

Original article

Phytochemical Profiling of Phenolic Compounds in *Ginkgo biloba* Extracts and Their Potential Modulatory Effects on CA 15-3 and CA 125 Tumor Markers: An Organic and Bio-Analytical Study

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Abstract

This study aimed to characterize the phenolic composition of *Ginkgo biloba* extract using HPLC-UV analysis and to evaluate its effect on selected tumor biomarkers, namely CA 15-3 and CA 125. The chromatographic analysis revealed the presence of five phenolic compounds with varying concentrations. Ellagic acid was identified as the predominant compound, with a retention time of 1.320 min, an area of 1.452 mAU·min, and a concentration of 147.8549 µg/mL, representing 75.85% of the total relative area. Trans-ferulic acid was the second most abundant compound, with a concentration of 9.1129 µg/mL and a relative area of 4.67%. In contrast, naringin acid, Hesperidin, and salicylic acid were detected at much lower concentrations, ranging from 0.6074 to 1.8555 µg/mL. The total identified compounds accounted for 82.61% of the relative chromatographic area, indicating a phenolic profile dominated by ellagic acid. In addition, the biological evaluation demonstrated a significant reduction in serum tumor markers following treatment with *Ginkgo biloba* phenolic extract. The level of CA 15-3 decreased from 28.45 ± 1.12 U/mL in the control group to 19.30 ± 0.85 U/mL in the treated group, corresponding to a reduction of 32.16% (p < 0.05). Similarly, CA 125 levels declined from 31.20 ± 1.45 U/mL to 22.15 ± 0.92 U/mL, reflecting a decrease of 29.00% (p < 0.05). These findings indicate that *Ginkgo biloba* extract is rich in bioactive phenolic compounds and exhibits a significant modulatory effect on tumor biomarkers.

Keywords. *Ginkgo Biloba*, HPLC-UV, Phenolic Compounds, Tumor Markers, Antioxidant Activity.

Introduction

Medicinal plants have long been recognized as a valuable source of bioactive compounds with significant therapeutic potential, particularly in the prevention and management of chronic diseases, including cancer [1-10]. In recent years, there has been growing scientific interest in plant-derived phenolic compounds due to their well-documented antioxidant, anti-inflammatory, and anticancer properties [11-20]. These compounds play a crucial role in neutralizing free radicals, modulating cellular signaling pathways, and inhibiting tumor progression, making them promising candidates for complementary and alternative medicine [21-25].

Ginkgo biloba, one of the oldest living tree species, has been widely used in traditional medicine for centuries and is currently among the most extensively studied herbal extracts [26]. The pharmacological activity of *Ginkgo biloba* is largely attributed to its rich composition of flavonoids, terpenoids, and various phenolic acids. These constituents are known to exert protective effects against oxidative stress and cellular damage, which are key factors in the initiation and progression of cancer [27]. Advanced analytical techniques such as High-Performance Liquid Chromatography coupled with UV detection (HPLC-UV) are commonly employed to identify and quantify these bioactive compounds, providing a detailed chemical profile of plant extracts [28].

Tumor markers such as CA 15-3 and CA 125 are widely used in clinical practice for monitoring cancer progression and therapeutic response, particularly in breast and ovarian cancers. Elevated levels of these biomarkers are often associated with tumor burden and disease severity [29]. Therefore, investigating natural compounds that can modulate these markers may offer new insights into potential therapeutic strategies. In this context, the present study was designed to analyze the phenolic composition of *Ginkgo biloba* extract using HPLC-UV and to evaluate its effect on serum levels of CA 15-3 and CA 125. By combining phytochemical analysis with biochemical assessment, this study aims to provide a clearer understanding of the potential role of *Ginkgo biloba* as a natural agent in regulating tumor-associated biomarkers.

Methods

Leaves of *Ginkgo biloba* were collected, cleaned, and air-dried under controlled laboratory conditions. The dried plant material was finely powdered using a mechanical grinder. The phenolic extract was prepared by solvent extraction using an appropriate organic solvent (e.g., ethanol or methanol). The mixture was subjected to continuous stirring, followed by filtration to remove plant debris. The filtrate was then concentrated using a rotary evaporator under reduced pressure to obtain a crude phenolic-rich extract, which was stored at 4°C until further analysis. The qualitative and quantitative analysis of phenolic

compounds in the *Ginkgo biloba* extract was performed using High-Performance Liquid Chromatography coupled with UV detection (HPLC-UV). The chromatographic separation was carried out using a reverse-phase C18 column under optimized conditions. The mobile phase consisted of a gradient system of solvent A (water with a small percentage of acid) and solvent B (acetonitrile or methanol). The flow rate was maintained at a constant rate, and the injection volume was fixed for all samples. Detection was performed at a suitable wavelength specific to phenolic compounds. Identification of peaks was achieved by comparing retention times with those of standard compounds, while quantification was based on peak area integration and expressed as $\mu\text{g/mL}$.

A total of 20 experimental animals ($n = 20$) were used and randomly divided into two groups ($n = 10$ per group). The control group received no treatment, while the treated group was administered the *Ginkgo biloba* phenolic extract at a specified dose for a defined experimental period. All experimental procedures were conducted under standard laboratory conditions. At the end of the experimental period, blood samples were collected from all animals under appropriate conditions. Serum was separated by centrifugation and stored at low temperature until analysis. The levels of tumor markers CA 15-3 and CA 125 were measured using standard immunoassay techniques, following the manufacturer's instructions. The results were expressed in U/mL.

Statistical Analysis

All data were expressed as mean \pm standard error (SE). Statistical analysis was performed using appropriate software. Differences between the control and treated groups were evaluated using an independent sample t-test. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

Results

The HPLC-UV analysis of the *Ginkgo biloba* extract, as presented in (Table 1), revealed the presence of five identifiable phenolic compounds with distinct quantitative variations. Among these, ellagic acid was the predominant compound, exhibiting a retention time of 1.320 min, with an area of 1.452 mAU·min and a height of 14.573 mAU. It accounted for the highest relative area (75.85%) and relative height (68.52%), with a quantified concentration of 147.8549 $\mu\text{g/ml}$. In contrast, naringin acid showed a minimal presence, with a retention time of 1.533 min, an area of 0.006 mAU·min, and a negligible height of 0.000 mAU. Its relative area and height were 0.31% and 0.00%, respectively, corresponding to a low concentration of 0.6074 $\mu\text{g/ml}$. Hesperidin was detected at a retention time of 2.385 min, with an area of 0.018 mAU·min and a height of 0.303 mAU. It represented 0.95% of the relative area and 1.42% of the relative height, with a concentration of 1.8555 $\mu\text{g/ml}$. Similarly, salicylic acid appeared at 3.765 min, with an area of 0.016 mAU·min and a height of 0.152 mAU, contributing 0.82% and 0.71% to the relative area and height, respectively, and a concentration of 1.6046 $\mu\text{g/ml}$. Trans-ferulic acid demonstrated a moderate presence, with a retention time of 4.723 min, an area of 0.089 mAU·min, and a height of 0.660 mAU. It accounted for 4.67% of the relative area and 3.10% of the relative height, with a measured concentration of 9.1129 $\mu\text{g/ml}$. Overall, the total integrated area reached 1.581 mAU·min, while the total peak height was 15.688 mAU. The identified compounds collectively contributed 82.61% of the total relative area and 73.76% of the total relative height, indicating that ellagic acid is the major phenolic constituent in the *Ginkgo biloba* extract, followed by trans-ferulic acid, whereas the remaining compounds were present in comparatively minor concentrations.

Table 1. HPLC-UV Quantitative Analysis and Integration Parameters of Phenolic Compounds Identified in *Ginkgo biloba* Extract

| Peak Name | Retention Time | Area | Height | Relative Area | Relative Height | Amount |
|--------------------|----------------|---------|--------|---------------|-----------------|------------------|
| | Min | mAU*min | mAU | % | % | $\mu\text{g/ml}$ |
| Ellagic acid | 1.320 | 1.452 | 14.573 | 75.85 | 68.52 | 147.8549 |
| Naringin acid | 1.533 | 0.006 | 0.000 | 0.31 | 0.00 | 0.6074 |
| Hesperidin | 2.385 | 0.018 | 0.303 | 0.95 | 1.42 | 1.8555 |
| Salicylic acid | 3.765 | 0.016 | 0.152 | 0.82 | 0.71 | 1.6046 |
| trans-Ferulic acid | 4.723 | 0.089 | 0.660 | 4.67 | 3.10 | 9.1129 |



Figure 1. Representative HPLC Chromatogram of the Phenolic Profile in *Ginkgo biloba* Extract, Showing High Concentrations of Ellagic Acid and Related Organic Acids



Figure 2. HPLC Chromatogram and Peak Identification for the Quantitative Analysis of Phenolic Acids in *Ginkgo biloba*.

The results presented in (Table 2) demonstrate a significant effect of the *Ginkgo biloba* phenolic extract on serum tumor markers. The level of CA 15-3 in the control group was 28.45 ± 1.12 U/mL, whereas it decreased to 19.30 ± 0.85 U/mL in the treated group, representing a reduction of 32.16%. This decrease was statistically significant ($p < 0.05$). Similarly, CA 125 levels showed a notable decline following treatment with the extract. The control group recorded a value of 31.20 ± 1.45 U/mL, while the *Ginkgo biloba* group exhibited a reduced level of 22.15 ± 0.92 U/mL, corresponding to a percentage decrease of 29.00%. This reduction was also statistically significant ($p < 0.05$). Overall, both tumor markers (CA 15-3 and CA 125) showed marked reductions in the treated group compared to the control, indicating a consistent response across the evaluated parameters.

Table 2. Effect of *Ginkgo biloba* Phenolic Extract on CA 15-3 and CA 125 Levels

| Parameter (U/mL) | Control Group (n=10) | <i>Ginkgo biloba</i> Group (n=10) | Percentage Change (%) | p-value |
|------------------|----------------------|-----------------------------------|-----------------------|---------|
| CA 15-3 | 28.45 ± 1.12 | 19.30 ± 0.85 * | -32.16 % | < 0.05 |
| CA 125 | 31.20 ± 1.45 | 22.15 ± 0.92 * | -29.00 % | < 0.05 |

All values are expressed as Mean \pm Standard Error (SE) to ensure clarity and precision in reporting. Statistically significant differences compared to the control group are indicated by an asterisk (*), with a threshold of $p < 0.05$. Biomarker concentrations are reported in U/mL (Units per milliliter), which represents the standard international unit for these parameters. In addition, the percentage change column highlights the relative efficacy of the extract in reducing biomarker levels. This measure provides a comparative perspective that strengthens the interpretation of findings and supports the discussion of therapeutic potential.

Discussion

The present study demonstrated that *Ginkgo biloba* extract possesses a phenolic profile dominated by ellagic acid, followed by trans-ferulic acid, while other compounds such as hesperidin, salicylic acid, and naringin acid were detected at comparatively low concentrations. This distribution highlights the richness of the extract in potent antioxidant phenolics, particularly ellagic acid, which is widely recognized for its strong free radical scavenging capacity and its ability to modulate key cellular pathways involved in carcinogenesis [30-40]. The predominance of ellagic acid in the extract may therefore play a central role in the observed biological effects [41-45]. The significant reduction in CA 15-3 levels (-32.16%) and CA 125 levels (-29.00%) following administration of *Ginkgo biloba* extract suggests a potential modulatory effect on tumor-associated biomarkers. CA 15-3 is commonly associated with breast cancer progression, while CA 125 is widely used as a marker for ovarian cancer.

Elevated levels of these biomarkers are often linked to tumor burden and disease activity [46]. Therefore, the observed decrease in their levels may indicate a suppressive effect of the extract on tumor-related

processes or biomarker expression [47]. The biological activity of *Ginkgo biloba* can be attributed to its diverse phytochemical composition, particularly flavonoids and phenolic acids, which have been reported to exert antioxidant, anti-inflammatory, and anti-proliferative effects. Ellagic acid, the major compound identified in this study, has been shown to inhibit cancer cell proliferation, induce apoptosis, and reduce oxidative DNA damage [48]. Similarly, ferulic acid has been reported to exhibit chemopreventive properties through its ability to neutralize reactive oxygen species and modulate signaling pathways involved in inflammation and tumor growth [49]. Moreover, oxidative stress plays a critical role in the initiation and progression of cancer by promoting DNA mutations, lipid peroxidation, and activation of oncogenic pathways [50].

Natural antioxidants such as those present in *Ginkgo biloba* may counteract these effects by enhancing the cellular antioxidant defense system. Previous studies have shown that *Ginkgo biloba* extract can significantly reduce oxidative stress markers and improve cellular integrity, thereby contributing to its protective role against cancer-related alterations [51]. In addition, the anti-inflammatory properties of *Ginkgo biloba* may contribute to the reduction in tumor markers, as chronic inflammation is closely associated with tumor development and progression. Bioactive compounds in *Ginkgo biloba* have been shown to inhibit pro-inflammatory cytokines and downregulate inflammatory signaling pathways, which may indirectly reduce the expression or release of tumor biomarkers such as CA 15-3 and CA 125 [52-54]. Overall, the findings of this study support the potential therapeutic role of *Ginkgo biloba* as a natural source of bioactive compounds capable of modulating tumor biomarkers. The combined antioxidant and anti-inflammatory properties of its phenolic constituents may underlie the observed reductions in CA 15-3 and CA 125 levels, suggesting its possible application in cancer prevention or supportive therapy.

Conclusion

The findings of this study demonstrate that *Ginkgo biloba* phenolic extract is rich in bioactive compounds, with ellagic acid identified as the predominant constituent. The extract exhibited a significant modulatory effect on tumor biomarkers, as evidenced by the marked reduction in serum levels of CA 15-3 and CA 125. These results indicate that the phenolic components of *Ginkgo biloba* may contribute to the regulation of tumor marker expression. Overall, the study highlights the potential value of *Ginkgo biloba* as a natural source of biologically active compounds with measurable effects on cancer-related biochemical parameters.

Conflict of interest. Nil

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