Original Research Article

Phytochemical, Mineral and Vitamin Profile of different aqueous layers of *Justicia carnea* and *Mucuna pruriens.*

**ABSTRACT**

For ages, plants have been utilized for both nutritional and medicinal purposes. *Mucuna pruriens* and *Justicia carnea* are two prominent examples, traditionally used in medicine. This study examined the phytochemical, mineral, and vitamin profiles of the supernatant and down layers of aqueous extract of both plants. Aqueous extracts of *Justicia carnea* and *Mucuna pruriens* were prepared by maceration and kept to separate into two distinct layers after 24 and 4 hours, respectively. The assays were conducted using standard biochemical methods. The results revealed the presence of various bioactive compounds, including total phenol, flavonoid, tannin, saponin, phytate, oxalate and alkaloid, in varying levels in all the layers of the extracts. The analyzed vitamins and minerals varied in quantity and were absent in some layers. *Mucuna* *pruriens*, specifically its down-layer, showed higher phytochemical levels, while the down-layer of *Justicia carnea* was found to have higher vitamins and mineral levels. These findings therefore suggest that all extract layers of *Mucuna pruriens* and *Justicia carnea* are good sources of vital phytochemicals, minerals and vitamins that are nutritional and therapeutically valuable. However, the phytochemicals are evenly distributed in both layers but some of the vitamins and minerals were found only in some layers.

Keywords: Mucuna pruriens; Justicia carnea; phytochemicals; vitamins; bioactive.

**INTRODUCTION**

For centuries, plants have been utilized for both nutritional and therapeutic purposes. These properties attributed to the presence of bioactive compounds within it. Secondary metabolites known as phytochemicals are one of these bioactive compounds. In plants, they carry out almost no immediate obvious growth or metabolic functions, but are non-nutritive substances that possess defensive properties, playing a crucial role in their protection against environmental stresses and predators [1]. In the human body, these phytochemicals carry out various physiological actions and possess potential disease inhibiting capabilities. They have been shown to exhibit a wide range of biological activities, including strong antioxidant, antimicrobial, antidiarrheal, anthelmintic, anti-allergic, antispasmodic, and antiviral effects [2, 3]. Some of these phytochemicals include flavonoids, tannins, alkaloids, terpenoids, steroids, cardiac glycosides and Saponins [4].

For years now, scientists have been working on understanding the molecular-level effects of various plant based nutrients on human health and disease [5-7]. *Mucuna pruriens* and *Justicia carnea* are prominent examples of these plants with established therapeutic properties. [8, 9]. *Mucuna pruriens* belongs to the family Leguminoseae and is a cover crop commonly known as Velvet bean and Common Cowitch [10]. It is widely cultivated across Asia, America, Africa, and the Pacific Islands, where its pods have been used for food and its young leaves as animal feeds. Additionally, it has been widely utilized in traditional medicine for centuries, particularly in the treatment of various ailments, including neurological disorders, reproductive health issues, and parasitic infections. [11, 12]. *Justicia carnea* on the other hand belongs to Acanthaceae family [13], it has been reported to possess diverse therapeutic benefits inclusive of hematinic, anti-inflammatory, anti-cancer, antimalalaria, Antisickling effects and Antidiabetic effects [13,14].

Though, previous research has been carried out to quantify the presence of these bioactive compounds in these plants. However, it was observed that aqueous extract of these plants, when left to stand, separates into layers. The number of layer is determined by the length of time. In this study, we used two layers which were obtained after 4 and 24 hours for *Mucuna pruriens* and *Justicia carnea* respectively. Therefore, it is pertinent to evaluate these layers and determine the concentration of phytochemicals, vitamin and mineral in the two layers of both plant extracts. This will help to identify the optimal extraction method that maximizes their therapeutic potential. Hence, this study aims to determine the distribution and concentration of phytochemicals, vitamins, and minerals in the two layers of *Mucuna pruriens* and *Justicia carnea* aqueous extracts.

**MATERIALS AND METHODS**

**2.1 Plant Materials**

Fresh Leaves of *Mucuna pruriens* and *Justicia carnea* were collected from Umu-Ugwaunta community in Akwuke-Awkunanaw village, Enugu South Local Government of Enugu state. It was then Identified and validated in Applied Biology department in Enugu State University of Science and Technology, and the Department of Forestry, Michael Okpara University of Agriculture Umudike, Abia State respectively.

**2.2 Preparation of Extract**

The aqueous extract of both plants was prepared by measuring Fifty (50) grams of *Mucuna pruriens and Justicia carnea.* Following washing, the samples were macerated with distilled water and filtered. The aqueous extract of *Mucuna pruriens* and *Justicia carnea* were then kept for 4 hours and 24 hours respectively to separate into two layers.

**2.3 Phytochemical Profiling**

Total Phenol was determined by the method of Barros et al. [15], using Folin Ciocalteu reagent and Gallic acid as standard, Flavonoid content was also determined using a colorimetric method with catechin as standard as described by Barros et al. [15]. Tannin and Saponin content were determined using the standard method of AOAC [16, 17]. Phytate content was determined with iron (III) chloride solution utilizing the method of Young and Greaves [18]. Oxalate Content was determined using the method of Osagie [19]. While, Alkaloid Content was determined using the Harborne method [20].

**2.4 Vitamin analysis**

Vitamins A, E, B1, B2, B3, B6, Ascorbic Acid and Folic Acid contents were all analysed by spectrophotometric techniques. Vitamin A and E was assessed by the method described by Rutkoski et al. [21]. Vitamins B1, B2 and Vitamin B3 (Nicotinamide) were assessed utilizing the method of Kirk and Sawyer [22]. Beta Carotene and Lycopene were quantified by the method of Barros et al. [15]. Vitamin B9 (Folic Acid) was assessed by the method of Padmarajaiah et al. [23]. Vitamin B6 was quantified by the method of Raeed and Azam using Cerium-IV ion and Arsenazo III reagent, [24] while Ascorbic Acid by the method of Klein and Perry [25].

**2.5. Mineral analysis**

Mineral level were assessed using Atomic Absorption Spectrometer as described by AOAC [26]

**2.6. Statistical Analysis.**

The data was statistically analyzed using SPSS version 20 statistical package and expressed as mean ± standard deviation (SD) of duplicate values. Statistical significance were evaluated using ANOVA and Turkey’s Post HOC test at p<0.05.

**3. RESULTS**

**3.1. Phytochemical content.**

Table 1 reveals the result of the phytochemical screening of the different groups of extracts; *Justicia carnea* (supernatant), *Mucuna pruriens* (supernatant), *Justicia carnea* (down-layer) and *Mucuna pruriens* (down-layer**)**. From the results, Total Phenol, Flavonoid, Tannin, Saponin, Phytate, Oxalate and Alkaloid was present in all groups in varying quantities. The Total Phenol content was significantly higher (p<0.05) than other phytochemicals, with Phytate being the least present. The phenol content of the down-layers of both plants were significantly higher (p<0.05) than that of the supernatant

**Table 1: Quantitative Phytochemical Composition of Aqueous Extract of Supernatant and Down-layer Of *Mucuna pruriens* and *Justicia carnea*.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Phytochemicals** | ***Justicia carnea* (supernatant)** | ***Mucuna pruriens* (supernatant)** | ***Justicia carnea* (downlayer)** | ***Mucuna pruriens* (downlayer)** |
| Total Phenol (mgGAE/ml) | 27.35 ± 1.93a | 29.85 ± 2.04a | 42.43 ± 4.74b | 50.14 ± 1.28b |
| Flavonoid (mgCE/ml) | 11.78 ± 0.46c | 12.01 ± 0.62c | 11.64 ± 2.62c | 11.80 ± 0.95c |
| Tanin (mgTAE/ml) | 0.17 ± 0.01d | 0.50 ± 0.06d | 0.61 ± 0.06e | 0.85 ± 0.19e |
| Phytate (%) | 0.10 ± 0.03d | 0.17 ± 0.01f | 0.22 ± 0.01f | 0.27 ± 0.00fg |
| Oxalate (mg/ml) | 1.08 ± 0.08d | 2.84 ± 0.36f | 1.16 ± 0.21g | 2.84 ± 0.24h |
| Alkaloids (%) | 0.84 ± 0.11d | 0.96 ± 0.12d | 1.42 ± 0.21d | 1.96 ± 0.12i |
| Saponin | 0.11 ± 0.01 | 0.30 ± 0.03 | 0.24 ± 0.02 | 0.29 ± 0.03 |

**GAE:** Gallic acid equivalent, **CE:** Catechin equivalent, **TAE:** Tannic acid equivalent. Values are mean ± SD (n=2). Values with the same superscripts vertically and horizontally are not significantly different (p>0.05).

3.2 Vitamin Content

From the result of the vitamin analysis as shown in Table 2, there were varying levels of Vitamin contents in all groups. Notably, Vitamin C level was significantly higher (p<0.05) than all the vitamins. There was an absence of Beta carotene in both supernatant of *Mucuna pruriens* and *Justicia carnea*, and Lycopene was absent in all extract groups. Additionally, Vitamin E and Folic Acid was absent in *Justicia carnea* (supernatant) and *Mucuna pruriens* (supernatant) respectively.

**Table 2: Vitamin Content of Aqueous Extract of Supernatant and Down-layer of *M. Pruriens* and *J. Carnea*.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Vitamins** | ***Justicia carnea* (supernatant)** | ***Mucuna pruriens* (supernatant)** | ***Justicia carnea* (downlayer)** | ***Mucuna pruriens* (downlayer)** |
| Vit A (µg/g) | 0.70 ± 0.03 | 0.67 ± 0.10 | 1.08 ± 0.06 | 0.59 ± 0.70 |
| B1 (mg/g) | 0.05 ± 0.01 | 0.19 ± 0.19 | 0.03 ± 0.01 | 0.08 ± 0.02 |
| B2 (mg/g) | 0.95 0.33 | 1.07 ± 0.12 | 0.91 ± 0.11 | 1.16 ± 0.10 |
| B3 (mg/g) | ND | 0.03 ± 0.04 | 0.17 ± 0.07 | 0.10 ± 0.02 |
| B6 (mg/g) | 0.01 ± 0.01 | 0.03 ± 0.02 | 0.04 ± 0.01 | 0.05 ± 0.01 |
| C (mg/g) | 18.19 ± 5.56b | 11.72 ± 0.84b | 3.66 ± 0.64c | 10.67 ± 0.77b |
| Folic Acid (mg/g) | 0.61 ± 0.09 | ND | 0.68 ± 0.04 | 0.10 ± 0.01 |
| Vit E (mg/g) | ND | 0.11 ± 0.02 | 0.22 ± 0.01 | 0.16 ± 0.01 |
| Lycopene (mg/ml) | ND | ND | ND | ND |
| Beta carotene (mg/ml) | ND | ND | 0.08 ± 0.02 | 0.10 ± 0.01 |

**ND:** Not detected. Values are mean ± SD (n=2). Values with different superscripts are significantly different (p<0.05).

3.3 Mineral Content

Table 3 displays the result of the mineral analysis in all aqueous Extract of Supernatant and down-layer of *M. Pruriens* and *J. Carnea*. From the result, calcium levels was the highest and significantly higher (p<0.05) in all the layers, and was more present in *Justicia carnea* (down layer). This was followed by Fe in the down-layer of *Justicia carnea.*  Zinc was the least present and was not detected in Mucuna pruriens supernatant. In addition, Co and Cu were not detected in both *Mucuna pruriens* and *Justicia carnea* supernatants.

**Table 3: Mineral Content of Aqueous Extract of Supernatant and Down-layer of *M. Pruriens* and *J. Carnea.***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Minerals (mg/Kg)** | ***Justicia carnea* supernatant** | ***Mucuna pruriens* (supernatant)** | ***Justicia carnea* (down layer)** | ***Mucuna pruriens* (down layer)** |
| Zn | 0.08 | ND | 0.015 | 0.01 |
| Co | ND | ND | 0.11 | 0.13 |
| Ca | 11.20a | 10.80a | 18.12b | 14.34a |
| Mg | 1.24 | 3.21 | 2.76 | 3.51 |
| Fe | 7.63c | 0.58c | 16.55b | 0.92c |
| Cu | ND | ND | 0.04 | 0.08 |
| Na | 6.71 | 5.12 | 1.65 | 1.12 |

**ND:** Not detected. Values with different superscripts across are significantly different (p<0.05).

**4. DISCUSSION**

**4.1 Phytochemical Screening.**

The presence of various bioactive compounds reveals the potential nutritional and therapeutic effect of plants. From this study, the results of the phytochemical screening reveals the presence of Phenol, Flavonoid, Tannin, Saponin, Phytate, Oxalate and Alkaloid in all aqueous extracts; the supernatant and down layer of *Mucuna pruriens* and *Justicia carnea*. These phytochemicals have been shown to possess various medicinal effect inclusive of antioxidant, anti-inflammatory, analgesic, anti-proliferative, anti-cancer, anti-angiogenic, anti-microbial, and anti-viral activity [27].

Phenol was present in high amount in all layers, though it was significantly higher (p<0.05) in the down layer of *Mucuna pruriens*. Phenols are believed to possess significant antioxidant potential, attributed to the hydroxyl groups attached to their phenyl ring, which is thought to confer antioxidative properties [28]. These antioxidants are known to play a significant role in preventing oxidative stress by neutralizing free radicals in the body cells, which is linked to cardiovascular diseases, neurodegenerative diseases and cancer [29-31]. Tannins which are polyphenols were also present though in the least amount in comparison to other phytochemicals detected. Specifically, *Justicia carnea*supernatant (0.17 ± 0.01) was quantified to possess the least tannin content in comparison to the other layerss, with the down-layer of *Mucuna pruriens* (0.85 ± 0.19 mgTAE/ml) being the highest. These tannins exhibit various biological activities, including antioxidant, antibacterial, antiviral, antiparasitic, anti-inflammatory, and antidiabetic effects [32 - 35]. Flavonoid, a polyphenol have also been found to display medicinal properties. They exhibit various beneficial biochemical, anti-oxidative, anti-inflammatory, anti-carcinogenic effects, and also regulate some enzyme function. Xanthine oxidase, cyclo-oxygenase, lipoxygenase, and phosphoinositide 3-kinase are example of these enzymes that are regulated by flavonoid [36 - 37]. The enzymes involved in the inflammatory, oxidative, and proliferative processes which are key players in the pathogenesis of cancer, Alzheimer's disease, and atherosclerosis [38-40]. From the results, flavonoid was present in all the layers, though in higher amount in the supernatant of *Mucuna pruriens*, followed by its down-layer. This shows the potential anti-cancer, neuroprotective, and cardio protective effects of all the layers, with potential higher effect in the supernatant of *Mucuna pruriens*.

Furthermore, alkaloids content was highest in the down-layer extract of *Mucuna pruriens* (1.96 ± 0.12%), followed by *Justicia carnea* down-layer extract (1.42 ± 0.21%), and Oxalate content was significantly higher in the supernatant and down-layer of *Mucuna pruriens* (2.84 ± 0.36 and 2.84 ± 0.24 mg/ml, respectively) in comparison to other layers. These Alkaloids have been reported to exhibit various pharmacological activities, including antihypertensive, antiarrhythmic, antimalarial, and anticancer effects [42]. On the other hand, oxalates have been a concern due to their antinutritive effects and potential nephrotoxicity [43, 44]. Oxalates can bind to minerals, reducing their absorption and bioavailability, and may lead to kidney stone formation, hyperoxaluria, and systemic oxalosis [45 - 50].

The analysis of the vitamin content revealed the varied presence of the vitamins in both layers of the plants. Both supernatant and down-layer aqueous extracts of *M. pruriens* and *J. carnea* revealed the presence of Vitamins A, B1, B2, B6, C, and E, in moderate amounts, and as well in varying quantities. These vitamins are highly needed in the human system, as they play crucial role in human health. From the research findings, vitamin B3 (Niacin) was not detected inthe supernatant of *Justicia carnea,* but quantified the highest in its down-layer. Niacin has been found to aid in the reduction of LDL cholesterol level, thereby reducing the risk of cardiovascular diseases [51]. In contrary, there was also an absence of Vitamin E *in* the supernatant of *Justicia carnea,* with the highest present in the down-layer of *Mucuna pruriens*. Vitamin E (α-tocopherol) is an antioxidant useful in blood vessels formation and boosting of immune function. [52, 53]. The supernatant of *Mucuna pruriens* also revealed total absence of folic acid. Specifically, Folic acid (Vitamin B9) is a water soluble vitamin that aids in vitamins and amino acid metabolism [54], and its absence has been linked to the development of megaloblastic anaemia [55, 56]. Additionally, lycopene was found totally absent in both supernatant and down layer of *Mucuna pruriens* and *Justicia carnea.* Lycopene is known for its antioxidant activity, which provides cellular protection against oxidative damage, regulating immune function, and inhibits the growth of cancer cell [57 - 61]. Though absent in all the layers, its antioxidant effect can be complemented for, by the presence of Vitamin E. Since both lycopene and Vitamin E were absent in the supernatant of *Justicia carnea,* thisstudy reveals that it potentially possesses no antioxidant activity.

Minerals are micronutrients that are essential and cannot be synthesized in the body but obtained from the diet. They are important in the human body for proper building-up, functioning and also in the biochemical processes [62]. Enzymatic activities and electrolyte balance relies deeply on the presence and level of these minerals. As revealed in this study, *Justicia carnea* (supernatant) had a significantly (p<0.05) higher calcium content (18.12 mg/kg) compared to other group of aqueous layers, which is essential for bone growth and development [63]. In the body, calcium also plays a crucial role in cellular signalling pathways, in the regulation of protein kinase C and calmodulin [64]. In the body cells, magnesium, a needed mineral in the regulation of muscular contraction and neuronal excitability [65], and is also involved in the regulation of ATP production and glycolysis [66]. From the result of this study, Mg was found present in all the layers, with higher levels in the supernatant of *Mucuna pruriens* (3.2 mg/kg). Iron is necessary for the formation of heme proteins, including hemoglobin and myoglobin [67]. It is also involved in the regulation of oxygen transport and energy production through the electron transport chain. From the results, iron content was also noticed to be higher in both supernatant and down-layer of *Justicia carnea*, but in very low levels in both *Mucuna pruriens* layers. Additionally, *Mucuna pruriens* and *Justicia carnea* may also aid in cell-mediated immunity and bone formation due to the presence of zinc. Zinc has also been found to be involved in the regulation of protein synthesis and wound healing through the activation of transcription factors and the regulation of collagen synthesis. [68]

**5.0. CONCLUSION**

The findings of this study revealed that *Mucuna pruriens* and *Justicia carnea* are good sources of vital phytochemicals, minerals and vitamins that are nutritional and therapeutically valuable. *Mucuna pruriens* may provide higher phytochemical levels, majorly in its down-layer, particularly for compounds like total phenol, flavonoid, and alkaloid. Additionally, the down-layer of the *Justicia carnea* may provide higher vitamins and mineral levels when utilized. However, these phytochemicals are evenly distributed in both layers but some of the vitamins and minerals were found only in some layers.

Authors Contributions:

Author ONA conceived the work, wrote the protocol, designed the study and performed the statistical analysis. Author EOC managed the literature searches and wrote the first draft of the manuscript. Authors IIIU and CEA managed the analyses of the study. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**REFERENCES**

1. Subhashini RUS, Mahadeva R, Sumathi P, Gayathri, G. A comparative phytochemical analysis of cocoa and green tea. Indian J. Sci. Tech. 2010; 2(3),
2. Sharma BR, Kumar V, Gat Y, Kumar N, Parashar A, Pinakin DJ. Microbial maceration: A sustainable approach for phytochemical extraction. Biotech. 2018; 8:401.
3. Jaeger R, Cuny E. Terpenoids with special pharmacological significance: A review. Nat. Prod. Commun. 2016; 11(9):1373-1390.
4. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. A’fr.J. Biotechnol. (2005). 4(7): 685-688.
5. Rasheed H, Shehzad M, Rabail R, Kowalczewski P, Kidoń M, Jeżowski P, Ranjha MMA, Rakha A, Din A, Aadil RM. Delving into the Nutraceutical Benefits of Purple Carrot against Metabolic Syndrome and Cancer. A Review. Appl. Sci. 2022; 12:3170.
6. Khalid W, Gill P, Arshad MS, Ali A, Ranjha MMAN, Mukhtar S, Afzal F, Maqbool Z. Functional Behavior of DHA and EPA in the Formation of Babies Brain at Different Stages of Age, and Protect from Different Brain-Related Diseases. Int. J. Food Prop. 2022; 25:1021–1044.
7. Mutch D, Wahli W, Williamson G. Nutrigenomics and Nutrigenetics: The Emerging Faces of Nutrition. FASEB J. 2005; 19:1602–1616.
8. Lucia RL, Alessio C, Roberto G, Claudia S, Giuseppe V. The Magic Velvet Bean of *Mucuna pruriens*. J Tradit Complement Med. 2011; 2(4):331-339.
9. Anarado CE, Ajiwe VIW, Anarado CJO, Obumselu OF, Umedum NL, Okafor SE. The phytochemisty, ethnomedicinal and pharmacologicy uses of *Justicia carnea* Linddl used in traditional medicine in Nigeria - A review. South Asian Res. J. Nat. Prod. 2021; 4(4) 85-93.
10. Buckles D. Velvet bean (*Mucuna pruriens* ): A “new” plant with a history. Economic Botany. 1995; 49(1): 13-25.
11. Warrier PK, Nambiar VKP, Ramankutty C. Indian medicinal plants, Vol.4 (Orient Longman, Chennai. 1996; pp.68-72.
12. Nadkarni KM. Indian plants and drugs with their medical properties and uses. Asiatic publishing House, Delhi. 2001; pp.242-243.
13. Corea GM. Chemical consistent and biological activities or species of Justicia: A review. Brazitia phamarcognosis 2012; 22:220 238.
14. Badami S, Aneesh R, Sankar S, Sathishkumar MN, Suresh B, & Rajan S. Antifertility activity of Derris brevipes variety coriacea. J Ethnopharmacol. 2003; 84:99 - 104.
15. Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, Baptista P. Total phenols, ascorbic acid, β-carotene and lycopene in portuguese wild edible mushrooms and their antioxidant activities. Food Chem. 2007; 103(2):413-419
16. AOAC. Official Methods of Analysis, 15th edn. Association of Official Analytical Chemists, Arlington, VA. 1990.
17. AOAC. Official methods of analysis, 16th edn. Association of Official Analytical Chemists. Arlington, V. A. USA. 1995.
18. Young SM., Greaves JS. Influence of variety and treatment on phytic acid content of wheat. J. Food Res. 1940 5: 103-105
19. Osagie, AU. Antinutritional Factors. In: Nutritional Quality of Plant Foods. Ambik Press Ltd, Benin City, Nigeria. 1998;1-40:221-244.
20. Harborne JB. Methods of plant analysis. In: Phytochemical Methods. Chapman and Hall, London. 1973
21. Rutkowski M, Grzegorczyk K, Paradowski MT. Colorimetricmethod of blood plasma total vitamin E determination – the own modification of Tsen method*. Diagn. Lab.* 2005; 41: 375
22. Kirk R, Sawyer R. Perason’s composition and analysis of foods, 9th edition. Longman Scientific and Technical. 1991; pp 647-648.
23. Padmarajaiah N, Ramanathapura AV, Hemmige SY. Spectrophotometric determination of folic acid in pharmaceutical preparations by coupling reactions with iminodibenzyl or 3-aminophenol or sodium molybdate-pyrocatechol. Anal Biochem. *2002;* 307(2):316-321
24. Raeed MQ, Azzam AM. Spectrophotometric Assay of Pyridoxine Hydrochloride (Vitamin B6) in Pharmaceutical Preparations and Serum Via Arsenazo III- Cerium (III) Reaction. *Raf. Jour. Sci.* 2008; 19(2):28 – 41.
25. Klein BP, Perry AK. Ascorbic acid and vitamin A activity inselected vegetables from different geographical areas of theUnited States. *J Food Sci*. 1982; 47: 941-945
26. AOAC. Association of Analytical Chemistry. Methods for Mineral Analysis. 2003; Pp 2319-2331.
27. Ngoci SN, Mwendia CM, Mwaniki CG. Phytochemical and cytotoxicity testing of Indigofera lupatana Baker F. J. Anim Plant Sci. 2011; 11(1), 1364-1373.
28. Hollman PCH. Evidence for health benefits of plant phenols: local or systemic effects? J Sci Food Agric. 2001; 81: 842 852
29. Forman HJ, Zhang H. Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nat. Rev. Drug Discov.* 2021; *20*: 689 - 709.
30. Rudrapal M, Khairnar SJ, Khan J, Bin Dukhyil A, Ansari MA, Alomary MN, Alshabrmi, FM, Palai S, Deb PK, Devi R. Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action. Front.Pharmacol.2022; *13*, 806470.
31. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. *Nutr. J.* 2016; *15*, 71.
32. Lall RK, Syed DN, Adhami VM, Khan MI, Mukhtar H. Dietary polyphenols in prevention and treatment of prostate cancer. Int J Mol Sci. 2015; 16:3350–76.
33. Minho AP, Gennari SM, Amarante AFTD, Ab Dala AL. Anthelmintic effects of condensed tannins on Trichostrongylus colubriformis in experimentally infected sheep. Semina Ciências Agrárias. 2010; 31:1009–16.
34. Wijesinghe W, Ahn G, Lee WW, Kang MC, Kim EA, Jeon YJ. Anti-inflammatory activity of phlorotannin-rich fermented Ecklonia cava processing by-product extract in lipopolysaccharide-stimulated RAW 264.7 macrophages. J Appl Phycol. 2013; 25:1207–13.
35. Bonelli F, Turini L, Sarri G, Serra A, Buccioni A, Mele M. Oral administration of chestnut tannins to reduce the duration of neonatal calf diarrhea. BMC Vet Res. 2018; 14:227.
36. Metodiewa D, Kochman A, Karolczak S. Evidence for antiradical and antioxidant properties of four biologically active N, N, diethylaminoethyl ethers of flavanone oximes: a comparison with natural polyphenolic flavonoid (rutin) action. Biochem Mol Biol Int. 1997; 41: 1067–1075.
37. Hayashi T, Sawa K, Kawasaki M, et al. Inhibition of cow's milk xanthine oxidase by flavonoids. J Nat Prod. 1988; 51: 345–348.
38. Walker E, Pacold M, Perisic O, et al. Structural determinations of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. Mol Cell. 2000; 6: 909–919.
39. Burak M, Imen Y. Flavonoids and their antioxidant properties. Turkiye Klin Tip Bil Derg. 1999; 19, 296–304.
40. Ovando C, Hernandez D, Hernandez E, et al. Chemical studies of anthocyanins: a review. Food Chem. 2009; 113, 859–871.
41. Lee Y, Yuk D, Lee J, et al. Epigallocatechin-3-gallate prevents lipopolysaccharide-induced elevation of β-amyloid generation and memory deficiency. Brain Res. 2009; 1250: 164–174.
42. Saxena M, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of Medicinal Plants. J Pharmacogn Phytochem. 2013; 8192 (1): 168-182.
43. Fatoki OS. Determination of Oxalic Acid in Vegetable. In: Linskens, H.F., Jackson, J.F. (eds) Vegetables and Vegetable Products. Modern Methods of Plant Analysis., vol 16. Springer, Berlin, Heidelberg, 1994; pp 161-167
44. Singh PP, Kothari LK, Sharma DC, Saxena SN. Nutritional Value of Foods in Relation to Their Oxalic Acid Content. Am. J. Clin. Nutr. 1972; 25:1147–1152.
45. Petroski W, Minich DM. Is There Such a Thing as “Anti-Nutrients”? A Narrative Review of Perceived Problematic Plant Compounds. Nutrients. 2020; 12:2929
46. Huynh NK, Nguyen DHM, Nguyen HVH. Effects of Processing on Oxalate Contents in Plant Foods: A Review. J. Food Compos. Anal. 2022; 112:104685.
47. Wang Z, Zhang Y, Zhang J, Deng Q, Liang H. Recent Advances on the Mechanisms of Kidney Stone Formation (Review) Int. J. Mol. Med. 2021; 48:149.
48. Witting C, Langman CB, Assimos D, Baum MA, Kausz A, Milliner D, Tasian G, Worcester E, Allain M, West M, et al. Pathophysiology and Treatment of Enteric Hyperoxaluria. Clin. J. Am. Soc. Nephrol. 2021; 16:487-495.
49. Sharma S, Rao RN, Pani KC, Paul P. Bone Marrow Oxalosis: An Unusual Cause of Cytopenia in End-Stage Renal Disease; Report of Two Cases. Indian J. Pathol. Microbiol. 2018; 61:268–270.
50. Fogo AB, Lusco MA, Najafian B, Alpers CE. AJKD Atlas of Renal Pathology: Oxalosis. Am. J. Kidney Dis. 2017; 69:e13–e14.
51. Lule VK, Garg S, Gosewade SC, Tomar SK. Niacin. In: Caballero B, Fingelas P, Toldra F (eds) The Encyclopedia of food and health, vol 4. Academic, Oxford. 2016; pp 63–72
52. Bellizzi MC et al. Vitamin E and coronary heart disease: the European paradox. Eur J Clin Nutr. 1994; 48: 822–831
53. WHO/FAO. Handbook on vitamin and mineral requirements in human nutrition, 2nd ed. 2004.
54. Kunisawa J, Hashimoto E, Ishikawa I, Kiyono H. A pivotal role of Vitamin B9 in the maintenance of regulatory T cells In Vitro and In Vivo. PLoS One. 2012; 7(2):e32094.
55. Lykstad J, Sharma S (2019) Biochemistry, Water Soluble Vitamins. Treasure Island (FL): StatPearls Publishing; 2019.
56. Ankar A, Kumar A. Vitamin B12 Deficiency (Cobalamin). Treasure Island (FL): StatPearls Publishing; 2019.
57. Rao AV, Ray MR, Rao LG. Lycopene. Adv Food Nutr Res. 2006; 51:99-164.
58. Agarwal S, Rao AV. Tomato lycopene and its role in human health and chronic diseases. CMAJ. 2000; 163:739–744.
59. Palozza P, Simone R, Catalano A, Boninsegna A, Böhm V, Fröhlich K, Mele MC, Monego G, Ranelletti FO. Lycopene prevents 7-ketocholesterolinduced oxidative stress, cell cycle arrest and apoptosis in human macrophages. J Nutr Biochem. 2010; 21:34–46.
60. Rao LG, Mackinnon ES, Josse RG, Murray TM, Strauss A, Rao AV. Lycopene consumption decreases oxidative stress and bone resorption markers in postmenopausal women. Osteoporosis Int. 2007; 18:109–115.
61. Palozza P, Colangelo M, Simone R, Catalano A, Boninsegna A, Lanza P, Monego G, Ranelletti FO. Lycopene induces cell growth inhibition by altering mevalonate pathway and Ras signaling in cancer cell lines. Carcinogenesis. 2010; 31:1813–1821.
62. Zhao A, Xue Y, Zhang Y, Li W, Yu K, Wang P. Nutrition concerns of insufficient and excessive intake of dietary minerals in lactating women: a cross-sectional survey in three cities of China. PLoS One. 2016; 11(1):e0146483.
63. Matkovic V, Ilich JZ. Calcium requirements for growth: are current recommendations adequate? Nutr Rev. 1993; 51(6):171–180
64. McCarron DA, Reusser ME. Finding consensus in the dietary calcium-blood pressure debate. J Am Coll Nutr. 1999;18(sup5):398S–405S
65. Allen MJ, Sharma S. Magnesium. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2019.
66. Gragossian A, Friede R. Hypomagnesemia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019.
67. Fairweather-Tait S, Hurrell RF. Bioavailability of minerals and trace elements: Members of EC flair concerted action no. 10: Measurements of micronutrient absorption and status. Nutr Res Rev. 1996; 9(1):295–324
68. Bagherani N, Smoller BR. An overview of zinc and its importance in dermatology- Part I: importance and function of zinc in human beings. Glob Dermatol. 2016; 3(5):330–336