WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 4, 1068-1078. Research Article ISSN 2277–7105

EVALUATION OF *IN-VITRO* ANTIOXIDANT AND HYPOGLYCEMIC POTENTIAL OF FLAVONOIDS FRACTION OF *PTERIDIUM AQUILINIUM* LEAF

Chizoba Ojimelukwe[1], Obinna Ajah[1], Cecilia Chika Unegbu[2] and Ikenna Elvis Nnaoma[1]*

[1]Department of Pharmaceutical Technology, Federal Polytechnic Nekede, Owerri, Nigeria.
[2]Postdoctoral Research Fellow, Bowie State University, 14000 Jericho Park Road, Bowie, Maryland, USA.

Article Received on
05 January 2024,
Revised on 26 Jan. 2024,
Accepted on 15 Feb. 2024
DOI: 10.20959/wjpr20244-31371

***Corresponding Author**
Ikenna Elvis Nnaoma
Department of Pharmaceutical Technology, Federal Polytechnic Nekede, Owerri, Nigeria.

ABSTRACT

Many of the world population has insight in the use of plants and herbs in their environs for treatment and management of different diseases. Herbal medicines are often used to provide first line and basic health services, both in people living in remote areas where it is the only available health service and to people living in poor areas where it offers the only affordable remedy. This present study evaluated the potential effect of extracted flavonoids fractions of *Pteridium aquilinium* leaf as antioxidant and hypoglycemic agent. The antioxidant activity was done using standard methods (inhibition of nitric oxide assay, inhibition of hydrogen peroxide, inhibition of lipid peroxidation, and reducing power assay) and the *in vitro* hypoglycemic activity was done using Alpha-Glucosidase Inhibitory activities assay, Lipase activity assay, and Glucose absorption Capacity assay. The result of the percentage alpha glucosidase inhibition ranged from 52.18 to 68.67%. The flavonoids fraction of *Pteridium aquilinium* showed significant ($p<0.05$) dose dependent inhibition of alpha glucosidase. The extract possess inhibition of lipase activity with percentage inhibition ranging from 57.10 - 82.36%. The glucose absorption capacity was also in a dose dependent manner. The flavonoids fraction of *Pteridium aquilinium* showed great antioxidant potential in a concentration dependent manner. The study revealed that flavonoids fraction of *Pteridium aquilinium* leaf could be used in the management of diabetes and oxidative stress mediated health challenges.

KEYWORDS: Diabetes, oxidative stress, hypoglycemic agent, antioxidant, *Pteridium aquilinium.*

INTRODUCTION

The use of herbal medicine to manage or cure diseases dates back to the Stone Age. There has been an advancement in pharmacological discoveries over the years that has resulted in the production of many synthetic drugs. Almost every part of plant is endowed with medicinal potential and can be useful in the management of diseases such as malaria, measles, diabetes mellitus, arthritis, and stomach disorders (Larayetan *et al*., 2019).

Diabetes mellitus (DM) is characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins; and an increased risk of complications from a vascular disease that affects 10 % of the population (Sengottaiyan *et al.*, 2016). Despite, the availability and extensive utilization of hypoglycemic agents, diabetes and the related complications continue to be major health concerns worldwide (Sengottaiyan *et al.*, 2016). According to International Diabetic Federation, the estimated diabetes prevalence in 2010 has risen to 285 million, representing 6.4 % of the world's adult population, with a prediction that by 2030, the number of people with diabetes will have risen to 438 million. A country-by-country summary table by IDF 2012 showed that 3,165.31 million Nigerians between the ages of 20 and 79 years have diabetes, while 2,532.25million Nigerians living with the conditions are unaware and undiagnosed. Nigeria lost 88.681million persons in 2012 due to diabetes related illnesses and has a 4.83% comparative prevalence according to World Health Organization (WHO) standard.

There is a great correlation between Diabetes mellitus and oxidative stress. Modern medications have little role to the alleviation of diseases associated with oxidative stress and plant-based preparations are chiefly available medicines employed for the treatment of diseases associated with oxidative stress. Most of the physiological impairments, tissues damages, pathological events, or diseases affecting humans have been attributed in recent scientific studies to be caused by unstable and extremely reactive chemical species called free radicals and/or reactive oxygen species (Anokwuru *et al*., 2011; Saeed *et al*., 2013).

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the rich chemical diversity (Afsar *et al*., 2018). Numerous studies have revealed that natural antioxidants possess

numerous pharmacological activities, including neuroprotective, anticancer, and anti-inflammatory activities, and these activities may be attributed to the properties of antioxidant compounds present in the natural plant products (Fuentes *et al*., 2005).

Bracken fern (*Pteridium aquilinum*) has a long history of traditional use in medicinal folklore. Researchers have identified numerous bioactive compounds in bracken fern that demonstrate potential pharmacological activities. Some studies suggest that specific compounds in bracken fern, including flavonoids, alkaloids, and phenolic compounds, exhibit antimicrobial, antibacterial, antioxidative, immunomodulatory, and anti-inflammatory properties (Malik *et al*., 2023). Hence this study evaluated the in-vitro hypoglycemic and antioxidant potential of methanol extract of *Pteridium aquilinum* leaf.

MATERIALS AND METHOD

Chemicals / Reagents

All chemicals and reagents used were good and of analytical grade.

Plant Collection

Fresh plant of *P. aquilinum* leaves was collected from Ekeapkara Osisioma, Abia State Nigeria. The plants were identified and authenticated by a Plant Taxonomist at the Michael Okpara University of Agriculture Umudike and deposited in the herbarium of the same institution.

Extract preparation

Five hundred grams (500g) of powdered leaves were macerated in 2.5L of methanol at room temperature for 72h. It was continuously mixed and then filtered using filter paper (Whatman size No.1). The filtrate was dried in a water bath at 45ºC and the concentrate was kept in air tight bottle at 4°C until used.

Extraction Flavonoids Fraction of *Pteridium aquilinium* (FFPA)

The method as described by Subramanian and Nagarajan (1969) was used, the crude extract was re-extracted in petroleum ether (fraction I), diethyl ether (fraction II) and ethyl acetate (fraction III) in succession. Fraction II and fraction lll were evaporated to dryness in water bath at 70^{o}c, combined and used as flavonoids fraction.

Evaluation of *in Vitro* Hypoglycemic Assay

The alpha-glucosidase inhibition assay was determined using a method described by Sancheti et al (2010). The lipase inhibition assay was determined according to the method described by Lewis and Liu (2012). Glucose adsorption capacity was determined by the method of Ou et al. (2001).

***In vitro* antioxidants**

The hydrogen peroxide (H_2O_2) scavenging activity of the extract was determined by the method of (Srinivasan *et al*., 2007) with slight modification. The method described by Kumaran and Karunakaran (2007) was used for reducing power assay. The NO scavenging activity of the extract was determined by the method of Daljit and Priyanka (2010). Inhibitory Capacity of Extract on Lipid Peroxidation by Malondialdehyde (MDA) assay was determined colorimetrically using thiobarbituric acid (TBA), as described by Okolie *et al*. (2009) with slight modifications.

Statistical Analysis

Statistical analysis of the data was carried out with SPSS version 22.0 using One Way Analysis of Variance (ANOVA) with Duncan post hoc test. The statistically analyzed data were reported as Mean ± SEM. A significant difference was accepted at 95% confidence level of probability ($P < 0.05$).

RESULT AND DISCUSSION

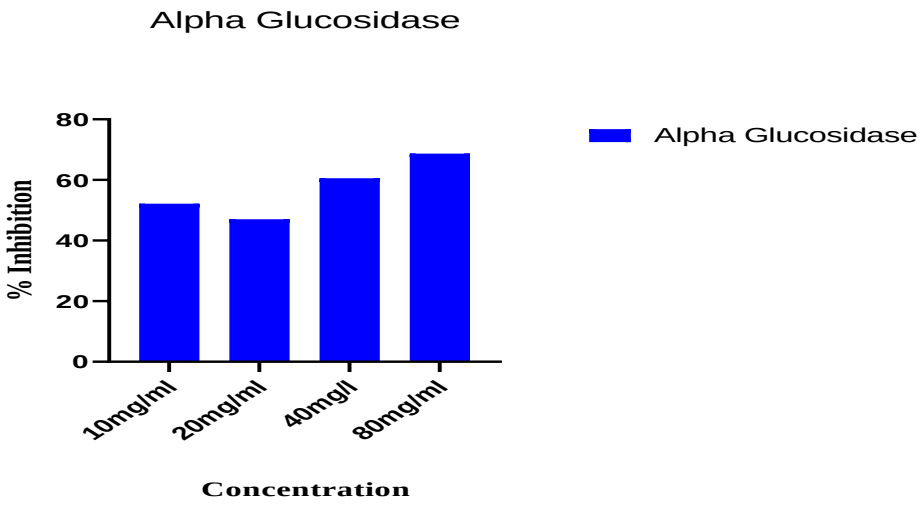

Figure 1: Alpha-glucosidase inhibitory activity of flavonoids fraction *Pteridium aquilinum* leaf. Values are mean±SEM of triplicate determinations.

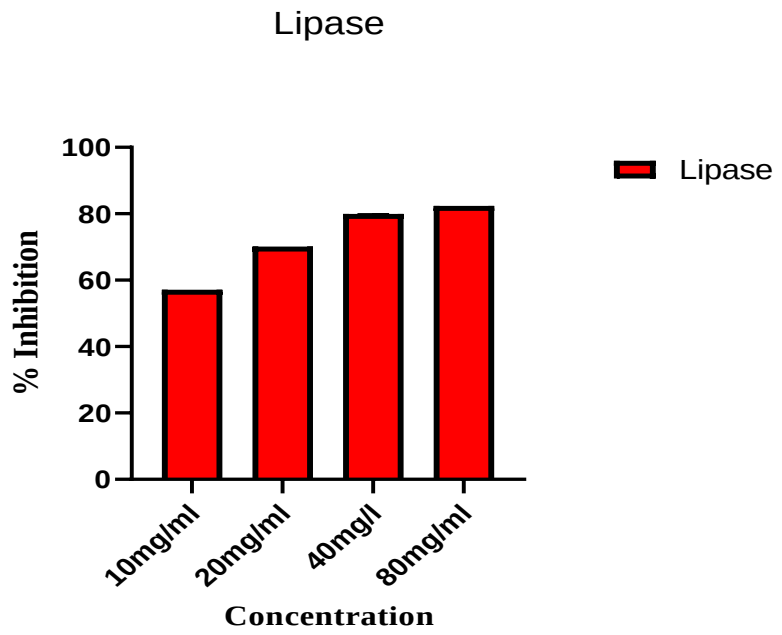

Figure 2: Lipase inhibitory activity of flavonoids fraction *Pteridium aquilinum* leaf. Values are mean±SEM of triplicate determinations.

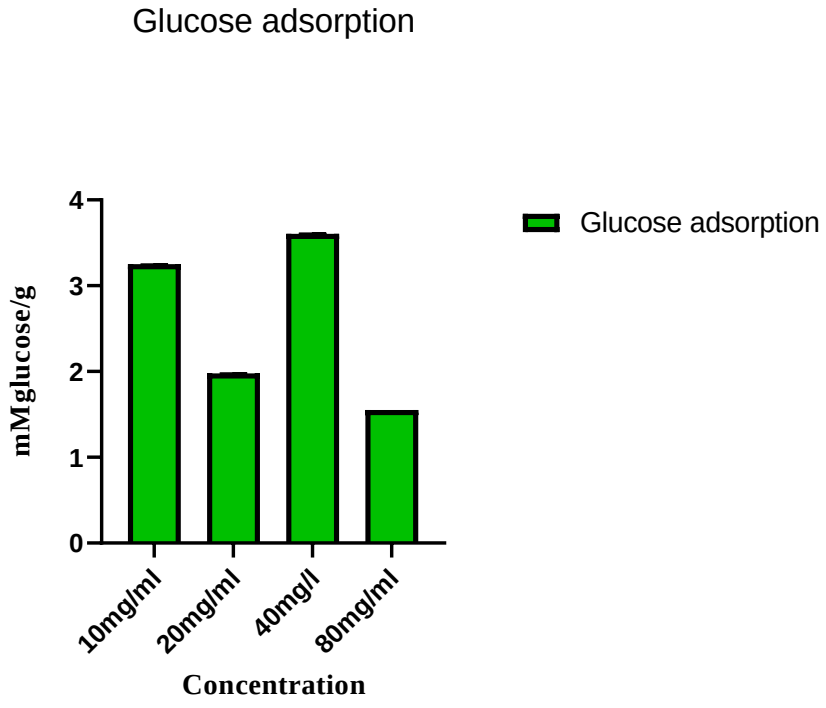

Figure 3: Glucose adsorption potential of flavonoids fraction *Pteridium aquilinum* leaf. Values are mean±SEM of triplicate determinations.

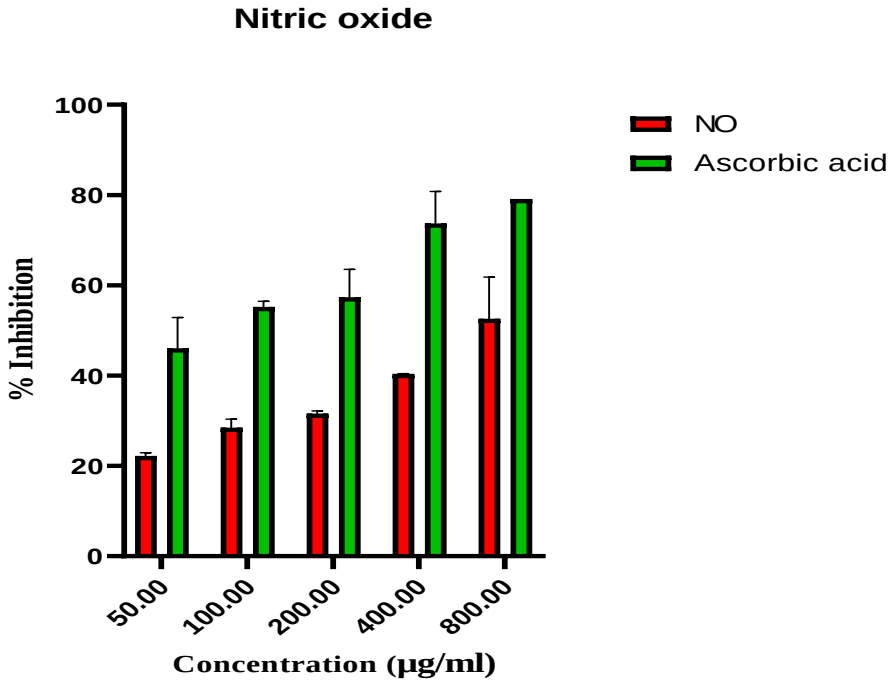

Figure 4: Nitric oxide scavenging activity of flavonoids fraction *Pteridium aquilinum* leaf.

The result showed some significant increase (P<0.05) in % nitric oxide inhibition activity of the methanol leaf extract in increasing concentration in comparison to the standard control.

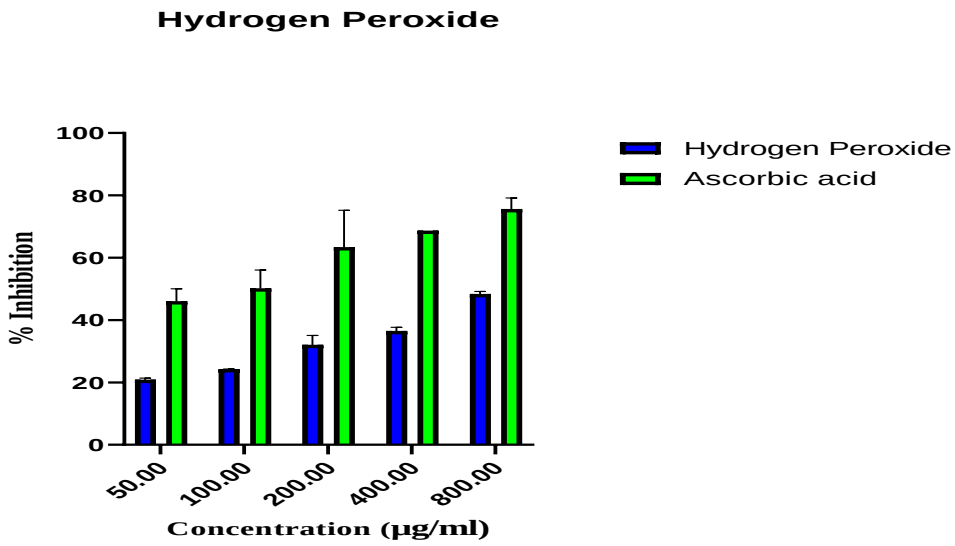

Figure 5: Hydrogen peroxide scavenging activity of flavonoids fraction *P. aquilinum* leaf.

The result showed some significant increase (P<0.05) in % hydrogen peroxide inhibition activity of the methanol leaf extract in increasing concentration in comparison to the standard control.

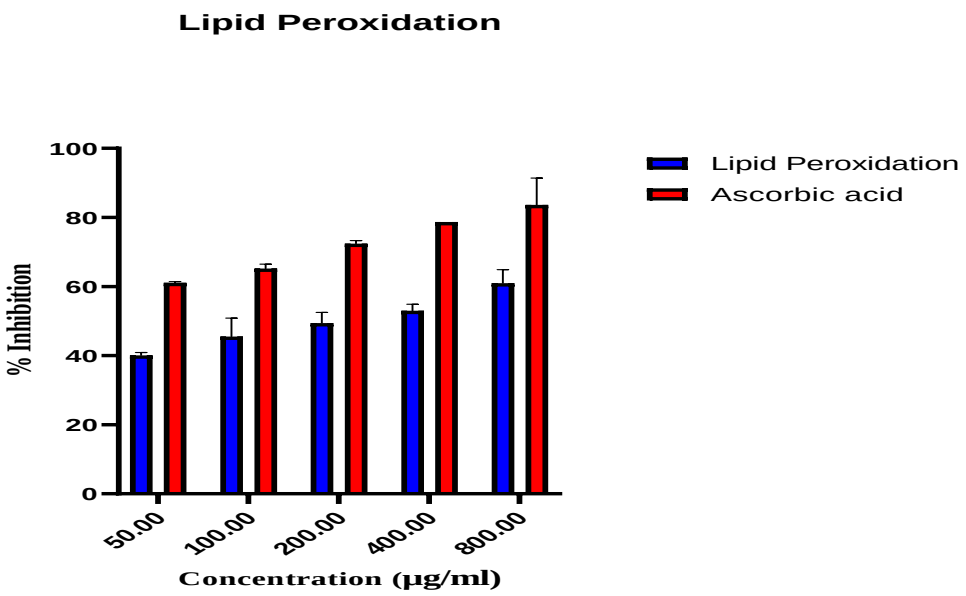

Figure 6: Lipid peroxidizing scavenging activity of flavonoids fraction *P. aquilinum* leaf.

The result showed some significant increase (P<0.05) in % lipid peroxidation inhibition activity of the methanol leaf extract in increasing concentration in comparison to the standard control.

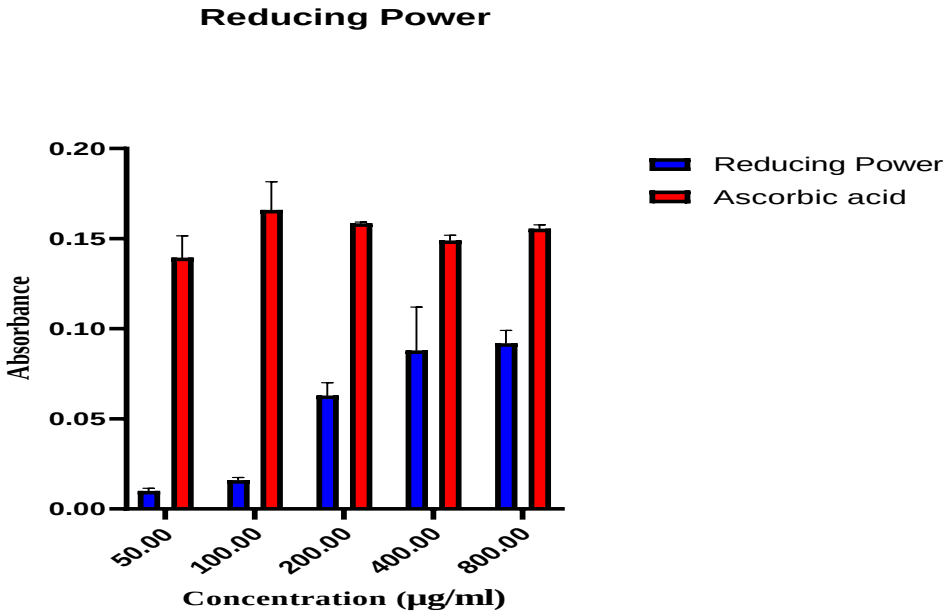

Figure 7: Reducing power potential of flavonoids fractions *P. aquilinum* leaves.

The result showed some significant increase (P<0.05) in reducing power scavenging activity of the methanol leaf extract in increasing concentration in at 50, 100, 200, 400 & 800µg/ml in comparison to the standard control.

DISCUSSION

Over the years, there has been an increasing search for natural products having potent bioactive compounds with low toxicity and possess ability to oxidize fats, control appetite, regulate levels of hormones related to obesity and modulate digestive enzymes involved in the absorption of carbohydrates and lipids (Cho et al., 2010; Rains et al., 2011).

Flavonoids and their derivatives such as luteolin and kaempferol possess the ability of blocking glucose absorption, inhibiting sodium dependent glucose transporter-1 which improves glucose tolerance (Thouri et al. 2017). Also, high levels of polyphenolic compounds have been shown to reduce the potency of alpha-glucosidase by either interacting or inhibiting specific position of the enzyme (Rohn et al., 2002). The digestive tracts glucosidase, which break down carbohydrates, are inhibited in order to delay the absorption of glucose. Inhibitors of these enzymes cause slower carbohydrate digestion and longer total carbohydrate digestion times, which slows down the rate of glucose absorption and dampens the post-prandial rise in plasma glucose. According to Unuofin et al. (2017b) in their study, the plant extracts have strong free radical scavenging potential and high polyphenolic contents which have been implicated in enzyme inhibitory capacity. In this present study, *P. aquilinum* leaves showed strong antioxidant potentials which may have been the basis for its strong inhibitory activities in the enzymes to assess its antidiabetic potentials.

The glucose adsorption capacity of the sample was also found to have a directly proportional relationship with the concentration of glucose. The adsorption property of the *P. aquilinum* leaves extract may be due to the presence of soluble and insoluble dietary fibers present in the extract. The flavonoids fraction of *P. aquilinum* possibly could bind to glucose efficiently even at low glucose concentrations, hence lowering the quantity of available glucose in the small intestine.

The reducing power ability of a chemical compound is based on its reductive capacity in a Fe^{3+}-Fe^{2+} system (Arika *et al*., 2019). The flavonoids fraction of *P. aquilinum* showed reducing potential. According to Parul et al. (2013), arginine is converted to citrulline via a 5-electron oxidative process, which produces nitric oxide (NO), a chemical that is used in cell signaling. In the hydrogen peroxide scavenging activity assay, the flavonoids fraction of *P. aquilinum* demonstrated a significant increase in inhibitory activity as the concentration increased. This suggests that the extract is effective in neutralizing hydrogen peroxide, a reactive oxygen species known to contribute to oxidative stress. Lipid peroxidation is the

process by which glycation and protein modification in cellular constituents are mediated by free radicals (Arika *et al*., 2019). Lipid hydroperoxides break down more quickly into peroxyl and alkoxyl radicals in biological systems, which in turn start the chain reaction in lipids. Lipid peroxidation is triggered by the production of hydroxyl and superoxide radicals (Hazra *et al*., 2008; Arika *et al*., 2019), hence the flavonoids fraction of *P. aquilinum* scavenged the formation of lipids peroxide in a dose dependent manner.

CONCLUSION

Overall, the findings suggest that the flavonoids fraction of *P. aquilinum* possesses significant antioxidant and hypoglycemic properties, particularly in scavenging nitric oxide, neutralizing hydrogen peroxide, inhibiting lipid peroxidation, and acting as a reducing agent, inhibiting alpha glucosidase and enhancing glucose absorption.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge Tertiary Education Trust Fund (TETFund) Nigeria for the financial support. We also acknowledge the Management of Federal Polytechnic Nekede Owerri for creating enabling environment for research.

REFERENCES

1. Afsar, T., Razak, S., Shabbir, M., & Khan, M. R. (2018). Antioxidant activity of polyphenolic compounds isolated from ethyl-acetate fraction of Acacia hydaspica R. Parker. *Chemistry Central Journal*, *12*: 1-13.
2. Anokwuru, C. P., Anyasor, G. N., Ajibaye, O., Fakoya, O., & Okebugwu, P. (2011). Effect of extraction solvents on phenolic, flavonoid and antioxidant activities of three nigerian medicinal plants. *Nature and Science*, *9*(7): 53-61.
3. Arika, W., Kibiti, C. M., Njagi, J. M., & Ngugi, M. P. (2019). In Vitro Antioxidant Properties of Dichloromethanolic Leaf Extract of Gnidia glauca (Fresen) as a Promising Antiobesity Drug. *Journal of Evidence-Based Integrative Medicine*, *24*. https://doi.org/10.1177/2515690X19883258.
4. Benrahou, K., Naceiri Mrabti, H., Bouyahya, A., Daoudi, N. E., Bnouham, M., Mezzour, H., & Faouzi, M. E. A. (2022). Inhibition of α-Amylase, α-Glucosidase, and Lipase, Intestinal Glucose Absorption, and Antidiabetic Properties by Extracts of Erodium guttatum. *Evidence-Based Complementary and Alternative Medicine*, *2022*.

5. Cho, A. S., Jeon, S. M., Kim, M. J., Yeo, J., Seo, K. I., Choi, M. S., & Lee, M. K. (2010). Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. *Food and chemical toxicology*, *48*(3): 937-943.
6. Fuentes, N.L., Sagua, H., Morale, G. & Bongue, Z, J. (2005). Experimental antihyperglycemic effect of diterpenoids of Hareta Azorella Compacta (Umbelliferae) Philinrats. *Phytotherapy Research*, 19: 713-716.
7. Hazra, B., Biswas, S., & Mandal, N. (2008). Antioxidant and free radical scavenging activity of Spondias pinnata. *BMC Complementary and Alternative Medicine*, *8*: 63. https://doi.org/10.1186/1472-6882-8-63.
8. Kardong, D., Upadhyaya, S., & Saikia, L. R. (2013). Screening of phytochemicals, antioxidant and antibacterial activity of crude extract of Pteridium aquilinum Kuhn. *Journal of pharmacy research*, *6*(1): 179-182.
9. Lavanya, R., Jairam V., Sudhakar C. Stephen H.S. and Millar Jnr J. C (2007). Anthocyanin fraction from potato extracts is cytotoxic to prostate cancer cells through activation of caspase- dependent and caspase- independent pathways. *Carcinogenesis*, 2B(10): 2227-2235
10. Malík, M., Mika, O. J., Navrátilová, Z., Killi, U. K., Tlustoš, P., & Patočka, J. (2023). Health and Environmental Hazards of the Toxic Pteridium aquilinum (L.) Kuhn (Bracken Fern). *Plants*, *13*(1): 18. https://doi.org/10.3390/plants13010018.
11. Parul, R., Kundu, S. K., & Saha, P. (2013). In vitro nitric oxide scavenging activity of methanol extracts of three Bangladeshi medicinal plants. *The pharma innovation*, *1*(12, Part A): 83.
12. Rains, T. M., Agarwal, S., & Maki, K. C. (2011). Antiobesity effects of green tea catechins: a mechanistic review. *The Journal of nutritional biochemistry*, *22*(1): 1-7.
13. Rehman, G., Hamayun, M., Iqbal, A., Ul Islam, S., Arshad, S., Zaman, K., & Lee, I. (2018). In vitro antidiabetic effects and antioxidant potential of Cassia nemophila pods. *BioMed research international*, *2018*.
14. Rohn, S., Rawel, H. M., & Kroll, J. (2002). Inhibitory effects of plant phenols on the activity of selected enzymes. *Journal of Agricultural and Food Chemistry*, *50*(12): 3566-3571.
15. Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N. et al (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition.

Diabetes Research and Clinical Practice, 157: 107843. https://doi.org/10.1016/j.diabres.2019.107843.

16. Sengottaiyan, A. *et al.* (2016) 'Synthesis and characterization of Solanum nigrum-mediated silver nanoparticles and its protective effect on alloxan-induced diabetic rats', *Journal of Nanostructure in Chemistry*, 6(1): 41–48. doi:10.1007/s40097-015-0178-6.
17. Subramanian, S.S and Nagaraja. (1965). Flavonoids of seed of crotoloriaretusa and C Straita; current science, 38: 365.
18. Thouri, A., Chahdoura, H., El Arem, A., Omri Hichri, A., Ben Hassin, R., & Achour, L. (2017). Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). *BMC complementary and alternative medicine*, *17*(1): 1-10.
19. Unuofin, J. O., Otunola, G. A., & Afolayan, A. J. (2018). In vitro α-amylase, α-glucosidase, lipase inhibitory and cytotoxic activities of tuber extracts of Kedrostis africana (L.) Cogn. *Heliyon*, *4*(9). https://doi.org/10.1016/j.heliyon.2018.e00810.