
**In Vitro Antioxidant Activity of Fruits of
*Salix alba***

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INTRODUCTION

Salix alba L., commonly known as white willow, is a deciduous tree native to Europe (Jack 1997; Mahdi et al. 2006). The white willow (*Salix alba*) has long been known for its therapeutic qualities, especially because of its high polyphenolic and flavonoid content. Thanks to active compounds such as salicin, salicylic acid, and acetylsalicylic acid found in willow, this plant has a wide range of pharmaceutical applications. The pharmacological effect of aspirin was revealed after its ability to inhibit prostaglandin (PG) synthesis was demonstrated. Studies have also shown that aspirin, known for its analgesic properties, reduces the risk of developing colorectal cancer (Mahdi et al. 2006).

Due in major part to its main ingredient, salicin, *Salix alba* has long been utilized for its analgesic, antipyretic, and anti-inflammatory properties. Its strong antioxidant activity, which is essential for reducing oxidative stress-related illnesses, has been highlighted by recent research that has broadened its pharmacological profile (Shivatare et al. 2021; Julkunen-Tiitto and Maeier, 1992).

It belongs to the Salicaceae family and has been widely used in traditional medicine. Numerous chronic illnesses, including as cancer, neurological diseases, and cardiovascular problems, are linked to oxidative stress, which is brought on by an imbalance between free radicals and antioxidants. Plant-based natural antioxidants have drawn a lot of interest because of their capacity to improve cellular defense systems and neutralize reactive oxygen species (ROS). The antioxidant activity of *Salix alba* fruits is enhanced by the presence of bioactive substances such flavonoids, phenolic acids, and tannins. Research has demonstrated that antioxidant phytochemicals are essential for preserving cellular health and for preventing and curing illnesses brought on by oxidative stress (Jomova et al. 2023; Shara and Stohs 2015).

In this study, the antioxidant capacity of *Salix alba* fruits was comprehensively evaluated using both DPPH and ABTS radical scavenging assays to assess their free radical neutralizing potential. Furthermore, the chemical composition of the extracts was thoroughly characterized through gas chromatography–mass spectrometry (GC-MS) analysis to identify the bioactive constituents responsible for the observed pharmacological activities. The results demonstrated that *Salix alba* fruits exhibit significant antioxidant activity, effectively scavenging free radicals in vitro. These findings support the potential use of *S. alba* as a natural source of antioxidants in pharmaceutical and nutraceutical formulations.

1. MATERIAL AND METHODS

1.1. Obtaining Extracts and Chemical Composition

The fruits of *Salix alba* were harvested from the natural flora of Sivas. After collection, the samples were oven-dried and subsequently ground into a fine powder using a laboratory mill. The powdered plant material was subjected to maceration with 80% ethanol. Following 24 hours of continuous shaking, the mixture was filtered to separate the plant residues, and the filtrate was evaporated by oven drying to obtain the crude extracts. These extracts were then analyzed using GC-MS to identify their chemical constituents and to determine their relative abundances, as described by Sacchetti et al. (2005).

1.2. Biological Activity Evaluation

1.2.1. *In vitro* antioxidant activity

The antioxidant properties of the extract were assessed through various spectrophotometric assays. The DPPH radical scavenging capacity was measured following a modified version of the Blois

protocol (1958), while the ABTS assay was performed using a slightly adapted method originally described by Re et al. (1999). Total phenolic content was quantified based on the procedure developed by Clarke et al. (1993), and results were expressed as gallic acid equivalents (GAE). Additionally, total flavonoid content was determined through the aluminum chloride colorimetric technique described by Molan and Mahd (2014), with outcomes reported as catechin equivalents per gram of dry extract.

2. RESULTS AND DISCUSSION

2.1. The Chemical Composition

The GC-MS analysis of the *S. alba* fruits extract revealed the presence of 18 distinct compounds, accounting for 77.14% of the total chromatographic area (Table 1). The chemical profile of the extract comprised various fatty acid esters, long-chain alkanes and alcohols, phenolic compounds, and bioactive amides. Notably, 1,2-Benzenedicarboxylic acid, bis(8-methylnonyl) ester (17.64%), n-Heneicosane (10.18%), 1-Heptacosanol (6.37%), Oleamide (5.74%), and Cyclododecane (5.30%) were detected as the major constituents. Additionally, methyl and ethyl esters of fatty acids such as hexadecanoic acid, oleic acid, and ethyl linoleate, which are widely recognized for their antioxidant and antimicrobial activities, were also identified in considerable amounts.

Table 1: Chemical components of 80% ethanol extracts of fruit of *Salix alba*

Peak no	Retention time	%Area	Compound name
1	28.281	1,44	n-Tridecanol
2	29.391	1,00	2,4-Di-tert-butylphenol
3	33.482	5,30	Cyclododecane
4	33.888	2,44	Cyclohexane, 1,5-diethenyl-3-methyl-2-methylene-, (1.alpha.,3.alpha.,5.alpha.)
5	35.628	2,52	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one
6	37.739	2,40	Hexadecanoic acid, methyl ester
7	38.861	3,24	Hexadecanoic acid, ethyl ester
8	40.554	2,03	Oleic acid, methyl ester
9	41.476	4,59	Ethyl linoleate
10	41.579	2,63	Ethyl 9,12,15-octadecatrienoate
11	43.398	2,52	Eicosane
12	44.411	5,74	Oleamide
13	44.714	1,15	Eicosanoic acid, ethyl ester
14	47.426	1,41	Docosanoic acid, ethyl ester
15	48.737	6,37	1-Heptacosanol
16	49.183	10,18	n-Heneicosane
17	52.124	4,54	Oxalic acid, allyl hexadecyl ester
18	52.588	17,64	1,2-Benzenedicarboxylic acid, bis(8-methylnonyl) ester
19	Total	77,14	

The detection of 2,4-Di-tert-butylphenol (1.00%), a phenolic compound with strong antioxidant activity, further supports the bioactive potential of the extract. Overall, the findings suggest that *Salix alba* harbors a diverse array of biologically active compounds, indicating its potential for applications in the pharmaceutical, cosmetic, and nutraceutical industries (Table 1). Flavonoids, phenolic acids and phenolic glycosides, are predominantly found in the leaves of *Salix*

species, whereas the stem bark is particularly rich in procyanidins (Tawfeek et al. 2021).

3.1. *In vitro* Antioxidant Activity

3.1.1. DPPH Radical Scavenging Activity (%)

The antioxidant activity results of *S. alba* fruit extract was tested by DPPH radical scavenging method was given in Figure 1.

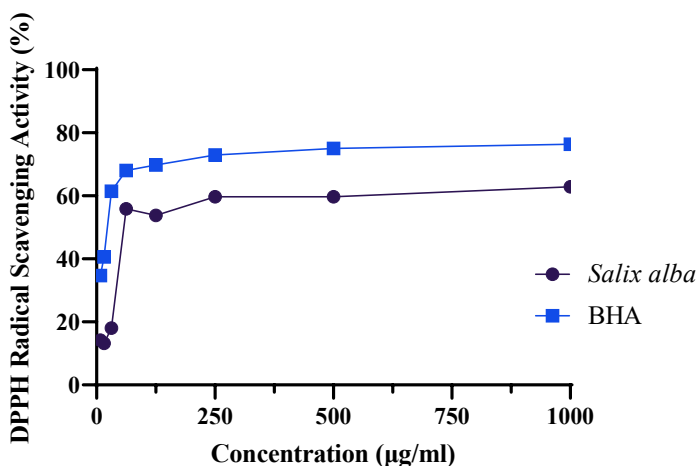


Figure 1: DPPH radical scavenging activity of 80% ethanol extract of *Salix alba* fruits

The DPPH radical scavenging assay was performed to evaluate the antioxidant potential of the *Salix alba* extract in comparison with the synthetic antioxidant BHA (butylated hydroxyanisole). As shown in the graph, both samples exhibited a concentration-dependent increase in DPPH radical scavenging activity. However, BHA consistently demonstrated stronger radical scavenging activity across all concentrations, reaching a plateau above 80%, while *Salix alba* showed a maximum inhibition of around 65%. The IC_{50} value for *Salix alba* was determined to be 2.129 ± 2.379 µg/mL, which is higher than that of BHA

($1.293 \pm 1.646 \mu\text{g/mL}$), indicating that a higher concentration of the extract is required to achieve 50% inhibition of DPPH radicals.

The observed antioxidant activity can be attributed to the presence of phenolic compounds and fatty acid esters identified through GC-MS analysis. Compounds such as 2,4-di-tert-butylphenol, a well-known phenolic antioxidant, and oleamide, which also exhibits radical scavenging activity, likely contribute significantly to the extract's activity. Additionally, the presence of methyl and ethyl esters of hexadecanoic acid and oleic acid, known for their moderate antioxidant effects, may support the overall radical scavenging performance of the extract. Although the extract does not outperform the synthetic antioxidant BHA, it demonstrates considerable natural antioxidant potential, likely due to the synergistic action of multiple phytochemical constituents. These results suggest that *Salix alba* could be a promising source of natural antioxidants for applications in functional foods, cosmetics, or therapeutic formulations.

3.1.2. ABTS Radical Scavenging Activity (%)

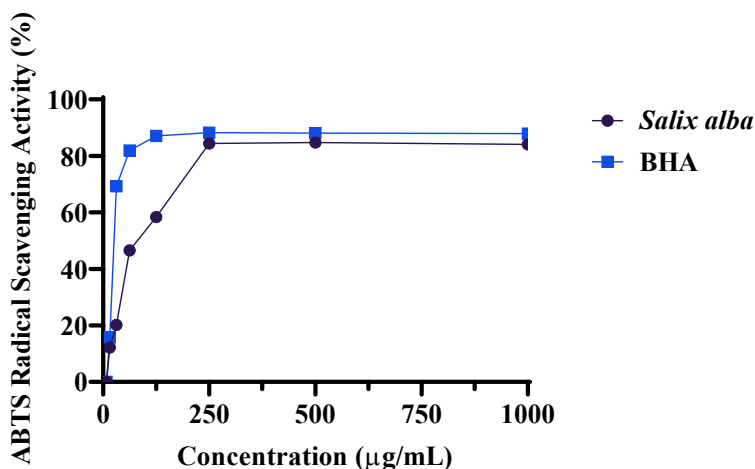


Figure 2: ABTS radical scavenging activity of 80% ethanol extract of *Salix alba* fruits

The ABTS radical scavenging assay was employed to assess the antioxidant potential of the *Salix alba* extract compared to the standard synthetic antioxidant, BHA. Both samples exhibited a rapid increase in radical scavenging activity with increasing concentration, reaching a plateau phase at relatively low concentrations. BHA achieved over 90% inhibition at lower concentrations, whereas *Salix alba* required slightly higher concentrations to attain similar levels of activity. The IC₅₀ value of *Salix alba* was calculated as 1.890 µg/mL, which is slightly higher than that of BHA (1.420 µg/mL) (Figure 2). This indicates that although BHA remains more potent, the *Salix alba* extract also exhibits strong radical scavenging efficiency. Notably, both IC₅₀ values fall within a low micromolar range, underscoring the extract's considerable antioxidant capacity. The Hill slope values, 4.949 for *Salix alba* and 6.737 for BHA, suggest a steep dose-response curve for both samples. Such high slope values imply a strong cooperative effect, where small increases in concentration lead to a sharp rise in antioxidant activity.

These findings are consistent with the previously observed DPPH results and further support the contribution of bioactive constituents identified via GC-MS analysis, including 2,4-di-tert-butylphenol, oleamide, and fatty acid esters such as ethyl linoleate and hexadecanoic acid derivatives. These compounds are known for their electron-donating ability, enabling them to effectively neutralize ABTS radicals.

In contrast to this finding, other research has shown that *Salix alba* extracts contains substantial levels of flavonoids and polyphenols, which support its antioxidant qualities. Its rather high IC₅₀ value is probably explained by the presence of these bioactive chemicals. However, the antioxidant potential of plant extracts can be impacted by differences in extraction techniques, solvent systems, and ambient conditions.

Synthetic antioxidants, such as BHA, on the other hand, are made to be highly stable and effective, frequently exceeding natural extracts in

controlled tests. Nevertheless, because of their biocompatibility and lower toxicity concerns, natural antioxidants like *Salix alba* are favored in pharmaceutical and nutraceutical applications. *Salix alba* extracts have been shown to be capable of scavenging free radicals in vitro, especially using tests like hydroxyl radical inhibition, reducing power, and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging (Sulaiman et al., 2013). According to these results, the fruits of *S. alba* may be a useful natural antioxidant source that might be used in pharmaceutical and nutraceutical compositions. This chapter explores the antioxidant properties of *Salix alba* fruits, focusing on their phytochemical composition, mechanisms of action, and potential therapeutic applications.

3.1.3. TFC (Total Flavonoid Content) and TPC (Total Phenol Content)

The total phenol and total flavonoid content of ethanol extract from *Salix alba* fruits are presented in Figure 3.

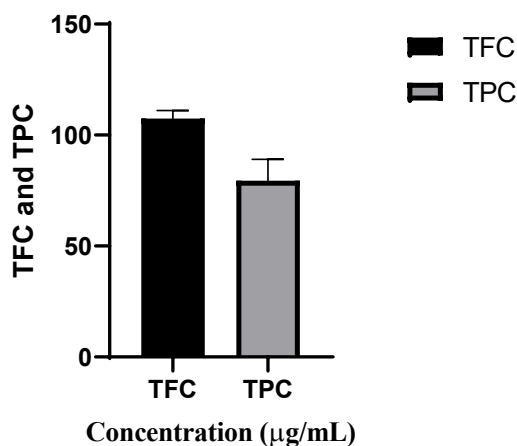


Figure 3: TPC and TFC of ethanol extracts of Fruits of *Salix alba*

The total flavonoid content (TFC) and total phenolic content (TPC) of the *Salix alba* fruit extract were quantitatively assessed and expressed in $\mu\text{g/mL}$. The results revealed that the TFC value ($107.48 \pm 3.55 \mu\text{g/mL}$) was significantly higher than the TPC value ($79.38 \pm 9.63 \mu\text{g/mL}$), indicating that flavonoids constitute a major portion of the phenolic compounds in the extract. This relatively high flavonoid concentration suggests that flavonoids may play a prominent role in the antioxidant activity of *Salix alba*, as observed in both DPPH and ABTS assays. Flavonoids such as quercetin, kaempferol, and their glycosides are known to possess strong radical scavenging properties due to their hydroxyl groups and conjugated structures, which enable them to donate electrons or hydrogen atoms to neutralize free radicals (Rice-Evans et al. 1996; Ferrali et al. 1997; Heim et al. 2002).

This aligns with previous studies that have examined the phenolic composition of *Salix alba* and related species. For instance, research on *Salix alba* leaves and bark has identified a substantial presence of flavonoids, particularly flavanols and procyanidins, which contribute to the plant's antioxidant properties (Piątczak et al. 2020). Another study reviewing phenolic compounds across different *Salix* species highlights the prevalence of flavonoids as key bioactive components (Petruk, 2019). Additionally, quantitative analysis of various *Salix* species has shown that flavan-3-ols and flavonols are among the most abundant phenolic compounds, reinforcing the idea that flavonoids play a dominant role in the plant's phytochemical profile (Curtasu et al., 2024).

Moreover, the substantial levels of both TFC and TPC are consistent with the presence of phenolic compounds such as 2,4-di-tert-butylphenol identified in the GC-MS analysis. These findings collectively support the antioxidant potential of *Salix alba* and highlight its value as a natural source of polyphenolic antioxidants, which could be beneficial in pharmaceutical and nutraceutical applications.

4. CONCLUSION

In summary, *Salix alba* demonstrated potent ABTS and DPPH radical scavenging effects, comparable to that of BHA, confirming its potential as a natural antioxidant source suitable for use in pharmaceutical, cosmetic, and food formulations.

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