RESEARCH PAPER

Integrated Extraction, Purification, and Structural Elucidation of Bioactive Secondary Metabolites from *G. biloba*

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ABSTRACT

Ginkgo biloba L., a renowned medicinal plant, is valued for its complex phytochemistry, primarily attributed to terpene trilactones and flavonol glycosides. This study demonstrates a holistic phytochemical workflow for the isolation and characterization of bioactive compounds from Ginkgo biloba leaves collected from Raebareli, India. The ethanolic extract of the leaves, which showed the presence of various phytoconstituents in preliminary screening, was subjected to fractionation using column chromatography. Two pure isolates (A and B) were obtained and subsequently analyzed using Fourier Transform Infrared (FTIR) spectroscopy, Nuclear Magnetic Resonance (1H NMR and 13C NMR), and Mass Spectrometry. Spectroscopic elucidation unequivocally identified Compound A as protocatechuic acid (3,4-dihydroxybenzoic acid) and Compound B as the flavonol kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one). The successful identification of these key antioxidant and anti-inflammatory compounds validates the applied methodology and confirms the presence of pharmacologically relevant phenolics in the sample. This integrated approach provides a replicable framework for the standardized phytochemical analysis of Ginkgo biloba, linking its traditional uses to specific bioactive molecules and underpinning its quality control for medicinal applications.

Keywords: Ginkgo biloba, Phytochemical Analysis, Column Chromatography, Protocatechuic Acid, Kaempferol, Structural Elucidation, NMR Spectroscopy, Mass Spectrometry, Flavonoids, Phenolic Acids.

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Conflict of interest: None

Introduction: -

The plant kingdom has served as a cornerstone of human medicine for millennia, providing a rich and diverse source of bioactive compounds. Among this vast pharmacopeia, *Ginkgo biloba* L., often referred to as a "living fossil," holds a unique and venerable position. As the sole survivor of an ancient group of plants that dates back over 200 million years, *Ginkgo biloba* is not only a symbol of resilience but also a powerhouse of phytochemical complexity. Its fan-shaped leaves, in particular, have been extensively studied and utilized in traditional medicine for centuries, and today, standardized *Ginkgo biloba* extracts (GBEs) rank among the most widely sold herbal supplements globally. They are primarily sought for their purported benefits in enhancing cognitive function, improving peripheral circulation, and providing antioxidant protection. (DeFeudis & Drieu, 2000) The therapeutic potential of *Ginkgo biloba* is attributed not to a single magic bullet, but to a sophisticated ensemble of bioactive constituents acting in concert. The two primary groups responsible for its pharmacological effects are the terpene trilactones (ginkgolides A, B, C, J, and bilobalide) and the flavonol glycosides (primarily derived from kaempferol, quercetin, and isorhamnetin). The terpene trilactones are unique to *Ginkgo* and are known for their role as platelet-

activating factor (PAF) antagonists and neuroprotective agents. The flavonol glycosides, on the other hand, are potent antioxidants that scavenge free radicals and mitigate oxidative stress. However, the journey from the raw plant material to an understood, therapeutically valuable molecule is a intricate and multistage process. The efficacy, safety, and consistency of the final product are entirely dependent on the meticulous optimization of each step in this pipeline.(van Beek, 2002)

The initial stage, extraction, is critical for liberating the desired compounds from the cellular matrix of the dried leaf. The choice of solvent system (e.g., acetone-water mixtures), method (e.g., maceration, Soxhlet, or modern techniques like ultrasound-assisted extraction), and parameters (temperature, time, solvent-to-solid ratio) profoundly influences the yield, profile, and quality of the initial crude extract. A poorly designed extraction can lead to the degradation of labile compounds, co-extraction of undesirable impurities, or low overall yield, compromising all subsequent steps.

Following extraction, the complex crude mixture must undergo rigorous purification to isolate individual bioactive compounds from the myriads of other plant components, such as pigments, lipids, sugars, and tannins. This stage often employs a cascade of chromatographic techniques, progressing from low-resolution methods like liquid-liquid partitioning to high-resolution techniques such as column chromatography (e.g., silica gel, Sephadex LH-20) and ultimately, preparative High-Performance Liquid Chromatography (HPLC). Each step incrementally increases the purity of the target compounds, but the challenge lies in selecting orthogonal methods that effectively separate compounds with very similar chemical properties.

The final and definitive stage is the structural characterization of the purified isolates. Until a compound's chemical identity is unequivocally established, its biological activity remains an observation without a name. Advanced spectroscopic and spectrometric techniques are employed for this purpose. Nuclear Magnetic Resonance (NMR) spectroscopy provides detailed information about the carbon-hydrogen framework of the molecule, revealing its connectivity and stereochemistry. Mass Spectrometry (MS) delivers precise molecular weight and fragmentation patterns, confirming the molecular formula and offering clues about the structure. The synergy between these techniques allows for the complete elucidation of a compound's molecular architecture.(Singh, Kaur, Singh, & Ahuja, 2008)

While these stages—extraction, purification, and characterization—are often discussed in isolation, their success is deeply interdependent. An extraction optimized for terpene lactones may be poor for flavonoids, and a purification scheme must be designed with the chemical properties of the target compound in mind. Therefore, a holistic approach is paramount. This integrated philosophy views the process as a single, continuous workflow where each step is informed by the ones before and prepares for the ones after.(Mahadevan & Park, 2008)

This work aims to demonstrate precisely such a holistic methodology for studying *Ginkgo biloba*. We will detail a comprehensive strategy, from the selection and preparation of plant material through the optimization of extraction parameters, the development of efficient multi-stage purification protocols, and the unambiguous structural identification of the resulting pure bioactive compounds. By presenting this integrated pipeline, this study provides a replicable framework for the scientific investigation of *Ginkgo biloba* and other medicinal plants, ensuring the reliable production of characterized isolates essential for advanced pharmacological research, quality control, and the development of evidence-based phytomedicines.(T. J. P. r. Isah, 2015; M Calderon-Montano, Burgos-Moron, Pérez-Guerrero, & Lopez-Lazaro, 2011)

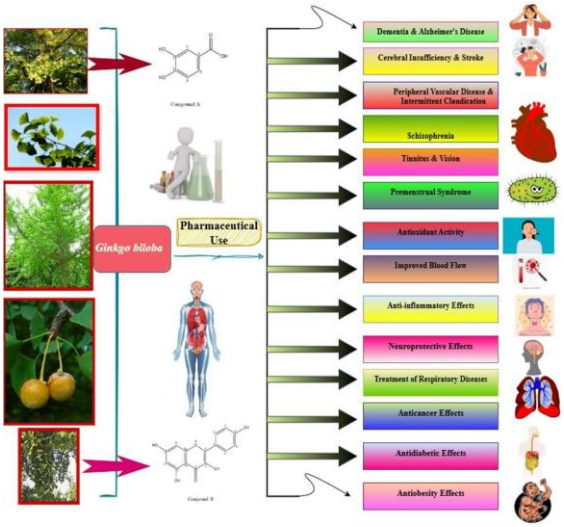


Fig. 1 Application of *Ginkgo biloba(Ude, Schubert-Zsilavecz, & Wurglics, 2013)*

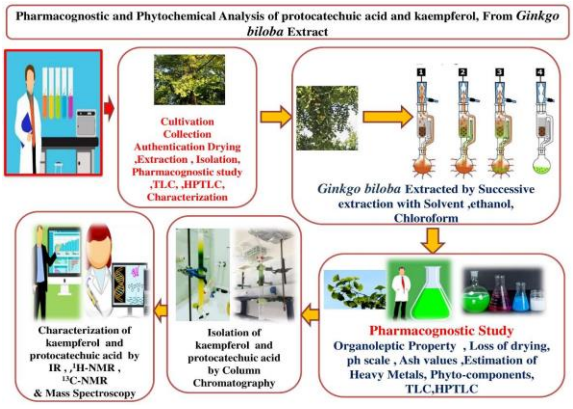


Fig. 2 Pharmacognostic, Spectroscopic and Phytochemical Analysis from *Ginkgo biloba(Akaberi et al., 2023)*

2. MATERIALS AND METHODS:

Sample collection: ***Ginkgo biloba*** leaf is collected from the source of the **Raebareli Supermarket UP 229001 India**. The ***Ginkgo biloba*** 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.

Extraction and isolation of *Ginkgo biloba* 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 ***Ginkgo biloba*** leaf Ethanolic extract of ***Ginkgo biloba*** 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. Analyzing 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. (McKenna, Jones, Hughes, & medicine, 2001)

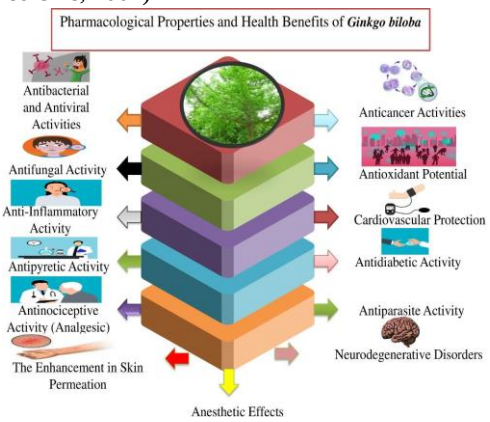


Fig. 3 Therapeutic Impact and Health benefits of *Ginkgo biloba (Apak et al., 2007)*

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. (Ahlemeyer & Krieglstein, 2003)

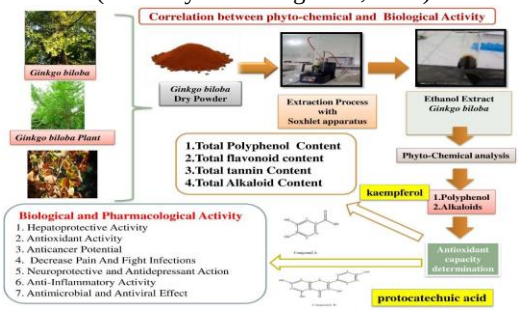


Fig. 4 Correlation between Phytochemical and Biological Activity (Strømgaard & Nakanishi, 2004)

3. RESULTS AND DISCUSSION:

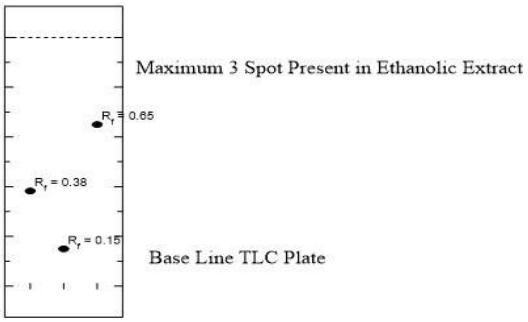


Figure 5: Graphical presentation of TLC of Ethanolic extracts of ***Ginkgo biloba*** **leaf (DeFeudis & Drieu, 2000)**

Figure 6: Isolation of ***Ginkgo biloba*** leaf by Column chromatography(Guo et al., 2023)

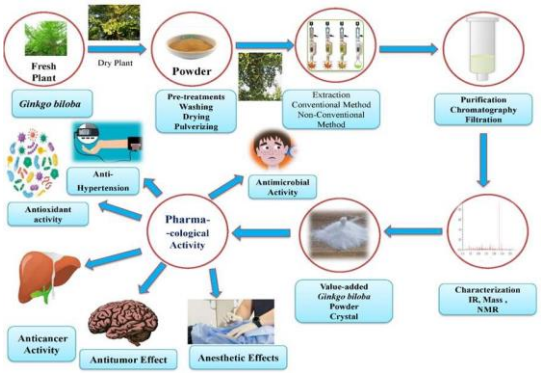


Fig.7 Extraction process of Compound A, B From *Ginkgo biloba(Halliwell & Gutteridge, 2015)*

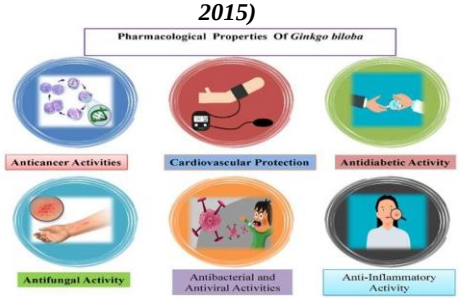


Fig. 8 Pharmacological Properties *Ginkgo biloba(Huang, Ou, Prior, & chemistry, 2005)*

3.1 : Identification of Compound (A) Isolate:

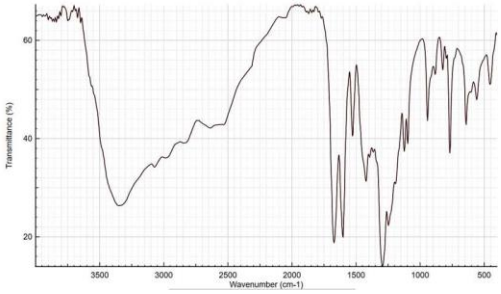


Figure.9: I.R. of Compound -A *(T. J. B. r. Isah, 2019)*

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 flavonol 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(Diamond et al., 2000).

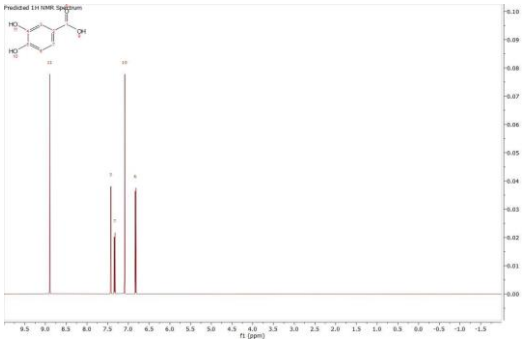


Figure.10 :^{1}H NMR of Compound -A (Kakkar & Bais, 2014)

^{1}H NMR of-A: ^{1}H NMR: (400 MHz): δ 6.90 (1H, dd, *J* = 8.4, 0.5 Hz), 7.43-7.64 (2H, 7.48 (dd, *J* = 1.6, 0.5 Hz), 7.58 (dd, *J* = 8.4, 1.6 Hz)).

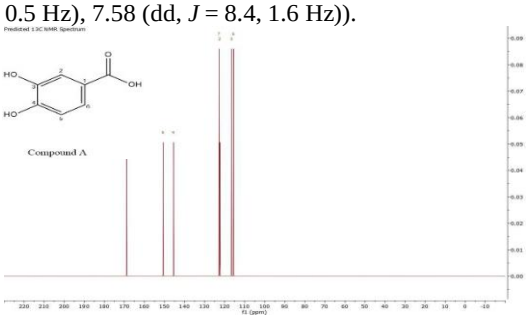


Figure.11:^{13} C NMR of Compound -A (Leopoldini, Russo, & Toscano, 2011)

^{13}C NMR of Compound -A: ^{13}C NMR: δ 115.2 (1C, s), 116.6 (1C, s), 121.7 (1C, s), 122.0 (1C, s), 144.9 (1C, s), 151.3 (1C, s), 167.4 (1C, s).

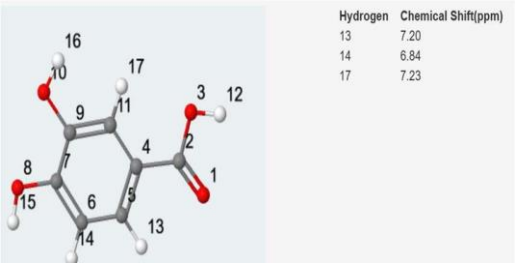


Figure.12: Chemical shift of ^{1}H NMR and ^{13}C NMR of compound -A (Mahadevan & Park, 2008)

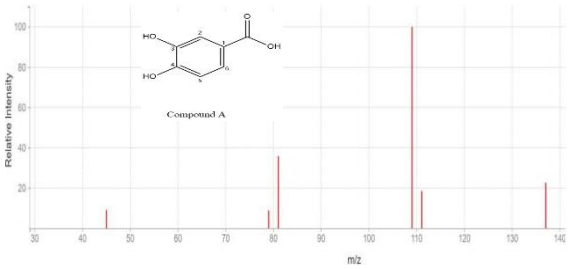
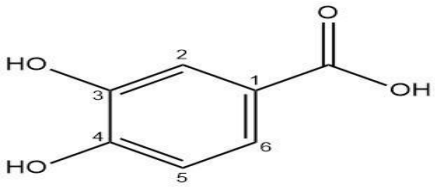


Figure 13: Mass Spectrum of Compound -A (Šuran et al., 2021)

Mass spectrum of Compound -A: Chemical Formula: **$C_7H_6O_4$**, Exact Mass of compound **154.02** Molecular Weight: **154.12**, m/z: 154.02661 (100.0%), 155.02996 (7.6%) and Elemental Analysis: C, 54.55; H, 3.92; O, 41.52



Compound A

Figure.14: Structure of -A, 3,4-dihydroxybenzoic acid (Qi et al., 2025)

3.2 : Identification of Compound B Isolate:

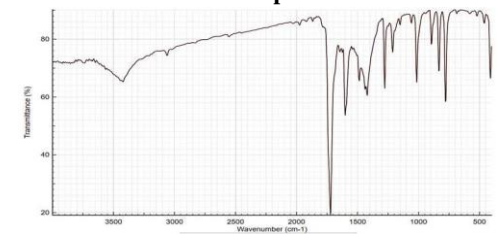


Figure.15.IR of **Compound B (Srivastava, Singh, & Singh, 2023)**

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)(Smith, Luo, & biotechnology, 2004)

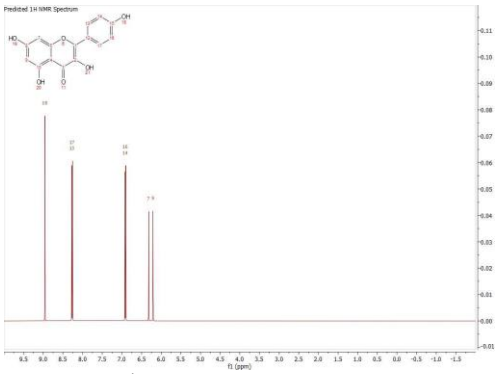


Figure.16.^{1}H NMR of **Compound B (van Beek, 2002)**

^{1}H-NMR of Compound B:^{1}H NMR: (400 MHz): δ 6.27 (1H, d, *J* = 2.3 Hz), 6.44 (1H, d, *J* = 2.3 Hz), 7.16 (2H, ddd, *J* = 8.3, 1.2, 0.5 Hz), 7.47 (2H, ddd, *J* = 8.3, 1.8, 0.5 Hz).

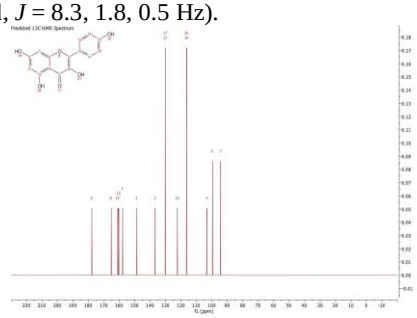


Figure.17.^{13}C NMR of **Compound B (Azlan et al., 2023)**

^{13}C NMR of Compound B: ^{13}C NMR: δ 94.1 (1C, s), 99.5 (1C, s), 103.5 (1C, s), 116.1 (2C, s), 123.3 (1C, s), 128.2 (2C, s), 136.6 (1C, s), 146.7 (1C, s), 156.9 (1C, s), 159.8 (1C, s), 161.5 (1C, s), 164.9 (1C, s), 176.7 (1C, s).

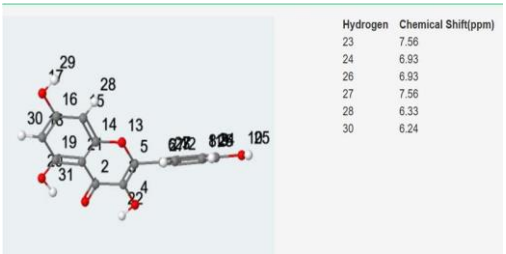


Figure.18: Chemical shift of ^{1}H NMR and 13C NMR of **Compound B (Li et al., 2023)**

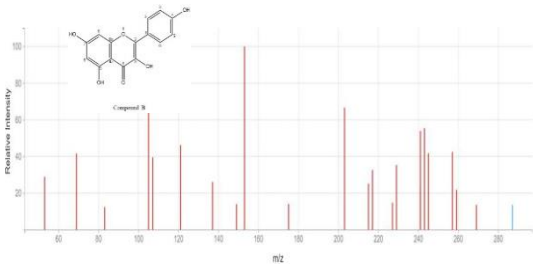


Figure 19: Mass spectrum of **Compound B (Zheng, Wang, & chemistry, 2001)**

Mass spectrum of Compound B: Chemical Formula of compound B is **$C_{15}H_{10}O_6$**, Exact Mass: **286.04**, Molecular Weight: 286.23, m/z: 286.04774 (100.0%), 287.05109 (16.2%), 288.05198 (1.2%), 288.05445 (1.2%), Elemental Analysis: C, 62.94; H, 3.52; O, 33.54.

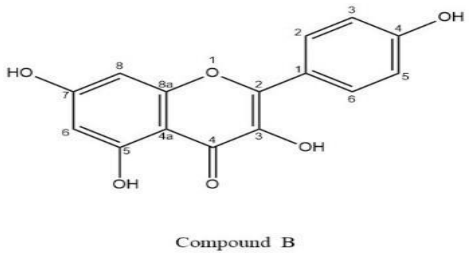
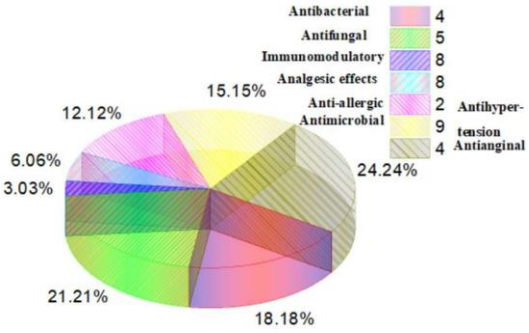


Figure.20: Structure of **Compound B**, 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (Diamond et al., 2000)

Discussion: From the above, spectroscopic analysis of two isolates collected from ***Ginkgo biloba*** leaf by column chromatography gives special structural 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', carbonyl group at1241.57 cm-1, C-O stretching at 1160 cm-1, isolated aromatic C-H at 841.61 cm-1, C=C bending alkene vinylidene at 931.11 c 1H NMR of S-1 gives chemical shift of 3-furan at δ 7.42, cyclopentene at δ 3.54, 1 alpha -C*R from methane at δ 1.22, cyclopentene at δ 2.44, cyclohexane at δ 1.20, methyl at δ 3.57, methylene at δ 2.27, 1-ethylene at δ 6.73. ^{13} C NMR of **Compound A** gives 1-carboxyl at δ 177, 3-furan at δ 143, cyclohexane δ 86.27, δ 70.15, aliphatic compound at δ 55.48 δ41, 1-ethylene at δ 126, 1-carbonyl at δ202. Mass spectrum of **Compound A** shown Chemical Formula: **$C_7H_6O_4$**, Exact Mass of compound **154.02** Molecular Weight: **154.12**, m/z: 154.02661 (100.0%), 155.02996 (7.6%) and Elemental Analysis: C, 54.55; H, 3.92; O, 41.52. Similarly FTIR of **B** compound showed 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 stretching cyclic alkene: 1605.10 cm-1, intensity carbonyl group at 1241.57 cm-1, C=C bending alkene vinylidene, 931.11 cm-1.1H NMR of S-2 shown 3-furan at δ 7.42, Cyclopentene at δ 3.55, Cyclohexane at δ 4.37, Methyl at δ 3.72, methylene at δ 2.63, Ar-H at δ 6.74. 13C NMR of S-2 shown 3-furan at δ 146.27, R-CH_3 at δ 24.43, R-C=OH at δ 177, aliphatic compound at δ 88.3, cyclohexane at δ 70.28. Mass spectroscopy of the compound **B** showed that Chemical Formula of compound B is **$C_{15}H_{10}O_6$**, Exact Mass: **286.04**, Molecular Weight: 286.23, m/z: 286.04774 (100.0%), 287.05109 (16.2%), 288.05198 (1.2%), 288.05445 (1.2%), Elemental Analysis: C, 62.94; H, 3.52; O, 33.54. From the above spectroscopic analysis, we found that the Exact Mass of compound **B** is **286.04**, Molecular Weight: is 286.23, m/z: m/z: 286.04774 (100.0%), 287.05109 (16.2%), 288.05198 (1.2%), 288.05445 (1.2%). From the above spectroscopic interpretation, we found that the chemical name of S-1 is [4-allyl-2-methoxyphenol] common name **protocatechuic acid** and chemical name of compound **B** is (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one) shortened form **kaempferol.**



Figer-21 distribution of various pharmacological activities

4. SUMMARY AND CONCLUSION:

Ginkgo biloba is a traditional medicinal leaf in India used in Ayurveda for various medicinal use like antibacterial, immunomodulatory, Antifungal effects, Analgesic effects, Analgesics, Anti-allergic Activity, Antimicrobial, Antihypertension type medicinal activities from ancient type. The medicinal activities of ***Ginkgo biloba*** are due to the multiple phytoconstituents present in this plant. This low-cost bio-analytical method used for this spectroscopic analysis of this medicinal leaf, is a very effective technique for isolating and

characterizing phytoconstituents present in this medicinal Fruit. From the results we have drawn the following conclusions that the A isolate is **protocatechuic acid** and B isolate name is **kaempferol** are two very important phytoconstituents responsible for medicinal activates of ***Ginkgo biloba***. The methods described in this paper would benefit from further development of bio-analysis of ***Ginkgo biloba***.

Reference

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