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THE BALANCE OF PLANT EXTRACTS POTENCY TO SCAVENGE MODEL RADICAL AND TO REDUCE FERRIC ION IN MODEL COMPLEX

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ABSTRACT

Fifty randomly selected plant extracts were evaluated for their ability to scavenge a model radical and to reduce ferric ions in a model complex, using the DPPH and FRAP assays applied simultaneously in a microplate format. The DPPH assay measures the capacity of extracts to scavenge the stable DPPH• radical, broadly reflecting antioxidant potential. The FRAP assay assesses the ability to reduce Fe^{3+} in complex with TPTZ, which also indicates antioxidant activity but, under certain conditions, may reflect the potential to generate more reactive pro-oxidant metal ion species. The ratio of these two activities was expressed as the Pro-oxidant/Antioxidant Balance Index (PABI). Most extracts showed higher activity in the FRAP assay, suggesting a greater potential for potential pro-oxidant metal ion formation than radical scavenging. However, some taxa, including *Aesculus hippocastanum* L., *Clematis vitalba* L., *Mespilus germanica* L., and *Piper nigrum* L., displayed predominant radical scavenging activity. Both properties followed a normal distribution well-fitted by a Gaussian function, allowing extrapolation of frequency for chosen $DPPH_{50}$ and $FRAP_{50}$ values. Plant extracts with borderline PABI values, after systematic research, could be valuable for applications in the food industry, therapeutics, and cosmetics.

Keywords: Balance, Plant extracts, Radical scavenging, Ferric ion reduction, Study *in silico*

INTRODUCTION

The demand for high-quality substances with antioxidant efficacy remains highly relevant, particularly in key sectors such as pharmaceuticals, cosmetics, and the food industry **(Chongtham *et al.*, 2018; Jaykumar and Claudete, 2023)**. Antioxidants represent a significant commodity, with a growing preference for natural over synthetic sources (Perez *et al.*, 2022; McCarthy *et al.*, 2001). However, recent studies have revealed that many antioxidants independently of an origin (and their natural sources) can exhibit both antioxidant and pro-oxidant effects **(Maliar *et al.*, 2023)**. This phenomenon can be described as the balance between two opposing activities: the ability to scavenge or neutralize free radicals and the capacity to reduce transition metals to more pro-oxidative forms **(Perron *et al.*, 2011)**.

Radical scavenging may be mediated by various atoms, including oxygen **(Lang *et al.*, 2024)**, sulfur **(Ulrich and Jakob, 2019)**, nitrogen and carbon **(Schöneich *et al.*, 1992)**, in specific structural arrangement. The oxygen atom is particularly significant, for instance in hydroxyl groups on double bonds or aromatic rings, as in ascorbic acid and vitamin E **(Jiang *et al.*, 2022; Mahmoud *et al.*, 2013)**. Sulfur atoms can act through the interconversion of certain thiols to disulfides **(Luque-Ceballos *et al.*, 2023)**, while nitrogen atoms within or adjacent to aromatic systems, such as in berberine, also contribute **(Imenshahidi and Hosseinzadeh, 2020)**. Paradoxically, even carbon atoms can play a role—specifically those in conjugated systems between two double bonds—as demonstrated by the process of lipid peroxidation **(Babbs and Steiner, 1990)**. Conversely, these antioxidant-functional moieties also interact with transition metals via coordination bonds, thus contributing to their pro-oxidant activity **(Nemeikaitė-Čėnienė *et al.*, 2005)**. Plant extracts are complex mixtures of primary and especially secondary metabolites, many of which may still be unknown or insufficiently characterized. Each plant source thus displays a unique profile in its ability to scavenge radicals and to modulate the oxidation state of transition metals.

The goal of the present study is to explore the diversity of both antioxidant and pro-oxidant properties in 50 randomly selected plant extracts, aiming to better understand the balance between these activities across different botanical sources.

MATERIAL AND METHODS

Chemicals

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH•); 1,1-Diphenyl-1-picrylhydrazin (DPPH); 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ); sodium acetate; acetic acid; $FeCl_2.4H_2O$; $FeCl_3.6H_2O$; Merck /Sigma/ (USA).

Plant material and extract preparation

Randomly selected plant material from 50 plant species was chosen based on their bioactivity in our current research. A total of 2 g of dried plant matter was disintegrated into small pieces (under 5 mm particle size). The plant matter was then extracted in screwed-up tubes with 20 mL of a 48% ethanol solution in the dark at room temperature for 24 h. Afterward, the extract was filtered and stored in Eppendorf tubes at 4 °C in the dark.

Determination of antioxidant activity/AOXAP/ and prooxidant activity /PROOXA/

Antioxidant activity was determined using the DPPH assay, and pro-oxidant/antioxidant activity was assessed using the FRAP assay. Both were performed in 96 wells microplates with equal concentrations (0.4 $mmol \cdot dm^{-3}$) of the key reagents DPPH, TPTZ, and $FeCl_3$. The detailed methodology was described previously **(Maliar *et al.*, 2023)**.

Briefly, a 0.4 $mmol \cdot dm^{-3}$ DPPH• radical solution was prepared in ethanol, and the FRAP reagent was freshly prepared by combining solution A (0.0187 g TPTZ in 10 mL ethanol) with solution B (0.338 g sodium acetate in 88.3 mL water and 1.748 mL acetic acid). In addition, 1.2 $mol \cdot dm^{-3}$ solutions of $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$ were freshly prepared before each experiment. The microplate layout used for the assays has been published previously. Dilution of the tested samples (plant extracts) was carried out directly on the microplate by serial two-fold

dilution. Conversion standard solutions were then added to the respective wells. The assay was initiated by adding 150 µL of DPPH reagent to rows A–C and 100 µL of FRAP reagent to rows D–F. Subsequently, 100 µL of the reaction mixture was removed from the wells in rows A–C. The microplate was incubated for 10 min at room temperature, after which absorbance was measured at 520 nm for DPPH and at 630 nm for FRAP. The optical density (OD) values were corrected by subtracting the background readings and converted into percentage conversion values using 0% and 100% conversion standards.

Calculation of final variables

$DPPH_{50}$ (X_1) and $FRAP_{50}$ (X_3) parameters (expressed in µmol.dm^{-3}) were calculated from the following function plot: percentage of conversion = f (concentration of dry matter weight of the extract sample). The pro-oxidant antioxidant balance index /PABI/ was calculated according to Equation (1), using the micromolar units for compounds and dry matter for extracts:

$$/PABI/ = FRAP_{50}/DPPH_{50} \quad (1)$$

To improve visualization of sample efficacy and to enable clearer comparative interpretation, the $DPPH_{50}$ and $FRAP_{50}$ values were transformed into their inverse forms, defined as $X_2 = 1/DPPH_{50}$ and $X_4 = 1/FRAP_{50}$. These transformed parameters were applied to the dataset of experimentally determined values. Frequency histograms were then generated in Microsoft Excel, where frequency (F) represents the percentage of samples exhibiting activity within a given interval. The histograms for each variable were fitted with a Gaussian function according to Equation (2):

$$F= a*\exp\{ [- (X -b)^{2}] /2c\} \quad (2)$$

where *X* corresponds to X_1 and X_2 for $DPPH_{50}$ values, X_3 and X_4 for $FRAP_{50}$ values, and X_5 and X_6 for PABI index values.

RESULTS AND DISCUSSION

Table 1 presents the primary results obtained from 50 plant extract samples. The original dataset published by **Maliar *et al.* (2023)** was extended to cover a broader diversity of plant extracts containing the main classes of secondary metabolites, including polyphenols, flavonoids, anthocyanins, alkaloids, thiols, carotenoids, conjugated dienes, and others. The cited publication from our research team indicates that the ability to scavenge DPPH$^{\bullet}$ radical is primarily influenced by the presence of double bonds and aromatic hydroxyl groups, whereas the capacity to reduce ferric ions in complex with TPTZ depends on the presence of heteroatoms, the degree of hybridization, and the magnitude of the negative partial charge. The detailed structural features of secondary metabolites that govern these properties will be addressed in a separate scientific article.

Specifically, Table 1 includes the following parameters: $DPPH_{50}$ and $FRAP_{50}$ values, correlation coefficients reflecting the accuracy of parameter determination, and the calculated PABI index values. The $DPPH_{50}$ and $FRAP_{50}$ results are expressed in milligrams of dry matter per milliliter of extract (mg DM/mL).

Table 1 $DPPH_{50}$ and $FRAP_{50}$ values, corresponding correlation coefficients, and Pro-oxidant/Antioxidant Balance Index (PABI) values for an extended collection of 50 plant extract samples

No.:	Plant Species	*Latin name and botanical classifier*	Plant Part	$DPPH_{50}$ ± SD (mg dm/mL)	r^2	$FRAP_{50}$ ± SD (mg dm/mL)	r^2	PABI ± SD
1	Anise	*Pimpinella anisum*, L.	fruits	0.94 ± 0.04	0.92	1.23 ± 0.05	0.95	1.31 ± 0,06
2	Apple	*Malus domestica*	peels	6.12 ± 4.15	0.91	32.90 ± 4.73	0.94	5.38 ± 0,44
3	Baltic pine	*Pinus sylvestris*	bark	0.54 ± 0.15	0.91	0.79 ± 0.04	0.96	1.46 ± 0,09
4	Barley, var. Zlatan	*Hordeum vulgare,* L.	grain	over	-	over	-	-
5	Basil	*Ocimum basilicum*, L.	leaves	over	-	over	-	-
6	Black elderberry,	*Sambuicus nigra*, L.	flower	1.09 ± 0.10	0.97	1.61 ± 0.2	0.97	1.47 ± 0,11
7	Black pepper	*Piper nigrum*, L.	fruits	0.52 ± 0.04	0.99	5.13 ± 0.36	0.95	9.86 ± 0,56
8	Carrot	*Daucus carota*, L.	radix	over	-	31.49 ± 0.63	0.94	-
9	Chamomile,	*Matricaria chamomilla*, L.	flower	5.06 ± 0.30	0.98	5.03 ± 0.20	0.98	0.99
10	Chives	*Allium schoenoprasum*	leaves	50.25 ± 6.9	0.93	1172.39 ± 229.70	0.90	23.33
12	Chives	*Allium schoenoprasum*	flower	over	-	14.22 ± 0.60	0.99	-
13	Citrus	*Citrus medica* L.	Peels	0.56 ± 0.07	0.97	0.41 ± 0.07	0.95	0.73 ± 0,08
14	Common wormwood,	*Artemisia absinthium*, L.	leaves	1.73 ± 0.10	0.98	1.25 ± 0.10	0.96	0.73 ± 0,07
15	Fig	*Ficus carica*, L.	fruits	over		over		
16	Garlic	*Allium sativum*, L.	bulb	0.76 ± 0.16	0.95	5.00 ± 2.14	0.99	6.53 ± 0,52
17	Garlic	*Allium sativum*, L.	Leaves	10.28 ± 1.15	0.99	over	-	-
18	Ginger,	*Zingiber officinale*, Roscoe.	root	4.62 ± 0.20	0.99	4.43 ± 0.30	0.99	0.95 ± 0,11
19	Grape	*Vitis vinifera*, L.	berries	0.06 ± 0	0.97	0.08 ± 0	0.97	1.26 ± 0,08
20	Grape pomace	*Vitis vinifera*, L.	tassel, nucleus	0.34 ± 0.13	0.97	0.16 ± 0	0.99	0.47 ± 0,02
21	Grape wine,	*Vitis vinifera*, L.	frost dried grapes	3.52 ± 0.10	0.97	40.63 ± 1.80	0.96	11.53 ± 1,08
22	Green tea,	*Camelia sinensis*, (L.) Kuntze.	flower	1.93 ± 0.10	0.92	0.21 ± 0.10	0.99	0.11 ± 0,02
11	Horse chestnut,	*Aesculus hippocastanum*, L.	leaves	1.82 ± 0.10	0.98	9.09 ± 0.04	0.93	4.98 ± 0,44
23	Horseradish	*Armoracia rusticana*	leaves	4.02 ± 0.23	0.92	over	-	-
24	Leek	*Allium porum*	Leaves	over	-	over	-	-
25	Ligure	*Levisticum officinale*, L.	leaves	3.71 ± 0.42	0.98	6.93 ± 0.38	0.92	1.87 ± 0,09
26	Liquorice,	*Glycyrrhiza glabra*, L.	root	5.62 ± 0.20	0.95	3.41 ± 0.20	0.97	0.61 ± 0,07
27	Marigold	*Calendula officinalis*, L.	flower	2.46 ± 0.32	0.91	8.24 ± 1.64	0.96	3.34 ± 0,21
28	Medlar	*Mespilus germanica*, L.	Fruit	0.50 ± 0.07	0.95	2.84 ± 1.26	0.98	5.60 ± 0,31
29	Oat, var. RACOON	*Avena sativa,* L.	grains	over	-	over	-	-
30	Oat, var. TAIDON	*Avena sativa,* L.	grains	over	-	over	-	-
31	Old man's beard,	*Clematis vitalba*, L.	bark	7.72 ± 0.30	0.91	32.33 ± 0.20	0.94	4.18 ± 0,03
32	Onion,	Allium cepa, L.	Bulb	0.19 ± 0.02	0.99	0.15 ± 0	0.99	0.79 ± 0,08
33	Orange	*Citrus sinensis*, L.	peels	3.39 ± 0.14	0.91	7.66 ± 0.98	0.94	2.26 ± 0,18

34	Parsley	*Petroselinum crispum, L.*	leaves	over	-	over	-	-
35	Pedunculate oak,	*Quercus robur*, L.	bark	0.50 ± 0.10	0.97	0.30 ± 0.10	0.94	0.61 ± 0,05
36	Plum	*Prunus domestica*	fruits	0.92 ± 0.08	0.99	2.93 ± 0.32	0.91	3.18 ± 0,29
37	Pumpkin	*Cucurbita pepo*, L.	grains	over	-	over	-	-
38	Rapeseed,	*Brassica napus*, L.	grains	145.15 ± 0.10	0.96	11.64 ± 0.30	0.96	0.08 ± 0,01
39	Rhubarb,	*Rheum rhabarbarum*, L.	root	1.39 ± 0.20	0.96	0.58 ± 0.10	0.93	0.42 ± 0,04
40	Rose	*Rosa canina*, L.	flower	0.87 ± 0.05	0.94	0.66 ± 0.07	0.98	0.76 ± 0,07
41	Rosmary	*Rosmarinus officinalis*, L.	leaves	1.34 ± 0.06	0.92	0.95 ± 0.09	0.96	0.71 ± 0,06
42	Sage,	*Salvia officinalis*, L.	leaves	0.27 ± 0.10	0.975	1.73 ± 0.10	0.96	6.27 ± 0,55
43	Sessile oak,	*Quercus petraea*, (Matt.) Liebl.	leaves	2.54 ± 0.20	0.95	1.81 ± 0.22	0.98	0.71 ± 0,07
44	Silver birch,	*Betula pendula*, Roth.	leaves	1.60 ± 0.20	0.99	2.37 ± 0.30	0.99	1.48 ± 0,12
45	Spearmint	*Mentha piperita*, L.	leaves	0.33 ± 0.02	0.96	0.38 ± 0.04	0.99	1.15 ± 0,12
46	Star anise	*Illicium verum*, L.	fruits	2.22 ± 0.04	1.00	3.37 ± 0.09	0.99	1.52 ± 0,11
47	Thistle,	*Silybum marianum*, (L.) Gaertn.	grain	2.22 ± 0.40	0.99	1.61 ± 0.10	0.97	0.73 ± 0,07
48	Tomato fruit	*Solanum lycopersicum*, L.	fruits	over		12.11 ± 0.74	0.98	-
49	Turmeric,	*Curcuma longa*, L.	root	17.52 ± 0.50	0.99	10.44 ± 0.40	0.98	0.59 ± 0,05
50	Woundwort,	*Prunella vulgaris*, L.	flower	1.57 ± 0.10	0.97	1.27 ± 0. 10	0.96	0.81 ± 0,06

As shown in Table 1, there are evident differences among the plant extracts. Out of the 41 plant extract samples prepared according to the described method, 6 samples (14.63%) exhibited antioxidant activity—measured by the DPPH and FRAP assays— that exceeded the selected concentration range.

The most potent extracts, those with $DPPH_{50}$ values below 1 mg/mL (indicating strong antioxidant capacity by the DPPH method), included: *grape (Vitis vinifera L.), onion (Allium cepa L.), sage (Salvia officinalis L.), spearmint (Mentha piperita L.), grape pomace (Vitis vinifera L.), citrus (Citrus medica L.), pedunculate oak (Quercus robur L.), medlar (Mespilus germanica L.), black pepper (Piper nigrum L.), garlic (Allium sativum L.), rose (Rosa canina L.), plum (Prunus domestica L.), and anise (Pimpinella anisum L.).*

On the other hand, plant taxa with $FRAP_{50}$ values below 1 mg/mL (indicating a strong ferric-reducing capacity with potential pro-oxidant effect) included: *grape (Vitis vinifera L.), onion (Allium cepa L.), spearmint (Mentha piperita L.), grape pomace (Vitis vinifera L.), citrus (Citrus medica L.), pedunculate oak (Quercus robur L.), and rose (Rosa canina L.),* as well as additional taxa not observed among the top DPPH-active extracts, namely *green tea (Camellia sinensis L. Kuntze), rhubarb (Rheum rhebarbarum L.), and rosemary (Rosmarinus officinalis L.).*

Taxa with favorable PABI index values, indicating significantly higher $FRAP_{50}$ values relative to $DPPH_{50}$, included: *medlar (Mespilus germanica L.) = 5.60, sage (Salvia officinalis L.) = 6.27, garlic (Allium sativum L.) = 6.53, black pepper (Piper nigrum L.) = 9.09, and grape (Vitis vinifera L.) = 11.53.*

The aim of this study was to assess whether the investigated properties follow a normal (Gaussian) distribution in a limited set of plant extract samples (n = 50). Specifically, the study examined the ability to scavenge the model radical DPPH$^{\bullet}$, the ability to reduce ferric ions (Fe^{3+}) in complex with TPTZ (FRAP assay), and the PABI index derived from these two properties. For both activity parameters, inverse values were also calculated, as transformations: $X_2 = 1/DPPH_{50}$ and $X_4 = 1/FRAP_{50}$ to offer better graphical interpretation of Gauss curve. Frequency histograms were generated for both the original and transformed variables and subsequently fitted using a Gaussian function. The results obtained are presented in Figures 1 and 2, including the exact Gaussian equation parameters along with statistical parameters R^2 and F-ratio.

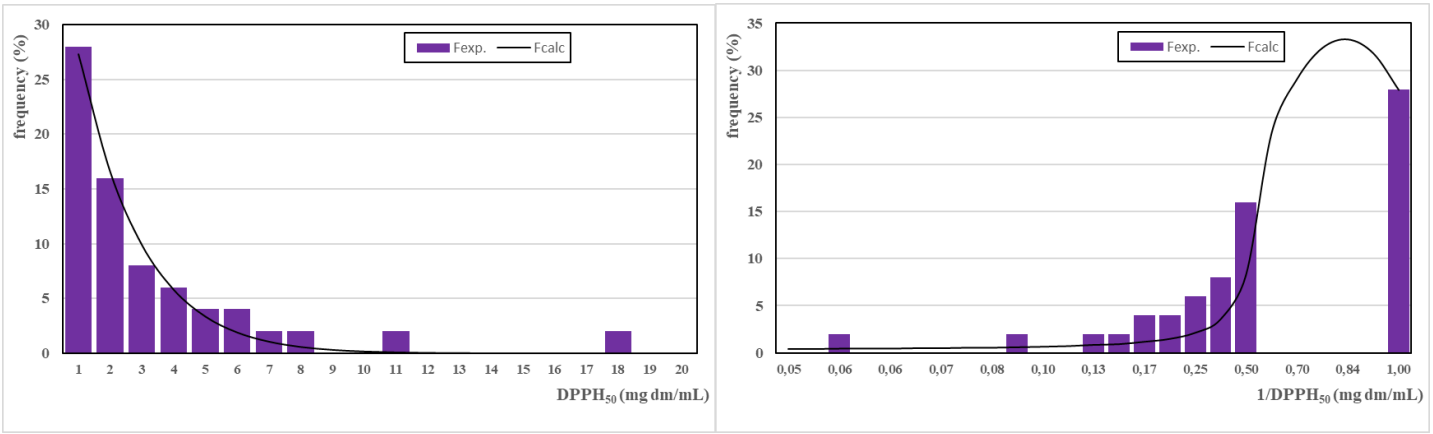

Figure 1 Normal distribution of the DPPH$^{\bullet}$ radical scavenging activity for a collection of 50 plant extract samples, displayed as a frequency histogram fitted with a Gaussian function; fitting coefficients are provided. (A, F= ($DPPH_{50}$): r^2= 0.978; F= 383.64; a= 25767.11; b= -29.67, c= 7.90. (B, F=($1/DPPH_{50}$): r^2= 0.977; F= 357.23; a= 16.27; b= 0.84; c= 0.29.

It is evident from Figures 1 and 2 that the distribution of both properties presented follows a normal distribution, which is well described and fitted by the Gaussian function. The correlation coefficients (r^2) for all cases fall within the interval 0.93–0.98. Interpretation of the Gaussian function equations for both parameters shows that the use of inverse values, $X_2=1/DPPH_{50}$ and $X_4=1/FRAP_{50}$, is statistically justified and provides improved graphical visualization of the curve's peak position, represented by parameter *b*. The performance of the PABI index parameter in terms of its normal distribution, fitted using the Gaussian function (Figure 3).

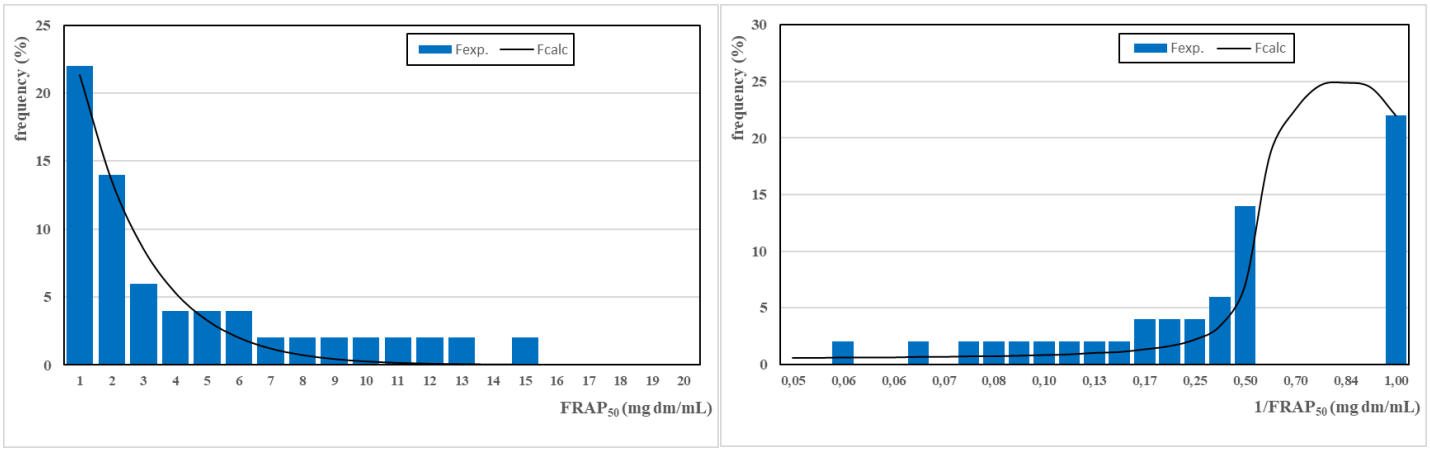

Figure 2 Normal distribution of the Fe^{3+} reducing capacity (in complex with TPTZ) for a collection of 50 plant extract samples, displayed as a frequency histogram fitted with a Gaussian function; fitting coefficients are provided. (A, F= ($FRAP_{50}$): r^2= 0.934; F= 120.96; a= 81868.48; b= -39.04, c= 9.47. (B, F=($1/FRAP_{50}$): r^2= 0.977; F= 358.76; a= 12.44; b= 0.84; c= 0.3

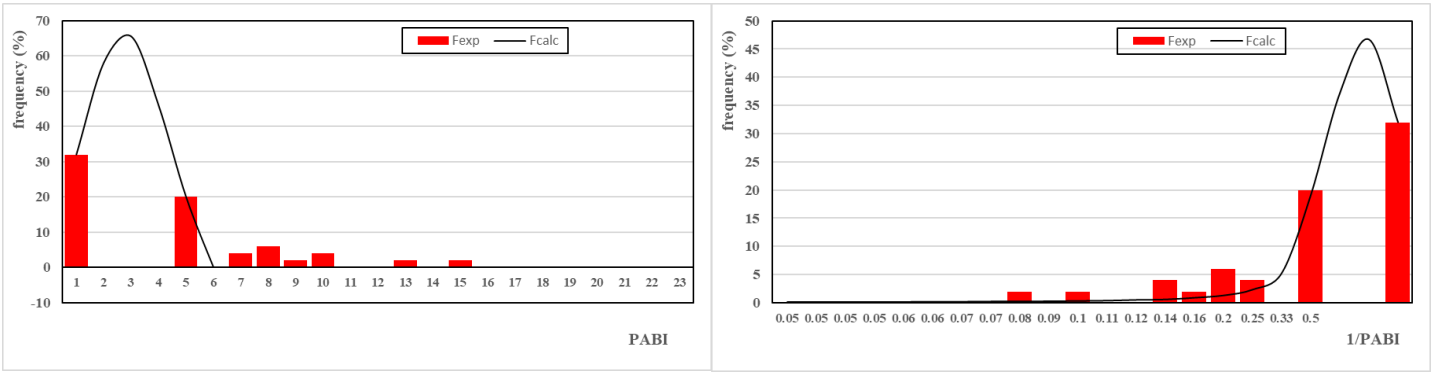

Figure 3 Normal distribution of the final parameter – PABI index for a collection of 50 plant extract samples, displayed as a frequency histogram fitted with a Gaussian function; fitting coefficients are provided. (A, F= (PABI): r^2= 0.936; F= 123.75; a= 66.61, b= 1.44, c= 0.36. (B, F=(1/PABI): r^2= 0.942; F= 138.6; a= 46.71; b= 0.8; c= 0.23.

It is evident that applying the Gaussian function to the frequency histogram of the PABI index yields a different outcome. While this parameter also follows a normal distribution and shows a strong fit to the Gaussian function (r^2= 0.936) the resulting curve is noticeably narrower, reflected by a lower value of parameter *c*. This suggests a reduced variability of PABI values and a lower potential diversity within the examined set of plant extract samples.

CONCLUSIONS

These findings provide a framework for predicting both the antioxidant–pro-oxidant profile of novel plant extracts and the likelihood of encountering specific activity levels. Extracts with low $FRAP_{50}$ or low PABI indices—indicative of strong ferric-reducing capacity—are preferable when a pro-oxidative effect is desired, for example to suppress microbial growth or inhibit neoplastic cell proliferation. Conversely, after resolution of such conditions, extracts with low $DPPH_{50}$ or high PABI indices should be prioritized to counteract oxidative stress and promote cellular repair, reflecting a predominance of radical scavenging over ferric ion–reducing activity.

The Gaussian function equations enable estimation of the probability (frequency) of encountering extracts with specific activity levels, such as a $DPPH_{50}$ or $FRAP_{50}$ of 0.5 mg DM/mL. For DPPH• radical scavenging, this probability is <0.1%, indicating that statistically robust detection would require screening ≥1000 samples; a similar trend applies to Fe^{3+}-reducing capacity in the TPTZ complex. In contrast, both activities occur most frequently (~30%) at moderate levels, around 1 mg DM/mL. The narrower distribution of the PABI index indicates reduced variability, making extracts with extreme values (< or > thresholds) relatively rare (~1% frequency) and requiring screening of >100 samples for reliable identification. Such extracts, however, are of particular interest for targeted biotechnological, therapeutic, and cosmetic applications.

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