Volume 8, Issue 3, 2018, 3259 - 3264 ISSN 2069-5837

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Review Article Open Access Journal

Received: 30.04.2018 / Revised: 02.06.2018 / Accepted: 05.06.2018 / Published on-line: 15.06.2018

Interactions of natural products and antimicrobial drugs: investigations of a dark matter in chemistry

Marcus Vinícius Dias-Souza [1, *], Cleiton Gonçalves Dias [2], Pedro Henrique Ferreira-Marçal [3]

[1]Head, Integrated Pharmacology and Drug Interactions Research Group (GPqFAR), Biochemistry Laboratory, Faculty of Health Sciences, University Vale do Rio Doce, Governador Valadares, Brazil

[2]BScPharm, Faculty of Health Sciences, University Vale do Rio Doce, Governador Valadares, Brazil

[3]Integrated Pharmacology and Drug Interactions Research Group (GPqFAR), Immunology Laboratory, Faculty of Health Sciences, University Vale do Rio Doce, Governador Valadares, Brazil

*corresponding author e-mail address: ***marcus.souza@univale.br***

ABSTRACT

Bacterial infectious diseases are each time more difficult to manage due to the limited options of antimicrobial drugs and due to the multidrug resistance profile of pathogenic strains. Several works on natural products of vegetable sources have provided evidence of the antimicrobial potential of different phytomolecules such as flavonoids. Thus, they represent an important strategy to overcome the microbial resistance scenario. Beyond the use of natural products alone in popular medicine, they are commonly combined to synthetic drugs of different pharmacological groups such as antimicrobials in several ways, including interactions with industrially-manufactured or artisanally prepared phytotherapics, nutritional supplements, food, juices, and teas. Such interactions are often ignored in the clinical context. Synergistic interactions can improve treatment outcomes, however, enough evidences of efficacy and safety must be provided before being clinically used. Here, interactions of natural products with antimicrobial drugs against bacterial pathogens are discussed, aiming to provide contributions for advancements on the field towards a safe clinical use of such combinations. The data discussed here might open doors for improvements on phytotherapy for infectious bacterial diseases.

Keywords: *natural products; interactions; antimicrobial.*

1. INTRODUCTION

Natural products (NPs), especially the ones of vegetable sources, are used since ancient times for the treatment of several diseases. The traditional knowledge diffused in different populations is of great ethnopharmacological interest: pharmaceutical formulations of NPs and varied drugs currently available for clinical comprise results of investigations in this field. According to the World Health Organization, more than 80% of the global drug market relies on medicinal NPs [1]. Generally, NPs formulations are low-cost products, thus, they tend to more accessible than conventional formulations for clinical treatments. Beyond financial and accessibility issues, patients are generally interested in using NPs for clinical treatments based on a common belief that they are devoid of side effects and more safe to use than conventional medication [2]. Pharmacologically active phytomolecules of NPs may cause toxic effects that are not easily predictable either *in vitro* or *in vivo*, notwithstanding the several health benefits which NPs are associated with [3].

The interactions of drugs and NPs of vegetable sources, the so-called drug-herb interactions (DHI), are an old and neglected clinical concern in this context. DHI can happen due to the simultaneous intake of synthetic drugs (SD) and phytotherapics, food, dietary supplements and nutraceutical formulations, and they have been described to be more frequent than drug-drug interactions (DDI) [4, 5]. Patients usually explore DHI mostly as a complementary strategy to achieve the desired clinical outcomes in a shorter period of time when compared to the conventional treatment, expecting the combination to be synergistic [5]. However, at least three main problems (out of many) can be pointed here: firstly, NPs must meet quality standards that include quantitative assays for detection of bioactive and eventually toxic compounds, microbiological safety, and so on. Due to the influence of factors such as preparation methods and seasonality, these standards might be not met by either artisanal or manufactured phytotherapics [6].

Fluctuations in the presence and levels of determined phytomolecules may strongly influence DHI, and when treatments are conducted with phytotherapics only, the expected clinical outcomes might not be reached. This becomes even more complicated when considering, for instance, the combination of plant food *in natura* or as teas or juices, rich in certain molecules like flavonoids, and synthetic drugs, as nutrients can alter drug absorption, distribution, metabolism and/or elimination by physiologic and physicochemical mechanisms of interactions [4, 7]. Moreover, advanced age, chronic liver disease, kidney failure, polymedication, long-term drug use, and pharmacogenetics issues are examples of relevant known risk factors for DDI; however, the extension in which they affect DHI remains unclear [5, 8].

In the latest years, studies on DDI and DHI have been focused and unfortunately limited to investigate the modulation of drug metabolism and eventual safety aspects of these events. Thus, most of our approaches on researches on this subject should be reviewed, as the safety of NPs for clinical use is still mostly dependent on empirical experiences. In Brazil, data from the Council of Phytotherapy indicate that 38% of physicians would prescribe more phytotherapics [9]; however, DHI clinical studies

are scarce, and the methods for studying them are generally based on clinical and mechanistic investigations and in extrapolations obtained from animal models and *in vitro* studies, which are often incipient and/or not fully representative of the reality.

In spite of this complex negative scenario of DHI, a bright side on this topic has been explored by different research groups. DHI may improve the outcomes of clinical treatments with conventional medication if the interactions result in synergism and are physiologically safe [10].

This approach can show several benefits. For instance, it can be possible in some situations to associate a lower dose of the drug and of the phytocompound of interest when they are combined, what may decrease the cost of the medication for patients. Also, the onset of action might be shorter when compared to the drug combined alone, what is especially important for diseases in which symptoms are rapidly triggered. These points are also relevant to be explored to improve patients' adherence to drug therapy. Regarding the treatment of infectious diseases, DHI represents a relevant alternative to overcome microbial resistance to the currently available drugs, as researches for new antimicrobials in pharmaceutical industries remain scarce.

Some of such combinations might be alternatively prepared in compounding pharmacies with a proper prescription, and in a near future, they might be prepared in industrial scale. Here, DHI interactions with antimicrobial drugs against bacterial pathogens will be discussed. Relevant data for formulation developers and prescribers of phytotherapics (such as pharmacists, nutritionists, physicians and other professionals) will be reviewed, with the aim of integrating clinically useful information, and to open doors for researchers and prescribers to review their methods and practices, in order to open doors for improvements on phytotherapy for infectious bacterial diseases and other clinical areas from now on.

2. OVERVIEW OF DHI INVESTIGATIONS: WHAT IS KNOWN?

A study combined plant extracts prepared in 70% methanol that present antimicrobial and other pharmacological effects, which were guaco (*Mikania glomerata*), guava (*Psidium guajava*), clove (*Syzygium aromaticum*), garlic (*Allium sativum*), lemongrass (*Cymbopogon citratus*), ginger (*Zingiber officinale*), carqueja (*Baccharis trimera*), and mint (*Mentha piperita*), with disks of penicillin, oxacillin, vancomycin, ampicillin, cephalothin, cefoxitin, chloramphenicol, gentamicin, netilmicin, tetracycline, erythromycin, cotrimoxazole, and ofloxacin, against *Staphylococcus aureus* strains [11]. All the extracts were active against the strains, and clove, guava, and lemongrass extracts exhibited the highest synergism rate with the antimicrobials. However, poor synergism was observed for ginger and garlic extracts [11].

The aqueous extracts of *Punica granatum* and *Plantago major* were combined to amoxicillin against *S. aureus* and *Escherichia coli* strains using the disk diffusion test [12]. *P. granatum* extract was somehow active against the strains in a broth microdilution test, but *P. major* extract was not active. Antagonistic interactions were detected for *P. granatum* extract and amoxicillin against the strains, mostly against *E. coli* [12].

The essential oils of *Origanum compactum*, *Thymus willdenowii*, and *Melissa officinalis* were combined to disks of gentamicin, tobramycin, imipenem and ticarcillin against *S. aureus, E. coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Pseudomonas putida, Salmonella enteritidis* and *Enterobacter aerogenes* [13]. *O. compactum* combination with tobramycin showed a synergistic effect against *K. pneumoniae, P. putida, P. aeruginosa,* and *P. mirabilis*. When combined to imipenem, a synergistic effect was confirmed against *P. putida* and *S. enteritidis* and an antagonist effect in the rest of bacteria. *T. willdenowii* combination with tobramycin was synergistic against *K. pneumoniae, P. aeruginosa, P. putida* and *E. aerogenes*. Following, when this extract was combined to imipenem, it had an antagonistic effect against *K. pneumoniae, E. aerogenes,* and *P. mirabilis,* and resulted in a synergistic effect against *E. coli, P. putida, P. aeruginosa,* and *S. enteritidis*. *Melissa oficinalis* extract combination with gentamicin resulted in synergistic effect against *P. mirabilis,* and when combined to and imipenem resulted in synergistic effect against *S. enteritidis*. The extract in combination with tobramycin had no different effect against *S. aureus* [13].

The need for outlining pharmacological parameters such as dose selection, dosing regimen, and administration route makes the use of experimental models highly relevant following *in vitro* assays. In this context, chloramphenicol, oxacillin, amoxicillin, norfloxacin, rifampicin, and vancomycin were combined to a fraction of a commercial cranberry preparation in checkerboard assays against *S. aureus*, including a MRSA strain [14]. A synergistic activity was detected, and molecular data pointed that the mechanism is associated to peptidoglycan synthesis.

For MRSA, a 512-fold drop of amoxicillin MIC was detected when the drug was combined to the fraction. The authors explored a murine model of mastitis to investigate the *in vivo* efficacy of this combination. The mammary glands infected with *S. aureus* were treated with the cranberry fraction and amoxicillin alone, and with the combination. Although the treatments with the fraction and amoxicillin alone decreased the bacterial counts, only the combination resulted in a significantly reduced counting when compared to untreated animals [14].

The antimicrobial properties of olive leaf extract were evaluated alone and combined to ampicillin against *E. coli* and *S. aureus* strains [15]. The extract was effective against both bacteria but presented more activity on S. aureus. Some metabolites were obtained from the extract, and when combined to ampicillin, hydroxytyrosol resulted in synergic effect, and caffeic acid, verbascoside, and oleuropein resulted in additive effects, as well as the extract itself [15].

An interesting study involving *Poincianella pyramidalis* extract provided important evidence on how results of combinations of antimicrobials and NP can differ due to variations of the species, and also due to variations on the susceptibility

profile among isolates of the same species [16]. *P. pyramidalis* is a plant from Brazilian savannah and is popularly used against diarrhea, dysentery, and respiratory and urinary infections.

The mentioned plant extract (ethanol-water 50:50 v/v) and gallic acid, one of its bioactive metabolites, were tested alone and combined to different drugs against ATCC strains and clinical isolates of *E. coli*, *P. aeruginosa* and *K. pneumoniae*. The combination of the extract and gallic acid to gentamicin significantly increased the activity of the drug against *E. coli*, and the combination of the drug and the extract decreased the MIC of gentamicin. The combination of the extract and gallic acid to chloramphenicol was synergic against *E. coli* and MIC value was reduced. Synergism was also detected for combinations of gallic acid and nitrofurantoin, ampicillin, and norfloxacin against *E. coli*. However, an additive effect was observed when the extract or gallic acid was combined to gentamicin, ceftriaxone, ciprofloxacin, and cefepime against *P. aeruginosa* strains. For *K. pneumoniae*, synergism was only detected for combinations of gentamicin to the extract and gallic acid [16].

Salvia officinalis and *Cichorium intybus* extracts were prepared with different solvents and combined to amoxicillin and chloramphenicol using the checkerboard method against *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and clinical isolates of *Staphylococcus aureus, Bacillus subtilis, Enterobacter cloacae, Klebsiella pneumoniae, Escherichia coli* and *Proteus mirabilis* [17]. Synergistic interactions were observed for combinations of amoxicillin and acetone or ethyl acetate extract of *S. officinalis* and for combinations of chloramphenicol and ethyl acetate extract of *S. officinalis*. In general, MIC values of the antimicrobials decreased from 2 to 10-fold [17].

Most of the studies discussed in this section were conducted using drugs in antibiogram disks and aliquots of the NP of interest. Advantages of this method include its simple carrying out and the relative low cost, as antimicrobial disks are cheaper than antimicrobial drugs as dry powders. However, this approach has some limitations, including the impossibility of modifying the concentration of the antimicrobial drugs (unless the researcher prepares the disks rather than purchasing them from industrial manufacturers), and the fact that some drugs are often not available as disks.

Thus, another method for studying DHI is the checkerboard method. Advantages of this method include the possibility of exploring several and unique combinations of concentrations of antimicrobials and NPs, and the possibility of determining synergism or antagonism with mathematical parameters based on experimental data obtained with the antimicrobials and microbial strains being tested, rather than using breakpoints assumed for the species. Limitations of the method include the often elevated cost of the antimicrobial drugs as dry powders and an eventual formation of precipitates on the culture media due to interactions with NP.

Considering these methodological approaches, how can the researchers start an exploratory study on combinations of NP and antimicrobials? An interesting strategy is to combine crude plant extracts with antimicrobial disks for a primary assessment of DHI behavior. Then, chromatographic methods should be employed to isolate potential antimicrobial molecules. Disk diffusion should be then repeated. If, for instance, synergism is expected and observed, the checkerboard method should then be used for confirmation of this result. Following, *in vivo* studies can be planned. Most of the studies cited here provided evidence of DHI against pathogenic bacteria that deserve further studies. Now, I will present data from our research group in Brazil, which has explored not only botanical extracts but also extracts obtained from plant food in a different perspective regarding the way we analyze DHI data using statistical methods.

3. BRIEF OVERVIEW OF THE ROLE OF STATISTICS IN DHI STUDIES

In general, DHI studies using antimicrobial disks establish a determined numerical parameter to describe results as synergism, indifference or antagonism, based on the results of control disks (free of the addition of NP). However, most of the investigations do not provide any statistical analysis of their results, although some numerical considerations are often provided. One might ask why statistics is so important for DHI studies. Statistics are essential to provide a better description and understanding of phenomena such as synergism and antagonism. Several problems of poor reproducibility in science are associated with weak (or even lack) statistical tests to fully investigate the hypothesis of the study [18, 19]. Nevertheless, the so called "significosis" must be avoided.

An important point ofstatistical analysis is the adequate selection of the number of microbial isolates for the study. The higher the number of microorganisms used in the study, the greater are the chances of the results being representative of reality. This number should be calculated by adequate sampling methods, for instance, when evaluating microorganisms belonging to a broad collection of a laboratory. In such cases, the research team should calculate the sample size before starting the experiments and adequately describe the details in the manuscript. Fixation on sample size should be replaced by adequate sampling calculations lacking bias, such as the use of clonal strains. Inappropriate experimental designs and lack of statistical rigor are common explanations for underpowered studies with reports of false positive and false negative data [18]. However, when sampling is not possible and there are few microorganisms available for the experiments, it is paramount to report the limitations of the study due to the lack of representativeness of the sample size.

P value is the critical final result of statistical analysis. It indicates whether an observation is statistically significant, generally by setting $P < 0.05$ as significant and $P < 0.01$ as highly significant. It is important to mention that *P* value is not only an index on the chances of occurrence of a synergism or antagonism, but is more directed to the interpretation of the degree of improbability of acceptance of the opposite hypothesis of the study [19]. A common mistake of researchers is to conclude or predict biological phenomena based only on statistics. Statistical

significance does not necessarily reflect biological significance, and lack of statistical significance does not necessarily indicate lack of biological meaning. Experimental observations should always be analyzed in light of adequate evidence. For instance, in experiments using drug disks, if DHI istending to antagonism and no statistical significance is found, it does not mean that the combination is completely free of risks. Also, synergistic interactions detected with statistical support must be evaluated *in vivo* for confirming the observations, given that *in vitro* systems are not fully representative of a living organism. Thus, it is important to be aware that although statistical significance is relevant for interpreting DHI data, conclusions should not be withdrawn only based on statistic P values [20].

4. DHI STUDIES CONDUCTED THE GPqFAR TEAM

Our group (GPqFAR – the acronym in Portuguese for *Integrated Pharmacology and Drug Interactions Research Group*) has been investigating synergic DHI with statistical support, in order to generate evidence to start the development of pharmaceutical formulations for the treatment of infectious diseases. Nevertheless, we have reported antagonistic interactions as well, given that they are equally important and represent a potential risk of hampering drug therapy (Fig. 1).

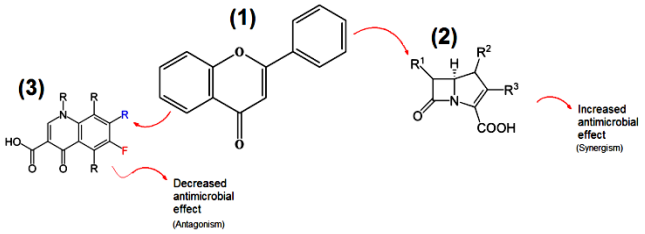

Figure 1. Hypotheses for the observations of synergism and antagonism when combining phytomolecules such as flavonoids (1) to antimicrobials (2-general structure of β-lactams; 3-general structure of quinolones). Synergism might be a result of an increased entrance of the antimicrobial molecules to the bacterial cytoplasm by unspecific mechanisms of flavonoids such as damage to membrane phospholipids and transmembrane proteins, with eventual pore formation. Antagonism might be a result of the bacteriostatic effect of the phytomolecules or even due to complexation of molecules, what makes the antimicrobial drug unavailable for binding to the bacterial target.

The Dias-Souza method of investigation of DHI was standardized and published by our group in 2013 [21]. The method is based on the combination of NPs such as hydroalcoholic plant extracts and chromatographic fractions, or a specific potentially antimicrobial phytomolecule, at their minimal bactericidal concentration (MBC) with antimicrobial drug disks (summarized at Fig. 2). MBC is an important parameter related to the ability of the antimicrobial drug to provide adequate clinical outcomes in immunocompromised patients, what can be useful for immunocompetent patients as well.

After the experiments are concluded and the data is collected (inhibition zones of disks with and without the addition of NP in its MBC should preferably be expressed in mm rather than in cm), our first step on statistical analysis is to determine the homoscedasticity or the heteroscedasticity of the results.

Homoscedasticity is the property of data to show equal statistical variance, indicating that they are scattered to the same extent. In spite of the robustness of ANOVA to support some deviation in this sense for the analysis of parametric data, the same is not applicable for non-parametric data. Heteroscedasticity of data must be checked carefully to avoid selecting wrong tests and thus, obtaining inaccurate results, mainly if the samples are of different sizes. Following, the normality of data should be tested. Parametric or non-parametric tests are then employed for comparing the inhibition zones of disks with and without the addition of NP for all the strains of the study.

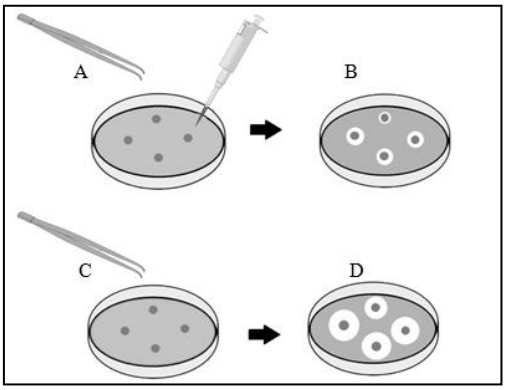

Figure 2. Summarization of the Dias-Souza method for DHI studies. Here is illustrated an experiment in which four antimicrobial drugs are being assessed for DHI with a hypothetical plant extract against a bacterial isolate. For the DHI tests, (A) each bacterial strain is plated onto Mueller Hinton agar and disks are equally distributed. Following, briefly, 10 µL of the hypothetical plant extract in its MBC is added to each disk. The plate is then incubated overnight (37 °C), and (B) the inhibition zones are measured. Results are then compared to the control: each bacterial strain is plated onto Mueller Hinton agar and disks are equally distributed (C). The plate is then incubated overnight (37 °C), and (D) the inhibition zones are measured. When comparing plates in (B) and (D), it is possible to suggest that the DHI was antagonistic, as the inhibition zones are shorter than the ones observed in the control.

On the first DHI study conducted by our group using this methodology, we combined the cashew stembark extract and different antimicrobial drugs against clinical isolates of *S. aureus* [21]. The cashew stembark extract is popularly used and clinically prescribed in Brazilian and Indian phytotherapy for the treatment of infectious diseases, thus, we assumed that it would be of interest to verify whether interactions with antimicrobial drugs would be synergic. The extract was combined at 30 mg/mL to gentamycin, ampicillin, ciprofloxacin, penicillin, neomycin, rifampicin, and vancomycin. Most of the interactions were antagonistic, in spite of the antimicrobial and anti-biofilm activities of the extract against the same bacterial strains.

Following, we have drawn our attention to the influences of diet and lifestyle on DHI, which is also a critical point on the safety of antimicrobial drug therapy beyond the combined use of NPs such as hydroalcoholic plant extracts. Thus, we sought to investigate whether carotenoids and flavonoids could influence the effectiveness of antimicrobial drugs *in vitro* against *E. coli* and *S. aureus* [22]. These classes of phytomolecules are widely

consumed and studied worldwide; however, patients and professionals are generally unaware of the potential risks related to DHI involving these molecules.

Hence, we have adapted the Dias-Souza method to investigate the combination of lycopene, β-carotene, resveratrol and routine *in vitro* against clinical isolates of uropathogenic *E. coli* (tested drugs: gentamicin, amoxicillin, cephalexin, ciprofloxacin) and *S. aureus* (tested drugs: nitrofurantoin, penicillin, erythromycin, oxacillin). Given that none of the flavonoids and carotenoids we selected for this study presented antimicrobial activity against any of the strains, we used the estimated average of their intake in Brazil, especially because they are widely used in nutraceutical products. Most of the DHI tested against *E. coli* isolates were antagonistic, and the antimicrobial effect of the drugs tested against *S. aureus* isolates was abrogated for most of the tested DHI [22]. Such results were unexpected, as the phytomolecules were not active against the bacterial strains. Therefore, we presumed that the DHI is more probably related to chemical interactions with the antimicrobial drugs than any possible effect on bacterial membranes. Mechanistic studies would be of interest to solve this puzzle.

We investigated DHI involving carotenoids and flavonoids against clinical isolates of *P. aeruginosa* [23] using the Dias-Souza interference method with slight modifications. We explored lycopene, β-carotene, diosmin and curcumin combined to chloramphenicol, aztreonan, and meropenem. Again, none of the flavonoids and carotenoids we selected for this study were active against the strains, and we used their estimated average of intake in Brazil. For the first time in GPqFAR investigations, we found synergistic interactions. Both chloramphenicol and aztreonam had their antimicrobial activity increased with the combination of all the four phytomolecules. The antimicrobial effects of meropenem were weakly affected, although some antagonistic interactions were detected for some strains [23].

As mentioned, GPqFAR group has explored the antimicrobial potential of the cashew stem bark extract, which is widely prescribed and popularly used against infectious diseases. Given that the stem bark extract was bioactive against *S. aureus* [21], we hypothesized that extracts of edible parts of the plant could also present antimicrobial activity, and that possibly would result in synergistic interactions when combined to antimicrobial drugs, differently from the stem bark. Hence, we explored the 80% methanolic extract of the Brazilian cashew juice pulp against *S. aureus* [24]. The investigation of DHI using juice pulps is important due to the use of juices to administer (mainly solid) oral dosage forms in order to (at least partially) mask their flavor and make them more palatable. However, DHI is possible in such cases, and thus, oral dosage forms should be administrated using water. In that study, we have used pulps manufactured in Brazil, available for sales at local markets. The juice pulp extract was more potent than the stem bark extract against planktonic cells and biofilms of the isolates. Flavonoids were detected in the extract by HPLC, and they probably have contributed to these results. Nevertheless, when we combined the juice pulp extract in its MBC to ampicillin, chloramphenicol, gentamicin, and meropenem, we detected antagonistic interactions [24], like for the stem bark extract [21].

We also investigated the antimicrobial activity of *Vaccinium myrtillus* (Blueberry) juice pulp extract against *S. aureus* and its possible DHI with levofloxacin, amoxicillin, and gentamicin [25]. The extract was effective against the bacterial isolates, and interestingly, it did not interfere significantly on the activity of the antimicrobial drugs at its MBC, albeit a slight tendency of antagonism was detected [25]. Curiously, in our previous studies, this picture of lack of interference was detected mostly for meropenem [21-24], but here, other drugs were not affected as well.

Preliminary data of our group indicated that the red pepper extract also did not interfere significantly on the activity of different antimicrobial drugs against *S. aureus* and *E. coli* isolates [Santos et al., submitted for publication], but some tendency of antagonism was detected. As mentioned in the statistics section, the lack of statistical significance does not imply lack of biological meaning. Thus, we are planning further studies to investigate the interference of these extracts in a broader range of antimicrobials and bacterial strains. On the other hand, the Black tea extract was effective against *S. aureus*, *P.aeruginosa*, and *E.coli*, and its interaction with antimicrobial drugs against these species resulted in synergism for ciprofloxacin and azitromicin and clindamicin (Santos et al., submitted for publication).

More recently, our group demonstrated by the first time the antimicrobial potential of an 80% methanolic extract of *Euterpe oleracea* (Açaí) juice pulp [26]. *E. oleracea* is widely consumed in Brazil mostly as juice and frozen desserts. The pulp extract was effective against planktonic cells and biofilms of clinical isolates of *S. aureus*. When we tested the interference of the extract in its MBC on the activity of ciprofloxacin, chloramphenicol, and erythromycin, we detected statistically significant synergism except for erythromycin. Flavonoids were detected in the extract by UPLC and it's possible that they have a role in such results. Important volatile molecules were also detected by GC-MS, including 2-Pyrrolidinone, which was demonstrated to present antitumor activity in different models [27], and was possibly involved in the antitumor results observed using HepG2 cells [26].

5. CONCLUSION

Several combinations of NPs and antimicrobial drugs were effective against bacterial strains *in vitro*, but also, varied combinations resulted in negative effects that suggest their potential to impair positive clinical outcomes by hampering drug therapy probably in a molecular level, what raises the need for mechanistic studies on DHI. Patients should be advised that the safety premise that NPs of clinical interest are safe due to their non-synthetical origin is not accurate. Although phytomolecules are associated to several health benefits, they may lead to toxic effects as well as synthetic medication. In general, DHI remains

poorly predictable, speculative and lacking many answers, for antimicrobials and other therapeutic groups as well. Nevertheless, results of synergistic interactions deserve further study with animal models, in order to better understand the behavior of such combinations when treating infectious diseases with and without the participation of the immune system. In spite of the difficult endeavor that such investigations may be, they represent a faster strategy to develop formulations of combinations of NPs and antimicrobial drugs to overcome bacterial resistance with the existing antimicrobials.

6. REFERENCES

[1] World Health Organization, WHO. Traditional Medicine Strategy Document 2002–2005, Geneva, Switzerland. Available at:http://www.wpro.who.int/health_technology/book_who_traditional_medicine_strategy_2002_2005.pdf

[2] Zhou S.F., Zhou Z.W., Li C.G., *et al*., Identification of drugs that interact with herbs in drug development, *Drug Discov Today*, 12, 664–73, **2007**.

[3] Magro L., Moretti U., Leone R., Epidemiology and characteristics of adverse drug reactions caused by drug–drug interactions, *Expert Opin. Drug Saf.*, 11, 83–94, **2012.**

[4] Boullata J., Natural health product interactions with medication, *Nutr. Clin. Pract.*, 20, 33–51, **2005**.

[5] Shaw D., Graeme L., Pierre D., Elizabeth W., Kelvin C., Pharmacovigilance of herbal medicine, *J. Ethnopharmacol*., 140, 513–518, **2012.**

[6] Harrigan J., Patient disclosure of the use of complementary and alternative medicine to their obstetrician/gynaecologist, *Aust. N. Z. J. Obstet. Gynaecol.*, 31, 59–61, **2011**.

[7] Ohnishi N., Yokoyama T., Interactions between medicines and functional foods or dietary supplements, *Keio J. Med.*, 53, 137–150, **2004**.

[8] Heuberger R., Polypharmacy and food-drug interactions among older persons: a review. *J. Nutr. Gerontol. Geriatr*., 31, 325–403, **2012.**

[9] Conselho Brasileiro de Fitoterapia, CONBRAFITO. Conheça a CONBRAFITO (Conselho Brasileiro de Fitoterapia). Available at: http://www.conbrafito.org.br/index.php?option=com_content&view=article&id=45&Itemid=57

[10] Won C.S., Oberlies N.H., Paine M.F., Mechanisms underlying food-drug interactions: inhibition of intestinal metabolism and transport, *Pharmacol. Ther.*, 136, 186–201, **2012**.

[11] Betoni J.E.C., Mantovani R.P., Barbosa L.N., Di Stasi L.C., Fernandes Junior, A. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases, *Mem. Inst. Oswaldo Cruz* 101, 387–390, **2006**.

[12] Teles D.G., Costa M.M., Estudo da ação antimicrobiana conjunta de extratos aquosos de Tansagem (*Plantago major* l., Plantaginaceae) e Romã (*Punica granatum* l., Punicaceae) e interferência dos mesmos na ação da amoxicilina *in vitro*., *Rev. Bras. Plantas Med.*, 16, S1, 323–328, **2014**.

[13] Moussaoui F., Alaoui T., Evaluation of antibacterial activity and synergistic effect between antibiotic and the essential oils of some medicinal plants, *Asian Pacific J Trop Biomed*, 6, 32–37, **2016.**

[14] Diarra M.S., Block G., Rempel H., *et al*., *In vitro* and *in vivo* antibacterial activities of cranberry press cake extracts alone or in combination with β-lactams against *Staphylococcus aureus*, *BMC Compl. Altern. Med.*, **2013**.

[15] Lim A., Subhan N., Jazayeri J.A., *et al*., Plant Phenols as Antibiotic Boosters: *In Vitro* Interaction of Olive Leaf Phenols with Ampicillin, *Phytother. Res.*, 30, 503-9, **2016**.

[16] Chaves T.P., Fernandes F.H., Santana C.P., *et al*., Evaluation of the Interaction between the *Poincianella pyramidalis* (Tul.) LP Queiroz Extract and Antimicrobials Using Biological and Analytical Models, *PLoS One*, 11, e0155532, **2016**.

[17] Stefanović O.D., Stanojević D.D., Comić L.R., Synergistic antibacterial activity of *Salvia officinalis* and *Cichorium intybus* extracts and antibiotics, *Acta Pol Pharm*, 69, 457–63, **2012.**

[18] Riffenburgh R.H., Statistics in Medicine, *San Diego: Academic Press*, 1–10, **1999.**

[19] Lachin J.M., Biostatistical Methods: The Assessment of Relative Risks, *New York: Wiley*, 20–30, **2000**.

[20] Kuzma J.W., Bohnenblust S.E., Basic Statistics for the Health Sciences, 4th Edition. Mayfield, *California: Mountain View*, 5–20, **2000**.

[21] Dias-Souza M.V., Caldoncelli J.L., Monteiro A.S., *Annacardium occidentale* Stem Bark Extract can Decrease the Efficacy of Antimicrobial Drugs, *Rev. Ciências Med. Biol.*, 12, 161–165, **2013.**

[22] Dos Santos R.M., Pimenta G., Dias-Souza M.V., Carotenoids and Flavonoids can Impair the effectiveness of some Antimicrobial Drugs against Clinical Isolates of *Escherichia coli* and *Staphylococcus aureus*, *Int. Food Res. J.*, 5, 1777–1782, **2015**.

[23] Dos Santos R.M., Pimenta G., Figueiredo F.J.B., Dias-Souza M.V., Interference of flavonoids and carotenoids on the antimicrobial activity of some drugs against clinical isolates of *Pseudomonas aeruginosa*, *Int. Food Res. J.*, 23, 1268–1273, **2016**.

[24] Dias-Souza M.V., Dos Santos R.M., Siqueira E.P., Marçal P.H.F., Antibiofilm 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. Drug Food. Anal.*, 25, 589-596, **2017.**

[25] Costa G.J., Dos Santos R.M., Figueiredo F.J.B., Dias-Souza M.V., *Vaccinium myrtillus* extract is effective against *Staphylococcus aureus* and does not interfere on the activity of antimicrobial drugs, *J. Appl. Pharm. Sci.*, 4, 6–8, **2017**.

[26] Dias-Souza M.V., Dos Santos R.M., Cerávolo I.P., Cosenza G., Marçal P.H.F., Figueiredo F.J.B., *Euterpe oleracea* pulp extract: Chemical analyses, antibiofilm activity against *Staphylococcus aureus*, cytotoxicity and interference on the activity of antimicrobial drugs, *Microbial Pathogenesis*, 114, 29-35, **2018**.

[27] Thangam R., Suresh V., Rajkumar M., *et al.*, Antioxidant and *in vitro* anticancer effect of 2-pyrrolidinone rich fraction of *Brassica oleracea* var. *capitata* through induction of apoptosis in human cancer cells, *Phytother. Res.*, 27, 1664-70, **2013**.

7. ACKNOWLEDGEMENTS

The authors are thankfull to Professor Flávio Figueiredo (Faculdades Santo Agostinho, Brazil) for the continuous colaboration at the GPqFAR research network. We are also thankful to Silas Neto (BSc, Federal University of Juiz de Fora, Brazil), for providing the Euterpe olereacea fruit pulp for chemical studies.