International Journal of Chemical and Biological Sciences 2022; 4(2): 10-14

E-ISSN: 2664-6773
P-ISSN: 2664-6765
Impact Factor: RJIF 5.6
IJCBS 2022; 4(2): 10-14
www.chemicaljournal.org/
Received: 27-07-2022
Accepted: 13-08-2022

Cleiton Gonçalves Dias
University Vale do Rio Doce, Governador Valadares, MG, Brazil

Washington de Souza Leal
Única Faculty, Ipatinga, MG, Brazil

Marcus Vinícius Dias-Souza
Pitágoras College, Ipatinga, MG, Brazil

Corresponding Author:
Marcus Vinícius Dias-Souza
Pitágoras College, Ipatinga, MG, Brazil

Interference of nutraceuticals on the activity of antibacterial drugs against clinical isolates of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*

Cleiton Gonçalves Dias, Washington de Souza Leal and Marcus Vinícius Dias-Souza

DOI: https://doi.org/10.33545/26646765.2022.v4.i2a.40

Abstract
Drug-food interactions may be beneficial for the treatment of several diseases, mostly due to the increased absorption effect resulting from the combinations to nutrients. However, they may also pose risks to treatments: drug-nutrient complexes may be not properly absorbed, hampering pharmacokinetics and pharmacodynamics parameters. Here we used a standardized method of our group to investigate the effect of nutraceuticals compounds (melatonin, vitamin C, vitamin D and lutein) on the activity of clinically relevant antimicrobial drugs used against clinical isolates of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. We noticed that the interactions did not follow a regular or predictable pattern. For Gram-negative species, most of the synergistic interactions were observed with melatonin and vitamin C. Vitamin D produced synergism in some combinations against *S. aureus*.

Keywords: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, nutraceuticals, antibacterial drugs

Introduction
Nutraceuticals are pharmaceutical formulations prepared with nutrients of vegetable, mineral, biosynthetic and other relevant sources, which behave like active substances in medications to offer different health benefits [1]. Most governmental regulatory institutions for drugs and food worldwide do not recognize the term "nutraceuticals", and characterize them as nutritional supplements [2]. Nutraceuticals can be of interest as complementary therapeutic tools to treat different diseases, and can be purchased without need of a valid prescription. These formulations usually require regular administration, more than once a day, in fasting or following food intake, to make its benefits noticeable (clinically or by laboratorial exams)[3]. This is due to factors that include low concentration of nutrients (as to authorize sales without prescription) and poor oral absorption of some molecules, like flavonoids [3, 4]. Given that the effects of nutraceuticals are dependent of prolonged use, interactions with drugs that also require frequent administration, such as antimicrobials, are highly probable.
The use of antimicrobial drugs is necessary to treat infectious diseases [5]. These drugs can be antibacterial, antiviral and antifungal. Drug interactions with anti-bacterial are widely described in the literature and gathered in relevant databases [6], whereas their interactions with nutrients are poorly described. In several cases, nutrients may hamper the activity of anti-bacterial [6]. This might contribute to make the picture of bacterial resistance even more complex and life- threatening. Given the exposed, we investigated the effects of combining different molecules explored in nutraceuticals formulations to different clinically relevant antibacterial drugs.

Materials and Methods
Nutraceuticals
We used four different molecules that are widely explored in nutraceuticals products (Table 1). They were prepared as aqueous solutions in concentrations that correspond to international or national recommended daily intakes [7-9].

Table 1: nutrients used in the study as aqueous solutions and their concentrations

Nutrient	Concentration	Manufacturer
Melatonin	3 mg/mL	Sigma Aldrich (St. Lous, USA)
Lutein	10 mg/mL	Eurofarma (Brazil)
Vitamin C	60 mg/mL	União Química (Brazil)
Vitamin D	200 IU/mL	Purifarma (Brazil)

Preparation of bacterial strains

Clinical isolates of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the microorganism's collection from Pit Agoras College (Pirating, MG, and Brazil). *S. aureus* strains are from indwelling catheters tips of hemodialysis, *E. coli* strains are uropathogenic, and *P. aeruginosa* strains were from tracheal secretions. A total of 10 isolates from each species was used. All strains were cultured in Brain Heart Infusion (BHI) broth (Difco) before being tested for identity confirmation with VITEK 2 system (version R04.02, bio Meraux), following the manufacturer's instructions.

Interference of nutraceuticals on the activity of antibacterial drugs

The possible interference of the nutrients in table 1 on antibacterial drugs was assessed in duplicate using a method standardized by our group [10], with some modifications. Overnight-grown bacterial isolates (0.5 Mac Farmland scale, 100 µL) were inoculated in Mueller Hinton agar petri dishes, and antimicrobial disks (Table 2, all from Sensifar, Brazil) were distributed as for performing a susceptibility test. Then, briefly, 10 µL of the solution of each nutrient was dispensed in each disk. The plates were incubated overnight at 37 °C, and the inhibition zone mean diameter was compared with control plates (disks free of nutrients). Results were considered as synergism or antagonism following the parameters previously described [10].

Table 2: Antimicrobial disks used in this study

Disks used against *S. aureus*	Disks used against *E. coli* and *P. aeruginosa*
Amoxicillin (20 µg)	Amoxicillin (20 µg)
Ceftriaxone (30 µg)	Ampicillin (10 µg)
Chloramphenicol (30 µg)	Ceftriaxone (30 µg)

Statistics

The interference of the nutrients on the activity of the antibacterial drugs was analyzed using Bio stat for Windows. We used Kreskas-Wallis tests followed post hoc Student-Newman-Keels test. Results were considered significant if $p < 0.05$.

Results

The combination of nutrients and antibacterial drugs used in this study resulted in mixed interactions, but most of them were antagonistic (Figures 1-3). The activity of ceftriaxone against *E. coli* isolates was significantly decreased ($p<0.05$) by the addition of the tested nutrients (Fig.1). Lutein decreased the activity of ceftriaxone as well, but it was not significant ($p = 0.077$). Melatonin significantly increased the activity of ampicillin and amoxicillin against *E. coli* ($p<0.05$). The other nutrients affected the drugs without statistical significance.

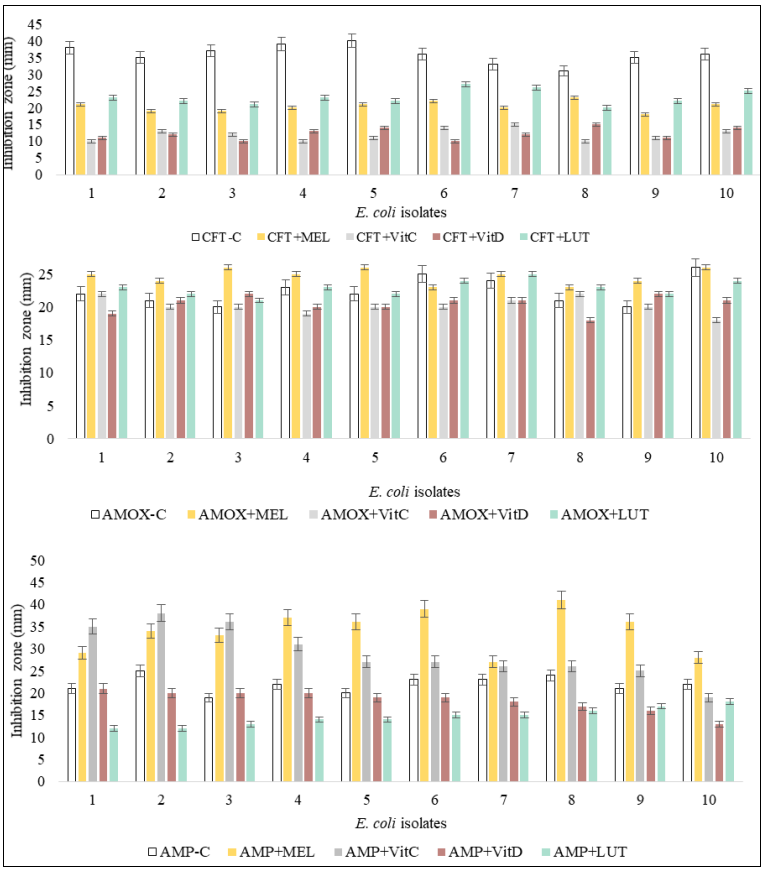

Fig 1: Interference of nutrients on antimicrobial drugs used against *E. coli* isolates (data is expressed as inhibition zones in mm). CFT: ceftriaxone, AMOX: amoxicillin, AMP: ampicillin. C: control, +MEL: addition of melatonin, +Vit C: addition of vitamin C, +Vit D: addition of vitamin D, +LUT: addition of lutein. For *S. aureus* (Figure 2), lutein, vitamin C and melatonin significantly decreased the activity of chloramphenicol. Melatonin and vitamin D significantly decreased the activity of amoxicillin. Interestingly, vitamin D and lutein had no interference on the activity of ceftriaxone for this specie

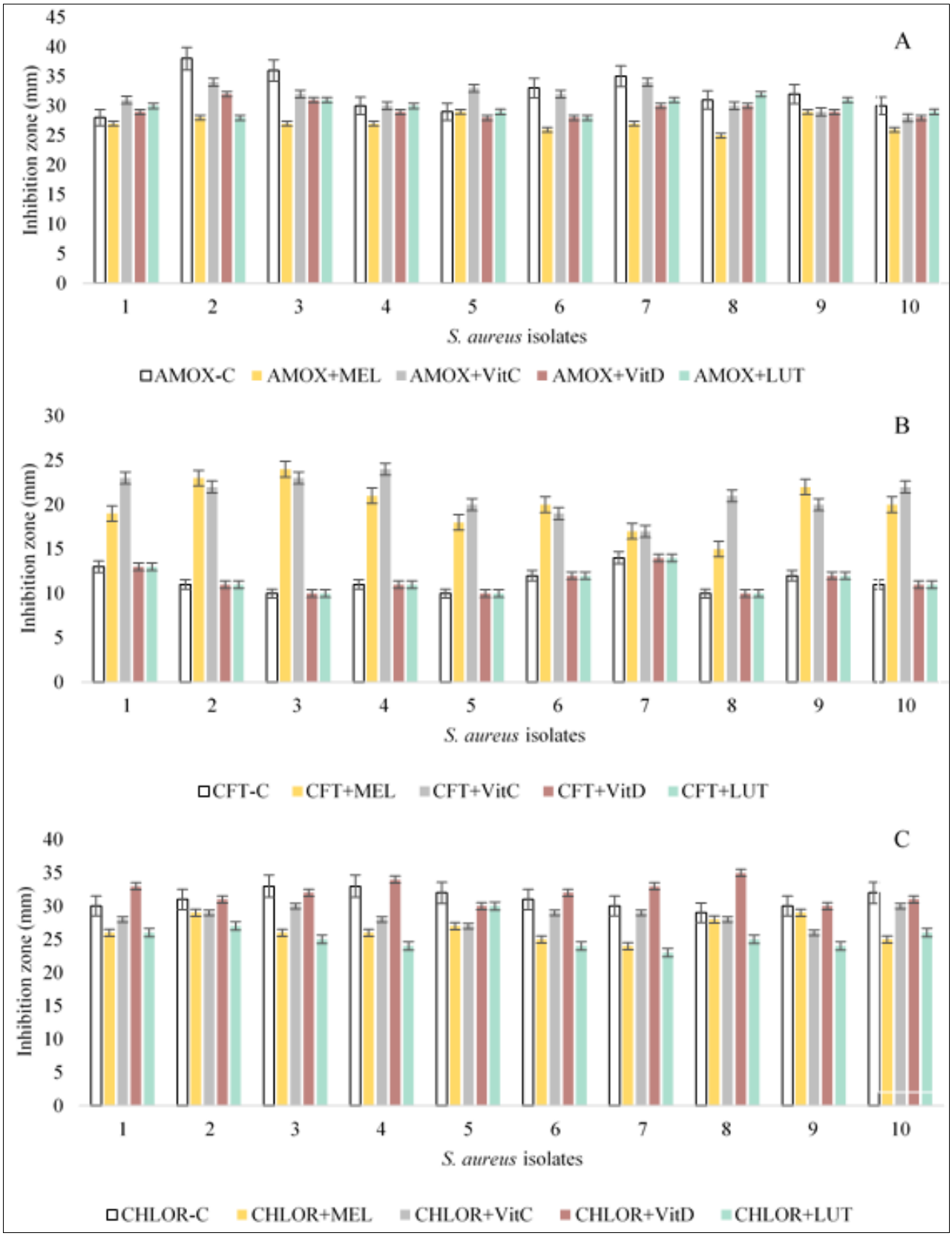

Fig 2: Interference of nutrients on antimicrobial drugs used against *S. aureus* isolates (data is expressed as inhibition zones in mm). AMOX: amoxicillin, CFT: ceftriaxone, CHLOR: chloramphenicol. C: control, +MEL: addition of melatonin, +VitC: addition of vitamin C, +Vit D: addition of vitamin D, +LUT: addition of lutein. Melatonin significantly increased the activity of ceftriaxone against *P. aeruginosa* (Figure 3), and vitamin C significantly increased the activity of amoxicillin. However, the addition of lutein significantly decreased the activity of amoxicillin and ampicillin.

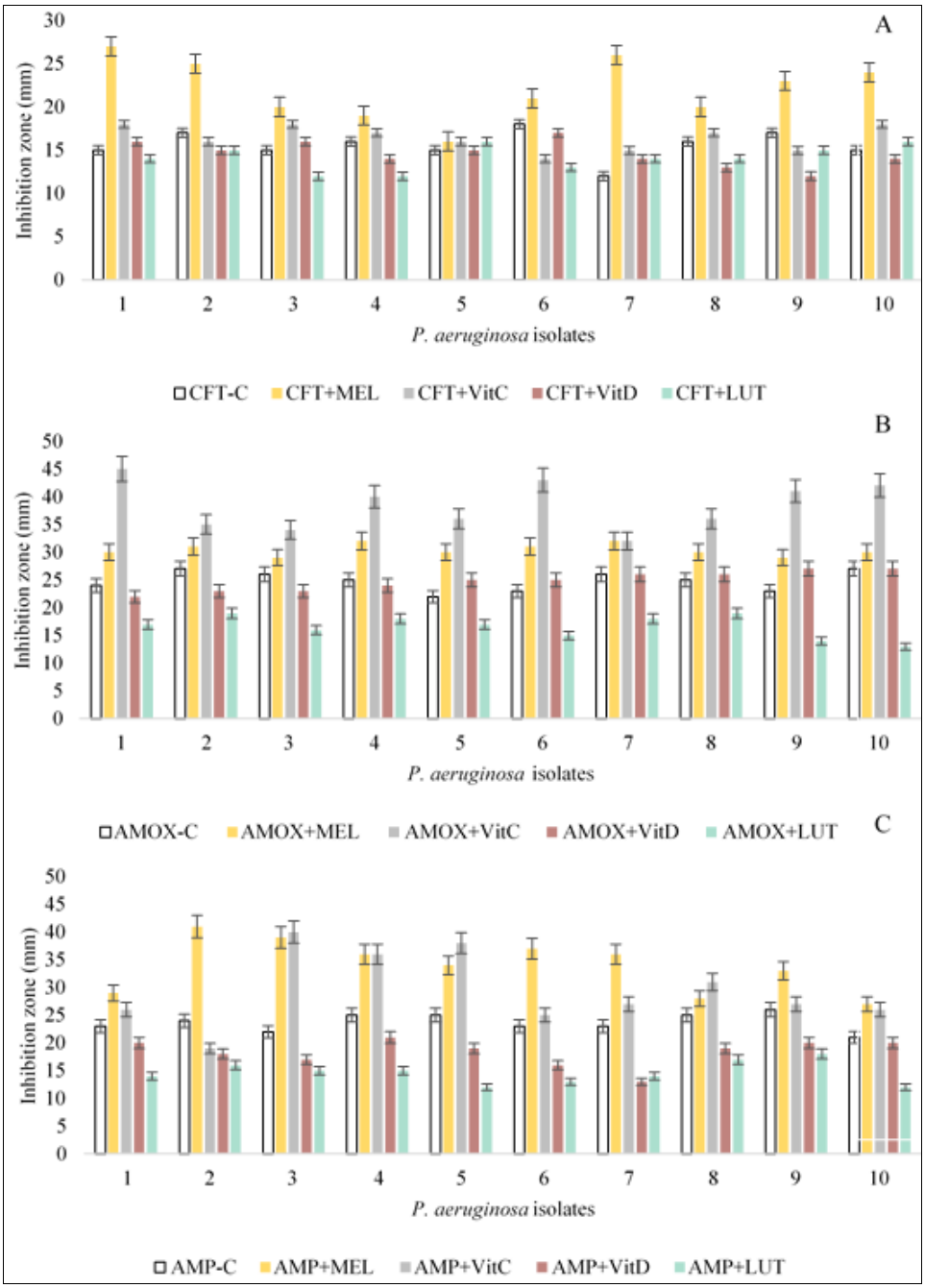

Fig 3: Interference of nutrients on antimicrobial drugs used against *P. aeruginosa* isolates (data is expressed as inhibition zones in mm). CFT: ceftriaxone, AMOX: amoxicillin, AMP: ampicillin. C: control, +MEL: addition of melatonin, +Vit C: addition of vitamin C, +Vit D: addition of vitamin D, +LUT: addition of lutein.

Discussion

Food and drugs are two essential elements for human health, for nourishing and transferring essential nutrients for life, and for treating diseases [11]. A good balance regarding adequate intake of them can provide benefits to health [12]. However, drug-food interactions tend to pose risk to clinical treatments, due to the chemical interactions that might impair pharmacokinetics stages and hamper the mechanism of action of drugs [13]. The combinations investigated in this study did not present a clear pattern. For Gram-negative species, we noticed that most of the synergistic interactions were for combinations of the drugs with melatonin and vitamin C. Most of the antagonistic interactions, on the other hand, were Observed for combinations of the drugs with vitamin D and lutein. For *S. aureus,* the combinations of drugs and nutrients Resulted mostly in antagonistic interactions. Vitamin D, however, produced synergism in some combinations. Such Interactions are poorly predictable and are dependent on Experimental procedures to be confirmed. Lutein is a nutrient detectable in green leafy vegetables such as kale, spinach, broccoli and lettuce. Its distribution in the Body is not uniform, with greater concentration in the central retina, contributing to visual acuity and central vision [14]. In addition, it also has anti-inflammatory properties [14]. Vitamin D is known as a steroid hormone, with relevant role in the Immune system [15]. The deficiency of vitamin D is considered a factor for diseases such as osteoporosis and increased risk of bone fractures, as it influences the metabolism of calcium [15]. Melatonin is produced in the pineal gland and is relevant for proper neural mechanism of sleep and awake cycles [7]. It can also be obtained through supplementation. Vitamin C intake is necessary for collagen metabolism and general skin health [16]. Its effectiveness on the treatment of infectious diseases is still being investigated [16]. Previous studies from our group

support the observation of the poorly predictable behavior of drug-nutrient interactions. Nutrients such as β-carotene and lycopene were combined to antimicrobials such as amoxicillin and cephalexin against *S. aureus* and *E. coli*, and statistically significant antagonism was detected [17]. Furthermore, flavonoids such as routine and resveratrol also produced statistically significant antagonism when combined to drugs such as gentamycin and ciprofloxacin against these species [17]. On the other hand, β-carotene and lycopene produced statistically significant synergism when combined to drugs such as chloramphenicol and aztreonam against *P. aeruginosa* strains [18]. Therefore, evidence such as the provided here are necessary to clarify the risks or potential benefits of combinations of nutrients and drugs.

Conclusion

This study showed that nutraceuticals products may interfere negatively on the activity of antimicrobial drugs. In some situations, the interactions may result in increased activity of the drugs. However, it is still safer to conduct pharmacological treatments considering the need of avoiding drug-food interactions whenever possible and necessary – unless these combinations are undeniably beneficial for the treatment. Our study is not without limitations: not only more strains are necessary in further tests as to increase the representativeness of the assay, but also, *in vivo* studies are necessary as to investigate the effects of the immune system and hepatic metabolism.

Conflict of Interest

Not available

Financial Support

Not available

References

1. Chandra S, Saklani S, Kumar P, Kim B, Coutinho HDM. Nutraceuticals: Pharmacologically Active Potent Dietary Supplements. Biomed Res Int. 2022;2022:2051017.
2. Santini A, Cammarata SM, Capone G, et al. Nutraceuticals: opening the debate for a regulatory framework. Br J Clin Pharmacol. 2018;84(4):659-672. doi:10.1111/bcp.13496
3. Siddiqui RA, Moghadasian MH. Nutraceuticals and Nutrition Supplements: Challenges and Opportunities. Nutrients. 2020;12(6):1593. doi:10.3390/nu12061593
4. Andrew R, Izzo AA. Highlights into the pharmacology of nutraceuticals. Br J Pharmacol. 2020;177(6):1209-1211. doi:10.1111/bph.15006
5. Becker DE. Antimicrobial drugs. Anesth Prog. 2013;60(3):111-123.
6. Vivithanaporn P, Kongratanapasert T, Suriyapakorn B, et al. Potential drug-drug interactions of antiretrovirals and antimicrobials detected by three databases. Sci Rep. 2021;11(1):6089. doi:10.1038/s41598-021-85586-8
7. Lewis SR, Pritchard MW, Schofield-Robinson OJ, Alderson P, Smith AF. Melatonin for the promotion of sleep in adults in the intensive care unit. Cochrane Database Syst Rev. 2018;5(5):CD012455. doi:10.1002/14651858.CD012455.pub2
8. Evans JR, Lawrenson JG. Antioxidant vitamin and mineral supplements for slowing the progression of age-related macular degeneration. Cochrane Database Syst Rev. 2017;7(7):CD000254. doi:10.1002/14651858.CD000254.pub4
9. Brasil. Ministério da Saúde, Secretaria de Vigilância Sanitária. PORTARIA N º 33, DE 13 DE JANEIRO DE 1998. Available at: https://bvsms.saude.gov.br/bvs/saudelegis/svs1/1998/prt0033_13_01_1998.html
10. Dias-Souza MV, Dos Santos RM, de Siqueira EP, Ferreira-Marçal PH. Anti-biofilm activity of cashew juice pulp against *Staphylococcus aureus*, high performance liquid chromatography/diode array detection and gas chromatography-mass spectrometry analyses, and interference on antimicrobial drugs. J Food Drug Anal. 2017;25(3):589-596.
11. Quigley EMM. Nutraceuticals as modulators of gut microbiota: Role in therapy. Br J Pharmacol. 2020;177(6):1351-1362. doi:10.1111/bph.14902
12. Daliu P, Santini A, Novellino E. From pharmaceuticals to nutraceuticals: bridging disease prevention and management. Expert Rev Clin Pharmacol. 2019;12(1):1-7. doi:10.1080/17512433.2019.1552135
13. Ronis MJJ, Pedersen KB, Watt J. Adverse Effects of Nutraceuticals and Dietary Supplements. Annu Rev Pharmacol Toxicol. 2018;58:583-601. doi:10.1146/annurev-pharmtox-010617-052844
14. Eisenhauer B, Natoli S, Liew G, Flood VM. Lutein and Zeaxanthin-Food Sources, Bioavailability and Dietary Variety in Age-Related Macular Degeneration Protection. Nutrients. 2017;9(2):120. Published 2017 Feb 9. doi:10.3390/nu9020120
15. Alia E, Kerr PE. Vitamin D: Skin, sunshine, and beyond. Clin Dermatol. 2021;39(5):840-846. doi:10.1016/j.clindermatol.2021.05.025
16. Lykkesfeldt J. On the effect of vitamin C intake on human health: How to (mis)interprete the clinical evidence. Redox Biol. 2020;34:101532. doi:10.1016/j.redox.2020.101532
17. Dos Santos RM, Pimenta G, DIAS-SOUZA MV. Carotenoids and Flavonoids can Impair the effectiveness of some Antimicrobial Drugs against Clinical Isolates of Escherichia coli and Staphylococcus aureus. International Food Research Journal, 2015;5:1777-1782.
18. Dos Santos RM, Pimenta G, Figueiredo FJB, DIAS-SOUZA MV. Interference of flavonoids and carotenoids on the antimicrobial activity of some drugs against clinical isolates of Pseudomonas aeruginosa. International Food Research Journal, 2016;23:1268-1273.