

Extraction, isolation and characterization

Fresh bark of *Carya illinoinensis* was subjected to continuous hot percolation using ethanol as solvent. Ethanolic extract was dried and column chromatography was performed to isolate different fractions. CI-01 was obtained from one of the eleven fractions as light yellow crystalline compound (Figure 1). The compound was characterized using 1H-NMR, 13C-NMR, mass spectra and proposed to have chemical formula $C_{21}H_{40}O_8$. The compound CI-01 was identified as Farnasonoic acid α-1 glucoside, which has not been reported earlier from this plant. On the bases of data from NMR and fragmentation pattern of the compound, the chemical structure of the compound has been established.

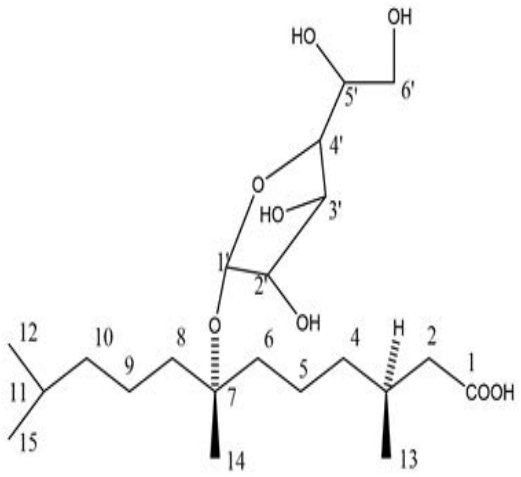

Figure 1: Proposed structure of compound CI-01.

HPTLC is an important separating technique particularly useful for isolating components of herbal extract and nowadays used for fingerprinting of plant species and varieties. As developed a simple HPTLC method for the ethanolic extract using chloroform-methanol (90:10) as mobile phase and standard Quercetin as marker. The developed chromatogram exhibited ten peaks distributed over three peak regions that can be marked as 'A', 'B' and 'C' depending on distribution and resolution (Figure 2). Although, used ethanolic extract along with quercetin, there is no mention of isolated principle CI-01. It could have also been incorporated for ease of identification. The chromatogram showed four peaks in region 'A' with Rf value below 0.30. Peak '1' and '4' are prominent however peak '2' and '3' are not quiet resolved. Another five peaks can be observed distributed in region "B' and show poor degree of resolution. This region contains peak '8' for quercetin which could be easily verified with the similar peak for standard quercetin chromatogram. Region 'C' has well resolved single peak 10. Peak 9 in region 'B' might be compound CI-01 since the eluent used for the isolation of CI-01 and the mobile phase used for developing chromatogram have identical composition which could have been justified if the isolated pure compound was also used as second marker in HPTLC [11,12].

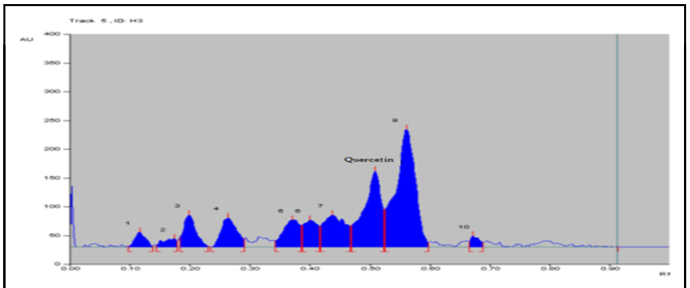

Figure 2: Chromatogram of CI extract.

Modern and essential tools like Supercritical fluid and flash chromatography could have been employed for the better isolation of various fractions from ethanolic extract over traditional column chromatography which is time consuming, hazardous and less eco-friendly. Supercritical fluid chromatography which uses liquefied CO_2 as mobile phase to extract a particular component based on its solubility at a particular temperature and pressure. By varying the temperature and pressure, pure components can be easily isolated from plant extracts. This would have helped in developing better HPTLC profile of the ethanolic extract of *Carya illinoinensis* [10].

Biological studies

Authors have performed and exhibited a new biological activity of ethanolic extract of *Carya illinoinensis* which has not been reported earlier. Anti-inflammatory activity along with analgesic activities was carried out and suggests that CI-01 has the potential to become potential lead compound. The study suggests that compound CI-01 has much better activity as compared to the standard NSAID drug indomethacin. Even the ethanolic extract at a dose of 100 and 200 mg per kg body weight showed better activity than indomethacin (5 mg/kg). However, this cannot be concluded that ethanolic extract is better than indomethacin in preventing inflammatory response. Similarly, this can also be not inferred from the data that the compound CI-01 is better than indomethacin in preventing anti-inflammatory response even though the compound exhibited better response than indomethacin at a dose of 10 mg/kg body weight. A careful observation of data from anti-inflammatory activity further suggests that the drug response is not directly dose dependent and might involve other mechanisms as well [13, 14].

The fact is further supported by in silico study performed on various targets associated with inflammatory response. Analgesic activity of the drugs was determined by acetic acid induced writhing using diclofenac as standard drug. Data provided could not be analyzed due inaccessibility to the supplementary data link. One important aspect noticed was that none of the COX-2 drugs/standards were included in the study while the data from in silico studies suggest COX-2 mediated anti-inflammatory and analgesic responses of the ethanolic extract as well as CI-01. Another study involving inhibition of COX-2 might help in confirming the mechanism of action of the drug.

Acute toxicity study of the isolated active principle CI-01 has been carried out in accordance with the guidelines laid by OECD and suggests that the compound has good safety profile

with no toxic effects on vital organs. The compound resulted in no mortality in any of the group of animals under study. Since most of the NSAIDs have unpleasant side effects such as gastric irritation, study related to this should also be included for the safety of the compound CI-01.

RESULTS AND DISCUSSION

Molecular docking and simulation studies

Their work has been further supported by *in silico* studies which included molecular docking followed by molecular docking simulation study. Results obtained from *in vivo* and *in silico* studies not only favour and justify their biological role in inflammation and pain but also suggest their targets and mechanism of action. Molecular docking study of the drug CI-01 was performed on various targets associated with inflammatory and analgesic response such as s COX-1, COX-2, iNOS, TNFα and μ-opioid receptors. The study supported the probable role of COX 2, iNOS and μ-opioid receptors for the activity based on respective docking scores and interactions with different residues in the receptor binding sites.

One important aspect with respect to molecular docking study is the inclusion of standard drugs such as ibuprofen or celecoxib or protein bound ligands, which should have been taken care of with each target for a better comparative study. Nowadays drug design and development is incomplete without the use of MD Simulation, an essential tool and technique to determine the stability of the interaction with the target receptor under physiological conditions. The work involved MD simulation study for 50 ns on COX-2 receptor to understand the kinetics and thermodynamics of the biological system with the isolated compound CI-01. This study gives an insight that the compound remained in close contact with the target protein over specified time which further supports the role of CI-01 as anti-inflammatory agent through COX-2 inhibition. But since the data from molecular docking study also support the involvement of iNOS receptor, MD simulation study should have been carried out for the isolated compound bound with iNOS receptor as well to rule out exact mechanism of action of the compound under study [15, 16].

CONCLUSION

The work report has laid down another milestone by deciphering a completely independent study on isolation of a new active principle CI-01 from the bark of Carya illinoinensis Plant and its biological activity. Since most of the NSAIDs have unpleasant side effects, study related to gastric irritation could also be performed to further establish the safety of the compound CI-01. The isolated active principle CI-01 identified as Farnesoic acid α-L-glucoside might act as natural lead compound in future to design and develop a new class of anti-inflammatory and analgesic agents.

ACKNOWLEDGMENT

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding of this work through Small Groups Project under grant Number (RGP. 1/218/43).

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Azam F, Abodabos HS, Taban IM, Rfieda AR, Mahmood D, Anwar MJ, et al. Rutin as promising drug for the treatment of Parkinson's disease: An assessment of MAO-B inhibitory potential by docking, molecular dynamics and DFT studies. Mol Simul. 2019;45(18):1563-1571.
2. Wakeling JM, Pascual SA, Nigg BM, Tscharner V. Surface EMG shows distinct populations of muscle activity when measured during sustained sub-maximal exercise. Eur J Appl Physiol. 2001;86(1): 40-47.
3. Bottari NB, Lopes LQ, Pizzuti K, dos Santos Alves CF, Corrêa MS, Bolzan LP, et al. Antimicrobial activity and phytochemical characterization of *Carya illinoensis*. Microb Pathog. 2017;104:190-195.
4. Villasante J, Girbal M, Metón I, Almajano MP. Effects of pecan nut (*Carya illinoiensis) and roselle flower (Hibiscus sabdariffa) as antioxidant and antimicrobial agents for sardines (Sardina pilchardus)*. Molecules. 2018;24(1):85.
5. Gad HA, Ayoub NA, Al-Azizi MM. Phenolic constituents with promising antioxidant and hepatoprotective activities from the leaves extract of Carya illinoinensis. Nat Prod Indian J. 2007;3:151-158.
6. Abdallah HM, Salama MM, Abd-Elrahman EH, El-Maraghy SA. Antidiabetic activity of phenolic compounds from Pecan bark in streptozotocin-induced diabetic rats. Phytochem Lett. 2011;4(3): 337-341.
7. Rábago-Panduro LM, Morales-de la Peña M, Romero-Fabregat MP, Martín-Belloso O, Welti-Chanes J. Effect of pulsed electric fields (PEF) on extraction yield and stability of oil obtained from dry pecan nuts (*Carya illinoinensis (Wangenh. K. Koch)*). Foods. 2021;10(7):1541.
8. Hussain MS, Azam F, Ahamed KN, Ravichandiran V, Alkskas I. Anti-endotoxin effects of terpenoids fraction from Hygrophila auriculata in lipopolysaccharide-induced septic shock in rats. Pharm Biol. 2016;54(4):628-636.
9. Hussain MS, Azam F, Eldarrat HA, Alkskas I, Mayoof JA, Dammona JM, et al. Anti-inflammatory, analgesic and molecular docking studies of Lanostanoic acid 3-O-α-D-glycopyranoside isolated from Helichrysum stoechas. Arabian J Chem. 2020;13(12): 9196-9206.
10. Hussain MS, Azam F, Mezogi J, Enwij FA, Benhusein GM, Haque A, et al. A simple validated HPTLC method for the analysis of flavonoids and molecular docking studies of novel tri-terpenoid glycoside isolated from *Carya illinoinensis bark with potential anti-inflammatory and antinociceptive activities*. South African J Bot. 2022;147:596-607.
11. Hussain MS, Fareed S, Ansari S, Rahman MA, Ahmad IZ, Saeed M. Current approaches toward production of secondary plant metabolites. J Pharm Bioallied Sci. 2012;4(1):10.
12. Hussain MS, Fareed S, Ali M. Hyphenated chromatographic analysis of bioactive gallic acid and quercetin in Hygrophila auriculata (K.

Schum) Heine growing wildly in marshy places in India by validated HPTLC method. Asian Pacific J Trop Biomed. 2012;2(2):S477-83.

13. Mukherjee PK, Saha K, Das J, Pal M, Saha BP. Studies on the anti-inflammatory activity of rhizomes of Nelumbo nucifera. Planta medica. 1997;63(04):367-369.
14. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. 2020;83(3):770-803.
15. Azam F. Elucidation of teicoplanin interactions with drug targets related to COVID-19. Antibiotics. 2021;10(7):856.
16. Azam F. Ginger components as anti-Alzheimer drugs: Focus on drug design. Neuroprotective effects of phytochemicals in neurological disorders. 2017:149.