

***In vitro* Antioxidant and Free Radical Scavenging Activity of the Bark of *Dillenia indica* L.**

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Abstract

The aim of the study was to explore the antioxidant and free radical scavenging activity of bark of *Dillenia indica* L. The methanolic extract (CME) of coarse powder of the dried bark was fractionated with pet-ether (PETF), chloroform (CHF), and water (AQF) and each fraction was investigated for antioxidant and free radical potentials. The phenolic contents of CME, CHF, PETF and AQF were found to be 90.2, 171.1, 52.1 and 158.1 and flavonoid contents 182.6, 340.2, 285.9, and 362.6 mg of GAE/g of dried extract, respectively. The total antioxidant activity of CME, PETF, CHF, AQF and standard catechin in terms of absorbance were 0.373, 0.707, 0.88, 1.051 and 0.804, respectively at 160 µg/ml concentration indicating AQF possessing the highest total antioxidant activity. In DPPH radical scavenging activity, the AQF and CHF showed potential radical scavenging activity followed by CME and PETF with IC₅₀ values of 1.5, 2.5, 23 and 61 µg/ml, respectively, whereas standard ascorbic acid showed IC₅₀ at 8 µg/ml. Among the extractives, AQF and CHF exhibited potent hydroxyl radical scavenging activity with IC₅₀ values of 49.0 and 42 µg/ml followed by CME and PETF with IC₅₀ of 86 and 168 µg/ml, respectively. In ferric-reducing antioxidant assay, AQF, CHF and CME (absorbance 2.262, 2.418 and 2.157, respectively) showed higher activity than that of standard (1.793) followed by PETF (absorbance 0.593). Since polyphenols like flavonoids play a vital role in controlling antioxidants, polyphenol rich extracts are potent source of antioxidants. Our data showed that the chloroform fraction of the bark of *D. indica* provided highest polyphenols and antioxidant activities, hence, can be used further as a source of natural antioxidants.

Key words: *Dillenia indica*, Dilleniaceae, Polyphenols, Free radicals, Antioxidant activity.

Introduction

Generation of free radicals (reactive oxygen species, ROS) inside biological system gives rise to oxidative stress (OS) that plays a role in a variety of diseases, including cancer, neurodegenerative disorder, heart disease, rheumatoid arthritis and in the aging process (Das *et al.*, 2021; Alam *et al.*, 2016). Exogenous as well as endogenous antioxidants are free radical scavenging agents that play a role against the development of chronic, age-related degenerative diseases, hence, dietary antioxidants lower the risk of

these disease (Sharifi-Rad *et al.*, 2020). In addition to health benefit, antioxidants prevent or delay oxidation of food that are initiated by free radical formation during their exposure to environmental factors such as light, air and temperature (Franco *et al.*, 2019). At present a significant number of antioxidants are synthesized as medicines that produce various side effects *in vivo* (Xu *et al.*, 2021).

From ancient time, plants have been used in traditional medicine in most of the civilized cultures and tribes and are proven to be the potential source of

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natural antioxidants (Reza *et al.*, 2021). Flavonoids, polyphenols, carotenoids, folic acid, trans-cinnamic acids, tocopherols, etc., are some of the important antioxidants produced by the plant kingdom for their sustenance. The plant *Dillenia indica* L. (family Dilleniaceae), available in various parts of Bangladesh and are locally called Chalta, has a wide variety of folkloric reputation (Akter *et al.*, 2021). It is used by the rural people in the treatment of fever, constipation, dysentery, stomach ache, snake bite, burning sensation in the chest, food poisoning, bacterial infection, diabetes, etc. (Saiful and Armania, 2014). A growing body of evidence has revealed the potential of whole plant of *D. indica* as a source of various antioxidants, including polyphenols (Rahayu *et al.*, 2020), flavonoids (Saha *et al.*, 2009), triterpenoids and steroids (Kumar *et al.*, 2011), etc. There is no complete data on total phenolic and flavonoid content, antioxidant capacity, radical scavenging activity and ferric reducing activity of extracts from *D. indica* bark. Hence, the aim of the current study was to determine the total phenolic and flavonoid content and total antioxidant activity of the extracts and their free radical scavenging activity.

Materials and Methods

Collection and preparation of plant materials: *D. indica* bark was purchased from Dinajpur, Bangladesh, in October-November, 2020 and was identified by taxonomist. A voucher specimen (JU/32234) is deposited at the Bangladesh National Herbarium. The collected stem bark was washed with tap water, shade dried for several days with occasional sun drying followed by oven drying at 45 °C for 24 hours and was ground into coarse powder. The powdered bark was kept in air tight container at room temperature.

Preparation of extract: Powdered bark (500 g) was soaked in about 1500 ml methanol in an amber-colored container. The sealed container was occasionally shaken and stirred for 6-7 days and after 7 days extract was filtered through cotton filter followed by filter paper, concentrated by rotary evaporator in reduced pressure and preserved at 4 °C

(40 g, CME). After dilution with water, the CME was partitioned with pet-ether followed by chloroform to get petroleum ether (PETF), chloroform (CHF), and finally aqueous (AQF) fractions (Rahman *et al.*, 2015).

Determination of total phenolics: The total phenolic content (TPC) of the samples was determined using method described by Wolfe *et al.*, 2003. An aliquot was mixed with 2 ml (75 g/l) of Na₂CO₃ in a tube and diluted 2 ml Folin-Ciocalteu reagent. The tube was vortexed (15 sec.) and allowed to stand for 20 minutes at 25 °C for color development. Absorbance (at 760 nm) was measured using Shimadzu spectrophotometer and the result was expressed in terms of standard gallic acid equivalent (mg of GA per gram of bark extract). Each experiment was performed in triplicate.

Determination of total flavonoids: Total flavonoids (TFC) were estimated using AlCl₃ colorimetric assay described by Ordonez *et al.*, 2006. 100 µl of 10% AlCl₃, 1.5 ml of MeOH, 100 µl of 1 M potassium acetate solution and 2.8 ml of distilled water were added to 0.5 ml of sample. The absorbance (at 420 nm) was measured in UV spectrophotometry after 90 minutes of incubation at 25 °C. The result was expressed in terms of catechin equivalent (mg of catechin per gram of dry bark extract).

Determination of total antioxidant capacity: Total antioxidant capacity (TAC) was calculated by method described by Prieto *et al.*, (1999). In brief, samples at different concentrations (each 0.5 ml) was mixed with 3 ml of H₂SO₄ (0.6 M), Na₃PO₄ (28 mM) and ammonium molybdate (1%) mixture. The absorbance (at 695 nm) of samples was measured after incubation at 95 °C for 10 min. Increased absorbance is the indication of increased total antioxidant capacity.

DPPH free radical scavenging assay: DPPH, common abbreviation for 2,2-diphenyl-1-picrylhydrazyl is a chemical used to determine free radical scavenging potential (DRSA). The assay was performed using modified method described by Kedare and Singh, 2011. 2.4 ml of DPPH (0.1 mM)

in MeOH solution was mixed with 1.6 ml of methanolic samples of various concentrations. The mixture was vortexed, left in the dark (25-30 minutes) and the absorbance (at 517 nm) were measured to calculate % of DRSA by “inhibition % = $[(A_0 - A_1) / A_0] \times 100$ ” equation, where A_0 is the absorbance of control and A_1 is that of extractives. From % of inhibition vs concentration curve “ IC_{50} ” was calculated.

Hydroxyl radical-scavenging assay: Hydroxyl radical-scavenging assay (HRSA) was performed by modified method of Klein *et al.* (1981). 500 μ l of extract (1 mg/ml in 70% MeOH) was taken in test tube and following reagents were added to it: 100 μ l of H_2O_2 (1.0 mM), 100 μ l of 2-deoxy D-ribose (28 mM), 100 μ l of EDTA (1.04 mM), 100 μ l of $FeCl_3$ (200 μ M) and 100 μ l of ascorbic acid (1mM). The mixtures were incubated for 1 hr at 37 °C and 10% TCA and 1% TBA (1 ml each) were added in each test tube. Finally, mixtures are incubated at 100 °C (for 20 minutes) and the absorbance (at 532 nm) were taken to calculate % HRSA by “% = $[(A_0 - A_1) / A_0] \times 100$ ”, where A_0 is the absorbance of control and A_1 , extractive. From curve IC_{50} was calculated.

Determination of ferric-reducing antioxidant capacity: Oyaizu (1986) described the ferric reducing capacity (FRC) method. In the method, 0.25 ml of different diluted samples were added to 0.625 mL of 0.2 M potassium buffer and 0.625 mL of 1% $[K_3Fe(CN)_6]$ solution and the mixture was incubated for 20 min at 50 °C. After that, 0.625 ml TCA (10% solution) was added followed by centrifuged at 3000

rpm (for 10 min). Lastly, 1.8 ml supernatant was withdrawn and mixed with distilled water (1.8 ml) and 0.1% $FeCl_3$ (0.36 ml) solution. The absorbance (700 nm) of solution was measured and found an increased absorbance is the indication of increased ferric reducing capacity.

Results and Discussion

Amount of bark extract and its fractions: The dried coarse powder of *D. indica* (500 g) was extracted with 1500 ml methanol for 7 days (3 times) and the mixtures were combined, filtered and concentrated to get 40 g concentrated brownish mass. After dilution with adequate amount of water, the extract was partitioned with pet-ether, chloroform and water to get PETF (9.8 g, 24.5%), CHF (10.15 g, 25.3%) and AQF (20 g, 50%) fractions. The data indicates that around half of the phytochemicals were polar in nature.

Total phenolic (TPC) and flavonoid (TFC) contents of D. indica: TPC and TFC of the bark extracts were calculated on the basis of standard gallic acid curve and the results were expressed as GAE/g of dried extractives. TPC of CME, CHF, PETF and AQF was found to be 90.2, 171.1, 52.1 and 158.1 mg of GAE/g of dried bark extract, respectively (Figure 1A). From the data it was shown that the phenolic content of CHF as well as AQF was higher than original extract CME, hence chloroform fraction might serve as a good source for phenolics as well as antioxidants (Alam *et al.*, 2016).

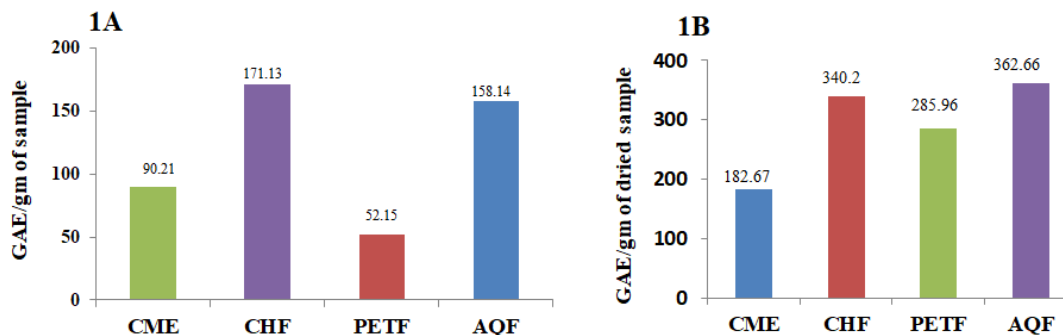


Figure 1. (A) Total phenolic and (B) Total flavonoid contents of CME and three fractions of *D. indica* (mg/g plant extract in GAE).

The TFC of CME, PETF, CHF and AQF were 182.6, 285.9, 340.2 and 362.6 mg of GAE/gm of dried bark extract, respectively (Figure 1B). The data indicates that CHF contained two times higher amounts of flavonoids than that of CME and the results demonstrated that *D. indica* is a significant source of flavonoids.

Total antioxidant capacity (TAC) of CME and various fractions of D. indica: The TAC of CME and

standard CA are represented in Figure 2A. The results showed that CME possessed comparable antioxidant activity to the standard CA (0.373 vs 0.804) at a concentration of 160 $\mu\text{g/ml}$. Among the fractions, AQF showed the highest TAC (1.051) followed by CHF (0.88), and PETF (0.707) at 160 $\mu\text{g/ml}$ (Figure 2B). Our results demonstrated that all the extractives of *D. indica* possessed appreciable antioxidant activity.

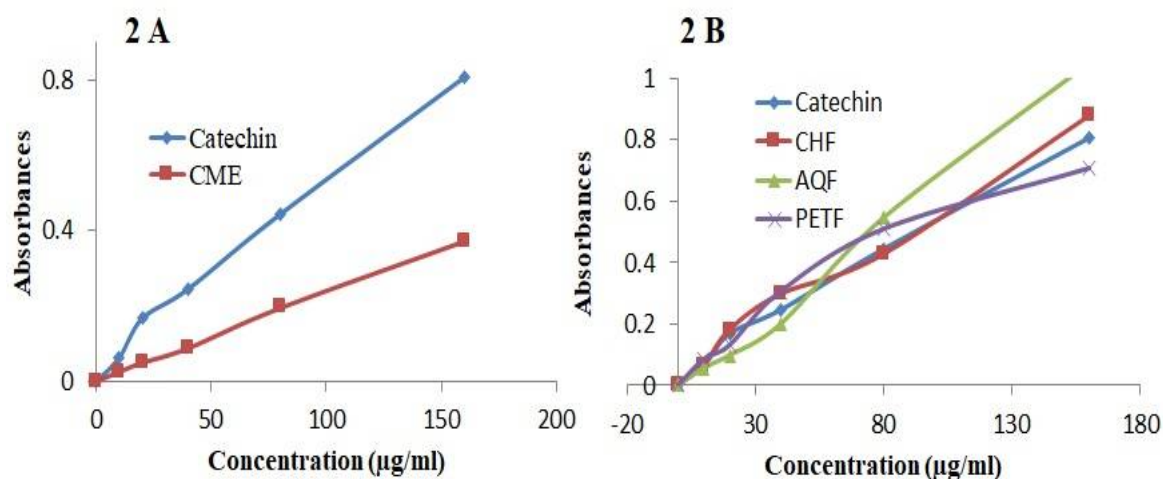


Figure 2. Total antioxidant activity of (A) CME and standard CA and (B) PETF, CHF, AQF and standard CA.

DPPH Free radical scavenging assay (DRSA) of CME and various fractions of D. indica: The results of DRSA of various fractions are shown in Table 1 where ascorbic acid was used as standard. From the table, the IC_{50} values of CME and fraction PETF, CHF, AQF and ascorbic acid were found to be 23, 61, 2.5, 1.5 and 8 $\mu\text{g/ml}$, respectively. Among the extractives, the significant scavenging activity was found in AQF and CHF. Our observation demonstrated that all the extractives of bark of *D. indica* possessed significant DPPH free radical scavenging activity.

Hydroxyl radical scavenging activity (HRSa) of various fraction of D. indica: In the HRSa, scavenging activity percentage was calculated. It was observed that the activity was concentration dependent (Figure 3A). The IC_{50} values of CME,

PETF, CHF, AQF and standard AA were found to be 86, 168, 42, 49 and 17.5 $\mu\text{g/ml}$, respectively (Figure 3B). Among the fractions, the highest scavenging activity was found in CHF and AQF. Our observation demonstrated that all the extractives of bark of *D. indica* possessed significant hydroxyl free radical scavenging activity.

Ferric-reducing antioxidant capacity: The ferric reducing capacity (FRC) of the different extractives of *D. indica* is represented in Table 2. Among the fractions, CHF (2.418), AQF (2.262) and CME (2.157) showed higher reducing capacity than standard AA (1.793) at 160 $\mu\text{g/ml}$. The reducing power of different extractives and standard AA showed the following order: CHF > AQF > CME > AA > PETF.

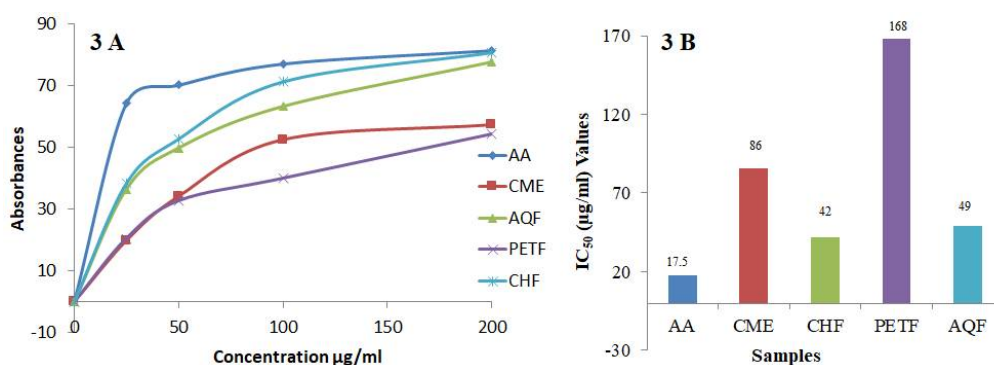


Figure 3. (A) Hydroxyl radical scavenging activity of standard AA, CME and various fractions at different concentrations and (B) IC₅₀ (µg/ml) values of different extractives and standard for hydroxyl radical scavenging activity.

Table 1. DPPH free radical scavenging activity (DRSA) of AA, CME and various fractions at different concentrations.

Conc. (µg/ml)	Sample with % of scavenging (Mean ± STD)*				
	AA	CME	PETF	CHF	AQF
100	96.3±0.20	93.6±0.38	86.7±2.58	91.4±0.40	91.6±0.14
50	96.2±0.20	94.3±0.45	39.6±5.87	93.1±0.52	93.4 ±0.56
25	96.2±0.68	53.7±9.31	14.8±3.25	94.9±0.22	93.2±0.39
12.5	95.7±0.26	28.7±2.94	8.4±1.58	94.4±0.3	87.2±2.26
6.25	34.7±16.03	13.5±0.67	5.5±0.56	90.5±0.68	86.0±2.16
3.124	23.7±1.93	7.2±0.89	3.6±0.87	75.6±1.32	81.4±0.82
IC ₅₀ (µg/ml)	8±0.93	23±1.13	61±2.41	2.5±0.99	1.5±0.22

*The values are the mean± SD of triplicate experiment. AA, CME, PETF, CHF and AQF represent ascorbic acid, methanolic extract, pet-ether fraction, chloroform fraction and water fraction, respectively.

Table 2. Ferric reducing power capacity of ascorbic acid, CME and its three fractions at different concentrations.

Conc. (µg/ml)	Sample with Absorbance (Mean ± STD)*				
	AA	CME	PETF	CHF	AQF
160	1.79± 0.09	2.15± 0.08	0.77± 0.05	2.41± 0.19	2.26± 0.09
80	1.50± 0.15	1.57± 0.19	0.39± 0.08	2.23± 0.11	2.09± 0.05
40	0.74± 0.06	0.87± 0.05	0.24± 0.07	1.96± 0.13	1.74±0.10
20	0.55± 0.05	0.48± 0.15	0.12± 0.05	1.15± 0.01	1.15± 0.10
10	0.27± 0.01	0.19± 0.05	0.05± 0.05	0.73± 0.12	0.59± 0.04

*The values are the mean± SD of triplicate experiment.

Conclusion

The crude methanolic extract of the bark of *D. indica* and its several fractions, especially chloroform fraction CHF showed remarkable antioxidant activity

by inhibiting DPPH, hydroxyl and reducing power activities when compared with standards. Moreover, the chloroform fraction was found to have a noticeable quantity of polyphenols and flavonoids

which play a major role in controlling antioxidant potentials of plant. In conclusion, the findings with significant radical scavenging as well as antioxidant activity of chloroform and aqueous fractions may provide valuable information for bioassay guided isolation of active principle responsible for particular activity.

References

- Akter, M. J., Ali, S., Hasnin, I., Khatune, N. A. and Rahman, M. A. A. 2021. Cytotoxicity and sedative activity of steam bark of *Dillenia indica* L. *Bang. Pharm. J.* **24**, 105-110.
- Alam, A. H. M. K., Hossain, A. S. M. S., Khan, M. A., Kabir, S. R., Reza, M. A., Rahman, M. M., Islam, M. S., Rahman, M. A. A., Rashid, M. and Sadik, M. G. 2016. The antioxidative fraction of white mulberry induces apoptosis through regulation of p53 and NFκB in EAC cells. *PLoS One* **11**, 0167536.
- Das, R. R., Rahman, M. A., Al-Araby, S. Q., Islam, M. S., Rashid, M. M., Babteen, N. A., Alnajeebi, A. M., Alharbi, H. F. H., Jeandet, P., Rafi, M. K. J., Siddique, T. A., Uddin, M. N. and Zakaria, Z. A. 2021. The antioxidative role of natural compounds from a green coconut mesocarp undeniably contributes to control diabetic complications as evidenced by the associated genes and biochemical indexes. *Oxid. Med. Cell. Longev* **2021**, 2021.
- Franco, R., Navarro, G. and Martinez-Pinilla, E. 2019. Antioxidants versus food antioxidant additives and food preservatives. *Antioxidants (Basel)* **8**, 542.
- Kedare, S. B. and Singh, R. P. 2011. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol* **48**, 412-422.
- Klein, S. M., Cohen, G. and Cederbaum, A. I. 1981. Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical-generating systems. *Biochemistry* **20**, 6006-6012.
- Kumar, S., Kumar, V. and Prakash, O. 2011. Microscopic evaluation and physiochemical analysis of *Dillenia indica* leaf. *Asian Pac. J. Trop. Biomed.* **1**, 337-340.
- Ordóñez, A., Gomez, J., and Vattuone, M. 2006. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food chem.* **97**, 452-458.
- Oyaizu, M. 1986. Studies on products of browning reaction: Antioxidant activities of products of browning reaction prepared from glucoamine. *Jpn. J. Nutr. Diet.* **44**, 307-315.
- Prieto, P., Pineda, M. and Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* **269**, 337-341.
- Rahayu, S., Zahara, I., Afifah, A., P. K. and Supriyatin, S. 2020. Antioxidant capacity of *Dillenia* sp. leaf extract against DPPH (1,1-Diphenyl-2picryl Hidrazil) radical Antioxidant capacity of *Dillenia* sp. leaf extract against DPPH (1,1-Diphenyl-2picryl Hidrazil) radical. *J. Phys. Conf.* **1402**, 1-8.
- Rahman, M. M., Islam, M. B., Biswas, M. and Khurshid Alam, A. H. M. 2015. *In vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Res. Notes* **8**, 1-9.
- Reza, A. S. M. A., Haque, M. A., Sarker, J., Nasrin, M. S., Rahman, M. M., Tareq, A. M., Khan, Z., Rashid, M., Sadik, M. G., Tsukahara, T. and Alam, A. K. 2021. Antiproliferative and antioxidant potentials of bioactive edible vegetable fraction of *Achyranthes ferruginea* Roxb. in cancer cell line. *Food Sci. Nutr.* **9**, 3777-3805.
- Saha, M. R., Alam, M., Hasan, R., Hossain, M., Akter, R. and Rana, E. 2009. *In-vitro* anti-oxidant activity of the leaves of *Dillenia indica*. *Orient. Pharm. Exp. Med.* **9**, 277-284.
- Saiful Yazan, L. and Armania, N. 2014. *Dillenia* species: A review of the traditional uses, active constituents and pharmacological properties from pre-clinical studies. *Pharm. Biol* **52**, 890-897.
- Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M. E., Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., Setzer, W. N., Calina, D., Cho, W. C. and Sharifi-Rad, J. 2020. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Front. Physiol.* **11**, 694.
- Wolfe, K. Wu, X. and Liu, R. H., 2003. Antioxidant activity of apple peels. *J. Agric. Food Chem.* **51**, 609-614.
- Xu, X., Liu, A., Hu, S., Ares, I., Martínez-Larrañaga, M.-R., Wang, X., Martínez, M., Anadón, A. and Martínez, M.-A. 2021. Synthetic phenolic antioxidants: Metabolism, hazards and mechanism of action. *Food Chem.* **353**, 129488.