Arch. Bas. App. Med.13 (2025): 42-46
www.archivesbamui.com
www.ojshostng.com/index.php/abam

Research Article

In vitro Antioxidant, Total Phenolic Content and Cytotoxicity study of Selected Herbal Medicinal Products in Ilorin Metropolis

***Sidiq L.O.[1,2], Nkumah A.O.[2] Quadri M.T.[1] and Ogbole O.O.[2]**

[1&2]Department of Plant and Environmental Biology, Kwara State University, Malete, Nigeria.
[2]Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Nigeria.

Accepted: January 18, 2025

Abstract

There are no doubts herbal medicines are endowed with bioactive constituents considered to have many therapeutic effects. The usage of herbal medicines increased sporadically as a result of the belief that herbal medicines have a higher safety margin than conventional medicines. In other to improve acceptance of herbal remedies in the treatment of diseases, the study aimed to assess the antioxidant capability, total phenolic content and cytotoxicity of five (5) herbal medicinal products that were sourced from the city of Ilorin. The products were labelled A, B, C, D and E in this study. The Folin-Ciocalteu method was used to determine the total phenolic content, Brine Shrimp Lethality Assay (BSLA) was used to determine the toxicity of the medicinal products, and the 1, 1-diphenyl, 2-picrylhydrazyl (DPPH) radical scavenging assay was applied to evaluate the antioxidant activity of the selected herbal medicinal products. Products C, D and E had higher DPPH radical scavenging activities (IC_{50} = 54.51± 0.22, 42.30± 0.11 and 39.17± 0.24 µg/mL respectively), which was lower in products A and B (129.53± 0.21 and 180.06± 0.34 µg/mL) compared with Ascorbic acid (7.32± 0.12 µg/mL). Total phenolic content was high in products D and E (315.86±0.22 and 205.13±0.31 mg GAE/g extract. Brine shrimp lethality assay (BSLA) revealed products A, C, D, E were found to be non-toxic to *Artemia salina* larvae, with LC_{50} > 1000 µg/mL, except extract of product B which was perceived to be moderately toxic with LC_{50} = 418.80 ± 0.24 µg/mL. The selected herbal medicinal products possessed reasonable safety, antioxidant capacity and phenolic content.

Key Words: *Radical scavenging, Toxicity, Herbal medicinal products, Ilorin metropolis*

INTRODUCTION

Herbal remedies are complex mixtures derived from biological sources. They are widely perceived as natural and safe, and are believed to possess therapeutic properties, including the ability to prevent and treat various diseases (Sarkar *et al*., 2024). As a form of complementary and alternative medicine, herbal medicine is gaining popularity in both developed and developing nations (Komolafe *et al*., 2021). According to the World Health Organization (WHO), traditional medicine remains one of the most reliable approaches to achieving universal access to healthcare (Hoenders *et al*., 2024). In support of this goal, the WHO has developed technical guidelines for the evaluation of herbal medicines and continues to promote their prudent uses among member countries to ensure affordable and culturally appropriate healthcare (WHO2014–2023, 2021; Zhang *et al*., 2021). Despite the expansion of pharmaceutical industries producing conventional drugs, many nations, continents, and cultures continue to hold strong beliefs in the efficacy of herbal remedies (Pranskuniene *et al*., 2022). In numerous rural communities across Africa and Asia, herbal medicines form an integral part of primary healthcare delivery. Furthermore, many rural Africans trust the safety of herbal treatments, citing historical uses by their ancestors without apparent adverse effects (systems (Ikhoyameh *et al*., 2024). However, this assumption has unfortunately contributed to instances of organ damages and fatalities linked to herbal medicine use (Frenzel and Teschke, 2016).

In Nigeria, there has been a significant increase in the use of herbal medicines for the prevention and treatment of diseases. Factors such as limited availability and the high cost of conventional drugs have been associated with this rising trend (Aina *et al*., 2020). Herbal remedies are now commonly sold in traffic jams, on roadsides, at bus stops and stations, during festivals, and even within some conventional medical facilities. Although commercialization of these products is widespread, the therapeutic properties and safety profiles of many have not been rigorously evaluated, raising concerns among health authorities, scholars, and researchers. Vendors of herbal products make extensive therapeutic claims, marketing remedies for malaria ("Agbo iba"), hemorrhoids ("Agbo jedi"), hypertension ("Agbo eje riru"), and multi-purpose treatments ("gbogbo nise"). Despite their widespread uses and claimed therapeutic benefits, herbal medicines also pose potential toxicological risks due to contamination with heavy metals, adulterants, and chemical toxins (Okaiyeto and Oguntibeju, 2021). Therefore, continuous validation of their safety and therapeutic potentials is essential.

**Author for Correspondence:* Tel: +2348039619276 E-mail: *anuoluwanimorigba@gmail.com*

Five (5) herbal remedies were selected in Ilorin metropolis. The selection of these herbal remedies was based on criteria such as regular advertisement on major media channels (Radio and Television station) in the city, high sales in pharmaceutical stores, sale outlets and street hawking. The five herbal remedies were coded as follows; Products A, B C, D and E. Information regarding each product was sourced from its label. Plant species that form the constituents of the herbal products were identified using their native and English names, with full botanical names obtained through the International Plant Names Index. All the selected herbal remedies were presented in liquid form. Among them, three products had manufacturing and expiry dates, two declared their constituent ingredients. All products carried specific therapeutic claims ranging from treatment of pile, body pain, low sperm count, normalising irregular menstrual period, general body wellness. These broad claims are often attributed to herbal products' purported abilities to scavenge free radicals, which play a crucial role in biological processes that can lead to disease development (Engwa *et al*., 2022). Herbal remedies contain array of constituents that exhibit therapeutical effects. Numerous studies have reported the free radical scavenging abilities of various plant-derived products (Yasmin *et al*., 2024; Sanpinit *et al*., 2024; Nurudeen *et al*., 2024; Petchpoung *et al*., 2024). It is believed that therapeutic effects of bioactive constituents manifest by their ability to counteract the effect of oxidants in the body (Santhi *et al*., 2021). Reports have also indicated that some plant bioactive constituents may be toxic (Tavakkoli *et al*., 2017; Kamran *et al*., 2018; Sharma *et al*., 2021; Xu *et al*., 2022; Okafor *et al*., 2025). This study aimed to examine their antioxidant ability and evaluated their toxicity. This study represents the first investigation into the antioxidant activity, total phenolic content, and toxicity of five (5) selected herbal products from Ilorin metropolis.

MATERIALS AND METHODS

Materials: 1, 1-diphenyl, 2-picrylhydrazyl radical (DPPH), Ascorbic acid (Sigma Aldrich, USA), Folin-Ciocalteu reagent, gallic acid, anhydrous sodium carbonate (Lobs chemie Laboratory, India) five (5) herbal medicinal products, 96-well microplates, UV Spectrophotometer microplate reader (Paul Baucher, SPECTRA max PLUX, Analytik and Biotechnologie, 4051 Basel, Germany*), Artemia salina* eggs. All other reagents and chemicals used in this study were of analytical grades.

Samples Selection and Preparation: The selection of five (5) herbal remedies was based on criteria such as regular advertisement on major media channels (Radio and Television station) in the city, high sales in pharmaceutical stores, sale outlets and street hawking. The herbal products were purchased from traditional medicine sales/pharmacy outlets in Ilorin Metropolis. Products were coded as; A, B, C, D and E. One hundred millilitres (100 mL) of each herbal product was sieved using Whatman filter paper No. 1 and evaporated to dryness to obtain solid extract. The extracts were packed into sample bottles and stored in refrigerator (4ºC) and used for analysis when needed.

1, 1-diphenyl, 2-picrylhydrazyl radical (DPPH) radical scavenging assay: Radical scavenging activity was evaluated according to the method described by Krishnan *et al*. (2015). One milligram per millilitre (1 mg/mL) stock solution of each sample was prepared in methanol. Two-fold serial dilution of each sample were prepared to obtain working concentrations of; 1000 - 15.63 µg/mL, respectively. Ascorbic acid was used as positive control at concentrations of; 50 - 0.781 µg/mL, while negative control contained all the reagents except extract or standard drugs. Freshly prepared DPPH (0.1 mmol in methanol; 900 µL) solution was mixed with 100 µL each of the extract or standard. The mixtures were incubated in the dark for 30 min at room temperature. The change in colour from deep violet to light yellow was measured at 517 nm using UV Spectrophotometer microplate reader (Paul Baucher, SPECTRA max PLUX, Analytik and Biotechnologie, 4051 Basel, Germany). The experiment was conducted in triplicate. The equation below was used to determine the herbal extracts' capacity to scavenge DPPH radicals:

$$\text{Percentage (\%) Inhibition} = \frac{\text{Mean Absorbance Control} - \text{Mean Absorbance sample}}{\text{Mean Absorbance Control}} \times 100$$

The IC_{50} values were calculated as the concentration of the extracts that resulted in 50% DPPH radical inhibition during the triplicate experiment.

Total phenolic content: Folin-Ciocalteu reagent was used to assess total phenolic content (TPC) of the herbal extracts as described by Laličić-Petronijević *et al*. (2016) with slight modification. Herbal extracts were prepared at a concentration of 1 mg/mL for the experiment. Gallic acid was prepared at concentrations of 50, 40, 30, 20, 10, and 5 µg/mL. Calibration curve was constructed to be used for result calculation. The reaction mixtures were prepared by mixing 2.5 mL of 10% Folin-Ciocalteu's reagent with 0.5 mL of herbal extract solution (1 mg/mL). After 5 minutes of incubation, 2.5 mL of 7.5% $NaCO_3$ was added. The blank included 2.5 mL of 7.5% $NaCO_3$, 2.5 mL of 10% Folin-Ciocalteu's reagent, and 0.5 mL of methanol. The reaction mixtures were left for 2 h at room temperature to incubate. Absorbance was measured at 765 nm with UV Spectrophotometer microplate reader (Paul Baucher, SPECTRA max PLUX, Analytik and Biotechnologie, 4051 Basel, Germany). The calibration curve was used to calculate the phenolic content, which was expressed in gallic acid equivalent (mg of GA/g of extract). The experiment were conducted in triplicate.

Brine shrimp lethality assay: The assay was conducted using Asaduzzaman *et al*. (2015) methodology. The natural saline water used in hatching of Brine shrimp eggs was fetched from Bar Beach, Lagos, Nigeria. Eggs were hatched in a hatching tank of two unequal halves partitioned. One litre (1L) of seawater was poured into the hatching tank and water level was equilibrated on both sides. Twenty milligram (20 mg) of Artemia salina eggs (Dohse Aquaristik GmbH and CO. KG, Germany) was gently poured to one side of the tank and covered with aluminium foil to protect from light whereas other side was exposed to light for hatched motile larvae to move toward the direction of light through a hole in the partition wall to the large side of tray. The resultant nauplii (larvae) were collected using a Pasteur pipette and used for the experiment following a 48-hour incubation period at room temperature and under light. To get working concentrations, a stock solution (2000 µg/mL) was prepared and used for serial

two-fold dilutions (1% methanol): 1000 - 3.9 µg/mL. Ten 10 motile nauplii were added in glass vial which already contained l mL solution of each concentration of extracts/drug. A reference cytotoxicity drug cyclophosphamide served as the positive control, while 1% methanol served as the negative control. The test was conducted in three replicates. The vials were exposed to light at room temperature for twenty-four (24) hours. Percentage (%) mortality at each concentration after counting the number of dead and live larvae after 24 hours was determined.

Data Analysis: Each experiment was conducted in triplicates and the results were reported as mean ± standard error of means (SEM). The percentage inhibition was plotted against concentrations to generate the dose-response curve. GraphPad Prism software version 9.0® (GraphPad Inc, San Diego, CA, USA) was employed to calculate IC_{50} values in the antioxidant assay. The 50% lethal concentration (LC50 value) was calculated for each plant extract in the BSLA (brine shrimp lethality assay) using Graph Pad prism 9.0® software.

RESULTS

The product label provided information about the herbal products. Table1. The native and English names of the plant species were used to identify their full botanical names. All selected herbal remedies were in solution. Three (3) of the products (A, D and E) had manufacturing and expiring dates. Products (A and E) declared the contents used in preparation. All products presented therapeutic claims (Table1).

Table 1:
Product information on selected herbal medicines used in the study

Product Code	Product form	Date of manufacture	Expiry Date	Content	Therapeutic claim
A	Solution	Feb., 2023	Feb., 2025	*Aristolochia, ringes* *Curculigo pilosa* *Phyllanthus amarus*	Man extra power, pile, waist pain, low sperm count
B	Solution	-	-	-	Body pain, dysentery, Stomach pain, skin rashes
C	Solution	-	-	-	Low sperm count, man power, chronic pile, body pain, waist pain, gonorrhea, diabetes, menstrual pain
D	Solution	Sep., 2023	Aug., 2025	_	Irregular menstrual period, man dysfunction, general body pain
E	Solution	Sep., 2022	Aug., 2024	*Tetrapleura tetraptera* *Axonopus compresus* *Cnestic ferruginea* *Sorghum bicolor*	For overall body wellness

Table 2:
Antioxidant activity, total phenolic content (TPC) and Brine Shrimp Lethality Assay of the selected herbal products

Product code	DPPH IC_{50} (µg/mL)	TPC (mg GAE/g extract)	BSLA LC_{50} (µg/mL)
A	129.53± 0.21	20.13±0.44	> 1000
B	180.06± 0.34	37.15±0.24	418.80 ± 0.26
C	54.51± 0.22	46.18±0.36	> 1000
D	42.30± 0.11	315.86±0.22	> 1000
E	39.17± 0.24	205.13±0.31	> 1000
Control	7.32± 0.12*	-	16.31 ± 0.15**

Values expressed as mean ± SEM; n = 3; BSLA- Brine Shrimp Lethality Assay *Ascorbic acid, **Cyclophosphamide

Extracts of the herbal products exhibited promising radical scavenging activity. High DPPH radical scavenging exhibited by products D (IC_{50} = 42.30± 0.11 µg/mL) and E (IC_{50} = 39.17± 0.24 µg/mL) correlate with their total phenolic content (D = 315.86±0.22 mg GAE/g extract; E = 205.13±0.31 mg GAE/g extract). Product C had moderate DPPH radical scavenging of IC_{50} = 54.51± 0.22 µg/mL and TPC of 46.18±0.36 mg GAE/g extract. Product A and B had DPPH radical scavenging of IC_{50} = 129.53± 0.21 and 180.06± 0.34 µg/mL, and TPC of 20.13±0.44 and 37.15±0.24 mg GAE/g extract, respectively. The results of the brine shrimp lethality assay (BSLA) of products A, C, D, E were found to be safe, with LC_{50} > 1000 µg/mL, except herbal product B which might be moderately toxic with LC_{50} < 1000 (Table 2).

DISCUSSION

Herbal medicines contain agents which are important to be evaluated for their bioactivity, efficacy, safety, among others. It has been reported that free radicals trigger oxidative stress, which can result in cell damage and a number of disorders, including diabetes, atherosclerosis, ischemia injury, inflammation, and carcinogenesis (Melaku *et al*., 2020; Zhazykbayeva *et al*., 2020). Extracts of the selected herbal products exhibited promising radical scavenging activity, which is higher in product C, product D, product E, compared with product A and product B. The antioxidant action of plant

extracts could be attributed to their phenolic and flavonoid components (Kaurinovic *et al*., 2019; Tanleque-Alberto *et al*., 2020). Product D and product E had high phenolic content compared to product A, B and C. High DPPH radical scavenging exhibited by products D and E correlate with their total phenolic content. The antioxidant capacity of phytochemicals derived from medicinal plants likely reduce the chance of developing chronic diseases (Barghout *et al*., 2020; Ciampi *et al*., 2020). For this reason, there is a greater interest in finding new therapeutic and antioxidant agents from natural sources that can prevent disease. (Ben Mrid *et al*., 2019).

Ensuring safety of plant products and assess any possible hazards related to the use of herbal remedies, plant toxicity studies are crucial. The harmful consequences of using substandard herbal remedies vary from nausea to diverse complications affecting the gastrointestinal, hematological, cardiovascular and neurological systems which can ultimately result to death. The severity of these effects is contingent on the quantity and duration of use of the herbal products (Chambial *et al*., 2017; Fernandez-Acenero *et al*., 2019). Plant extracts and compounds are among the many substances whose toxicity can be evaluated using the popular and cost-effective Brine Shrimp Lethality Assay (BSLA) (Santos *et al*., 2022). Brine shrimp lethality assay results were compared with the toxicity scale of; LC_{50} < 100 ug/mL is considered extremely toxic, LC_{50} < 1000 µg/mL is moderately toxic, and LC_{50} > 1000 ug/mL is considered non-toxic (Santos *et al*., 2022). Products A, C, D, and E were found to be non-toxic, with LC_{50} > 1000 µg/mL, with the exception of the extract of product B, which was perceived to be moderately toxic with an LC_{50} < 1000. In an effort to pursue the safety and quality of herbal medicinal products, Ogbole *et al*. (2023) assessed the microbial and elemental content of herbal remedies in Ibadan metropolis, pointed out potential health hazards associated with the use of herbal remedies despite their rich phytochemical content. In another study, Balogun *et al*. (2021) investigated safety of herbal medicines in Nigeria, appraised the state of PVG (pharmacovigilance) system for the safety monitoring of herbal medicines using WHO core PVG indicators. Results of this study showcased herbal remedies have low toxicity and should not be considered totally non-toxic. Evaluation of herbal remedies in this study offers information for users and public health interventions.

CONCLUSION

The selected herbal medicinal products possessed reasonable non-toxicity effects on *Artemia salina* larvae, radical scavenging strength and phenolic content. The safety, effectiveness, and quality control of herbal medicines produced, marketed and utilized in Nigeria require continuous evaluation.

ACKNOWLEDGEMENTS

Authors appreciate the technical support rendered by the technologists in the Department of Plant and Environmental Biology, Kwara State University, Malete, Nigeria.

REFERENCES

Aina, O., L. Gautam, P, Simkhada, and S. Hall. 2020. Prevalence, determinants and knowledge about herbal medicine and non-hospital utilisation in southwest Nigeria: a cross-sectional study. *BMJ open*, 10(9), e040769.

Asaduzzaman, M., M.S. Rana, S.M. Hasan, M.M. Hossain, and N. Das. 2015. Cytotoxic (brine shrimp lethality bioassay) and antioxidant investigation of Barringtonia acutangula (L.). *Int. J. Pharm. Sci. Res*. 6, 1179–1185.

Barghout, N., N. Chebata, S. Moumene, S. Khennouf, A. Gharbi, and D.I. El Had. 2020. Antioxidant and antimicrobial effect of alkaloid bulbs extract of Polianthes tuberosa L. (Amaryllidaceae) cultivated in Algeria. *J. Drug Deliv. Ther.* 10, 44-48, https://doi.org/10.22270/jddt.v10i4.4134

Ben Mrid, R., N. Bouchmaa, Y. Bouargalne, B. Ramdan, K. Karrouchi, I. Kabach, M. El Karbane, A. Idir, A. Zyad, and M. Nhiri. 2019. Phytochemical Characterization, Antioxidant and In Vitro Cytotoxic Activity Evaluation of Juniperus oxycedrus Subsp. oxycedrus Needles and Berries. Molecules. 24, 502-520, https://doi.org/10.3390/molecules24030502.

Chambial, S, P. Bhardwaj, A.A. Mahdi, and Sharma P. 2017. Lead Poisoning Due to Herbal Medications. *Indian J Clin Biochem.* 32(2):246-7.

Ciampi, F., L.M. Sordillo, J.C. Gandy, M. Caroprese, A. Sevi, M. Albenzio, and A. Santillo. 2020. Evaluation of natural plant extracts as antioxidants in a bovine in vitro model of oxidative stress. *J. Dairy Sci.* 103, 8938-8947, https://doi.org/10.3168/jds.2020-18182.

Engwa, G. A., F.N. EnNwekegwa, and B.N. Nkeh-Chungag. 2022. Free Radicals, Oxidative Stress-Related Diseases and Antioxidant Supplementation. *Alternative therapies in health and medicine*, 28(1), 114–128.

Fernandez-Acenero, M.J., L. Ortega Medina, and M. Maroto. 2019. Herbal Drugs: Friend or Foe? *J Clin Exp Hepatol*. 9(3):409-11.

Frenzel, C., and R. Teschke, R. 2016. Herbal hepatotoxicity: Clinical characteristics and listing compilation. *Int. J. Mol. Sci.* 17, 588.

Hoenders R., R. Ghelma, C, Portella, S. Simmons, A. Locke, H. Cramer, D. Gallego-Perez, and M. A. Jong. 2024. A review of the WHO strategy on traditional, complementary, and integrative medicine from the perspective of academic consortia for integrative medicine and health. *Front Med (Lausanne).* 2024 Jun 11;11:1395698. doi: 10.3389/fmed.2024.1395698. PMID: 38933107; PMCID: PMC11201178.

Ikhoyameh, M., W. E. Okete, R. M. Ogboye, O. K. Owoyemi and O. S. Gbadebo. 2024. Integrating traditional medicine into the African healthcare system post-Traditional Medicine Global Summit: challenges and recommendations. *The Pan African medical journal*, 47, 146. https://doi.org/10.11604/pamj.2024.47.146.43011.

Kamran, M., M. R. Khan, H.U. Khan, M. Abbas, M. Iqbal, and A. Nazir. 2018. Phytochemical and cytotoxic evaluation of Medicago monantha: In vivo protective potential in rats. *Biomedicine and pharmacotherapy = Biomedecine and pharmacotherapie*, 102, 1052–1063. https://doi.org/10.1016/j.biopha.2018.03.160.

Kaurinovic, B., and D. Vastag. 2019. Flavonoids and phenolic acids as potential natural antioxidants. *IntechOpen*., https://doi.org/10.5772/intechopen.83731.

Komolafe, K., T.R. Komolafe, T.H. Fatoki, A.C. Akinmoladun, B.I.C. Brai, M.T. Olaleye, A.A Akindahunsi. 2021. Coronavirus Disease 2019 and Herbal Therapy: Pertinent Issues Relating to Toxicity and Standardization of Phytopharmaceuticals. *Revista brasileira de farmacognosia : orgao oficial da Sociedade Brasileira de Farmacognosia*,

31(2), 142–161. https://doi.org/10.1007/s43450-021-00132-x.

Krishnan, V., S. Ahmad, and M. Mahmood. 2015. Antioxidant potential in different parts and callus of Gynura procumbens and different parts of Gynura bicolor. *BioMed Research International*, https:// doi. org/ 10. 1155/ 2015/147909.

Laličić-Petronijević, J., D. Komes, S. Gorjanović, A. Belščak-Cvitanović, L. Pezo, F. Pastor, S. Ostojić, J. Popov-Raljić, and D. Sužnjević. 2016. Content of total phenolics, flavan-3-ols and proanthocyanidins, oxidative stability and antioxidant capacity of chocolate during storage. *Food technology and biotechnology,* 54(1), p.13.

Melaku, L.S., and D. Worku. 2020. Role of long-term oxidative stress in pathobiology of atherosclerosis with evaluation of enzymes involved in the production of endogenous reactive oxygen species. *J. Med. Allied Sci*. 10, 43-51, https://doi.org/10.5455/jmas.91137.

Nurudeen, Q.O., M.R. Asinmi, M.B. Falana, M.A. Dikwa, Z.M. Yusuf, and M.O. Lambe. 2024. Antioxidant activity and toxicological implications of the aqueous extract of Azanza garckeana fruit pulp in female wistar rats. *Nigerian Journal of Biochemistry and Molecular Biology,* 39(1), pp.1-7.

Ogbole, O. O., T.O. Ajayi, T.O. Ajala, Z.A.Molik, A.E. Ipingbemi, E. Akinbobobola, and K.M. Akinsiku, 2023. Microbial content and elemental analysis of some locally prepared herbal remedies (Agbo) in Ibadan metropolis. *Journal of Pharmacy and Bioresources*, 20(3), 117-124.

Okafor, A. L., T. O. Azeez, S. C. Iwuji, E.C. Chikelu, and F. M. Arukalam. 2025. Bioactive constituents and acute toxicity of Blighia sapida capsule extracts using wistar rats. *Journal of Ethnopharmacology*, 337(Pt 1), 118790. https://doi.org/10.1016/j.jep.2024.118790.

Okaiyeto, K., and O.O. Oguntibeju. 2021. African Herbal Medicines: Adverse Effects and Cytotoxic Potentials with Different Therapeutic Applications. *International Journal of Environmental Research and Public Health*, 18(11), 5988. https://doi.org/10.3390/ijerph18115988.

Petchpoung, K., S, Soiklom, and W. Siri-Anusornsak. (2024). Estimation of Andrographolide and Antioxidant Activities in Andrographis peniculata Commercial Products by Color Parameters. *Trends in Sciences*, 21(3), pp.7393-7393.

Pranskuniene, Z., R. Balciunaite, Z. Simaitiene, and J. Bernatoniene 2022. Herbal Medicine Uses for Respiratory System Disorders and Possible Trends in New Herbal Medicinal Recipes during COVID-19 in Pasvalys District, Lithuania*. International journal of environmental research and public health*, 19(15), 8905. https://doi.org/10.3390/ijerph19158905.

Sanpinit, S., J. A. Goon, and P. Wetchakul. 2024. Characterization of the antioxidant activity, identified free radical-relieving components by LC/QTOF/MS and acute oral toxicity studies of Tri-Tharn-Thip tea. *Journal of Agriculture and Food Research,* 16, p.101131.

Santhi, V. P., P. Masilamani, V. Sriramavaratharajan, R. Murugan, S. S. Gurav, V. P. Sarasu, S. Parthiban, and M. Ayyanar. 2021. Therapeutic potential of phytoconstituents of edible fruits in combating emerging viral infections. *Journal of food biochemistry*, 45(8), e13851. Advance online publication. https://doi.org/10.1111/jfbc.13851.

Santos Filipe, M., V.S. Isca, N.E. Ntungwe, S. Princiotto, A.M. Díaz-Lanza, and P. Rijo. 2022. Lethality Bioassay using Artemia salina L. *Journal of Visualized Experiments*: JoVE, (188), 10.3791/64472. https://doi.org/10.3791/64472.

Sarkar, B., N. Rana, C. Singh, and A. Singh, (2024). Medicinal herbal remedies in neurodegenerative diseases: an update on antioxidant potential. *Naunyn-Schmiedeberg's archives of pharmacology,* 397(8), 5483–5511. https://doi.org/10.1007/s00210-024-03027-5.

Sharma, A., A. Kumar, A. K. Singh, K. J. Kumar, B. Narasimhan, and P. Kumar. 2024. Ethnomedicinal Uses, Phytochemistry, Pharmacology, and Toxicology of Ruellia tuberosa L.: A Review. *Chemistry and biodiversity,* 21(8), e202400292. https://doi.org/10.1002/cbdv.202400292.

Sharma, M., I. Dhaliwal, K. Rana, A. K. Delta, and P. Kaushik. 2021. Phytochemistry, Pharmacology, and Toxicology of Datura Species-A Review. *Antioxidants (Basel, Switzerland),* 10(8), 1291. https://doi.org/10.3390/antiox1008129.

Tanleque-Alberto, F., M. Juan-Borrás, and L. Escriche. 2020. Antioxidant characteristics of honey from Mozambique based on specific flavonoids and phenolic acid compounds. *J Food Compost Anal.* 86, htt ps://doi.org/10.1016/j.jfca.2019.103377.

Tavakkoli, A., A. Ahmadi, B. M. Razavi, and H. Hosseinzadeh. 2017. Black Seed (Nigella Sativa) and its Constituent Thymoquinone as an Antidote or a Protective Agent Against Natural or Chemical Toxicities. Iranian journal of pharmaceutical research: *IJPR*, 16(Suppl), 2–23.

World Health Organization, WHO Traditional Medicine Strategy: 2014–2023, 2021. https://www.who.int/medicines/publications/traditional/trm_strategy 14_23/en/.

Xu, Y., J. Liu, Y. Zeng, S. Jin, W. Liu, Z. Li, X. Qin, and Y. Bai. 2022. Traditional uses, phytochemistry, pharmacology, toxicity and quality control of medicinal genus Aralia: A review. *Journal of Ethnopharmacology*, 284, 114671. https://doi.org/10.1016/j.jep.2021.114671.

Yasmin, A., M.J. Sumi, K. Akter, R.H.M. Rabbi, H.S. Almoallim, M.J. Ansari, A. Hossain, and S. Imran. 2024. Comparative analysis of nutrient composition and antioxidant activity in three dragon fruit cultivars. *PeerJ*, 12, p.e17719.

Zhang, M., C. Wang, R. Zhang, Y. Chen, C. Zhang, H. Heidi, and M. Li. 2021. Comparison of the guidelines on good agricultural and collection practices in herbal medicine of the European Union, China, the WHO, and the United States of America. *Pharmacological research*, 167, 105533. https://doi.org/10.1016/j.phrs.2021.105533

Zhazykbayeva, S., S. Pabel, A. Mügge, and S. Sossalla, and N. Hamdani. 2020. The molecular mechanisms associated with the physiological responses to inflammation and oxidative stress in cardiovascular diseases. *Biophys. Rev*. 12, 947-968, https://doi.org/10.1007/s12551-020-00742-0.