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# Antioxidant Profiling, *In Silico Toxicity*, and Virtual Bioactivity Assessment of Selected Plant-Derived Phenolics

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**Abstract**

**Background and Aim:** Oxidative stress is a major contributing factor in the pathogenesis of many diseases. However, approaches to reduce oxidative stress, such as the use of antioxidants, are thought to be potentially protective. Towards this goal, the antioxidant potential of 38 plant phenolics from various chemical classes was screened through *in vitro* methods in the current work.

**Methods:** The phenolic compounds were tested for their metal chelation and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity along with copper-reducing antioxidant capacity (CUPRAC) and ferric-reducing antioxidant power (FRAP) assays at 10 mM. *In silico* toxicity and adverse effect estimations, as well as the potential bioactivity probabilities of the compounds, were assessed through PASS and Swiss prediction programs.

**Results:** All the tested compounds displayed metal chelation activity below 50%, while the highest chelating capacity was exhibited by vitexin (44.12 ± 0.09%). Twenty-two of the compounds displayed a scavenging effect of over 50%, where the highest scavenging effect was caused by oleuropein (87.05 ± 0.05%). In the CUPRAC and FRAP assays, all compounds exhibited remarkable levels of antioxidant activity. The most active ones in the CUPRAC assay were lined up according to their absorbance values as compared to quercetin (3.99 ± 0.01, the reference) as isoquercitrin (3.99 ± 0.01), luteolin (3.96 ± 0.05), myricetin (3.78 ± 0.11), kaempferol (3.60 ± 0.28), morin (3.48 ± 0.26), catechin (3.33 ± 0.28), and hyperoside (3.11 ± 0.50). All compounds possessed FRAP values close to or better than the reference (*i.e.*, quercetin). Our data reveals that the tested phenolics match well with the *in silico* estimations considering their antioxidant effects, which is the *in silico* first data on them in this regard.

**Conclusion:** Our extensive screening indicated oleuropein, gallic acid, taxifolin, rosmarinic acid, catechin, quercetin, kaempferol, luteolin, myricetin, hyperoside, morin, isoquercitrin, fisetin, epigallocatechin gallate, and vitexin as the most promising phenolic antioxidants in the current work.

**Keywords** Antioxidant activity · Phenolic Compounds · *In Silico* Toxicity · Adverse Effect

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## INTRODUCTION

An imbalance between the body's antioxidant defence systems and the generation of reactive oxygen species (ROS), also known as free radicals, is called oxidative stress (Sies, 2015). Under normal physiological conditions, a certain level of free radical production is necessary for cellular functions (Tan et al., 2018). However, when there is excessive production of free radicals or a weakening of antioxidant defence mechanisms, oxidative stress occurs. Understanding the mechanisms of oxidative stress and developing strategies to reduce cellular damage may be important in the prevention and treatment of many diseases.

Plant phenolics are a broad class of chemicals with strong antioxidant properties that are widely distributed in vegetables, fruits, seeds, nuts, grains, and other plant-based foods (Dai & Mumper, 2010). These compounds possess antioxidant properties due to their ability to donate hydrogen atoms or electrons to ROS, thereby neutralising their harmful effects on cells. The antioxidant potential of plant phenolics can be attributed to several mechanisms, including radical scavenging activity, metal chelation, regeneration of other antioxidants such as tocopherols and ascorbic acid, enhancing the overall antioxidant defence system in cells, and modulation of signalling pathways (Sadef et al., 2022). Plant phenolics with potent antioxidant activity mainly include flavonoids, phenolic acids, stilbenes, and lignans (Shen et al., 2022). Owing in part to their antioxidant qualities, eating foods high in phenolics has been linked to many health benefits, including reduced risk of chronic illnesses like cancer, cardiovascular disease, and neurodegenerative disorders. One of the most common preliminary methods used to evaluate the antioxidant capacity of phenolic compounds is the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (RSA) due to its simplicity, rapidity, and reliability in screening the antioxidant capacity of phenolic compounds and other natural antioxidants (Brand-Williams et al., 1995). An approach that is frequently used to assess the antioxidant potential of different substances, such as natural products, plant extracts, and synthetic antioxidants, is the cupric ion-reducing antioxidant capacity assay (CUPRAC) (Munteanu & Apetrei, 2021; Gulcin, 2020). The CUPRAC assay provides a measurement of the total antioxidant activity of a sample by assessing its ability to reduce cupric ions ($Cu^{2+}$) to cuprous ions ($Cu^{+}$). This method evaluates the combined contribution of all the antioxidants present in the sample, including both lipophilic and hydrophilic antioxidants. On the other hand, a ferric-reducing antioxidant power (FRAP) assay was applied for the measurement of the antioxidant activity of the samples by assessing their capacity to reduce ferric ions ($Fe^{3+}$) to ferrous ions ($Fe^{2+}$). The total antioxidant capacity of a sample is determined using the FRAP assay, which considers the combined effect of all the present antioxidants (Munteanu & Apetrei, 2021; Gulcin, 2020). This includes both hydrophilic and lipophilic antioxidants, making it a comprehensive method for assessing the overall antioxidant potential. It is highly sensitive and can detect antioxidants at low concentrations.

Natural products have always been cited as inspiring lead molecules in pharmaceutical research. Thus, plant phenolics are essential for preserving general health and shielding cells from oxidative damage. In the current study, our inspiration from pertinent previous outcomes of plant phenolics led us to screen 38 phenolic compounds, as presented in Table 1, for their antioxidant effect, whose selection was mainly based on their wide existence in medicinal and edible plants. Our goal in this study was to test them simultaneously under the same conditions using the ELISA microtiter methods available in our laboratory. Their antioxidant effect was assessed by DPPH RSA, metal chelation activity, CUPRAC, and FRAP assays. Their possible toxicity and adverse effects, along with potential antioxidant activity match, were searched *in silico* using PASS software and Swiss Prediction programs.

## MATERIALS AND METHODS

### Chemicals

Sigma-Aldrich (St. Louis, Missouri, USA) provided the references for the phenolic compounds used in this study (Table 1), which were over 95% purity.

### DPPH Radical Scavenging Activity Assay

The stable DPPH RSA of the phenolic compounds was assessed using Blois's method (1958). The samples (10 µL) that had been dissolved in ethanol (75%) were combined with 90 µL of freshly prepared DPPH solution ($1.5 \times 10^{-4}$ M). Utilizing a 96-well microplate reader (SpectraMax i3x microplate reader, USA), the remaining quantity of DPPH was determined at 520 nm. The reference was quercetin (98% of purity, Sigma, St. Louis, MO, USA). To calculate the percentage (%) of DPPH inhibition represented as RSA, the following formula was employed:

$RSA\% = [(A_{control} - A_{sample}) / A_{control}] \times 100$, where $A_{sample}$ is the absorbance of the extracts/reference and $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test sample). Three parallel sets of experiments were conducted, and the data were reported as the average values and standard deviation (S.D.).

Table 1. Antioxidant activity and metal-chelating capacity results of the phenolic compounds tested at 10 mM

| No. | Phenolic compounds | Chemical class | DPPH radical scavenging effect (Scavenging% ± S.D.[a]) | CUPRAC (Absorbance at 450 nm ± S.D.) | FRAP (Absorbance at 700 nm ± S.D.) | Metal-chelating capacity (Chelating% ± S.D.) |
|---|---|---|---|---|---|---|
| 1 | Gallic acid[b] | | 85.16 ± 0.06 | 3.40 ± 0.01 | 1.61 ± 0.02 | 2.60 ± 0.03 |
| 2 | Syringic acid | Hydroxybenzoic acid | 83.08 ± 0.07 | 1.57 ± 0.03 | 1.80 ± 0.17 | 4.84 ± 0.03 |
| 3 | Vanillic acid | | 26.38 ± 0.30 | 1.61 ± 0.07 | 1.17 ± 0.01 | 3.47 ± 0.07 |
| 4 | Chlorogenic acid | Ester of caffeic acid and (−)-quinic acid | 83.57 ± 0.07 | 2.37 ± 0.08 | 1.57 ± 0.11 | 11.99 ± 0.05 |
| 5 | Ellagic acid | Dimeric derivative of gallic acid | 78.72 ± 0.09 | 0.52 ± 0.08 | 1.59 ± 0.43 | 39.82 ± 0.02 |
| 6 | *Trans*-Ferulic acid | | 82.41 ± 0.07 | 1.58 ± 0.01 | 1.57 ± 0.09 | 1.90 ± 0.01 |
| 7 | *p*-Coumaric acid | Hydroxycinnamic acid | 16.95 ± 0.34 | 1.07 ± 0.09 | 1.08 ± 0.04 | 14.51 ± 0.07 |
| 8 | Caffeic acid | | 83.66 ± 0.07 | 2.23 ± 0.16 | 1.72 ± 0.10 | -[c] |
| 9 | Rosmarinic acid | Caffeic acid ester | 85.35 ± 0.06 | 2.71 ± 0.12 | 1.83 ± 0.05 | - |
| 10 | D-(-)-Quinic acid | Cyclohexanecarboxylic acid | 19.19 ± 0.33 | 0.31 ± 0.01 | 1.14 ± 0.23 | 26.82 ± 0.08 |
| 11 | Oleuropein | | 87.05 ± 0.05 | 1.39 ± 0.10 | 1.68 ± 0.04 | 2.61 ± 0.05 |
| 12 | *p*-Tyrosol | Phenethyl alcohol | 33.27 ± 0.27 | 0.67 ± 0.01 | 1.11 ± 0.12 | 14.71 ± 0.09 |
| 13 | 3-Hydroxytyrosol | | 83.01 ± 0.07 | 1.80 ± 0.04 | 1.86 ± 0.05 | 2.84 ± 0.05 |
| 14 | Pelargonidin | Anthocyanidin | 38.74 ± 0.25 | 1.30 ± 0.10 | 1.31 ± 0.03 | - |
| 15 | *Trans*-Resveratrol | Stilbene | 84.81 ± 0.06 | 1.69 ± 0.04 | 1.51 ± 0.03 | 11.00 ± 0.05 |
| 16 | Catechin | Flavan-3-ol | 82.57 ± 0.07 | 3.33 ± 0.28 | 1.69 ± 0.08 | 14.89 ± 0.06 |
| 17 | Epigallocatechin gallate (EGCG) | | 84.73 ± 0.06 | 1.96 ± 0.20 | 1.97 ± 0.06 | 4.44 ± 0.07 |
| 18 | Quercetin[d] | | 86.20 ± 0.06 | 3.99 ± 0.01 | 1.84 ± 0.03 | 13.87 ± 0.12 |
| 19 | Kaempferol | | 85.77 ± 0.06 | 3.60 ± 0.28 | 1.63 ± 0.05 | 8.88 ± 0.01 |
| 20 | Myricetin | Flavonol | 78.42 ± 0.09 | 3.78 ± 0.11 | 1.78 ± 0.23 | 10.06 ± 0.08 |
| 21 | Fisetin | | 81.50 ± 0,08 | 2.66 ± 0.36 | 1.93 ± 0.05 | 22.64 ± 0.06 |
| 22 | Morin | | 83.15 ± 0.07 | 3.48 ± 0.26 | 1.98 ± 0.03 | 7.95 ± 0.03 |
| 23 | Hyperoside | Flavonol glycoside | 65.81 ± 0.14 | 3.11 ± 0.50 | 1.52 ± 0.05 | 38.96 ± 0.12 |
| 24 | Luteolin | Flavone | 82.28 ± 0.07 | 3.96 ± 0.05 | 1.70 ± 0.22 | 23.62 ± 0.03 |
| 25 | Apigenin | | 24.36 ± 0.31 | 0.98 ± 0.30 | 1.10 ± 0.04 | 32.55 ± 0.02 |
| 26 | Taxifolin | Flavononol | 85.72 ± 0.06 | 1.46 ± 0.11 | 2.00 ± 0.10 | 5.15 ± 0.05 |
| 27 | Tricin | | 40.51 ± 0.24 | 0.07 ± 0.01 | 0.93 ± 0.05 | 37.13 ± 0.05 |
| 28 | Tangeretin | Methoxyflavone | 11.78 ± 0.03 | 0.26 ± 0.01 | 1.14 ± 0.03 | 5.46 ± 0.05 |
| 29 | Diosmetin | | 10.36 ± 0.37 | 0.43 ± 0.04 | 1.40 ± 0.10 | 5.65 ± 0.02 |
| 30 | Hesperetin | Flavanone | 60.07 ± 0.16 | 1.79 ± 0.17 | 1.20 ± 0.10 | 10.69 ± 0.01 |
| 31 | Naringin | Flavanone 7-O-glycoside | 9.93 ± 0.37 | 0.19 ± 0.01 | 1.16 ± 0.03 | 10.44 ± 0.07 |
| 32 | Isoquercitrin | Flavone 3-O-glycoside | 83.50 ± 0.07 | 3.99 ± 0.01 | 1.76 ± 0.06 | 8.33 ± 0.11 |
| 33 | Orientin | Flavone 8-C-glycoside | 20.46 ± 0.33 | 1.74 ± 0.24 | 1.30 ± 0.23 | 9.51 ± 0.05 |
| 34 | Vitexin | | 46.15 ± 0.22 | 0.17 ± 0.01 | 0.98 ± 0.05 | 44.12 ± 0.09 |
| 35 | Daidzein | | 15.50 ± 0.35 | 0.47 ± 0.06 | 1.23 ± 0.06 | 13.77 ± 0.09 |
| 36 | Genistein | Isoflavone | 16.53 ± 0.01 | 1.56 ± 0.14 | 1.02 ± 0.01 | 13.11 ± 0.07 |
| 37 | Glycitein | | 14.12 ± 0.35 | 0.27 ± 0.01 | 1.09 ± 0.02 | 14.99 ± 0.03 |
| 38 | Amentoflavone | Biflavonoid | 15.80 ± 0.35 | 2.5 ± 0.18 | 1.37 ± 0.04 | 32.54 ± 0.05 |
| | EDTA[e] | | | | | 93.35 ± 0.01 |

[a]Standard deviation (n: 3), [b]Reference for CUPRAC assay, [c]No activity, [d]Reference for DPPH and FRAP assays, [e]Reference for metal chelation capacity

## Metal Chelation Assay

The metal (ferrous ion) chelation effect of the compounds was calculated using the method of Chua *et al.* (2008). In short, a 2 mM $FeCl_2$ solution was used to incubate the compounds at different dilutions. The mixture was mixed with 5 mM of ferrozine to initiate the reaction, which was then left to stand at room temperature for ten minutes. A UV-visible spectrophotometer (SpectraMax i3x microplate reader, USA) was used to quantify the absorbance of the reaction mixture at 562 nm. The following equation was applied to determine the ratio of the inhibition of ferrozine-$Fe^{2+}$ complex formation:

$I\% = [(A_{blank}-A_{sample}) / A_{blank}] \times 100$, where $A_{sample}$ is the absorbance of the compounds/reference and $A_{blank}$ is the control reaction's absorbance (consisting just of ferrozine and $FeCl_2$). The average values with S.D. were provided after the analyses were carried out in triplicate. In this experiment, ethylenediamine tetraacetic acid (EDTA) served as the reference.

## Cupric-Reducing Antioxidant Capacity (CUPRAC) Assay

Using the modified method of Apak *et al.* (2008), the CUPRAC of the phenolic compounds and the reference (*i.e.,* gallic acid) was assessed. In brief, the antioxidant compounds cause the copper-neocuproin complex [Cu(II)-Nc] to decrease to Cu (I)-Nc. To ascertain the reduction, a 96-well microplate well was filled with 25 µL of 10 mM copper (II) chloride (Sigma, USA), 7.5 mM neocuproin (Sigma, USA), 1 M ammonium acetate (pH 7), compound, and distilled water (100 µL). After that, the microplate was incubated for 30 min. At a wavelength of 450 nm, the absorbance was measured at the end of the period with an ELISA microplate reader (SpectraMax i3x microplate reader, USA). Every sample was examined three times, and the results are shown as the average ± S.D. of the absorbances acquired from three consecutive experiments.

## Ferric-Reducing Antioxidant Power (FRAP) Assay

The FRAP of the compounds was examined using Oyaizu's assay (1986). The compounds were combined with 2.5 mL of potassium ferricyanide (10%, w/v) and phosphate buffer (0.2 mol/L, pH 6.6) at varying concentrations. The mixture was mixed with trichloroacetic acid (10%, w/v) after it had been incubated for 20 min at 50°C. This solution was combined with ferric chloride (0.1%, w/v) and distilled water after the combination was given a vigorous shake. The absorbance was measured at 700 nm using a UV-visible spectrophotometer (SpectraMax i3x microplate reader, USA) after 30 min of incubation. Three parallels of each experiment were completed. A higher absorbance of the reaction is interpreted as a higher reducing power relative to the reference (*i.e.*, quercetin).

## *In Silico* Estimation of the Biological Activity

An examination for the *in silico* estimation of biological activity was performed to demonstrate the ways in which the chosen phenolic compounds and antioxidant activity overlap. To this end, *in silico* screening programs such as "Swiss Target Prediction" and "Prediction of Activity Spectra for Substances" (PASS), which can analyse approximately 3600 different biological activity types, were used. The PASS program (version 2.0) was used to analyse computer-aided biological activity evaluations of the compounds investigated in this study (Brus, 2014). In the analysis, the values with a higher probability of being active ($P_a$) in the relevant effect than being inactive ($P_i$) for the effect under investigation were taken into account. It was possible to compare the likelihood of the compounds exhibiting antioxidant action based on the $P_a$-$P_i$ estimation results. Furthermore, the Swiss Target Prediction program, created by the Swiss Bioinformatics Institute, was utilised to estimate the relevant bioactivity of the substances under investigation (Borodina et al., 1996; Gfeller et al., 2013).

## *In Silico* Toxicity and Adverse Effect Estimation

In accordance with the International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use, ICH Harmonised Guideline M7(R1) (2017), the potential mutagenicity of the compounds was examined by combining two methodologies that offer analysis with statistical and knowledge-based methods (https://database.ich.org/sites/default/files/M7_R1_Guideline.pdf). The ultimate determination was made through professional study utilising the VEGA QSAR Mutagenicity (Ames test) CONSENSUS model (version 1.0.3) and the ToxTREE SAR model *in vitro* mutagenicity (Ames; ISS) model (version 3.1.0). Using the VEGA hepatotoxicity model (IRFMN) (version 1.0.0), the hepatotoxicity potential of the drugs was assessed. Furthermore, PASS (version 2.0) was used to analyse computer-aided general toxicity evaluations of the drugs. $P_a > P_i$ values were considered in the analysis, and by assuming $P_a > 0.7$, estimates with a high probability of adverse effects were obtained.

# RESULTS

## Antioxidant Activity

Among the phenolic acid derivatives tested, gallic and rosmarinic acids were the most active. In the DPPH RSA assay, except for vanillic, *p*-coumaric, and (D)-(-)-quinic acids, all phenolic acids had a strong scavenging effect (Table 1). Besides, pelargonidin as the anthocyanin derivative, along with methoxyflavones and isoflavones, were revealed to be the low to moderately effective (below 50%) scavengers of DPPH. In the CUPRAC and FRAP assays, all compounds

exhibited remarkable levels of antioxidant activity. In the CUPRAC assay, the most active ones as compared to the reference (*i.e.*, gallic acid) were quercetin, isoquercitrin, luteolin, myricetin, kaempferol, morin, catechin, and hyperoside (Table 1). Our findings indicated that quercetin, luteolin, myricetin, kaempferol, morin, and isoquercitrin exhibited higher CUPRAC activity than that of the reference. The FRAP values of the compounds were found to be close to or better than the reference (*i.e.*, quercetin), where the highest FRAP value was owned by taxifolin.

In the FRAP assay, many of the tested compounds displayed close or higher FRAP activity than that of the reference (*e.g.*, quercetin) (Table 1). In this assay, taxifolin exerted a markedly higher activity than the reference. All the compounds possessed a chelation capacity below 50%, while the most active one was vitexin (44.12 ± 0.09%), followed by hyperoside (38.96 ± 0.12%).

### *In Silico* Bioactivity Outcomes

Structure–activity relationship (SAR) and adverse effect analyses were conducted to assess the mutagenic effects of the phenolic compounds. In the PASS software, probabilities are given over $P_a$ (probability of being active) and $P_i$ (probability of being inactive) values. The likelihood of the biological activity in question is increased by the fact that the values are "$P_a > P_i$" and that there is a significant difference between the two values. The screening results for the selected phenolic compounds are tabulated in Tables 2 and 3. The *in silico* screening results matched well with our *in vitro* data. The computational prediction analyses indicated likely genotoxicity, trembling, vascular toxicity, and diarrhoea, alongside rarer possibilities such as urine discolouration and inflammation for these compounds.

## DISCUSSION

Among the most prevalent classes of secondary metabolites found in medicinal plants are phenolic compounds. The literature survey has indicated that most of the studies on the antioxidant activity of plant phenolics have focused on the antioxidant effects of the plant extracts, in which they are usually present, and the attribution of the antioxidant effect to these compounds by determining their presence in the extract as a result of phytochemical analysis. The number of studies on the antioxidant capacity of single plant phenolics is quite limited. All the compounds screened herein were revealed to exert a chelation capacity of less than 50% and lower than that of EDTA (92.56 ± 1.24%), the most accepted reference for the mentioned activity. According to our literature survey, this is the first data on the metal chelation activity of tangerine, tricin, and vanillic acid, despite their low metal (iron) chelating capacity. The compounds we screened herein against the DPPH radical have been reported by various researchers (Zheng et al., 2010; Stankevičius et al., 2011; Jing et al., 2012), which compiled well with our relevant data. Doubtlessly, the DPPH RSA effect of phenolics is dependent on the electron-donating substituent groups on the phenol ring, the hydrogen-bond donor/electron-donating groups at the ortho-position, the electron-donating groups at the meta-position, and/or the hydrophobic groups at the meta-/ortho-position (Jing et al., 2012).

All compounds with no or weak chelation compared to EDTA are consistent with our relevant data. Chan *et al.* (2016) reported gallic acid as well as propyl gallate, gallamide, and epigallocatechin gallate (EGCG), which have the pyrogallol moiety among the strong Fe(II) chelators. Nevertheless, their data are incompatible with our results on gallic acid and EGCG. In the same study, EGCG was revealed to be the notable chelator of Cu(II), which is consistent with our relevant data. Nevertheless, gallic acid possessed a strong effect in our CUPRAC assay, and it was described as a marked Cu(II) chelator by Chan *et al.* (2016). That variance might be associated with the use of different methods, buffers, and solvents used, and concentrations as underlined by Andjelković *et al.* (2006). Besides, Andjelkovic *et al.* (2006) indicated that hydroxycinnamic acids were better ligands for Fe(II) than hydroxybenzoic acids related to their binding constant values. Additionally, the occurrence of the ethylene group in phenolic acids may have an impact on their chelation capacity. On the other hand, the presence of a methoxy group in phenolic acids as vanillic acid, syringic acid, and ferulic acid tested herein hindered their Fe(II)-chelating capacity, whereas it contributed greatly to their antioxidant effect in the CUPRAC and FRAP assays. The authors also commented that the Fe(II)-chelating capacity of 3-hydroxytyrosol was much lower than that of EDTA, a well-known chelator, which is consistent with our data.

Among the tested phenolic compounds in the present work, phenolic acids, including gallic, vanillic, *p*-coumaric, syringic, caffeic, ferulic, and rosmarinic acids, displayed a noted activity in the FRAP assay in a recent study, where the modest FRAP value was obtained with *p*-coumaric acid (Skroza et al., 2022), consistent with our data. Invariably, quinic acid was recently shown to have low CUPRAC and FRAP values (Ercan & Doğru, 2022). Hydroxytyrosol was reported to have a better CUPRAC value than that of oleuropein, which was moderately active in our identical assay (Zorić et al., 2021). Fernandez-Pasto *et al.* reported an matching level of FRAP value (0.87 mM) to that of the reference, *e.g.* α-tocopherol (0.87 mM) (Fernandez-Pastor et al., 2016), whereas it had a modest activity in our

FRAP assay. This might be due to the use of a lipophilic type of antioxidant reference compound in that study, while the reference (*e.g.,* quercetin) had a hydrophilic nature in our assay, which means a more suitable reference. Most of the phenolics screened herein were shown to exert remarkable CUPRAC and FRAP values in various studies, except tricin and orientin, as well as CUPRAC values of morin and amentoflavone (Firuzi et al., 2005; Apak et al., 2008; Ben Sghaier et al., 2011).

Our findings also underlined the lower *in vitro* antioxidant potential of isoflavones and methoxyflavones compared with the phenolics from other chemical classes. Isoflavones and methoxyflavones have slightly different chemical structures than other phenolic compounds such as flavonols, flavanols, and phenolic acids (Ross et al., 2002; de Souza Farias et al., 2021). These differences can influence their antioxidant activity. The position and number of hydroxyl groups on the phenolic rings of flavonoids contribute to their antioxidant potential. Isoflavones and methoxyflavones often have fewer hydroxyl groups than other phenolics, which can result in lower antioxidant activity.

The potential biological activity estimates of the phenolic compounds screened in our study with *in silico* biological activity (PASS) and Swiss prediction programs displayed a noteworthy consistency with our *in vitro* antioxidant data (Table 2). The *in silico* toxicity/adverse effect predictions indicated that they might possess probabilities of mainly genotoxicity, trembling, vascular toxicity, and diarrhoea, whereas some possibilities such as discolouration urine, inflammation, respiratory toxicity, reproductive dysfunction, hyperthermia, irritation, haematemesis might rarely occur (Table 3). These virtual predictions underline once again the importance of careful use of naturally occurring molecules for health.

## CONCLUSION

The current study presented data for 38 plant phenolics that were tested for their antioxidant activity using the metal chelation capacity assay, in addition to the DPPH RSA effect, CUPRAC, and FRAP methods. All of the phenolics were also evaluated *in silico* for their toxicity and adverse effect estimations as well as potential bioactivity probabilities *via* PASS and Swiss prediction programs. Our findings disclosed that the tested antioxidant effects of the phenolics overlapped consistently with the *in silico* estimations. The *in silico* part of our study constitutes the first investigation into the toxicity and adverse effects of the screened phenolic compounds and their virtual biological activity predictions. According to our results, the most favourable antioxidants were highlighted as oleuropein, gallic acid, taxifolin, rosmarinic acid, catechin, quercetin, kaempferol, luteolin, myricetin, hyperoside, morin, isoquercitrin, fisetin, epigallocatechin gallate, and vitexin. While this study assessed the antioxidant activity, future work could delve deeper into the specific mechanisms by which these phenolics exert their antioxidant effects. This may include investigating interactions with specific enzymes, signalling pathways, or cellular components. Another future perspective for this study could be exploring potential synergistic effects between different phenolics or between phenolics and other antioxidants.

Table 2. Estimation of potential biological activity of the phenolic compounds *via* the antioxidant mechanisms

| Phenolic compounds tested | Estimated biological activity | Probability[a] | |
|---|---|---|---|
| | | $P_a$ | $P_i$ |
| **Kaempferol** | Peroxidase inhibitor | 0.956 | 0.001 |
| | Antioxidant | 0.856 | 0.003 |
| | Lipid peroxidase inhibitor | 0.783 | 0.004 |
| | Free radical scavenger | 0.771 | 0.003 |
| **Fisetin** | Peroxidase inhibitor | 0.953 | 0.001 |
| | Antioxidant | 0.787 | 0.004 |
| | Lipid peroxidase inhibitor | 0.755 | 0.004 |
| | Free radical scavenger | 0.753 | 0.003 |
| **Vitexin** | Peroxidase inhibitor | 0.538 | 0.034 |
| | Antioxidant | 0.780 | 0.004 |
| | Lipid peroxidase inhibitor | 0.741 | 0.004 |
| | Free radical scavenger | 0.901 | 0.002 |
| **Naringin** | Antioxidant | 0.851 | 0.003 |
| | Lipid peroxidase inhibitor | 0.978 | 0.002 |
| | Free radical scavenger | 0.981 | 0.001 |
| **Myricetin** | Peroxidase inhibitor | 0.966 | 0.001 |
| | Antioxidant | 0.924 | 0.003 |
| | Lipid peroxidase inhibitor | 0.836 | 0.003 |
| | Free radical scavenger | 0.832 | 0.002 |
| **Resveratrol** | Peroxidase inhibitor | 0.825 | 0.004 |
| | Lipid peroxidase inhibitor | 0.585 | 0.010 |
| | Antioxidant | 0.546 | 0.005 |
| | Free radical scavenger | 0.572 | 0.007 |
| **Rosmarinic acid** | Peroxidase inhibitor | 0.550 | 0.032 |
| | Antioxidant | 0.539 | 0.005 |
| | Lipid peroxidase inhibitor | 0.719 | 0.005 |
| | Free radical scavenger | 0.745 | 0.003 |
| **Epigallocatechin gallate** | Peroxidase inhibitor | 0.759 | 0.007 |
| | Antioxidant | 0,814 | 0.003 |
| | Lipid peroxidase inhibitor | 0.946 | 0.002 |
| | Free radical scavenger | 0.934 | 0.001 |
| **Genistein** | Peroxidase inhibitor | 0.842 | 0.004 |
| | Antioxidant | 0.765 | 0.004 |
| | Lipid peroxidase inhibitor | 0.667 | 0.006 |
| | Free radical scavenger | 0.458 | 0.013 |
| **Daidzein** | Peroxidase inhibitor | 0.797 | 0.005 |
| | Antioxidant | 0.623 | 0.004 |
| | Lipid peroxidase inhibitor | 0.537 | 0.013 |
| | Free radical scavenger | 0.353 | 0.022 |
| **Gallic acid** | Peroxidase inhibitor | 0.891 | 0.003 |
| | Antioxidant | 0.520 | 0.006 |
| | Lipid peroxidase inhibitor | 0.554 | 0.012 |
| | Free radical scavenger | 0.570 | 0.007 |

| Phenolic compounds tested | Estimated biological activity | Probability[a] | |
|---|---|---|---|
| | | $P_a$ | $P_i$ |
| **Apigenin** | Peroxidase inhibitor | 0.924 | 0.002 |
| | Antioxidant | 0.732 | 0.004 |
| | Lipid peroxidase inhibitor | 0.695 | 0.005 |
| | Free radical scavenger | 0.719 | 0.004 |
| **Ellagic acid** | Peroxidase inhibitor | 0.861 | 0.004 |
| | Antioxidant | 0.699 | 0.004 |
| | Lipid peroxidase inhibitor | 0.536 | 0.013 |
| | Free radical scavenger | 0.596 | 0.006 |
| **Syringic acid** | Peroxidase inhibitor | 0.846 | 0.004 |
| | Antioxidant | 0.403 | 0.012 |
| | Lipid peroxidase inhibitor | 0.559 | 0.012 |
| | Free radical scavenger | 0.619 | 0.005 |
| **Caffeic acid** | Peroxidase inhibitor | 0.753 | 0.007 |
| | Antioxidant | 0.603 | 0.005 |
| | Lipid peroxidase inhibitor | 0.570 | 0.011 |
| | Free radical scavenger | 0.647 | 0.005 |
| **Ferulic acid** | Peroxidase inhibitor | 0.708 | 0.011 |
| | Antioxidant | 0.540 | 0.005 |
| | Lipid peroxidase inhibitor | 0.618 | 0.008 |
| | Free radical scavenger | 0.731 | 0.004 |
| **Quinic acid** | Peroxidase inhibitor | 0.424 | 0.048 |
| | Antioxidant | 0.830 | 0.003 |
| | Lipid peroxidase inhibitor | 0.627 | 0.008 |
| | Free radical scavenger | 0.541 | 0.008 |
| **Coumaric acid** | Peroxidase inhibitor | 0.740 | 0.008 |
| | Antioxidant | 0.553 | 0.005 |
| | Lipid peroxidase inhibitor | 0.529 | 0.014 |
| | Free radical scavenger | 0.627 | 0.005 |
| **Oleuropein** | Antioxidant | 0.497 | 0.007 |
| | Lipid peroxidase inhibitor | 0.437 | 0.026 |
| | Free radical scavenger | 0.659 | 0.005 |
| ***p*-Tyrosol** | Peroxidase inhibitor | 0.798 | 0.005 |
| | Antioxidant | 0.341 | 0.018 |
| | Lipid peroxidase inhibitor | 0.452 | 0.023 |
| | Free radical scavenger | 0.380 | 0.019 |
| **3-Hydroxytyrosol** | Peroxidase inhibitor | 0.809 | 0.004 |
| | Antioxidant | 0.411 | 0.011 |
| | Lipid peroxidase inhibitor | 0.487 | 0.018 |
| | Free radical scavenger | 0.425 | 0.015 |
| **Chlorogenic acid** | Peroxidase inhibitor | 0.141 | 0.140 |
| | Antioxidant | 0.785 | 0.004 |
| | Lipid peroxidase inhibitor | 0.855 | 0.003 |
| | Free radical scavenger | 0.856 | 0.002 |
| | Peroxidase inhibitor | 0.676 | 0.015 |

| Phenolic compounds tested | Estimated biological activity | Probability[a] | |
|---|---|---|---|
| | | $P_a$ | $P_i$ |
| **Isoquercitrin** | Antioxidant | 0.913 | 0.003 |
| | Lipid peroxidase inhibitor | 0.976 | 0.002 |
| | Free radical scavenger | 0.978 | 0.001 |
| **Hyperoside** | Peroxidase inhibitor | 0.676 | 0.015 |
| | Antioxidant | 0.913 | 0.003 |
| | Lipid peroxidase inhibitor | 0.976 | 0.002 |
| | Free radical scavenger | 0.978 | 0.001 |
| **Hesperetin** | Peroxidase inhibitor | 0.812 | 0.004 |
| | Antioxidant | 0.746 | 0.004 |
| | Lipid peroxidase inhibitor | 0.885 | 0.003 |
| | Free radical scavenger | 0.878 | 0.002 |
| **Orientin** | Lipid peroxidase inhibitor | 0.760 | 0.004 |
| | Antioxidant | 0.828 | 0.003 |
| | Peroxidase inhibitor | 0.558 | 0.031 |
| | Free radical scavenger | 0.955 | 0.001 |
| **Glycitein** | Peroxidase inhibitor | 0.791 | 0.005 |
| | Antioxidant | 0.607 | 0.004 |
| | Lipid peroxidase inhibitor | 0.734 | 0.004 |
| | Free radical scavenger | 0.506 | 0.010 |
| **Amentoflavone** | Peroxidase inhibitor | 0.859 | 0.004 |
| | Antioxidant | 0.652 | 0.004 |
| | Lipid peroxidase inhibitor | 0.582 | 0.010 |
| | Free radical scavenger | 0.658 | 0.005 |
| **Luteolin** | Peroxidase inhibitor | 0.936 | 0.002 |
| | Antioxidant | 0.775 | 0.004 |
| | Lipid peroxidase inhibitor | 0.740 | 0.004 |
| | Free radical scavenger | 0.755 | 0.003 |
| **Morin** | Peroxidase inhibitor | 0.439 | 0.047 |
| | Antioxidant | 0.850 | 0.003 |
| | Lipid peroxidase inhibitor | 0.783 | 0.004 |
| | Free radical scavenger | 0.759 | 0.003 |
| **Tangeretin** | Peroxidase inhibitor | 0.736 | 0.009 |
| | Antioxidant | 0.645 | 0.004 |
| | Lipid peroxidase inhibitor | 0.682 | 0.005 |
| | Free radical scavenger | 0.715 | 0.004 |
| **Diosmetin** | Peroxidase inhibitor | 0.922 | 0.002 |
| | Lipid peroxidase inhibitor | 0.767 | 0.004 |
| | Free radical scavenger | 0.808 | 0.003 |
| **Tricin** | Peroxidase inhibitor | 0.920 | 0.002 |
| | Antioxidant | 0.737 | 0.004 |
| | Lipid peroxidase inhibitor | 0.760 | 0.004 |
| | Free radical scavenger | 0.797 | 0.003 |
| **Taxifolin** | Peroxidase inhibitor | 0.826 | 0.004 |
| | Antioxidant | 0.938 | 0.002 |

| Phenolic compounds tested | Estimated biological activity | Probability[a] | |
|---|---|---|---|
| | | $P_a$ | $P_i$ |
| **Catechin** | Lipid peroxidase inhibitor | 0.915 | 0.002 |
| | Free radical scavenger | 0.877 | 0.002 |
| | Peroxidase inhibitor | 0.721 | 0.010 |
| | Antioxidant | 0.810 | 0.003 |
| | Lipid peroxidase inhibitor | 0.888 | 0.003 |
| | Free radical scavenger | 0.842 | 0.002 |

[a]Estimated probability, $P_a$: active, $P_i$: inactive

Table 3. Estimates of potential toxic or adverse effects in humans of the phenolic compounds tested

| Phenolic compounds tested | Toxic or advers effect | Probability[a] | |
|---|---|---|---|
| | | $P_a$ | $P_i$ |
| **Kaempferol** | Genotoxicity | 0.843 | 0.003 |
| | Vascular toxicity | 0.773 | 0.023 |
| | Trembling | 0.783 | 0.044 |
| **Fisetin** | Trembling | 0.823 | 0.028 |
| | Genotoxicity | 0.761 | 0.004 |
| | Vascular toxicity | 0.755 | 0.026 |
| **Vitexin** | Diarrhea | 0.903 | 0.010 |
| | Vascular toxicity | 0.845 | 0.009 |
| | Inflammation | 0.803 | 0.015 |
| **Naringin** | Inflammation | 0.965 | 0.004 |
| | Diarrhea | 0.906 | 0.010 |
| | Neurotoxicity | 0.886 | 0.007 |
| **Myricetin** | Genotoxicity | 0.877 | 0.003 |
| | Inflammation | 0.763 | 0.019 |
| | Hematotoxicity | 0.774 | 0.031 |
| **Resveratrol** | Discoloration of urine | 0.905 | 0.004 |
| | Hematemesis | 0.901 | 0.004 |
| | Trembling | 0.899 | 0.006 |
| **Rosmarinic acid** | Hematemesis | 0.797 | 0.014 |
| | Discoloration of urine | 0.739 | 0.015 |
| | Trembling | 0.748 | 0.059 |
| **Epigallocatechin gallate** | Inflammation | 0.811 | 0.014 |
| | Vascular toxicity | 0.804 | 0.017 |
| | Hyperthermia | 0.671 | 0.022 |
| **Genistein** | Carcinogenicity (IARC Group 3) | 0.832 | 0.004 |
| | Hypothermia | 0.798 | 0.006 |
| | Diarrhea | 0.762 | 0.031 |
| **Daidzein** | Hypocalcemia | 0.775 | 0.002 |
| | Trembling | 0.783 | 0.044 |
| | Carcinogenicity (IARC Group 3) | 0.715 | 0.005 |
| **Gallic acid** | Hematemesis | 0.939 | 0,003 |
| | Aft | 0.934 | 0.003 |
| | Gastrointestinal bleeding | 0.887 | 0.003 |
| **Apigenin** | Vascular toxicity | 0.833 | 0.011 |
| | Trembling | 0.835 | 0.024 |
| | Reproductive dysfunction | 0.805 | 0.018 |
| **Ellagic acid** | Hematemesis | 0.830 | 0.010 |
| | Respiratory toxicity | 0.829 | 0.023 |
| | Trembling | 0.829 | 0.026 |
| **Syringic acid** | Gastrointestinal bleeding | 0.907 | 0.003 |
| | Hematemesis | 0.897 | 0.004 |
| | Aft | 0.888 | 0.004 |
| | Discoloration of urine | 0.902 | 0.004 |

| Phenolic compounds tested | Toxic or advers effect | Probability[a] | |
|---|---|---|---|
| | | $P_a$ | $P_i$ |
| **Caffeic acid** | Hematemesis | 0.885 | 0.005 |
| | Aft | 0.877 | 0.005 |
| **Ferulic acid** | Discoloration of urine | 0.839 | 0.007 |
| | Irritation | 0.819 | 0.004 |
| | Hypercholesterolemia | 0.812 | 0.004 |
| **Quinic acid** | Respiratory toxicity | 0.932 | 0.014 |
| | Trembling | 0.871 | 0.013 |
| | Aft | 0.855 | 0.008 |
| ***p*-Coumaric acid** | Hematemesis | 0.901 | 0.004 |
| | Trembling | 0.896 | 0.007 |
| | Discoloration of urine | 0.853 | 0.005 |
| **Oleuropein** | Hypercholesterolemia | 0.761 | 0.009 |
| | Inflammation | 0.646 | 0.032 |
| | Nausea | 0.630 | 0.055 |
| ***p*-Tyrosol** | Hematemesis | 0.904 | 0.004 |
| | Discoloration of urine | 0.899 | 0.004 |
| | Trembling | 0.888 | 0.008 |
| **3-Hydroxytyrosol** | Discoloration of urine | 0.930 | 0.003 |
| | Hematemesis | 0.889 | 0.004 |
| | Orthostatic hypotension | 0.884 | 0.005 |
| **Chlorogenic acid** | Neurotoxicity | 0.874 | 0.008 |
| | Sensitization | 0.832 | 0.004 |
| | Irritation | 0.801 | 0.005 |
| **Isoquercitrin** | Inflammation | 0.930 | 0.005 |
| | Hematotoxicity | 0.919 | 0.009 |
| | Diarrhea | 0.905 | 0.010 |
| **Hyperoside** | Inflammation | 0.930 | 0.005 |
| | Hematotoxicity | 0.919 | 0.009 |
| | Diarrhea | 0.905 | 0.010 |
| **Hesperetin** | Inflammation | 0.796 | 0.015 |
| | Vascular toxicity | 0.791 | 0.019 |
| | Genotoxicity | 0.687 | 0.005 |
| **Orientin** | Diarrhea | 0.907 | 0.010 |
| | Vascular toxicity | 0.853 | 0.009 |
| | Neurotoxicity | 0.834 | 0.014 |
| **Glycitein** | Hypothermia | 0.801 | 0.006 |
| | Hypocalcemia | 0.730 | 0.003 |
| | Peptic ulcer | 0.589 | 0.011 |
| **Amentoflavone** | Genotoxicity | 0.886 | 0.003 |
| | Vascular toxicity | 0.806 | 0.016 |
| | Reproductive dysfunction | 0.767 | 0.023 |
| **Luteolin** | Genotoxicity | 0.824 | 0.004 |
| | Reproductive dysfunction | 0.819 | 0.016 |
| | Trembling | 0.823 | 0.028 |

| Phenolic compounds tested | Toxic or advers effect | Probability[a] | |
|---|---|---|---|
| | | $P_a$ | $P_i$ |
| **Morin** | Genotoxicity | 0.800 | 0.004 |
| | Vascular toxicity | 0.773 | 0.023 |
| | Trembling | 0.783 | 0.044 |
| **Tangeretin** | Vascular toxicity | 0.793 | 0.019 |
| | Deprivation | 0.638 | 0.037 |
| | Hypothermia | 0.625 | 0.027 |
| **Diosmetin** | Vascular toxicity | 0.858 | 0.008 |
| | Genotoxicity | 0.780 | 0.004 |
| | Reproductive dysfunction | 0.785 | 0.020 |
| **Tricin** | Vascular toxicity | 0.822 | 0.013 |
| | Reproductive dysfunction | 0.782 | 0.021 |
| | Genotoxicity | 0,713 | 0.004 |
| **Taxifolin** | Diarrhea | 0.830 | 0.020 |
| | Vascular toxicity | 0.796 | 0.018 |
| | Inflammation | 0.728 | 0,022 |
| **Catechin** | Inflammation | 0.810 | 0.014 |
| | Vascular toxicity | 0.789 | 0.020 |
| | Hypertension | 0.786 | 0.017 |

[a]Estimated probability, $P_a$: active, $P_i$: inactive

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