RESEARCH ARTICLE

ANTIOXIDANT ACTIVITY OF *Rhizophora apiculata* STEM BARK EXTRACT

T. Manoranjan*, S. Kalubovila, G. Sashikesh

Department of Chemistry, Faculty of Science, University of Jaffna, Thirunelvely, Jaffna 40000, Sri Lanka

ABSTRACT

Oxidative stress, a pathological condition stemming from an imbalance between the production of harmful free radicals and the body's antioxidant defenses, is a critical factor in the pathogenesis of numerous diseases. The overproduction of reactive oxygen and nitrogen species exacerbates this imbalance, contributing to cellular damage and tissue injury. *Rhizophora apiculata*, a mangrove plant with a rich ethnomedical tradition in Asia and Africa, has been employed for centuries in treating diverse diseases. Given its historical use and potential therapeutic benefits, this research study aimed to evaluate the antioxidant properties of its stem bark. The phenolic content of the methanolic stem bark extract from *R. apiculata* was quantified, and its antioxidant potential was assessed using the DPPH radical scavenging assay and ascorbic acid used as a standard. There was a positive correlation between phenolic content and antioxidant activity, suggesting that phenolic compounds are the principal contributors to the extract's antioxidant characteristics. This research finding indicates a direct relationship between the amount of methanolic extract and its total phenolic content as its ability to act as an antioxidant. The methanolic extract demonstrated antioxidant properties with an IC_{50} value of 271.2 ppm. Furthermore, the methanolic extract exhibited a total phenolic content of 10.86 ± 0.21%.

Keywords: Antioxidant activity, phenolic content, IC_{50} value, DPPH assay.

DOI. https://doi.org/10.4038/jsc.v15i2.72

*Corresponding author: tmranjan@univ.jfn.ac.lk

1. INTRODUCTION

Normal physiological activities produce oxygen-centered free radicals and other reactive oxygen species (ROS) [1]. While synthetic antioxidants have been used to reduce oxidative effects, concerns about their toxicity have sparked an increased interest in the study of natural antioxidant sources derived from plants [2, 3].

Antioxidant compounds play a critical role in mitigating a variety of diseases, including chronic inflammation, atherosclerosis, cancer, and cardiovascular disease, as well as attenuating the aging process [4-6]. Consequently, they hold significant commercial value in the pharmaceutical [7, 8], food [9, 10], and cosmetic industries [11, 12]. The growing consumer inclination towards natural products, coupled with concerns over the potential toxicity of synthetic antioxidants, has fostered a sensitive interest in the development of natural antioxidant-based solutions.

Mangroves constitute a diverse group of halophytic plants thriving in the intertidal regions of tropical and subtropical coastal ecosystems. These plants have developed specialized morphological and physiological adaptations to endure the challenging conditions imposed by tidal inundation and high salinity. *R. apiculata*, a prominent mangrove species, has been traditionally utilized by local populations for various purposes, including sterilization [13], deodorization [13-15], and growth promotion [16]. Previous investigations have reported the isolation of triterpenes and diterpenoids from this plant [14], while phenolic compounds have been identified in its pyroligneous acid [17]. Furthermore, previous scientific studies have confirmed the antioxidant potential of *R. apiculata* bark extract [17, 18].

The compounds responsible for the antioxidant activity within the methanolic crude extract of *R. apiculata* stem bark have yet to be elucidated. The expanding interest in phenolic-rich diets has spurred significant research into the potential health benefits of these compounds in nutrition and food science. Phenolic compounds, characterized by the presence of at least one hydroxyl group on an aromatic ring, are recognized for their antioxidant properties. These compounds can directly contribute to antioxidant action through electron donation or indirectly by stimulating the endogenous antioxidant defense system [19, 20]. Extensive literature supports the role of phenolic compounds in mitigating oxidative stress through various mechanisms, including free radical

scavenging [21], peroxide decomposition [22], metal chelation [23, 24], and oxygen quenching.

2. MATERIAL AND METHODS

2.1 Preparation of plant extracts

The stem bark of *R. apiculta* plants was collected from Kadolkele area in Thaladuwa, Negombo, Sri Lanka (7°11'N, 79°50'E) and the herbrium sample perpared and deposited in the depatment of chemistry, University of Jaffna. Stem bark samples were washed thoroughly with tap water, followed by a rinse with deionized water. Subsequently, the samples were air-dried under ambient light conditions. The collected bark was cleaned, dried in the open air, and ground into a fine powder. This powder was subjected to methanol extraction using a soxhlet apparatus for 24 hours. The resulting methanol extract was concentrated through rotary evaporation, and the mass of the crude extract was determined.

2.2 DPPH Free Radical Scavenging Capacity

The free radical scavenging capacity (RSC) of *R. apiculta* stem bark extracts was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. A DPPH stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol. The solution was filtered to obtain a working solution with an absorbance of 517 nm. 1.0 mL of bark extract solution was combined with 3.0 mL of DPPH working solution in a test tube for the RSC determination. A control solution was made by mixing 3.0 mL of DPPH solution with 1.0 mL of methanol. The reaction mixtures were incubated in the dark at 25°C for 30 minutes before measuring absorbance at 517 nm. The discoloration was measured at 517 nm at least in triplicate and radical scavenging capacity, which can be expressed as the percentage of antioxidants or radical scavenging capacity (RSC) was calculated using the following formula [27]:

$$\text{DPPH free radical scavenging capacity (\%)} = \frac{(A_c - A_s)}{A_c} \times 100\ \%$$

where A_c is the absorbance for the control reaction and A_s is the absorbance for the experimental samples.

2.3 Determination of the total phenol content

A 10 mg of crude extract was transferred to a 100 mL volumetric flask and dissolved in distilled water to prepare a 100 ppm stock solution. Subsequent serial dilutions were performed to obtain a 50 ppm extract solution.

A volume of 5.0 mL of 10 ppm phenolic solution was transferred to a boiling tube. The solution was subsequently mixed with 0.2 mL of buffer solution (pH=10) and 0.2 mL of 4-aminoantipyrine (4-AAP) reagent. Finally, 0.2 mL of potassium ferricyanide was added and the mixture was thoroughly homogenized. The reaction mixture was allowed to equilibrate. Subsequently, a 0.4 mL aliquot of the supernatant was transferred to a quartz cuvette and its absorbance was measured using a Jasco V-570 UV-VIS-NIR spectrophotometer within the spectral range of 425 nm-725 nm [28, 29]. The entire experiment was carried out with phenolic solutions containing 7.5, 5.0, 2.5, 1.0, 0.5, 0.25 and 0.1 ppm.

10.0 mg of crude was added to 100 mL of distilled water. The resultant suspension was shaken vigorously at 100 rpm for 5 minutes in an orbital shaker, and then it was allowed to settle. The supernatants were filtered using a Buchner funnel. The filtrate was subjected to spectrophotometric analysis using a Jasco V-570 UV-VIS-NIR spectrophotometer. The 5.0 mL of the filtrate was combined with 2.0 mL of pH 10 buffer, 0.2 mL of 4-aminoantipyrine (4-AAP) solution, and 0.2 mL of potassium ferricyanide [29]. The absorbance of the antipyrine dye complex, characterized by its intense reddish colour, was measured at a wavelength of 510.5 nm. This experimental procedure was replicated using a 50 ppm extract solution.

3. RESULTS AND DISCUSSION

3.1 DPPH-free radical scavenging capacity

Several chemical species can interact with DPPH radicals by donating hydrogen atoms or transferring electrons [30]. Phenolic compounds, in particular, have a strong reactivity with DPPH, rendering them important contributors to antioxidant capacity [31]. The hydrogen atom abstraction process, also known as the hydrogen atom transfer (HAT) mechanism, usually includes sequential or contemporaneous electron and proton transfer

phases [30]. As a result, the DPPH test provides a quantitative measure of plant extracts' overall reduction capability.

The antioxidant mechanisms of phenolic compounds are generally based on HAT or single-electron transfer followed by proton transfer. However, an unambiguous distinction between these paths is not always feasible [32]. HAT-based assays quantify the ability of antioxidants to donate a hydrogen atom to quench free radicals. In contrast, SET-based assays measure the capacity of antioxidants to transfer an electron, thereby reducing various species including radicals, metals, and carbonyl compounds [33]. In addition to these mechanisms, the sequential proton loss electron transfer (SPLET) pathway has also been identified [34].

The primary mechanism underlying the DPPH radical scavenging activity of antioxidants involves hydrogen atom donation from the antioxidant to the stable DPPH radical, as illustrated in Figure 1. Multiple factors, including the molecular structure and concentration of the antioxidant, influence this reaction's efficiency.

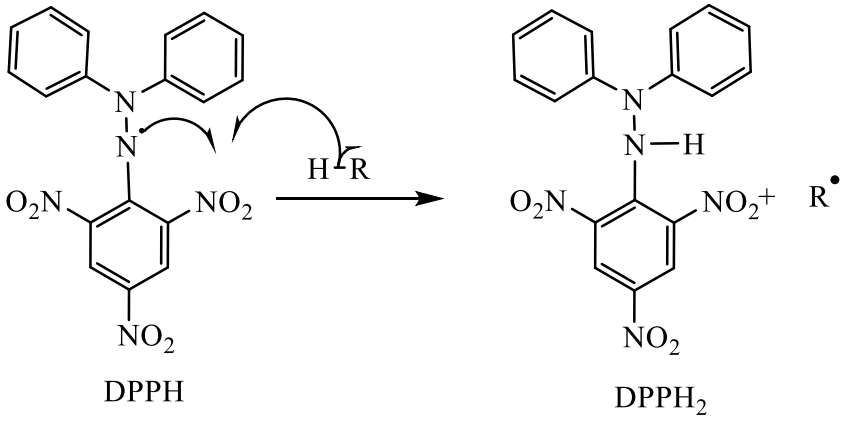

Figure 1: DPPH reduction by an antioxidant compound. RH hydrogen atom donor

The DPPH radical is characterized by a strong absorbance at 517 nm, resulting in a deep purple colour [35]. Upon interaction with an antioxidant, DPPH undergoes reduction to DPPH-H, accompanied by a decrease in absorbance at 517 nm due to reduced hydrogen content. This reduction manifests as decolorization, transitioning from purple to yellow. As antioxidant concentration increases, the degree of decolorization becomes more pronounced. The antioxidant activity of *R. apiculta* stem bark extract (500 ppm), as

determined by its ability to scavenge DPPH radicals, yielded an absorbance of 0.1365 at 517 nm, corresponding to a calculated antioxidant percentage of 55.27%.

Table 1. Antioxidant activity of the 500 ppm *R. apiculta* stem bark extract with DPPH

	Absorbance at 517 nm	DPPH free radical scavenging capacity (%)
Control	0.3052	- -
Sample	0.1365	55.28

3.1.1 Effect of the Concentration of *R. apiculta* stem bark extract

Antioxidant compounds were efficiently extracted from *R. apiculata* stem bark using methanol. The antioxidant capacity of these extracts was evaluated using the DPPH free radical scavenging assay. As illustrated in Figure 2, the antioxidant activity exhibited a concentration-dependent manner, with a clear positive correlation between extract concentration and free radical inhibition over time. These findings provide insights into the temporal dynamics of the extracts' antioxidant properties. Notably, the 500 ppm extract solution demonstrated much greater levels of free radical scavenging ability than the other concentrations evaluated with the particular incubation time.

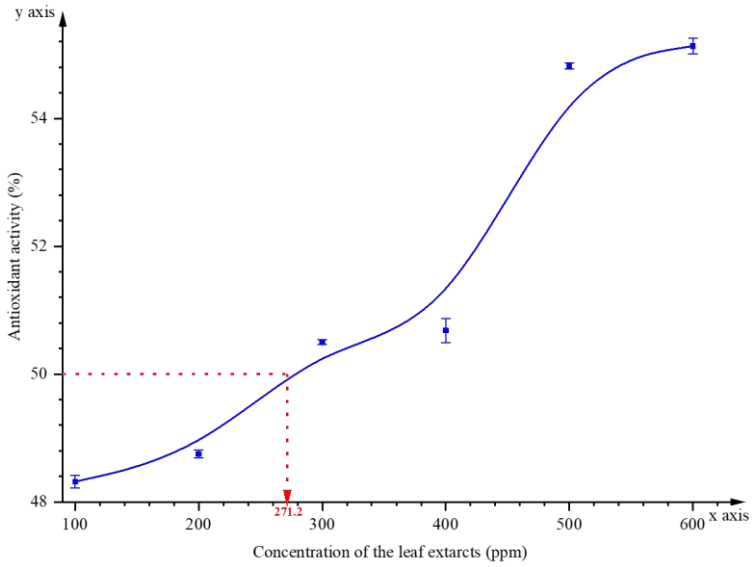

Figure 2: The correlation between antioxidant activity and R. apiculata stem bark extract concentration at a constant incubation duration of 5 minutes.

3.1.2 Effect of the incubation period on the antioxidant activities of *R. apiculta* stem bark extract

Figure 3 demonstrates that the radical scavenging capacity (RSC) of *R. apiculata* stem bark extract is unaffected by variations in incubation time when the extract concentration remains constant. This consistent RSC across different time points suggests that the extract's antioxidant properties are primarily influenced by its concentration rather than the duration of the assay. These findings highlight the concentration-dependent nature of the antioxidant activity exhibited by the *R.apiculata* stem bark extract and underscore the importance of concentration as a critical factor in determining its radical scavenging capacity

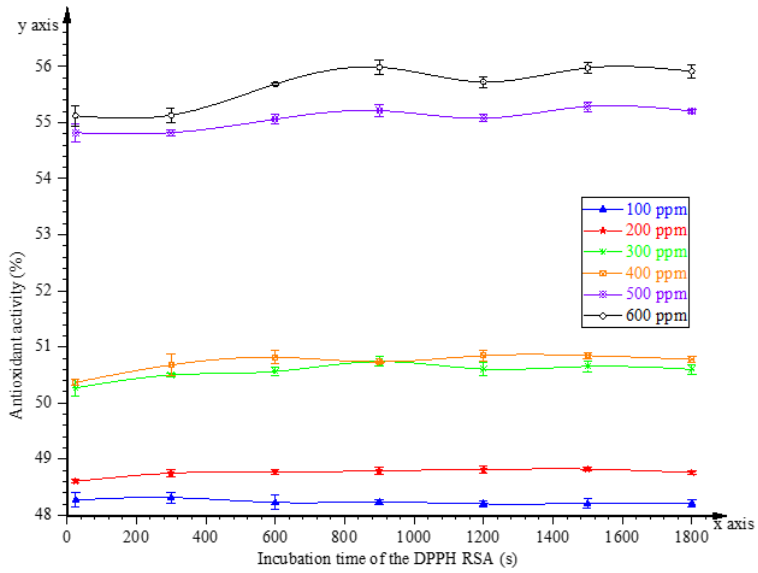

Figure 3: The correlation between antioxidant activity and incubation period for various R. apiculata stem bark extract concentrations.

3.1.3 Importance of the IC_{50} Value in DPPH Radical Scavenging Activity

Various antioxidant concentrations are used to determine the amount of antioxidants required to neutralize 50% of the initial DPPH radical species in a particular period. This quantity is often known as the inhibitory concentration (IC_{50}). Lower IC_{50} values suggest that the antioxidant is capable of scavenging DPPH radicals more efficiently. The IC_{50} is a widely used statistic in biochemistry that compares the antioxidant effectiveness of

various substances [30]. The IC_{50}, as a quantitative measure of radical scavenging affinity, it is an essential tool for assessing antioxidant activity.

The present investigation focused on evaluating the IC_{50} values of the *R. apiculata* stem bark extract, which was shown to have an IC_{50} value of 271.2 ppm (see figure 2), suggesting antioxidant activity. In comparison, the reference standard, L-ascorbic acid, has a substantially lower antioxidant ability, as revealed by an IC_{50} value of 41.25 ppm [36].

3.2 Estimation of the total Phenolic Content

In this research study, the visible absorption spectrum for the various concentrations (25.0 ppm – 0.1 ppm) of standard phenol solution was recorded in the wavelength range of 425 nm - 725 nm to find the optimum wavelength for the maximum absorbance. Figure 4(a) clearly shows that the noticeably higher absorbance was found at 510.5 nm for the above-mentioned concentration of standard phenol solution. The standard calibration curve was plotted for the absorbance at the optimum wavelength 510.5 nm as a function of the concentration of phenol solution, it is shown in Figure 4(b).

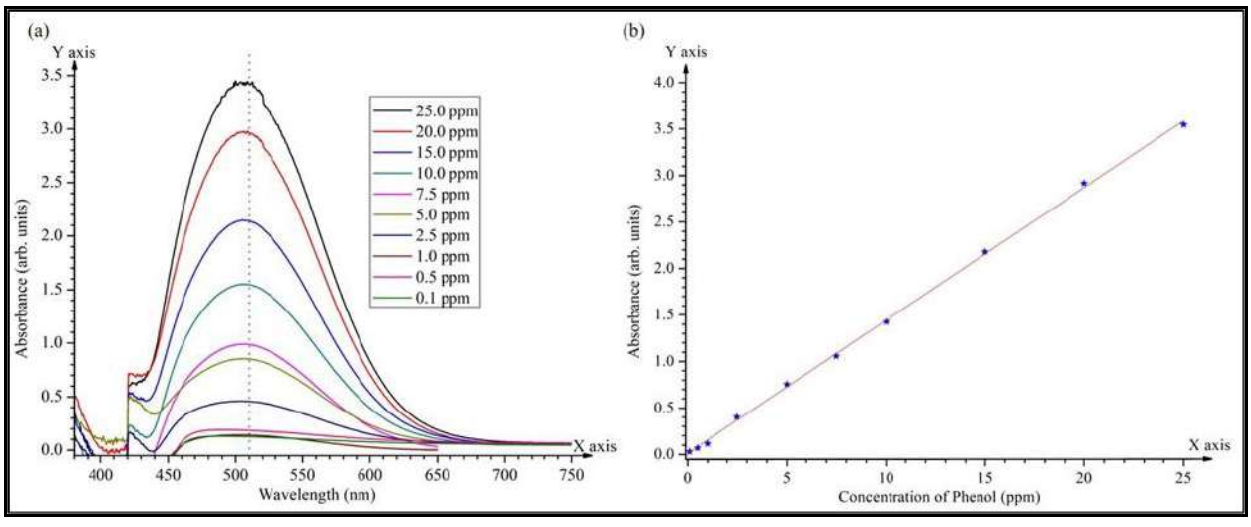

Figure 4: (a) The absorbance spectra of various concentrations of standard phenol solutions in the presence of potassium ferricyanide and 4-APP reagent within the wavelength range of 425 nm - 725 nm and (b) The standard calibration curve of the standard phenol solution at the optimum wavelength 510.5 nm.

The amount of total phenolic content was determined using the Folin-Ciocalteu method [29]. A standard calibration curve was constructed for phenol, exhibiting a linear relationship between absorbance and concentration (Y= 0.14312 X= + 0.01088) as depicted in Figure 4(b). The methanol extract from *R. apiculata* stem bark contained 10.86 ± 0.21 of total phenols using the established standard calibration curve, as shown in Table 2.

Table 2. Phenolic content of R. apiculta stem bark extract

R. apiculata stem bark extract (ppm)	Absorbance at 517 nm	Obtained phenolic content (ppm)	Percentage of phenolic content (%)
50 ppm	0.81664	5.63 ppm	11.26
100 ppm	1.49789	10.39 ppm	10.39
200 ppm	3.05075	21.24 ppm	10.62
300 ppm	4.79824	33.45 ppm	11.15

4. CONCLUSION

Phenolic compounds constitute a diverse group of aromatic secondary metabolites widely distributed in plants. This study investigated the antioxidant capacity of polyphenolic compounds derived from the *R. apiculata* stem bark. Methanolic extracts of the stem bark were analyzed for total phenolic content, free radical scavenging activity, and overall antioxidant potential. Antioxidant activity was assessed using the DPPH radical scavenging assay. The results indicate a positive correlation between the concentration of the methanolic extract and total phenolic content and antioxidant activity. The IC_{50} value was evaluated by antioxidant activity and was reported to be 271.2 ppm. The extract exhibited a total phenolic content of 10.86 ± 0.21 %.

R. apiculata stem bark demonstrated only moderate antioxidant activity, these findings still highlight its potential as a natural source of antioxidants. Further investigation into the specific phenolic compounds responsible for the observed antioxidant activity could provide valuable insights for developing novel antioxidant-based products.

5. REFERENCES

[1]. Bozidarka, L.Z., Mirjana, T.M., and Esma, R.I., (2023) *Free radicals: Relationship to Human Diseases and Potential Therapeutic applications.* The International Journal of Biochemistry & Cell Biology. **154**: p. 106346.

[2]. Wanyi, W., Ping, X., He, Z., Qingqing, Z,. Chunyang, L,. and Guibin, J, (2021) *Analysis, occurrence, toxicity and environmental health risks of synthetic phenolic antioxidants: A review.* Environmental Research. **201**: p. 111531.

[3]. Liu, R. and Mabury, S.A., (2020) *Synthetic Phenolic Antioxidants: A Review of Environmental Occurrence, Fate, Human Exposure, and Toxicity.* Environmental Science \& Technology. **54**(19): p. 11706-11719.

[4]. García-Sánchez, A., Miranda-Díaz, A.G., and Cardona-Muñoz, G.G., (2020) *The Role of Oxidative Stress in Physiopathology and Pharmacological Treatment with Pro- and Antioxidant Properties in Chronic Diseases.* Oxidative Medicine and Cellular Longevity. **2020**(1): p. 2082145.

[5]. Kohen, R. and Nyska, A., (2002) *Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification.* Toxicol Pathol,. **30**(6): p. 620-50.

[6]. Aurelia, M., Pisoschi, A.P., Florin, I., Loredana, S., Gabriel, P., and Andreea, I.S,. (2021). *Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status.* European Journal of Medicinal Chemistry, **209**: p. 112891.

[7]. Aline, F.B., Daiele, D.S, Jaqueline, P.S., and Jessica, F.H., (2022) *Phenolic compounds from coffee by-products: Extraction and application in the food and pharmaceutical industries.* Trends in Food Science and Technology. **123**: p. 172-186.

[8]. Sanjeet, M., Rahul, K.G., Obulisamy, P.K., and Pradeep, V., (2021) *Microalgae for high-value products: A way towards green nutraceutical and pharmaceutical compounds.* Chemosphere,. **280**: p. 130553.

[9]. João P. Trigo, E.M.C.A.J.A.S., and Manuela, E.P., (2020) *High value-added compounds from fruit and vegetable by-products – Characterization, bioactivities, and application in the development of novel food products.* Critical Reviews in Food Science and Nutrition,. **60**(8): p. 1388--1416.

[10]. Alirezalu, A., Nima, A., Peyman, S., Ali, S., Kazem, A., Amin, M.K., Francisco, J.B, Paulo, E.S.M., and Jose, M.L., (2020) *Physicochemical Characterization, Antioxidant Activity, and Phenolic Compounds of Hawthorn (Crataegus spp.) Fruits Species for Potential Use in Food Applications.* Foods,. **9**(4): p. 436.

[11]. Hoang, H.T., Moon, Y.-T., and Lee, Y.-C., (2021) *Natural Antioxidants from Plant Extracts in Skincare Cosmetics: Recent Applications, Challenges and Perspectives.* Cosmetics,. **8**(4): p. 106.

[12]. Çağla, Y., Ceren, G., and Suphi, S.O., (2020) *Potential of microalgal compounds in trending natural cosmetics: A review.* Sustainable Chemistry and Pharmacy,. **17**: p. 100304.

[13]. Loo, A.Y., Jain, K., and Darah, I., (2007) *Antioxidant and radical scavenging activities of the pyroligneous acid from a mangrove plant, Rhizophora apiculata.* Food Chemistry,. **104**(1): p. 300-307.

[14]. Gao, M. and Xiao, H., (2012) *Activity-guided isolation of antioxidant compounds from Rhizophora apiculata.* Molecules, **17**(9): p. 10675-82.

[15]. Nattaya, P., Monthon, N., Buppha, S., Nootsara, N., Nattadon, R., and Rettanaphol, M., (2013) *Silk fabric dyeing with natural dye from mangrove bark (R. apiculata Blume) extract.* Industrial Crops and Products,. **49**: p. 122-129.

[16]. Tangapo, A.M., Mambu, S.M., Colondam, B.J., Pelealu, J., and Maabuat, P.V., (2024) *The potency of endophytic bacteria isolated from Rhizophora apiculata as plant growth-promoting*. in *AIP Conference Proceedings*. AIP Publishing.

[17]. Loo, A.Y., Jain, K., and Darah, I., (2008) *Antioxidant activity of compounds isolated from the pyroligneous acid, Rhizophora apiculata.* Food Chemistry,. **107**(3): p. 1151-1160.

[18]. Afidah, A.R., Emmanuel, R., Jean, S., Jain, S., and Sani, I., (2008) *Antioxidant activities of mangrove Rhizophora apiculata bark extracts.* Food Chemistry, **107**(1): p. 200-207.

[19]. Kruk, J., Aboui-Enein, B.H., Duchnik, E., and Marchlewicz, M., (2022) *Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise.* J Physiol Sci,. **72**(1): p. 19.

[20]. Andrés, C.M.C., Lstra, J.M.P., Juan, C.A., Pluo, F.J., and Perez-Lebena, E., (2023) *Polyphenols as Antioxidant/Pro-Oxidant Compounds and Donors of Reducing Species: Relationship with Human Antioxidant Metabolism.* Processes,. **11**(9): p. 2771.

[21]. Rudrapal, M., Khairnar, S.J., Khan, J., Dukhyil, A.B., Ansari, M.A., Alomary, M.N., Alshabrmi, F.M., Palai, S., Deb, P.K., and Devi, R., (2022) *Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action.* Front Pharmacol,. **13**: p. 806470.

[22]. Akagawa, M., Shigemitsu, T., and Suyama, K., (2003). *Production of Hydrogen Peroxide by Polyphenols and Polyphenol-rich Beverages under Quasi-physiological Conditions.* Bioscience, Biotechnology, and Biochemistry, **67**(12): p. 2632-2640.

[23]. Scarano, A., Laddomada, B., Blando, F., De Santis, S., Verna, G., Chieppa, M., and Santino, A., (2023). *The Chelating Ability of Plant Polyphenols Can Affect Iron Homeostasis and Gut Microbiota.* Antioxidants, **12**(3): p. 630.

[24]. Lakey-Beitia, J., Burillo, G.M., La Penna, G., Hegde, M.L., and Rao, K.S., (2021), *Polyphenols as Potential Metal Chelation Compounds Against Alzheimer's Disease.* J Alzheimers Dis,. **82**(s1): p. S335-s357.

[25]. Sharma, P., Jha, A.B., Dubey, R.S., and Pessarakli, M., (2012) *Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions.* Journal of Botany,. **2012**(1): p. 217037.

[26]. Petrou, A.L., Petrou, P.L., Ntanos, T., and Liapi,A., (2018) *A Possible Role for Singlet Oxygen in the Degradation of Various Antioxidants. A Meta-Analysis and Review of Literature Data.* Antioxidants,. **7**(3): p. 35.

[27]. Monika, S., Sukanya, P., and Rajesh Kumar, S., (2021) *15 - Common techniques and methods for screening of natural products for developing of anticancer drugs.*: p. 323-353.

[28]. Varadaraju, C., Tamilselvan, G., Enoch, I.V.M.V., and Selvakumar, P.M., (2018) *Phenol Sensing Studies by 4-Aminoantipyrine Method–A Review.* Organic & Medicinal Chemistry International Journal,. **5**(2): p. 001-007.

[29]. Ganeshalingm, S., Anushkkaran,P., Praveena, Y., Mancheevan, A., Kugamoorthy, V., and Kandasamy, V., (2023) *.A comparison study of the efficacy of different activated charcoals derived from Palmyra kernel shell in removing phenolic compounds.* Current Research in Green and Sustainable Chemistry, **6**: p. 100355.

[30]. Gulcin, İ. and Alwasel, S.H. (2023) *DPPH Radical Scavenging Assay.* Processes,. **11**(8): p. 2248.

[31]. Chao, Z., Xiaoxue, G., Ruijia, G., Lin, Z., Xinrong, Q., Xiaohan, Y., Jun, C., Chunhe, G., and Zhen, F., (2023) *Insights into the effects of extractable phenolic compounds and Maillard reaction products on the antioxidant activity of roasted wheat flours with different maturities.* Food Chemistry: X,. **17**: p. 100548.

[32]. Apak, R., Calokerinos, A., Gorinstein, S., Segundo, M.A., Hibbert, D.B., Cekic, I.G.S.D., Guclu, K., Ozyurek, M., Celik, S.E., Magalhaes, L.M., and Arancibia-Avila, P., (2022) *Methods to evaluate the scavenging activity of antioxidants toward reactive oxygen and nitrogen species (IUPAC Technical Report).* Pure and Applied Chemistry,. **94**(1): p. 87-144.

[33]. Fereidoon, S., and Priyatharini, A., (2015) *Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review.* Journal of Functional Foods,. **18**: p. 820-897.

[34]. Liang, S., Hong-Yu, Z., and Hong-Fang, j., (2008) *A thermodynamic investigation of DPPH radical-scavenging mechanisms of folates.* Journal of Molecular Structure: THEOCHEM,. **856**(1): p. 119-123.

[35]. Contreras-Guzmán, E.S., and Strong III, F.C., (1982) *Determination of tocopherols (vitamin E) by reduction of cupric ion.* Journal of the Association of Official Analytical Chemists,. **65**(5): p. 1215-1221.

[36]. Matuszewska, A., Jaszek, M., Stefaniuk, D., Ciszewski, T., and Matuszewska, L., (2018) *Anticancer, antioxidant, and antibacterial activities of low molecular weight bioactive subfractions isolated from cultures of wood degrading fungus Cerrena unicolor.* PLoS One,. **13**(6): p. e0197044.