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The effect of cranberry juice on bone growth and development using an animal model

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The aim of the study was to analyze the effect of cranberry juice on bone microstructure. The research was carried out on an animal model (experimental animals, Windstar rats). The structure of the proximal tibial metaphysis was analyzed using micro CT, and X-ray microtomography equipment with a VDS 1.3Mp FW camera. It was found that cranberry juice increased bone mineral density (BMD). It also increased the thickness of the bone trabeculae (Tb.Th). The highest BMD value was observed in group drinking a 10% concentration of cranberry juice. In rats drinking 25% concentration, an increase in Tb.Th was observed compared to the two other groups, including the control group.

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Cranberry (*oxycoccus*) is a widely spread plant of the heath family (*Ericineae*). There are two main varieties of it, which are the large-fruited cranberry (Vaccinium macrocarpon) and the bog cranberry (*Vaccinium oxycoccos*). The genus Vaccinium in the family Ericaceae includes more than 450 species growing in North America, Central America, Europe, Asia, Japan, Central and Southeast Africa and Madagascar. However, the large-fruited cranberry in particular is cultivated almost all over the world [Jurikowa *et al*. 2019, Adamczak *et al*. 2011, Pappas *et al*. 2009, Baranowska *et al*. 2016]. Importantly, it is known not only for its organoleptic but also for its health-promoting properties, as reflected in a centuries-old tradition of use in folk medicine. For years, the juice of this plant has been used for urinary tract infections and the efficacy of this treatment is emphatically confirmed by recent studies [Harris 2023, Xia *et al*. 2021, Meena *et al*. 2021]. The juice's effects on cardiovascular disease, diabetes, cancer, obesity, metabolic syndrome, Helicobacter pylori bacterium, tooth decay or periodontitis, among others, are also being studied [Nemzer *et al*. 2022].

The unique composition of a cranberry fruit: the presence of not only vitamins and minerals but also phytochemicals, i.e. polyphenols, i.e. anthocyanins, phenolic acids and flavonoids, as well as alkaloids, carotenoids, nitrogen-containing compounds and organosulfur compounds accounts for its healing effect. D-mannose, an epimer of glucose, contained in cranberries and their derivatives has beneficial effects on the human body. The lack of mannose receptors negatively affects bone marrow and increases the risk of inflammation [Nour et *al*. 2022]. In addition, cranberry juice contains proanthocyanidins, which are not found in many fruits, which may distinguish this plant [Pappas *et al*. 2009, Nemzer *et al.* 2022]. Phytonutrients have great potential for a future therapeutic application in modulating bone health, and have many advantages over modern conventional pharmaceutical preparations, including low cost and least negative side effects [Huminiecki *et al*. 2017, 2018, Tewari *et al*. 2017, Mozos *et al*. 2018, 2021, Wang *et al*. 2018, 2020, Pieczyńska *et al*. 2020, Yeung *et al*. 2018, 2019, 2020abc, 2021ab, 2022, 2023, Zaffar *et al*. 2020, Li *et al*. 2021, Chopra *et al*. 2022].

However, although cranberries and their preparations, i.e. cranberry juice among others, are increasingly the subject of research in many other areas than just the above, there is a lack of literature data that deals with the effects of cranberry juice on bone growth and development. This is a topic of great importance given the high frequency of diagnoses of bone diseases, i.e. osteoporosis, osteomalacia or rickets, among others. Osteoporosis is a widespread bone disease that increases the risk of bone fractures through deterioration of bone microarchitecture, reduced density and loss of bone mass [Cooper *et al*. 2008]. For instance, an estimated 10 million Americans over the age of 50 have osteoporosis, and another 34 million are at risk [Clynes *et al*. 2020]. Osteomalacia and rickets are also common bone diseases with defective mineralization as their common denominator. These diseases, moreover, can occur simultaneously [Gu *et al*. 2023]. With the above in mind, it is reasonable to look for and study various factors that can potentially improve bone growth, development and composition to enhance the health and quality of life of millions of people worldwide.

Consequently, the purpose of this study was to investigate the effects of cranberry juice as a potentially beneficial factor in bone growth and development. The paper presents pioneering research and is the first publication to closely examine and describe this area.

Material and methods

Wistar (male) rats aged 10 weeks were kept in cages meeting the statutory requirements (in concordance with the Law Gazette of 2006, No 50 item 368). Method of killing animals: decapitation, after anaesthesia. Method of anaesthesia: 1.) agent: ketamine; method of administration: intraperitoneally; dose 100 mg/kg body weight; 2) xylazine, method of administration: intraperitoneally, dose 10 mg/kg body weight; 3) sodium pentobarbital, route of administration: intraperitoneal: 200 mg/kg body weight. Efforts to alleviate suffering: anaesthesia. The animals were kept under standard farm conditions with constant access to fodder and water in controlled environment conditions, which meant a stable temperature of 23±2°C, regular light cycle 12/12 hours, air humidity of 40-60% and 20 air exchanges in the farm room. The animals were fed standard fodder (Altromin Spezialfutter GmbH & Co) dedicated to the maintenance of animals with an energy value of 3625.85 kcal/kg.

After a 2-week quarantine, the animals were divided into 3 experimental groups (6 individuals each). The groups differed in the concentration of cranberry juice administered.

- Group 1 (Control) received only water,
- Group 2 received cranberry juice with a concentration of 10%, and
- Group 3 received cranberry juice with a concentration of 25%.

After two months of the experiment, the animals were euthanised and study material was harvested. All the procedures were conducted in conformance with institutional guidelines as well as national and international laws and policies. The study was approved by the appropriate Local Ethics Committee for Experimentation on Animals based in Warsaw. The reference document is Resolution No 27/200.

Throughout the experiment, the same environmental conditions were maintained and the same feed as during the quarantine period was maintained.

The proximal end of the left tibia was the subject of the analysis. The measurement site was determined separately for each bone. To examine bone structure Skyscan 1174 X-ray microtomograph (Bruker, Belgium) with a VDS 1.3 Mp FW camera was used. The resolution of the scan was 1024 x 1024 (Im-age pixel size: 21.74 µm). The intensity and voltage of the lamp were 50 KV and 850 µA, respectively. The exposure duration was 2400 ms. The rotation angle was 0.7° with 3 frames per rotation and the aluminium filter was 0.5 mm thick. The scanning duration was 50 minutes. To analyse the designated tibia part appropriate ROIs were distinguished. The following software was used for the studies: Software=Version 1.5 (build 7), NRecon Version 1.7.4.2 reconstruction software, CT Analyser, version: 1.18.4.0 software.

The following osseous tissue morphological parameters were analysed: 3D morphometric parameters integrated for the whole volume of interest (VOI), BMD (Bone Mineral Density, mm^3), BS/BV (bone surface/volume ratio, mm^{-1}), Tb.Th. (trabecular thickness, mm), TB.N (trabecular number), TB.Sp (trabecular separation, mm), VOI (Total volume of the volume of interest, tissue volume). The 3D volume measurement is based on the marching cubes (volume model of the VOI mm^3).

Statistical analysis of the findings

The results were statistically processed by means of one-way analysis of variance ($p \leq 0.05$). The significance of differences was checked using Tukey's test at $p \leq 0.05$. Before starting the analysis of variance, it was checked with the Bartlet's and Leven's tests whether the assumptions for using this analysis were met and the normality of the distribution was also assessed. For each of the features, these assumptions were met.

The r-Pearson correlation coefficient was used to examine the relationship between bone parameters and body weight. Regression equations were calculated for the correlated variables.

All calculations were done using STATISTICA 13.0 software.

Results and discussion

Statistical analyzes have shown that the administration of cranberry juice increases bone mineral density (BMD). Juice administration in both 10% and 25% concentrations significantly increased BMD compared to the control group. The highest BMD was obtained in the group 2 that received 10% cranberry juice (0.957 mm^3), compared to 0.770 mm^3 in the control group. These differences were statistically significant

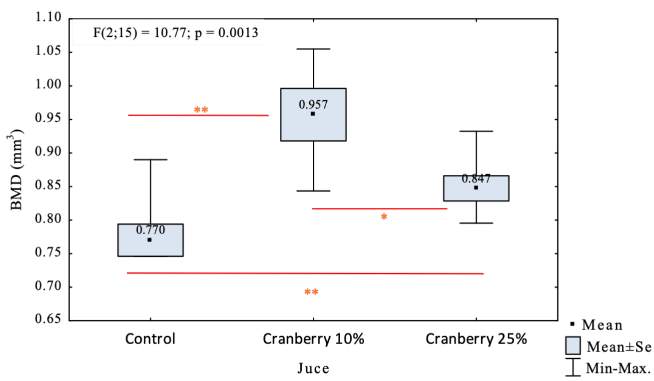

Fig 1. Mean values of bone mineral density (BMD) depending on juice concentration (F- value of the F test obtained in ANOVA; *- significant differences between the means at $p \leq 0.05$; **- significant differences between the means at $p \leq 0.01$).

(p≤0.01). Interestingly, in group 3 with 25% juice solution, BMD was significantly lower (0.847 mm^3) compared to animals that received 10% concentration, but still higher than in the control group (0.770 mm^3) - Figure 1.

Cranberry juice also increased the thickness of the bone trabeculae (Tb.Th). Tb.Th in individuals consuming 25% juice was the greatest (5,018 mm) and the smallest in the control group (4,238 mm). These differences were statistically significant. Figure 2 shows that Tb.Th in group 2 on 10% cranberry juice was also greater than in the control group (4,749 mm), but these differences were not statistically confirmed. There was also no statistical difference in Tb.Th between the individuals from group 3 with 25% cranberry juice (5.018 mm) and from group 2, which was dosed with 10% juice (4.749 mm).

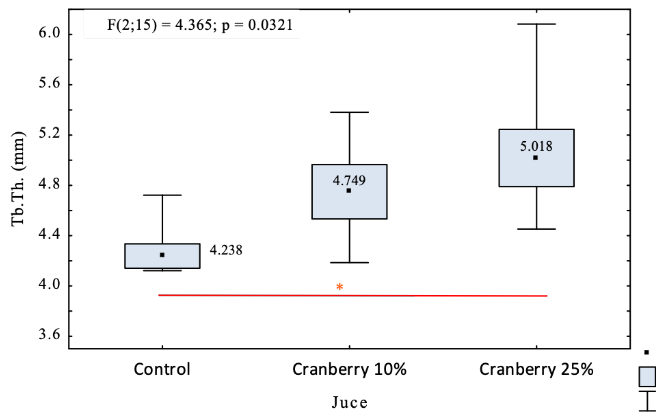

Fig 2. Average values of Tb.Th. depending on the type of juice served (F-value of the F test obtained in ANOVA; *-significant differences between the means at p≤0.05).

Statistical analysis showed that the distance (mm) between the trabeculae (Tb. Sp.) did not differ between the control group and the cranberry juice-treated groups (F=1.178) - Figure 3. However, some trends can be observed. With increasing concentration of cranberry juice Tb.Sp. decreased slightly.

As in the case of the Tb.Sp. parameter, cranberry juice did not significantly affect the number of trabeculae (Tb.N) parameter. Although Tb.N was the highest (0.025 mm^{-1}) in the bones of the individuals from group 3 consuming juice with a concentration of 25%, it did not differ statistically from those observed in the control group (0.024 mm^{-1}) or group 2 consuming juice with a concentration of 10% (0.024 mm^{-1}) - Figure 4.

The highest value of the bone surface/volume ratio (BS/BV) parameter was obtained after using cranberry juice with a concentration of 10% (0.434 mm^{-1}). This value was significantly higher than in the control group (0.333 mm^{-1}). Cranberry juice with a 25%-concentration also increased the value of this parameter compared to the

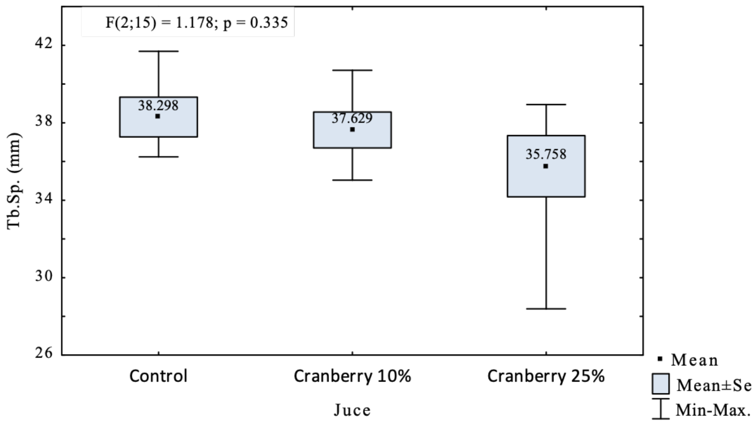

Fig 3. Mean Tb. Sp. values depending on the type of juice (F- F test value obtained in ANOVA).

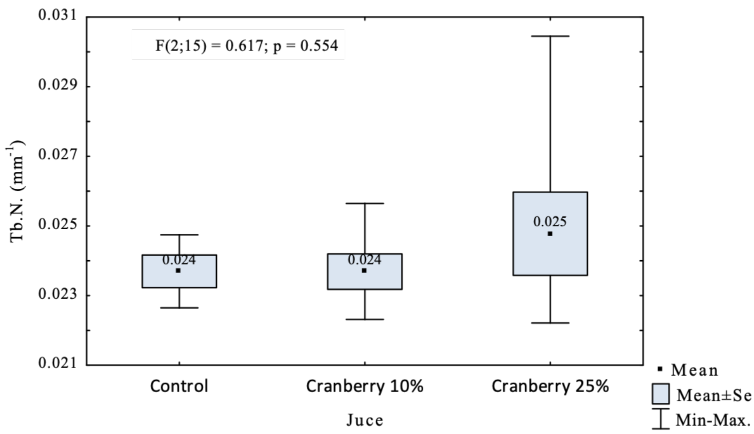

Fig 4. Average Tb.N values. depending on the concentration of juice (F- F test value obtained in ANOVA)

control (0.402 mm^{-1}). The BS/BV in the 10% and 25% cranberry juice groups was not statistically different (Fig 5).

Consumption of cranberry juice did not significantly affect the body weight of the animals. The body weight of individuals from the individual study groups was similar and ranged from 119.833 g (control group) to 121.667 g in the group receiving 25% cranberry juice (Fig. 6).

Table 1 presents the values of correlation coefficients between bone parameters and body weight for the control group receiving only water. In Table 2, the regression equations for the correlated variables are listed. The analysis showed a positive correlation between Tb.N and the BS/BV parameters and a negative correlation

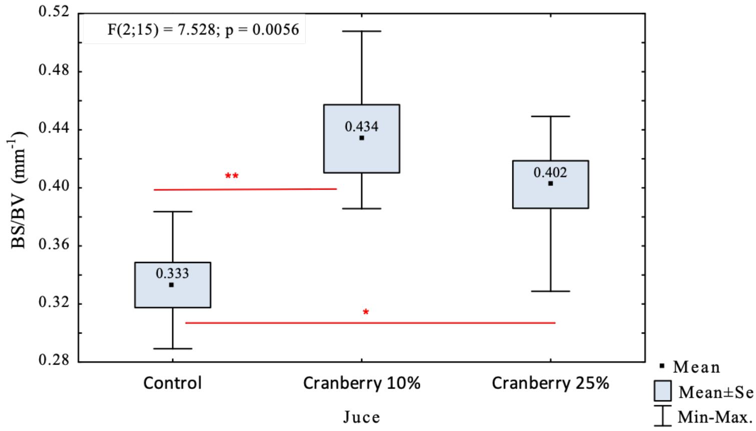

Fig 5. Average values of the BS/BV parameter depending on the type of juice served (F-value of the F test obtained in ANOVA; *-significant differences between the means at p≤0.05; **-significant differences between the means at p≤0, 01).

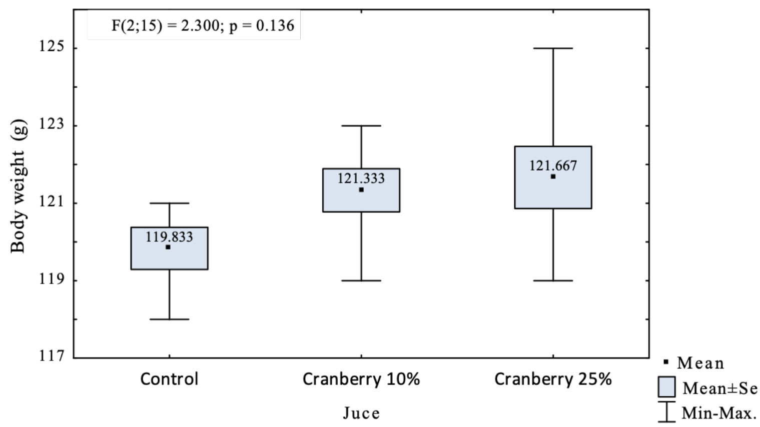

Fig 6. Mean values of body weight depending on the type of juice served (F-value of the F test obtained in ANOVA).

between body weight and Tb.N (Tab. 1). With an increase in BS/BV ratio by 1 mm^{-1}, Tb.N increased by 0.025 on average. On the other hand, an increase in body weight by 1 g resulted in a decrease in the number of trabeculae by 0.008 (Tab. 2).

In group 2 with cranberry juice concentration of 10%, the relationship between body weight and BMD as well as between Tb.N and Tb.Sp was proved. Table 3 shows it was a negative relationship. An increase in body weight by 1 g resulted in a decrease in bone density by 0.057 mm^3, while an increase in the number of trabeculae by one unit resulted in a decrease in the distance between them by 6.31 mm (Tab. 4).

Table 1. Values of correlation coefficients between bone parameters for the control group

Control	BMD	BS/BV	Tb.TH	Tb.N.	Tb.SP.	Body weight
BMD	1.000					
BS/BV	-0.050	1.000				
Tb.TH	-0.173	0.208	1.000			
Tb.N	-0.447	**0.878***	0.388	1.000		
Tb.SP	0.573	0.366	-0.335	0.171	1.000	
Body weight	0.430	-0.804	-0.626	**-0.962****	-0.045	1.000

*Significant relationship at $p \leq 0.05$; **significant relationship at $p \leq 0.01$.

Table 2. Regression equations describing the relationship between BS/BV and Tb.N and body weight and Tb.N in the control group

Dependent variable	Independent variable	Regression equation
Tb.N	BS/BV	y = 0.014 + 0.025x
Body weight	Tb.N	y = 0.123 – 0.0008x

Table 3. Values of correlation coefficients between bone parameters for the group administered cranberry juice with a concentration of 10%

Control	BMD	BS/BV	Tb.TH	Tb.N.	Tb.SP.	Body weight
BMD	1.000					
BS/BV	0.304	1.000				
Tb.TH	0.186	0.369	1.000			
Tb.N	-0.029	0.166	-0.017	1.000		
Tb.SP	0.266	0.155	0.308	**-0.895***	1.000	
Body weight	**-0.820***	-0.053	-0.241	-0.285	0.164	1.000

*Significant relationship at $p \leq 0.05$.

Table 4. Regression equations describing the relationship between Tb.N and Tb.Sp as well as body weight and BMD in group 2 receiving 10% cranberry juice

Dependent variable	Independent variable	Regression equation
BMD	Body weight	y = 7.94 – 0.057x
Tb.Sp	Tb.N.	y = 76.28 – 6.31x

In subjects with 25%-cranberry-juice concentration, a significant negative relationship between the BS/BV parameter and BMD was observed. (Tab. 5). If BMD increased by one unit, the bone surface area to volume ratio decreased by 0.87mm^{-1} (Tab. 6). BMD was positively correlated with Tb.Th (Tab. 5). With an increase in

Table 5. Values of correlation coefficients between bone parameters for the group receiving 25% cranberry juice

25%	BMD	BS/BV	Tb.TH	Tb.N.	Tb.SP.	Body weight
BMD	1.000					
BS/BV	**-0,999****	1.000				
Tb.TH	**0.998****	**-0.995****	1.000			
Tb.N	-0.686	0.702	-0.648	1.000		
Tb.SP	0.642	-0.657	0.606	**-0.996****	1.000	
Body weight	0.223	-0.235	0.196	-0.732	0.771	1.000

**Significant relationship at $p \leq 0.01$.

Table 6. Regression equations describing the relationship between Tb.N and Tb.Sp as well as body weight and BMD in group 2 receiving 10% cranberry juice

Dependent variable	Independent variable	Regression equation
BS/BV	BMD	y = 1.142 – 0.873x
BMD	Tb.Th.	y = 0.435 + 0.082x
BS/BV	Tb.Th.	y = 0.761 – 0.0716x
Tb.Sp	Tb.N.	y = 76.12 – 7.16x

Tb.Th by 1 mm, their density increased by 0.082 mm^3 (Tab. 6). However, Tb.Th was negatively correlated with the BS/BV parameter (Tab. 5). An increase of 1 mm in Tb.Th resulted in a decrease of 0.071 mm^{-1} in BS/BV (Tab. 6). As in group 2, it was observed that there is a significant relationship between the number of barrels and the distance between them. Increasing the number of trabeculae by one unit reduced the distance between them by 7.16 mm (Tab. 5 and 6).

The results obtained are pioneering and shed new light on another property of cranberry juice, as they show for the first time its effect on bone growth and development. (Fig. 7). We did not have similar studies conducted before, especially because our work focused on multiple facets of the influence of cranberry juice on bones. We examined not only its impact on bone mineral density, but also on bone trabeculae thickness (Tb. Th), trabeculae separation (Tb.Sp), number of trabeculae (Tb.N) or the ratio of bone surface area to bone volume (BS/BV). No such publications are currently available. Counterintuitively, while we showed that 25% concentration had a better effect on increasing Tb.Th compared to the 10% group and the control group (which seems quite intuitive), the 10% concentration had a better effect on BMD compared to the 25% concentration. This finding suggests that perhaps the amount of each compound alone (reflected in the concentration value) is not responsible for the effect and that there is a more multifaceted range of factors at play. Further investigation should seek to clarify and confirm this proposition.

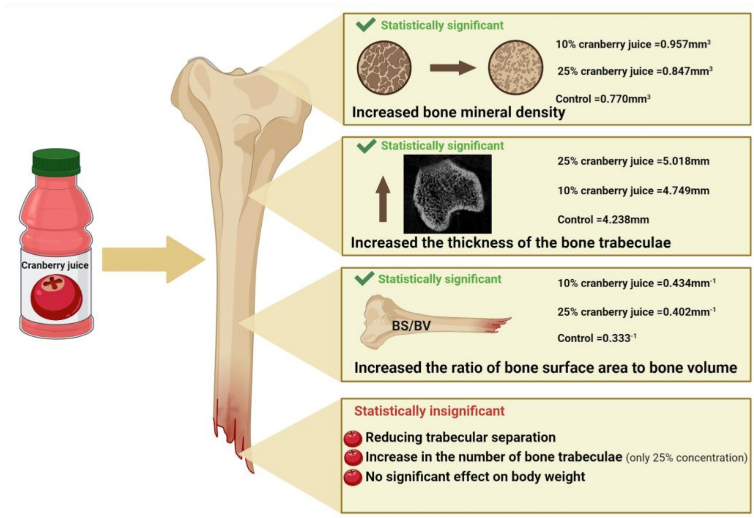

Fig. 7. Effect of cranberry juice on bone growth and development.

Despite the current lack of data on the direct effects of cranberry juice on bone growth and development, there is indirect evidence worth citing to put our results in context. Gu *et al*. in a recent 2023 study showed an interesting effect of D-mannose, which is abundant in cranberries. The authors studied the effects of D-mannose, which was administered intragastrically to rats in a weightless state. The goal was to evaluate D-mannose as a potential antiresorptive drug to prevent bone mass loss during astronauts' space missions [Gu *et al*. 2023]. They found that D-mannose improves BMD and inhibits osteoclast proliferation and fusion. The authors indicate that this may be a potential strategy to protect against bone mass loss. Vivian Alonso-Garcia's research confirms the role of mannose in treating fractures, thereby increasing bone density [Vivian *et al*. 2020]. D-mannose given with drinking water can even mitigate periodontal bone loss [Yang *et al.* 2023]. This overlaps with the results of our study, as both studies showed an increase in BMD. Another study also showed a protective effect of D-mannose. The authors studied its effects on preventing and treating osteoporosis in mice. They found that D-mannose inhibited bone loss caused by ageing and oestrogen deficiency. Supplementation with D-mannose increased the volume of cortical bone and the microarchitecture of trabecular bone. The authors suggest that these effects may take place through the proliferation of regulatory T cells and microbiota-dependent anti-inflammatory effects [Liu *et al.* 2020]. Importantly, given the composition of cranberry juice, it is D-mannose that seems to hold the most promise in the context of bone health. In addition, it may also be helpful in osteoarthritis, even to such an extent that the authors of the publication suggest that it may represent a new therapeutic agent for treating the disease. Thus, current research

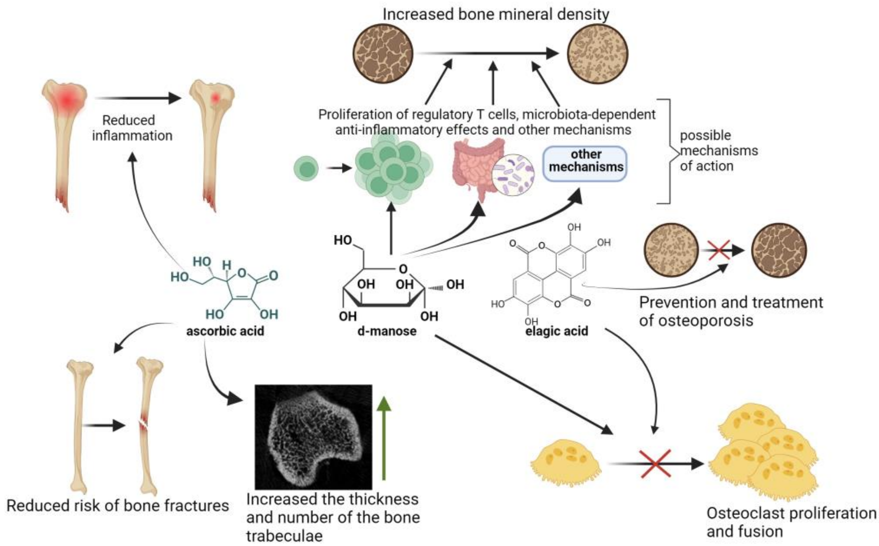

Fig. 8. Potential mechanisms of action of d-mannose, vitamin C and elagic acid on bones.

focuses mainly on this monosaccharide and directly suggests it may mitigate bone loss. Importantly, given our puzzling results, in which the best effect on bone mineral density was shown by a 10% concentration of cranberry juice, in the cited study, the average dose of d-mannose was also found to be more effective in inhibiting the development of osteoarthritis compared to a higher and lower dose [Lin *et al*. 2021].

Consequently, it is possible that D-mannose in cranberry juice is mainly responsible for our results. Future studies should examine this assumption.

While the effect of the medium concentration of cranberry juice (and the medium concentration of D-mannose) is most efficient in increasing BMD, our highest juice concentration has a better effect on improving the thickness and Tb.N Many compounds contained in cranberry juice, including mainly vitamin C, may be responsible for the improvement in Tb.Th This vitamin is worth looking into, as it has a beneficial effect on bones (including reducing their susceptibility to fracture) and is involved in the production of collagen, which is their integral part [Viguet *et al*. 2006, Dephillipo *et al*. 2018]. Vitamin C deficiencies have been shown to reduce the number and thickness of trabeculae in bone. Specifically, our study showed that the highest concentration of cranberry juice, and so the highest content of vitamin C, significantly increased Tb.Th and also Tb.N (although this change was not statistically significant) - Table 8. Just as other authors showed that vitamin C deficiency increases the spacing of the tibial bone trabeculae [Aghajanian *et al*. 2015], in our study we showed that as the concentration of cranberry juice (thus the vitamin C content) increased, the distance between the trabeculae decreased slightly. In view of this, vitamin C may be responsible for the effect on bone trabecular thickness, although certainly not solely, given the richness of cranberry juice composition.

Reports on the effects of proanthocyanidins, which are also present in cranberries, imply they may prevent alveolar bone destruction and connective tissue breakdown. Feghali *et al.* [2012] suggest their anti-inflammatory property, inhibition of bacterial proteolytic enzymes, osteoclast activity and differentiation may contribute to the findings.

Apart from studies indirectly confirming our results, a publication by Villareal et al. found no such effects. The authors evaluated the effect of drinking cranberry juice on bone quality in rats undergoing orchidectomy. In this case, cranberry juice did not affect bone mineral status and quality, although it did increase plasma antioxidant status. It is worth noting, however, that these animals were not healthy individuals as in our study, but the ones that had undergone orchidectomy [Villarreal *et al*. 2007]. Thus, it should be kept in mind that this factor may make the results of the study differ.

Other components of cranberry juice that could potentially be responsible for beneficial effects on bone growth and development include ellagic acid, which cranberries are rich in. The properties of this ingredient have been studied for years, and its therapeutic effect in the treatment and prevention of osteoporosis has been directly described. This is because ellagic acid affects the weakening of osteoclast formation and function [Lin *et al*. 2020]. Studies suggest that the beneficial effect is due to, among other things, blocking the interaction of receptor activator of nuclear factor (NF)-κB ligand (RANKL) and its receptor RANK, resulting in blocking osteoclastogenesis [Xu *et al*. 2020]. Antioxidants present in cranberry juice may also have a beneficial effect. Indeed, it has been shown that there is a lower risk of osteoporosis if the total antioxidant capacity (TAC) of the body is higher [Malekian *et al*. 2023]. It is also known that increased oxidative stress contributes to an increased risk of osteoporosis, as, among other things, it induces osteocyte apoptosis and affects the levels of specific factors, i.e. RANKL, sclerostin or fibroblast growth factor 23. The result is high bone resorption and impaired bone remodelling. As it is described, antioxidants in foods can inhibit oxidative stress and reduce the risk of osteoporosis [Marcucci *et al*. 2023]. The mere presence of one of the more potent antioxidants, resveratrol, in cranberry juice may improve bone parameters [Yeung *et al*. 2019]. Resveratrol has been shown to mitigate bone mass loss and deterioration of bone trabecular structure in rats [Tou 2015]. In a randomized controlled trial (RCT) among obese men with metabolic syndrome, resveratrol was shown to increase BMD and stimulate bone formation [Ornstrup *et al*. 2014].

Considering the pioneering nature of our research, the individual mechanisms of action of cranberry juice on bone growth and development are still unknown and not confirmed in the literature. They should thus be the subject of future studies that aim at investigating the individual components and their synergies. The concentration-dependent effects are also intriguing and need to be studied. We showed that the thickness of bone trabeculae was better affected by a concentration of 10% compared to 25%, while bone mineral density itself is better increased by a 25% concentration of cranberry juice. This may be due, among other things, to the presence of D-mannose and ascorbic acid, however, these are issues that require further research. The development of research on the effects of cranberry juice on bone growth and development may contribute to a better treatment of bone diseases and a reduction in the risk of their occurrence.

REFERENCES

1. ADAMCZAK A., BUCHWALD W., KOZLOWSKI J., 2011 - Variation in the content of flavonols and main organic acids in the fruit of European cranberry (Oxycoccus palustris Pers.) growing in peatlands in peatlands of North-Western Poland. ***Herba Po-lonica*** 54.
2. AGHAJANIAN P., HALL S., WONGWORAWAT M.D., MOHAN S., 2015 - The roles and mechanisms of actions of vitamin c in bone: new developments. ***Journal Bone Mineral Research*** 30, 1945-55.
3. BARANOWSKA M., BARTOSZEK A., 2016- Antioxidant and antimicrobial properties of bioactive phytochemicals from cranberry. ***Postępy Higieny i Medycyny Doświadczalnej*** (Online).31, 1460-1468.
4. CHOPRA A.S., LORDAN R., HORBAŃCZUK O.K., ATANASOV,A.G., CHOPRA I., HORBAŃCZUK J.O., JÓŹWIK A., ARKELLS N., 2022-The current use and evolving landscape of nutraceuticals. ***Pharmacological Research*** 175.
5. CLYNES M.A., HARVEY N.C., CURTIS E.M., FUGGLE N.R., DENNISON E.M., COOPER C., 2020-The epidemiology of osteoporosis. ***British Medical Bulletin***133, 105-117.
6. COOPER R.G., NARANOWICZ H., MALISZEWSKA E., TENNETT A., HORBANCZUK J.O., 2008 - Sex-based comparison of limb segmentation in ostriches aged 14 months with and without tibiotarsal rotation. ***Journal of the South African Veterinary Assosiation*** 79, 142-144.
7. DEPHILLIPO N.N., AMAN Z.S., KENNEDY M.I., BEGLEY J.P., MOATSHE G., LAPRADE R.F., 2018- Efficacy of vitamin C supplementation on collagen synthesis and oxidative stress after musculoskeletal injuries: a systematic review. ***Orthopaedic Journal of Sports Medicine*** 25. 6, 2325967118804544.
8. FEGHALI K., FELDMAN M., LA V.D., SANTOS J., GRENIER D., 2012-Cranberry proanthocyanidins: natural weapons against periodontal diseases. ***Journal of Agricultural and Food Chemistry*** 60, 5728-35.
9. GU R., LIU H., HU M., ZHU Y., LIU X., WANG F., WU L., SONG D., LIU Y. D., 2023 - Mannose prevents bone loss under weightlessness. ***Journal of Translational Medicine*** 9, 21, 8.
10. HARRIS E., 2023 - Updated Meta-analysis: Cranberry Products Reduced UTI Risk. JAMA. 329, 1730.
11. HUA YANG, NANNAN HAN, ZHENHUA LUO , JUNJI XU, LIJIA GUO, YI LIU., 2023-D-mannose alleviated alveolar bone loss in mice with experimental periodontitis via regulating the anti-inflammatory effect of amino acids. ***Journal of Periodontology*** 94, 542-553.
12. HUMINIECKI L., HORBAŃCZUK J., 2018 - The functional genomic studies of resveratrol in respect to its anti-cancer effects. ***Biotechnology Advances*** 36, 1699-1708.
13. HUMINIECKI L., HORBANCZUK J., ATANASOV A.G., 2017 - The functional genomic studies of curcumin. ***Seminar Cancer in Biology*** 46, 107-118.
14. JURIKOVA T., SKROVANKOVA S., MLCEK J., BALLA S., SNOPEK L., 2019- Compounds, Antioxidant Activity, and Biological Effects of European Cranberry (Vaccinium oxycoccos). ***Molecules*** 24, 24.

15. LI CH., LI J., JIANG F., LI Y., TZVETKOV N.T., HORBANCZUK J.O., ATANASOV A.G., WANG D., 2021 - Vasculoprotective effects of ginger (Zingiber officinale Roscoe) and underlying molecular mechanisms. ***Food and Function*** 12, 1897-1913.
16. LIN X., YUAN G., LI Z., ZHOU M., HU X., SONG F., SHAO S., FU F., ZHAO J., XU J., LIU Q., FENG H., 2020- Ellagic acid protects ovariecto-my-induced bone loss in mice by inhibiting osteoclast differentiation and bone resorption. ***Journal of Cellular Physiology*** 235, 5951-5961.
17. LIN Z,. MIAO J., ZHANG T., HE M., ZHOU X., ZHANG H., GAO Y., BAI L., 2021- d-Mannose suppresses osteoarthritis development in vivo and delays IL-1β-induced degeneration in vitro by enhancing autophagy activated via the AMPK pathway. ***Biomed Pharma-cother*** 135, 111199.
18. LIU H., GU R., ZHU Y., LIAN X., WANG S., LIU X., PING Z., LIU Y., ZHOU Y., 2020- D-mannose attenuates bone loss in mice via Treg cell proliferation and gut microbiota-dependent anti-inflammatory effects. ***Therapeutic Advances in Chronic Disease*** 11:2040622320912661.
19. MALEKIAN S., MIRGHAFOURVAND M., NAJAFIPOUR F., OSTADRAHIMI A., GHASSAB-ABDOLLAHI N., FARSHBAF-KHALILI A., 2023- The Associations between bone mineral density and oxidative stress biomarkers in postmenopausal women. ***Korean Journal of Family Medicine*** 44, 95-101.
20. MARCUCCI G., DOMAZETOVIC V., NEDIANI C., RUZZOLINI J., FAVRE C., BRANDI M.L., 2023- Oxidative stress and natural antioxidants in osteoporosis: novel preventive and therapeutic approaches. ***Antioxidants*** 12, 373.
21. MEENA J., THOMAS C.C., KUMAR J., RAUT S., HARI P., 2021-Non-antibiotic interventions for the prevention of urinary tract infections in children: a systematic review and meta-analysis of randomized controlled trials. ***European Journal of Pediatrics*** 180, 3535-3545.
22. MOZOS I., FLANGEA C., VLAD D.C., GUG C., MOZOS C., STOIAN D., LUCA C.T., HORBAŃCZUK J.O., HORBAŃCZUK O.K., ATANASOV A.G., 2021-Effects of anthocyanins on vascular health. ***Biomolecules*** 11, 811.
23. NEMZER B.V., AL-TAHER F., YASHIN A., REVELSKY I., YASHIN Y., 2022- Cranberry: Chemical Composition, Antioxidant Activity and Impact on Human Health: Overview. ***Molecules*** 23, 27, 1503.
24. NOUR J., MOREGOLA A., SVECLA M., DA DALT L., BELLINI R., NEYROLLES O., FADINI G.P., ROMBOUTS Y., ALBIERO M., BONACINA F., NORATA G.D., 2022- Mannose receptor deficiency impacts bone marrow and circulating immune cells during high fat diet induced obesity. ***Metabolites*** 1, 12, 1205.
25. ORNSTRUP M.J., HARSLØF T., KJÆR T.N., LANGDAHL B.L., PEDERSEN S.B., 2014- Resveratrol increases bone mineral density and bone alkaline phosphatase in obese men: a randomized placebo-controlled trial. ***The Journal of Clinical Endocrinology and Metabolism*** 99, 4720-9.
26. PAPPAS E., SCHAICH KM., 2009- Phytochemicals of cranberries and cranberry products: characterization, potential health effects, and processing stability. ***Critical Reviews in Food Science and Nutrition*** 49, 741-81.
27. PIECZYNSKA M.D., YANG Y., PETRYKOWSKI S., HORBANCZUK O.K., ATANASOV A.G., HORBAŃCZUK J.O., 2020 - Gut microbiota and its metabolites in atherosclerosis development. ***Molecules*** 25(3), 594.
28. TEWARI D., MOCAN A., PARVANOV E.D., SAH A,N., NABAVI S.N. HUMINIECKI L, M,A, Z.,F., LEE Y.,Y., HORBAŃCZUK J.O, ATANASOV A.,G., 2017- Ethnopharmacological approaches for therapy of jaundice: Part II. Highly used plant species from acanthaceae, euphorbiaceae, asteraceae, combretaceae, and fabaceae families. ***Frontiers in Pharmacology*** 15, 8, 518.
29. TOU JC. , 2015- Evaluating resveratrol as a therapeutic bone agent: preclinical evidence from rat models of osteoporosis. ***Annals of the New York Academy of Sciences Journal*** 1348, 75-85.

30. VIGUET-CARRIN S., GARNERO P., DELMAS P.D., 2006 - The role of collagen in bone strength. ***Osteoporosis International*** 17, 319-36.
31. VILLARREAL A., STOECKER B.J., GARCIA C., GARCIA K., RIOS R., GONZALES C., MANDADI K., FARAJI B., PATIL B.S., DEYHIM F., 2007- Cranberry juice improved antioxidant status without affecting bone quality in orchidectomized male rats. Phytomedicine. 14: 815-20.
32. VIVIAN ALONSO-GARCIA, CUTTER CHABOYA, QIONGYU LI , BRYAN LE, TIMOTHY J CONGLETON , JOSE FLOREZ, VICTORIA TRAN, GANG-YU LIU, WEI YAO, CARLITO B LEBRILLA , FERNANDO A FIERRO., 2020- High Mannose N-Glycans Promote Migration of Bone-Marrow-Derived Mes-enchymal Stromal Cells. ***International Journal of Molecular Sciences*** 21, 7194.
33. WANG D., ÖZEN C., ABU-REIDAH I.M., CHGURUPATI S., PATRA J.K., HORBAŃCZUK J.O., JÓŹWIK A., TZVETKOV N.T., UHRIN P., ATANASOV A.G., 2018 - Vasculoprotective Effects Of Pomegranate (Punica Granatum L.). ***Frontiers In Pharmacolog***y 9, 544.
34. WANG D., ZHANG L., HUANG J., HIMABINDU K., TEWARI D., HORBAŃCZUK J.O, XU S., CHEN Z., ATANASOV A.G., 2020 - Cardiovascular protective effect of black pepper (Piper nigrum L.) and its major bioactive constituent piperine, ***Trends in Food Science & Technology*** https://doi.org/10.1016/j.tifs.2020.11.024.
35. XIA J.Y., YANG C., XU D.F., XIA H., YANG L.G., SUN G.J., 2021- Consumption of cranberry as adjuvant therapy for urinary tract infections in susceptible populations: A systematic review and meta-analysis with trial sequential analysis. ***PLoS One*** 2. 16.9.
36. XU H., CHEN F., LIU T., XU J., LI J., JIANG L., WANG X., SHENG J., 2020-Ellagic acid blocks RANKL-RANK interaction and suppresses RANKL-induced osteoclastogenesis by inhibiting RANK signalling pathways. ***Chemico-Biological Interactions Journal*** 331:109235.
37. YEUNG A.W.K., AGGARWAL B., BARRECA D., BATTINO M., BELWAL T., HORBAŃCZUK O., BERINDAN-NEAGOE I., BISHAYEE A., DAGLIA M., DEVKOTA H., ECHEVERRÍA J., EL-DEMERDASH A., ORHAN I., GODFREY K., GUPTA V., HORBAŃCZUK J., MODLIŃSKI J., HUBER L., HUMINIECKI L., JÓŹWIK A., MARCHEWKA J., MILLER M., MOCAN A., MOZOS I., NABAVI S., NABAVI S., PIECZYNSKA M., PITTALÀ V., RENGASAMY K., SILVA A., SHERIDAN H., STANKIEWICZ A., STRZAŁKOWSKA N., SUREDA A., TEWARI D., WEISSIG V., ZENGIN G., ATANASOV A., 2018 - Dietary natural products and their potential to influence health and disease including animal model studies. ***Animal Science Papers and Reports***. 36, 345-358.
38. YEUNG A.W.K., AGGARWAL B.B., ORHAN I.E., HORBAŃCZUK O.K., BARRECA D., BATTINO M., BELWAL T., BISHAYEE A., DAGLIA M., DEVKOTA H. P., ECHEVERRÍA J., EL-DEMERDASH A., BALACHEVA A., GEORGIEVA M., GODFREY K., GUPTA V., HORBAŃCZUK J. O., HUMINIECKI L., JÓŹWIK A., STRZAŁKOWSKA N., MOCAN A., MOZOS, I., NABAVI S. M., PAJPANOVA T., PITTALA V., FEDER-KUBIS J., SAMPINO S., SILVA A. S., SHERIDAN H., SUREDA A., TEWARI D., WANG D., WEISSIG V., YANG Y., ZENGIN G., SHANKER K., MOOSAVI M. A., SHAH M. A., KOZUHAROVA E., ALRIMAWI F., DURAZZO A., LUCARINI M., SOUTO E. B., SANTINI A., MALAINER C., DJILIANOV D., TANCHEVA L.P., LI H.B., GAN R.Y., TZVETKOV N.T., ATANASOV A. G. 2019 - Resveratrol, a popular dietary supplement for human and animal health: Quantitative research literature analysis - a review. ***Animal Science Papers and Reports*** 37, 103-118.
39. YEUNG A.W.K., CHOUDHARY N., TEWARI D., EL-DEMERDASH A., HORBAŃCZUK O.K., DAS N., PIRGOZLIEV V., LUCARINI M., DURAZZO A., SOUTO E.B., SANTINI A., DEVKOTA H.P., UDDIN M. S., ECHEVERRIA J., WANG D., GAN R. Y., BRNCIC M., KALFIN R. E., TZVETKOV N. T., JOZWIK A., SOLKA,M., STRZALKOWSKA N., HORBANCZUK J.O., ATANASOV A.G., 2021a - Quercetin: total-scale literature landscape analysis of a valuable nutraceutical with numerous potential applications in the promotion of human and animal health - a review. ***Animal Science Papers and Reports*** 39, 199-212.

40. YEUNG A.W.K., CHOUDHARY N., TEWARI D., EL-DEMERDASH A., TOMCZYK M., DAS N., PIRGOZLIEV V., LUCARINI M., DURAZZO A., SOUTO E.B., SANTINI A., DEVKOTA H.P., UDDIN M.S., ECHEVERRÍA J., WANG D., GAN R-Y., BRNČIĆ M., KALFIN R.E., De R., CENANOVIC M., SAI C.S., KAPOOR B., KIRILOV K., TZVETKOV N.T., BELAKOVA B., UHRIN P., JÓŹWIK A., HORBANCZUK O.K., STRZAŁKOWSKA N., KOSZARSKA M., CHARUTA A., HORBAŃCZUK J.O., ATANASOV A.G., 2022 – Lycopene: total-scale literature landscape analysis of a valuable nutraceutical with numerous potential applications in the promotion of human and animal health. ***Animal Science Papers and Reports*** 40 (2), 119-134.
41. YEUNG A.W.K, KSEPKA N., MATIN M.,WANG D., SOUTO E.,STOYANOV J., ECHEVERRÍA J., TEWARI D., HORBANCZUK J., LUCARINI M., DURAZZO A., MARCHEWKA J., PIRGOZLIEV V., GAN R.,TZVETKOV N., WYSOCKI K., MATIN F., LITVINOVA O., BISHAYEE A., DEVKOTA H., EL-DEMERDASH A., BRNČIĆ M., SANTINI A., HORBAŃCZUK O., MICKAEL M., ŁAWINSKI M., DAS N., SIDDIQUEA B., HRG D., ATANASOV A.G., 2023 – Dietary factors in nonalcoholic fatty liver disease: impacts on human and animal health - a review. ***Animal Science Papers and Reports*** 41, 3, 179-194.
42. YEUNG A.W.K., ORHAN I.E., AGGARWAL B.B., BATTINO M., BELWAL T., BISHAYEE A., DAGLIA M., DEVKOTA H.P., EL-DEMERDASH A., BALACHEVA A.A., GEORGIEVA M.G., GUPTA V.K., HORBAŃCZUK J.O., JOZWIK A., MOZOS I., NABAVI,S. M., PITTALÀ V., FEDER-KUBIS J., SANCHES SILVA A., SHERIDAN H., SUREDA A., WANG D., WEISSIG V., YANG Y., ZENGIN G., SHANKER K., MOOSAVI M.A., SHAH M.A., AL-RIMAWI F., DURAZZO A., LUCARINI M., SOUTO E. B., SANTINI A., DJILIANOV D., DAS N., SKOTTI E., WIECZOREK A., LYSEK-GLADYSINSKA M. W., MICHALCZUK M., HORBAŃCZUK O.K., TZVETKOV N. T., ATANASOV A.G., 2020a - Berberine, a popular dietary supplement for human and animal health: Quantitative research literature analysis-a review. ***Animal Science Papers and Reports*** 38, 5-19.
43. YEUNG A W. K., TZVETKOV N. T., EL-DEMERDASH A., HORBANCZUK O. K., DAS N., PIRGOZLIEV V., LUCARINI M., DURAZZO A., SOUTO E. B., SANTINI A., DEVKOTA H. P., UDDIN M., ECHEVERRÍA J., WANG D., GAN R. Y., BRNČIĆ M., KALFIN R., TANCHEVA L. P., TEWARI D., BERINDAN-NEAGOE I., SAMPINO S., STRZAŁKOWSKA N., MARCHEWKA J., JOZWIK A., HORBANCZUK J. O., ATANASOV A. G. 2021b - Apple polyphenols in human and animal health. ***Animal Science Papers and Reports*** 39, 105-118.
44. 17. YEUNG A.W.K., TZVETKOV N..T, BALACHEVA A.A., GEORGIEVA M.G., GAN R.Y., JOZWIK A., PYZEL B., HORBAŃCZUK J.O., NOVELLINO E., DURAZZO A., LUCARINI M., CAMILLI E., SOUTO E.B., ATANASOV A.G., SANTINI A., 2020b - Lignans: quantitative analysis of the research literature. Frontiers in Pharmacology 11, 37. doi: 10.3389/fphar.2020.00037. PMID: 32116713; PMCID: PMC7020883.
45. YEUNG A.W.K., TZVETKOV N.T., DURAZZO A., LUCARINI M., SOUTO E.B., SANTINI A., GAN R.-Y., JOZWIK A., GRZYBEK W., HORBAŃCZUK J.O., MOCAN A., ECHEVERRÍA J., WANG D., ATANASOV A.G., 2020c - Natural products in diabetes research: quantitative literature analysis. ***Natural Product Research*** doi: 10.1080/14786419.2020.1821019.
46. ZAFFAR A., VIKAS PANDEY, NAVITA GUPTA, LEENA SAPRA, HAMID Y. DAR, NITI SHOKEEN, ASHA BHARDWAJ, AYAAN AHMAD, VANDANA SONI, RUPESH K. SRIVASTAVA., 2020 - Phytoconstituents as novel osteo-protective agents: Implications in bone health. ***Frontiers in. Bioscience*** (Landmark Ed). 25:1259-1296.