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# PHYTOCHEMICAL PROFILING AND *IN VITRO* ANTIOXIDANT POTENTIAL OF *PRUNUS PERSICA* SEED EXTRACT: AN LC-MS AND GC-MS BASED INVESTIGATION

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**Abstract:**
Natural products are a significant resource for bioactive compounds that could have antioxidant activity. *Prunus persica* (L.) These are rich in various phytochemicals, which could account for their biological activities, but there is a lack of detailed information on the chemical composition and antioxidant properties of these seeds. The objective of the present work was to study in detail the phytochemical profile and *in vitro* antioxidant activity of *Prunus persica* seed extract by chromatographic and antioxidant evaluation techniques. The unsaponifiable fraction was obtained through Soxhlet extraction and saponification of *Prunus persica* seeds followed by liquid–liquid fractionation. Thin-layer chromatography (TLC) was used for preliminary phytochemical characterization. The phytochemical profile of the extract was comprehensively analyzed using liquid chromatography coupled with mass spectrometry (LC–MS) and gas chromatography–mass spectrometry (GC–MS). The antioxidant potential was subsequently determined employing DPPH, Ferric Reducing Antioxidant Power (FRAP) and ABTS radical scavenging assay with ascorbic acid serving as the reference antioxidant. 12 phytoconstituents (neohesperidin dihydrochalcone, synephrine, bilobalide, tetrahydropalmatine, sclareol) were tentatively identified by LC–MS analysis. The results showed eighteen compounds with varying chemical classes through it was found using GC–MS profiling, which includes phenolic derivatives, esters and fatty acid-related constituents. The extract showed low levels of DPPH radical scavenging activity and strong ferric reducing activity in FRAP assay. The antioxidant activity of the extract was also confirmed by the ABTS assay. The activity observed may be due to the synergistic effects of several bioactive phytochemicals found in the plant by chromatographic analysis. The results revealed that the extract from *Prunus persica* seeds contains significant antioxidant activity and is rich in phytochemically diverse constituents. The findings give a scientific basis for further investigations to isolate the active compounds and to assess the importance of the biological aspects of oxidative stress related diseases.

**KEYWORDS:** *Prunus persica;* Peach seed; LC–MS; GC–MS; Phytochemical profiling; Antioxidant activity; DPPH; FRAP; ABTS; Natural products

## INTRODUCTION

Digital transformation has changed the healthcare landscape around the globe. Mobile health (mHealth) technologies
Oxidative stress has been recognized as a key determinant of the pathogenesis and progression of various metabolic and endocrine diseases. An excess amount of reactive oxygen species (ROS) may result in loss of cellular homeostasis, lipid peroxidation, protein oxidation, and impaired physiological functions.[1] It is becoming clear that natural products that enriches in bioactive phytochemicals can be protective against oxidative damage, leading to their ability to inhibit production of free radicals and to have reducing properties [2,3] Medicinal plants thus have been a topic of great interest as a source of antioxidant compounds of therapeutic relevance. Of these, the *Prunus persica* (L.) is the most significant. Several *in vitro* antioxidant assays have been reported to estimate the antioxidant potential of plant extracts such as evaluation of antioxidant activity through multiple *in vitro* assays. The combination of DPPH, FRAP and ABTS assays allows a fuller evaluation of the antioxidant activity since the mechanism of action for the different reactive species and reaction pathways could be different [2]. In spite of the study of *Prunus persica* for different pharmacological activities, there are scanty data about phytochemical configuration and antioxidant efficacy of the unsaponifiable fraction obtained from the seeds. In addition, there are few studies that combine chromatographic characterization with antioxidant assessment. Identification of bioactive constituents with antioxidant activity is crucial for elucidating the therapeutic potential of this plant and for further pharmacological studies. Hence, the existing work was designed to analyze the phytochemical composition of *Prunus persica* seed extract by LC–MS and GC–MS and its antioxidant activity was assessed by radical DPPH, FRAP and ABTS assays. The results of this study could be useful in the expanding knowledge about antioxidants derived from plants, and could serve as a scientific foundation for continued biological and pharmacological research into these plants. [4–6]. All plant parts have been used traditionally in traditional medicine to

control inflammatory and metabolic disorders. In spite of its ethnopharmacological significance, however, detailed phytochemical analysis of extracts of *Prunus persica* seeds and their antioxidant activity is still not comprehensive. Thus, systematic investigation of its chemical profile and antioxidant activity is warranted, which would help in providing greater basis for its potential biological applications [7–9]

The seed of *Prunus persica* is an under-exploited plant material that is rich in biologically active substances with therapeutic applications. Previous phytochemical studies have established the presence of fatty acids, phytosterols, phenolics, flavonoids, terpenoids and other secondary metabolites in various parts of the plant [6]. Many of such compounds are linked to antioxidant, suppressing inflammation and metabolic regulatory assets, which could be related to the traditional therapeutic properties of the plant. The unsaponifiable fraction after saponification and solvent partitioning procedures is particularly rich in lipophilic seed constituents which can be concentrated. These phytochemicals can be identified and characterized by advanced analytical methods, such as LC–MS and GC–MS, which will aid to understand the chemical diversity and potential biological relevance of these compounds [4]. Profiling of phytochemical components is thus important for establishing quality control parameters and for determining possible bioactive compounds that may be accountable for the therapeutic action of the extracts of *Prunus persica* seeds.

2Materials and methods

**2.1 Collection of plant material and authentication**

Seeds of the *Prunus persica* (L.) were obtained from local sources of Moradabad, Uttar Pradesh, India during the harvesting spell. The collected seeds were properly cleaned to get rid of any foreign materials and impurities which might be present on them. The endocarp was removed from the kernels and they were dried under shade at room temperature.

The dried kernels were powdered using a mechanical grinder to get coarse powder and placed in airtight containers, away from the moisture and light till use. The plant part was identified by a taxonomist and a voucher sample was kept for future reference in herbarium of the plant source institution. Authentications were made using the normal taxonomic features and published floristic literature. The authentic plant material was then used to extract, analyze for phytochemicals, chromatograph and test for antioxidant activity.

**2.2 Preparation of *Prunus persica* Seed Extract**

The collected seeds of *Prunus persica* were cleaned and the endocarp was removed from the nucleus. The kernels were grounded coarsely in a mechanical pounder. The powdered material was extracted by Soxhlet extraction and the lipid fraction was obtained using n-hexane as an extraction solvent. Extraction continued until the plant material was exhausted (when the siphon tube was colorless). The filtered concentrated extract obtained was dried under reduced pressure to give a crude seed extract. [10,11]

The crude extract was treated with 2 N alcoholic KOH in order to enrich the phytosterol fraction. The mixture was heated for 1 hour with stirring at 60℃ to hydrolyze the fatty acid esters and to remove the saponifiable constituents. The reaction mixture was then cooled down, and the unsaponifiable portion was extracted with hexane. The organic part was removed, washed with distilled water to neutrality, filtered over anhydrous sodium sulfate and evaporated under reduced pressure to give the unsaponifiable fraction. The extract thus obtained was stored in airtight amber colored glass bottles at 4°C for further phytochemical and antioxidant evaluation.[11]

**2.3 Fractionation of the Unsaponifiable Fraction and Thin-Layer Chromatographic Analysis**

After saponification, the unsaponifiable fraction was partitioned into polar and non-polar components using liquid–liquid partitioning method. The extract was extracted in aqueous methanol and then using a separating funnel was partitioned with hexane. The upper hexane layer (non-polar constituents) was collected after thorough mixing and phase separation. The extraction was repeated to obtain the maximum number of phytosterol-rich constituents. The combined hexane fractions were concentrated with the help of the reduced pressure to give a semi-solid residue, which was then chromatographed. Preliminary phytochemical profiling of the obtained fraction was conducted by thin-layer chromatography (TLC). Silica gel 60 F254 precoated aluminum plates were used as the stationary phase.

The extract was dissolved in methanol and applied as individual spots using a capillary tube as the sample solution. Plates were developed in an appropriate solvent system—Hexane and ethyl acetate in optimized proportions. The plates were then air dried and the plates were illuminated under UV light at 254 and 366 nm after the development. The plates were further visualization by spraying with anisaldehyde–sulfuric acid reagent and then heated at 105°C for 2–5 min. The relative retention points (Rf) of the separated spots were determined and noted down to get a chromatographic fingerprint of the extract [12].

**2.4 Liquid Chromatography-Mass Spectroscopy (LC-MS) analysis**

The chemical composition of *Prunus persica* seed extract has been analyzed by LC–MS. The Waters Alliance e2695 HPLC system was used in conjunction with a Waters triple quadrupole mass spectrometer with an electrospray ionization (ESI) source for analysis. The separation was carried out using an Accucore C18 (150 mm × 2.1 mm, 2.6 μm) column and the flow rate was 1.0 mL/min with gradient elution. [13,14]

The extract was dissolved in solvents (methanol) and filtered through 0.22 μm nylon membrane filters prior to analysis and loaded into LC vials. The prepared sample was injected into the chromatographic system in 2 μL amount. MS spectra were collected in positive and negative mode with mass scan range m/z 150-2000. Nebulizing and drying gas was nitrogen and the source temperature was kept at 150°C. The gas temperature and flow rate in the drying gas was kept constant at 350°C and 950 L/h, respectively. The detection of the compounds was done by matching the resulting mass spectrum to

those stored in the spectral libraries and published literature. The identified phytoconstituents were tentatively recognized by associating their retention time, molecular ion and fragmentation pattern. [14]

### 2.5 Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

GC-MS was used to analyze the volatile and semi-volatile constituents present in the extract of *Prunus persica* seed. The extract was dissolved in chromatographic grade solvent and filtered through 0.22 µm membrane filter before analysis. The prepared sample was then aliquoted and injected into the GC–MS system with an appropriate capillary column for the separation of the phytochemical constituents.

The separation was performed by chromatography with a helium flow rate of 10 ml/min. The oven temperature was ramped up from initial temperature to final temperature to aid separation of compounds of different volatilities. Optimized instrumental conditions for the injector and transfer line temperatures were kept. Electron ionization (EI) mass spectral data were collected within a suitable mass scan range.

The compounds were detected by associating the acquired mass spectra with the National Institute of Standards and Technology (NIST) mass spectral library and published literature reports. Peak area normalization was used to determine the relative abundance of individual constituents. For compounds with substantial peak areas, further research on their reported biological and antioxidant properties was performed based on literature analysis and they were regarded as the major ingredients of the extract [14]

### 2.6 *In vitro* Antioxidant Evaluation

#### 2.6.1 DPPH Radical Scavenging Assay

*Prunus persica* seed extract showed free radical scavenging activity to be measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. In short, DPPH solution in methanol was freshly prepared and used as the source of radical. Different concentrations of the extract were prepared and mixed with the DPPH solution. The reaction mixtures were allowed to stand in darkness at room temperature for 30 min to allow the reaction to happen completely between antioxidants from the extract and DPPH radicals. The absorbance was then read at 517 nm on a UV–Visible spectrophotometer. The reference standard used was ascorbic acid. The percentage inhibition of DPPH radicals was determined from the absorbance value of the control and test sample. The dose response curve was used to calculate the concentration needed to inhibit 50% of the DPPH radicals ($IC_{50}$). The $IC_{50}$ values with lower score were assumed to have higher free radical scavenging activity [7].

#### 2.6.2 Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay was done by mixing extract with phosphate buffer and potassium ferricyanide solution and the mixture was incubated at 50°C for 20 min, after which the reaction was completed by incorporating trichloroacetic acid and the mixture was centrifuged to get a clear supernatant. Supernatant was taken and added to distilled water and ferric chloride solution. This blue complex was allowed to develop and absorbance was measured at 700 nm. The ascorbic acid was taken as the positive control. The antioxidant activity was expressed as $IC_{50}$ values based on the concentration–response relationship [15].

#### 2.6.3 ABTS Radical Scavenging Assay

The antioxidant activity of the extract was also evaluated by decolorization of the radical cation 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The ABTS radical cation solution was prepared by mixing ABTS with an appropriate oxidizing agent and allowing to remain in the dark until formation of the stable radical. The ABTS solution was diluted prior to the analysis to give an absorbance of 0.70 at 734 nm. The extract was placed in the solution of ABTS radical at various concentrations and allowed to stand for a particular time at room temperature. The decrease in absorbance was measured at 734 nm against a reagent blank. The reference antioxidant was ascorbic acid. The percentage inhibition of the ABTS radicals was calculated and $IC_{50}$ values were determined to compare the antioxidant effect of the extract with the standard [16].

## 3 RESULTS AND DISCUSSION

### 3.1 Extraction Yield and TLC Profiling

The extraction and fractionation processes extracted an unsaponifiable fraction from *Prunus persica* seeds that could be used for further chromatographic and antioxidant study. After Soxhlet extraction and saponification, it was feasible to recover the unsaponifiable fraction by liquid–liquid partitioning with hexane and aqueous methanol. The fraction obtained was yellowish in colour and semi-solid in nature, further suggesting the presence of non-polar components (phytosterols, terpenoids and other lipophilic metabolites). These extraction and fractionation methods were found to be appropriate to remove most of the saponifiable lipids, thus enriching compounds of potential biological significance. The preliminary phytochemical characterization was done by TLC.

The separation by chromatograph yielded clear and separate spots having different Rf values indicating the presence of more than one phytoconstituent of different polarities. The bands were well resolved under u/v light and after derivatization with anisaldehyde – sulfuric acid reagent and the bands were representative of sterols and related terpenoid compounds. An initial qualitative assessment of the chemical complexity of the extract was carried out by TLC and the results indicated the suitability of the fraction for advanced chromatographic analysis. In phytosterol rich fractions derived from medicinal plants, TLC is also used as a quick and effective method for preliminary phytochemical screening and quality control [17].

### 3.2 LC–MS Characterization of *Prunus persica* Seed Extract

The chemical configuration of the unsaponifiable fraction of the seeds of *Prunus persica* was elucidated using LC–MS analysis (Figure 1). The chromatographic profile showed several peaks with different retention times suggesting chemical diversity of the extract. Twelve compounds were tentatively identified from the mass fragmentation pattern, the molecular ion and the retention time. Table 1 shows the identified constituents, which included indole-3-pyruvate, neohesperidin dihydrochalcone, 3-methyl-3,4-dihydro-2H-1,4-benzoxazine, 1-O-hexyl-2,3,5-trimethylhydroquinone, bilobalide, synephrine, N-methylanthranilate, portoamide A, nornicotine, tetrahydropalmatine, kukoline, and sclareol (Table 1).
Some of the compounds identified have been previously linked to antioxidant, anti-inflammatory, neuroprotective, and metabolic regulatory effects. In turn, neohesperidin dihydrochalcone is a flavonoid derivative with antioxidant potential, while synephrine has been shown to have metabolic effects in experimental studies [18] bilobalide and sclareol have been reported to have antioxidant and cytoprotective properties in experimental studies. This antioxidant activity observed in the present investigation may be in part due to the occurrence of these bioactive metabolites. Based on the results of the LC-MS analysis, the extract of the seeds of *Prunus persica* seems to contain a wide array of phytochemical components that may act synergistically to provide the biological activity. All these findings also corroborate the traditional medicinal usage of the plant and give a chemical basis for further pharmacological investigation of the bioactive components of the plant [13].

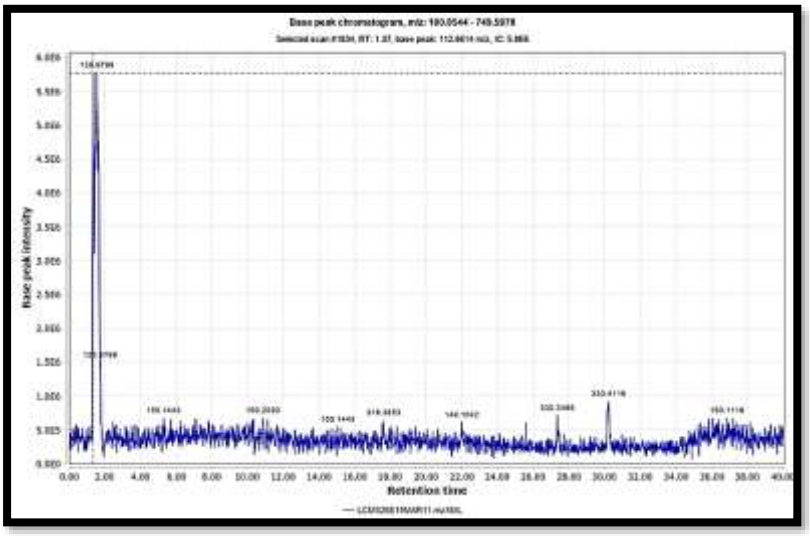


**Figure 1** LC-MS spectra of the extract

**Table 1 Tentatively Identified Phytoconstituents in *Prunus persica* Seed Extract by LC–MS Analysis**

| Peak No. | RT (min) | Compound Name | Molecular Formula | Precursor m/z | Ion Mode | Tentative Biological Activity |
|---|---|---|---|---|---|---|
| 1 | 1.37 | Indole-3-pyruvate | $C_{11}H_9NO_3$ | 202.0 | Positive | Antioxidant precursor, metabolic regulation |
| 2 | 1.56 | Neohesperidin dihydrochalcone | $C_{28}H_{36}O_{15}$ | 613.21 | Positive | Antioxidant, anti-inflammatory [19] |
| 3 | 2.46 | 3-Methyl-3,4-dihydro-2H-1,4-benzoxazine | $C_9H_{11}NO$ | 150.09 | Positive | Reported bioactive heterocycle |
| 4 | 5.30 | 1-O-Hexyl-2,3,5-trimethylhydroquinone | $C_{15}H_{24}O_2$ | 235.17 | Positive | Antioxidant potential |
| 5 | 5.71 | (-)-Bilobalide | $C_{15}H_{18}O_8$ | 325.09 | Positive | Neuroprotective, antioxidant [20] |
| 6 | 6.28 | Synephrine | $C_9H_{13}NO_2$ | 168.0 | Positive | Metabolic regulation [21] |
| 7 | 10.85 | N-Methylanthranilate | $C_8H_9NO_2$ | 150.0 | Positive | Aromatic bioactive compound [22] |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | 13.69 | Portoamide A | $C_{74}H_{109}N_{13}O_{22}$ | 1532.79 | Positive | Reported biological activity |
| 9 | 21.99 | Nornicotine | $C_9H_{12}N_2$ | 149.11 | Positive | Alkaloid constituent |
| 10 | 25.59 | Tetrahydropalmatine | $C_{21}H_{25}NO_4$ | 356.0† | Positive | Neuroactive alkaloid [23] |
| 11 | 27.34 | Kukoline | $C_{19}H_{23}NO_4$ | 330.30 | Positive | Alkaloid derivative |
| 12 | 30.19 | Sclareol | $C_{20}H_{36}O_2$ | 331.26 | Positive | Antioxidant, anti-inflammatory [24] |

### 3.3 GC-MS Characterization of *Prunus persica* Seed Extract

To gain more insights into the chemical composition of the *Prunus persica* seed extract and to identify volatile and semi-volatile constituents, the sample was analyzed by GC–MS. A complex phytochemical profile consisting of eighteen compounds with different retention time and relative peak area was identified on chromatogram (Figure 2). The identified constituents were representatives of different chemical classes such as ketones, esters, fatty acid derivatives, phenolic compounds and phosphate-containing molecules. The presence of different number of compounds with various chemical structures indicates the metabolic complexity of the seed extract and the existence of several bioactive constituents.

The major components of the extract were identified as two compounds namely Tris(2,4-di-tert-butylphenyl) phosphate (25.03% peak area) and Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) (17.68% peak area). Glycerol tricaprylate, 1,2-benzenedicarboxylic acid, and 2-(decanoyloxy) propane-1,3-diyl dioctanoate were some other significant compounds (Table 2). Some of these compounds have been reported to have antioxidant, antimicrobial, anti-inflammatory, or membrane-protective properties. Phenolic derivatives, especially, have been recognized to be a major source of antioxidants because of their ability to act as electron or hydrogen donors and thereby neutralizes the free oxygen species. Thus, the presence of such compounds may, at least partially, explain the antioxidant activity seen herein.

**Table 2 Tentatively Identified Chemical Constituents of *Prunus persica* Seed Extract by GC–MS Analysis**

| | **RT (min)** | **Compound Name** | **Peak Area (%)** | **Chemical Class** |
|---|---|---|---|---|
| 1 | 5.511 | 2-Heptanone, 3-methyl- | 5.92 | Ketone |
| 2 | 8.701 | Benzaldehyde, 2,4-dimethyl- | 0.98 | Aromatic aldehyde |
| 3 | 8.964 | Neral | 2.21 | Monoterpene aldehyde |
| 4 | 9.383 | 2,6-Octadienal, 3,7-dimethyl-, (E)-(α-Citral) | 2.44 | Monoterpene aldehyde |
| 5 | 11.189 | Dodecane | 0.36 | Alkane hydrocarbon |
| 6 | 11.616 | Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4E,9S*)]- | 0.43 | Sesquiterpene hydrocarbon |
| 7 | 12.612 | Phenol, 2,4-bis(1,1-dimethylethyl)- | 3.52 | Phenolic compound |
| 8 | 13.593 | 1,2-Benzenedicarboxylic acid, diethyl ester | 3.82 | Phthalate ester |
| 9 | 14.770 | Cyclooctane, methyl- | 0.45 | Cycloalkane |
| 10 | 17.112 | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | 2.32 | Oxygenated aromatic compound |
| 11 | 17.606 | N-Hexadecanoic acid (Palmitic acid) | 17.20 | Saturated fatty acid |
| 12 | 19.518 | Octadecanoic acid (Stearic acid) | 2.98 | Saturated fatty acid |
| 13 | 22.510 | Octadecanoic acid, 2,3-dihydroxypropyl ester | 1.80 | Fatty acid ester |
| 14 | 22.635 | 1,2-Benzenedicarboxylic acid | 2.68 | Aromatic dicarboxylic acid |

| | | | | |
|---|---|---|---|---|
| 15 | 25.557 | Glycerol tricaprylate | 5.21 | Triglyceride ester |
| 16 | 27.156 | 2-(Decanoyloxy)propane-1,3-diyl dioctanoate | 4.98 | Fatty acid ester |
| 17 | 30.456 | Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) | 17.68 | Phenolic phosphite derivative |
| 18 | 33.667 | Tris(2,4-di-tert-butylphenyl) phosphate | 25.03 | Organophosphate derivative |

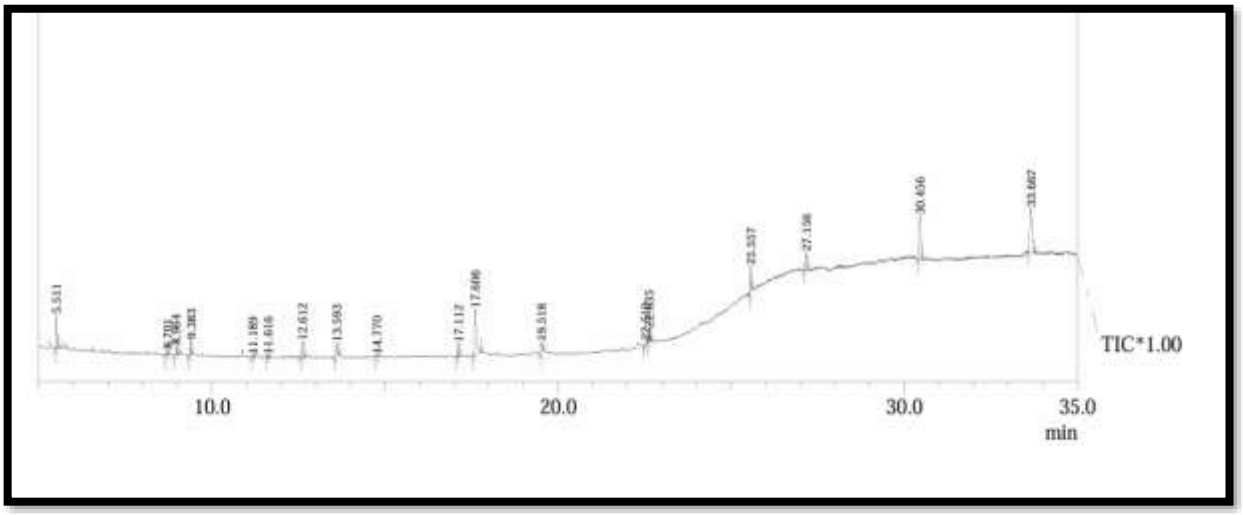


**Figure 2 GC-MS analysis of extract**

The GC–MS data further supports the LC–MS data as it gives some extra information on the low molecular weight and volatile constituents present in the extract. The combination of the above analytical tools shows that the seeds of *Prunus persica* are rich in different phytochemicals with biological relevance. The discovery of these components offers a chemical basis for further research to isolate particular bioactive constituents and study their pharmacologic properties. In previous studies, phytochemical diversity is generally tied to increased biological activity, which is found to be due to synergistic interactions between various constituents [19,25,26]

### 3.4 *In vitro* Antioxidant Activity

#### 3.4.1DPPH Radical Scavenging Activity

The scavenging activity of the *Prunus persica* -seed extract (sample name-NF01) was determined through DPPH assay, which is a commonly used method for measuring hydrogen-donating ability of antioxidant compounds (Figure 3). The antioxidant activity was estimated as $IC_{50}$, the concentration needed to inhibit 50% of the DPPH radicals and was compared to Ascorbic acid (Vitamin C) as the reference standard. The standard showed strong radical

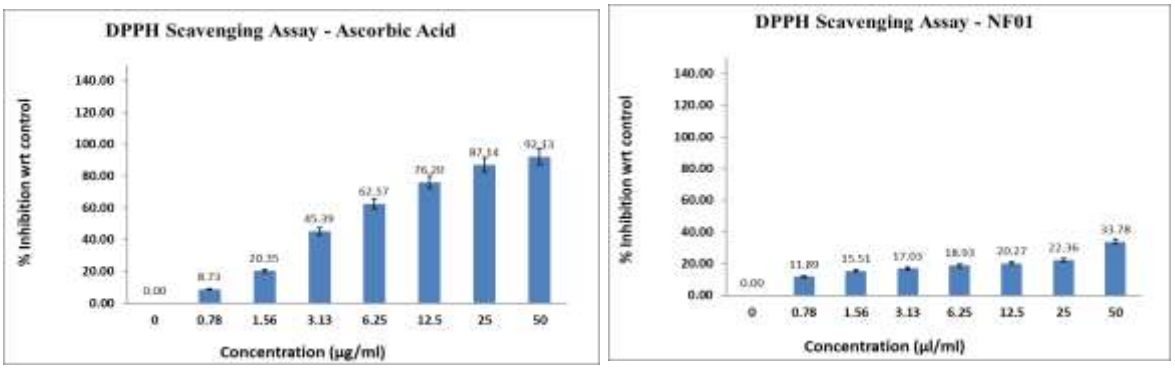


**Figure 3 DPPH scavenging assay of Ascorbic acid and sample (NF01)**

scavenging activity ($IC_{50}$ 4.28 ± 0.02 µg/mL) while the extract showed much less activity ($IC_{50}$ > tested range 985.9 ± 0.35 µL/mL).

The relatively low DPPH scavenging activity of the extract indicates a reduced ability to directly donate hydrogen atoms to scavenge DPPH radicals. This may be due to the chemical composition of the unsaponifiable fraction that has mainly non-polar components, which might not be able to interact well with the DPPH radical system. Moreover, the structural properties, concentration and interaction of each phytochemical in the extract can affect the antioxidant activity. Accordingly, the decrease in activity in the DPPH assay cannot be interpreted as a lack of antioxidant activity per se, but rather represents the mechanism of action being measured by this assay. Interestingly, some of the phytochemicals

identified using LC–MS and GC–MS have been previously reported for their antioxidant and cytoprotective effects. Thus, other mechanisms of antioxidant activity like electron transfer, metal ion reduction or modulation of endogenous antioxidant system could be more representative of the antioxidant potential of the extract. This is similar to what has been described with plant fractions rich in phytosterols and lipophilic fractions in which moderate or weak activity has been reported in DPPH assays, but which have shown antioxidant activity in other experimental models [7]

### 3.4.2 Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing antioxidant power (FRAP) assay was used to assess the electron-donating properties of the seed extract from *Prunus persica* (sample name-NF01). The antioxidant capacity was expressed as the concentration required to achieve 50% activity ($IC_{50}$) and compared with the reference antioxidant, ascorbic acid. The extract exhibited significant reducing activity with $IC_{50}$ value of 12.75 ± 0.14 µL/mL which is similar to ascorbic acid (13.46 ± 0.02 µg/mL) as observed in Figure 4.

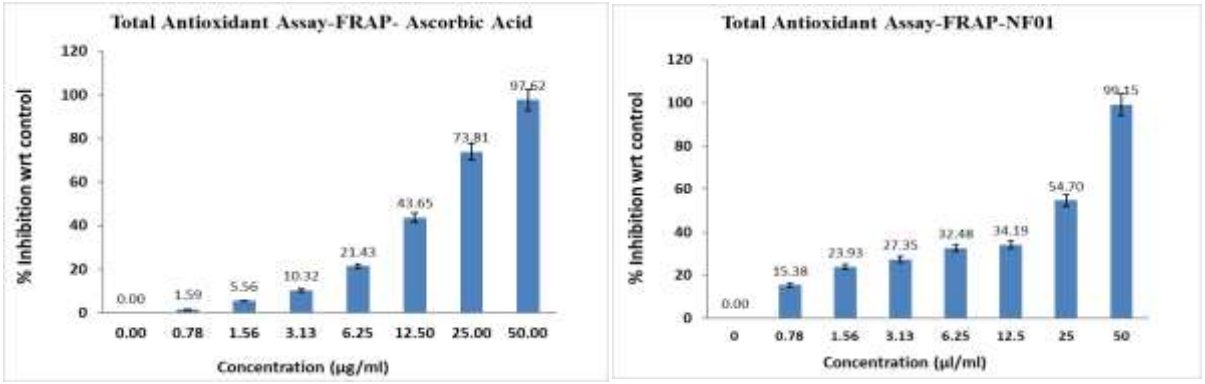


**Figure 4 FRAP assay of Ascorbic acid and sample (NF01)**

The reducing capacity observed suggest that the phytochemicals available in the extract can donate electrons to $Fe^{3+}$ to reduce it to $Fe^{2+}$. This mechanism is one of the significant aspects of antioxidant activity, as electron donation can break the chain of oxidative reactions and help stabilize the ROSs. The in-vitro antioxidant activity of extract was moderate in the FRAP assay but low in the DPPH assay indicating that the antioxidant effect may be mainly due to electron transfer mechanisms rather than to direct hydrogen atom donation. The bioactive constituents detected using LC–MS and GC–MS analyses (phenolic derivatives, flavonoid-related compounds, and others which are reducing agents) could be responsible for the FRAP activity exhibited. Neohesperidin dihydrochalcone and other phenolic components have other reported significant reducing and antioxidant properties. The differences noted between DPPH and FRAP further highlights the need for using several antioxidant assays that measure the different types of antioxidant action. Comparable results were obtained for plant extracts containing lipophilic and phenolic compounds; the reducing power assays, for example, sometimes showed an antioxidant activity that did not match with the DPPH radical scavenging activity. In general, FRAP results indicate that the antioxidant activity of P. persica seeds extract is moderate and it can be used as a source of naturally occurring reducing agents. More studies are needed on the isolation of active constituents and *in vivo* antioxidant studies, to confirm the biological significance of these findings though.[9]

### 3.4.3 ABTS Radical Scavenging Activity

The antioxidant activity of *Prunus persica* seed extract (NF01) was also determined by ABTS radical cation scavenging assay that is generally used to evaluate the antioxidant activity of both hydrophilic and lipophilic antioxidants. It is a pre-formed ABTS•+ radical cation measurement, where antioxidant compounds in the extract are responsible for reducing the radical cation, and hence causing a decrease in absorbance. The antioxidant activity of the extract was compared with ascorbic acid (a standard antioxidant) and the $IC_{50}$ value was calculated as given in Figure 5

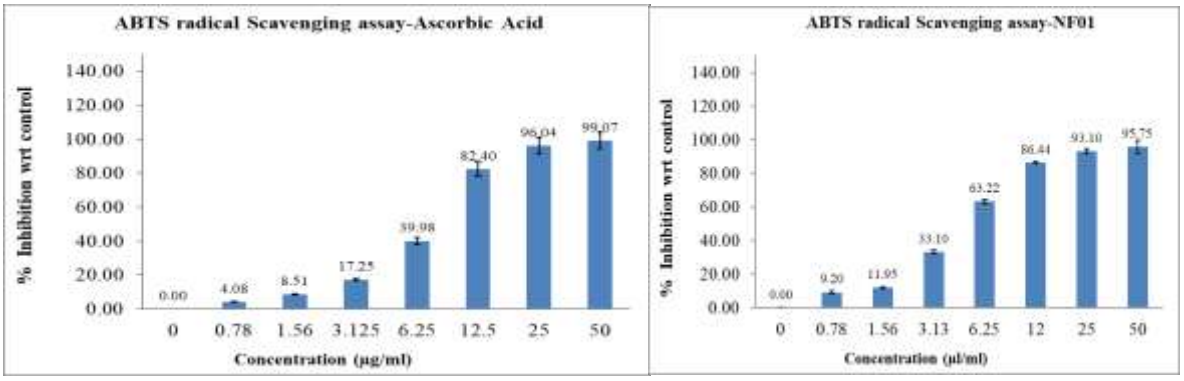


**Figure 5 ABTS assay of Ascorbic acid and sample (NF01)**

The scavenging of ABTS radicals was concentration dependent, suggesting the presence of compounds with electron or

hydrogen atom donation capacity to stabilize reactive radical species. The results of the LC–MS and GC–MS analyses might be due to the synergistic effect of the various phytochemicals detected. The flavonoid associated compounds & phenolic derivatives are a few of the constituents tentatively identified previously that have been reported to have radical scavenging properties; may contribute to antioxidant response observed in this assay. In contrast to the DPPH method, the ABTS method can be used to assess the antioxidant activity of a wider variety of antioxidant compounds, and it is especially useful for assessing extracts with both polar and non-polar components.
Hence, the ABTS assay also offers some additional data about the antioxidant activity of the extract. The overall results of DPPH, FRAP and ABTS indicated that the extract from P. persica seeds have measurable antioxidant activity via multiple mechanisms such as free radical scavenging and electron transfer. Further studies using isolated compounds and biological systems are required, however, to establish the exact contribution of each compound to the total antioxidant activity [16].

## 4. CONCLUSION

The present study aimed to explore the phytochemical profile and *in vitro* antioxidant activity of the extract of seed of *Prunus persica*, applying chromatographic assays and antioxidant activity tests. The tentative identification of several bioactive components such as flavonoid-related compounds, alkaloids, terpenoids and other secondary metabolites was made possible by LC–MS whereas, GC–MS profiling showed the presence of multiple VOCs and VSVCs that are likely to be of biological significance. The results of the combined chromatographic analysis revealed the chemical complexity of the extract and its phytochemicals which might be responsible for the biological activity of the extract.
The antioxidant activity was assessed by three methods: DPPH, FRAP, and ABTS and showed that the extract is anti-oxidative in different ways. The DPPH assay showed moderate scavenging activity, while the FRAP assay showed that the extract had good ferric reducing power indicating there were constituents in the extract that could donate electrons and modulate the redox activity. The results as a whole support the antioxidant effect of the P. persica seed extract and justify the traditional and potential therapeutic uses.
In the present investigation, however, only phytochemical characterization and *in vitro* antioxidant assessment were carried out. Further studies on the isolation of individual bioactive compounds, mechanistic studies, toxicity evaluation and *in vivo* pharmacological models are needed to confirm the biological significance of the identified constituents. These studies could contribute to understanding the therapeutic value of P. persica and promote its use as a natural source of antioxidants to treat disorders related to oxidative stress.

### Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this work.


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