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Identification of Bioactive Chemical Compounds in Ginkgo and Evaluation of its Antioxidant Activity using GC–MS and DPPH Assays

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ABSTRACT

Medicinal plants serve as a significant natural source of bioactive compounds with potential pharmaceutical and therapeutic applications. Among these, *Ginkgo* has garnered substantial scientific interest due to its diverse chemical composition and potential biological activities. This study aimed to identify the chemical constituents of a *Ginkgo* sample using gas chromatography-mass spectrometry (GC-MS) and to evaluate its antioxidant activity via the DPPH free radical scavenging assay.GC-MS analysis enabled the identification of twelve chemical compounds with distinct chemical properties. Among the identified constituents, 2,4-di-tert-butylphenol was detected with a peak area percentage of 4.28% and a spectral match quality of 93. Other identified compounds included methyl hex decanoate, methyl stearate, 3-tridecylphenol, unsaturated alkylphenols, di-n-decyl sulfone, eicosane, siloxane derivatives, and an anthracene derivative. The antioxidant activity of the *Ginkgo* sample was evaluated at concentrations of 0.12, 0.25, 0.5, and 1 mg/mL. An increase in free radical scavenging activity (DPPH) was observed with rising sample concentration; the percentage rose from 72.365% at a concentration of 0.12 mg/mL to 82.188% at 1 mg/mL, while the corresponding absorbance values decreased from 0.2895 to 0.1866. These results indicate that the analyzed Ginkgo sample exhibits significant antioxidant activity under the experimental conditions employed.

KEYWORDS: *Ginkgo*; GC–MS; DPPH; antioxidant activity; phenolic compounds; free-radical scavenging; bioactive compounds.

1. INTRODUCTION

Historically, medicinal plants have been a vital source of natural products used in traditional medicine and continue to attract significant interest in modern scientific research[1]. The biological properties of plants are closely linked to their complex chemical composition, which may include phenolic compounds, flavonoids, terpenoids, fatty acids, esters, hydrocarbons, and other secondary metabolites[2]. These constituents can contribute—either individually or synergistically—to various biological activities, such as antioxidant, antimicrobial, and anti-inflammatory effects, as well as other pharmacological actions[3] .

Oxidative stress is associated with the formation of reactive oxygen species (ROS) and free radicals capable of interacting with biomolecules. When the generation of these reactive species exceeds the capacity of endogenous antioxidant systems, oxidative damage may occur. Consequently, the search for natural sources of antioxidant compounds has become a significant area of research, particularly given that plant extracts contain a wide array of compounds capable of interacting with free radicals [4].

Phenolic compounds are among the most extensively studied plant constituents; many phenolic molecules possess the ability to donate hydrogen atoms or electrons to reactive species. Through these interactions, unstable free radicals can be converted into more stable chemical species, thereby limiting the propagation of oxidative reactions. Thus, the antioxidant capacity of a plant extract is influenced by the concentration and nature of the phenolic compounds and other antioxidant constituents present within it. The *Ginkgo* plant is a subject of significant scientific interest due to its complex chemical composition; identifying its chemical constituents is a crucial step in understanding the relationship between its chemical profile and biological activity. Since plant extracts typically contain numerous compounds, analytical techniques capable of separating and identifying individual components are required[1].

Gas chromatography-mass spectrometry (GC-MS) is a key analytical technique for studying volatile and semi-volatile organic compounds. Gas chromatography (GC) enables the separation of compounds based on their interactions with the chromatographic column and their physicochemical properties, while mass spectrometry (MS) provides distinctive mass spectra used for compound identification. Combining these two techniques allows for the effective analysis of the chemical profile of complex plant samples[5] Compound identification via GC-MS typically involves evaluating various parameters—such as retention time, chromatographic peak area, area percentage, and spectral match quality—and determining chemical identity through comparison with reference spectra or standard materials. In this study, GC-MS analysis enabled the identification of twelve chemical constituents in the

analyzed *Ginkgo* sample. The identified compounds belonged to diverse chemical classes, including phenolic compounds, fatty acid derivatives, esters, hydrocarbons, sulfones, siloxane derivatives, and an anthracene derivative. This chemical diversity indicates that the analyzed plant sample contains a complex mixture of organic constituents [6].

Among the identified compounds was 2,4-di-tert-butylphenol, which exhibited a retention time of 18.376 minutes, a peak area of 1,412,553, an area percentage of 4.28%, and a match quality of 93. This compound was identified by comparing its mass spectrum with a reference standard, and its significance as an antioxidant among the detected compounds was highlighted[6] .

Other compounds detected in the sample included methyl hexadecanoate, methyl stearate, and 3-tridecylphenol. Additionally, unsaturated phenolic compounds were identified, such as (Z)-3-(pentadec-8-en-1-yl)phenol, (E)-3-(pentadec-8-en-1-yl)phenol, and (Z)-3-(heptadec-10-en-1-yl)phenol. The latter compound showed the highest area percentage among the identified compounds, at 27.31%.

The detection of phenolic compounds is of particular importance when studying antioxidant activity. However, the presence of a single compound in the gas chromatography-mass spectrometry (GC-MS) chromatogram does not, in itself, prove that this compound is responsible for the total antioxidant activity of the plant extract; Given that the sample contains diverse chemical constituents, the observed antioxidant response may result from the combined contribution of several compounds.

The DPPH radical scavenging assay is a method[7].

2. MATERIALS AND METHODS

2.1 Plant Material

A sample of *Ginkgo* seeds was used for chemical analysis and the evaluation of antioxidant activity; the plant material was prepared prior to analysis in accordance with established laboratory procedures.

2.2 GC-MS Analysis

The chemical constituents of the *Ginkgo* sample were examined using gas chromatography-mass spectrometry (GC-MS).

Compound identification was based on chromatographic and mass spectrometric characteristics. Key parameters recorded during the analysis included retention time (RT), peak area, area percentage (Area%), Chemical Abstracts Service (CAS) number, and match quality.

Compound identification was supported by comparing mass spectra with reference spectra or standard materials; a total of twelve chemical compounds were identified in the analyzed sample.

Table 1. Chemical compounds identified in the *Ginkgo* sample by GC–MS

No	RT (min)	Area (Ab*s)	Area%	Name	Quality	CAS Number
1	18.376	1412553	4.28	2,4-Di-tert-butylphenol	93	000096-76-4
2	25.978	1917798	5.81	Hexadecanoic acid, methyl ester	95	000112-39-0
3	29.091	1616492	4.89	Methyl stearate	98	000112-61-8
4	31.275	1764518	5.34	3-Tridecylphenol	80	072424-02-3
5	33.735	3209557	9.72	(Z)-3-(pentadec-8-en-1-yl)phenol	96	000501-26-8
6	33.875	3729010	11.29	(E)-3-(pentadec-8-en-1-yl)phenol	91	000501-26-8
7	36.49	9021700	27.31	(Z)-3-(Heptadec-10-en-1-yl)phenol	96	111047-33-7
8	37.751	1147342	3.47	Di-n-decylsulfone	46	111530-37-1
9	40.703	1380087	4.18	Methyltris(trimethylsiloxy)silane	43	017928-28-8
10	41.435	1765227	5.34	Eicosane	53	000112-95-8
11	45.97	1944679	5.89	Cyclotrisiloxane, hexamethyl-	35	000541-05-9
12	49.82	4122507	12.48	Anthracene, 9,10-dihydro-9,9,10-trimethyl-	27	014923-29-6

The values in Table 1 were transcribed from the GC–MS results supplied in the original document.

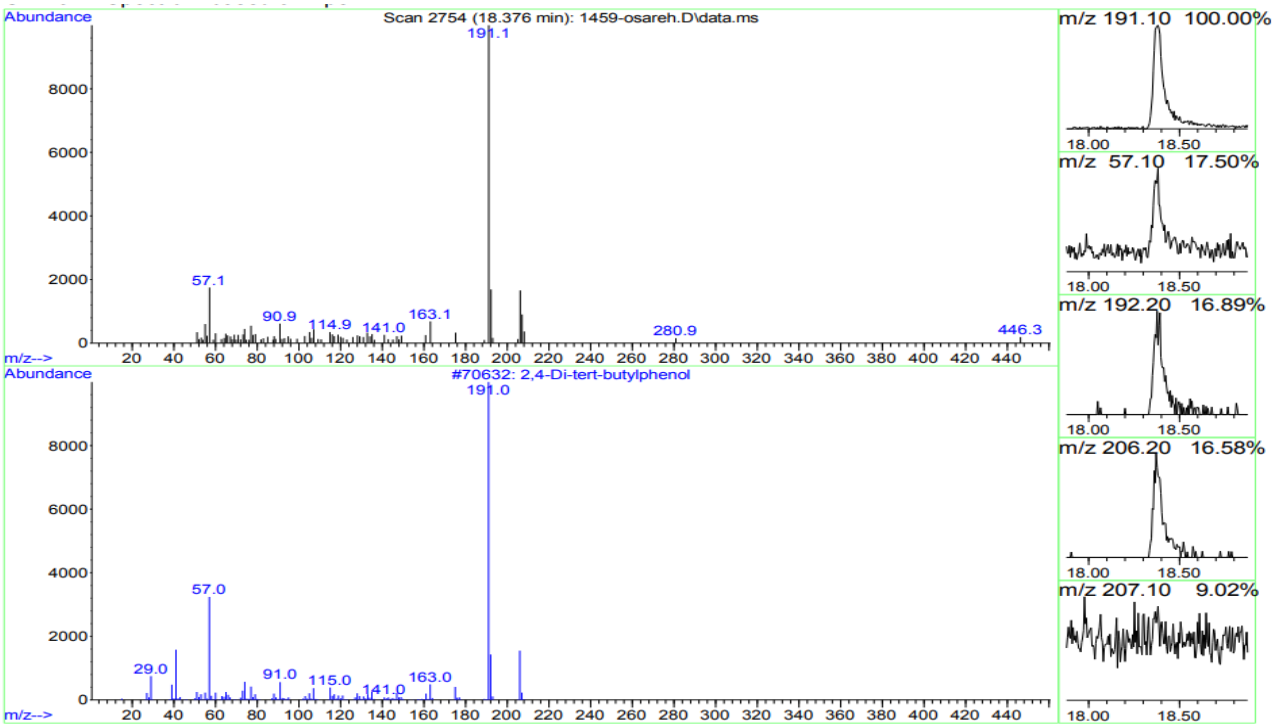

Figure 1. GC–MS mass spectrum of 2,4-Di-tert-butylphenol.

2.3 DPPH Radical-Scavenging Assay

The antioxidant activity of the *Ginkgo* sample was evaluated using the DPPH radical-scavenging method[8].

Four sample concentrations were investigated:

- 0.12 mg/mL
- 0.25 mg/mL
- 0.50 mg/mL
- 1.00 mg/mL

The absorbance values and corresponding radical-scavenging percentages obtained from the supplied experimental data are presented in Table 2.

Table 2. DPPH radical-scavenging activity of the *Ginkgo* sample

sample name	concentration	absorbency	scavenging %
1	0.12 mg/ml	0.2895	72.365
2	0.25 mg/ml	0.2686	74.360
3	0.5 mg/ml	0.2033	80.594
4	1 mg/ml	0.1866	82.188
	control	1.0476	

The original document records these measurements and graphically displays their trend as a function of concentration.

3. RESULTS AND DISCUSSION

3.1 GC–MS Chemical Profile

Gas chromatography-mass spectrometry (GC-MS) analysis revealed that the *Ginkgo* sample under study contained a chemically diverse mixture comprising twelve identified compounds. These compounds varied significantly in terms of retention time, chromatographic peak area, area percentage, and match quality.

The chromatogram was characterized by the presence of numerous phenolic compounds. Among them, 2,4-di-tert-butylphenol was identified, exhibiting an area percentage of 4.28% and a match quality of 93; this relatively high match quality supports the proposed identification of the compound.

The sample also contained several lipid-like compounds, including methyl hexadecanoate and methyl stearate. The detection of these compounds indicates the presence of fatty acid-derived constituents in the analyzed sample.

Additionally, several alkyl-substituted phenolic compounds were detected. Notably, the compounds (Z)-3-(pentadec-8-en-1-yl)phenol and (E)-3-(pentadec-8-en-1-yl)phenol showed area percentages of 9.72% and 11.29%, respectively. The compound (Z)-3-(heptadec-10-en-1-yl)phenol recorded the highest area percentage among the identified components, reaching 27.31%. The detection of these phenolic compounds is particularly significant, given that phenolic structures can contribute to free-radical scavenging activity. However, data derived from GC-MS analysis should be interpreted primarily as evidence of the sample's chemical composition, rather than as direct evidence of the biological activity of individual compounds.

3.2 Identification of 2,4-Di-tert-butylphenol

The identification of 2,4-di-tert-butylphenol stands out as a key result of the chemical analysis; the compound exhibited a retention time of 18.376 minutes, a peak area of 1,412,553, an area percentage of 4.28%, and a match quality score of 93.

The study indicates that this identification was based on comparing the mass spectrum with a reference standard, providing more robust evidence of the compound's identity than relying solely on retention time.

It is worth noting that the phenolic nature of 2,4-di-tert-butylphenol makes its detection relevant to the assessment of antioxidant activity. However, given the simultaneous detection of other compounds, the antioxidant activity observed in the DPPH assay cannot be attributed exclusively to this compound without further isolation and individual testing.

3.3 Antioxidant Activity

The DPPH assay demonstrated measurable antioxidant activity at all four concentrations studied. The lowest concentration tested (0.12 mg/mL) exhibited a free radical scavenging activity of 72.365%. Increasing the concentration to 0.25 mg/mL resulted in a moderate rise in activity to 74.360%.

A more pronounced increase was observed at a concentration of 0.50 mg/mL, where scavenging activity reached 80.594%. The highest concentration studied (1 mg/mL) demonstrated the greatest activity, achieving a free radical scavenging rate of 82.188% .

Conversely, the corresponding absorbance values showed an inverse trend; absorbance decreased from 0.2895 at 0.12 mg/mL to 0.2686, 0.2033, and 0.1866 at concentrations of 0.25, 0.50, and 1 mg/mL, respectively.

This inverse relationship between absorbance and free radical scavenging activity aligns with the general principle of the DPPH assay: as antioxidant components react with DPPH radicals, the measured absorbance decreases, leading to an increase in the calculated scavenging percentage.

The relatively high scavenging rates observed across the tested concentrations indicate that the *Ginkgo* sample under study possesses significant free radical scavenging capacity under the applied experimental conditions

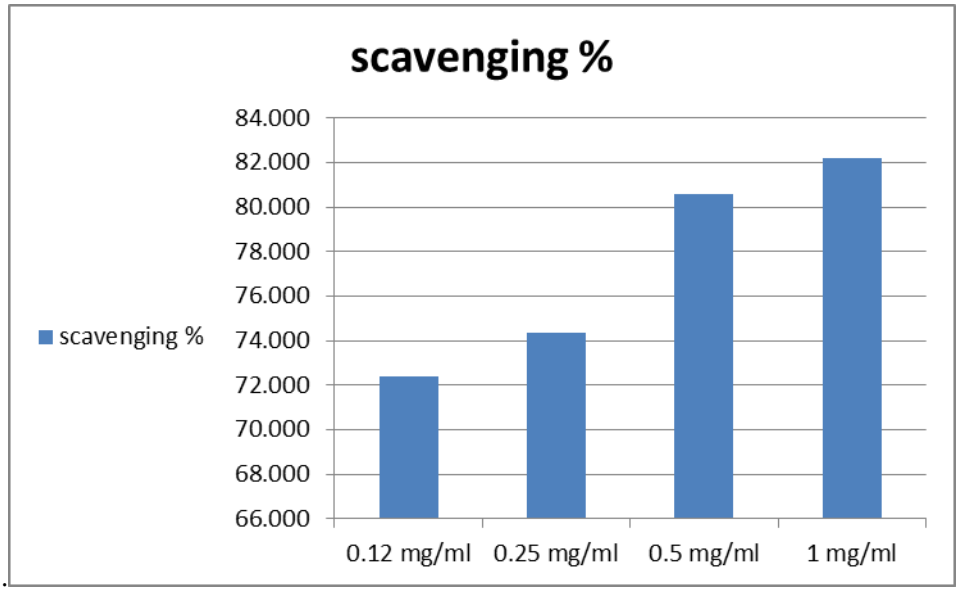

Figure 2. DPPH radical-scavenging activity of the Ginkgo sample at different concentrations.

3.4 Concentration-Dependent Antioxidant Response

The graph included in the original document shows a gradual increase in DPPH free radical scavenging activity as the sample concentration increases; the activity rose from approximately 72% at a concentration of 0.12 mg/mL to over 82% at 1 mg/mL.

This increase can be logically attributed to the greater availability of antioxidant components at higher sample concentrations, as elevated concentrations provide a larger number of antioxidant molecules capable of reacting with DPPH radicals.

However, the range of concentrations studied does not allow for a determination of whether the maximum antioxidant response has been reached. Since the highest concentration tested exhibited the highest measured activity, further studies at additional concentrations are required to determine whether the activity eventually reaches a plateau (steady state).

3.5 Relationship between chemical composition (determined by GC-MS) and antioxidant activity

The results from the GC-MS and DPPH techniques provide complementary evidence regarding the chemical and biological properties of the analyzed *Ginkgo* sample.

GC-MS analysis revealed the presence of various phenolic compounds and other organic compounds, while the DPPH test demonstrated significant free radical scavenging activity. The detection of phenolic compounds offers a plausible chemical basis for explaining the observed antioxidant activity, at least in part.

Of particular note is the presence of the compound 2,4-di-tert-butylphenol; The study classifies it as an active component and indicates that it was identified by comparing mass spectra against a reference standard.

However, the antioxidant activity should not be attributed solely to the compound 2,4-di-tert-butylphenol; the sample contained multiple components—both phenolic and non-phenolic—and interactions between these compounds may contribute to the overall antioxidant response.

Furthermore, the area percentage (% area) obtained via GC-MS analysis represents a relative chromatographic response and should not be interpreted as an absolute concentration unless quantitative calibration using appropriate standards is performed. 4. Conclusion

This study demonstrated that the analyzed *Ginkgo* sample contains a diverse range of chemical constituents. GC-MS analysis enabled the identification of twelve compounds belonging to various chemical classes, including phenolic compounds, fatty acid derivatives, esters, hydrocarbons, sulfonates, siloxanes, and an anthracene derivative. Among the identified components, 2,4-di-tert-butylphenol was detected with an area percentage of 4.28% and a match quality of 93.

The DPPH assay demonstrated significant antioxidant activity at all concentrations tested; The free radical scavenging activity increased from 72.365% at a concentration of 0.12 mg/mL to 82.188% at 1 mg/mL, accompanied by a decrease in absorbance from 0.2895 to 0.1866.

Overall, the results indicate that the studied *Ginkgo* sample possesses significant free radical scavenging capacity under the applied experimental conditions. Furthermore, GC-MS analysis reveals the presence of several compounds that may contribute to its biological properties. However, further research—including compound isolation, quantitative analysis, supplementary antioxidant assays, and individual testing of specific components—is required to pinpoint the specific compounds responsible for the observed antioxidant activity.

4. RECOMMENDATIONS

1. Quantitative analysis of the identified major compounds should be conducted using standard reference materials and calibration curves.
2. Major phenolic compounds should be isolated, and their antioxidant activity evaluated individually.
3. Additional antioxidant assays, such as ABTS and FRAP, should be performed to corroborate and confirm the results obtained via the DPPH method.
4. The DPPH assay should be repeated using independent replicates to enable statistical analysis.
5. A wider range of concentrations should be investigated to determine whether the antioxidant response reaches a plateau.
6. More comprehensive chemical characterization, using complementary analytical techniques, can provide a more complete chemical profile of a *Ginkgo* sample.

5. CONCLUSION

This study presents a comprehensive chemical and functional characterization of the analyzed *Ginkgo* sample, combining a metabolite profile obtained via gas chromatography-mass spectrometry (GC-MS) with an assessment of DPPH free radical scavenging capacity. GC-MS analysis revealed a chemically diverse matrix comprising twelve identified compounds belonging to various structural classes; phenolic compounds constitute a prominent and significant portion of this chemical profile. The presence of a diverse array of phenolic constituents and lipid derivatives highlights the sample's chemical complexity and provides a plausible molecular basis for the observed antioxidant activity.

Notably, the compound 2,4-di-tert-butylphenol was identified with a spectral match index of 93 and a relative chromatographic area of 4.28%, whereas (Z)-3-(heptadec-10-en-1-yl)phenol exhibited the highest relative chromatographic contribution among the identified components (27.31%). These results confirm the presence of structurally diverse phenolic constituents in the analyzed sample. However, the antioxidant response—in terms of mechanism—cannot be attributed to any single compound based solely on its detection by GC-MS or its relative peak area; Rather, the observed activity is more accurately interpreted as a collective response

resulting from the extract's chemical composition, potentially involving additive, synergistic, or antagonistic interactions among its constituents.

The DPPH test results demonstrated a clear, concentration-dependent free radical-scavenging response; activity increased from 72.365% (at a concentration of 0.12 mg/mL) to 82.188% (at 1.00 mg/mL), accompanied by a gradual decrease in absorbance from 0.2895 to 0.1866. The consistency of this inverse relationship between absorbance and scavenging percentage provides empirical evidence of significant DPPH radical-neutralizing capacity under the studied conditions. It is important to note that the progressive increase in activity across the tested concentration range suggests that the antioxidant response did not necessarily reach a plateau at the highest concentration examined; consequently, the current data do not allow for the determination of maximum scavenging capacity or the establishment of a definitive limit for the concentration-response relationship. Overall, the results from the GC-MS and DPPH techniques confirm a consistent correlation between the chemical complexity of the analyzed *Ginkgo* sample and its measurable *in vitro* free radical-scavenging capacity. However, this correlation should be viewed as an association rather than a direct causal relationship. Specifically, the relative peak areas obtained via GC-MS provide information regarding chemical composition but do not constitute absolute quantitative measurements; At the same time, the DPPH assay represents a specific chemical model for free radical scavenging and should not be viewed in isolation as definitive evidence of biological or therapeutic efficacy. Consequently, the current results provide a chemical and functional basis for future research, rather than offering conclusive attribution of antioxidant activity to specific molecular constituents.

Therefore, future studies should shift from qualitative to quantitative and mechanistic characterization. Quantifying key constituents using reliable reference standards and appropriate calibration procedures—followed by the isolation of significant phenolic compounds and the individual assessment of their biological activity—would enable the establishment of more robust relationships between chemical composition and biological activity. Furthermore, employing complementary antioxidant assays (such as ABTS and FRAP), alongside independent experimental replicates and appropriate statistical analysis, would further enhance result reproducibility and the accuracy of biological interpretation. Additionally, expanding the range of concentrations tested would help elucidate the concentration-response relationship and determine whether the activity reaches a plateau or a maximum level.

In summary, these results support the view of the analyzed *Ginkgo* sample as a chemically complex natural source that demonstrates significant *in vitro* free radical scavenging capacity in the DPPH assay. Most importantly, the study highlights the value of combining characterization via chromatography and mass spectrometry with the functional assessment of antioxidant activity; this represents a primary strategy for linking a plant's chemical composition to its biological activity. There remains a need for comprehensive research—characterized by quantitative and instrumental approaches and relying on compound isolation—to identify the key bioactive constituents and determine the extent to which the observed antioxidant properties can be translated into...

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