Bibliography

Abd El-Aziz, A. B., & Abd El-Kalek, H. H. (2011). Antimicrobial proteins and oil seeds from pumpkin (Cucurbita moschata). *Nature and Science*, *9*(3), 105-119.

Abd El-Ghany, M., Dalia, A.H., & Soha, M. (2010). Biological study on the effect of pumpkin seeds and zinc on reproductive potential of male rats. Faculty of Specific Education Mansoura University. The 5th Arab and 2nd International Annual Scientific Conference, 2384-2403.

Abdalla, A.E., Ejaz, H., Mahjoob, M.O., Alameen, A.A.M., Abosalif, K.O.A., Elamir, M.Y.M., Mousa, M.A. (2020). Intelligent Mechanisms of Macrophage Apoptosis Subversion by *Mycobacterium*. *Pathogens*, *9*(3):218.

Abdel-Moemin, A. R. (2014). Oxalate content of Egyptian grown fruits and vegetables and daily common herbs.

Abdelnour, S. A., Metwally, M. G., Bahgat, L. B., & Naiel, M. A. (2023). Pumpkin seed oil–supplemented diets promoted the growth productivity, antioxidative capacity, and immune response in heat-stressed growing rabbits. *Tropical Animal Health and Production*, *55*(1), 55.

Abed, N. M., & Alkalby, J. M. (2018). Protective Effect of Pumpkin Seed Oil on Reproductive System in Chlorpyrifos Treated Adult Male Rats. *Basrah Journal of Veterinary Research*, *17*(2).

Abiola, T. (2018). Proximate Composition, Phytochemical Analysis and in vivo Antioxidant Activity of Pomegranate Seeds (*Punica granatum*) in Female Albino Mice. *Reviews*, 439.

Abouelghar, G. E., El-Bermawy, Z. A., & Salman, H. M. (2020). Oxidative stress, haematological and biochemical alterations induced by sub-acute exposure to fipronil (COACH®) in albino mice and ameliorative effect of selenium plus vitamin E. *Environmental Science and Pollution Research*, *27*, 7886-7900.

Abou-Shehema, B. M., & Shahba, H. (2023). Enhancing Antioxidant Status, Semen Quality and Fertility of V-Line Rabbit Bucks Fed on a Diet Supplemented with Pumpkin Seed Powder. *Egyptian Poultry Science Journal*, *43*(4), 683-700.

Adamkovicova, M., Toman, R., Cabaj, M., Massanyi, P., Martiniakova, M., Omelka, R., ... & Duranova, H. (2014). Effects of subchronic exposure to cadmium and diazinon on testis and epididymis in rats. *The Scientific World Journal*, *2014*(1), 632581.

Adnan, M., Gul, S., Batool, S., Fatima, B., Rehman, A., Yaqoob, S., & Aziz, M. A. (2017). A review on the ethnobotany, phytochemistry, pharmacology and nutritional composition of *Cucurbita pepo* L. *The Journal of Phytopharmacology*, *6*(2), 133-139.

Adyanthaya, S., & Jose, M. (2013). Quality and safety aspects in histopathology laboratory. *Journal of Oral and Maxillofacial Pathology*, *17*(3), 402-407.

Agarwal, A., & Majzoub, A. (2017). Role of antioxidants in assisted reproductive techniques. *The World Journal of Men's Health*, *35*(2), 77-93.

Agarwal, A., Cho, C. L., Esteves, S. C., & Majzoub, A. (2017). Reactive Oxygen Species and sperm DNA fragmentation. *Translational Andrology and Urology*, *6*(Suppl 4), S695.

Agarwal, A., Leisegang, K., Majzoub, A., Henkel, R., Finelli, R., Selvam, M. K. P., ... & Shah, R. (2021). Utility of antioxidants in the treatment of male infertility: clinical guidelines based on a systematic review and analysis of evidence. *The World Journal of Men's Health*, *39*(2), 233.

Agarwal, A., Mulgund, A., Hamada, A., & Chyatte, M. R. (2015). A unique view on male infertility around the globe. *Reproductive Biology and Endocrinology*, *13*, 1-9.

Agati, G., Brunetti, C., Fini, A., Gori, A., Guidi, L., Landi, M., ... & Tattini, M. (2020). Are flavonoids effective antioxidants in plants? Twenty years of our investigation. *Antioxidants*, *9*(11), 1098.

Aghaei, S., Nikzad, H., Taghizadeh, M., Tameh, A. A., Taherian, A., & Moravveji, A. (2014). Protective effect of Pumpkin seed extract on sperm characteristics, biochemical parameters and epididymal histology in adult male rats treated with Cyclophosphamide. *Andrologia*, *46*(8), 927-935.

Agnihotri, S. K., Agrawal, A. K., Hakim, B. A., Vishwakarma, A. L., Narender, T., Sachan, R., & Sachdev, M. (2016). Mitochondrial membrane potential (MMP) regulates sperm motility. *In Vitro Cellular & Developmental Biology-Animal*, *52*, 953-960.

Ahin, S. S., Ari, F., Demir, C., & Ulukaya, E. (2014). *Journal of Food Biochemistry*, 38:248–257.

Ahmad, G., & Khan, A. A. (2019). Pumpkin: horticultural importance and its roles in various forms; a review. *International Journal of Horticulture and Agriculture*, *4*(1), 1-6.

Aitken, R. J. (2020). Impact of oxidative stress on male and female germ cells: implications for fertility. *Reproduction*, *159*(4), R189-R201.

Ajanal, M., Gundkalle, M. B., & Nayak, S. U. (2012). Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. *Ancient Science of Life*, *31*(4), 198-201.

Akbari, B., Baghaei- Yazdi, N., Bahmaie, M., & Mahdavi Abhari, F. (2022). The role of plant-derived natural antioxidants in reduction of oxidative stress. *BioFactors*, *48*(3), 611-633.

Akinmoladun, Afolabi F., et al. (2018). Protective effects of pumpkin seed extract against lead-induced nephrotoxicity in rats. *Journal of Environmental Toxicology and Pharmacology,* 62*:* 68-74.

Aksu, D. S., Sağlam, Y. S., Yildirim, S., & Aksu, T. (2017). Effect of pomegranate (Punica granatum L.) juice on kidney, liver, heart and testis histopathological changes, and the tissues lipid peroxidation and antioxidant status in lead acetate-treated rats. *Cellular and Molecular Biology*, *63*(10), 33-42.

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, *21*(2), 143-152.

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2021). Extraction of phenolic compounds: A review. *Current research in food science*, *4*, 200-214.

Aldaddou, W. A., Aljohani, A. S., Ahmed, I. A., Al-Wabel, N. A., & El-Ashmawy, I. M. (2022). Ameliorative effect of methanolic extract of Tribulus terrestris L. on nicotine and lead-induced degeneration of sperm quality in male rats. *Journal of Ethnopharmacology*, *295*, 115337.

Alelign, T., & Petros, B. (2018). Kidney stone disease: an update on current concepts. *Advances in Urology*, *2018*(1), 3068365.

Alidoust, L., Akhoondian, M., homayoun Atefi, A., Keivanlou, M. H., Ch, M. H., & Jafari, A. (2023). Stem cell-conditioned medium is a promising treatment for Alzheimer's disease. *Behavioural Brain Research*, *452*, 114543.

Al-Masri, S. A. (2015). Effect of pumpkin oil and vitamin E on lead induced testicular toxicity in male rats. *JAPS: Journal of Animal & Plant Sciences*, *25*(1).

Al-Okbi, S. Y., Mohamed, D. A., Kandil, E., Ahmed, E. K., & Mohammed, S. E. (2014). Functional ingredients and cardiovascular protective effect of pumpkin seed oils. *Grasas Aceites*, *65*(1), e007.

Al-Salhie, K. C. K., Al-Hummod, S. K., & Abbas, R. J. (2017). Effect of supplementation different levels of vitamin E and pumpkin seed oil to the diet on productive, physiological and reproductive performance of Japanese quail. *Basrah Journal of Agricultural Sciences*, *30*(2), 50-58.

Amorati, R., & Valgimigli, L. (2015). Advantages and limitations of common testing methods for antioxidants. *Free radical research*, *49*(5), 633-649.

Anjum, M. R., Madhu, P., Reddy, K. P., & Reddy, P. S. (2017). The protective effects of zinc in lead-induced testicular and epididymal toxicity in Wistar rats. *Toxicology and Industrial Health*, *33*(3), 265-276.

Anwar, H., Hussain, G., & Mustafa, I. (2018). Antioxidants from natural sources. *Antioxidants in Foods and Its Applications*, *3*.

AOAC, (2005). Official method of Analysis. 18th Edition, Association of Officiating Analytical Chemists, Washington DC.

Apak, R. (2019). Current issues in antioxidant measurement. *Journal of Agricultural and Food Chemistry*, *67*(33), 9187-9202.

Archana, P., Samatha, T., Mahitha, B., Chamundeswari, N. and Ramaswamy, N. (2012). Preliminary phytochemical screening from leaf and seed extracts of *Senna alata* L. Roxb-an Ethnomedicinal plant. *International Journal of Pharmaceutical and Biological Research*, *3*(3): 82-89.

Asif, M., Yousaf, H. M., Saleem, M., Hussain, L., Mahrukh, Zarzour, R. A., ... & Tahir, M. A. (2022). Raphanus sativus seeds oil arrested in vivo inflammation and angiogenesis through down-regulation of TNF-α. *Current Pharmaceutical Biotechnology*, *23*(5), 728-739.

Asowata-Ayodele, A. M., Afolayan, A. J., & Otunola, G. A. (2016). Ethnobotanical survey of culinary herbs and spices used in the traditional medicinal system of Nkonkobe Municipality, Eastern Cape, South Africa. *South African Journal of Botany*, *104*, 69-75.

Assi, M. A., Hezmee, M. N. M., Abba, Y., Rajion, M. A., Wahid, H., & Yusof, M. S. M. (2017). Assessment of therapeutic effects of *Nigella sativa* against chronic lead acetate-induced reproductive dysfunction in male Sprague-Dawley rats. *Comparative Clinical Pathology*, *26*, 87-97.

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., ... & Stuppner, H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances*, *33*(8), 1582-1614.

Badr, S. E., Shaaban, M., Elkholy, Y. M., Helal, M. H., Hamza, A. S., Masoud, M. S., & El Safty, M. M. (2011). Chemical composition and biological activity of ripe pumpkin fruits (*Cucurbita pepo* L.) cultivated in Egyptian habitats. *Natural Product Research*, *25*(16), 1524-1539.

Badu, M., Pedavoah, M. M., & Dzaye, I. Y. (2020). Proximate composition, antioxidant properties, mineral content and anti-nutritional composition of *Sesamum indicum*, *Cucumeropsis edulis* and *Cucurbita pepo* seeds grown in the savanna regions of Ghana. *Journal of Herbs, Spices & Medicinal Plants*, *26*(4), 329-339.

Bahrami, G., Hosseinzadeh, L., Azadi, Z., Mohammadi, B., Mahdiyan, Z., Hajialyani, M., & Farzaei, M. H. (2018). The Anti-Atherosclerotic Effect of Prosopis Farcta is Associated with the Inhibition of VCAM-1 and ICAM-1. *Journal of Reports in Pharmaceutical Sciences*, *7*(3), 357-367.

Bakrim, S., Benkhaira, N., Bourais, I., Benali, T., Lee, L. H., El Omari, N., ... & Bouyahya, A. (2022). Health benefits and pharmacological properties of stigmasterol. *Antioxidants*, *11*(10), 1912.

Barati, E., Nikzad, H., & Karimian, M. (2020). Oxidative stress and male infertility: current knowledge of pathophysiology and role of antioxidant therapy in disease management. *Cellular and Molecular Life Sciences*, *77*, 93-113.

Bardaa, S., Turki, M., Ben Khedir, S., Mzid, M., Rebai, T., Ayadi, F., & Sahnoun, Z. (2020). The effect of prickly pear, pumpkin, and linseed oils on biological mediators of acute inflammation and oxidative stress markers. *BioMed Research International*, *2020*(1), 5643465.

Barros, S. L., Frota, M. M., de Menezes, F. L., de Brito Araújo, A. J., dos Santos Lima, M., Fernandes, V. B., ... & de Vasconcelos, L. B. (2024). Physical–chemical, functional and antioxidant properties of dehydrated pumpkin seeds: Effects of ultrasound time and amplitude and drying temperature. *Waste and Biomass Valorization*, *15*(2), 1123-1140.

Batool, M., Ranjha, M. M. A. N., Roobab, U., Manzoor, M. F., Farooq, U., Nadeem, H. R., ... & Ibrahim, S. A. (2022). Nutritional value, phytochemical potential, and therapeutic benefits of pumpkin (Cucurbita sp.). *Plants*, *11*(11), 1394.

Benatta, M., Kettache, R., Buchholz, N., & Trinchieri, A. (2020). The impact of nutrition and lifestyle on male fertility. *Archivio Italiano Di Urologia e Andrologia*, *92*(2).

Bhalodia, N. R., Acharya, R. N., & Shukla, V. J. (2011). Evaluation of in vitro Antioxidant Activity of hydroalcoholic seed extracts of *Cassia fistula* linn. *Free Radicals and Antioxidants*, *1*(1), 68-76.

Bhongade, M.B., Prasad, S., Jiloha, R.C., Ray, P.C., Mohapatra, S., & Koner, B.C. (2015). Effect of psychological stress on fertility hormones and seminal quality in male partners of infertile couples. *Andrologia*, *47*, 336–342.

Białek, M., Rutkowska, J., Adamska, A., & Bajdalow, E. (2016). Partial replacement of wheat flour with pumpkin seed flour in muffins offered to children. *CyTA-Journal of Food*, *14*(3), 391-398.

Bidanchi, R. M., Lalrindika, L., Khushboo, M., Bhanushree, B., Dinata, R., Das, M., ... & Gurusubramanian, G. (2022). Antioxidative, anti-inflammatory and anti-apoptotic action of ellagic acid against lead acetate induced testicular and hepato-renal oxidative damages and pathophysiological changes in male Long Evans rats. *Environmental Pollution*, *302*, 119048.

Boitrelle, F., Shah, R., Saleh, R., Henkel, R., Kandil, H., Chung, E., ... & Agarwal, A. (2021). The sixth edition of the WHO manual for human semen analysis: a critical review and SWOT analysis. *Life*, *11*(12), 1368.

Buccellato, F. R., D’Anca, M., Fenoglio, C., Scarpini, E., & Galimberti, D. (2021). Role of oxidative damage in alzheimer’s disease and neurodegeneration: From pathogenic mechanisms to biomarker discovery. *Antioxidants*, *10*(9), 1353.

Bui, A. D., Sharma, R., Henkel, R., & Agarwal, A. (2018). Reactive Oxygen Species impact on sperm DNA and its role in male infertility. *Andrologia*, *50*(8), e13012.

Busari, M. B., Hamzah, R. U., Muhammad, H. L., Yusuf, R. S., Madaki, F. M., Adeniyi, J. O., ... & Berinyuy, E. B. (2021). Phenolic rich-extracts from Nauclea latifolia fruit restored Lead acetate-induced liver and kidney damaged in Wistar rats. *Clinical Phytoscience*, *7*, 1-10.

Caili, F. U., Huan, S., & Quanhong, L. I. (2006). A review on pharmacological activities and utilization technologies of pumpkin. *Plant Foods for Human Nutrition*, *61*, 70-77.

Carola, C. M. (2020). Heavy Metal Exposure and Its Effects in Male Reproductive Health: An *in vivo* and *in vitro* Study.

Çelik, S. E., Özyürek, M., Güçlü, K., & Apak, R. (2010). Solvent effects on the antioxidant capacity of lipophilic and hydrophilic antioxidants measured by CUPRAC, ABTS/persulphate and FRAP methods. *Talanta*, *81*(4-5), 1300-1309.

Chahal, A., Saini, A. K., Chhillar, A. K., & Saini, R. V. (2018). Natural antioxidants as defense system against cancer. *Asian Journal of Pharmaceutical and Clinical Research*, 38-44.

Chari, K., Polu, P., & Shenoy, R. (2018). An Appraisal of Pumpkin Seed Extract in 1, 2-Dimethylhydrazine Induced Colon Cancer in Wistar Rats. *Journal of Toxicology*. 1-12.

Chen, L., & Huang, G. (2019). Antioxidant activities of sulfated pumpkin polysaccharides. *International Journal of Biological Macromolecules*, *126*, 743-746.

Chen, M., Chen, Y., Zhu, W., Yan, X., Xiao, J., Zhang, P., ... & Li, P. (2023). Advances in the pharmacological study of Chinese herbal medicine to alleviate diabetic nephropathy by improving mitochondrial oxidative stress. *Biomedicine & Pharmacotherapy*, *165*, 115088.

Chhikara, N., Kaur, R., Jaglan, S., Sharma, P., Gat, Y., & Panghal, A. (2018). Bioactive compounds and pharmacological and food applications of *Syzygium cumini*–a review. *Food & function*, *9*(12), 6096-6115.

Chibuye, B., Singh, I. S., Chimuka, L., & Maseka, K. K. (2024). Phytochemical profiling and bioactivity study of *Adenia panduriformis* in Zambia using UHPLC-MS/MS-MZmine3, GNPS, and METLIN Gen2. *Scientific African*, *24*, e02151.

Choudhary, R., Chawala, V. K., Soni, N. D., Kumar, J., & Vyas, R. K. (2010). Oxidative stress and role of antioxidants in male infertility. *Pakistan Journal of Physiology*, *6*(2), 54-59.

Choudhury, S., Garg, A., Anand, L., Bharath, M. S., Yadav, R., & Alladi, P. A. (2024). Strategies in Parkinson's Disease Therapeutics-A Need for Synergy of Ayurveda, Small Molecules and Nanoparticles aided Approaches. *Current Topics in Medicinal Chemistry*.

Clemensen, A. K., Provenza, F. D., Hendrickson, J. R., & Grusak, M. A. (2020). Ecological implications of plant secondary metabolites-phytochemical diversity can enhance agricultural sustainability. *Frontiers in Sustainable Food Systems*, *4*, 547826.

Creasy, D.M., Foster, P.M., Haschek, W.M., Rousseaux, C.G., & Wallig M.A. (2002). Male reproductive system. *Handbook of Toxicologic Pathology*, San Diego, CA Academic Press 785-846.

Dai, Y., Huo, X., Cheng, Z., Faas, M. M., & Xu, X. (2020). Early-life exposure to widespread environmental toxicants and maternal-fetal health risk: A focus on metabolomic biomarkers. *Science of the Total Environment*, *739*, 139626.

Darbandi, M., Darbandi, S., Agarwal, A., Sengupta, P., Durairajanayagam, D., Henkel, R., & Sadeghi, M. R. (2018). Reactive Oxygen Species and male reproductive hormones. *Reproductive Biology and Endocrinology*, *16*, 1-14.

Darenskaya, M., Kolesnikov, S., Semenova, N., & Kolesnikova, L. (2023). Diabetic nephropathy: significance of determining oxidative stress and opportunities for antioxidant therapies. *International Journal of Molecular Sciences*, *24*(15), 12378.

David, B., Wolfender, J. L., & Dias, D. A. (2015). The pharmaceutical industry and natural products: historical status and new trends. *Phytochemistry Reviews*, *14*, 299-315.

Dcunha, R., Hussein, R. S., Ananda, H., Kumari, S., Adiga, S. K., Kannan, N., ... & Kalthur, G. (2022). Current insights and latest updates in sperm motility and associated applications in assisted reproduction. *Reproductive sciences*, 1-19.

de Carvalho, L. M. J., Ortiz, G. M. D., de Carvalho, J. L. V., Smirdele, L., & de Souza Neves Cardoso, F. (2017). *Carotenoids in yellow sweet potatoes, pumpkins and yellow sweet cassava*. IntechOpen: London, UK.

de Ligny, W., Smits, R. M., Mackenzie-Proctor, R., Jordan, V., Fleischer, K., de Bruin, J. P., & Showell, M. G. (2022). Antioxidants for male subfertility. *Cochrane Database of Systematic Reviews*, (5).

Dehelean, C. A., Marcovici, I., Soica, C., Mioc, M., Coricovac, D., Iurciuc, S., ... & Pinzaru, I. (2021). Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy. *Molecules*, *26*(4), 1109.

Devi, N. M., Prasad, R. V., & Sagarika, N. (2018). A review on health benefits and nutritional composition of pumpkin seeds. *International Journal of Chemical Studies*, *6*(3), 1154-1157.

Di Meo, S., & Venditti, P. (2020). Evolution of the knowledge of free radicals and other oxidants. *Oxidative Medicine and Cellular Longevity*, *2020*(1), 9829176.

Dimitriadis, F., Borgmann, H., Struck, J. P., Salem, J., & Kuru, T. H. (2023). Antioxidant supplementation on male fertility—a systematic review. *Antioxidants*, *12*(4), 836.

Docea, A. O., Gofita, E., Goumenou, M., Calina, D., Rogoveanu, O., Varut, M., ... & Tsatsakis, A. (2018). Six months exposure to a real-life mixture of 13 chemicals' below individual NOAELs induced non monotonic sex-dependent biochemical and redox status changes in rats. *Food and Chemical Toxicology*, *115*, 470-481.

Domínguez-Oliva, A., Hernández-Ávalos, I., Martínez-Burnes, J., Olmos-Hernández, A., Verduzco-Mendoza, A., & Mota-Rojas, D. (2023). The importance of animal models in biomedical research: current insights and applications. *Animals*, *13*(7), 1223.

Đorđević, I., Milutinović, M., Kostić, M., Đorđević, B., Dimitrijević, M., Stošić, N., ... & Kitić, D. (2016). Phytotherapeutic Approach to Benign Prostatic Hyperplasia Treatment by Pumpkin Seed (*Cucurbita Pepo* L., Cucurbitaceae). *Acta Medica Medianae*, *55*(3).

Dotto, J. M., & Chacha, J. S. (2020). The potential of pumpkin seeds as a functional food ingredient: A review. *Scientific African*, *10*, e00575.

Durante, M., Lenucci, M.S., & Mita, G. (2014). Supercritical carbon dioxide extraction of carotenoids from pumpkin (Cucurbita spp.): a review. *International Journal of Molecular Sciences*. 15:6725e6740.

Dutta, S., Majzoub, A., & Agarwal, A. (2019). Oxidative stress and sperm function: A systematic review on evaluation and management. *Arab journal of urology*, *17*(2), 87-97.

Egbuna, C., Kumar, S., Ifemeje, J. C., Ezzat, S. M., & Kaliyaperumal, S. (Eds.). (2019). Phytochemicals as lead compounds for new drug discovery. *Elsevier*.

Ekeleme-Egedigwe, C. A., Famurewa, A. C., David, E. E., Eleazu, C. O., & Egedigwe, U. O. (2019). Antioxidant potential of garlic oil supplementation prevents cyclophosphamide-induced oxidative testicular damage and endocrine depletion in rats. *Journal of Nutrition & Intermediary Metabolism*, *18*, 100109.

El Alami El Hassani, N., Baraket, A., & Alem, C. (2024). Recent advances in natural food preservatives: a sustainable solution for food safety and shelf-life extension. *Journal of Food Measurement and Characterization*, 1-23.

Elfiky, S.A., Elelaimy, I.A., Hassan, A.M., Ibrahim H.M., & Elsayad R.I. (2012). Protective effect of pumpkin seed oil against genotoxicity induced by azathioprine. *The Journal of Basic and Applied Zoology*, *65*(5): 289-298.

Elinge, C. M., Muhammad, A., Atiku, F. A., Itodo, A. U., Peni, I. J., Sanni, O. M., & Mbongo, A. N. (2012). Proximate, mineral and anti-nutrient composition of pumpkin (*Cucurbita pepo* L) seeds extract. *International Journal of plant research*, *2*(5), 146-150.

Elmastas, M., Çinkiliç, S., & Aboul-Enein, H. Y. (2015). Antioxidant capacity and determination of total phenolic compounds in daisy (*Matricaria chamomilla*, Fam. Asteraceae). *World Journal of Analytical Chemistry*, *3*(2), 9-14.

El-Mosallamy, A. E., Sleem, A. A., Abdel-Salam, O. M., Shaffie, N., & Kenawy, S. A. (2012). Antihypertensive and cardioprotective effects of pumpkin seed oil. *Journal of Medicinal Food*, *15*(2), 180-189.

Engwa, G. A., Ferdinand, P. U., Nwalo, F. N., & Unachukwu, M. N. (2019). Mechanism and health effects of heavy metal toxicity in humans. *Poisoning in the modern world-new tricks for an old dog*, *10*, 70-90.

Ethiraj, S. U. M. A. T. H. I., & Sridar, V. (2018). Phytochemical Screening, Antioxidant Activity and Extraction of Active Compound (Anonaine) From Fruit Peel Extract of *Annona reticulata* L. *Asian Journal of Pharmaceutical and Clinical Research*, *11*(11), 372-377.

Ethiraj, S., & Balasundaram, J. (2016). Phytochemical and biological activity of Cucurbita seed extract. *Journal of Advances in Biotechnology*, *6*(1), 813-821.

Fainberg, J., & Kashanian, J. A. (2019). Recent advances in understanding and managing male infertility. *F1000Research*, *8*.

Fan, L., Fan, W., Mei, Y., Liu, L., Li, L., Wang, Z., & Yang, L. (2022). Mechanochemical assisted extraction as a green approach in preparation of bioactive components extraction from natural products-A review. *Trends in Food Science & Technology*, *129*, 98-110.

Fenga, C., Gangemi, S., Di Salvatore, V., Falzone, L., & Libra, M. (2017). Immunological effects of occupational exposure to lead. *Molecular Medicine Reports*, *15*(5), 3355-3360.

Floegel, A., Kim, D.O., Chung, S.J., Koo, S.I., & Chun, O.K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition Analysis, 24,* 1043–1048.

Fountoucidou, P., Veskoukis, A. S., Kerasioti, E., Docea, A. O., Taitzoglou, I. A., Liesivuori, J., ... & Kouretas, D. (2019). A mixture of routinely encountered xenobiotics induces both redox adaptations and perturbations in blood and tissues of rats after a long-term low-dose exposure regimen: The time and dose issue. *Toxicology Letters*, *317*, 24-44.

Gatimel, N., Moreau, J., Parinaud, J., & Léandri, R. D. (2017). Sperm morphology: assessment, pathophysiology, clinical relevance, and state of the art in 2017. *Andrology*, *5*(5), 845-862.

Geng, S., Liu, Y., Ma, H., & Chen, C. (2015). Extraction and antioxidant activity of phenolic compounds from okra flowers. *Tropical Journal of Pharmaceutical Research*, *14*(5), 807-814.

Ghaffar, F., Kainat, B., Shah, H., & Akram, M. (2018). Nutritional, Physico-Chemical, Antimicrobial and Antioxidant Screening of Seed and Seed Oil of *Cucurbita pepo* Grown in KPK, Pakistan. *FUUAST Journal of Biology*, *8*(1).

Ghanbari, E., Nejati, V., & Khazaei, M. (2016). Antioxidant and protective effects of Royal jelly on histopathological changes in testis of diabetic rats. *International Journal of Reproductive BioMedicine, 14*(8), 519.

Gianazza, E., Brioschi, M., Martinez Fernandez, A., Casalnuovo, F., Altomare, A., Aldini, G., & Banfi, C. (2021). Lipid peroxidation in atherosclerotic cardiovascular diseases. *Antioxidants & Redox Signaling*, *34*(1), 49-98.

Gill, N. S., & Bali, M. (2011). Isolation of anti-ulcer cucurbitane type triterpenoid from the seeds of *Cucurbita pepo*.

Gomathi, D., Ravikumar, G., Kalaiselvi, M., Vidya, B., & Uma, C. (2015). In vitro free radical scavenging activity of ethanolic extract of the whole plant of *Evolvulus alsinoides* (L.) L. *Chinese Journal of Integrative Medicine*, *21*, 453-458.

Gossell-Williams, M., Davis, A., & O'Connor, N. (2006). Inhibition of testosterone-induced hyperplasia of the prostate of Sprague Dawley rats by pumpkin seed oil. *Journal of Medicinal Food*, *9*(2):284–6.

Goto, T., Hirabayashi, M., Watanabe, Y., Sanbo, M., Tomita, K., Inoue, N., Tsukamura, H., & Uenoyama, Y. (2020). Testosterone Supplementation Rescues Spermatogenesis and In Vitro Fertilizing Ability of Sperm in Kiss1 Knockout Mice. *Endocrinology*, *161*, bqaa092.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of Toxicology*, *94*(3), 651-715.

Gulcin, İ., & Alwasel, S. H. (2023). DPPH radical scavenging assay. *Processes*, *11*(8), 2248.

Gundidza, G.M., Mmbengwa, V.M., Magwa, M.L., Ramalivhana, N.J., & Mukwevho, N.T. (2009). Aphrodisiac properties of some Zimbabwean medicinal plants formulations. *African Journal of Biotechnology*, *8*(22):6402e6407.

Gupta, K., & Rajalakshmi Walavalkar, D. K. U. (2020). Male infertility. *4*, 33.

Gupta, R. K., Gangoliya, S. S., & Singh, N. K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *Journal of Food Science and Technology*, *52*, 676-684.

Gusti, A. M., Qusti, S. Y., Alshammari, E. M., Toraih, E. A., & Fawzy, M. S. (2021). Antioxidants-related superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST), and nitric oxide synthase (NOS) gene variants analysis in an obese population: a preliminary case-control study. *Antioxidants*, *10*(4), 595.

Gutierrez, R.M.P. (2016). Review of *Cucurbita pepo* (pumpkin) its phytochemistry and pharmacology. *Medicinal Chemistry*, 6:012e021.

Guvvala, P. R., Sellappan, S., & Parameswaraiah, R. J. (2016). Impact of arsenic (V) on testicular oxidative stress and sperm functional attributes in Swiss albino mice. *Environmental Science and Pollution Research*, *23*, 18200-18210.

Hadwan, M. H., Hussein, M. J., Mohammed, R. M., Hadwan, A. M., Saad Al-Kawaz, H., Al-Obaidy, S. S., & Al Talebi, Z. A. (2024). An improved method for measuring catalase activity in biological samples. *Biology Methods and Protocols*, *9*(1), bpae015.

Handy, D. E., & Loscalzo, J. (2022). The role of glutathione peroxidase-1 in health and disease. *Free Radical Biology and Medicine*, *188*, 146-161.

Hasona, N. A. (2018). Grape seed extract attenuates dexamethasone- induced testicular and thyroid dysfunction in male albino rats. *Andrologia*, *50*(5), e13002.

Hassan, E., El-Neweshy, M., Hassan, M., & Noreldin, A. (2019). Thymoquinone attenuates testicular and spermotoxicity following subchronic lead exposure in male rats: Possible mechanisms are involved. *Life Sciences*, *230*, 132-140.

Henkel, R., Maab, G., Bodeker, R.H., Scheibelhut, C., & Stalf, T. (2005). Sperm function and assisted reproduction technology. *Reproductive Medicine and Biology*, *4*(1):7-30.

Hosseini, F. S., Abedini, A. A., Chen, F., Whitfield, T., Ude, C. C., & Laurencin, C. T. (2023). Oxygen-Generating biomaterials for translational bone regenerative engineering. *ACS Applied Materials & Interfaces*, *15*(44), 50721-50741.

Hou, S., Zheng, N., Tang, L., Ji, X., Li, Y., & Hua, X. (2019). Pollution characteristics, sources, and health risk assessment of human exposure to Cu, Zn, Cd and Pb pollution in urban street dust across China between 2009 and 2018. *Environment International*, *128*, 430-437.

Huang, D. (2018). Dietary antioxidants and health promotion. *Antioxidants*, *7*(1), 9.

Huang, W. Y., Cai, Y. Z., Corke, H., & Sun, M. (2010). Survey of antioxidant capacity and nutritional quality of selected edible and medicinal fruit plants in Hong Kong. *Journal of Food Composition and Analysis*, *23*(6), 510-517.

Hussein, R. A., & El-Anssary, A. A. (2019). Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. *Herbal Medicine*, *1*(3), 11-30.

Hwang, S.Y., Kim, W.J., Wee J.J., Choi, J.S., & Kim, S.K. (2004). Panax ginseng improves survival and sperm quality in guinea pigs exposed to 2,3,7,8-tetrachlorodibenzop-dioxin. *BJU International*, *94*(4):663-668.

Ibrahim, N. I., & Naina Mohamed, I. (2021). Interdependence of anti-inflammatory and antioxidant properties of squalene–implication for cardiovascular health. *Life*, *11*(2), 103.

Ismael, Z. K., AL-Anbari, L. A., & Mossa, H. A. (2017). Relationship of FSH, LH, DHEA and testosterone levels in serum with sperm function parameters in infertile men. *Journal of Pharmaceutical Sciences and Research*, *9*(11), 2056-2061.

Iyengar, M.A. (1995). Study of Crude Drugs. 8th Edn., Manipal Power Press, Manipal, India.

Jabeen, S., & Noor, S. (2024). Oxidative stress and chronic kidney disease. *Fundamental Principles of Oxidative Stress in Metabolism and Reproduction*, 151-165.

Jain, S., Kedia, S., Bopanna, S., Sachdev, V., Sahni, P., Dash, N. R., ... & Ahuja, V. (2017). Faecal calprotectin and UCEIS predict short-term outcomes in acute severe colitis: prospective cohort study. *Journal of Crohn's and Colitis*, *11*(11), 1309-1316.

Jideani, A. I., Silungwe, H., Takalani, T., Omolola, A. O., Udeh, H. O., & Anyasi, T. A. (2021). Antioxidant-rich natural fruit and vegetable products and human health. *International Journal of Food Properties*, *24*(1), 41-67.

Jimoh, O. A., Oyeyemi, W. A., Okin-Aminu, H. O., & Oyeyemi, B. F. (2021). Reproductive characteristics, semen quality, seminal oxidative status, steroid hormones, sperm production efficiency of rabbits fed herbal supplements. *Theriogenology*, *168*, 41-49.

Juan, C. A., Pérez de la Lastra, J. M., Plou, F. J., & Pérez-Lebeña, E. (2021). The chemistry of Reactive Oxygen Species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *International Journal of Molecular Sciences*, *22*(9), 4642.

Kakkar, P., Das, B., & Viswanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase.

Karbel Hadeel, A., Al-Bdairi Adnan, A., Khairullah Ahmed, R., & Al-Humairi Ameer, K. (2020). Histopathological evaluation of non-obstructive azoospermic males using testicular aspirate (TESA) biopsy. *Indian Journal of Forensic Medicine & Toxicology*, *14*(4), 2993.

Kashyap, K., Hait, M., Roymahapatra, G., & Vaishnav, M. M. (2022). Proximate and elemental analysis of Careya arborea Roxb plant’s root. *ES Food & Agroforestry*, *7*, 41-47.

Kasperczyk, A., Dobrakowski, M., Czuba, Z. P., Horak, S., & Kasperczyk, S. (2015). Environmental exposure to lead induces oxidative stress and modulates the function of the antioxidant defense system and the immune system in the semen of males with normal semen profile. *Toxicology and Applied Pharmacology*, *284*(3), 339-344.

Kaur, S., Panghal, A., Garg, M. K., Mann, S., Khatkar, S. K., Sharma, P., & Chhikara, N. (2020). Functional and nutraceutical properties of pumpkin–a review. *Nutrition & Food Science*, *50*(2), 384-401.

Kaurinovic, B., & Vastag, D. (2019). *Flavonoids and phenolic acids as potential natural antioxidants* (pp. 1-20). London, UK: IntechOpen.

Kawanishi, S., Ohnishi, S., Ma, N., Hiraku, Y., & Murata, M. (2017). Crosstalk between DNA damage and inflammation in the multiple steps of carcinogenesis. *International Journal of Molecular Sciences*, *18*(8), 1808.

Khalaf, A. A., Moselhy, W. A., & Abdel-Hamed, M. I. (2012). The protective effect of green tea extract on lead induced oxidative and DNA damage on rat brain. *Neurotoxicology*, *33*(3), 280-289.

Khalil, W. A., Hassan, M. A., Ibrahim, S., Mohammed, A. K., El-Harairy, M. A., & Abdelnour, S. A. (2024). The beneficial effects of quinoa seed extract supplementation on ram sperm quality following cryopreservation. *Animal Reproduction Science*, *264*, 107472.

Kibui, A.N., Owaga, E., & Mburu, M. (2018). Proximate composition and nutritional characterization of Chia enriched yoghurt. *African Journal of Food, Agriculture, Nutrition and Development*, *18*(1).

Kolesnikova, L. I., Kolesnikov, S. I., Kurashova, N. A., & Bairova, T. A. (2015). Causes and factors of male infertility. *Annals of the Russian Academy of Medical Sciences*, *70*(5), 579-584.

Kostoff, R. N., Aschner, M., Goumenou, M., & Tsatsakis, A. (2020). Setting safer exposure limits for toxic substance combinations. *Food and Chemical Toxicology*, *140*, 111346.

Kostyuk, V. A., Potapovich, A. I., Kostyuk, T. V., & Cherian, M. G. (2007). Metal complexes of dietary flavonoids evaluation of radical scavenger properties and protective activity against oxidative stress in vivo. *Cellular and Molecular Biology*, 53, 61-68.

Krimer-Malešević, V. (2020). Pumpkin seeds: Phenolic acids in pumpkin seed (*Cucurbita pepo* L.). *Nuts and Seeds in Health and Disease Prevention* (pp. 533-542). Academic Press.

Kubincová, P., Sychrová, E., Raška, J., Basu, A., Yawer, A., Dydowiczová, A., ... & Sovadinová, I. (2019). Polycyclic aromatic hydrocarbons and endocrine disruption: role of testicular gap junctional intercellular communication and connexins. *Toxicological sciences*, *169*(1), 70-83.

Kuhad, A., Pilkhwal, S., Sharma, S., Tirkey, N., & Chopra, K. (2007). Effect of curcumin on inflammation and oxidative stress in cisplatin-induced experimental nephrotoxicity. *Journal of Agriculture and Food Chemistry*, 55:10150–10155.

Kumar, M., Dahuja, A., Tiwari, S., Punia, S., Tak, Y., Amarowicz, R., ... & Kaur, C. (2021). Recent trends in extraction of plant bioactives using green technologies: A review. *Food Chemistry*, *353*, 129431.

Kumar, T., Jha, K., Zabihullah, M., Neelu, K., Kumar, Y., & Siddharth, K. (2023). Effects of the COVID-19 pandemic on semen quality in male partners of infertile couples: a hospital-based observational study. *Asian Journal of Andrology*, *25*(2), 240-244.

Kurutas, E. B. (2015). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition Journal*, *15*, 1-22.

Laoung-On, J., Jaikang, C., Saenphet, K., & Sudwan, P. (2021). Phytochemical screening, antioxidant and sperm viability of Nelumbo nucifera petal extracts. *Plants*, *10*(7), 1375.

Lapenna, D. (2023). Glutathione and glutathione-dependent enzymes: from biochemistry to gerontology and successful aging. *Ageing Research Reviews*, 102066.

Lateef, M. A., Abdulhadi, H. L., & Ali, L. H. (2024). Study of the Therapeutic Efficacy of Pumpkin Seed in Improving the Liver's Activity in Rats with Stz-Induced Diabetes. *Frontiers in Health Informatics*, 5336-5351.

Lee, J., Jang, J., Park, S. M., & Yang, S. R. (2021). An update on the role of Nrf2 in respiratory disease: molecular mechanisms and therapeutic approaches. *International Journal of Molecular Sciences*, *22*(16), 8406.

Lee, K. J., Sok, D. E., Kim, Y. B., & Kim, M. R. (2002). Protective effect of vegetable extracts on oxidative stress in brain of mice administered with NMDA. *Food Research International*, *35*(1), 55-63.

Lee, S. M., Lim, H. J., Chang, J. W., Hurh, B. S., & Kim, Y. S. (2018). Investigation on the formations of volatile compounds, fatty acids, and γ-lactones in white and brown rice during fermentation. *Food chemistry*, *269*, 347-354.

Levine, H., Jørgensen, N., Martino-Andrade, A., Mendiola, J., Weksler-Derri, D., Mindlis, I., ... & Swan, S. H. (2017). Temporal trends in sperm count: a systematic review and meta-regression analysis. *Human Reproduction Update*, *23*(6), 646-659.

Leyva-Porras, C., Román-Aguirre, M., Cruz-Alcantar, P., Pérez-Urizar, J. T., & Saavedra-Leos, M. Z. (2021). Application of antioxidants as an alternative improving of shelf life in foods. *Polysaccharides*, *2*(3), 594-607.

Li, H., Wang, X. R., Hu, Y. F., Xiong, Y. W., Zhu, H. L., Huang, Y. C., & Wang, H. (2024). Advances in immunology of male reproductive toxicity induced by common environmental pollutants. *Environment International*, 108898.

Li, X., Li, Z., Lin, C. J., Bi, X., Liu, J., Feng, X., ... & Wu, T. (2018). Health risks of heavy metal exposure through vegetable consumption near a large-scale Pb/Zn smelter in central China. *Ecotoxicology and Environmental Safety*, *161*, 99-110.

Liang, Q., Yang, J., He, J., Chen, X., Zhang, H., Jia, M., ... & Wei, J. (2020). Stigmasterol alleviates cerebral ischemia/reperfusion injury by attenuating inflammation and improving antioxidant defenses in rats. *Bioscience Reports*, *40*(4), BSR20192133.

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., ... & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, 757-772.

Lin, L., Wu, Q., Lu, F., Lei, J., Zhou, Y., Liu, Y., ... & Hu, M. (2023). Nrf2 signaling pathway: current status and potential therapeutic targetable role in human cancers. *Frontiers in Oncology*, *13*, 1184079.

Lipnick, R. L., Cotruvo, J. A., Hill, R. N., Bruce, R. D., Stitzel, K. A., Walker, A. P., Chu, I., Goddard, M., Segal, L., & Springer, J. A. (1995). Comparison of the up-and-down, conventional LD50, and fixed-dose acute toxicity procedures. *Food And Chemical Toxicology*, *33*(3), 223–231.

Liu, K. S., Mao, X. D., Pan, F., & An, R. F. (2021). Effect and mechanisms of reproductive tract infection on oxidative stress parameters, sperm DNA fragmentation, and semen quality in infertile males. *Reproductive Biology and Endocrinology*, *19*, 1-12.

Liu, X., Zhang, J., Li, J., Song, C., & Shi, Y. (2022). Pharmacological inhibition of ALCAT1 mitigates amyotrophic lateral sclerosis by attenuating SOD1 protein aggregation. *Molecular Metabolism*, *63*, 101536.

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, *4*(8), 118.

Lorenzo, J. M., Munekata, P. E. S., Baldin, J. C., Franco, D., Domínguez, R., Trindade, M. A., & Tindade, M. (2017). The use of natural antioxidants to replace chemical antioxidants in foods. *Strategies for Obtaining Healthier Foods; Lorenzo, JM, Carballo, FJ, Eds*, 205-228.

Maghraoui, S., Florea, A., Ayadi, A., Matei, H., & Tekaya, L. (2023). Changes in Organ Weight, Sperm Quality and Testosterone Levels After Aluminum (Al) and Indium (In) Administration to Wistar Rats. *Biological Trace Element Research*, *201*(2), 766-775.

Maiti, S., Nazmeen, A., Medda, N., Patra, R., & Ghosh, T. K. (2019). Flavonoids green tea against oxidant stress and inflammation with related human diseases. *Clinical Nutrition Experimental*, *24*, 1-14.

Majid, A. K., Ahmed, Z., & Khan, R. (2020). Effect of pumpkin seed oil on cholesterol fractions and systolic/diastolic blood pressure. *Food Science and Technology*, *40*, 769-777.

Makni, M., Fetoui, H., Gargouri, N. K., Garoui, E. M., & Zeghal, N. (2011). Antidiabetic effect of flax and pumpkin seed mixture powder: effect on hyperlipidemia and antioxidant status in alloxan diabetic rats. *Journal of Diabetes and its Complications*, *25*(5), 339-345.

Makni, M., Fetoui, H., Gargouri, N. K., Garoui, E. M., Jaber, H., Makni, J., ... & Zeghal, N. (2008). Hypolipidemic and hepatoprotective effects of flax and pumpkin seed mixture rich in ω-3 and ω-6 fatty acids in hypercholesterolemic rats. *Food and Chemical Toxicology*, *46*(12), 3714-3720.

Manshi., Chaturvedi, N., & Sahrawat, N. (2023). Effect of processing on nutraceutical profile and amino acid content on pumpkin (*Cucurbita pepo* L.) seeds.

Martha, R. & Gutierrez, P. Medicinal chemistry Review of *Cucurbita pepo* (Pumpkin) its Phytochemistry and Pharmacology. 6, 12–21 (2016).

Martins Gregório, B., Benchimol De Souza, D., Amorim de Morais Nascimento, F., Matta, L., & Fernandes-Santos, C. (2016). The potential role of antioxidants in metabolic syndrome. *Current Pharmaceutical Design*, *22*(7), 859-869.

McClements, D. J. (2023). Ultraprocessed plant‐ based foods: Designing the next generation of healthy and sustainable alternatives to animal‐ based foods. *Comprehensive Reviews in Food Science and Food Safety*, *22*(5), 3531-3559.

McIntyre, C., Li, X.F., de Burgh, R., Ivanova, D., Lass, G., & O'Byrne, K.T. (2022). GABA Signaling in the Posterodorsal Medial Amygdala Mediates Stress-induced Suppression of LH Pulsatility in Female Mice. *Endocrinology,164*, 197.

Mehmood, H., Khan, A. S., Saeed, A., Zainab, A., ud Din, N., Khan, A. U. H., ... & Din, A. U. (2024). Ameliorative Potential of Pumpkin Seeds Against Lead-Induced Toxicity in Poultry Chicken (*Gallus domesticus*). *Indus Journal of Bioscience Research*, *2*(02), 1433-1439.

Mehmood, N., Zubaır, M., Rızwan, K., Rasool, N., Shahid, M., & Ahmad, V. U. (2012). Antioxidant, antimicrobial and phytochemical analysis of cichoriumintybus seeds extract and various organic fractions. *Iranian Journal of Pharmaceutical Research: IJPR*, *11*(4), 1145.

Meli, R., Monnolo, A., Annunziata, C., Pirozzi, C., & Ferrante, M. C. (2020). Oxidative stress and BPA toxicity: an antioxidant approach for male and female reproductive dysfunction. *Antioxidants*, *9*(5), 405.

Micera, M., Botto, A., Geddo, F., Antoniotti, S., Bertea, C. M., Levi, R., ... & Querio, G. (2020). Squalene: more than a step toward sterols. *Antioxidants*, *9*(8), 688.

Mitra, A. K. (2020). Antioxidants: a masterpiece of mother nature to prevent illness. *Journal of Chemical Reviews*, *2*(4), 243-256.

Mohammad Azmin, S. N. H., Abdul Manan, Z., Wan Alwi, S. R., Chua, L. S., Mustaffa, A. A., & Yunus, N. A. (2016). Herbal processing and extraction technologies. *Separation & Purification Reviews*, *45*(4), 305-320.

Mohammad, N., Matinhomaee, H., & Hosseini, S. A. (2021). Antioxidant effects of resistance training with pumpkin seed extract consumption in heart tissue of rats exposed to H2O2-induced oxidative damage. *Zahedan Journal of Research in Medical Sciences*, *23*(3).

Mohanty, S. K., & Kastor, A. (2017). "Seven: Occupational class and chronic diseases in India". In *Work and Health in India*. Bristol, UK: Policy Press.

Moussa, Z., Judeh, Z. M., & Ahmed, S. A. (2019). Nonenzymatic exogenous and endogenous antioxidants. *Free Radical Medicine and Biology*, *1*, 11-22.

Ndam, L. M., Mih, A. M., Fongod, A. G. N., Tening, A. S., Tonjock, R. K., Enang, J. E., & Fujii, Y. (2014). Phytochemical screening of the bioactive compounds in twenty (20) Cameroonian medicinal plants. *International Journal of Current Microbiology and Applied Sciences*, *3*(12), 768-778.

Neelamma, G., Rasheed, S. H., Sulthana, K., & Srilekha, P. (2021). In-Vitro Assessment, Isolation and Spectral Analysis of the Petroleum Ether Extract of Pumpkin Seed. *International Journal of Pharmaceutical Sciences Review and Research*, *12*, 356-362.

Nworgu, F., Ekemezie, A.A.O., Ladele, A.O., & Akinrolabu, B.M. (2007). Performance of broiler chickens served heat-treated fluted pumpkin (*Telfaria occidentalis*) leaves extract supplement. *African Journal of Biotechnology*, *6*(6):818-825.

Ochie, J. & Kolhatkar, A. (2000). Medical Laboratory Science. Theory and Practice Tata McGraw-Hill Company Limited.

Oduwole, O. O., Huhtaniemi, I. T., & Misrahi, M. (2021). The roles of luteinizing hormone, follicle-stimulating hormone and testosterone in spermatogenesis and folliculogenesis revisited. *International journal of molecular sciences*, *22*(23), 12735.

OECD, (2000). OECD Annual Report 2000, OECD Publishing, Paris. https://www.oecd.org/en/publications/oecd-annual-report-2000_annrep-2000-en.html

OECD, (2001). OECD Annual Report 2001, OECD Publishing, Paris. https://doi.org/10.1787/annrep-2001-en

Offor, S. J., Mbagwu, H. O., & Orisakwe, O. E. (2017). Lead induced hepato-renal damage in male albino rats and effects of activated charcoal. *Frontiers in Pharmacology*, *8*, 107.

Ofoedu, C. E., You, L., Osuji, C. M., Iwouno, J. O., Kabuo, N. O., Ojukwu, M., ... & Korzeniowska, M. (2021). Hydrogen peroxide effects on natural-sourced polysacchrides: free radical formation/production, degradation process, and reaction mechanism—a critical synopsis. *Foods*, *10*(4), 699.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351-358.

Olszowy, M. (2019). What is responsible for antioxidant properties of polyphenolic compounds from plants? *Plant Physiology and Biochemistry*, *144*, 135-143.

Omotayo, F.O., & Borokini, T.I. (2012). Comparative phytochemical and ethnomedicinal survey of selected medicinal plants in Nigeria. *Scientific Research Essays*, *7*(9): 989-999.

Onyesom, I., & Okoh, P. N. (2006). Quantitative analysis of nitrate and nitrite contents in vegetables commonly consumed in Delta State, Nigeria. *British Journal of Nutrition*, *96*(5), 902-905.

Oomah, D., Caspar, F., Macolmson, L.J., & Bellido, A.S. (2011). *Food Research International*, 44:436-441.

Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. *Japan Journal of Nutrition,* 44: 307–315.

Oyetayo, F. L., Akomolafe, S. F., & Osesanmi, T. J. (2020). Effect of dietary inclusion of pumpkin (*Cucurbita pepo* L) seed on nephrotoxicity occasioned by cisplatin in experimental rats. *Journal of Food Biochemistry*, *44*(10), e13439.

Oyeyem, M., Olukole, S., & Esan, O. (2008). Sperm morphological studies of West African Dwarf Bucks treated with pumpkin plant (*Cucurbita pepo*). *International Journal of Morphology*, 26:121-126.

Oyeyemi, W. A., Daramola, O. O. O., Akinola, A. O., Idris, A. O., & Aikpitanyi, I. (2020). Hepatic and reproductive toxicity of sub-chronic exposure to dichlorvos and lead acetate on male Wistar rats. *Asian Pacific Journal of Reproduction*, *9*(6), 283-290.

Özbek, Z. A., & Ergönül, P. G. (2020). Cold pressed pumpkin seed oil. In *Cold pressed oils* (pp. 219-229). Academic Press.

Ozuna, C., & León-Galván, M. F. (2017). Cucurbitaceae seed protein hydrolysates as a potential source of bioactive peptides with functional properties. *BioMedical Research International*, *2017*(1), 2121878.

Pallab, K., Barman, T.K., Pal, T. and Kalita, R. (2013). Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* linn. *Journal of Drug Delivery and Therapeutics*, *3*(4), 33-37.

Panahi, Y., Khalili, N., Sahebi, E., Namazi, S., Karimian, M. S., Majeed, M., & Sahebkar, A. (2017). Antioxidant effects of curcuminoids in patients with type 2 diabetes mellitus: a randomized controlled trial. *Inflammopharmacology*, *25*, 25-31.

Parasuraman, S. (2011). Toxicological screening. *Journal of Pharmacology and Pharmacotherapy*, *2*(2):74-79.

Parimelazhagan, T. (2016). Pharmacological Assays of Plant-Based Natural Products. *Progress in Drug Research*, Vol 71, ISSN: 2297-4555.

Paris, H. S. (2016). Germplasm enhancement of *Cucurbita pepo* (pumpkin, squash, gourd: Cucurbitaceae): progress and challenges. *Euphytica*, *208*, 415-438.

Paris, H. S. (2018). Consumer-oriented exploitation and conservation of genetic resources of pumpkins and squash, Cucurbita. *Israel Journal of Plant Sciences*, *65*(3-4), 202-221.

Paris, H. S., & Lust, T. A. (2019). Origin of the zucchini squash, *Cucurbita pepo* subsp. pepo Zucchini group. In *VI International Symposium on Cucurbits 1294* (1-8).

Patani, A., Balram, D., Yadav, V. K., Lian, K. Y., Patel, A., & Sahoo, D. K. (2023). Harnessing the power of nutritional antioxidants against adrenal hormone imbalance-associated oxidative stress. *Frontiers in Endocrinology*, *14*, 1271521.

Patel, K., Soni, A., & Tripathi, R. (2023). Pumpkin seed: Nutritional composition, health benefits. *Magnesium*, *3*(12), 190-92.

Patil, A., Daniel, H., & Deshmukh, V. (2023). Testosterone: Male Reproductive Health. In *Encyclopaedia of Sexual Psychology and Behaviour* (pp. 1-4). Cham: Springer International Publishing.

Peiretti, P. G., Meineri, G., Gai, F., Longato, E., & Amarowicz, R. (2017). Antioxidative activities and phenolic compounds of pumpkin (*Cucurbita pepo*) seeds and amaranth (*Amaranthus caudatus*) grain extracts. *Natural Product Research, 31*(18), 2178-2182.

Perlman, R. L. (2016). Mouse models of human disease: An evolutionary perspective. *Evolution, Medicine, and Public Health*, *2016*(1), 170-176.

Petkova, Z., Antova, G., Angelova-Romova, M., & Vaseva, (2019). A comparative study on chemical and lipid composition of amaranth seeds with different origin. *Bulgarian Chemical Communications*, *51*, 262-267.

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, *30*, 11-26.

Pickering, A. M., Vojtovich, L., Tower, J., & Davies, K. J. (2013). Oxidative stress adaptation with acute, chronic, and repeated stress. *Free Radical Biology and Medicine*, *55*, 109-118.

Pisoschi, A. M., Pop, A., Iordache, F., Stanca, L., Predoi, G., & Serban, A. I. (2021). Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status. *European Journal of Medicinal Chemistry*, *209*, 112891.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., ... & Bitto, A. (2017). Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, *2017*(1), 8416763.

Pooja, G., Preethi, S., & Ramya, T. (2024). Antioxidant Effect of Pumpkin Flower. *Edited Text Book on Functional Foods for Disease Prevention*, 89.

Pound, P., & Ritskes-Hoitinga, M. (2018). Is it possible to overcome issues of external validity in preclinical animal research? Why most animal models are bound to fail. *Journal of Translational Medicine*, *16*(1), 304.

Preiser, J. C. (2012). Oxidative stress. *Journal of Parenteral and Enteral Nutrition*, *36*(2), 147-154.

Rajasree, R.S., Sibi, P.I., Femi, F., & Helen, W., (2016). Phytochemicals of Cucurbitaceae family – a review. *International Journal of Pharmacognosy and Phytochemistry Research*, 8, 113–123.

Raji, Y., Akinsomisoye, O. S., & Salman, T. M. (2005). Antispermatogenic activity of *Morinda lucida* extract in male rats. *Asian Journal of Andrology*, *7*(4), 405–410.

Rakass, S., Babiker, H., & Oudghiri-Hassani, H. (2018). Comparative evaluation of total phenolic content, total flavonoids content and antioxidants activity in skin and pulp extracts of *Cucurbita maxima*. *Moroccan Journal of Chemistry*, 6:218-226.

Ramachandran, P., Dhiman, A. K., & Attri, S. (2017). Extraction of pectin from ripe pumpkin (Cucurbita moschata Duch ex. Poir) using eco-friendly technique. *Indian Journal of Ecology*, *44*(6), 685-689.

Ratnam, N., Naijibullah, M., & Ibrahim, M. D. (2017). A review on *Cucurbita pepo*. *International Journal of Pharmacognosy and Phytochemical Research*, *9*, 1190-1194.

Recchia, K., Jorge, A. S., Pessôa, L. V. D. F., Botigelli, R. C., Zugaib, V. C., de Souza, A. F., ... & Pieri, N. C. G. (2021). Actions and roles of FSH in germinative cells. *International Journal of Molecular Sciences*, *22*(18), 10110.

Reddy, P. S., Begum, N., Mutha, S., & Bakshi, V. (2016). Beneficial effect of Curcumin in Letrozole induced polycystic ovary syndrome. *Asian Pacific Journal of Reproduction*, *5*(2), 116-122.

Retana-Márquez, S., Juárez-Rojas, L., Ávila-Quintero, A., Rojas-Maya, S., Perera, G., Casillas, F., Betancourt, M., & Gómez-Quiroz, L. (2020). Neuroendocrine disruption is associated to infertility in chronically stressed female rats. *Reproductive Biology, 20*, 474–483.

Rezig, L., Chouaibi, M., Ojeda-Amador, R. M., Gomez-Alonso, S., Salvador, M. D., Fregapane, G., & Hamdi, S. (2018). Cucurbita maxima pumpkin seed oil: From the chemical properties to the different extracting techniques. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *46*(2), 663-669.

Ritchie, C., & Ko, E. Y. (2021). Oxidative stress in the pathophysiology of male infertility. *Andrologia*, *53*(1), e13581.

Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science*, *179*(4073), 588-590.

Rouag, M., Berrouague, S., Djaber, N., Khaldi, T., Boumendjel, M., Taibi, F., ... & Messarah, M. (2020). Pumpkin seed oil alleviates oxidative stress and liver damage induced by sodium nitrate in adult rats: biochemical and histological approach. *African Health Sciences*, *20*(1), 413-425.

Ruch, R. J., Cheng, S. J. & Klaunig, J. E., 1989. Carcinogenesis, Rely. Chim. Acta., 10, 1003-1008.

Rudrapal, M., Khairnar, S. J., Khan, J., Dukhyil, A. B., Ansari, M. A., Alomary, M. N., ... & 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. *Frontiers in pharmacology*, *13*, 806470.

Sá, A. G. A., Moreno, Y. M. F., & Carciofi, B. A. M. (2020). Plant proteins as high-quality nutritional source for human diet. *Trends in Food Science & Technology*, *97*, 170-184.

Saddiq, A. A. N., & Awedh, M. H. et al. (2019). Pumpkin (Cucurbita moschata) against Aspergillus flavus and aflatoxin B1 induced lung cyto-morphological damage in rats. *Pakistan Journal of Pharmaceutical Sciences*, *32*(2), 575-579.

Saeed, S., Hasan, S., & Choudhury, P. (2017). Lead poisoning: A persistent health hazard-general and oral aspects. *Biomedical and Pharmacology Journal*, *10*(1), 439-445.

Saganuwan, S. A. (2017). Toxicity studies of drugs and chemicals in animals: an overview. *Bulgarian Journal of Veterinary Medicine*, *20*(4).

Samplaski, M.K., Loai, Y., Wong, K., Lo, K.C., Grober, E.D., & Jarvi, K.A. (2014). Testosterone use in the male infertility population: Prescribing patterns and effects on semen and hormonal parameters. *Fertility and Sterility, 101*, 64–69.

Sardana, R. K., Chhikara, N., Tanwar, B., & Panghal, A. (2018). Dietary impact on esophageal cancer in humans: a review. *Food & function*, *9*(4), 1967-1977.

Sarikaya, S. B. O., Sisecioglu, M., Cankaya, M., Gulcin, I., & Ozdemir, H. (2015). Inhibition profile of a series of phenolic acids on bovine lactoperoxidase enzyme. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *30*(3), 479-483.

Sarkar, S., & Guha, D. (2008). Effect of ripe fruit pulp extract of *Cucurbita pepo* Linn. in aspirin induced gastric and duodenal ulcer in rats.

Savage, G., & Klunklin, W. (2018). Oxalates are found in many different European and Asian foods-effects of cooking and processing.

Schieber, M., & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current Biology*, *24*(10), R453-R462.

Schreck, J. O. & Loffredo, W. M. (2017). 'Qualitative tests for carbohydrates', in Neidig, H. A. (ed.) Essentials of Practical Biochemistry. Pennsylvania: Chemical Education Resources, Inc.

Segal, T. R., & Giudice, L. C. (2019). Before the beginning: environmental exposures and reproductive and obstetrical outcomes. *Fertility and Sterility*, *112*(4), 613-621.

Selvaraju, V., Baskaran, S., Agarwal, A., & Henkel, R. (2021). Environmental contaminants and male infertility: Effects and mechanisms. *Andrologia*, *53*(1), e13646.

Sen, S., & Chakraborty, R. (2017). Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. *Journal of Traditional and Complementary Medicine*, *7*(2), 234-244.

Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y.S.R. & De, B. (2010). Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospects. *International Journal of Pharmaceutical Sciences Review and Research*, 3: 91-100.

Sener, B., Orhan, I., Ozcelik, B., Kartal, M., Aslan, S., & Ozbilen, G. (2007). Antimicrobial and antiviral activities of two seed oil samples of *Cucurbita pepo* L. and their fatty acid analysis. *Natural Product Communications*, *2*(4), 1934578X0700200409.

Sengupta, P., Dutta, S., & Irez, T. (2024). Oxidants and antioxidants in male reproduction: roles of oxidative and reductive stress. *Journal of Integrated Science and Technology*, *12*(3), 753-753.

Shaban, A., & Sahu, R. P. (2017). Pumpkin seed oil: an alternative medicine. *International Journal of Pharmacognosy and Phytochemical Research*, *9*(2).

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of Functional Foods*, *18*, 820-897.

Shahidi, F., & Zhong, Y. (2010). Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews*, *39*(11), 4067-4079.

Shahin, M. A., Khalil, W. A., Saadeldin, I. M., Swelum, A. A., & El-Harairy, M. A. (2021). Effects of vitamin C, vitamin E, selenium, zinc, or their nanoparticles on camel epididymal spermatozoa stored at 4 C. *Tropical Animal Health and Production*, *53*, 1-9.

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., ... & Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology*, *11*, 694.

Sharma, A. (2017). Male infertility; evidences, risk factors, causes, diagnosis and management in human. *Annal Clinical Lab Research*, *5*(3), 188.

Sharma, N., & Jacob, D. (2001). Inhibition of fertility and functional alteration in the genital organs of male Swiss albino mouse after administration of *Calotropis procera* flower extract. *Pharmaceutical Biology*, *39*(6), 403-407.

Sheel, R., & Nisha, K. (2014). Qualitative phytochemical analysis for isolation of terpenes from *Clerodendron infortunatum* leaves. *ISOR Journal of Applied Chemistry*, *7*(7), 14-18.

Shraim, A. M., Ahmed, T. A., Rahman, M. M., & Hijji, Y. M. (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *Lwt*, *150*, 111932.

Siddiqui, A.A., & Ali, M. (1997). Practical pharmaceutical chemistry, 1st edn, CBS Publishers and distributors, New Delhi. pp. 126, 1317.

Sies, H., Berndt, C., & Jones, D. P. (2017). Oxidative stress. *Annual Review of Biochemistry*, *86*(1), 715-748.

Sihag, S., Pal, A., & Saharan, V. (2022). Antioxidant properties and free radicals scavenging activities of pomegranate (*Punica granatum* L.) peels: An in-vitro study. *Biocatalysis and Agricultural Biotechnology*, *42*, 102368.

Singh, A., & Kumar, V. (2022). Nutritional, phytochemical, and antimicrobial attributes of seeds and kernels of different pumpkin cultivars. *Food Frontiers*, *3*(1), 182-193.

Sinha, A. K. (1972). Colorimetric assay of catalase. *Analytical Biochemistry*, *47*(2), 389-394.

Sinkovic, L., & Kolmanic, A. (2021). Elemental composition and nutritional characteristics of *Cucurbita pepo* subsp. pepo seeds, oil cake and pumpkin oil. *Journal of Elementology*, *26*(1), 97-107.

Sivakumar, P., Brundha, M. P., & Sharma, S. (2020). Awareness of contraception and sexually transmitted diseases among the general population. *Drug Invention Today*, *13*(1), 38.

Skoracka, K., Eder, P., Łykowska-Szuber, L., Dobrowolska, A., & Krela-Kaźmierczak, I. (2020). Diet and nutritional factors in male (In)fertility—underestimated factors. *Journal of Clinical Medicine*, *9*(5), 1400.

Soave, I., Occhiali, T., Assorgi, C., Marci, R., & Caserta, D. (2020). Environmental toxin exposure in polycystic ovary syndrome women and possible ovarian neoplastic repercussion. *Current Medical Research and Opinion*, *36*(4), 693-703.

Soliman, H.A.M., Hamed, M., Lee, J.S., & Sayed, A.H. (2019). Protective effects of a novel pyrazolecarboxamide derivative against lead nitrate induced oxidative stress and DNA damage in *Clarias gariepinus*. *Environmental Pollution*, 247:678–684.

Soni, R., & Bali, M. A. N. O. J. (2019). Evaluation of antioxidant, antimicrobial, and antifungal potential of *Cucurbita pepo* var. Fastigata seed extracts. *Asian Journal of Pharmaceutical and Clinical Research*, *12*(2).

Sorita, G. D., Favaro, S. P., Ambrosi, A., & Di Luccio, M. (2023). Aqueous extraction processing: An innovative and sustainable approach for recovery of unconventional oils. *Trends in Food Science & Technology*, *133*, 99-113.

Srinivasan, M., Padmaja, B., & Nair, S. (2014). GC-MS profiling and in vitro radical scavenging effect of *Adhatoda beddomei*. *Journal of Pharmacognosy and Phytochemistry*, *2*(5), 55-59.

Su, L., Qu, H., Cao, Y., Zhu, J., Zhang, S. Z., Wu, J., & Jiao, Y. Z. (2022). Effect of antioxidants on sperm quality parameters in subfertile men: a systematic review and network meta-analysis of randomized controlled trials. *Advances in Nutrition*, *13*(2), 586-594.

Sudjarwo, S. A., & Sudjarwo, G. W. (2017). Protective effect of curcumin on lead acetate-induced testicular toxicity in Wistar rats. *Research in Pharmaceutical Sciences*, *12*(5), 381-390.

Sun, Q., Li, Y., Shi, L., Hussain, R., Mehmood, K., Tang, Z., & Zhang, H. (2022). Heavy metals induced mitochondrial dysfunction in animals: Molecular mechanism of toxicity. *Toxicology*, *469*, 153136.

Sun, Q., Ma, H., Zhang, J., You, B., Gong, X., Zhou, X., ... & Bai, Y. (2023). A self- sustaining antioxidant strategy for effective treatment of myocardial infarction. *Advanced Science*, *10*(5), 2204999.

Swallah, M. S., Yu, H., Piao, C., Fu, H., Yakubu, Z., & Sossah, F. L. (2021). Synergistic two-way interactions of dietary polyphenols and dietary components on the gut microbial composition: Is there a positive, negative, or neutralizing effect in the prevention and management of metabolic diseases? *Current Protein and Peptide Science*, *22*(4), 313-327.

Syed, Q. A., Akram, M., & Shukat, R. (2019). Nutritional and therapeutic importance of the pumpkin seeds. *Seed*, *21*(2), 15798-15803.

Takahashi, M., Suzuki, K., Kim, H. K., Otsuka, Y., Imaizumi, A., Miyashita, M., & Sakamoto, S. (2014). Effects of curcumin supplementation on exercise-induced oxidative stress in humans. *International Journal of Sports Medicine*, *35*(06), 469-475.

Tan, B. L., Norhaizan, M. E., Liew, W. P. P., & Sulaiman Rahman, H. (2018). Antioxidant and oxidative stress: a mutual interplay in age-related diseases. *Frontiers in Pharmacology*, *9*, 1162.

Taslimi, P., & Gulçin, İ. (2018). Antioxidant and anticholinergic properties of olivetol. *Journal of Food Biochemistry*, *42*(3), e12516.

Tawheed, A. M. T. (2013). *Cucurbita mixta* (pumpkin) seeds: A general overview on their health benefits. *International Journal of Recent Scientific Research*, 4:846-854

Tian, T., Wang, Z., & Zhang, J. (2017). Pathomechanisms of oxidative stress in inflammatory bowel disease and potential antioxidant therapies. *Oxidative Medicine and Cellular Longevity*, *2017*(1), 4535194.

Tietz, N.W. (1995). Clinical Guide to Laboratory Tests (ELISA). 3rd Edition, W.B. Saunders, Co., Philadelphia, 22-23.

Tietz, N.W. (1995). Clinical Guide to Laboratory Tests, third ed. W.B. Saunders, Co., Philadelphia.

Tiwari, P., Kaur, M. and Kaur, H. (2011) Phytochemical Screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia*, 1, 98-106.

Tournaye, H., Krausz, C., & Oates, R. D. (2017). Concepts in diagnosis and therapy for male reproductive impairment. *The Lancet Diabetes & Endocrinology*, *5*(7), 554-564.

Valenzuela, G. M., Soro, A. S., Tauguinas, A. L., Gruszycki, M. R., Cravzov, A. L., Giménez, M. C., & Wirth, A. (2014). Evaluation polyphenol content and antioxidant activity in extracts of Cucurbita spp. *Open Access Library Journal*, *1*(3), 1.

Van Norman, G. A. (2019). Limitations of animal studies for predicting toxicity in humans: part 1: Our current system. *JACC: Basic to Translational Science*, *4*(7), 845-854.

Van Vugt, R. M., Rijken, P. J., Rietveld, A. G., Van Vugt, A. C., & Dijkmans, B. A. (2008). Antioxidant intervention in rheumatoid arthritis: results of an open pilot study. *Clinical Rheumatology*, *27*, 771-775.

Velmurugan, G., & Anand, S. P. (2017). Phytochemical Analysis of *Phyllodium pulchellum* L. Desv. Leaf by UV-Visible Spectroscopy and FTIR. *International Journal of Pharmacy and Biological Sciences*, *7*(3), 61-64.

Velu, G., Palanichamy, V., & Rajan, A. P. (2018). Phytochemical and pharmacological importance of plant secondary metabolites in modern medicine. *Bioorganic Phase in Natural Food: An Overview*, 135-156.

Venkidasamy, B., Subramanian, U., Samynathan, R., Rajakumar, G., Shariati, M. A., Chung, I. M., & Thiruvengadam, M. (2021). Organopesticides and fertility: where does the link lead to? *Environmental Science and Pollution Research*, *28*, 6289-6301.

Vidhya, C. S., Loganathan, M., Bhuvana, S., Wadje, P., & Rajamani, M. (2022). A study on the evaluation of proximate, fatty acid and amino acid profile of two species of pumpkin using advanced techniques. *Uttar Pradesh Journal of Zoology*, *43*(4), 74-83.

Vigeh, M., Smith, D. R., & Hsu, P. C. (2011). How does lead induce male infertility? *Iranian Journal of Reproductive Medicine*, *9*(1), 1.

Vladimir-Knežević, S., Blažeković, B., Štefan, M. B., & Babac, M. (2012). Plant polyphenols as antioxidants influencing the human health. *Phytochemicals as nutraceuticals-Global approaches to their role in nutrition and health*. IntechOpen.

Vyas, N. Y., & Raval, M. A. (2016). Aphrodisiac and spermatogenic potential of alkaloidal fraction of *Hygrophila spinosa* T. Ander in rats. *Journal of Ethnopharmacology*, *194*, 947-953.

Wagner, H., Cheng, J. W., & Ko, E. Y. (2018). Role of Reactive Oxygen Species in male infertility: An updated review of literature. *Arab Journal of Urology*, *16*(1), 35-43.

Wahab, O. A., Princely, A. C., Oluwadamilare, A. A., Oore-oluwapo, D. O., Blessing, A. O., & Alfred, E. F. (2019). Clomiphene citrate ameliorated lead acetate-induced reproductive toxicity in male Wistar rats. *JBRA Assisted Reproduction*, *23*(4), 336.

Wal, A., Singh, M. R., Gupta, A., Rathore, S., Rout, R. R., & Wal, P. (2024). Pumpkin Seeds (Cucurbita spp.) as a Nutraceutical Used in Various Lifestyle Disorders. *The Natural Products Journal*, *14*(1), 118-137.

Walke, G., Gaurkar, S. S., Prasad, R., Lohakare, T., & Wanjari, M. (2023). The impact of oxidative stress on male reproductive function: Exploring the role of antioxidant supplementation. *Cureus*, *15*(7).

Wan, Z. Z., Chen, H. G., Lu, W. Q., Wang, Y. X., & Pan, A. (2019). Metal/metalloid levels in urine and seminal plasma in relation to computer-aided sperm analysis motion parameters. *Chemosphere*, *214*, 791-800.

Wang, H. X., & Ng, T. B. (2003). Isolation of cucurmoschin, a novel antifungal peptide abundant in arginine, glutamate and glycine residues from black pumpkin seeds. *Peptides*, *24*(7), 969-972.

Wang, L., Tang, J., Wang, L., Tan, F., Song, H., Zhou, J., & Li, F. (2021). Oxidative stress in oocyte aging and female reproduction. *Journal of Cellular Physiology*, *236*(12), 7966-7983.

Wang, R., Liang, L., Matsumoto, M., Iwata, K., Umemura, A., & He, F. (2023). Reactive Oxygen Species and NRF2 signaling, friends or foes in cancer? *Biomolecules*, *13*(2), 353.

Wang, Y., Qi, H., Liu, Y., Duan, C., Liu, X., Xia, T., ... & Liu, H. X. (2021). The double-edged roles of ROS in cancer prevention and therapy. *Theranostics*, *11*(10), 4839.

Wangchuk, P. (2018). Therapeutic applications of natural products in herbal medicines, biodiscovery programs, and biomedicine. *Journal of Biologically Active Products from Nature*, *8*(1), 1-20.

Warusavithana, S. T., & Safeena, M. I. S. (2022). Prevalence of secondary metabolites and antioxidants in selected commercially available medicinal plants of Sri Lanka. *Journal of Science-FAS-SEUSL*, *3*(01), 24-41.

Wehner, T. C., Naegele, R. P., Myers, J. R., Narinder, P. S., & Crosby, K. (2020). *Cucurbits*, 32, CABI.

World Health Organization. ICD-11. Available online: https://icd.who.int/en (accessed on 23 December 2021).

Xanthopoulou, M. N., Nomikos, T., Fragopoulou, E., & Antonopoulou, S. (2009). Antioxidant and lipoxygenase inhibitory activities of pumpkin seed extracts. *Food Research International*, *42*(5-6), 641-646.

Yadav, A., Kumari, R., Yadav, A., Mishra, J. P., Srivatva, S., & Prabha, S. (2016). Antioxidants and its functions in human body-A Review. *Research in Environment and Life Science, 9*(11), 1328-1331.

Yadav, M., Jain, S., Tomar, R., Prasad, G. B. K. S. & Yadav, H. (2017). Medicinal and biological potential of pumpkin: an updated review. *Nutrition Research Reviews*, 184–190.

Yadav, N., Pal, A., Sihag, S., & CR, N. (2020). Antioxidant activity profiling of acetonic extract of jamun (*Syzygium cumini* L.) seeds in different in-vitro models. *The Open Food Science Journal*, *12*(1).

Yeshi, K., Crayn, D., Ritmejerytė, E., & Wangchuk, P. (2022). Plant secondary metabolites produced in response to abiotic stresses has potential application in pharmaceutical product development. *Molecules*, *27*(1), 313.

Yoshinari, O., Udani, J., Moriyama, H., Shiojima, Y., & Chien, X. (2015). The efficacy and safety of a proprietary onion-pumpkin extract (OPtain120) on blood pressure: an open-label study. *Functional Foods in Health and Disease*, *5*(6), 224-242.

Zegers-Hochschild, F., Adamson, G. D., Dyer, S., Racowsky, C., De Mouzon, J., Sokol, R., ... & Van Der Poel, S. (2017). The international glossary on infertility and fertility care, 2017. *Human Reproduction*, *32*(9), 1786-1801.

Zhang, C., Hu, Y., Yuan, Y., Guo, J., Li, H., Li, Q., & Liu, S. (2023). Liposome-embedded SOD attenuated DSS-induced ulcerative colitis in mice by ameliorating oxidative stress and intestinal barrier dysfunction. *Food & Function*, *14*(9), 4392-4405.

Zhang, Q. F., Li, Y. W., Liu, Z. H., & Chen, Q. L. (2016). Reproductive toxicity of inorganic mercury exposure in adult zebrafish: Histological damage, oxidative stress, and alterations of sex hormone and gene expression in the hypothalamic-pituitary-gonadal axis. *Aquatic Toxicology*, *177*, 417-424.

Zhang, W., Li, L., Ma, Y., Chen, X., Lan, T., Chen, L., & Zheng, Z. (2022). Structural characterization and hypoglycemic activity of a novel pumpkin peel polysaccharide-chromium (III) complex. *Foods*, *11*(13), 1821.

Zhao, J., Jin, Y., Du, M., Liu, W., Ren, Y., Zhang, C., & Zhang, J. (2017). The effect of dietary grape pomace supplementation on epididymal sperm quality and testicular antioxidant ability in ram lambs. *Theriogenology*, *97*, 50-56.

Zhao, Y., Shao, C., Zhou, H., Yu, L., Bao, Y., Mao, Q., ... & Wan, H. (2023). Salvianolic acid B inhibits atherosclerosis and TNF-α-induced inflammation by regulating NF-κB/NLRP3 signaling pathway. *Phytomedicine*, *119*, 155002.

Zhao, Z. M., Mei, S., Zheng, Q. Y., Wang, J., Yin, Y. R., Zhang, J. J., & Wang, X. Z. (2023). Melatonin or vitamin C attenuates lead acetate-induced testicular oxidative and inflammatory damage in mice by inhibiting oxidative stress mediated NF-κB signaling. *Ecotoxicology and Environmental Safety*, *264*, 115481.

Zhou, J., Chen, L. I., Li, J., Li, H., Hong, Z., Xie, M., ... & Yao, B. (2015). The semen pH affects sperm motility and capacitation. *PloS one*, *10*(7), e0132974.

Zhu, L., Luo, M., Zhang, Y., Fang, F., Li, M., An, F., ... & Zhang, J. (2023). Free radical as a double-edged sword in disease: Deriving strategic opportunities for nanotherapeutics. *Coordination Chemistry Reviews*, *475*, 214875.

Zuhair, R. A., Aminah, A., Sahilah, A. M., & Eqbal, D. (2013). Antioxidant activity and physicochemical properties changes of papaya (*Carica papaya* L. cv. Hongkong) during different ripening stage. *International Food Research Journal*, *20*(4).

APPENDIX I

Procedure for Nutrient Analysis

i) DETERMINATION OF ASH CONTENT

Aim

To determine the ash content of the given food sample.

Principle

By continuous heating, the substance gets charred which can be used for the determination of minerals present.

Apparatus

Porcelain crucible, clay pipe triangle, muffles furnace, desiccators, weighing balance, asbestos sheet.

Procedure

- About 5g of the sample was weighed accurately into a tarred platinum or porcelain crucible (which had previously been heated to about 600°C and cooled).
- The crucible was then placed on a clay pipe triangle and heated in a muffle furnace for about 3-5 hours at 600°C, the crucible was then cooled in a desiccator and weighed.
- To ensure completeness of ashing, heated in a muffle furnace for half an hour, cooled and weighed. This was repeated till two consecutive weights were the same and ash was almost white or greyish white in colour.

Result

The ash content of food sample is ----------------gram.

ii) ESTIMATION OF MOISTURE

Aim

To estimate the amount of moisture present in the given sample.

Materials

Petri plate, weighing balance, desiccator and hot air oven

Procedure

- Take sterilized petri plates, weigh them and note the initial reading (W1).
- Place 5 g of the given sample in the petri plate, weigh them and note the reading (W2)
- Keep it in hot air oven at 100°C for 4 hours.
- After the sample has been dried, take out the petri plate from hot air oven and keep it inside the desiccator until it cools.
- Weigh the petri plate and note the final reading (W3).

Calculation

$$\% \text{ of moisture} = \frac{\text{weight of petri plate} + \text{weight of sample} - \text{final weight of the petri plate}}{\text{Weight of sample}} \times 100$$

iii) ESTIMATION OF FAT

Aim

To estimate the amount of fat present in 100 grams of the given food sample.

Principles

This method involves a partial drying of a weighed sample prior to a Soxhlet extraction. The extracted fat is weighed and the fat content is calculated. It is important that sand be incorporated with the sample before drying. The purpose of the sand is to create a greater surface area, necessary to remove moisture and prevent entrapment of fat.

Apparatus

Thimbles (33 x 80 mm), Soxhlet extraction apparatus, heating mantle, Filter paper, aluminium dishes, Glass beads, hot air oven, Analytical balance, weighing balance

Reagents and Solutions

Petroleum ether

Procedure

- Accurately weigh 3 – 4 grams of sample into a thimble lined with a circle of filter paper and containing a small amount of sand.
- Place thimble and contents in 50 mL beaker and keep it in Soxhlet apparatus for 1.5 hours at 125±1°C for 10 minutes.
- Upon completion of the extraction, separate the unit and pour off the ether (and thimble) from the extractor into a large filter (to collect the thimbles) positioned on a container
- Repeat until most of the ether is removed and the flask has very little ether left. Take apart the Soxhlet unit and place flask on a steam bath to evaporate the remaining petroleum ether.
- Dry flask and its contents in a mechanical convection oven at 100-102°C for time required to obtain constant weight. Cool to room temperature.

Calculations

$$\text{Fat content, percent} = \frac{100\,(B - C)}{A}$$

Where, A = Sample weight

B = Weight of flask after extraction

C = Weight of flask prior to extraction

iv) ESTIMATION OF PROTEIN

Aim

To estimate the amount of protein in the given sample.

Principle

The protein content is determined from the organic Nitrogen content by Kjeldahl method. The various nitrogenous compounds are converted into ammonium sulphate by boiling with concentrated sulphuric acid. The ammonium sulphate formed is decomposed with an alkali (NaOH) and the ammonia liberated is absorbed in excess of standard solution of acid and then back titrated with standard alkali.

Apparatus

Kjeldahl digestion flask - 500 or 800 mL

Kjeldahl distillation apparatus, - same digestion flask fitted with rubber stopper through which passes lower end of efficient rubber bulb or trap to prevent mechanical carryover of NaOH during distillation.

Conical flask, 250 mL

Burette 50 mL.

Reagents

Concentrated Sulphuric acid - special grade 1.84

Sodium Hydroxide solution - 45%. Dissolve 450 gm of Sodium Hydroxide in 1000 mL water

Standard Sulphuric acid solution – 0.1 N

Standard Sodium Hydroxide solution – 0.1 N

Methyl red Indicator solution - Dissolve 0.5 gram methyl red in 100 mL of alcohol

Procedure

Weigh about 1-2 grams of the sample and transfer to a 500 or 800 mL Kjeldahl flask taking care to see that no portion of the sample clings to the neck of the flask. Add 0.7 gram of Mercuric oxide, 15 grams of Potassium Sulphate and 40 mL of concentrated sulphuric acid (Mercuric oxide is added to increase the rate of organic breakdown during acid digestion). Because of environmental/safety concerns over handling and disposal of mercury, copper sulphate can be used. This is important from safety point of view as mercury vapours might escape into the environment during the distillation process. Missouri catalyst tablets known as Kjeldahl tablets (Composition: 48.8% Sodium sulphate & 48.9% Potassium sulphate & 0.3 % copper sulphate can also be used). Add two to three glass beads. Place the flask in an inclined position on the stand in the digestion chamber and digest. Heat the flask gently at low flame until the initial frothing ceases and the mixture boils steadily at a moderate rate. During heating rotate the flask several times. Continue heating for about an hour or more until the colour of the digest is pale blue. If black specs are present after 30 minutes of digestion, wrap the vessel with aluminium foil and keep for 2-3 minutes. By doing this black specs would move down from the walls in the digestion mixture. If the specs are still present, remove the vessel from heat and allow to cool for 10 minutes. Do not modify the heat intensity in the whole process. Alternatively, few drops of water may also be poured down across the side of the flask. Cool the digest and add slowly 200 mL of water. Cool, add a piece of granulated zinc or anti-bump granules and carefully pour down the side of the flask sufficient NaOH solution (450 gram/litre) to make the contents strongly alkaline (about 110 mL) before mixing the acid and alkaline layer.

Connect the flask to a distillation apparatus incorporating an efficient flash head and condenser. To the condenser fit a delivery tube which dips just below the surface of the pipette volume of standard acid contained in a conical flask receiver. (Precaution: The receiving solution must remain below 45°C to prevent loss of ammonia). Mix the contents of the digestion flask and boil until 150 mL have distilled into the receiver. Add 5 drops of methyl red indicator and titrate with 0.1 N NaOH solutions. Carry out a blank titration simultaneously.

1 mL of 0 .1 N (H_2 SO_4) = 0.0014 gram N.

Calculation

Nitrogen content (N) in %= $\frac{\text{(Blank – Titre value) x Normality x 1.4}}{\text{Sample weight}}$

Calculate protein %: N x Conversion factor

(Ideally the protein content of food stuff is calculated by multiplying its total nitrogen content by 6.25, This factor is used whenever the nature of the protein is unknown or when the product to be analyzed is a mixture of different proteins with different factors. However, use of different Nitrogen conversion factors for different matrices may lead to better accuracy of results.)

v) DETERMINATION OF CRUDE FIBER CONTENT

Aim

To determine the fiber content of the given food sample.

Principle

The term "crude fiber" ordinarily meant in agriculture and food analysis. It is the organic residue consisting largely of cellulose that is left after other carbohydrates and proteins have been removed by successive treatment with boiling acids and alkalis. The crude fiber obtained in this way is not cellulose but contains distinct properties of hemicelluloses, and nitrogenous substances. These however are not sufficient to prevent the results from being reasonably accurate and comparable.

Apparatus

Weighing balance, Beaker, Glass rod, Funnel, Muslin cloth, Burner and Wire gauze.

Reagents

0.255 N Sulphuric acid: 0.9 mL of Sulphuric acid in 99.1 mL water.

0.313 N Sodium hydroxide: 0.8 g Sodium hydroxide in 99.2 mL water.

Ether

Alcohol

Procedure

5 grams of the sample was weighed into a 500 mL beaker and 200 mL of boiling 0.255N sulphuric acid was added. The mixture was boiled for 30 minutes keeping the volume constant by adding water at frequent intervals (a glass rod inserted in the beaker helps smooth stirring and boiling). At the end of the period, the mixture was filtered through a muslin cloth and the residue was washed with hot water till free from acid. The mixture was then transferred to a beaker containing 200 mL of boiling 0.313 N sodium hydroxide. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth. The residue was washed with hot water till free from alkali following by washing with some alcohol and ether. It was then transferred into a crucible, dried overnight at 80-100°C and weighed. The crucible was heated in a muffle furnace at 600°C for 2-3 hours. Cooled and weighed again. The difference in the weight represents the weight of the fiber.

Results

100 grams of sample contains ----------- of fiber.

vi) <u>ESTIMATION OF CALCIUM</u>

Aim

To estimate the amount of calcium present in the given sample.

Principle

Calcium is determined by the precipitating it as calcium oxalate and titrating the oxalate solution in dilute sulphuric acid against standard potassium permanganate.

Apparatus

Beaker, burette, pipette flask and standard flask.

Reagents

Ammonium Oxalate (4%): Ammonium oxalate was dissolved in 200 mL of distilled water till it was saturated.

0.01N Oxalic acid: 0.063 gram oxalic acid crystals were weighed and dissolved in 100 mL of distilled water.

0.01N $KMnO_4$: 0.316 gram $KMnO_4$ was dissolved in 100mL of distilled water.

Strong ammonia.

Glacial acetic acid.

2 N sulphuric acid: 5.5 mL of sulphuric acid was dissolved in 94.5 mL of distilled water.

Procedure

2 mL of sample is taken into a 15 mL centrifuge tube. Add 2 mL of distilled water and 1 mL of 4% ammonium oxalate solution and mix thoroughly and leave overnight. Again, the contents are mixed and centrifuged for 5 min at 1500 rpm. The supernatant liquid is poured off and the centrifuge tube drained by inverting the tube for 5 min on a rack (care should be taken not to disturb the precipitate). The mouth of the centrifuge tube is wiped with a piece of filter paper. The precipitate is stirred and the sides of the tubes are washed with 3 mL of dilute ammonia. It is centrifuged again and drained as before. The precipitate is washed once more with dilute ammonia to ensure the complete removal of ammonium oxalate. The precipitate is dissolved in 2 mL of 1N H_2SO_4. The tube is heated by placing in it a boiling water bath for 1 min and titrated against 0.01N $KMnO_4$ solution to a definite pink colour persisting for at least 1 min.

Calculation

1 mL of 0.01N $KMnO_4$ is equivalent to 0.2004 mg of calcium

1 mg of calcium / 100 mL serum= (x-b) x 0.2004 x $\frac{100}{2}$

where,

X=number of mL of 0.01N $KMnO_4$ required to titrate of sample,

b= number of mL of 0.01N $KMnO_4$ required to titrate 2 mL of H_2SO_4 (Blank)

If the normality of $KMnO_4$ is N, the value obtained in the above formula should be multiplied by the factor N/0.01.

vii) ESTIMATION OF IRON

Aim

To estimate the amount of iron present in 100 g of the given food sample.

Principle

The food sample is oxidized with ignition or oxidation. Iron as ferric iron reacts with ammonium thiocyanate or with potassium thiocyanate to given ferric thiocyanate which is red in colour. The colour which is a measure of the concentration is measured calorimetrically.

Apparatus

Volumetric flask, test tubes, Klett pipettes.

Materials

Stock iron solution dissolved 0.0702 gm (70.2mg) of reagent grade crystalline ferrous ammonium sulphate (Mohr's salt) in 100 mL of water.

Working standard: prepared a working standard solution in a 100 mL volumetric flask by adding 100mL of the stock and diluted to the mark with distilled water.

Saturated potassium per sulphate solution stock 7 to 8 gram of reagent grade potassium per sulphate in 100 mL of water in a glass stopped flask. The undisclosed crystals settled to the bottom and com pen sate the loss by decomposition.

3N potassium thiocyanate dissolved 146 g of reagent potassium thiocyanate in water and diluted to 500 mL with water filtered if turbid added 20 mL of pure acetone to improve the keeping quality. Deterioration will be evident from the rapid fermentation of a yellow colour in the blank. Stored in brown bottles.

Procedure

2 g of the sample was ached by ignition when ashing had been com pelted 5mL of hydrochloric acid was added and made up to 100 mL in a volumetric flask. Took different aliquots of the standard solution (1 mL-5 mL) to corresponding to 10-50 grams in a series of the test tube. Added 1mL of 30% H_2So_4 1 mL of potassium per sulphate and 1.5mL of potassium thiocyanate to all the test tubes. This was made up to 10 mL with water. A blank was prepared by adding the reagents except the standard or the unknown solution. Allowed the colour to develop for 20 minutes and the intensity was read at 530-540µ filters in the colorimeter.

Result:

100 grams of sample contains--------------------------------milligram of iron.

viii) ESTIMATION OF PHOSPHOROUS

Aim

To estimate the amount of phosphorous present in the sample.

Principle

When the ash solution is treated with ammonium molybdate, phosphomolybdic acid is formed. Phosphomolybdic acid is reduced by the addition of 1,2,4 amino naphthol sulphonic acid reagent to produce a blue colour which is apparently a mixture of oxides of molybdenum. The intensity of the colour developed is the measure of phosphorous present.

Apparatus

Measuring cylinder, Klett, test tubes, pipette

Materials

Molybdate solution I: Dissolving 25 g of reagent grade ammonium molybdate in about 200 mL of water. In one-litre volumetric flask 500 mL of 10 N sulphuric acid wad added. The molybdate solution was added and was diluted with water to one litre. This solution is stable indefinitely.

Molybdate solution II: Dissolving 25 g of reagent grade ammonium molybdate in about 200 mL of water. In one-litre volumetric flask 300 mL of 10 N sulphuric acid wad added. The molybdate solution was added and was diluted with water to one litre. This solution is stable indefinitely.

ANSA: 195 mL of 15% sodium bisulphite solution was placed in a glass stoppered cylinder. 0.5 g of 1,2,4 ANSA was added followed by 5 mL of 20% sodium sulphite. Put the stopper and shook until the powder was dissolved. If the solution was not complete, added more sodium sulphite, 1mL at a time with shaking but avoided excess. This solution was transferred to a brown glass bottle and stored in the refrigerator.

Stock standard phosphorous solution: 35.1 mg of pure potassium di-hydration phosphate is weighted and dissolved in water. Added 10 mL of 10 N sulphuric acid and made up to 100 mL with water. 5 mL of the solution contains 0.4 mg of phosphorous. Prepared a working standard containing 8 grams of phosphorous in 1mL of the solution by making up to 5 mL of the standard solution to 50 mL with water.

Procedure

- 1 mL of the ash solution was taken in two test tubes.

- 1 mL of molybdate II and 0.4 mL of ANSA were added and the volume was made up to 10 mL with distilled water.
- To 1 mL, 2mL, 3 mL, 4 mL and 5 mL of standard solution, 1 mL of molybdate I solution and 0.4 mL of ANSA were added and made up to 10 mL.
- All the tubes containing 10 mL of the solution were mixed well and allowed to stand for 15 minutes.
- Simultaneous, a blank was prepared by mixing 8.6 mL of water, 1 mL of molybdate II and 0.4 mL of ANSA.
- The colour developed was read in the colorimeter using red filter of wavelength 660 nm.

Result

100 g of the sample contains -------------- mg of phosphorous.

ix) ESTIMATION OF ZINC

Aim

This standard prescribes the atomic absorption spectrophotometric method for the determination of zinc present in the sample.

Apparatus:

Atomic absorption spectrophotometer with air acetylene flame, hallow cathode lamp – 213.8 nm.

Reagents:

Zinc (NIST traceable), Nitric acid (1:499), Concentrated HCL.

Procedure:

Take 100 ml standard flask. Prepare Zn standards (Nist traceable) to 0.05, 0.075, 0.1, 0.125, 0.15&0.2 mg/L in nitric acid (1:499). Prepare a blank solution in 100 mL distilled water. Take 1-2gm of sample in a beaker and digest with 50 mL of conc. HCL till the volume reduced to $3/4^{th}$. Cool and filter and make up to 100 mL with distilled water. Process the blank also in the above manner. Set the AAS as per the specific work instruction. Aspirate the blank, standards and sample solutions. Measure the absorbance of the zinc at 213.8nm.

Calculation:

- Draw the standard calibration graph by plotting the absorbance Vs standard conc. for each Standard.
- Process a standard at detection level (0.01 ppm) as quality control check with every batch of samples and measure its conc. from the Calibration graph.

x) <u>ESTIMATION OF MAGNESIUM</u>

Aim

To determine the concentration of magnesium (Mg) in food samples

Principle

Magnesium is converted to magnesium pyrophosphate which is estimated gravimetrically.

Reagents

10 % Ammonium phosphate solution

10 % sodium citrate solution

0.1 % methyl red indicator

1:4 and 1:10 ammonia: water solution

Procedure

To the calcium free filtrate, 30 mL of concentrated nitric acid (HNO_3) is added and the solution evaporated completely on a boiling water bath. 5 mL of conc. HCl and 100 mL of water are then added and the solution is stirred well with a glass rod. it is followed by the addition of 10 ml of 10% ammonium phosphate solution and 5 ml of 10% sodium citrate solution and stirred the mixture. After adding 2-3 drops of methyl red indicator, the solution is neutralized with the addition of 1:4 diluted ammonia. Strong ammonia (25 mL) is added, stirred vigorously and the mixture left to stand overnight, filtered through Whatman No. 40 or 44 filter paper and washed free from chlorides using 1:10 diluted ammonia. The funnel with the precipitate on the filter paper is dried in an oven. The filter paper is then transferred to a weighed crucible (preheated and cooled) and made into ash slowly over a burner. It is then kept in a muffle furnace at 600°C for 2 hours. The crucible and contents are cooled in desiccators and weighed to get magnesium as its pyrophosphate.

Calculation

$$\text{mg of magnesium/100 g of sample} = \text{Wt. of ash} \times \frac{48.6}{222.6} \times \frac{100}{\text{Volume taken}} \times \frac{100}{\text{Wt. of sample}} \times 1000$$

xi) <u>ESTIMATION OF VITAMIN C BY DYE METHOD</u>

Aim:

To estimate the amount of vitamin C present in the given sample.

Principle:

Vitamin C is a good reducing agent and it reduces the dye 2,6 dichlorophenol indophenol. In this reaction, the ascorbic acid itself is oxidized to dehydro ascorbic acid. In the absence of interfering substances, the capacity of an extract of the sample to reduce a standard solution of the dye as determined by titration is directly proportional to the vitamin C content. Oxalic acid is not only used to reduce the pH of the extracting medium, there by establishing vitamin C but also form complexes with metals e.g. copper thereby preventing the catalytic oxidation of vitamin.

Apparatus:

Centrifuge, centrifuge tubes, mortar and pestle, beakers, pipette, 100 mL standard flask, burette and funnel.

Reagents:

1. 2, 6- Dichlorophenol indophenol dye: Dissolved 42 mg of bicarbonate and 52 mg of 2,6 dichloro phenol indophenol in about 50 mL of water. This was diluted to 200 mL, filtered, and stored in the refrigerator.
2. 4% oxalic acid: Dissolved 4 g of oxalic acid in 100 mL distilled water.
3. Standard Ascorbic acid: Dissolved 100mg of pure ascorbic acid crystals in 100 mL of 4% oxalic acid.

Standardization of the dye:

Pipetted out 10 mL of the standard ascorbic acid solution into a beaker and then added 25 mL of 4% oxalic acid. From this solution pipetted out 5 mL into a conical flask and placed in an ice container and the contents were titrated against the dye in the burette. The end point was the appearance of pink colour which persisted for 30 seconds. The amount of dye consumed is equivalent to the amount of ascorbic acid present.

Procedure:

5 g of the sample was weighed and soaked in 4% oxalic acid for 10 minutes. This was then ground in a mortar and transferred to centrifuge tubes adding more oxalic acid. The solution was centrifuged and the supernatant clear liquid was transferred to a 100 mL standard flask and repeated the extraction with oxalic acid three to four times. All the supernatants were collected in the same standard flask and this was finally made up to the mark with acid. The dye was taken in a microburette and titrated against 5 mL of the extract in a beaker. The end point was the appearance of pink colour which persisted for 30 sec. The titration was repeated till concordant values were obtained.

Determination of Dye Factor

Standard Ascorbic acid Vs 2,6 Dichlorophenol indophenol

Volume of ascorbic acid (mL)	Burette reading (mL)		Volume of the dye (mL)	Indicator
	Initial	Final		

1mL of standard ascorbic acid solution contains 1mg of ascorbic acid

10 mL of standard ascorbic acid solution contains 10 mg of ascorbic acid

10 mL of the standard ascorbic acid solution was made up to 35 mL

35 mL of the solution contains 10 mL of ascorbic acid

∴ 5.0 mL of the solution contains = 10 x 5 =1.428 mg of ascorbic acid

…………… mL of the dye is reduced by 1.428 mg of ascorbic acid

∴1 mL of the dye is reduced by ……… mg of ascorbic acid.

Dye factor

Extract Vs Dye

Volume of ascorbic acid (mL)	Burette Reading (mL)		Volume of the dye (mL)	Indicator
	Initial	Final		

1.0 mL of the dye is reduced by …………. mg of ascorbic acid

∴ ……. mL of the dye is reduced by …………. mg of ascorbic acid

5 mL of the extract contains =

∴ 50mL of the extract contains = ………………

50 mL extract made from ……….. g of food sample

∴ 3g of contains = …………. mg of ascorbic acid

Therefore 100 g contains = …………. mg of ascorbic acid

RESULT:

APPENDIX II

Quantitative Phytochemical Estimation

i) <u>TOTAL PHENOLIC CONTENT</u>

Preparation of standard solution

3 mg of Gallic acid dissolved in 3 mL of distilled water. Dilutions of this solution with distilled water were prepared to give the concentration of 25, 50, 75, 100, 200 and 250 μg/mL.

Preparation of test sample

Stock solutions of samples were prepared by dissolving 10 mg of dried methanolic extract in 10 ml of methanol to give concentration of 1mg/mL. Then prepares sample concentrations of 25, 50, 75, 100, 200 and 250 μg/mL.

Procedure

The powdered extract was dissolved in methanol to obtain a concentration of 1 mg/mL. The 100 μL of this solution was taken in to 25 mL volumetric flask, to which 10 mL of water and 1.5 mL of Folin-Ciocalteu's reagent were added. The mixture was then kept for 5 min and to it 4 mL of 20% w/v sodium carbonate solution was added. The volume was made up to 25 mL with double distilled water. The mixture was kept for 30 minutes until blue colour develops. The samples were then observed at 765 nm. The % of total phenolic was calculated from calibration curve of Gallic acid plotted by using similar procedure.

ii) <u>TOTAL FLAVONOID CONTENT</u>

Principle

Formation of acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols in addition with aluminium chloride. Aluminium chloride also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids.

Procedure

Extracts diluted with methanol to come under the linearity range (2 mL, 0.3 mg/mL) were individually mixed with 0.1 mL of 10% w/v aluminium chloride hexahydrate, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. After 40 minutes of incubation period, at room temperature, the absorbance of the reaction mixture was determined spectrophotometrically at 415 nm. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water. Sample and sample blank were prepared and their absorbance was measured at 415 nm with a double beam UV/Visible spectrophotometer, SHIMADZU UV 1800 (Japan). All prepared solutions were filtered through Whatman filter paper (no 41) before measuring. To perform the calculations of total flavonoid content in the plant extract, a standard calibration curve is needed which is obtained from a series of different concentrations of a standard reference flavonoid (like Quercetin, Rutin, Naringenin).

iii) TOTAL ALKALOID CONTENT

A part of extract residue was dissolved in 2 N HCl and then filtered. 1 mL of this solution was transferred to separatory funnel and washed with 10 mL chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 mL of BCG solution and 5 mL of phosphate buffer were added to this solution. The mixture was shaken and complex extracted with 1-, 2-, 3- and 4-mL chloroform by vigorously shaking, the extract was then collected in a 10 mL volumetric flask and diluted with chloroform.

Preparation of standard curve

Accurately measured aliquots (0.4, 0.6, 0.8, 1 and 1.2 mL) of Atropine standard solution was transferred to different separatory funnels. Then 5 mL of pH 4.7 phosphate buffer and 5 mL of BCG solution was taken and the mixture was shaken with extract with 1, 2, 3, and 4 mL of chloroform. The extracts were then collected in 10 mL volumetric flask and then diluted to adjust solution with chloroform. The absorbance of the complex in chloroform was measured at spectrum of 470 nm in UV-Spectrophotometer (SHIMADZU UV-1800) against the blank prepared as above but without Atropine.

APPENDIX III

Authentication Certificate for *Cucurbita pepo* L. Seeds

भारतसरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

दक्षिणीक्षेत्रीयकेन्द्र / Southern Regional Centre
टी.एन.ए.यूकैम्पस/ T.N.A.U. Campus
लाउलीरोड/ Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003

टैलीफोन / Phone: 0422-2432788, 2432123, 2432487
टैलीफैक्स/ Telefax: 0422- 2432835
ई-मेल/E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2021/Tech/२८२

दिनांक/Date: 04.03.2021

पौधा प्रमाणीकरण प्रमाणपत्र /PLANT AUTHENTICATION CERTIFICATE

The plant specimen which has been brought by you for authentication is identified as *Cucurbita pepo* L. – CUCURBITACEAE. The identified specimen is returned herewith for preservation in your College/ Department/ Institution Herbarium.

5/3

डॉ.एम. यू. शरीफ़/Dr. M.U. Sharief
वैज्ञानिक 'ई' एवं कार्यालयाध्यक्ष/
Scientist 'E' &Head of Office

वैज्ञानिक 'ई' एवं कार्यालयाध्यक्ष
Scientist 'E' & Head of Office
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
दक्षिणी क्षेत्रीय केन्द्र
Southern Regional Centre
कोयम्बत्तूर / Coimbatore - 641 003

सेवा में / To

Ms. Amrutha B. Nair
Ph.D. Research Scholar
Department of Food Science and Nutrition
Avinashilingam Institute for Home Science and Higher Education for Women
Coimbatore – 641 043, Tamil Nadu

APPENDIX IV

Animal Ethical Clearance Certificate

Avinashilingam Institute for Home Science and Higher Education for Women
(Deemed to be University under Category 'A' by MHRD, Estd. u/s 3 of UGC Act, 1956)
Re-accredited with 'A+' grade by NAAC, Recognised by UGC under Section 12 B
Coimbatore – 641 043, Tamil Nadu, India

Certificate

This is to certify that the project entitled **"Antioxidant Potential of *Curcurbita pepo* L. (Pumpkin) Seed Extract in the Treatment of Stress Induced Infertility in Male Wistar Rats"** has been approved by the IAEC having IAEC approval No **AIW:IAEC.2020:FSN:02**

Authorized by	Name	Signature	Date
Chairman:	Dr. S. KOWSALYA	S. Kowsalya	19/02/2020
Member Secretary:	Dr. R. NIRMALADEVI	[signature]	19/02/2020
Main Nominee of CPCSEA:	Dr. C. GUNASEKARAN	[signature]	19.02.2020

Avinashilingam Institute for HomeScience and Higher Education for Women
(Deemed to be University Estd. u/s 3 of UGC Act 1956, Category 'A' by MHRD
Re-accredited with A++ Grade by NAAC. CGPA 3.65/4, Category I by UGC
Coimbatore - 641 043, Tamil Nadu, India

Appendix L2

(Item No 5 of Check List) Details of Research Publications

S.No	Article	Journal	Other Details Vol/No/Page No/ Year	Published in UGC-CARE / Scopus Indexed/ Web of Science
1	Ameliorative effect of Cucurbita pepo L. Seed extract against lead induced effects on the serum and testicular oxidative status: An in-vivo validation	International Journal of Pharmaceutical Sciences and Drug Research	Vol. 15 Issue: 2 Page: 214-221 Year 2023	UGC CARE I
2	An Exploration of the nutritional, Phytochemical and In vitro Antioxidant Profile of Cucurbita Pepo L. (Pumpkin) Seeds	The Indian Journal of Home Science	Vol: 36 Issue: 1 Page: 298-306 Year: 2024	UGC CARE I

*Proof of list of Journals from Internet to be attached along with copies of reprints.

Scholar : [signature] 09/05/24

Supervisor : [signature] 9/5/24

[signature] 09/5/24
Checked By:

HoD/Dean of Respective School

The scholar Miss. Amrutha B. Nair (17PHFNF001) has published her research articles in the following journals:

1. International Journal of Pharmaceutical Sciences and Drug Research - indexed and active in UGC Care List Group I from June 2019 to present and
2. The Indian Journal of Home Science - indexed and active in UGC Care List Group I from July 2020 to present.

This may be considered.

[signature] 09.05.2024.

Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

journal home page : http://ijpsdr.com/index.php/ijpsdr

Research Article

Ameliorative Effect of *Cucurbita pepo* L. Seed Extract against Lead Induced Effects on the Serum and Testicular Oxidative Status: An *In-vivo* Validation

Amrutha B. Nair*, Raajeswari PA

Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India

ARTICLE INFO

Article history:

Received: 08 February, 2023

Revised: 12 March, 2023

Accepted: 16 March, 2023

Published: 30 March, 2023

Keywords:

Lead toxicity, Oxidative stress, *Cucurbita pepo* seeds, Antioxidants, Drug synthesis

DOI:

10.25004/IJPSDR.2023.150214

ABSTRACT

Lead (Pb) exposure is considered to be an alarming public health problem since evidence has mounted regarding its adverse impact on health and reproduction. The current research was intended to evaluate the ameliorative effects of *Cucurbita pepo* L. (pumpkin) against lead toxicity-induced oxidative stress in experimental rats. Before the animal study, a preliminary phytochemical screening was done to detect the presence of various phytoconstituents in the seed extract. Thirty adult male wistar rats were selected and randomly divided into five groups for the experimental study. Group 1 served as the control while groups 2, 3, 4 and 5 were treated with 30 mg/kg lead acetate, 1000 mg/kg seed extract alone (high dose), 30 mg/kg lead acetate and 100 mg/kg seed extract (low dose) and 30 mg/kg lead acetate and 1000 mg/kg seed extract (high dose), respectively. Enzymatic antioxidant concentrations in serum and testis were found to check the response of antioxidants to lead toxicity. In lead treated group, increased oxidative stress was observed which was indicated by a significant ($p<0.001$) decline in the concentration of the enzymatic antioxidants (SOD, CAT and GPx) coupled with a significant increase in lipid peroxidation marked by high MDA level. Interestingly, at high dosage of *C. pepo* seed extract, enzymatic antioxidant concentration was comparable to control and significantly higher compared to other experimental groups. The study revealed that even in low dosage, *C. pepo* administration could improve the antioxidant status in the lead-treated group. This investigation recommends *C. pepo* seeds as a potent natural product promising strong protection against lead toxicity-induced oxidative stress which could be pharmacologically explored for drug synthesis.

Introduction

Free radicals are natural unfavorable byproducts in our body created by various biological processes such as respiration, digestion, metabolism of alcohol and drugs, and the lipids conversion into energy. They can initiate multiple reactions in the body, including damage to cell membranes, block the functioning of key enzymes, hamper vital cellular processes, impair normal cell division, DNA, and obstruct energy generation.[1] If the natural antioxidant mechanism in our bodies that normally eliminates free radicals cannot cope properly, it may lead to oxidative stress, which is the underlying cause of a wide spectrum of diseases and disorders. The occurrence of oxidative stress can be accelerated by modern lifestyle, which includes an unhealthy diet, sedentary work, and exposure to a variety of toxic chemical substances, including pesticides, heavy metals, food additives, and environmental pollutants.[2]

Lead (Pb) is a heavy metal and a well-known environmental contaminant that poses a significant threat to mankind. The extensive use of lead in metal products, paints, batteries, medical appliances, and varnishes makes human exposure inevitable.[3] Lead has received considerable attention due to its bioaccumulative,

***Corresponding Author:** Ms. Amrutha B. Nair

Address: Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India

Email ✉: bnairamrutha93@gmail.com

Tel.: +91 8590515433

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

nonbiodegradable, and immutable characteristics. Lead poisoning causes unusual physiological, biochemical, and behavioral changes in the circulatory, metabolic, cardiovascular, neurological, excretory, and reproductive systems.[4] Conversely, little is known about the exact mechanism of action through which lead affects male fertility. Despite the fact that different theories have been put forth to elucidate the pathogenesis of lead toxicity, several studies declare that lead may partially intercede oxidative stress to repress male reproduction either by producing reactive oxygen species (ROS) or by affecting antioxidant enzymes, or sometimes both.[5]

The body possesses several enzymatic mechanisms to reduce radically generated damage and safeguard against the excess production of free radicals. Antioxidants play a crucial part in these defense mechanisms. A delicate balance between oxidants and antioxidants helps healthy organisms defend against reactive oxygen species' harmful effects. Therefore, the steady formation of free radicals must be balanced or neutralized by a constant intake of antioxidants. Antioxidants, irrespective of enzymatic or non-enzymatic, are chemicals that prevent the production of free radicals and strive to scavenge, neutralize, or repair the damage caused by them.[6] It is widely accepted that antioxidant supplementation against lead-induced oxidative stress is an established method to alleviate lead toxicity.[7] According to reports, antioxidant enzymes are essential for shielding cells from the oxidative stress brought on by lead exposure.[8] Natural antioxidants are available in various substances categorized as secondary plant metabolites, such as polyphenols (phenolic acids, flavonoids) and terpenoids (carotenoids) and consuming foods rich in these substances in significant amounts appears to play a crucial role in disease prevention.[9]

Since the dawn of human civilization, plants have served as a local repository of raw resources to isolate or produce several traditional medications.[10] Pumpkins are one of these plants, and because of its components and health-promoting phytochemicals, it is utilized to treat various diseases and disorders.[11] Pumpkins belong to the Cucurbitaceae family and are comprised of many edible species. They are mostly found in China, India, Pakistan, Argentina, Yugoslavia, America, Mexico and Brazil.[12] The macro-and micronutrient composition of pumpkin seeds-which includes proteins, phytosterols, lignans, triterpenes, polyunsaturated fatty acids, phenolic compounds, tocopherol, carotenoids, and minerals are all thought to be responsible for their health benefits.[13] Pumpkin seeds (*Cucurbita pepo* L.) are also rich sources of unsaturated fatty acids, fibers and antioxidants.[14]

Mechanisms of action must certainly be identified *in-vitro*; nonetheless, it is also necessary to demonstrate the efficacy of these same ingredients *in-vivo*.[15] In view of the scanty studies on the protective effect of *C. pepo* seeds against lead toxicity induced oxidative stress, the present study was designed to investigate on the efficacy of *C. pepo* seed extract in mitigating lead induced effects on the serum and testicular oxidative status in experimental rats and discover whether co-treatment with *C. pepo* seed extract could reverse these adverse physiological changes. The study also hopes it could lay a scientific foundation for exploring on multiple therapeutic applications of *C. pepo* seeds particularly in drug discovery.

Materials and Methods

Sample Collection and Identification

The *C. pepo* seeds were collected directly from farmers at Tudiyalur, locale in Coimbatore, Tamilnadu, India. The sample was confirmed and authenticated (voucher specimen number BSI/SRC/5/23/2021/Tech/282) at Botanical Survey of India, Southern Regional Centre, Tamilnadu Agricultural University, Coimbatore.

Preparation of *C. pepo* Seed Extract

The seeds were washed, cleaned to remove any debris followed by dehulling and shade drying for a week. Approximately 100 g of dried *C. pepo* seeds were coarsely powdered using mortar and pestle. Total of 50 gm of powder was subjected to extraction in 300 mL distilled water and kept in an incubator for 48 hours at 37°C. The slurry was intermittently stirred for 2 hours and kept overnight. The mixture was collected and filtered and the filtrate was dried in room temperature. Residue was collected and suspended in water at a fixed dose and used for experiments.

Phytochemical Screening of the Extract

Phytochemical screening of the extracts was carried out according to the methods of Trease[16] and Evans (1989) to discover the presence or absence of phytoconstituents such as carbohydrates, protein, flavonoids, alkaloids, tannins, steroids, terpenoids, saponins and quinones.

Dose Selection of the *C. pepo* Seed Extract

The dosage of *C. pepo* seed extract was fixed based on the literature available. Acute toxicity study conducted by Malgwi[17] *et al*., 2014 stated that aqueous *C. pepo* L. seed extract was safe up to 5000 mg/kg body weight. Hence the dosages 100 and 1000 mg/kg were selected for the current study.

Chemicals

Lead acetate trihydrate was obtained from Oxford Lab. Co., India. Lead acetate was dissolved in distilled water at concentration of 30 mg/kg body weight of 1% solution.

Animal Procurement

30 adult male wistar rats with mean weight of 120 ± 35g were procured from Biogen Animal Facility, Bangalore (971/PO/RcBiBt/S/06/CPCSEA).The animals were kept

for one week acclimatization in the experimental room having the temperature 28 ± 2°C, tolerable humidity conditions with 12:12 light and dark photoperiod. Animals were housed in clean cages bedded with sterile paddy husk. They had access to standard rat pellet diet and water *ad libitum*.[18] The procedures were carried out after receiving approval from the Institutional Animal Ethical Committee of the Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore (Ethical Approval No: AIW:IAEC.2020:FSN:02).

Experimental Design

After one week of acclimatization period, 30 male wistar rats were randomly allocated into 5 groups containing 6 rats each.
Group 1: Control (Normal saline)
Group 2: Lead acetate (LA) -30 mg/kg
Group 3: *Pepo* seed extract high dosage (PSE HD) -1000 mg/kg
Group 4: LA + *Pepo* seed extract low dosage (LA+PSE LD) -100 mg/kg
Group 5: LA + PSE HD -1000 mg/kg
Rats were treated by oral gavage once daily for 45 days excluding the acclimatization period. After giving last dosage, the animals were sacrificed within 24 hours by the mode of decapitation.

Serum Preparation

After the treatment period, the rats were anaesthetized using light ether anaesthesia in lethal chamber. Blood samples were collected from each rat through retro orbitral sinus puncture. Sample was allowed to clot at 37°C and centrifuged at 3000 rpm for 15 minutes. The serum (supernatant) was collected and stored at −20°C for antioxidant analyses.[19] The serum concentration of enzymatic antioxidants such as Superoxide dismutases (SOD) and glutathione peroxidase (GPx) was measured by standard biochemical methods.

Preparation of the Tissue Homogenate

The organ associated with reproduction which includes testes was excised out and cleaned thoroughly in phosphate buffer. Specimens from testis were homogenized in 1.17% potassium chloride (KCl) included ice cold phosphate buffer with molarity 0.1 and pH 7.4 was used in the preparation of 10% homogenate solution of each tissue sample. For estimating the lipid peroxidation levels, a part of this homogenate was used and the remaining solution was centrifuged at 10000 rpm for 10 minutes at a temperature of 4°C.[19] The supernatant was utilized for carrying out the antioxidant enzymatic assays consisting of SOD, catalase (CAT), GPx and lipid peroxidation (MDA) assays.

Determination of (SOD)

Testis homogenate (0.5 mL) was diluted with equal deionized water. 0.25 mL ethanol and 0.15 mL of chilled chloroform were added followed by shaking this mixture for 1-minute and centrifuging at 2000 rpm. The SOD enzyme in the supernatant was estimated. 1.5 mL of buffer was added to 0.5 mL of the supernatant. The reaction was initiated by adding 0.4 mL epinephrine and change in optical density per minute was detected at 480 nm using a double beam UV-vis spectrophotometer (UV 1700, Szhimadzhu). SOD activity was expressed as U/mg. Change in optical density per minute at 50% inhibition to adrenochrome transition by the enzyme was considered as one enzyme unit.[20]

Determination of CAT

To testis homogenate (0.1 mL), 1.0 mL of phosphate buffer and hydrogen peroxide were added. The reaction was arrested by adding 0.2 mL dichromate acetic acid reagent. Standard hydrogen peroxide between the ranges of 4 to 20 µL was treated in similar way. The tubes were kept in a boiling water bath for 10 minutes. The developed green colour was read at 570 nm in a double beam UV-vis spectrophotometer. Catalase activity was expressed as U/mg.[21]

Determination of GPx

The glutathione peroxidase activity was calculated according to the method given by Rotruck[22] *et al.*, 1973. EDTA (0.2 mL each), sodium azide, reduced glutathione, H_2O_2; 0.4 mL of buffer and 0.1 mL of enzyme (testis homogenate) were mixed and incubated at 37°C for 10 minutes. The reaction was arrested by adding 0.5 mL of TCA and the tubes were centrifuged. To 0.5 mL of supernatant, 3 mL of sodium hydrogen phosphate and 1-mL of DTNB were added and the developed color was read at 412 nm immediately in a double beam UV-vis spectrophotometer. Peroxidase activity in serum was expressed as µg/mg.

Determination of Lipid Peroxidation

In 1 mL of testis homogenate was mixed with 0.2 mL 4% (w/v) sodium dodecyl sulfate, 1.5 mL 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 15 mL of 0.8% thiobarbituric acid (TBA, pH 7.4). The mixture was kept in a hot water bath at 85°C for 1-hour. The developed pink colour was read against a reagent blank at 532 nm following centrifugation at 1200 g for 10 minutes. The concentration was expressed as n moles of malondialdehyde (MDA) per mg of protein using 1,1,3,3,-tetra-ethoxypropane as the standard.[23]

Statistical Analysis

Statistical analysis was carried out using one-way Analysis of Variance (ANOVA) followed by Dunnett's test for comparison between groups. *p*-values of <0.05, <0.01 and <0.001 were considered to be significant, very significant, and highly significant, respectively.

Results

Table 1 illustrates the preliminary phytochemical screening of *C. pepo* L. seeds aqueous extract which revealed appreciable levels of alkaloids and tannins, moderate levels of carbohydrates and steroids and remaining compounds in trace levels.

Fig 1 and 2 show the effect of *C. pepo* seeds on serum concentration of SOD and GPx respectively. The LA group caused a significant ($p<0.001$) drop in SOD and GPx levels in comparison with the control group whereas LA+ PSE LD group had a slight improvement when compared to LA group. From the graphical representation, it is clear that co-administration of *C. pepo* seed extract at high dose with lead acetate (LA+PSE HD) had a considerable significant improvement in SOD and GPx in comparison with LA and LA+ PSE LD group. Surprisingly, PSE HD group was found to have both SOD and GPx levels almost comparable to that of control. PSE HD group was found to have a highly significant improvement when compared to other treated groups indicating potent antioxidant capacity.

Table 2 reveals the effect of *C. pepo* seeds extract on testis concentration of SOD, CAT, GPx and MDA in experimental rats. LA group was found to have a highly significant decrease in SOD (0.525 ± 0.0086 to 0.4 ± 0.0115 U/mg protein), catalase (28.52 ± 0.987 to 19.30 ± 0.216 µmole/mg protein), considerably significant ($p<0.01$) decrease in GPx (4.605 ± 0.164 to 3.505 ± 0.1588 µg/mg protein) and a highly significant ($p<0.001$) increase in lipid peroxidation marked by high MDA level (13.35 ± 0.214 to 20.18 ± 0.736) when compared to control group. Even though LA+ PSE LD group exhibited a considerable significant decrease in SOD, CAT and GPx when compared to control, there was a slight improvement even at low dosage in comparison with LA group. Also, LA + PSE LD exhibited a significant decrease in MDA compared to LA group. Compared to other lead acetate treated groups, LA+ PSE HD showed highly significant improvement in SOD, CAT and GPx and also exhibited a highly significant decrease in MDA. In conclusion, treatment with *C. pepo* seed extract at 1000 mg/kg body weight (high dose) but not at 100mg/kg dose (low dose) markedly improved the antioxidant status and also reduced testis MDA which was significantly different from the lead acetate group (LA). Interestingly, PSE HD disclosed a notable enhancement in SOD than control itself and a highly significant improvement in CAT and GPx when compared to other treated groups. In addition, PSE HD group was found to have a remarkable reduction in lipid peroxidation marked by decreased MDA level compared to other treated groups.

Table 1: Phytochemical screening of *C. pepo* seed aqueous extract

Sl. no	*Phytochemicals*	*Inference*
1	Carbohydrates	++
2	Protein	+
3	Flavonoids	+
4	Alkaloids	+++
5	Tannins	+++
6	Steroids	++
7	Terpenoids	+
8	Saponins	+
9	Quinones	+

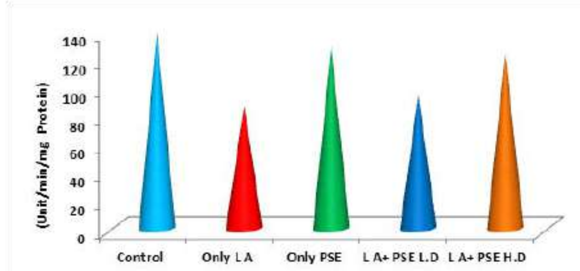

Fig. 1: Serum Concentration of SOD

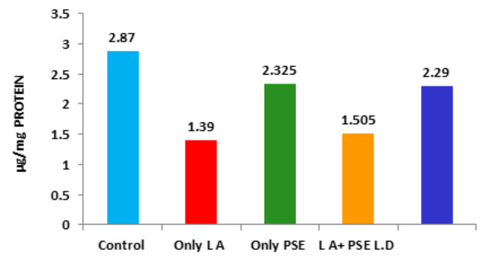

Fig. 2: Serum Concentration of Glutathione Peroxidase (GPx)

Discussions

Many studies have reported that the type of solvent used has a key role in determining the activity of the extract.[24] This is due to the disparity in the relative solubility of different phytochemicals in the solvents with different polarities. The systemic phytochemical analysis of plant extracts is a significant strategy to discover the new phytocompounds of therapeutic value.[25] The preliminary phytochemical screening of the aqueous extract of *C. pepo* seeds was carried out. Phytochemical analysis revealed that (Table 1) the *C. pepo* seeds had appreciable levels of alkaloids and tannins and moderate levels of carbohydrates and steroids whereas the other phytocompounds were present in trace amounts. The result of the current investigation was in accordance with the findings of Malgwi[17] *et al.*, (2014) and Rawat[26] and Garg (2021) who reported that the above phytochemicals were present in aqueous extract of the *C. pepo* seeds.

Lead acetate may induce oxidative stress characterized by excessive free radical generation which leads to modification in enzymatic antioxidant system and disruption of membrane structure.[27] Significant antioxidant enzymes together form a mutually beneficial defence system against free radicals. Therefore,

Table 2: Enzymatic Antioxidant Concentration in Testis

Parameter	*Control*	*Only LA*	*Only PSE H.D*	*LA+ PSE L.D*	*LA+ PSE H.D*
SOD (unit/min/mg protein)	0.525 ± 0.0086	0.400 ± 0.0115***	0.545 ± 0.0086^{ns}	0.45 ± 0.0058**	0.515 ± 0.0144^{ns}
CAT (µmole of H_2O_2 consumed/ Min/mg protein)	28.52 ± 0.987	19.30 ± 0.216***	23.92 ± 0.56**	19.85 ± 0.548***	21.11 ± 0.456***
GPx (µg/mg protein)	4.605 ± 0.164	3.505 ± 0.1588**	4.35 ± 0.133^{ns}	3.59 ± 0.173**	4.27 ± 0.167^{ns}
LPO (nmol of MDA/mg protein)	13.35 ± 0.214	20.18 ± 0.736***	12.52 ± 0.424^{ns}	18.62 ± 0.416***	13.17 ± 0.225^{ns}

*Statistical comparison: (***P<0.001, **P<0.01, *P<0.05) Control group was compared with standard group-2. Treated groups 3, 4 and 5 were compared with group 1.*

oxidative stress has been assessed using these enzymatic antioxidants activities.[28-30] In the study undertaken, protective role of *C. pepo* seed extract on the lead induced effects in the serum and testicular oxidative status has been investigated through *in-vivo* method. The biochemical pathways underlying the lead toxicity were investigated by monitoring the levels of MDA and the activity of primary antioxidant enzymes such SOD, CAT, and GPx.

Determination of serum levels of SOD and GPx indicated that lead acetate treated group had a highly significant reduction in both the enzymatic antioxidants when compared to that of control. This finding is in agreement with the results of El-Sherbini[31] *et al.*, 2017 who reported that the lead acetate-induced depletion of antioxidants is confirmed by the observed decrease in serum total antioxidants and circulating antioxidants. Lead acetate with seed extract at low dosage was found to have a considerable increase in SOD and GPx compared to lead acetate treated group whereas lead acetate with seed extract at high dosage had a highly significant improvement. *C. pepo* seed extract at high dosage when treated alone indicated SOD level almost same as control. This group also indicated a highly significant increase in SOD and GPx levels in comparison with other treated groups. The antioxidant activity exhibited by *C. pepo* seed extract in the reversal effect on the lead induced damage couldn't be overlooked. Hence, it can be deduced that *C. pepo* seed can exert potent antioxidant activity at higher doses and can reverse the lead induced oxidative damage.

Testis concentrations of SOD, CAT, GPx and MDA were estimated. The results revealed that lead acetate treated alone had a highly significant decrease in SOD, catalase and GPx and a highly significant increase in lipid peroxidation marked by high MDA level when compared to control group. A similar result was reported by Rania[32] and Heba, 2014 whose findings confirmed that SOD and CAT activities were significantly reduced (p< 0.001) in lead acetate treated rats. Rats exposed to lead experienced a substantial decrease in all antioxidant enzymes, including SOD and CAT, in the mitochondrial and post-mitochondrial fraction of the testis.[33] In the testes of lead-exposed rats, SOD and CAT activity levels were found to be significantly decreased.[34] Even though lead acetate with seed extract at low dosage had a highly significant reduction in the testis SOD, CAT and GPx when compared to that of control, MDA was significantly lower than that of lead acetate treated group. Co-administration of *C. pepo* seed extract and lead acetate led to an increase in the level of SOD, CAT and GPx when compared to its level in rats treated only with lead acetate. The rats treated with *C. pepo* seed extract high dosage alone had a remarkable increase in all the antioxidant enzyme concentrations when compared to other treated groups.

The present investigation revealed that MDA levels in the testis of the rats treated with lead acetate were substantially higher than the control group. This indicates that lipid peroxidation increased oxidative stress in the lead acetate-treated rats. It is understood that two processes, including enhanced ROS production and direct depletion of antioxidant reserves, might result in lead acetate-induced oxidative stress and tissue damage.[35] Lead exposure may generate intense lipid peroxidation that may disrupt the mitochondrial and cytoplasmic membranes, leading to more severe oxidative damage in the tissues and, as a result, releasing lipid hydroperoxides into circulation that represent the induction of oxidative stress.[30] At least in part, lead-induced testicular injury has been linked to toxicant-induced oxidative stress. A prolonged lead exposure results in increased lipid peroxidation and inhibited SOD function, and leads to testicular oxidative damage.[36, 37] In two distinct ways, lead acetate toxicity causes free radical damage, including the direct depletion of antioxidant reserves and the production of hydroperoxides, singlet oxygen, and hydrogen peroxides, which are measured by MDA levels as the end products of lipid peroxidation.[38] Rats treated with *C. pepo* seed extract at a dose of 1000 mg/kg body weight did not experience a rise in MDA when exposed to lead acetate. This indicates that the antioxidant activity of *C. pepo* seeds could minimalize the toxicity induced by lead acetate. Inhibiting lipid peroxidation as indicated by MDA levels, the antioxidant protection mechanism reduces oxidative stress and scavenges the free radical responsible for the testis injury. The significantly lower levels of MDA found in the tissues of the groups treated with *C. pepo* seed extract demonstrate that lipid peroxidation has been attenuated in comparison with the lead acetate group. It was confirmed that, *C. pepo* seeds work as a potent antioxidant and free radical scavenger to lower the MDA level perturbed by lead acetate.

A similar finding has shown that, in rats with lead acetate-induced testis injury, vitamin C and E improved the antioxidant status and reduced lipid peroxidation. These results suggest that the antioxidant activities of vitamin C and E are primarily focused on the lipid component of cells. In numerous biological systems, antioxidants like vitamins C and E have been shown to prevent the production of free radicals and to reduce lipid peroxidation.[39, 40]

Compared to untreated control rats, we noticed an increase in oxidative stress in lead administered rats evidenced by the markedly decreased antioxidant enzymes levels in both the serum and testis tissue. The oxidative injury besides any defects in the components of the free radical antioxidant enzyme defense system may serve as an upstream pathway of amplified oxidative stress,[41] leading to high inclination of lipid to peroxidation. An antioxidant works by delaying and hindering the process of oxidation by free radicals. Increased levels of antioxidant enzymes clearly forecasted the antioxidant potential of *C. pepo* seed. As the first line of defence against reactive oxygen species (ROS), SOD is a crucial endogenous antioxidant enzyme that converts superoxide radicals to H^2O^2 and protects against the harmful effects of radicals.[42] Hydroxyl radicals are created as a result of this interaction, which can also be harmful. These enzymes work as antioxidants by virtue of scavenging these hydroxyl radicals.[43] *C. pepo* seed extract at these doses (100 and 1000 mg/kg) brought about dose dependent changes on these antioxidant parameters. Co-administration of *C. pepo* at high dosage (1000 mg/kg) along with lead acetate resulted in the significant reversal of the effect of the lead acetate. The activities of antioxidant enzymes under study were restored to their normal level by *C. pepo* administration to lead acetate treated rats. This investigation reveals a ROS scavenging activity of *C. pepo* seeds.

The observed ameliorative effect could be owing to the numerous phytochemicals in the *C. pepo* seed extract as they are reported to possess certain antioxidant activity. Among these, flavonoids have frequently been linked to the ability of any plant extract to act as an antioxidant. According to the theory put forth by Ye[44] *et al., 2012,* flavonoids have a very strong efficiency to expel free radicals from blood and promote the activity of antioxidant enzymes including SOD and CAT. These actions of flavonoids are also dose-dependent. This background explains why there was an increase in the serum levels of antioxidant enzymes in our study. Apart from these, *C. pepo* seeds have been shown to contain substances including polyphenols, anthocyanins, tannins, saponins, alkaloids, glycosides, steroids, iron, and vitamins A, C, and E, all of which have been linked to their antioxidant capacity by numerous researchers. The potential antioxidant properties of tannins were previously suggested by Tsumbu[45] *et al.,* 2011. Ghani,[46] 1990 proposed that alkaloids and glycosides have antioxidant properties. Therefore, the presence of these antioxidant chemical components, which are known to lower oxidative stress by a variety of mechanisms, can be used to explain the antioxidant activity of *C. pepo* seed extract.

The study could infer that lead acetate treatment could significantly decrease the antioxidant enzymes and augment lipid peroxidation. The enzymatic antioxidant inhibition amplifies free radicals in testicular tissues and thereby affects male reproductive ability. Co-administration of *C. pepo* seeds with lead acetate exhibited defensive effects against lead toxicity induced oxidative stress. The enzymatic antioxidants were notably improved and lipid peroxidation was decreased after *C. pepo* seed extract administration. These activities could be attributed to the numerous phytoconstituents of *C. pepo* seeds as found in this study as well as previously reported by other researchers. The current research could pave a way to prove scientifically that *C. pepo* seeds can enhance enzymatic antioxidants; thereby counteract the deleterious effects of oxidative stress. Hence this study strongly recommends *C. pepo* seeds as a potent natural antioxidant source promising active protection against lead toxicity induced oxidative stress which could be pharmacologically explored for drug synthesis. Further researches are required to decode the probable mechanism through which *C. pepo* seeds exert their antioxidant potential.

Acknowledgements

We would like to express our gratitude to Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, India for providing the basic necessities to execute this study.

References

1. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr. J. 2015; 15(1):1-22. Available from: https://doi.org/10.1186/s12937-016-0186-5.
2. Pikula KS, Zakharenko AM, Aruoja V, Golokhvast KS, Tsatsakis AM. Oxidative stress and its biomarkers in microalgal ecotoxicology. Curr. Opin. Toxicol. 2019; 13:8-15. Available from: https://doi.org/10.1016/j.cotox.2018.12.006.
3. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Lead. US Department of Health and Human Services, 2007.
4. Pant N, Kumar G, Upadhyay AD, Gupta YK, Chaturvedi PK. Correlation between lead and cadmium concentration and semen quality. Andrologia. 2015; 47(8):887-891. Available from: https://doi.org/10.1111/and.12342.
5. Sainath SB, Meena R, Supriya CH, Reddy KP, Reddy PS. Protective role of *Centella asiatica* on lead-induced oxidative stress and suppressed reproductive health in male rats. Environ. Toxicol. Pharmacol. 2011; 32(2):146-154. Available from: https://doi.org/10.1016/j.etap.2011.04.005.
6. Clark IA, Hunt NH, Cowden WB. Oxygen-derived free radicals in the pathogenesis of parasitic disease. Adv. Parasitol. 1986; 25:1-44. Available from: https://doi.org/10.1016/s0065-308x(08)60341-3.

7. Flora G, Gupta D, Tiwari A. Toxicity of lead: a review with recent updates. Interdiscip. Toxicol. 2012; 5(2):47-58. Available from: https://doi.org/10.2478/v10102-012-0009-2.
8. Sivaprasad R, Nagaraj M, Varalakshmi P. Combined efficacies of lipoic acid and 2, 3-dimercaptosuccinic acid against lead-induced lipid peroxidation in rat liver. J. Nutr. Biochem. 2004; 15(1):18-23. Available from: https://doi.org/10.1016/j.jnutbio.2003.09.001.
9. Dru zyn ska B, Strzecha I, Wolosiak R, Worobiej E. The contents of selected biologically active compounds in the extracts of the dried apricots and their antioxidant properties. Z_ywnóśc Nauka Technol Jakóśc. 2008; 6 (61):77-87.
10. Rao MR, Palada MC, Becker BN. Medicinal and aromatic plants in agro-forestry systems. Agrofor. Syst. 2004; 61:107–22. Available from: http://dx.doi.org/10.1023/B:AGFO.0000028993.83007.4b.
11. Yadav M, Jain S, Tomar R, Prasad GBKS, Yadav H. Medicinal and biological potential of pumpkin: an updated review. Nutr. Res. Rev. 2010; 23(2):184-190. Available from: https://doi.org/10.1017/s0954422410000107.
12. Andrade-Cetto A, Heinrich M. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. J. Ethnopharmacol. 2005; 99(30):325-348. Available from: https://doi.org/10.1016/j.jep.2005.04.019.
13. Kim MY, Kim EJ, Kim YN, Choi C, Lee BH. Comparison of the chemical compositions and nutritive values of various pumpkin (Cucurbitaceae) species and parts. Nutr. Res. Pract. 2012; 6(1):21-27. Available from: https://doi.org/10.4162%2Fnrp.2012.6.1.21.
14. Makni M, Fetoui H, Gargouri NK, Garoui EM, Jaber H, Makni J, Zeghal N. Hypolipidemic and hepatoprotective effects of flax and pumpkin seed mixture rich in ω-3 and ω-6 fatty acids in hypercholesterolemic rats. Food Chem. Toxicol. 2008; 46(12):3714-3720. Available from: https://doi.org/10.1016/j.fct.2008.09.057.
15. Dillard CJ, Germen B. Phytochemicals: nutraceuticals and human health. J. Sci. Food Agric. 2000; 80(12):1744-1756. Available from: http://dx.doi.org/10.1002/1097-0010(20000915)80:12%3C1744::AID-JSFA725%3E3.0.CO;2-W.
16. Trease GE, Evans MC. Text book of pharmacognosy. Edn 13, Bailler Tindal, London, 1989, pp. 247-762.
17. Malgwi IS, Olorunshola KV, Hamman WO, Eze ED. and Onaadepo O. Effects of aqueous *Cucurbita pepo* Linn seed extract on some haematological parameters and serum electrolytes of lactating albino rats. Ann. Exp. Biol. 2014; 2(1):11-16.
18. Offor SJ, Mbagwu HO, Orisakwe OE. Lead induced hepato-renal damage in male albino rats and effects of activated charcoal. Front. Pharmacol. 2017; 8:107. Available from: https://doi.org/10.3389/fphar.2017.00107.
19. Ochei J, Kolhatkar A. Haemoglobin Structure and Synthesis. Medical laboratory science: theory and practice. McGraw Hill Education. 2000; 273-277.
20. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J. Biochem. Biophys.1984; 21:130-132.
21. Sinha AK. Colorimetric assay of catalase. Anal. Biochem. 1972; 47(2):389-394. Available from: https://doi.org/10.1016/0003-2697(72)90132-7.
22. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra W. Selenium: biochemical role as a component of glutathione peroxidase. J. Sci. 1973; 179(4073):588-590. Available from: https://doi.org/10.1126/science.179.4073.588.
23. Ohkawa H, Ohishi N, Yagi K. Assay for Lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 1979; 95(2):351 – 358. Available from: https://doi.org/10.1016/0003-2697(79)90738-3.
24. Bakht J, Tayyab M, Ali H, Islam A, Shafi M. Effect of different solvent extracted sample of *Allium sativum* (L.) on bacteria and fungi. Afr. J. Biotechnol. 2011; 10(31):5910–15. Available from: https://doi.org/10.5897/AJB11.232.
25. Swamy MK, Sudipta KM, Lokesh P, Neeki AM, Rashmi W, Bhaumik HS. Phytochemical screening and in vitro antimicrobial activity of *Bougainvillea spectabilis* flower extracts. Int. J. Phytomedicine. 2012; 4:375–79.
26. Rawat B, Garg AP. Characterization of Phytochemicals Isolated from *Cucurbita pepo* Seeds using UV–Vis and FTIR Spectroscopy. Plant Arch. 2021; 21(1):892-899. Available from: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.122.
27. Haleagrahara N, Jachie T, Chakravarthi S, Kulur AB. Protective effect of alpha-lipoic acid against lead-acetate induced oxidative stress in the bone marrow of rats. Int. J. Pharmacol. 2011; 7(2):217–227. Available from: https://doi.org/10.3923/ijp.2011.217.227.
28. Agarwal A, Said TM. Carnitine and male infertility. Reprod. Biomed. Online. 2004; 8(4):376–384. Available from: https://doi.org/10.1016/s1472-6483(10)60920-0.
29. Bello TH, Idris OA. The Effect of antioxidant (Gallic acid) on the testes of lead acetate induced Wistar rat. Toxicol. Environ. Health Sci. 2018; 10(5):261–267. Available from: http://dx.doi.org/10.1007/s13530-018-0374-0.
30. Khademi A, Safdarian L, Alleyassin A, Agha-Hosseini M, Hamed EA, Saeidabadi HS, Pooyan O. The effect of L-carnitine on sperm parameters in patients candidate for intracytoplasmic sperm injection. Iran. J. Reprod. Med. 2004; 2(2):65–69.
31. El-Sherbini E, El-Sayed G, El Shotory R, Gheith N, Abou-Alsoud M, Harakeh SM, Karrouf GI. Ameliorative effects of L-carnitine on rats raised on a diet supplemented with lead acetate. Saudi J. Biol. Sci. 2017; 24:1410–1417. Available from: https://doi.org/10.1016%2Fj.sjbs.2016.08.010.
32. Rania ARE, Heba MAA. Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect of cinnamon in albino rats. Toxicol. Rep. 2014; 795-801. Available from: https://doi.org/10.1016/j.toxrep.2014.10.010.
33. Doumouchtsis KK, Doumouchtsis SK, Doumouchtsis EK, Perrea DN. The effect of lead intoxication on endocrine functions. J. Endocrinol. Invest. 2009; 32:175–183. Available from: https://doi.org/10.1007/bf03345710.
34. El-Tohamy MM, El-Nattat WS. Effect of antioxidant on lead-induced oxidative damage and reproductive dysfunction in male rabbits. J. Am. Sci. 2010; 6(11):613–622.
35. Jegede AI, Offor U, Azu OO, Akinloye O. Red palm oil attenuates lead acetate induced testicular damage in adult male Sprague-Dawley rats. Evid-based Complement. Altern. Med. 2015; 1–7. Available from: https://doi.org/10.1155%2F2015%2F130261.
36. Griveau JF, Le Lannou D. Reactive oxygen species and human spermatozoa: Physiology and pathology. Int. J. Androl. 1997; 20:61–69. Available from: https://doi.org/10.1046/j.1365-2605.1997.00044.x
37. He Y, Zou Q, Chen H, Weng S, Luo T, Zeng X. Lead inhibits human sperm functions by reducing the levels of intracellular calcium, cAMP, and tyrosine phosphorylation. Tohoku J. Exp. Med. 2016; 238(4):295–303. Available from: https://doi.org/10.1620/tjem.238.295.
38. Abd-Allah AR, Helal GK, Al-Yahya AA, Aleisa AM, Al-Rejaie SS, Al-Bakheet SA. Pro-inflammatory and oxidative stress pathways which compromise sperm motility and survival may be altered by L-carnitine. Oxid. Med. Cell. Longev. 2009; 2(2):73-81. Available from: https://doi.org/10.4161/oxim.2.2.8177.
39. Kobayashi D, Goto A, Maeda T, Nezu JI, Tsuji A, Tamai I. OCTN2-mediated transport of carnitine in isolated Sertoli cells. Reprod. 2005; 129:729–736. Available from: https://doi.org/10.1530/rep.1.00507.
40. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Colorimetric method for determination of total antioxidant capacity. J. Clin. Pathol. 2001; 54:356–361.
41. Homma K, Fujisawa T, Tsuburaya N, Yamaguchi N, Kadowaki H, Takeda K, Ichijo H. SOD1 as a molecular switch for initiating the

homeostatic ER stress response under zinc deficiency. Mol. Cell. 2013; 52(1):75-86. Available from: https://doi.org/10.1016/j.molcel.2013.08.038.
42. Mahantesh SP, Gangawane AK, Patil CS. Free radicals, antioxidants, diseases and phytomedicines in human health: Future perspects. World Res. J. Med. Aromat. Plant. 2012; 1(1):6–10.
43. Olawale O, Ikechukwu NE, Grace TO, Chidiebere EU. Oxidative stress and superoxide dismutase activity in brain of rats fed with diet containing permethrin. Biokemistri. 2008; 20(2):93–8.
44. Ye Y, Guo Y, Luo YT. Anti-inflammatory and analgesic activities of a novel biflavonoid from shells of *Camellia oleifera*. Int. J. Mol. Sci. 2012; 13(10):12401–12411. Available from: https://doi.org/10.3390/ijms131012401.
45. Tsumbu CN, Deby-Dupont G, Tits M, Angenot L, Franck T, Serteyn D, Mouithys-Mickalad A. Antioxidant and antiradical activities of *Manihot esculenta* Crantz (Euphorbiaceae) leaves and other selected tropical green vegetables investigated on lipoperoxidation and phorbol-12-myristate-13-acetate (PMA) activated monocytes. Nutrients. 2011; 3(9):818–838. Available from: https://doi.org/10.3390/nu3090818.
46. Ghani A. Introduction to Pharmacognosy. Ahmadu. Bello University Press Ltd. Zaria, Nigeria. 1990; 45:187–97.

HOW TO CITE THIS ARTICLE: Nair AB, Raajeswari PA. Ameliorative Effect of *Cucurbita pepo* L. Seed Extract against Lead Induced Effects on the Serum and Testicular Oxidative Status: An In-vivo Validation. Int. J. Pharm. Sci. Drug Res. 2023;15(2):214-221. **DOI:** 10.25004/IJPSDR.2023.150214

The Indian Journal of Home Science 2024: 36(1)

30

AN EXPLORATION OF THE NUTRITIONAL, PHYTOCHEMICAL AND *IN VITRO* ANTIOXIDANT PROFILE OF *CUCURBITA PEPO* L. (PUMPKIN) SEEDS

Amrutha B. Nair[1] and Dr. P.A. Raajeswari[2],

Research Scholar[1] and Associate Professor[2],
Department of Food Science and Nutrition
Avinashilingam Institute for Home Science and Higher Education for Women,
Coimbatore-641043
Email ID[1]: bnairamrutha93@gmail.com, raajeswari_fsn@avinuty.ac.in
HSAI Membership ID[1]: HSAI-2019-TN-408-LF, HSAI-2019-TN-485-LF

ABSTRACT

The quest to discover the functional and health benefits of the underutilized seeds has exponentially increased in recent times. Although extensive research has been done on the fruits of *Cucurbita pepo* L., there is a dearth of data available on the nutritional, phytochemical and antioxidant profile of their seeds. Hence the current study was carried out with these objectives. The study revealed that *C. pepo* seeds have high calorific value, high protein and fat content proving it to be a rich source of energy. On the contrary, the carbohydrate content of the sample was not on par with its calorific value. The sample presented a low moisture content indicating the storage advantage of the seeds. There was an intriguing and notable mineral content whereas vitamins were found to be present in a fair amount. Quantitative phytochemical analyses were performed for quantifying the total phenolics, total flavonoids and total alkaloid contents in the sample which were found to be 32.7 ± 0.89 mg/100g GAE, 16.8 ± 0.63 mg/100g QE and 8.3± 1.12 mg/100g AE respectively. The current study is probably the first to have reported the total alkaloid content of *C. pepo* seed aqueous extract. The sample was found to be effective in inhibiting DPPH radicals almost comparable to that of reference standard, ascorbic acid. The presence of an assortment of potent secondary metabolites either alone or in combination, may be accountable for the observed antioxidant activity.

Keywords: Antioxidant profile, *Cucurbita pepo* L., Nutritional profile, Phytochemicals, Secondary metabolites

INTRODUCTION

Vegetable seeds are handy foods and most of them are edible. Protein, fiber, vitamins, minerals and phytochemicals contained in seeds are considered to be nutraceutical chemicals. They endorse general wellbeing and also boost immunity (Adeola and Anofi, 2021). Antioxidant supplements are the effective approaches to fight against the detrimental effects of reactive oxygen species (ROS) induced oxidative stress (Kasote *et al*., 2015). A diet enriched with plant foods will confer a milieu of phytochemicals which are non-nutritive compounds that possess health promoting properties. Vegetables are high in phenolic compounds, terpenoids, pigments, and other natural antioxidants that have been linked to disease prevention and therapy (Bloch and Thomson, 1995).

Pumpkin (*Cucurbita pepo*) is one of the widely cultivated and popular vegetable crops of high economical value. It is an annual plant bearing round shaped, deep yellow to orange coloured fruits with smooth, slightly ridged skin. The thick shell encloses the pulp and flat and dark green coloured seeds (Schieber *et al*., 2001). Its edible seeds are generally regarded as agro-waste and habitually discarded. The functional health benefits of *Cucurbita pepo* seeds need to be well explored in order to advocate their adequate consumption by the population and for the formulation of novel drugs for therapeutic purposes (Ayyildiz *et al*., 2019).

OBJECTIVES

- Determine the nutritional profile of *Cucurbita pepo* L. seeds
- Quantitatively estimate the phytochemical content in *Cucurbita pepo* L. seeds
- Analyze the antioxidant profile of *Cucurbita pepo* L. seeds *in vitro*

METHODOLOGY

Sample Collection and Preparation

Cucurbita pepo L. seeds were collected directly from the farmers at Tudiyalur, a locality in Coimbatore. The seeds were identified and authenticated (voucher specimen number BSI/SRC/5/23/2021/Tech/282) at Botanical Survey of India, Southern Regional Centre, Tamilnadu Agricultural University, Coimbatore.

Determination of Nutritional Composition

The seeds were washed and cleaned to remove any debris, dehulled and shade dried before powdering. Seeds were weighed and milled into fine flour and sieved. The *C. pepo* seeds were analyzed for its proximate composition and vitamins and mineral contents using standards procedures of Association of Official Analytical Chemists (AOAC, 2005) with slight modifications. The carbohydrate content was calculated by the following expression:

Carbohydrate content (%) = 100 - (moisture + fat + protein + ash + fiber) %

The energy value of the sample was calculated as (protein × 4) + (carbohydrate × 4) + (fat × 9) in kilo calories (kcal).

Quantitative Determination of Phytochemicals

10g seed powder was measured and dissolved in 100 ml deionized water and kept in a mechanical shaker overnight at 50ºC for 75-100 rpm. The solution was filtered using Whatman filter paper and the extract was collected and refrigerated until analyses.

a. Total Phenolics

3 mg of Gallic acid was dissolved in 3 ml of distilled water. Dilutions of this solution with distilled water were prepared to give the concentration of 25, 50, 75, 100, 200 and 250 µg/ml. Stock solutions of sample were prepared by dissolving 10 mg of seed extract in 10 ml of distilled water to give concentration of 1mg/ml. The 100µl of this solution was taken in to 25ml volumetric flask, to which 10 ml of water and 1.5 ml of Folin-Ciocalteau reagent were added. The mixture was

then kept for 5 min and to it 4ml of 20% w/v sodium carbonate solution was added. The volume was made up to 25 ml with distilled water. The mixture was kept for 30 minutes until blue colour developed and then observed at 765 nm. The standard curve of gallic acid was plotted between absorbance versus concentrations, and the unknown sample concentration was calculated in terms of mg GAE/100g (Bhalodia *et al*., 2011).

b. Total Flavonoids

10 mg of quercetin was dissolved in methanol and then diluted to 25, 50, 80, and 100 μg/ml. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm with a Shimadzu UV - 1800 spectrophotometer. 0.5ml of each extract stock solution, 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water. Sample and sample blank of extracts were prepared and their absorbance was measured at 415 nm. A linear calibration curve of quercetin was used to calculate the concentration of flavonoid content and expressed as milligrams of quercetin equivalent (mg QE/100 g) (Pallab *et al*., 2013).

c. Total alkaloids

Atropine standard solution was prepared by dissolving 1 mg of pure Atropine (AR-grade procured from Sigma Company) in 10 ml distilled water. Aliquots of Atropine standard solution (0.4, 0.6, 0.8, 1 and 1.2 ml) were accurately measured and transferred to different separatory funnels. 5 ml of phosphate buffer (pH 4.7) and 5 ml of Bromocresol Green (BCG) solution were taken and the mixture was shaken with extract with 1, 2, 3, and 4 ml of chloroform. The extracts were then collected into a 10 ml volumetric flask and diluted to adjust the solution with chloroform. The absorbance of the complex in chloroform was measured using UV-Spectrophotometer (470 nm) against the blank prepared as above but without Atropine (Ajanal *et al*., 2012).

Antioxidant Activity

DPPH scavenging assay

The DPPH solution was prepared in ethanol (control) and subsequently added to various concentrations of the sample extract (10, 20, 40, 60, 80 and 100 μg/ml). Ascorbic acid was taken as standard. Absorbance variations were read at 517 nm wavelength using a spectrophotometer. The percentage scavenging activities were plotted against varied concentrations of sample extract and represented in μg/ml to create the linear regression curve (Sadiq *et al*., 2015). Percentage inhibition was estimated by using the equation as follows.

Percentage inhibition = (AC-AS)/AC×100

Where, AC is the absorbance of control and AS is the absorbance of sample

The IC_{50} values were calculated using linear regression analysis and used to indicate scavenging activity of the sample extract.

Statistical Analysis

Data were analyzed using Microsoft EXCEL and IBM SPSS software version 21.0 and expressed as mean ± standard deviation (SD) of triplicate readings.

RESULTS AND DISCUSSION

The health benefits of *Cucurbita pepo* (Pumpkin) seeds are represented in **Fig. 1**.

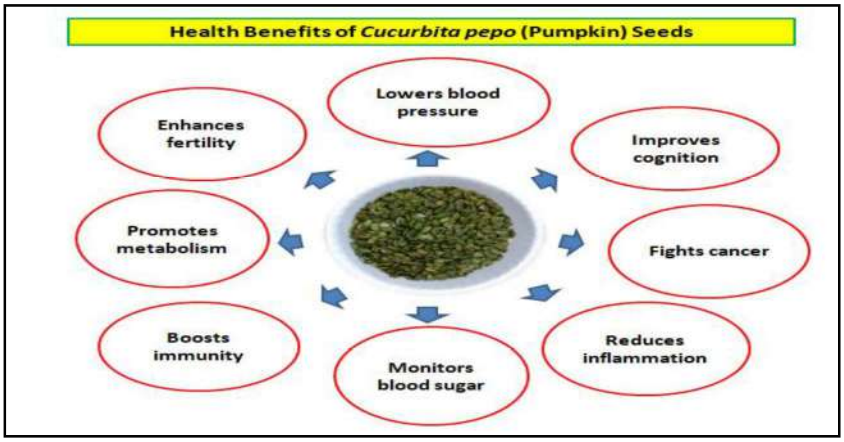

Fig.1: Health Benefits of *Cucurbita pepo* L. Seeds

Proximate Composition of *Cucurbita pepo* L. Seeds

In this study, the proximate composition of the *Cucurbita pepo* L. seeds was assessed and used as a criterion in determining the nutritional quality of the seeds. The proximate composition determined is furnished as a pie chart (**Fig. 2**) for a better understanding.

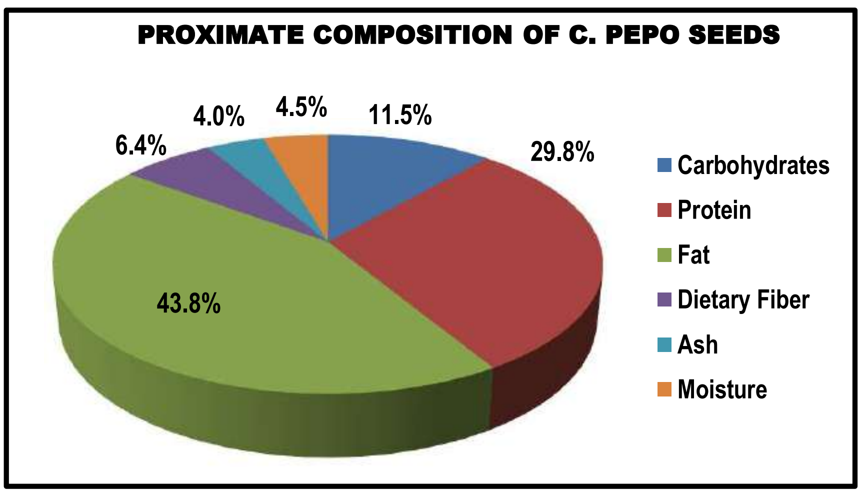

Fig. 2: Proximate composition of *Cucurbita pepo* L. seeds

The present study revealed that the *C. Pepo* seeds have moisture content which is lower than that of the vegetable seed like amaranth seeds (10.6± 0.2) and pomegranate seeds (*Punica granatum*) (6.84 ± 0.03) as reported by Zhana *et al*., (2019) and Abiola *et al*., (2018) respectively. The low moisture content indicated the storage benefit of the *C. pepo* seeds. The ash content of the seed was found to be closer to the ash content of chia seeds (4.45%) (Kibui *et al*., 2019). The ash content is a reflection of the quantity of minerals present in a food material (Gemede *et al*., 2016). The *C. pepo* seeds were found to be of high calorific value (559.4 kcal/100 gram). Lipids are essential for our body to sustain as they provide maximum energy, provide fat soluble vitamins and act as insulation. The lipid content of the sample indicated how pumpkin seeds could be regarded as oilseeds like sunflower, soybean, mustard etc. The lipid content was closer to the value reported by Badu *et al*., (2020). Chigwe and Saka (1991) indicated that pumpkins supply calcium, iron, vitamin A, fat (25 – 55%), and protein (25 – 35%). This is in accordance with the studies conducted by Achu *et al*., (2005) and Loukou *et al*., (2007) which revealed similar results on the analysis of *C. pepo* seeds. For example, their studies revealed oil and protein content within the ranges of 28 – 40.49% and 61 to 73.59% respectively, while this study revealed 43.8±0.098 and 29.8±0.103. Furthermore, the nutritional value of the *C. pepo* seed is species dependent and is also influenced by climate and geographic difference.

Most bodily tissues rely on dietary protein for their natural production and upkeep. The protein content of the seed showed that *C. pepo* seeds are a good source of protein. The available carbohydrate content of the seed was found to be poor and was not in agreement with the findings of Ghaffar *et al*., (2018) may be due to the difference in geographic location. These results conclusively confirmed the dependency of nutritional content of these seeds to regional climates. It was inferred that major contributors to calorific value of *C. pepo* seeds are fat and protein contents in comparison to carbohydrate. The fiber content of the seeds proved the *C. pepo* seeds are a good source of dietary fiber. Hence *C. pepo* seeds could be recommended for relieving constipation, protection from certain cancers in addition to lowering blood cholesterol level.

Vitamin and Mineral Content of *Cucurbita pepo* L. Seeds

Sample was screened for quantifying vitamin A and vitamin C contents. Further ash content was analyzed for quantifying certain macro and micro minerals (as shown in **Table 1**) present in the sample.

Table 1. Vitamins and minerals content of *Cucurbita pepo* L. seeds/100 gram

SL. No.	Element	Concentration
1.	Vitamin A (mcg)	92.4 ±0.23
2.	Vitamin C (mg)	2.6 ±0.05
3.	Calcium (mg)	48 ±1.19
4.	Iron (mg)	7.5 ±0.07
5.	Phosphorous (mg)	900±0.60
6.	Potassium (mg)	450 ± 0.12
7.	Magnesium (mg)	520 ±0.02
8.	Zinc (mg)	8.2± 0.10
9.	Sodium (mg)	24.2±0.05
10.	Copper (mg)	1.67±0.90

Note: The data are mean value ± standard deviation of triplicate results

Potassium and phosphorous content were found to be abundant in the sample. Potassium is required for fluid balance, nerve transmission, iron absorption and to control hypertension whereas phosphorous maintains acid-base balance and is essential for healthy bones and teeth. Thus, *C. pepo* seeds could be regarded as a rich source of potassium and phosphorous.

The sample presented a fairly high value for Zinc. Zinc is a part of many enzymes and is needed for genetic makeup, wound healing, growth and development and sexual maturation (Supasai *et al*., 2017). Dietary intake of iron is crucial for binding and transport of oxygen (Abbaspour *et al*., 2014). The sample was found to have a fair amount of iron present in it. *C. pepo* seeds had approximately 5 mg of magnesium for every 100 g of sample which imply that it is an excellent source. Magnesium is a constituent of bone and teeth and is directly associated with calcium and phosphorus absorption (Glasdam *et al*., 2016).

The concentration of sodium in the sample was found to be good as sodium is required to regulate blood pressure and blood volume and fluid balance. Study indicated that *C. pepo* seeds are a good source of calcium. Calcium ions are necessary for the normal functioning of nerves and muscles (Payne, *et al*., 1990). Copper acts as antioxidant and help in the prevention of free radical damage in living organisms (Blockhuys and Wittung-Stafshede, 2017). This study revealed that the sample under study is a good source of copper. Vitamin A in the present sample was found to be 92.4 mcg. Vitamin A is of high significance since it has a key role in vision, immunity and reproduction. Vitamin C content was found to be 2.6±0.05 mg/100 gram. Vitamin C or ascorbic acid is an indispensable factor required for growth and development, immunity, wound healing and iron absorption.

Quantitative Determination of Phytochemicals in *Cucurbita pepo* L. seeds

Qualitative phytochemical analysis of the aqueous extract revealed that *C. pepo* seeds had appreciable levels of alkaloids and tannins, moderate levels of carbohydrates and steroids and other compounds such as protein, flavonoids, saponins, terpenoids, quinones in trace levels (Nair and Raajeswari, 2023). Hence quantitative analyses were performed and the concentrations of total phenols, flavonoids and alkaloids of *C. pepo* seed extract are as furnished as in **Table 2**.

Table 2. Quantitative analyses of phytochemicals in *Cucurbita pepo* L. seeds

SL. No	Phytochemicals	Concentration	Regression coefficient (R^2)
1.	Total Phenolics (mg/100g GAE)	32.7± 0.89	0.964
2.	Total Flavonoids (mg/100g QE)	16.8 ± 0.63	0.976
3.	Total Alkaloids (mg/100 AE)	8.3 ±1.12	0.958

Note: GAE-Gallic Acid Equivalent, QE-Quercetin Equivalent, AE-Atropine Equivalent

The result revealed that the *C. pepo* seeds had a high concentration of total phenols (32.7± 0.89 mg/100g GAE) in it. Xanthopoulou *et al*., (2009) reported that pumpkin seed water extract was the richest in phenolic constituents (85–92% of total extractable phenolics) in comparison with other extracts. Sahin *et al*., (2014) revealed that the water extract of *Prunella grandiflora* has the highest total phenolic content (24.63 ± 0.55 mg GAE/g extract) when compared to alcoholic extracts. The high concentration of phenolics in the extract could be attributed to the presence of proteins and other water-soluble compounds containing phenolic rings.

Flavonoids are considered as the most imperative natural phenols. They have a wide spectrum of biological and chemical activities including radical scavenging properties. Total flavonoid content results revealed lower values than those obtained for the analysis of phenols. This is within expected, being that flavonoids are nothing but a subgroup of the phenolic compounds. Alkaloids are naturally occurring chemical compounds having a significant impact on plant medicine because of their vast application (Shamsa *et al*., 2008). The sample was found to have a high alkaloid content of 8.3 ±1.12 mg/100 AE. The current study is probably the first to have reported the total alkaloid content of *C. pepo* aqueous extract.

Quantitative phytochemical analysis of aqueous extract of *C. pepo* seeds exhibited that it has remarkable amount of total phenolics, flavonoids, and alkaloids. Polyphenols and flavonoids isolated from the plants are generally considered as potent antioxidants because of their ability to absorb and neutralize free radicals as well as quench reactive oxygen species. Therefore, *C. pepo* seeds could be recommended as excellent natural source of antioxidant agents.

Antioxidant activity of *Cucurbita pepo* L. seeds

DPPH scavenging activity

DPPH free radical scavenging activity is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% (IC_{50}). The highest antioxidant capacity is indicated by the lowest IC50 values (Stanković *et al*., 2010). **Fig. 3** indicates the increase of radical scavenging activity of extract which was deduced from the increase of percent inhibition as the extract concentration increases.

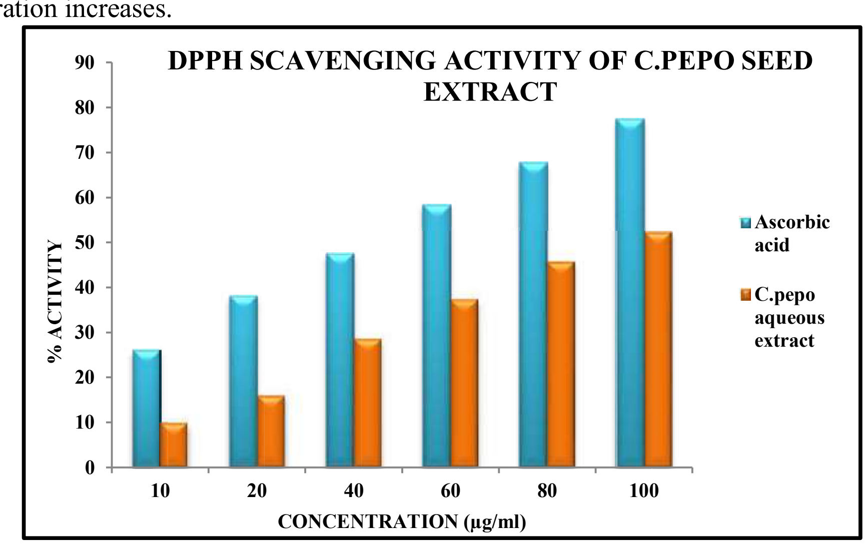

Fig. 3: DPPH scavenging activity of *Cucurbita pepo* L. Seed Extract

In the present study, the half-maximal inhibitory concentration (IC_{50}) of the *C. pepo* seed extract and the ascorbic acid (standard) were found to be 90.35µg/ml and 46.94µg/ml respectively. The *C. pepo* seed extract exhibited a high antioxidant activity with a lower IC_{50} value. From the investigation, it was evident that the *C. pepo* seed extract had a considerable DPPH radical scavenging activity almost similar to that of the standard. The inhibition of DPPH radical scavenging by the sample extract was found to be strictly proportional to the concentration of total phenolics. These results reflected that the aqueous extract had high scavenging activity which could be attributed to the presence of various bioactive compounds such as phenolics, flavonoids and alkaloids as found in quantitative phytochemical screening. Hence the study strongly implies a high correlation between the antioxidant activity and phenolic content of the *C. pepo* seeds under investigation.

Valenzuela *et al*., 2014 reported that in all varieties of seed Cucurbita spp studied, the highest antiradical activity was detected in the aqueous fractions. A study carried out by Rakass *et al*., (2018) also disclosed that among all extracts, water extract of *C. pepo* exhibited the highest antioxidant activity followed by ethanol, methanol and acetone extract. Numerous studies have proven that antioxidant activity of plant extracts is mainly associated to the total phenolic content in the plants. In this study, a strong correlation between total phenolic, total flavonoid and total alkaloid contents and radical scavenging activity of *C. pepo* seed extract were observed.

CONCLUSION

The present findings strongly recommend the daily intake of *Cucurbita pepo* L. seeds in the diet for numerous reasons i.e. it is a widely cultivated, cheap and cost-effective vegetable crop which is a nutrient dense pack and of high calorific value. Importantly, these results strongly suggest that the *C. pepo* seeds if consumed in sufficient quantities would immensely improve human nutritional status. Therefore, this alone can make a significant contribution to family food security and nutritional needs, enhancing normal growth and active protection against diseases and malnutrition in the most vulnerable members of society. The quantitative analyses of *C. pepo* seed extract indicated the high quantity of bioactive compounds such as total phenolics, total flavonoids and total alkaloids. The presence of various bioactive compounds in the *C. pepo* seeds make it a promising source of natural antioxidants which could be therapeutically utilized for the prevention and treatment of a diverse range of diseases and disorders.

REFERENCES

- Abbaspour, N., Hurrell, R., & Kelishadi, R. (2014). Review on iron and its importance for human health. Journal of Research in Medical Sciences, 19(2):164–174.
- Abiola, T., Falana, L.K. & Adediji, D.O. (2018). Proximate Composition, Phytochemical Analysis and in vivo Antioxidant Activity of Pomegranate Seeds (Punica granatum) in Female Albino Mice. Biochemistry and Pharmacology. 7(2).
- Achu, M.B., Fokou, E., Tchiégang, C., Fotso, M. & Tchouanguep, F.M. (2005). Nutritive value of some Cucurbitaceae oilseeds from different regions in Cameroon. African Journal of Biotechnology. 4(11).
- Adeola, A.O. & Anofi, O.A. (2021). Nutritional Composition of Grain and Seed Proteins. Grain and Seed Proteins Functionality. IntechOpen.
- Ajanal, M., Gundkalle, M.B. & Nayak, S.U. (2012). Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Ancient Science of Life, 31(4):198–201.
- AOAC (2005). Official Methods of Analysis. 16th Edn, Washington DC.

- Ayyildiz, H., Topkafa, M. & Kara, H. (2019). Pumpkin (Cucurbita pepo L.) Seed Oil. In: Ramadan M.F., editor. Fruit Oils: Chemistry and Functionality. Springer International Publishing; Cham, Switzerland.
- Badu, M., Pedavoah, M.M. & Dzaye, I.Y. (2020). Proximate composition, antioxidant properties, mineral content and anti-nutritional composition of Sesamum indicum, Cucumeropsis edulis and Cucurbita pepo seeds grown in the savanna regions of Ghana. Journal of Herbs, Spices and Medicinal Plants, 26(4):329-339.
- Bhalodia, N., Nariya, P., Acharya, R. & Shukla, V. (2011). Evaluation of in vitro antioxidant activity of flowers of Cassia fistula Linn. International Journal of PharmTech Research, 3(1):589-599.
- Bloch, A. & Thomson, C.A. (1995). Phytochemicals and functional foods. Journal of American Diet Association, 95(4):493-496.
- Blockhuys, S. & Wittung-Stafshede, P. (2017). Roles of Copper-binding Proteins in Breast Cancer. International Journal of Molecular Science, 18(4):1–10.
- Chigwe, C.F.B. & Saka, V.W. (1994). Collection and characterization of Malawi pumpkin germplasm. Zimbabwe Journal of Agricultural Research. 32:139-147.
- Gemede, H.F., Haki, G.D., Beyene, F., Woldegiorgis, A.Z. & Rakshit, S.K. (2016). Proximate, mineral, and antinutrient compositions of indigenous Okra (Abelmoschus esculentus) pod accessions: implications for mineral bioavailability. Wollega University.
- Ghaffar, F., Kainat, B., Shah, H. & Akram, M. (2018). Nutritional, physico-chemical, antimicrobial and antioxidant screening of seed and seed oil of Cucurbita pepo grown in Kpk, Pakistan. FUUAST Journal of Biology, 8(1):41-48.
- Glasdam, S.M., Glasdam, S. & Peters, G.H. (2016). The Importance of Magnesium in the Human Body: A Systematic Literature Review. Advances in Clinical Chemistry, 73:169–193.
- Kasote, D.M., Katyare, S.S., Hegde, M.V. & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. International Journal of Biological Sciences, 11:982-91.
- Kibui, A.N., Owaga, E. & Mburu, M. (2018). Proximate Composition and Nutritional Characterization of Chia Enriched Yoghurt. African Journal of Food, Agriculture, Nutrition and Development, 18(1):13239-13253.
- Loukou, A., Gnakri, D., Djè, Y., Kippré, A.V., Malice, M., Baudoin, J-P. & Bi, I. A. (2007). Macronutrient composition of three cucurbit species cultivated for seed consumption in Côte d'Ivoire. African Journal of Biotechnology, 6(5).
- Nair, A.B. & Raajeswari, P.A. (2023). Ameliorative Effect of Cucurbita pepo L. Seed Extract against Lead Induced Effects on the Serum and Testicular Oxidative Status: An In-vivo Validation. International Journal of Pharmaceutical Sciences and Drug Research, 15(2):214-221.
- Pallab, K., Tapan, B., Tapas, P. & Ramen, K. (2013). Estimation of total flavonoids content (TPC) and antioxidant activities of methanolic whole plant extract of Biophytum sensitivum Linn. Journal of Drug Delivery and Therapeutics, 3(4): 33-37.
- Payne, R., Flores, T.M. & Fein, A. (1990). Feedback inhibition by calcium limits the release of calcium by inositol trisphosphate in Limulus ventral photoreceptors. Neuron. 4(4):547-555.

- Zhana, P., Ginka, A., Maria. A.R. & Vaseva. (2019). A comparative study on chemical and lipid composition of amaranth seeds with different origin. Bulgarian Chemical Communications, 51:262-267.
- Rakass, S., Babiker, H.A.A. & Oudghiri-Hassani, H. (2018). Comparative evaluation of total phenolic content, total flavonoids content and antioxidants activity in Skin & Pulp extracts of Cucurbita maxima. Moroccan Journal of Chemistry, 6(2):218-226.
- Sahin, S., Ari, F., Demir, C. & Ulukaya, E. (2014). Isolation of major phenolic compounds from the extracts of Prunella L. species grown in Turkey and their antioxidant and cytotoxic activities. Journal of Food Biochemistry, 38:248-257.
- Sadiq, A., Mahmood, F., Ullah, F., Ayaz, M., Ahmad, S., Haq F.U., Khan, G. & Jan, M.S. (2015). Synthesis, anticholinesterase and antioxidant potentials of ketoesters derivatives of succinimides: A possible role in the management of Alzheimer's. Chemistry Central Journal, 9-31.
- Schieber, A., Stintzing, F. C., & Carle, R. (2001). By-products of plant food processing as a source of functional compounds: Recent developments. Trends in Food Science and Technology, 12:401-413.
- Shamsa, F., Monsef, H., Ghamooshi, R. & Verdian-rizi M. (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai Journal of Pharmaceutical Sciences. 32:17-20.
- Stanković, M., Topuzović, M., Marković, A., Pavlović, D., Solujić, S., Nićiforović, N., Mihailović, V. (2010). Antioxidant Activity, Phenol and Flavonoid Contents of Different Teucrium Chamaedrys L. Extracts. Biotechnology and Biotechnological Equipment, 24:82-86.
- Supasai, S., Aimo, L., Adamo, A.M., Mackenzie, G.G. & Oteiza, P.I. (2017). Zinc Deficiency Affects the STAT1/3 Signaling Pathways in Part through Redox-mediated Mechanisms. Redox Biology, 11:469–481.
- Valenzuela, G.M., Soro, A.S., Tauguinas, A.L., Gruszycki, M.R., Cravzov, A.L., Giménez, M.C. & Wirth, A. (2014). Evaluation of Polyphenol Content and Antioxidant Activity in Extracts of Cucurbita spp. Scientific Research.
- Xanthopoulou, M.N., Nomikos, T., Fragopoulou, E. & Antonopoulou, S. (2009). Antioxidant and lipoxygenase inhibitory activities of pumpkin seed extracts. Food Research International, 42:641-646.