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Evaluation of *In-Vitro* Antioxidant Activity in *Senna Alata* of Hydroalcoholic Extract and Methanolic Extract

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Abstract

Background: Free radicals, also known as highly reactive oxygen species, can cause oxidative damage to the human body. Antioxidants are molecules that avoid reactive species from attacking the body and reduce the risk of disease. Both *Senna alata* extracts are used to treat brain diseases in humans and have almost identical effects.

Objective: *Senna alata's* antioxidant properties were investigated in this review.

Results: The antioxidant function of *Senna alata* medicinal plants was assessed using DPPH, Reducing power assay, ABTS assay, and Nitric oxide assay methods to ascertain reducing potential and free radical scavenging capacity. Antioxidant inhibitory concentration percentages were also assessed in two extracts (Methanolic extract and hydroalcoholic extract) and found to vary significantly in antioxidant values. The hydroalcoholic extract of *Senna alata* whole leaf powder had slightly higher antioxidant activity than the methanolic extract.

Conclusion: According to the findings, daily supplementation with hydroalcoholic extract of *Senna alata* may be more beneficial than methanolic extract in the treatment of neurological disorders caused by free radical harm.

Key Words: *Senna alata*, DPPH, Reducing power assay, ABTS assay, Nitric oxide assay, Free radical scavenging activity, Antioxidant activity.

1. Introduction

Exogenous chemicals and endogenous metabolic pathways in the human body create free radicals, or highly reactive oxygen molecules. These are capable of oxidising biomolecules such as nucleic acids, proteins, lipids, which DNA, and can cause degenerative diseases such as neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, and arthritis, among others (Halliwell and Gutteridge, 1984; Maxwell, 1995). Antioxidants are

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molecules that stop free radicals from attacking and therefore reduce the risk of these diseases (Rice-Evans *et al.*, 1996). Almost all species are shielded from free radical destruction to some degree by antioxidant compounds such as ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids, and glutathione, as well as enzymes such as superoxide dismutase and catalase. According to Prior and Cao (Prior and Cao, 1999), antioxidant supplements or dietary antioxidants protect against free radical harm. Natural antioxidants are currently receiving a lot of attention as a way to protect the human body, particularly brain tissues, from oxidative damage caused by free radicals. Several medicinal plants have demonstrated such efficacy by conventional psycho-neuropharmacology approaches in the last two decades (Dhawan, 1995).

These positive results have been due to antioxidant-active compounds in the majority of instances. Free radicals and reactive oxygen species (ROS) have been discovered to play a crucial role in the production of significant chronic health issues such as diabetes, asthma, cancer, and malaria, among others (Tsao & Deng, 2004). High concentrations of reactive oxygen species (ROS) are generated in unfavourable environments for plants, such as excessive temperatures, drought, heavy metals, nutritional shortages, and high salinity, which can trigger oxidative stress. Cells have a complex antioxidant mechanism of enzymatic and non-enzymatic components to prevent this. Non-enzymatic system molecules have a number of action mechanisms, including enzyme inhibition, chelation of trace elements involved in free radical formation, reactive species uptake and activation, and an improvement in protection by other antioxidant defences (Barua *et al.*, 2014). Among these molecules, compounds derived from secondary metabolism, especially phenolic compounds, play an important role in oxidative stress resistance (Pang *et al.*, 2018). These compounds are considered to be antioxidants due to their ability to donate hydrogen or electrons, as well as the fact that they are stable radical intermediates (Niciforovic *et al.*, 2010). When plants are eaten as food, phenolic compounds have a defensive effect on humans (Niciforovic *et al.*, 2010). Plant extracts' antioxidant potential is typically effective at low concentrations, and it has been linked to the prevention of cardiovascular disease and cancer in humans (Duthie *et al.*, 2000; Li *et al.*, 2014; Balmus *et al.*, 2016). As a result, studies to determine the antioxidant function of various plant extracts may help to define the importance of these species as a source of new antioxidant compounds (Miliauskas *et al.*, 2004; Gouthamchandra *et al.*, 2010).

The mechanisms of action by which the applied compounds inhibit chain-breaking reactions can be used to classify the available methods for quantifying antioxidant operation.

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They are classified into two categories: hydrogen-atom transfer (HAT) reactions and single electron transfer (SET) reactions (compound reduction reactions involving electron transfer from an antioxidant) (Prior *et al.*, 2005; Perez Jimenez, 2007). The 2, 2-diphenyl-1-picrylhydrazyl (DPPH radical scavenging capacity assay), ferric reducing (FRAP) assay, Trolox equivalent antioxidant capacity (TEAC or ABTS) assay, reducing strength assay (RP), and nitric oxide assay (NOA) are the most widely utilised SET methods (Prior *et al.,* 2005; Huang *et al.,* 2005). The aim of this analysis was to compare the antioxidant properties of *Senna alata* antioxidant components such as ascorbic acid, complete phenol, and tannins.

2. Materials and Methods

2.1 DPPH Radical Scavenging Activity

The Molyneux procedure was used to test DPPH radical scavenging operation (2004). Equal volume of the test sample in methanol of varying concentrations was applied to 1.0 ml of 100.0 µM DPPH solution of methanol and incubated in the dark for 30 minutes. A spectrophotometer set to 514 nm was used to measure the colour transition in terms of absorbance. The monitoring tube was filled with 1.0 ml of methanol instead of the test sample.

Percentage of inhibition was calculated from the equation

[(Absorbance of control - Absorbance of test)/ Absorbance of control] × 100.

IC_{50} value was calculated using Graph pad prism 5.0.

2.2 ABTS radical scavenging activity

The extract's ABTS radical-scavenging operation was calculated according to Re *et al.,* 1999. The ABTS +cation radical was formed by reacting 5 ml of 14 mM ABTS with 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution in the dark for 16 hours at room temperature. This solution was diluted with ethanol to obtain an absorbance of 0.700 ± 0.020 at 734 nm before use. The plant extract was homogenised with 1ml of ABTS solution at different concentrations, and its absorbance was measured at 734 nm.Each assay contained ethanol blanks, and all measurements were completed after at least 6 minutes. Similarly, the regular group reaction mixture was made by combining 950 µl of ABTS solution with 50 µl of BHT. The antiradical behaviour of ABTS was measured using the IC_{50} (µg/ml) method. The following formula was used to measure the ABTS radical inhibition percentage:

ABTS scavenging activity (%) = (A0 –A1) /A0 ×100

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Where A0 is the absorbance of the control, and A1 is the absorbance of the sample

2.3 Reducing power assay

2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of 1 percent potassium ferricyanide were added to the sample along with Ascorbic acid solutions. For 20 minutes, the mixture was kept in a 50°C water tank. The resultant solution was quickly cooled before being spiked with 2.5ml of 10% trichloroacetic acid and centrifuged for 10 minutes at 3000rpm. The supernatant (5 mL) was blended with 5 mL purified water and 1 mL 0.1 percent ferric chloride, and incubated for 10 minutes. On a spectrophotometer, the absorbance was measured at 700nm. The graph of absorbance at 700 nm against extract concentration was used to measure the extract concentration that produced the absorbance. As a control, ascorbic acid was used. The higher the absorbance, the greater the reducing force (Oyaizu, 1986).

2.4 Nitric oxide scavenging activity

At physiological pH, sodium nitroprusside in aqueous solution releases nitric oxide (NO), which reacts with oxygen to create nitrite ions, which can be determined using the Griess Illosvosy reaction (Garrat, 1964). NO scavengers interact with oxygen, resulting in decreased NO development and the formation of a pink-colored chromophore. At 540 nm, the absorbance of these solutions was compared to that of blank solutions.

Percentage inhibition was calculated as NO scavenging activity (%) = (A0 –A1) /A0 ×100

The absorbance of the control is A0, and the absorbance of the sample is A1.

3. Results and Discussion

DPPH, Reducing power assay, ABTS assay, and Nitric oxide assay methods were used to test the antioxidant properties of *Senna alata* using hydroalcoholic and methanolic extracts. The antioxidant activity values measured for each extract lead to various concentrations. This concentration was chosen because it was the only one that existed in the absorbance values of the patterns for all four processes, out of all the concentrations tested. The IC_{50} of a compound is inversely related to its antioxidant ability, since it expresses the amount of antioxidant needed to reduce the concentration of ABTS, DPPH, and Nitric oxide by 50%, as determined by interpolation from a linear regression study (Liu *et al.,* 2009).

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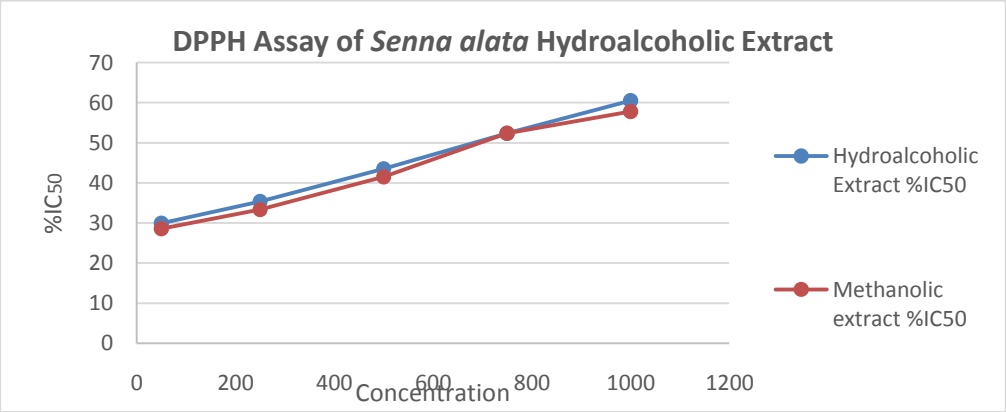
3.1 DPPH Assay

The Molyneux procedure was used to test DPPH radical scavenging operation (2004). Because of the radical's delocalization in aromatic rings, this radical is known for its extraordinary stability. It's a vibrant deep purple colour (Gupta, 2015). The radical is neutralised in assays by accepting a hydrogen atom or an electron from an antioxidant species (or reducing agents), after which it is reduced (DPPH or DPPH-H) at the end of the method. The DPPH radical's unpaired electron absorbs strongly at 517 nm, resulting in a deep purple colour. When an odd electron matches up with another electron, however, the original colour fades to a pale yellow. The colour ribbon below simulates decolorization. *Senna alata* hydroalcoholic extract (684.01 µg/ml) and methanolic extract (734.25 µg/ml) were tested using the DPPH process.

Table 1: DPPH Assay of *Senna alata*

S.No	Concentration	Hydroalcoholic Extract		Methanolic extract	
		%IC_{50}	IC_{50}	%IC_{50}	IC_{50}
1	50	29.93	684.01	28.57	734.25
2	250	35.37		33.33	
3	500	43.53		41.50	
4	750	52.38		52.38	
5	1000	60.54		57.82	

Figure 1: DPPH Assay of *Senna alata*



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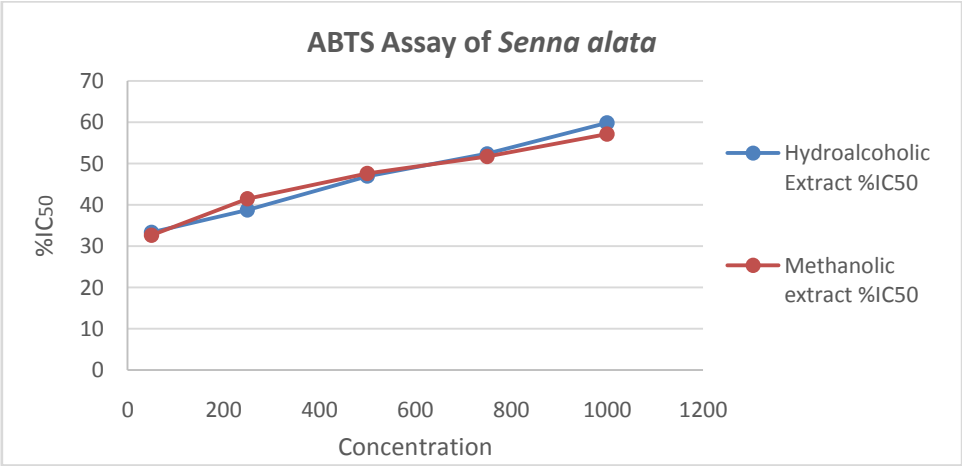
3.2 ABTS Assay

The $ABTS^{\bullet+}$ assay, also known as TEAC, was first recorded in 1993 by Miller and Rice-Evans. Later in 1999, Re and Colleagues improved this assay. The AO, for example, will decrease the $HO^{\bullet}$ radical present in the system along with metmyoglobin and $ABTS^{\bullet+}$, resulting in an overestimation of antioxidant ability and incorrect performance. To address this problem, an improved approach is suggested that does not include the use of the $HO^{\bullet}$ radical or metmyoglobin.

Table 2: ABTS Assay of *Senna alata*

S.No	Concentration	Hydroalcoholic Extract		Methanolic extract	
		%IC_{50}	IC_{50}	%IC_{50}	IC_{50}
1	50	33.33	643.95	32.65	667.75
2	250	38.78		41.50	
3	500	46.94		47.62	
4	750	52.38		51.70	
5	1000	59.86		57.14	

Figure 2: ABTS Assay of *Senna alata*



Prior to the incorporation of AOs, the improved process produces the ABTS•+ radical in just one reaction by reacting ABTS with ammonium or potassium persulfate ($(NH_4)_2\ S_2O_3$ or $K_2S_2O_3$, respectively). It's important to recall that ABTS has a 1:0.5 stoichiometry with persulfate salt, which ensures that not all ABTS are oxidised before AO is applied (Re *et al.*, 1999; Schaich *et al.*, 2015; Miller and Rice-Evans, 1997). *Senna alata* hydroalcoholic extract

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(643.95 µg/ml) and methanolic extract (667.75 µg/ml) were analysed using the ABTS process.

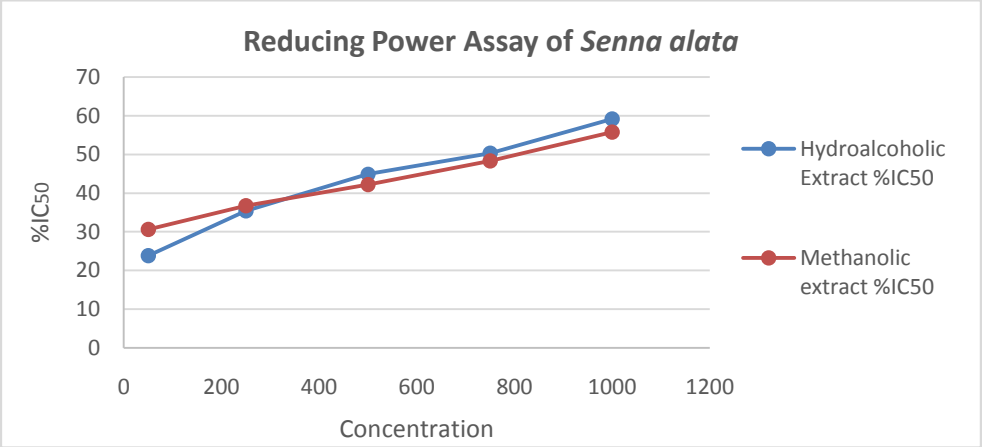
3.3 Reducing Power Assay

Oyaizu, 1986, developed the ferric ion reducing antioxidant force or ferric reducing potential of plasma, abbreviated as FRAP. Under acidic conditions, the FRAP process reduces ferric-tripyridyltriazine $[Fe^{III}(TPTZ)]^{3+}$ to form an extreme blue-colour ferrous complex $[Fe^{II}(TPTZ)]^{2+}$ (pH 3.6).

Table 3: Reducing Power Assay of *Senna alata*

S.No	Concentration	Hydroalcoholic Extract		Methanolic extract	
		%IC_{50}	IC_{50}	%IC_{50}	IC_{50}
1	50	23.80	715.26	30.61	791.58
2	250	35.37		36.73	
3	500	44.89		42.18	
4	750	50.34		48.30	
5	1000	59.18		55.78	

Figure 3: Reducing Power Assay of *Senna alata*



As seen in the colour wheel, the colour produced in this assay is intense blue, which is the complementary colour of orange. This is why the absorbance is estimated at 593 nanometres. Because of its simplicity, high reproducibility, and basic instrumentation, this approach was designed to be carried out in any laboratory (Benzie and

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Strain, 1996). According to Huang *et al* analysis, the redox potential of Fe (III) is approximately 0.70V, which is equivalent to the redox potential of $ABTS^{\bullet+}$ (0.68 V) (2005). Surprisingly, there is a fine line between the ABTS•+ and FRAP tests, with the difference that the ABTS assay is done at neutral pH whereas the FRAP procedure is performed under acidic conditions (Huang *et al.,* 2005). This assay, though, is non-specific. This is because any species in the reaction mixture with a redox potential smaller than Fe (III) (<0.70 V) would be responsible for the reduction in $[Fe^{III}(TPTZ)_2]^{3+}$, resulting in an underestimation (Benzie and Strain, 1996). *Senna alata* hydroalcoholic extract (715.26 µg/ml) and methanolic extract (791.58 µg/ml) were tested for reducing strength.

3.4 Nitric Oxide Assay

Enzymes manufacture nitric oxide from the amino acid L-arginine, and is present in vascular endothelial cells, individual neuronal cells, and phagocytes (Boora *et al.,* 2014; Thomas, 2015). $NO^{\bullet}$ plays an important role in biological activities such as antimicrobial activity, antitumor influence, vasodilation, and neuronal messenger at low concentrations. High levels of NO, on the other hand, can lead to a number of health issues, including inflammatory disorders including sclerosis, arthritis, and ulcerative colitis.

Table 4: Nitric Oxide Assay of *Senna alata*

S.No	Concentration	Hydroalcoholic Extract		Methanolic extract	
		%IC_{50}	**IC_{50}**	**%IC_{50}**	**IC_{50}**
1	50	25.17	856.79	23.81	899.67
2	250	31.29		29.93	
3	500	38.78		37.41	
4	750	44.90		45.58	
5	1000	55.78		53.06	

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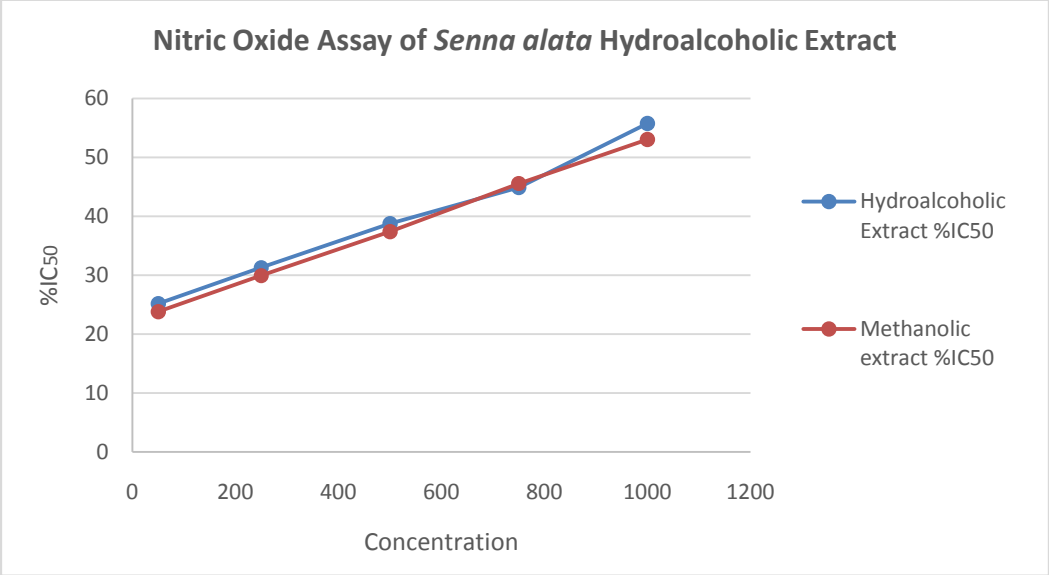
Figure 4: Nitric Oxide Assay of *Senna alata*



As NO reacts with superoxide radical to form the highly reactive anion peroxynitrite anion (ONOO−), its toxicity rises dramatically. Later on, we'll talk about the latter anion. Flavonoids have been shown in various experiments to easily scavenge $NO^{\bullet}$ radicals (Bhaskar and Balakrishnan, 2009; Lakhanpal *et al.*, 2007). The hydroalcoholic extract (856.79 µg/ml) and methanolic extract (899.67 µg/ml) of *Senna alata* were tested for nitric oxide.

The research of antioxidant activity in plants has exploded in recent years as a result of their widespread usage as a source of phytotherapeutic items (Davalos *et al.*, 2003; Moon and Shibamoto, 2009; Londono-Londono, 2012). The primary antioxidant compounds in plants are phenols, which have an aromatic ring that enables the unpaired electrons in their arrangement to be stabilised and relocated, allowing for the donation of hydrogen atoms and electrons from their hydroxyl classes (Rice-Evans *et al.*, 1997; Comert and Gokmen, 2017). The amount of total phenol in a plant depends on the organisms, tissue, developmental stage, and environmental factors including temperature, water stress, and light (Upadrasta *et al.*, 2011; Zlatic *et al.*, 2019).

4. Conclusion

According to the findings of this analysis, the four methods used can measure the antioxidant activity of the two *Senna alata* extracts, though the categorization formed among the species is dependent on the method used. When assessing variations in the antioxidant

function of the animals, the approaches vary in sensitivity. As a result, the separation between species obtained with some of these methods is smaller than that obtained with other methods. The most sensitive process, or the one that identified the most variations between species, was RP with the studied species. These findings highlight the importance of choosing the right method for determining the antioxidant activity of plant extracts, especially when deciding between a numbers of potential species.

5. References

1. Balmus, I.; Ciobica, A.; Trifan, A.; Stanciu, C. The implications of oxidative stress and antioxidant therapies in Inflammatory Bowel Disease: Clinical aspects and animal models. *Saudi J. Gastroenterol.* 2016, *22*, 3–17.
2. Barua, C.C.; Sen, S.; Das, A.S.; Talukdar, A.; Jyoti Hazarika, N.; Barua, A.; Barua, I. A comparative study of the in vitro antioxidant property of different extracts of *Acorus calamus* Linn. *J. Nat. Prod. Plant Resour.* 2014, *4*, 8–18.
3. Benzie, I.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* 1996, *239*, 70–76.
4. Bhaskar, H.; Balakrishnan, N. In vitro antioxidant property of laticiferous plant species from Western Ghats Tamilnadu, India. *Int. J. Health Res.* 2009, *2*, 163–170.
5. Boora, F.; Chirisa, E.; Mukanganyama, S. Evaluation of nitrite radical scavenging properties of selected Zimbabwean plant extracts and their phytoconstituents. *J. Food Process.* 2014, *2014*, 918018
6. Comert, E.D.; Gokmen, V. Antioxidants bound to an insoluble food matrix: Their analysis, regeneration behavior, and physiological importance. *Compr. Rev. Food Sci.* 2017, *16*, 382–399.
7. Davalos, A.; Gómez-Cordovés, C.; Bartolomé, B. Commercial Dietary Antioxidant Supplements Assayed for Their Antioxidant Activity by Different Methodologies. *J. Agric. Food Chem.* 2003, *51*, 2512–2519.
8. Dhawan BN. Centrally acting agents from Indian plants. In: Koslow SH, Murthy RS, Coelho GV, editors. *Decade of the Brain: India/USA Research in Mental Health and Neurosciences.* Rockville: National Institute of Mental Health; 1995. pp. 197–202.
9. Duthie, G.G.; Duthie, S.J.; Kyle, J.A.M. Plant polyphenols in cancer and heart disease: Implications as nutritional antioxidants. *Nutr. Res. Rev.* 2000, *13*, 79.
10. Garrat, D.C. The Quantitative Analysis of Drugs, Chapman and Hall Ltd, Japan; 1964; Vol 3; 456-458.

11. Gouthamchandra, K.; Mahmood, R.; Manjunatha, H. Free radical scavenging, antioxidant enzymes and wound healing activities of leaves extracts from *Clerodendrum infortunatum* L. *Environ. Toxicol. Pharmacol.* 2010, *30*, 11–18.
12. Gupta, D. Methods for determination of antioxidant capacity: A review. *Int. J. Pharm. Sci. Res.* 2015, *6*, 546–566.
13. Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and diseases. *Biochem J.* 1984;219:1–4.
14. Huang, D.; Ou, B.; Prior, R.L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* 2005, *53*, 1841–1856.
15. Huang, D.; Ou, B.; Prior, R.L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* 2005, *53*, 1841–1856
16. Lakhanpal, P.; Rai, D.K. Quercetin: A versatile flavonoid. *IJMU* 2007, *2*, 22–37.
17. Li, A.-N.; Li, S.; Zhang, Y.-J.; Xu, X.-R.; Chen, Y.-M.; Li, H.-B. Resources and biological activities of natural polyphenols. *Nutrients* 2014, *6*, 6020–6047.
18. Liu, S.C.; Lin, J.T.; Wang, C.K.; Chen, H.Y.; Yang, D.J. Antioxidant properties of various solvent extracts from lychee (Litchi chinenesis sonn.) flowers. Food Chemistry, 2009; 114:577-581.
19. Londono-Londono, J. Antioxidantes: Importancia biológica y métodos para medir su actividad. In *Desarrollo Y Transversalidad. Serie Lasallista Investigación Y Ciencia*; Corporación Universitaria Lasallista: Caldas, Colombia, 2012; pp. 129–162.
20. Maxwell SR. Prospect for the use of antioxidant therapies. *Drugs.* 1995;49:45–361.
21. Miliauskas, G.; Venskutonis, P.R.; van Beek, T.A. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.* 2004, *85*, 231–237.
22. Miller, N.J.; Rice-Evans, C.; Davies, M.J.; Gopinathan, V.; Milner, A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sci. 1993, 84, 407–412.
23. Miller, N.J.; Rice-Evans, C.A. Factors influencing the antioxidant activity determined by the ABTS•+ radical cation assay. *Free Radic. Res.* 1997, *26*, 195–199.
24. Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songlanakarin Journal of Science and Technology, 26, 211-219.
25. Moon, J.-K.; Shibamoto, T. Antioxidant assays for plant and food components. *J. Agric. Food Chem.* 2009, *57*, 1655–1666.

26. Niciforovic, N.; Mihailović, V.; Mašković, P.; Solujić, S.; Stojković, A.; Muratspahić, D.P. Antioxidant activity of selected plant species; potential new sources of natural antioxidants. *Food Chem. Toxicol.* 2010, *48*, 3125–3130.
27. Oyaizu, M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition; 1986; 44, 307–315.
28. Pang, Y.; Ahmed, S.; Xu, Y.; Beta, T.; Zhu, Z.; Shao, Y.; Bao, J. Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice. *Food Chem.* 2018, *240*, 212–221.
29. Perez Jimenez, J. Metodología Para la Evaluación de Ingredientes Funcionales Antioxidantes: Efectos de Fibra Antioxidante de Uva en Status Antioxidante Y Parámetros de Riesgo Cardiovascular en Humanos. Ph.D. Thesis, Universidad Autónoma de Madrid, Madrid, Spain, 2007.
30. Prior RL, Cao G. Variability in dietary antioxidant related natural product supplements: The need for methods of standardization. *J Am Nutraceutical Assoc.* 1999; 2: 46–56
31. Prior, R.L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* 2005, *53*, 4290–4302.
32. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Riceevans C. Antioxidant activity applying an improved ABTS radical decolorization assay. Free Radical Biology and Medicine 1999; 26, 1231–1237.
33. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* 1999, *26*, 1231–1237.
34. Rice-Evans CA, Miller NJ, Paganga G. Structure antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med.* 1996;20:933–56.
35. Rice-Evans, C.; Miller, N.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* 1997, *2*, 152–159.
36. Schaich, K.M.; Tian, X.; Xie, J. Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assays. *J. Funct. Foods* 2015, *14*, 111–125.
37. Thomas, D.D. Breathing new life into nitric oxide signaling: A brief overview of the interplay between oxygen and nitric oxide. *Redox Biol.* 2015, *5*, 225–233.

38. Tsao R, Deng Z (2004): Separation procedures for naturally occurring antioxidant phytochemicals. J Chromatogr-B Analyt Technol Biomed Life Sci 812: 85–99.
39. Upadrasta, L.; Mukhopadhyay, M.; Banerjee, R. Tannins: Chemistry, biological properties and biodegradation. In *Chemistry and Biotechnology of Polyphenols*; Sabu, A., Roussos, S., Aguilar, C.N., Eds.; Cibet Publishers: Thiruvananthapuram, India, 2011; pp. 5–32.
40. Zlatic, N.; Jakovljević, D.; Stanković, M. Temporal, plant part, and interpopulation variability of secondary metabolites and antioxidant activity of *Inula helenium* L. *Plants* 2019, *8*, 179.