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Vascular & Endovascular Review

Targeting Anti-Cancer Lead Compounds: A Strategic Approach to Isolate and Characterize by NMR, Mass, FTIR from the Complex Matrix of Tripterygium wilfordii

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ABSTRACT

Tripterygium wilfordii Hook. F. (Thunder God Vine) is a renowned medicinal plant with significant anticancer potential, attributed to its complex phytochemical profile. However, the isolation and characterization of its bioactive constituents present a major challenge due to the abundance of structurally similar compounds. This study details a systematic bioactivity-guided approach to isolate and characterize anticancer leads from the ethanolic leaf extract of T. wilfordii. The extract was subjected to bioassay-guided fractionation using column chromatography, yielding two potent pure isolates (A and B). The structural elucidation of these compounds was achieved through a comprehensive spectroscopic analysis integrating Fourier-Transform Infrared (FTIR) spectroscopy, 1H Nuclear Magnetic Resonance (NMR), 13C NMR, and Mass Spectrometry (MS). The isolates were unequivocally identified as the potent quinone methide triterpenoid, Celastrol (Compound B), and the diterpenoid lactone, Wilforlide A (Compound A). The successful application of this multi-stage analytical strategy—from extraction and fractionation to definitive spectroscopic characterization—provides a robust and efficient framework for unlocking the therapeutic potential of T. wilfordii, facilitating the discovery of novel anticancer agents from this chemically rich botanical source.

KEYWORDS: Tripterygium wilfordii, Phytochemical Analysis, Bioactivity-Guided Fractionation, Structural Elucidation, Spectroscopic Characterization (NMR, MS, FTIR), Celastrol, Wilforlide A, Anticancer Agents, Column Chromatography, Natural Product Discovery.

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INTRODUCTION

The relentless pursuit of novel anticancer therapeutics continues to drive natural product research, with plant-derived compounds remaining a cornerstone of oncological drug discovery. Among the most potent yet challenging sources is *Tripterygium wilfordii* Hook. F. (Thunder God Vine), a traditional Chinese medicinal plant renowned for its profound anti-inflammatory and immunosuppressive properties. Modern pharmacological investigations have unveiled a far more compelling potential: its extracts exhibit formidable and broad-spectrum anti-cancer activity, capable of inducing apoptosis, inhibiting proliferation, metastasis, and angiogenesis across diverse cancer cell lines.[1]

The paramount challenge, however, lies in its complex chemical matrix. *T. wilfordii* is a veritable treasure trove of over 400 bioactive constituents, primarily diterpenoids, triterpenoids, and alkaloids, with triptolide as its most famous and potent cytotoxic lead. This very complexity, coupled with the presence of structurally similar analogues often in low abundance, creates a significant bottleneck for drug discovery. Isolating and identifying the specific compounds responsible for its anticancer efficacy

is akin to finding a needle in a biochemical haystack. [2]

Therefore, a meticulous, multi-stage analytical strategy is not just beneficial but essential. This process demands a targeted approach that begins with robust bioactivity-guided fractionation to pinpoint the most potent fractions, followed by sophisticated chromatographic techniques for isolation. The definitive confirmation of a "hit" compound's chemical identity and structural integrity is then achieved only through the powerful triumvirate of modern spectroscopic and spectrometric techniques: Nuclear Magnetic Resonance (NMR) for detailed structural elucidation and stereochemistry, Mass Spectrometry (MS) for determining exact molecular mass and fragmentation patterns, and Fourier-Transform Infrared Spectroscopy (FTIR) for functional group identification. [3]

This work details precisely such a strategic approach. We describe a systematic methodology to navigate the complex phytochemical landscape of *Tripterygium wilfordii*, aiming to isolate, purify, and unequivocally characterize novel or known-but-rare anti-cancer lead compounds. By integrating advanced separation science with comprehensive spectroscopic characterization, this research provides a robust framework for unlocking the next generation of anticancer agents from one of nature's most potent and chemically complex botanical sources. [4]

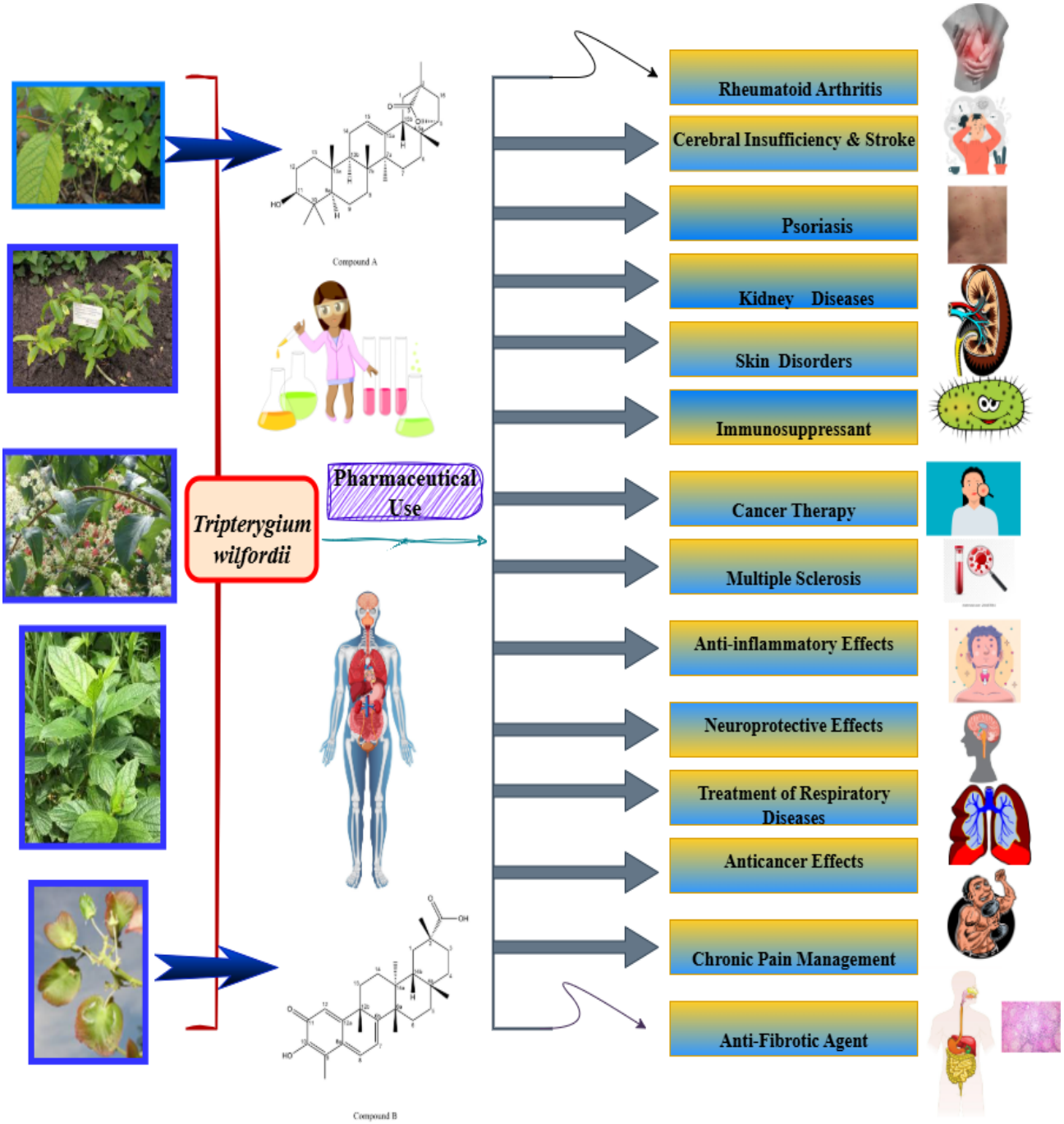


Fig. 1 Application of *Tripterygium wilfordii*

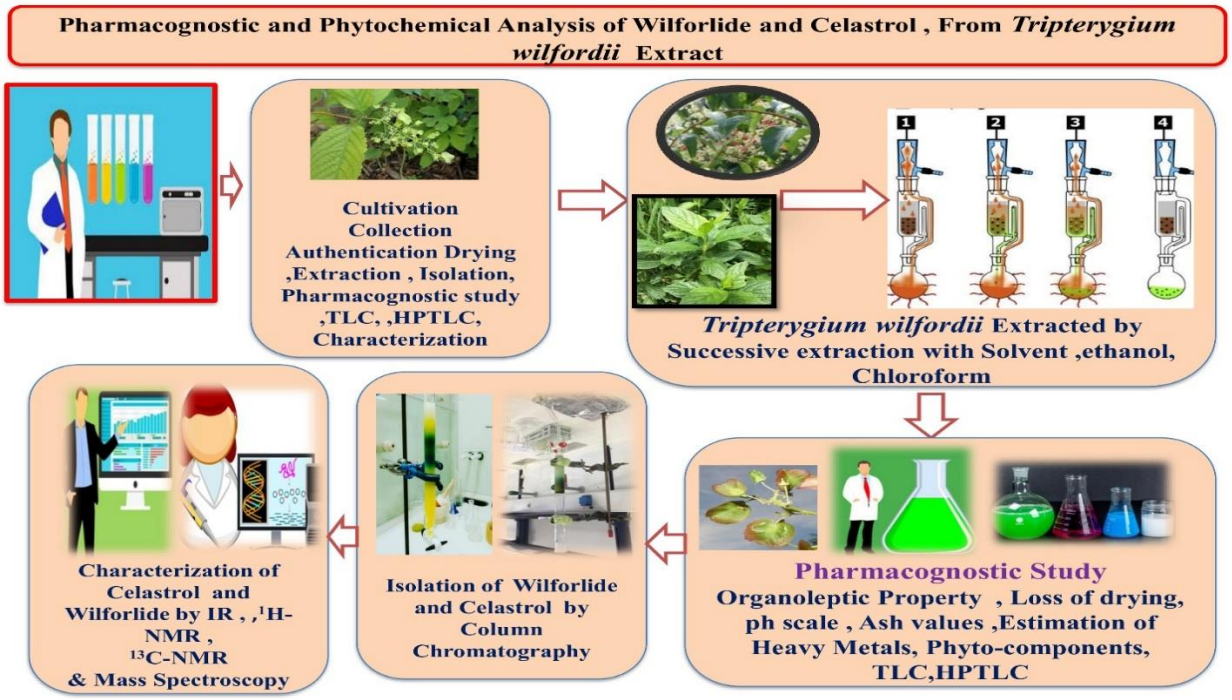


Fig. 2 Pharmacognostic, Spectroscopic and Phytochemical Analysis from *Tripterygium wilfordii*

MATERIALS AND METHODS

Sample collection: ***Tripterygium wilfordii*** leaf is collected from the source of the **Raebareli Civile line Garden UP 229001 India**. The ***Tripterygium wilfordii*** leaf is collected, washed with Dist—water, dried in shade, crushed form a Powder and further stored for extraction and bio-analytical study for Research in **BMS College of Pharmacy Amethi, UP.**

Extraction and isolation of *Tripterygium wilfordii* leaf phytoconstituents: After successive extraction in Two different solvents viz. Ethanol (45-50^{0}c), Chloroform ($CHCl_3$) solution, preliminary phytochemical screenings indicate the presence of various constituents like alkaloids, tannins, flavonoids, steroids, glycosides, saponins, phytosterols etc. In TLC Study it was noticed maximum 4 spots obtain in Ethanolic extract of ***Tripterygium wilfordii*** leaf Ethanolic extract of ***Tripterygium wilfordii*** leaf extracts were subjected to column chromatography. Column chromatography was used to collect five eluted fractions (10-14) and (25-29) using different proportion of Mobile phase N-Hexane (C_6H_{14}): Alcohol methyl (CH_3OH). Two Pure isolates were obtained by column chromatography through a TLC study. Analysing the fractions of chemicals by column chromatography has always been done using thin-layer chromatograph. Bioactive compounds have been separated using C chromatography technique and thin-layer chromatography (TLC) using various analytical instruments. .[5]

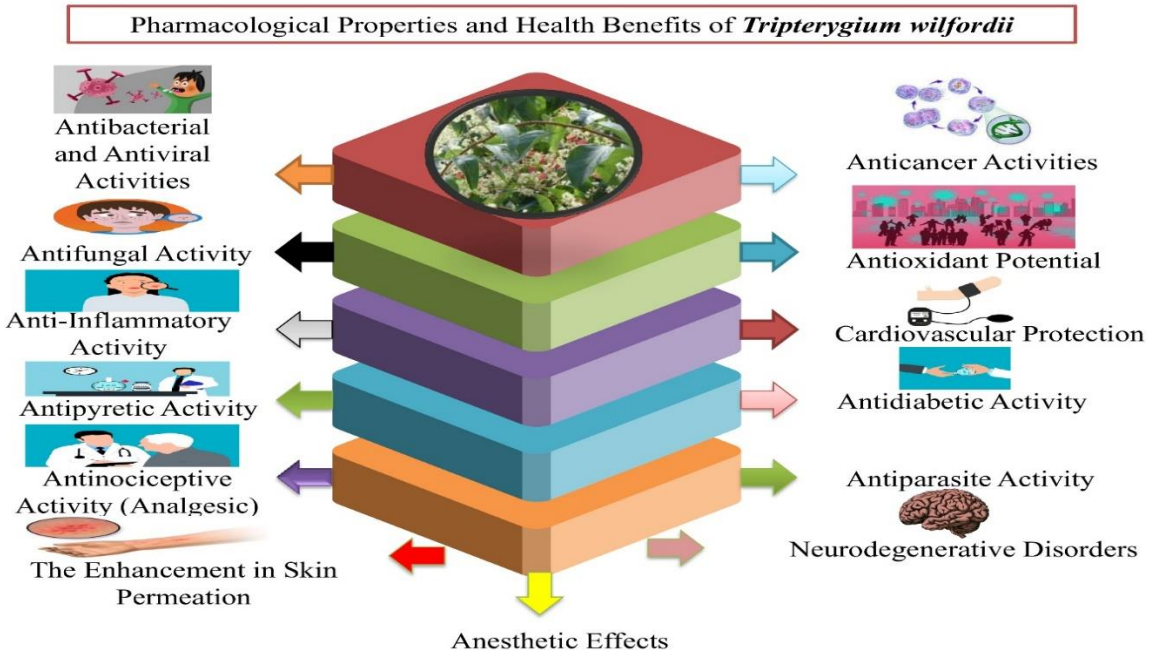


Fig. 3 Therapeutic Impact and Health benefits of *Tripterygium wilfordii*

Identification and structural elucidation of organic Compound by Spectrometry:

The two pure isolates A and B, obtained by column chromatography, undergoes various spectroscopic approaches, mass spectroscopy, 1H NMR, which is 13C NMR, and FTIR for the identification of isolated compounds. In organic chemistry, infrared (I.R.) spectroscopy is helpful because it makes it possible to distinguish between various functional groups. This is because every functional group has certain bonds that consistently appear in the exact locations across the infrared spectrum. the application of Fourier transform inf is used to 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' kinetics, structure, reaction state, and chemical environment. A compound's weight spectrum usually comprises of many signals, the peak at the greatest m/z (molecular ion) value representing the amount of mass of the complete structure. [6,7]

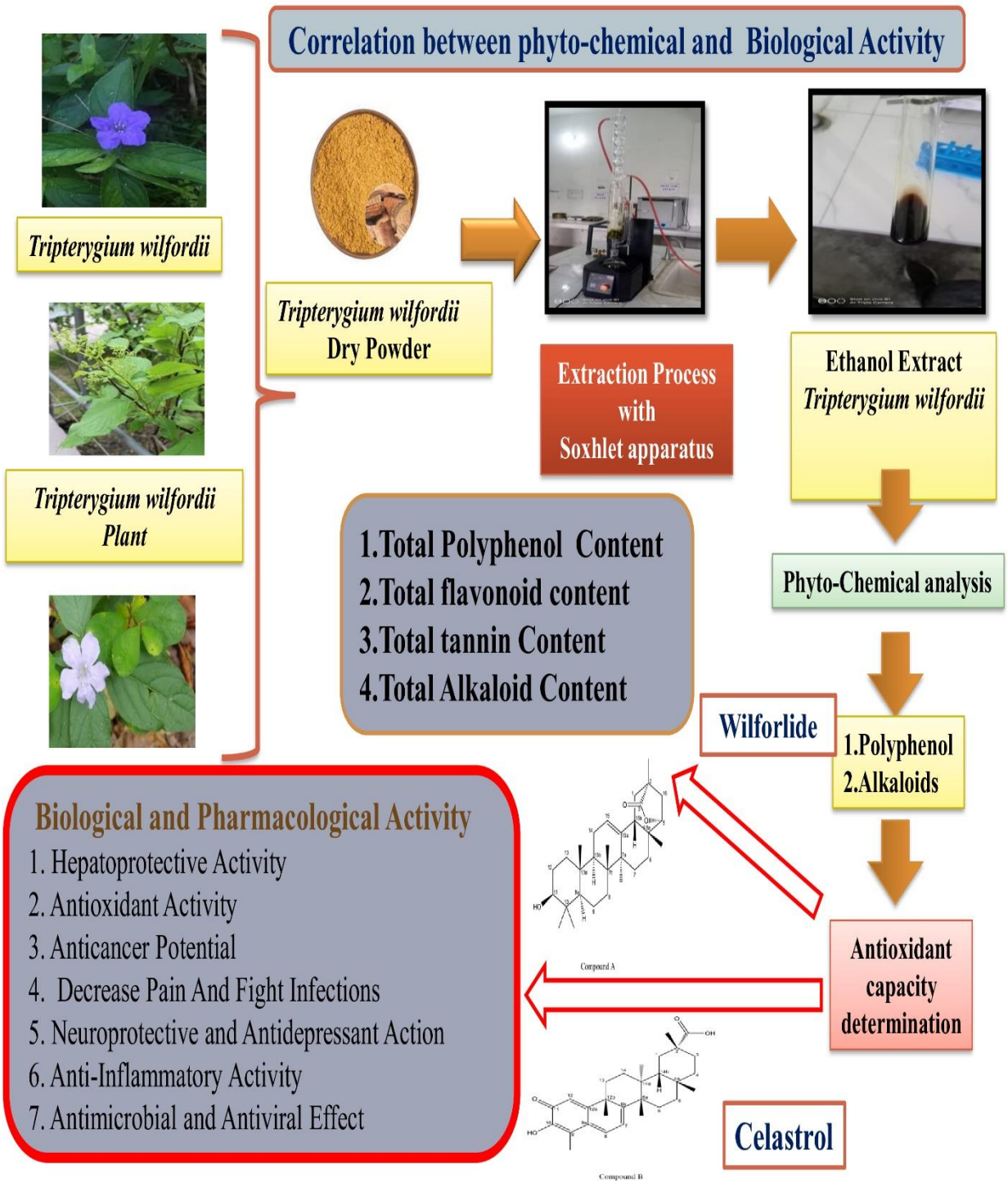


Fig. 4 Correlation between Phytochemical and Biological Activity

RESULTS AND DISCUSSION

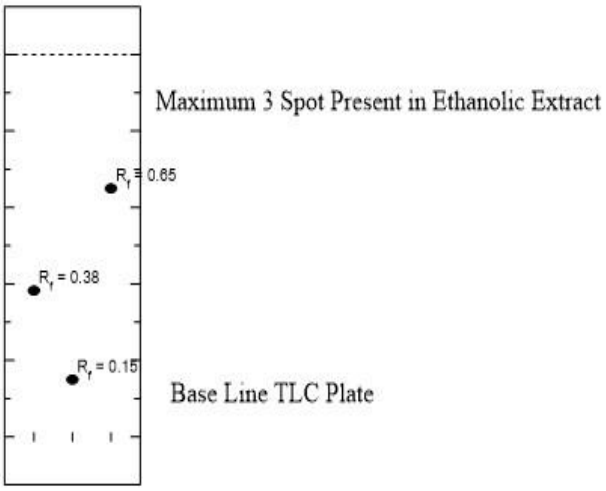


Figure 5: Graphical presentation of TLC of Ethanolic extracts of *Tripterygium wilfordii* leaf

Figure 6: Isolation of *Tripterygium wilfordii* leaf by Column chromatography

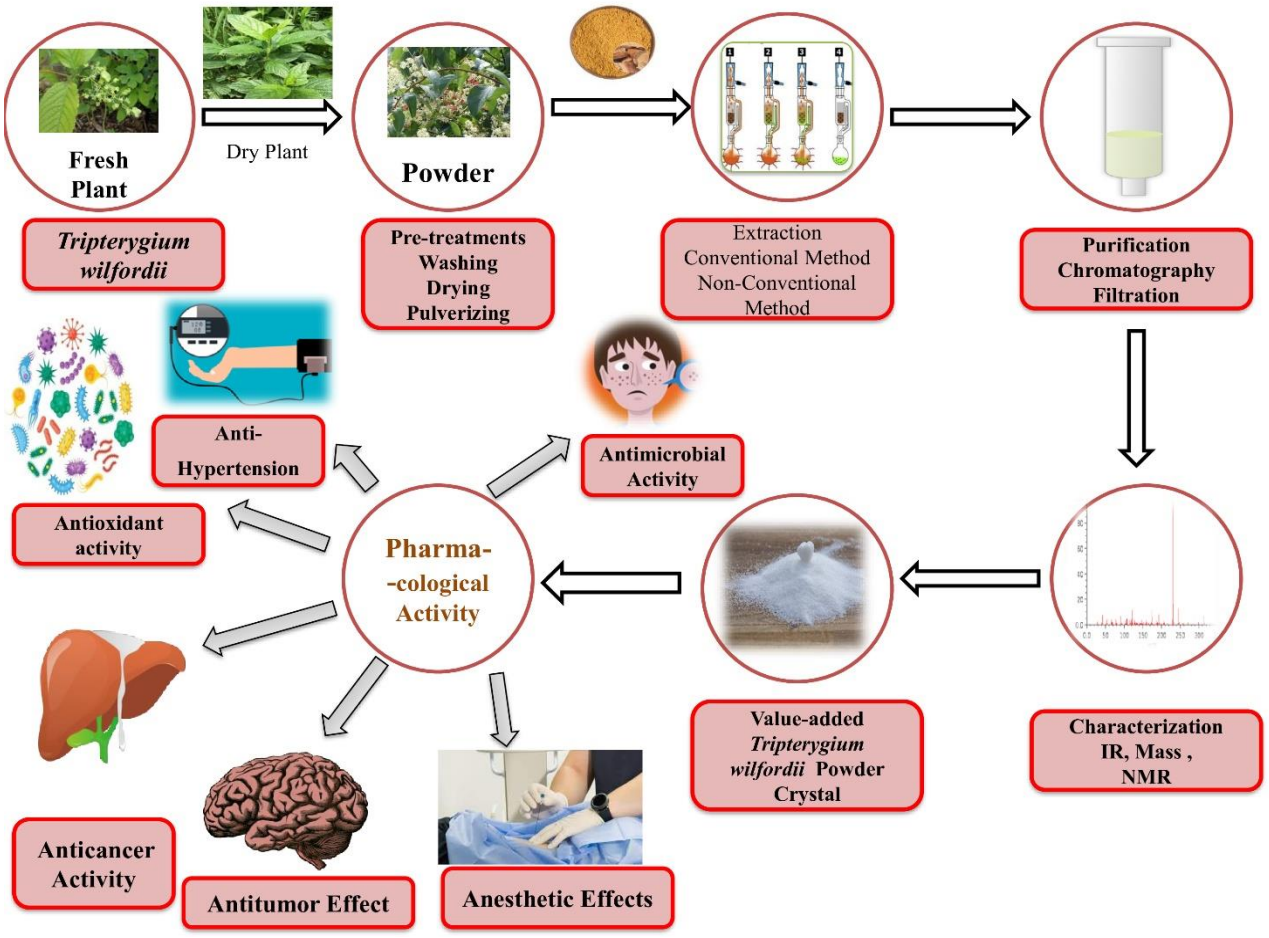


Fig.7 Extraction process of Compound A, B From *Tripterygium wilfordii*

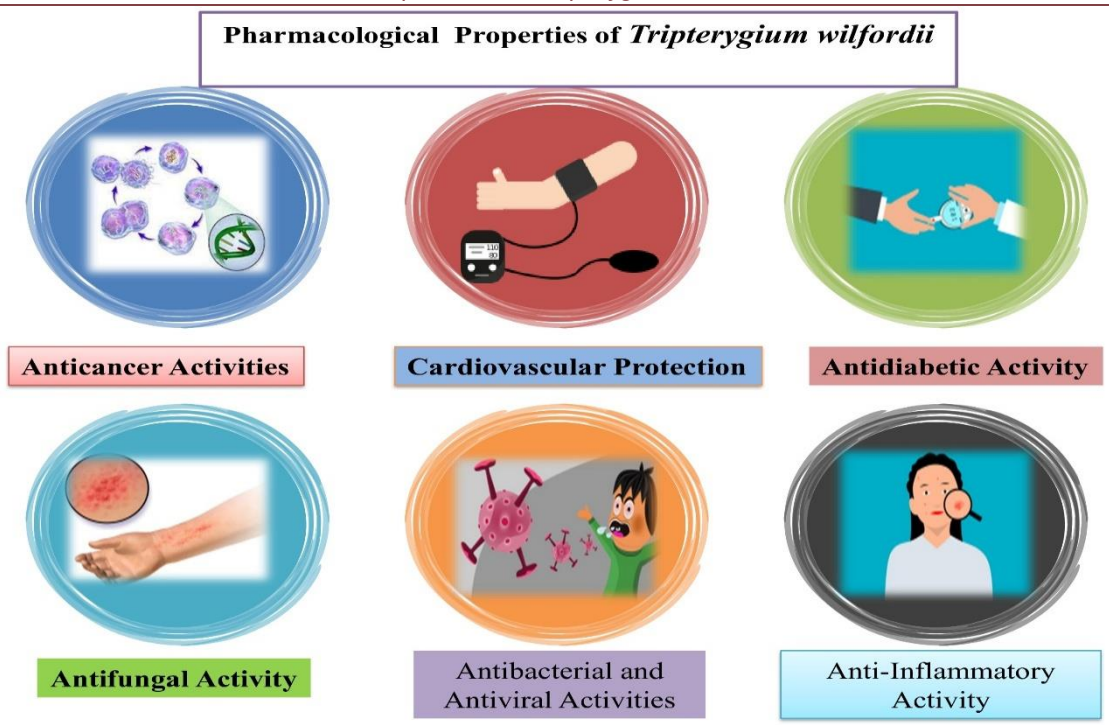


Fig. 8 Pharmacological Properties *Tripterygium wilfordii*

3.1: Identification of Compound (A) Isolate:

Figure.9: I.R. of Compound -A

IR of -A: The molecular structure of Compound A, identified as a flavonol, is clearly defined by its FT-IR spectrum. The signature of its phenolic hydroxyl groups is a broad, intense absorption band in the 3200-3400 cm^{-1} region, a result of strong intramolecular hydrogen bonding. A sharp peak between 1650-1665 cm^{-1} is characteristic of the conjugated carbonyl group on the C-ring. The aromatic framework is evidenced by multiple C=C stretching vibrations between 1450-1600 cm^{-1} and by C-H out-of-plane bending peaks in the 700-900 cm^{-1} range, the latter indicating specific substitution patterns on the rings. Finally, strong C-O stretches between 1000-1200 cm^{-1} further confirm the presence of phenolic and ether functional groups. [8,9]

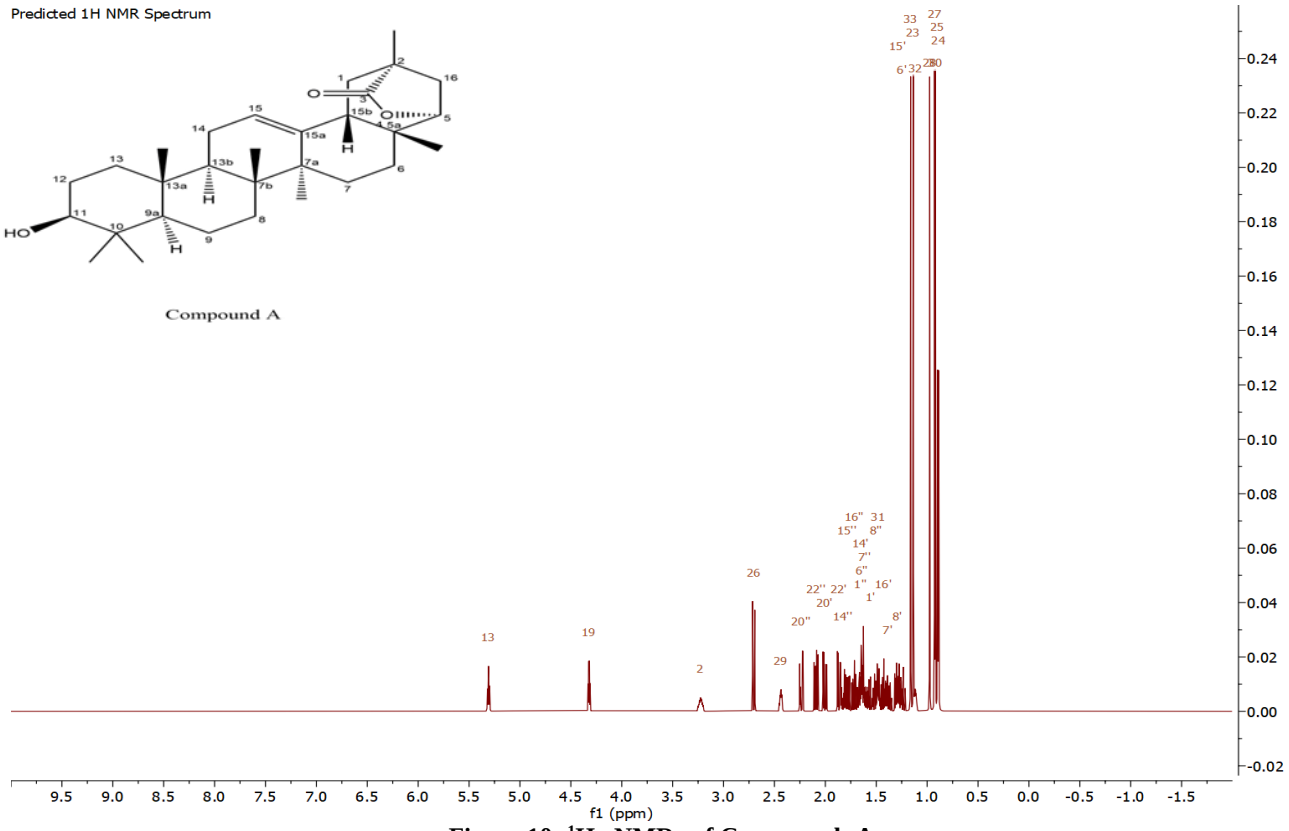


Figure.10 :^{1}H NMR of Compound -A

^{1}H NMR of-A: ^{1}H NMR: (400 MHz): δ 0.84-1.17 (18H, 0.89 (s), 0.90 (s), 0.93 (s), 1.03 (s), 1.03 (s), 1.12 (s)), 1.24-2.03 (20H, 1.30 (s), 1.36 (ddd, *J* = 13.5, 9.8, 3.8 Hz), 1.38 (dd, *J* = 9.9, 1.9 Hz), 1.43 (dddd, *J* = 13.1, 10.2, 9.9, 3.0 Hz), 1.46 (ddd, *J* = 13.3, 9.8, 4.1 Hz), 1.47 (ddd, *J* = 13.1, 10.2, 3.0 Hz), 1.46 (dd, *J* = 8.4, 5.9 Hz), 1.48 (dddd, *J* = 13.2, 3.1, 2.9, 2.6 Hz), 1.49 (dd, *J* = 13.6, 10.2 Hz), 1.55 (ddd, *J* = 13.1, 2.9, 2.9 Hz), 1.62 (ddd, *J* = 13.5, 4.1, 2.2 Hz), 1.63 (ddd, *J* = 13.1, 10.3, 2.6 Hz), 1.66 (ddd, *J* = 13.3, 3.8, 2.2 Hz), 1.73 (dddd, *J* = 13.1, 3.0, 2.8, 1.9 Hz), 1.80 (dddd, *J* = 13.2, 10.3, 10.2, 2.9 Hz), 1.80 (ddd, *J* = 13.1, 3.0, 2.8 Hz), 1.85 (dd, *J* = 13.8, 4.6 Hz), 1.95 (dd, *J* = 13.6, 3.3 Hz)), 2.03-2.42 (4H, 2.10 (dd, *J* = 13.8, 1.5 Hz), 2.22 (ddd, *J* = 12.2, 6.7, 5.9 Hz), 2.26 (dd, *J* = 10.2, 3.3 Hz), 2.33 (ddd, *J* = 12.2, 8.4, 6.7 Hz)), 3.45 (1H, dd, *J* = 10.2, 3.1 Hz), 4.95 (1H, dd, *J* = 4.6, 1.5 Hz), 5.09 (1H, dd, *J* = 6.7, 6.7 Hz).

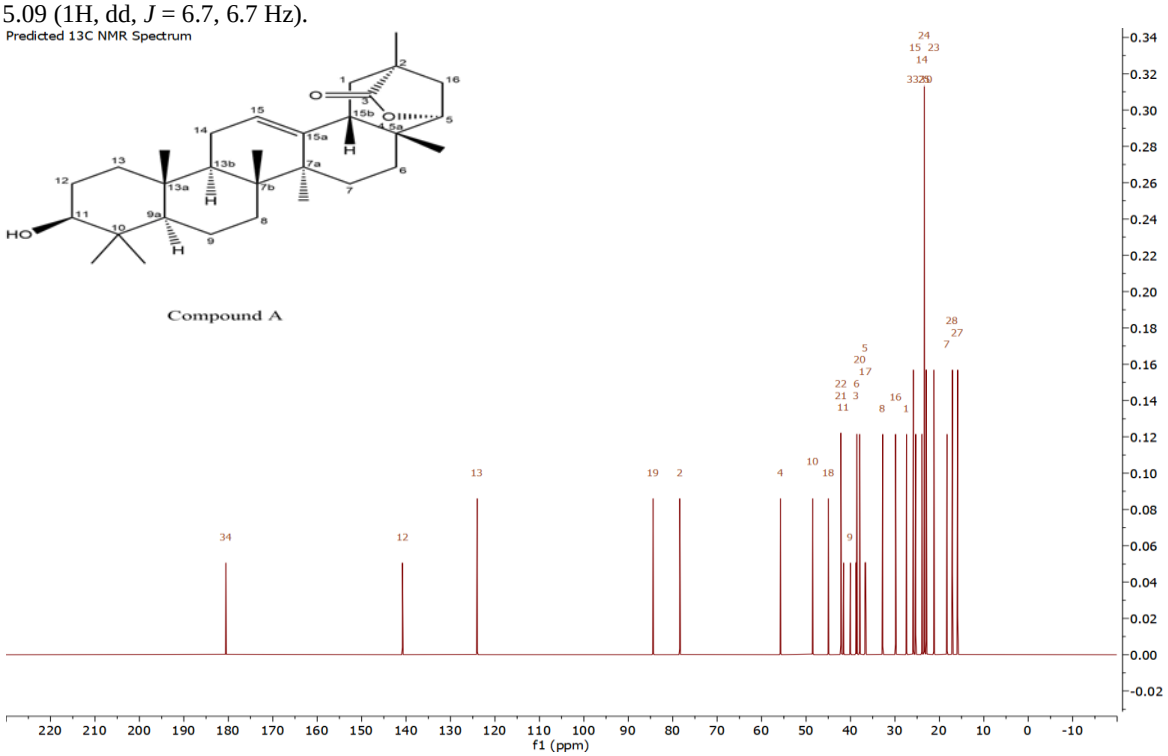
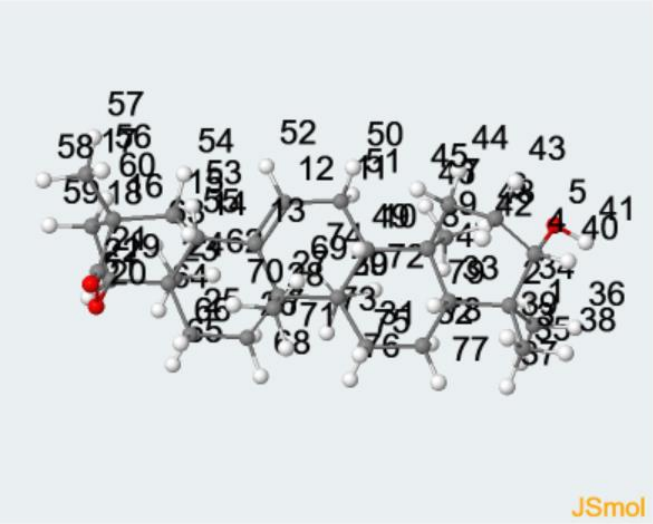


Figure.12:^{13} C NMR of Compound -A

^{13}C NMR of Compound -A: ^{13}C NMR: δ 15.4-15.7 (2C, 15.5 (s), 15.6 (s)), 16.8 (1C, s), 18.1 (1C, s), 20.4 (1C, s), 20.5 (1C, s), 23.9 (1C, s), 25.1 (1C, s), 25.8 (1C, s), 26.6 (1C, s), 27.1 (1C, s), 27.6 (1C, s), 32.8 (1C, s), 35.4 (1C, s), 36.7 (1C, s), 38.5-38.5 (2C, 38.5 (s), 38.5 (s)), 38.7 (1C, s), 38.9 (1C, s), 39.8 (1C, s), 40.9 (1C, s), 42.3 (1C, s), 45.2 (1C, s), 47.4 (1C, s), 55.1 (1C, s), 78.3 (1C, s), 84.7 (1C, s), 122.2 (1C, s), 140.9 (1C, s), 180.6 (1C, s).



Hydrogen	Chemical Shift(ppm)
34	0.87
35	0.87
36	0.87
37	0.96
38	0.96
39	0.96
40	3.40
42	1.67
43	1.59
44	1.66
45	1.16
46	0.85
47	0.85
48	0.85
49	1.78
50	1.80
51	1.89
52	5.62
53	2.60
54	1.58
55	1.92
56	1.18
57	1.18
58	1.18
59	1.95
60	2.03
61	4.70
62	0.95
63	0.95
64	0.95
65	1.58
66	1.80
67	1.66
68	1.52
69	0.99
70	0.99
71	0.99
72	0.81
73	0.81
74	0.81
75	1.70
76	1.38
77	1.31
78	1.35
79	1.26

Figure.13: Chemical shift of ^{1}H NMR of compound -A

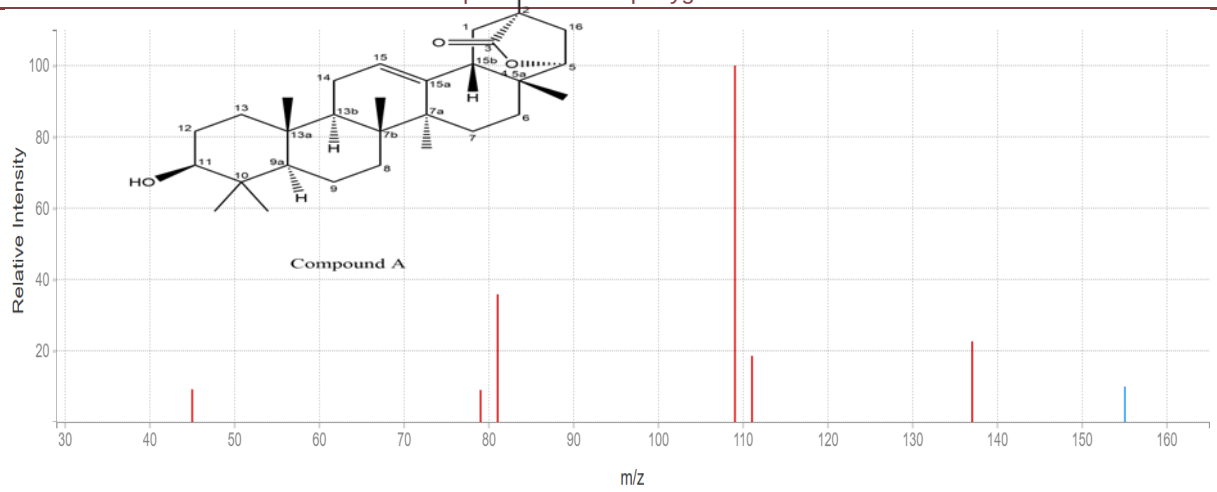


Figure 14: Mass Spectrum of Compound -A

Mass spectrum of Compound -A: Chemical Formula: $C_{30}H_{46}O_3$ Exact Mass: 454.34470 Molecular Weight: 454.69500 m/z: 454.34470 (100.0%), 455.34805 (32.4%), 456.35141 (2.7%), 456.35141 (2.4%) Elemental Analysis: C, 79.25; H, 10.20; O, 10.56 **Figure.15**: Structure of -A, (2S,5S,5aR,7aS,7bR,9aR,11S,13aR,13bR,15bS)-11-hydroxy-2,5a,7a,7b,10,10,13a-heptamethyl-1,5,5a,6,7,7a,7b,8,9,9a,10,11,12,13,13a,13b,14,15b-octadecahydro-2,5-methanochryseno[2,1-c]oxepin-3(2H)-one

3.2: Identification of Compound B Isolate:

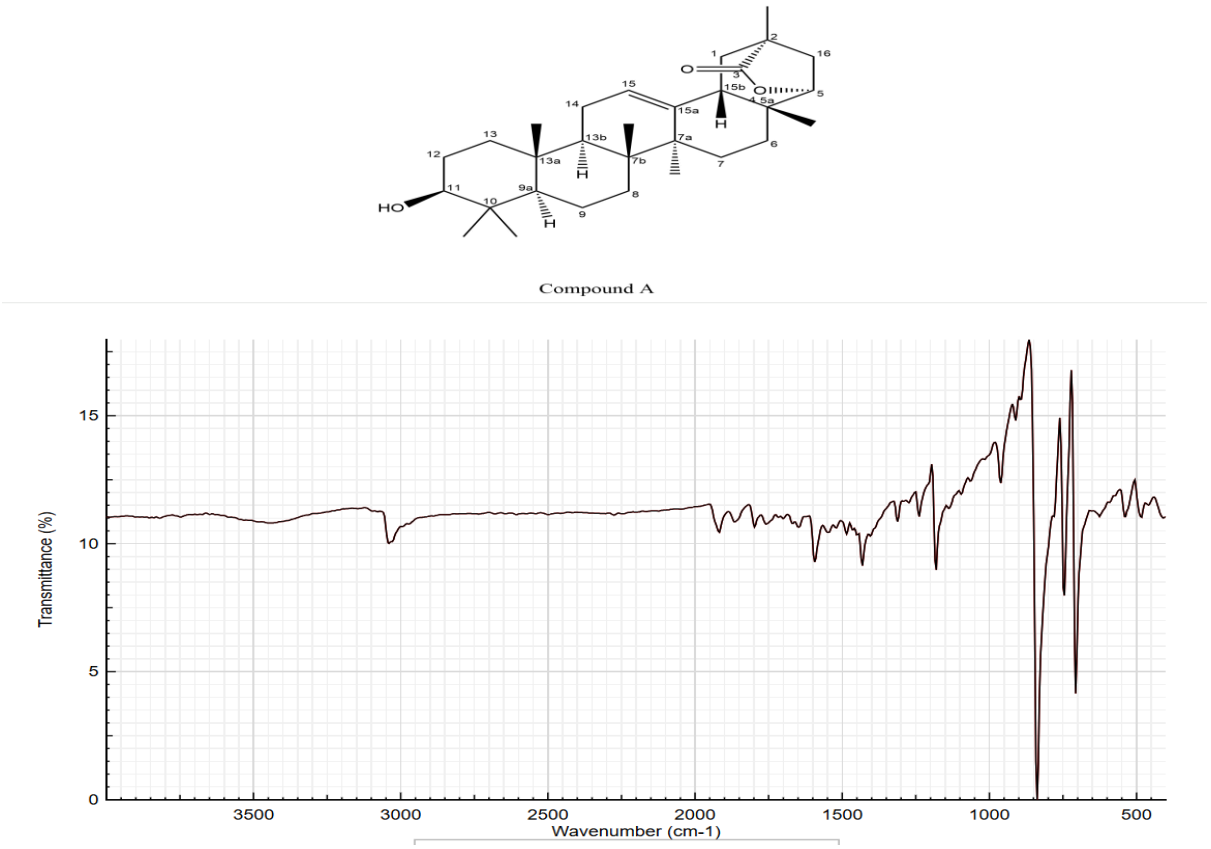


Figure.16 .IR of Compound B

IR of Compound B : 1828.92 cm-1 (w intensity C-H bending aromatic compound), 2306.48 cm-1 (s intensity O=C=O stretching), and 3649.77 cm-1 (v intensity free O-H) 1747.90 cm-1 (stretching with an intensity of C=O) and 1655.51 cm-1 (wrestling with an intensity of C=C)m intensity C=C stretching cyclic alkene: 1605.10 cm-1; s intensity carboxylate ions: 1312.38 cm-1'; s intensity carbonyl group: 1241.57 cm-1; s intensity C-O stretching ester: 1160 cm-1 841.61 cm-1 (w intensity isolated aromatic C-H), 931.11 cm-1 (m intensity C = C bending alkene viny idene) .[10,11,12]

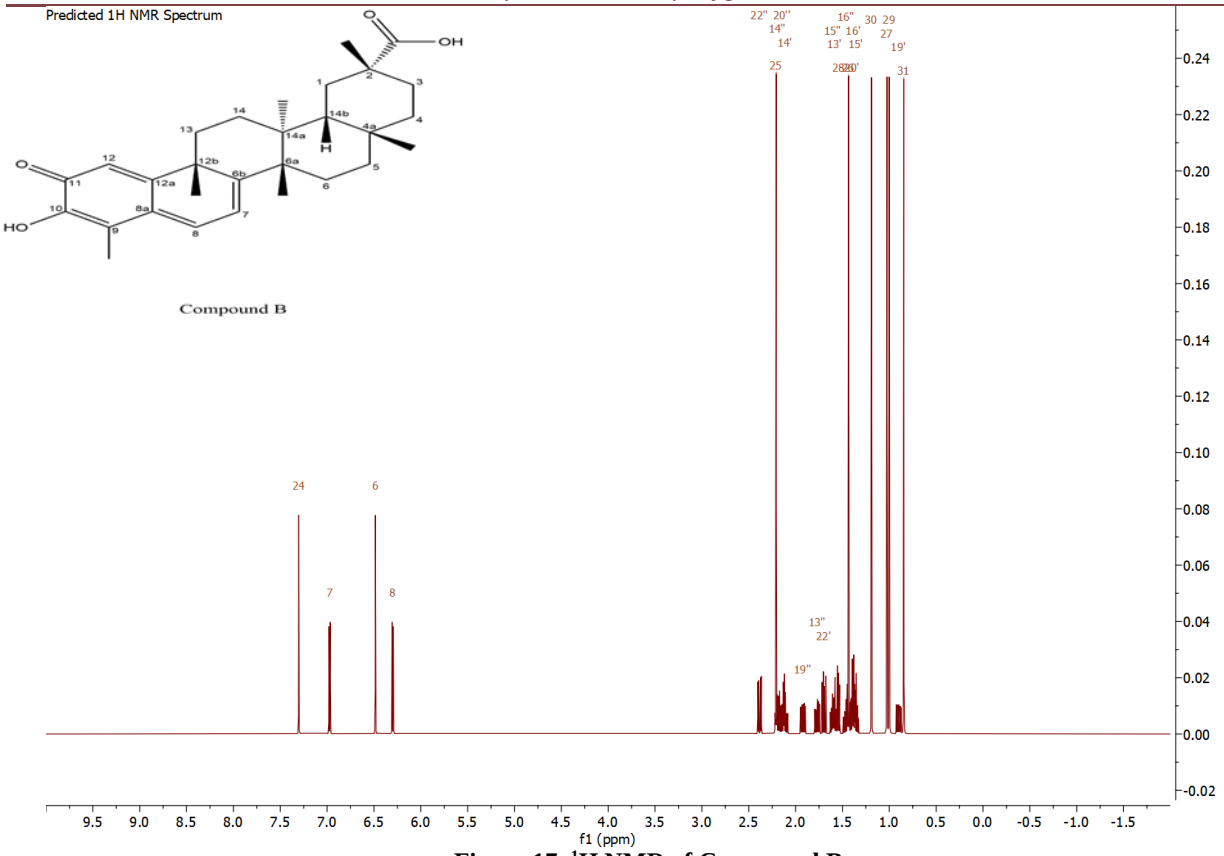


Figure.17 .[1]H NMR of Compound B

[1]H-NMR of Compound B:[1]H NMR: (400 MHz): δ 0.90 (3H, s), 1.03 (3H, s), 1.14 (3H, s), 1.28 (3H, s), 1.34-2.00 (18H, 1.42 (ddd, *J* = 13.1, 9.9, 3.9 Hz), 1.43 (ddd, *J* = 13.5, 10.0, 3.6 Hz), 1.46 (ddd, *J* = 13.1, 9.2, 4.7 Hz), 1.45 (dd, *J* = 6.5, 1.4 Hz), 1.50 (s), 1.57 (ddd, *J* = 13.4, 9.9, 3.7 Hz), 1.58 (ddd, *J* = 13.2, 10.0, 3.7 Hz), 1.60 (ddd, *J* = 13.5, 3.7, 2.3 Hz), 1.69 (ddd, *J* = 13.2, 3.6, 2.3 Hz), 1.69 (dd, *J* = 13.1, 6.5 Hz), 1.73 (ddd, *J* = 13.4, 9.2, 4.9 Hz), 1.79 (ddd, *J* = 13.1, 4.9, 1.7 Hz), 1.79 (ddd, *J* = 13.4, 4.7, 1.7 Hz), 1.86 (ddd, *J* = 13.1, 3.7, 2.2 Hz), 1.91 (dd, *J* = 13.1, 1.4 Hz), 1.93 (ddd, *J* = 13.4, 3.9, 2.2 Hz)), 2.07 (3H, s), 5.82 (1H, s), 6.27 (1H, d, *J* = 1.9 Hz), 6.50 (1H, d, *J* = 1.9 Hz). [13,14,15]

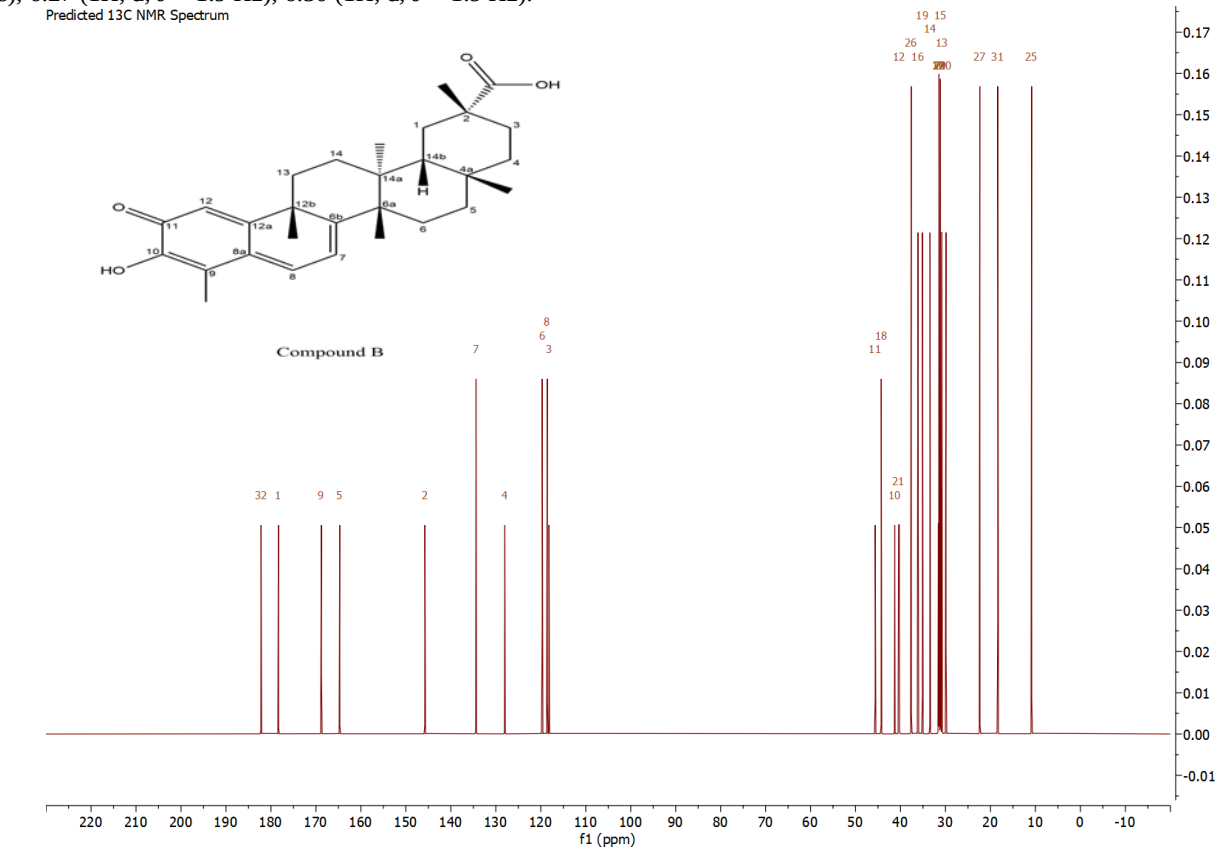
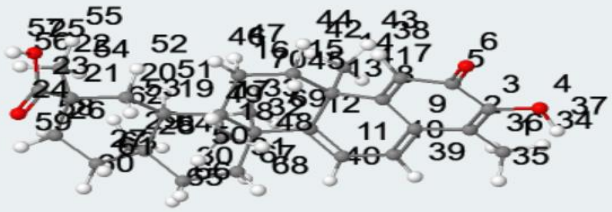


Figure.18 .[13]C NMR of Compound B

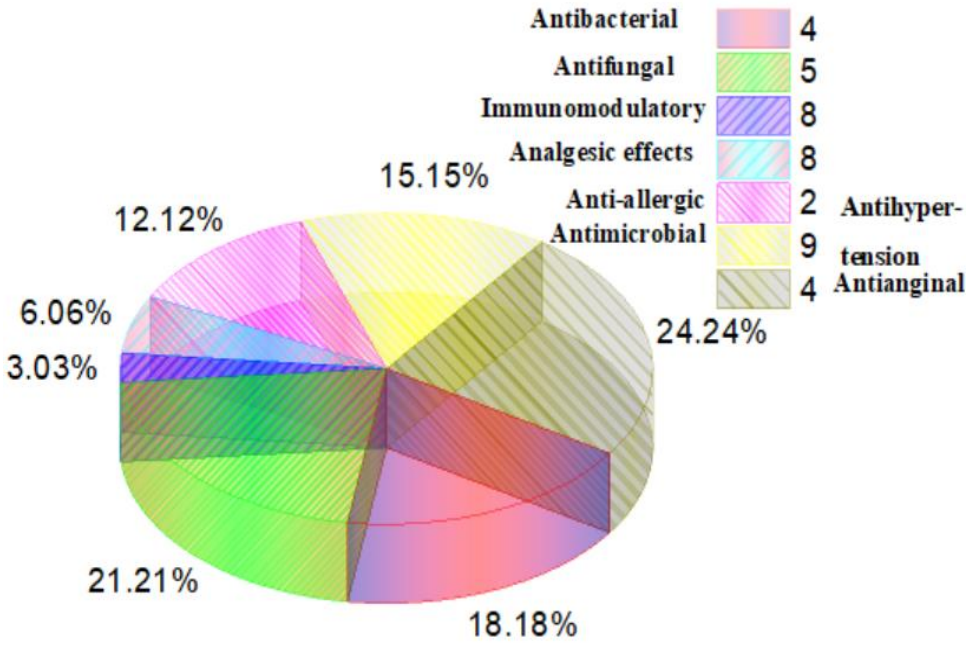
[13]C NMR of Compound B: [13]C NMR δ 10.6 (1C, s), 16.9 (1C, s), 23.9-24.1 (2C, 24.0 (s), 24.0 (s)), 25.9 (1C, s), 27.6 (1C, s), 27.7-27.9 (3C, 27.8 (s), 27.8 (s), 27.8 (s)), 30.8-30.9 (2C, 30.8 (s), 30.8 (s)), 35.2 (1C, s), 35.7 (1C, s), 36.8 (1C, s), 39.9 (1C, s), 41.9 (1C, s), 42.1-42.2 (2C, 42.2 (s), 42.2 (s)), 49.8 (1C, s), 115.4-115.5 (2C, 115.4 (s), 115.4 (s)), 124.0 (1C, s), 127.7 (1C, s), 135.4-135.6 (3C, 135.5 (s), 135.5 (s), 135.5 (s)), 150.9 (1C, s), 173.8 (1C, s), 183.6 (1C, s). [17,18,19]



Hydrogen	Chemical Shift(ppm)
34	2.31
35	2.31
36	2.31
38	6.51
39	7.13
40	6.59
41	1.25
42	1.25
43	1.25
44	2.39
45	1.81
46	1.78
47	1.92

Figure.19: Chemical shift of [1]H NMR and 13C NMR of Compound B

48	0.73
49	0.73
50	0.73
51	1.65
52	1.84
53	1.62
54	1.19
55	1.19
56	1.19
58	1.53
59	1.71
60	1.49
61	1.45
62	0.94
63	0.94
64	0.94
65	1.42
66	1.76
67	1.59
68	1.87
69	1.10
70	1.10
71	1.10



Figer-20 Shows the percentage contribution of a specific pharmacological effect.

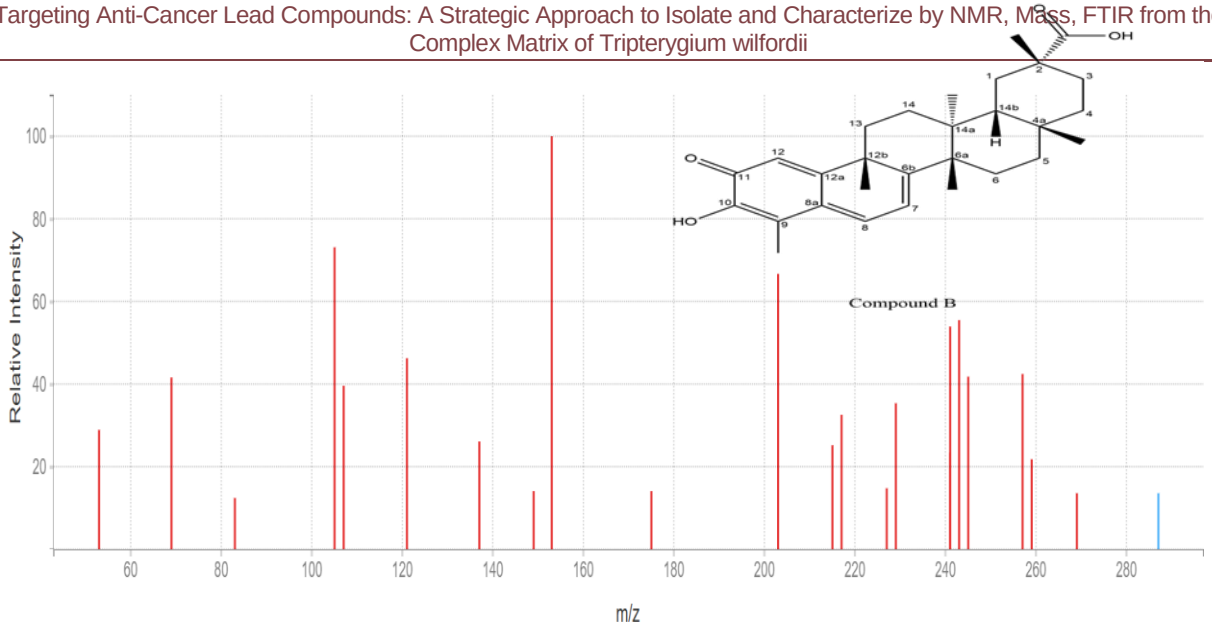
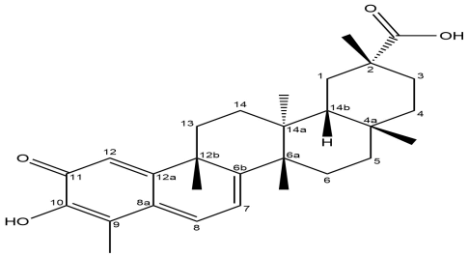


Figure 21: Mass spectrum of Compound B

Mass spectrum of Compound B: Chemical Formula: $C_{29}H_{38}O_4$, Exact Mass: 450.27701, Molecular Weight: 450.61900, m/z: 450.27701 (100.0%), 451.28036 (31.4%), 452.28372 (2.7%), 452.28372 (2.0%), Elemental Analysis: C, 77.30; H, 8.50; O, 14.20.[20,21,22]

Figure.22: Str ucture of **Compound B**, (2R,4aS,6aS,12bR,14aS,14bR)-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydropicene-2-carboxylic acid. [23,24,25]



Discussion: Spectroscopic analysis was performed on two isolates (A and B) purified from *Tripterygium wilfordii* leaves via column chromatography. The combined data from FTIR, 1H NMR, 13C NMR, and Mass Spectrometry led to their structural characterization.

Characterization of Compound A (Wilforlide A):

- **Proposed Structure:** (2S,5S,5aR,7aS,7bR,9aR,11S,13aR,13bR,15bS)-11-hydroxy-2,5a,7a,7b,10,10,13a-heptamethyl-1,5,5a,6,7,7a,7b,8,9,9a,10,11,12,13,13a,13b,14,15b-octadecahydro-2,5-methanochryseno[2,1-c] oxepin-3(2H)-one
- **Common Name:** Wilforlide A
- **Spectroscopic Evidence:**
  - **FTIR** indicated key functional groups: hydroxyl (-OH), carbonyl (C=O), and aromatic/alkene (C=C) stretches.
  - **1H NMR** signals were consistent with furan, cyclopentene, cyclohexane, methyl, methylene, and ethylene protons.
  - **13C NMR & MS** confirmed the molecular formula as **$C_{30}H_{46}O_3$** (Exact Mass: 454.34). [26,27]

Characterization of Compound B (Celastrol):

- **Proposed Structure:** (2R,4aS,6aS,12bR,14aS,14bR)-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydropicene-2-carboxylic acid. [28,29]
- **Common Name:** Celastrol
- **Spectroscopic Evidence:**
  - **FTIR** showed absorptions characteristic of carboxylic acid (-OH), ketone (C=O), and aromatic rings.
  - **NMR spectra** displayed signals for furan, aromatic protons (Ar-H), methyl groups, and aliphatic chains.
  - **Mass Spectrometry** established the molecular formula as **$C_{29}H_{38}O_4$** (Exact Mass: 450.28). [30]

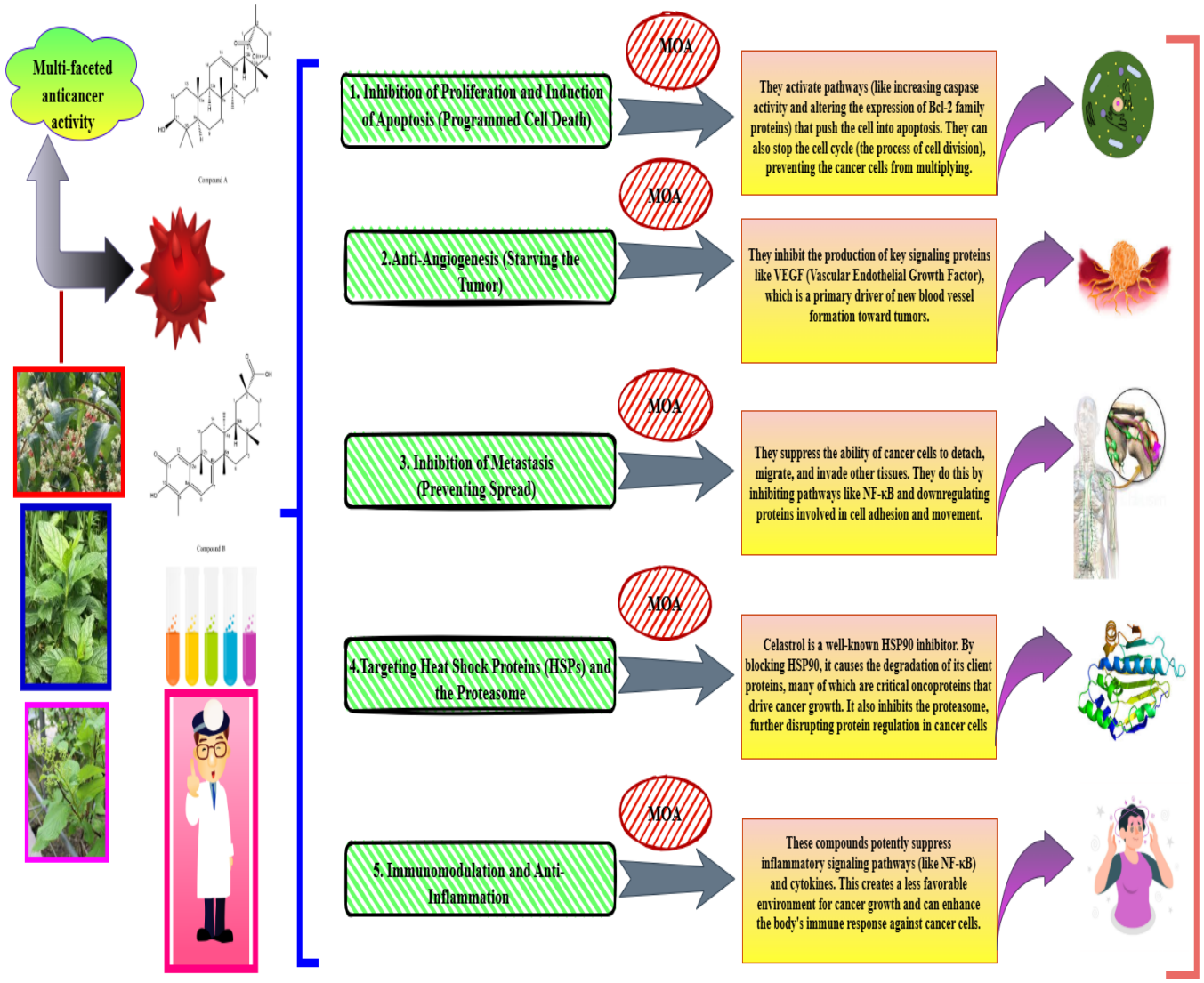


Fig.23 Application and Mechanism of Wilforlide A, Celastro (*Tripterygium wilfordii*)

SUMMARY AND CONCLUSION

Tripterygium wilfordii, a plant used in traditional Indian Ayurvedic medicine, possesses numerous therapeutic properties, including anticancer, immunomodulatory, and antimicrobial effects. These benefits are attributed to its diverse phytoconstituents. This study presents a cost-effective spectroscopic bio-analytical method for isolating and characterizing these compounds. The analysis successfully identified two key bioactive constituents: **Wilforlide A** (Isolate A) and **Celastrol** (Isolate B). The developed methodology provides a strong foundation for future bio-analytical research on ***Tripterygium wilfordii***.

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