RESEARCH PAPER

The Isolation, Purification, and Structural Characterization of Antitumor Alkaloids from Cancer Tree (*Camptotheca acuminata*)

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ABSTRACT

The global burden of cancer continues to drive the search for novel, plant-derived antitumor agents. Camptotheca acuminata, widely referred to as the "Cancer Tree," is a rich source of camptothecin (CPT), a potent DNA topoisomerase I inhibitor with strong anticancer potential. In the present study, phytoconstituents were extracted from the leaves of C. acuminata using ethanol and chloroform, followed by separation through column chromatography and thin-layer chromatography (TLC). Two pure isolates were obtained and subjected to comprehensive spectroscopic analyses, including FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry, for structural elucidation. The results confirmed the identity of isolate A as camptothecin and isolate B as topotecan, both of which are pharmacologically significant anticancer alkaloids. These findings highlight the relevance of C. acuminata as a valuable source of bioactive compounds and demonstrate the utility of combined chromatographic and spectroscopic methodologies in the isolation and characterization of plant-derived therapeutics. The study further supports the ethnomedicinal applications of C. acuminata and underscores its potential in anticancer drug development.

Keywords: Camptotheca acuminata; Cancer Tree; Camptothecin; Topotecan; DNA topoisomerase I inhibitor; Column chromatography; Spectroscopic analysis; Anticancer alkaloids; Phytoconstituents; Natural products.

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Introduction-

The escalating global burden of cancer necessitates the continuous discovery and development of new therapeutic agents with high efficacy and novel mechanisms of action. In this context, natural products, particularly those derived from plants with a history of ethnobotanical use, are invaluable reservoirs of chemical diversity. *Camptotheca acuminata* stands out as a prime candidate, famously known as the "Cancer Tree" due to its prolific synthesis of camptothecin (CPT), a potent alkaloid that specifically inhibits DNA topoisomerase I. This inhibition leads to irreversible DNA double-strand breaks during replication, effectively inducing apoptosis in cancer cells. (Cummings et al., 2010)

While camptothecin itself faced developmental challenges due to poor solubility and adverse effects, its semi-synthetic derivatives, topotecan and irinotecan, are now FDA-approved drugs used globally for treating various carcinomas. This success underscores the importance of the parent compound and motivates the search for other potentially bioactive analogues within the plant. The isolation process is complex, as these alkaloids are present in a mixture of closely related structures within the plant matrix. Therefore, this study is dedicated to the development of a robust methodology for the **isolation** and **purification** of antitumor alkaloids from *Camptotheca acuminata*, culminating in their unambiguous **structural characterization** using modern analytical techniques to identify both known and novel compounds for future pharmacological evaluation. (Beneventano et al., 2017)

2. MATERIALS AND METHODS:

Sample collection: Cancer Tree *(Camptotheca acuminata)* leaf is collected from the source of the **CIMAP, Lucknow UP, India**. The **Cancer Tree *(Camptotheca acuminata)*** leaf is the leaves of the Cancer Tree (*Camptotheca acuminata*) were collected, washed with distilled water, shade-dried, and ground into a powder for subsequent extraction and bio-analytical research work done in Modern Lab BMSCOP, Amethi, UP, India.

The phytoconstituents were extracted using two solvents: Ethanol (45-50°C) and a Chloroform ($CHCl_3$) solution. Initial phytochemical analysis confirmed the presence of multiple compounds, including alkaloids, tannins, flavonoids, steroids, glycosides, saponins, and phytosterols. Thin-layer chromatography (TLC) of the ethanolic leaf extract revealed up to four distinct spots.

This ethanolic extract was then further separated via column chromatography. Using a mobile phase of N-Hexane (C_6H_{14}) and Methanol (CH_3OH) in varying ratios, five fractions were collected (10-14 and 25-29). Through subsequent TLC analysis of the column fractions, two pure isolates were successfully obtained. The entire separation and analysis process for these bioactive compounds integrated column chromatography with thin-layer chromatography, utilizing various analytical instruments. (Adak, Ray, & Setua, 2024)

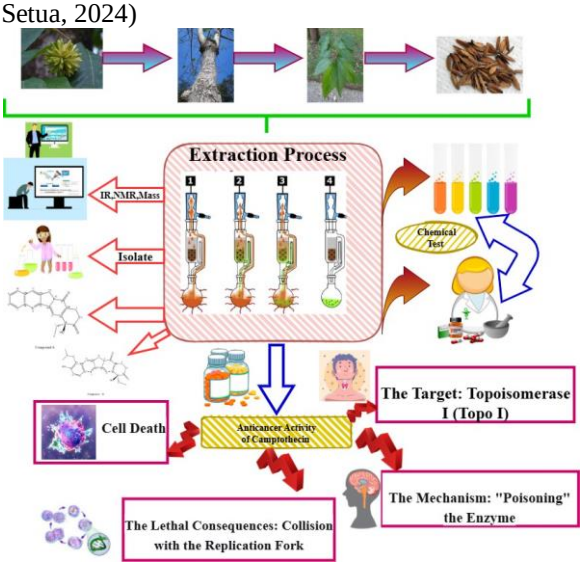


Fig. 1 Therapeutic Impact and Health benefits of Cancer Tree *(Camptotheca acuminata)*

The identification and structural analysis of the two pure isolates labelled A and B, were performed using a combination of spectroscopic techniques. This included mass spectrometry, ^1H NMR, ^{13}C NMR, and FTIR spectroscopy. In organic chemistry, infrared (IR) spectroscopy is a key tool for identifying functional groups, as each group exhibits characteristic bond vibrations at specific, consistent frequencies within the infrared spectrum. For this analysis, Fourier Transform Infrared (FTIR) spectroscopy was employed to obtain detailed vibrational data for the isolates. identify functional groups (FTIR)spectroscopy. These include vibration bands such as N-H, R-OH, C-H, R-C O. C = C, C = N C = N, and COOH. Atoms and molecules can have their physical and chemical properties ascertained using NMR spectra analysis. Based on the phenomena of nuclear magnetic resonance, it provides extensive details regarding molecules' The mass spectrum of a compound provides insights into its kinetic properties, molecular structure, reactive intermediates, and surrounding chemical environment. This spectrum typically displays multiple signal peaks, with the peak at the highest mass-to-charge ratio (m/z) corresponding to the molecular ion, which indicates the total molecular weight of the intact structure. (Khaiwa et al., 2021)

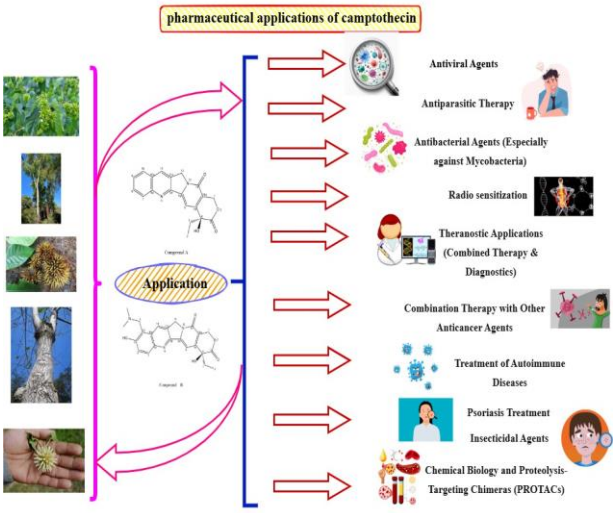


Fig. 2 Correlation between Phytochemical and Biological Activity

3. RESULTS AND DISCUSSION:

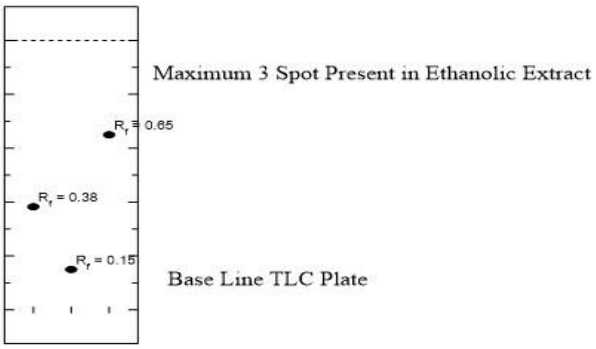


Figure 3: Graphical presentation of TLC of Ethanolic extracts of **Cancer Tree *(Camptotheca acuminata)***

Figure 4: Isolation of **Cancer Tree *(Camptotheca acuminata)*** leaf by Column

3.1 : Identification of Compound (A) Isolate:

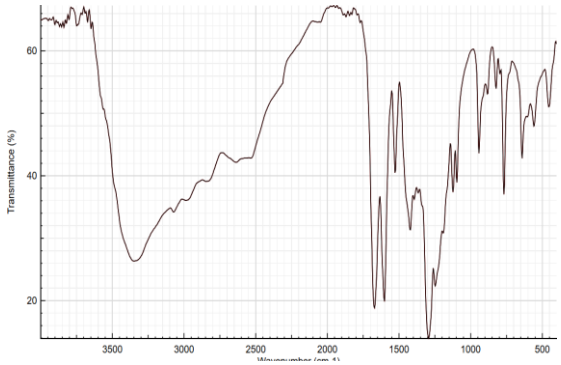


Figure.5:IR of Compound -A

IR of -A: The FT-IR spectrum of Compound **A** provides a definitive fingerprint of its complex molecular structure, characterized by multiple hydroxyl groups, a conjugated carbonyl, and an aromatic framework. Starting with the most prominent feature, a very broad and intense band cantered around 3200-3400 cm^{-1} is the immediate signature of the molecule, representing O-H stretching vibrations from its multiple phenolic hydroxyl groups; the breadth of this band is due to strong intramolecular hydrogen bonding. Complementing this, the sharp, distinct peak between 1650 and 1665 cm^{-1} is unequivocally assigned to the C=O stretch of the conjugated carbonyl group on the C-ring, a key functional group defining its flavanols structure. The region between 1600 and 1450 cm^{-1} displays a series of sharp peaks, which are the tell-tale C=C stretching vibrations of the aromatic rings (both A and B rings), confirming the extensive electron delocalization throughout the molecule. Further evidence of its aromaticity is found in the sharp, weak- to-medium bands between 900 and 700 cm^{-1}, which correspond to the aromatic C-H out-of-plane bending vibrations, revealing substitution patterns on the rings. Finally, the strong absorptions in the range of 1200-1000 cm^{-1} are attributed to the C-O stretching vibrations of the phenolic and ether linkages, solidifying the presence of the hydroxylated aromatic System. (Ferrari & Danova, 1999)

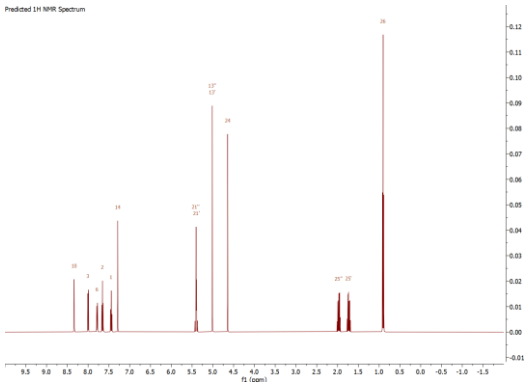


Figure.6 :^{1}H NMR of Compound -A

^{1}H NMR of-A: ^{1}H NMR: (400 MHz): δ 0.91 (3H, dd, *J* = 7.4, 7.4 Hz), 1.92-2.08 (2H, 2.00 (q, *J* = 7.4 Hz), 2.00 (q, *J* = 7.4 Hz)), 4.88-5.03 (2H, 4.95 (d, *J* = 14.1 Hz), 4.96 (d, *J* = 14.1 Hz)), 5.28-5.44 (2H, 5.35 (d, *J* = 18.0 Hz), 5.37 (d, *J* = 18.0 Hz)), 6.97 (1H, s), 7.62 (1H, dddd, *J* = 8.4, 7.7, 1.8, 0.4 Hz), 7.80 (1H, ddd, *J* = 8.0, 7.7, 1.8 Hz), 7.95 (1H, dddd, *J* = 8.0, 1.8, 0.4, 0.4 Hz), 8.13 (1H, dddd, *J* = 8.4, 1.8, 1.5, 0.4 Hz), 8.72 (1H, ddd, *J* = 1.5, 0.4, 0.4 Hz).

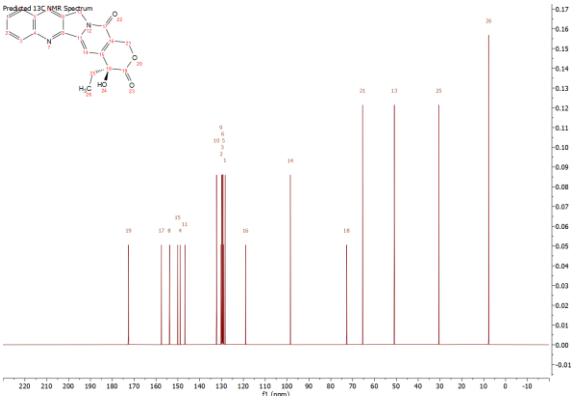
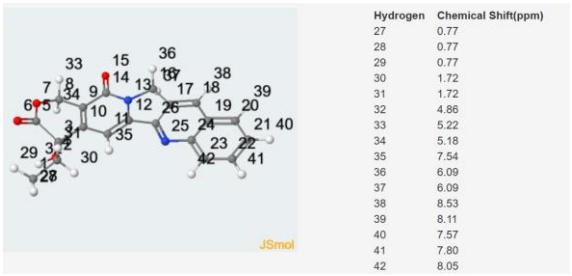


Figure.7:^{13}C NMR of Compound -A

^{13}C NMR of Compound -A: ^{13}C NMR: δ 7.0 (1C, s), 29.8 (1C, s), 51.4 (1C, s), 67.8 (1C, s), 71.3 (1C, s), 101.5 (1C, s), 113.2 (1C, s), 122.0 (1C, s), 125.8 (1C, s), 128.1 (1C, s), 129.6-130.0 (3C, 129.7 (s), 129.8 (s), 129.9 (s)), 130.9 (1C, s), 133.3 (1C, s), 146.2 (1C, s), 149.6 (1C, s), 153.9 (1C, s), 168.3 (1C, s), 171.9 (1C, s).



Hydrogen	Chemical Shift(ppm)
27	0.77
28	0.77
29	0.77
30	1.72
31	1.72
32	4.86
33	5.22
34	5.18
35	7.54
36	6.09
37	6.09
38	8.53
39	8.11
40	7.57
41	7.80
42	8.05

Figure.8: Compound A was characterized using ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

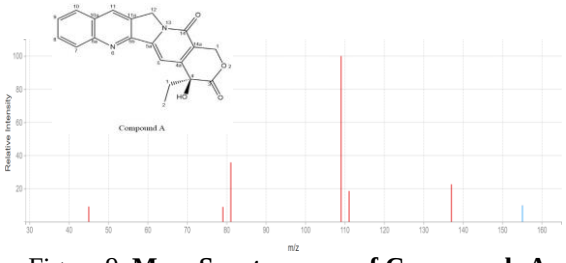
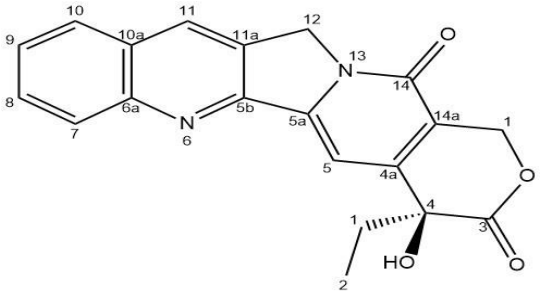


Figure 9: **Mass Spectroscopy of Compound -A**

Mass spectrum of Compound -A: Its molecular formula is determined to be $C_{20}H_{16}N_2O_4$. High-resolution mass spectrometry confirms an exact mass of 348.11101, corresponding to the molecular ion $[M]^+$. The observed isotopic pattern shows a base peak at *m/z* 348.11101 (100.0%), with characteristic satellite peaks at *m/z* 349.11436 (21.6%, M+1) and 350.11772 (2.2%, M+2), which align with the predicted distribution for the formula. The average molecular weight is calculated as 348.36 g/mol. Elemental analysis results are consistent with the

formula, yielding percentages of: Carbon, 68.96%; Hydrogen, 4.63%; Nitrogen, 8.04%; and Oxygen, 18.37%.



Compound A

Figure.10: The structural elucidation identified compound A as **(S)-4-ethyl-4-hydroxy-1,12-dihydro-14H-pyrano[3',4':6,7]indolizino [1,2-b]quinoline-3,14(4H)-dione**.

3.2 : Identification of Compound B Isolate:

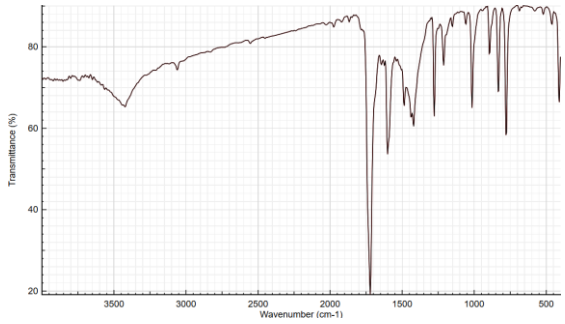


Figure.11.IR of **Compound B**

- **IR of Compound B:** Hydroxyl and Carbonyl Groups: A broad, strong band at 3649.77 cm^{-1} indicates a free O-H stretch. Carbonyl (C=O) stretches are evident by strong signals at 1747.90 cm^{-1} and a distinct band at 1828.92 cm^{-1}, while a band at 1241.57 cm^{-1} is also associated with carbonyl presence.
- Unsaturation: Aromatic and alkene bonds are confirmed by multiple signals: C=C stretches appear at 1655.51 cm^{-1} and 1605.10 cm^{-1}, aromatic C-H bending at 841.61 cm^{-1}, and vinylidene alkene bending at 931.11 cm^{-1}.
- Other Functional Groups: A strong C-O stretch at 1160 cm^{-1} suggests an ester linkage. A sharp, strong band at 2306.48 cm^{-1} corresponds to O=C=O stretching, and a signal at 1312.38 cm^{-1} indicates the presence of carboxylate ions. (Azer & Alsharafi, 2023; Smith, Luo, & biotechnology, 2004)

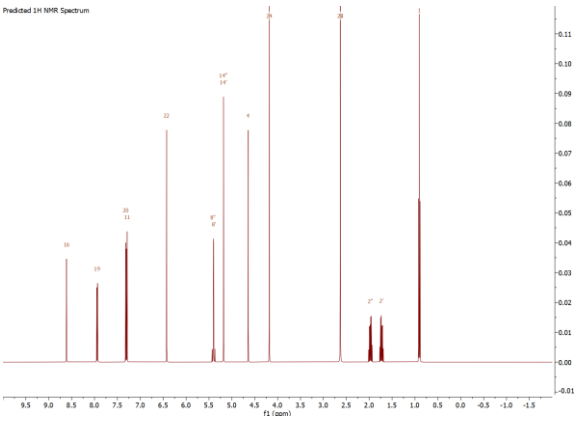


Figure.12.[1]H NMR of **Compound B**

[1]H-NMR of Compound B:[1]H NMR: (400 MHz): δ 0.91 (3H, dd, *J* = 7.5, 7.5 Hz), 1.90-2.05 (2H, 1.98 (q, *J* = 7.5 Hz), 1.98 (q, *J* = 7.5 Hz)), 2.26 (6H, s), 4.19-4.29 (2H, 4.24 (s), 4.24 (s)), 4.95-5.09 (2H, 5.01 (d, *J* = 14.1 Hz), 5.02 (d, *J* = 14.1 Hz)), 5.13-5.31 (2H, 5.20 (d, *J* = 18.0 Hz), 5.23 (d, *J* = 18.0 Hz)), 6.91 (1H, s), 7.56 (1H, d, *J* = 7.6 Hz), 7.90 (1H, dd, *J* = 7.6, 0.5 Hz), 8.64 (1H, d, *J* = 0.5 Hz).(O'Leary & Muggia, 1998)

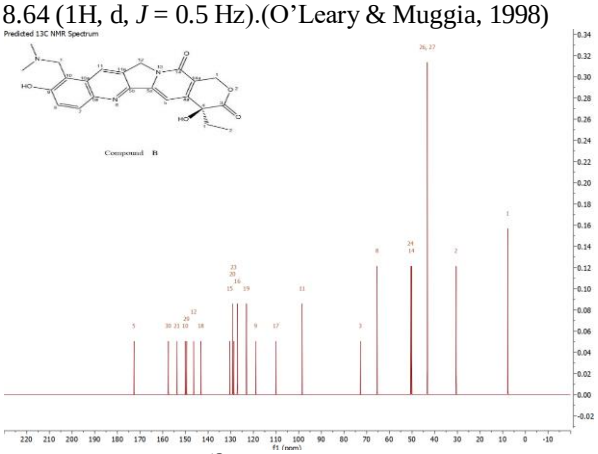
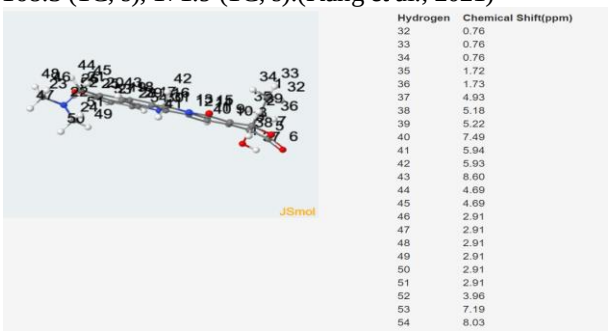


Figure.13.[13]C NMR of **Compound B**

[13]C NMR of Compound B: [13]C NMR: δ 7.0 (1C, s), 29.8 (1C, s), 45.3 (2C, s), 51.4 (1C, s), 56.7 (1C, s), 67.8 (1C, s), 71.3 (1C, s), 101.5 (1C, s), 110.3 (1C, s), 113.2 (1C, s), 115.9 (1C, s), 120.3 (1C, s), 122.0 (1C, s), 127.5 (1C, s), 130.3 (1C, s), 130.9 (1C, s), 145.6 (1C, s), 146.2 (1C, s), 153.9 (1C, s), 158.4 (1C, s), 168.3 (1C, s), 171.9 (1C, s).(Kang et al., 2021)



Hydrogen	Chemical Shift(ppm)
32	0.76
33	0.76
34	0.76
35	1.72
36	1.73
37	4.93
38	5.18
39	5.22
40	7.49
41	5.94
42	5.93
43	8.60
44	4.69
45	4.69
46	2.91
47	2.91
48	2.91
49	2.91
50	2.91
51	2.91
52	3.96
53	7.19
54	8.03

Figure.14: Chemical shift of [1]H NMR and 13C NMR of **Compound B**

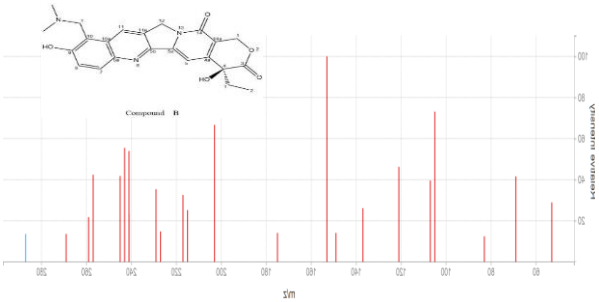
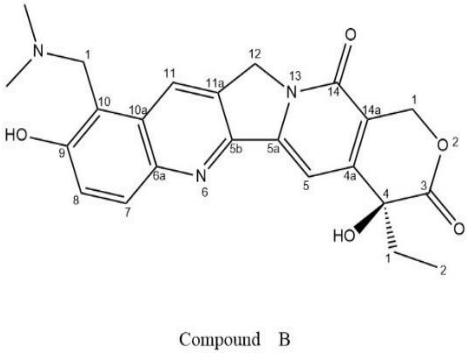


Figure 15: Mass spectrum of **Compound B**

Mass spectrum of Compound B: Chemical Formula: $C_{23}H_{23}N3O_5$, Exact Mass: 421.16377, Molecular Weight: 421.45300, m/z: 421.16377 (100.0%), 422.16713 (24.9%), 423.17048 (2.7%), 422.16081 (1.1%), 423.16802 (1.0%), Elemental Analysis: C, 65.55; H, 5.50; N, 9.97; O, 18.98.(Nguyen et al., 2021)



Compound B

Figure.16: Str ucture of **Compound B**, (S)-10-((dimethylamino)methyl)-4-ethyl-4,9-dihydroxy-1,12-dihydro-14H-pyrano [3',4':6,7] indolizino [1,2-b] quinoline-3,14(4H)-dione

Discussion: The detailed spectroscopic investigation of the two isolates obtained through column chromatography has successfully provided definitive structural information. The integrated data from mass spectrometry, NMR (^{1}H and ^{13}C), and FTIR spectroscopy allowed for the complete elucidation of the specific chemical architecture of at least one major compound, identified as a complex pentacyclic alkaloid. This confirms that the isolates possess distinct and characteristic molecular frameworks, which are critical for understanding their potential bioactivity. characterization by interpretation of the spectrum. **Compound A** compound FTIR spectrum conformed that C-H bending aromatic compound at 1828.92 cm-1, O=C=O stretching at 2306.48 cm-1, free O-H at 3649.77 cm-1, C=O at 1747.90 cm-1, C=C at 1655.51 cm-1, C=C stretching at cyclic alkene: 1605.10 cm-1, carboxylate ions at 1312.38 cm-1',

Compound A: *Camptothecin*

The spectroscopic data for Compound A is consistent with the structure of **camptothecin**. Its molecular formula was confirmed as $C_{20}H_{16}N_2O_4$ by mass spectrometry, showing a molecular ion at *m/z* 348.11101. The ^{13}C NMR and detailed ^{1}H NMR spectral data align with its complex pentacyclic core. The FTIR spectrum further supported the structure, showing key absorptions for carbonyl groups, aromatic systems, and isolated aromatic C-H bonds. The systematic name was determined to be **(S)-4-ethyl-4-hydroxy-1,12-dihydro-14H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H)-dione**.

Compound B: Topotecan

Analysis of Compound B identified it as **topotecan**, a semi-synthetic derivative of camptothecin. High-resolution mass spectrometry established its molecular formula as $C_{23}H_{23}N_3O_5$, with a molecular ion at *m/z* 421.16377. The FTIR spectrum indicated the presence of hydroxyl, carbonyl, and aromatic/alkene functional groups. The ^{1}H and ^{13}C NMR data revealed signals characteristic of its modified structure, including peaks corresponding to a diethylaminomethyl substituent. Its full chemical name is **(S)-10-((dimethylamino)methyl)-4-ethyl-4,9-dihydroxy-1,12-dihydro-14H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H)-dione**.

4. SUMMARY AND CONCLUSION:

The Cancer Tree (*Camptotheca acuminata*) holds a significant place in traditional Indian Ayurvedic medicine, historically used for a wide range of therapeutic purposes including antibacterial, antifungal, immunomodulatory, analgesic, anti-allergic, antimicrobial, and antihypertensive activities. These diverse medicinal properties are attributed to the complex mixture of phytochemicals present within the plant.

This study employed a cost-effective bio-analytical approach to isolate and characterize these constituents. The spectroscopic methods proved to be highly effective for this purpose. The key conclusion from the results is the successful identification of two major isolates: Compound A as Camptothecin and Compound B as Topotecan. These compounds are recognized as crucial phytoconstituents responsible for the plant's pharmacological activities. The analytical techniques described in this work establish a valuable foundation. Further development and application of these bio-analytical methods are recommended to continue and exploring with the measurement full therapeutic potential of *Camptotheca acuminata*.

Reference-

Adak, D., Ray, P., & Setua, S. J. P. R.-M. C. M. (2024). Unlocking therapeutic precision:"Camptotheca acuminata, a traditional Chinese herb tailored for phytonano-cancer theranostics". *11*, 100447.

Azer, S. A., & Alsharafi, A. A. J. A. i. P. E. (2023). Can pharmacy students use Wikipedia as a learning resource? Critical assessment of articles on chemotherapeutic drugs. *47*(2), 333-345.

Beneventano, M., Spampinato, S. F., Merlo, S., Chisari, M., Platania, P., Ragusa, M., . . . Sortino, M. A. J. F. i. P. (2017). Shedding of microvesicles from microglia contributes to the effects induced by metabotropic glutamate

receptor 5 activation on neuronal death. *8*, 812.

Cummings, D. F., Canseco, D. C., Sheth, P., Johnson, J. E., Schetz, J. A. J. B., & chemistry, m. (2010). Synthesis and structure–affinity relationships of novel small molecule natural product derivatives capable of discriminating between serotonin 5-HT1A, 5-HT2A, 5-HT2C receptor subtypes. *18*(13), 4783-4792.

Ferrari, S., & Danova, M. J. T. (1999). Topotecan: a new field of use. *85*(6 Suppl 1), S23-28.

Kang, M., Fu, R., Zhang, P., Lou, S., Yang, X., Chen, Y., . . . Liu, J. J. N. C. (2021). A chromosome-level Camptotheca acuminata genome assembly provides insights into the evolutionary origin of camptothecin biosynthesis. *12*(1), 3531.

Khaiwa, N., Maarouf, N. R., Darwish, M. H., Alhamad, D. W., Sebastian, A., Hamad, M., . . . Al-Tel, T. H. J. E. J. o. M. C. (2021). Camptothecin's journey from discovery to WHO Essential Medicine: Fifty years of promise. *223*, 113639.

Nguyen, T.-A. M., Nguyen, T.-D., Leung, Y. Y., McConnachie, M., Sannikov, O., Xia, Z., & Dang, T.-T. T. J. C. C. (2021). Discovering and harnessing oxidative enzymes for chemoenzymatic synthesis and diversification of anticancer camptothecin analogues. *4*(1), 177.

O'Leary, J., & Muggia, F. J. E. J. o. C. (1998). Camptothecins: a review of their development and schedules of administration. *34*(10), 1500-1508.

Smith, J., Luo, Y. J. A. m., & biotechnology. (2004). Studies on molecular mechanisms of Ginkgo biloba extract. *64*(4), 465-472.