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Anti-bacterial activity of *Annona muricata* Linnaeus extracts: a systematic review

Ricardo Mendes da SILVA[1]*, Isabella de Matos Mendes da SILVA[1], Maria Manuela ESTEVINHO[2], Leticia M. ESTEVINHO[3]

Abstract

Current analysis systematically reviews data available in the literature on the anti-bacterial activity of *Annona muricata* extracts (AME) against *Staphylococcus aureus* and *Escherichia coli*. Search was undertaken at four electronic databases and studies' quality were assessed. Results were summarized and Minimum Inhibitory Concentration (MIC) of AME (versus antimicrobial control) against *S. aureus* and *E. coli* was the main datum analyzed. The initial database search yielded 2,433 results. We selected 14 studies and four were used for meta-analysis. The MIC ranged between 156 µg/mL and 1,024 µg/mL against *S. aureus* and between 256 µg/mL and 1,024 µg/mL against *E. coli*. The different strains of *E. coli* studied were more sensitive to AME than those of *S. aureus*. The differences mean concentrations and standard deviations between AME and antimicrobials were 101.91 and 946.05 for *E. coli*, and 388.90 and 970.61 for *S. aureus* respectively. The heterogeneity of data is highlighted. Different methodologies were used, several studies didn't include antibiotics as control, antibiotics were different and various concentrations of extracts were tested. Studies showed possibilities of the plant under analysis as a source of new phytochemical compounds against microorganisms.

Keywords: soursop; anti-bacterial agents; plant extracts; antimicrobial resistance.

Practical Application: Development of phytotherapics and indication of a methodological script for research on antimicrobial action with plant extracts.

1 Introduction

It is common knowledge that novel pathogens resistance mechanisms against antimicrobials are emerging and spreading worldwide increasing bacterial resistance due to the dissemination of antibiotic resistance genes (ARGs) via plasmids and transposable elements between microbial communities, hindering the effectiveness of the treatment of common infectious diseases and causing prolonged illness, disability and death In recent years, antibiotic treatment for common infectious (Le et al., 2018; World Health Organization, 2019). Over-usage of antimicrobial agents in hospitals and by the community is a strong impetus for antimicrobial-resistant pathogens (Nagel et al., 2016).

Further, the use of antimicrobial agents in stockbreeding and agriculture contributes towards the selection of potentially resistant bacteria transferred to humans, directly or indirectly, through the food chain, representing a public health hazard (Lhermie et al., 2019). Indeed, sub-therapeutic antimicrobial concentrations may promote the development of acquired bacterial resistance by non-specific mutagenesis (Kohanski et al., 2010; Le et al., 2018).

The emergence and spread of antibiotic resistance and the evolution of new strains of pathogenic agents are a great concern to community health worldwide and entail the development of new antimicrobials or potential sources of novel drugs. Commonly used medicinal plants are promising sources of biologically active and safe compounds (Manandhar et al., 2019).

Plants are not merely chemically complex compounds, but their components may act synergistically on multiple targets. They may not only increase the efficacy but also minimize the possibility of resistance-developing pathogens (Wagner & Ulrich-Merzenich, 2009). Over the last decades, various plant-derived compounds and their active principles have been analyzed for phytochemicals with anti-bacterial activity (Chowdaiah et al., 2019).

The genus *Annona* comprises over 70 species among which *A. muricata* is the most widely grown. *A. muricata* has been empirically employed in tropical regions to prevent and alleviate diverse ailments such as fever, pain, respiratory and skin diseases, parasites, bacterial infections, hypertension, inflammation, diabetes and cancer. *In vitro* studies have characterized *A. muricata* as a valuable antimicrobial, anti-inflammatory, anti-protozoan, anti-neoplastic and antioxidant agent (Coria-Téllez et al., 2018).

Studying methanolic extracts of *A. muricata*, Pinto et al. (2017) found antimicrobial activity in a broad spectrum of action, on bacterial membranes (both plasma and outer membranes, Gram positive and Gram negative) and Uchegbu et al. (2017) scanning antibacterial properties of *A. muricata* leaves ethanolic

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[1]*Centro de Ciências da Saúde, Universidade Federal do Recôncavo da Bahia - CCS/UFRB, Santo Antonio de Jesus, BA, Brasil*
[2]*Departmento de Biomedicina, Unidade de Farmacologia e Terapêutica, Faculdade de Medicina, Universidade do Porto, Porto, Portugal*
[3]*Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus Santa Apolónia, Bragança, Portugal*
*Corresponding author: ricardomendes@ufrb.edu.br

extracts, detected inhibition in all the tested organisms (*S. aureus*, *P. mirabilis*, *K. pneumoniae*, *Salmonella* and *E. coli*).

The present analysis systematically reviews the data available in the literature on the antibacterial activity of the extracts of *A. muricata*, regardless of the part of the plant and the solvent, against Gram positive (*S. aureus*) and Gram negative (*E. coli*), microorganisms most frequently found in our systematic review. Accessing current knowledge, following a reproducible methodology, this research aims to show gaps in the subject, review methodologies, stimulate new researches and induce faster practical applications.

2 Material and methods

2.1 Search strategy

Search on the literature was undertaken at four electronic databases (PubMed , Web of Science, ScienceDirect, and Scopus, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses [PRISMA] Guidelines, as recommended by Moher et al. (2009) and Higgins & Green (2011). Search was carried out from inception to September 2019 using the following terms: [("Annona muricata" OR graviola OR guanabana OR soursop) (extract* OR extrato OR Anti-bacterial OR antibacterian? OR Bacteria OR Antimicrobial OR antimicrobian?)]. No restriction on publication dates was applied and search included surveys in English, French, Portuguese and Spanish. Reference lists of the papers selected from the databases were manually reviewed to ensure that all pertinent articles were included.

2.2 Eligibility and inclusion/exclusion criteria

Only basic research articles were eligible for current systematic review. Inclusion criteria comprised i) studies on the anti-bacterial activity of *Annona muricata* L. extracts, and ii) studies on dilution in microplates and/or diffusion disc methods. Exclusion criteria comprised i) reviews; ii) studies which did not use *A. muricata* in microbiological tests, and iii) studies that did not use dilution in microplates and/or diffusion disc methods in their microbiological tests. Reviews and duplicates were excluded.

2.3 Study selection and data collection process

The studies identified through electronic or manual search were independently screened by two authors (RMS and IMMS). In the first phase, titles and abstracts were carefully analyzed. Whenever assessment clearly indicated that a particular study failed to meet the inclusion criteria, it was immediately excluded. In the second phase, for all the remaining potentially relevant studies, the full text was evaluated to determine its inclusion or exclusion. The lists of publications which met inclusion criteria were compared by each author and disagreements were discussed and consensus reached. The following information was collected from the selected studies: authors' names, publication year, country, impact factor, plant´s origin, plant´s material employed, voucher herbarium specimen (record of exsiccate deposit and taxonomic identification in an herbarium), antimicrobial susceptibility methods, microorganisms tested, antimicrobial used as control, use and identification of reference strains, extraction solvent type and concentration, re-dissolution solvent, mass volume ratio and major results obtained. Whereas one author completed the evidence table, the second author verified the data´s accuracy. Results from each trial obtained by different methods (DDT and MAST) using the same microorganism (*S. aureus* and *E. coli*) were combined and polled together. The authors carefully confirmed that no statistical differences were observed between the combined groups, for each study.

2.4 Quality assessment

The quality of the included studies was independently assessed by two investigators, after adapting quality assessment tool [QATSDD] described by Sirriyeh et al. (2012). In the case of basic science, contrastingly to clinical studies, checklists and scores are rare to evaluate prior literature within a rigorous and quantitative manner. Therefore, the QATSDD scale was adapted to research aims by the authors. The developed tool included 13 items, scored from 0 to 3, which reflected, among others, the definition of the issue, the identification of purpose and hypothesis, the study design, the quality of the methodology for data collection, data analysis and manuscript drafting. For each paper, the sum of the scores of all items was divided by maximum score (39 points) to obtain the paper's overall quality score.

2.5 Statistical analysis

Minimum Inhibitory Concentration (MIC) of *A. muricata* extracts (versus antimicrobial control) against *S. aureus* and *E. coli* was the main datum analyzed. The microorganisms were chosen since they had been evaluated in most studies and are important representatives of Gram positive and Gram negative groups. Data were extracted from each study and descriptive statistics (mean and standard deviation) were calculated. The two groups (extract and control) were then compared to evaluate the difference between mean MIC rate obtained for *A. muricata* extracts against *E. coli* and *S. aureus* strains when compared to control (conventional antimicrobial agent). This last analysis was performed in only four studies (Yasunaka et al., 2005, Bento et al., 2013, Dzotam et al., 2016; Pinto et al., 2017). Other studies were very heterogeneous and could not be quantitatively pooled.

3 Results

3.1 Bibliographic search and study selection

The initial electronic database search yielded 2,433 results (552 studies identified in PubMed; 853 in ScienceDirect; 520 in SCOPUS; 508 in Web of Science) as shown in Figure 1.

The manual bibliographic search did not retrieve any additional study. Moreover, 2,392 studies were excluded, including duplicates (n = 23) and 25 out of 41 texts fully analyzed were excluded since they failed to employ MAST or DDT methodologies and two did not employ *A. muricata*. In all, 14 studies were selected for inclusion in current systematic review.

3.2 Description of the 14 publications selected

Supplementary Table 1 provides an overview of the key characteristics of the 14 publications available as full texts. The studies were published between 2005 and 2019, with the largest number of articles (n = 3) published in 2017 (Aruan et al.,

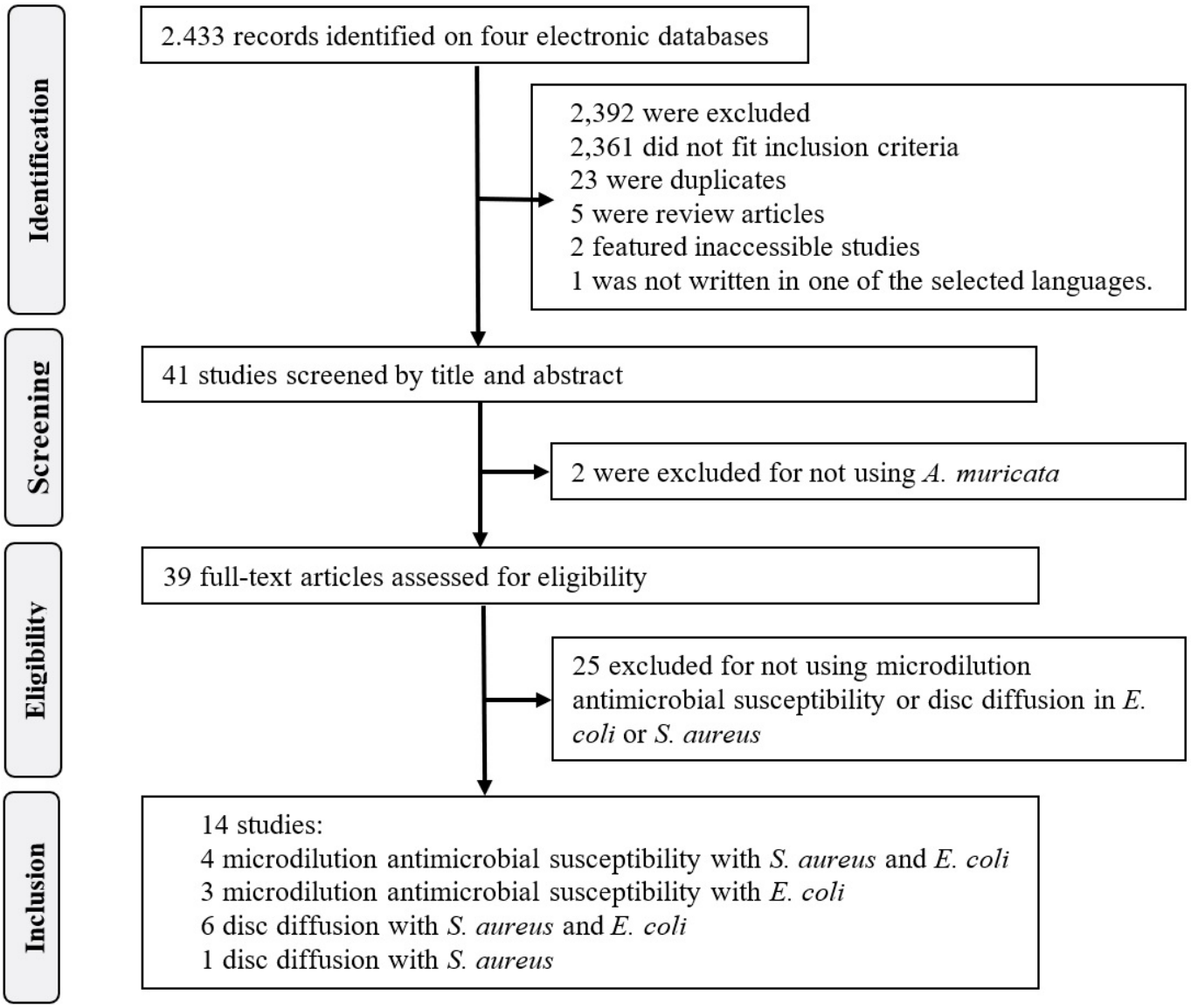

Figure 1. Flow diagram of current study´s selection.

2017; Pinto et al., 2017; Ezealisiji et al., 2017) and in 2019 (Andrade et al., 2019; Nugraha et al., 2019; Sebastiammal et al., 2019). Most experiments (64.29%) were performed with only *A. muricata* extracts (Aruan et al., 2017; Bento et al., 2013; Andrade et al., 2019; Pinto et al., 2017; Ezealisiji et al., 2017; Haro et al., 2014; Nugraha et al., 2019; Sánchez-Navarro et al., 2018; Sebastiammal et al., 2019). Most of the tested plants (35.71%) hailed from Brazil (Bento et al., 2013; Andrade et al., 2019; Pinto et al., 2017; Takahashi et al., 2006; Viera et al., 2010).

The leaf was the most studied morphological part of the plant, featuring in eight studies (Aruan et al., 2017; Bento et al., 2013; Andrade et al., 2019; Pinto et al., 2017; Dzotam et al., 2016; Haro et al., 2014; Sánchez-Navarro et al., 2018; Takahashi et al., 2006) and root was the second most evaluated part (Nugraha et al., 2019 and Ezealisiji et al., 2017).

Most studies (n = 8) reported voucher herbarium specimen and taxonomic identification of *A. muricata* (Bento et al., 2013; Bussmann et al., 2010; Andrade et al., 2019; Pinto et al., 2017; Dzotam et al., 2016; Nugraha et al., 2019; Takahashi et al., 2006; Yasunaka et al., 2005).

Most authors employed methanol and ethanol as extraction solvents (n = 5, each) as a methodology for extract preparation. Ethanol was used by Pinto et al. (2017), Dzotam et al. (2016), Haro et al. (2014), Nugraha et al. (2019) and Yasunaka et al. (2005) and methanol was used by Aruan et al. (2017), Bento et al. (2013), Bussmann et al. (2010), Viera et al. (2010) and Takahashi et al. (2006) Fifty percent of the studies revealed the concentration of extraction solvent (Bento et al., 2013; Pinto et al., 2017; Ezealisiji et al., 2017; Sánchez-Navarro et al., 2018; Sebastiammal et al., 2019; Takahashi et al., 2006; Yasunaka et al., 2005), of which 100% was the most prevalent (35.71%) (Andrade et al., 2019; Dzotam et al., 2016; Haro et al., 2014; Nugraha et al., 2019; Viera et al., 2010). The mass/volume ratio for extraction was not reported in 57.14% of the studies

(Aruan et al., 2017; Bento et al., 2013; Ezealisiji et al., 2017; Haro et al., 2014; Sánchez- Navarro et al., 2018; Sebastiammal et al., 2019; Takahashi et al., 2006; Yasunaka et al., 2005). Among the selected studies, 57.14% used the MAST method (Bento et al., 2013; Bussmann et al., 2010; Andrade et al., 2019; Pinto et al., 2017; Dzotam et al., 2016; Nugraha et al., 2019; Sánchez-Navarro et al., 2018; Yasunaka et al., 2005), whilst 42.86% used the DDT method (Aruan et al., 2017; Ezealisiji et al., 2017; Haro et al., 2014; Sebastiammal et al., 2019; Takahashi et al., 2006; Viera et al., 2010). *S. aureus* was the most tested Gram positive bacterium (92.86%) (Aruan et al., 2017; Bento et al., 2013; Bussmann et al., 2010; Andrade et al., 2019; Pinto et al., 2017; Ezealisiji et al., 2017; Haro et al., 2014; Nugraha et al., 2019; Sánchez-Navarro et al., 2018; Sebastiammal et al., 2019; Takahashi et al., 2006; Viera et al., 2010; Yasunaka et al., 2005). On the other hand, *E. coli* was the most tested Gram negative bacterium (85.71%) (Bento et al., 2013; Andrade et al., 2019; Pinto et al., 2017; Dzotam et al., 2016; Ezealisiji et al., 2017; Haro et al., 2014; Nugraha et al., 2019; Sánchez-Navarro et al., 2018; Sebastiammal et al., 2019; Takahashi et al., 2006; Viera et al., 2010; Yasunaka et al., 2005). Reference strains were not employed in 21.43% of the tests (Aruan et al., 2017; Ezealisiji et al., 2017; Nugraha et al., 2019; Sebastiammal et al., 2019). The most used reference strain of *S. aureus* was ATCC25923 (n = 4) (Bussmann et al., 2010; Andrade et al., 2019; Viera et al., 2010; Takahashi et al., 2006) and the most used reference strain of *E. coli* was ATCC25922 (n = 5) (Andrade et al., 2019; Haro et al., 2014; Nugraha et al., 2019; Sánchez-Navarro et al., 2018; Takahashi et al., 2006).

Bacteria were tested against commercial antimicrobial agents in 57.14% of the studies (Bento et al., 2013; Bussmann et al., 2010; Pinto et al., 2017; Dzotam et al., 2016; Ezealisiji et al., 2017; Sánchez-Navarro et al., 2018; Takahashi et al., 2006; Yasunaka et al., 2005).

The Impact Factor (IF) provided by the Journal Citation Reports (JCR) was absent in 21.43% of the articles (Aruan et al., 2017; Haro et al., 2014; Viera et al., 2010) and in 14.29%, according to SCOPUS (Bento et al., 2013; Haro et al., 2014). The JCR Impact Factor ranged between 1.40 (Bento et al., 2013) and 24.37 (Sebastiammal et al., 2019), and in SCOPUS between 1.04 (Aruan et al., 2017) and 4.58 (Pinto et al., 2017).

The last column of the Supplementary Table 1 lists the methodological quality scores of the publications, whilst Supplementary Table 2 presents classifications attributed to each quality criteria. Scores ranged between 27.00% and 77.00%, with a mean 54.29 ± 15.42% [± standard deviation]. In general, highest scores were obtained for items "accessible and transparent presentation of data throughout the paper" and "presentation, justification and relevance of the research problem" and the lowest for "statistical assessment of reliability and validity of measurement tools" and "draw consistent conclusions based on the evidence presented in the paper.

3.3 Antimicrobial activity - synthesis of results

In the case of MAST, the MIC ranged between 132 (Nugraha et al., 2019) and 128,000 μg/mL (Bussmann et al., 2010) for *S. aureus* and between 132 (Nugraha et al., 2019) and 1,024 μg/mL for *E. coli* (Bento et al., 2013; Dzotam et al., 2016; Yasunaka et al., 2005). The inhibition of halo growth in the analyzed publications which performed DDT ranged between 0.0 (Takahashi et al., 2006) and 14.0 mm (Viera et al., 2010) for *S. aureus,* and between 0.0 (Ezealisiji et al., 2017; Takahashi et al., 2006) and 8.0 mm (Haro et al., 2014) for *E. coli*.

Four out of the 14 publications were used for meta-analysis (Bento et al., 2013; Pinto et al., 2017; Dzotam et al., 2016; Yasunaka et al., 2005). The four studies tested the antibacterial activity of *A. muricata* extracts and it was compared with commercial antimicrobials against *E. coli* and *S. aureus* strains by using the MAST method. In studies for meta-analysis, 17 tests, or rather, six for *S. aureus* and 11 for *E. coli*, were performed. Dzotam et al. (2016) only tested *E. coli*, while the other three articles analyzed *E. coli* and *S. aureus*.

Bento et al. (2013) tested four commercial antimicrobial agents against *S. aureus* and *E. coli* and reported that the activity of *A. muricata* extract in three tests was more effective than antimicrobials, or rather, better than kanamycin against *E. coli* and *S. aureus* (1,024 μg/mL extract concentration when compared to 2,500 μg/mL kanamycin concentration against the two bacteria), and better than gentamicin against *S. aureus* (1.024 μg/mL extract concentration when compared to 2,500 μg/mL gentamicin concentration).

However, commercial antimicrobial agents performed better in 14 tests conducted by Bento et al. (2013), Pinto et al. (2017), Dzotam et al. (2016) and Yasunaka et al. (2005). Amikacin, neomycin, tetracycline and chloramphenicol performed better against *S. aureus* and *E. coli* than *A. muricata* extracts, while gentamicin performed better than *A. muricata* extracts only against *E. coli*.

Figure 2 gives the difference between mean MIC rate obtained for the different antimicrobial agents and *A. muricata* extracts against *E. coli* and *S. aureus* strains. The activity of *A. muricata* extracts was closer to the commercial antimicrobial agents against *E. coli* than against *S. aureus*. The MIC ranged between 156 μg/mL to 1,024 μg/mL against *S. aureus* and from 256 μg/mL to 1,024 μg/mL against *E. coli*. Mean 101.91 and standard deviation 946.05 for *E. coli* were lower than data obtained when comparing the activity of extracts with commercial antimicrobial agents challenged by *S. aureus*, 388.90 and 970.61, mean and standard deviation, respectively.

4 Discussion

Interest in the biological properties of *A. muricata* has increased in recent years. There are currently several reports on the anticancer, anticonvulsant, anti-arthritic, anti-parasite, antimalarial, hepato-protective and anti-diabetic activities of *A. muricata* (Adewole & Caxton-Martins, 2006; Sousa et al., 2010; Moghadamtousi et al., 2015). Several studies have been developed on the evaluation of antimicrobial activity, albeit with a limited number of samples. Therefore, results from existing studies were combined to increase their statistical capacity.

Brazil was the country of origin of most of the tested plants, probably because the graviola soursop is the second Annonaceae

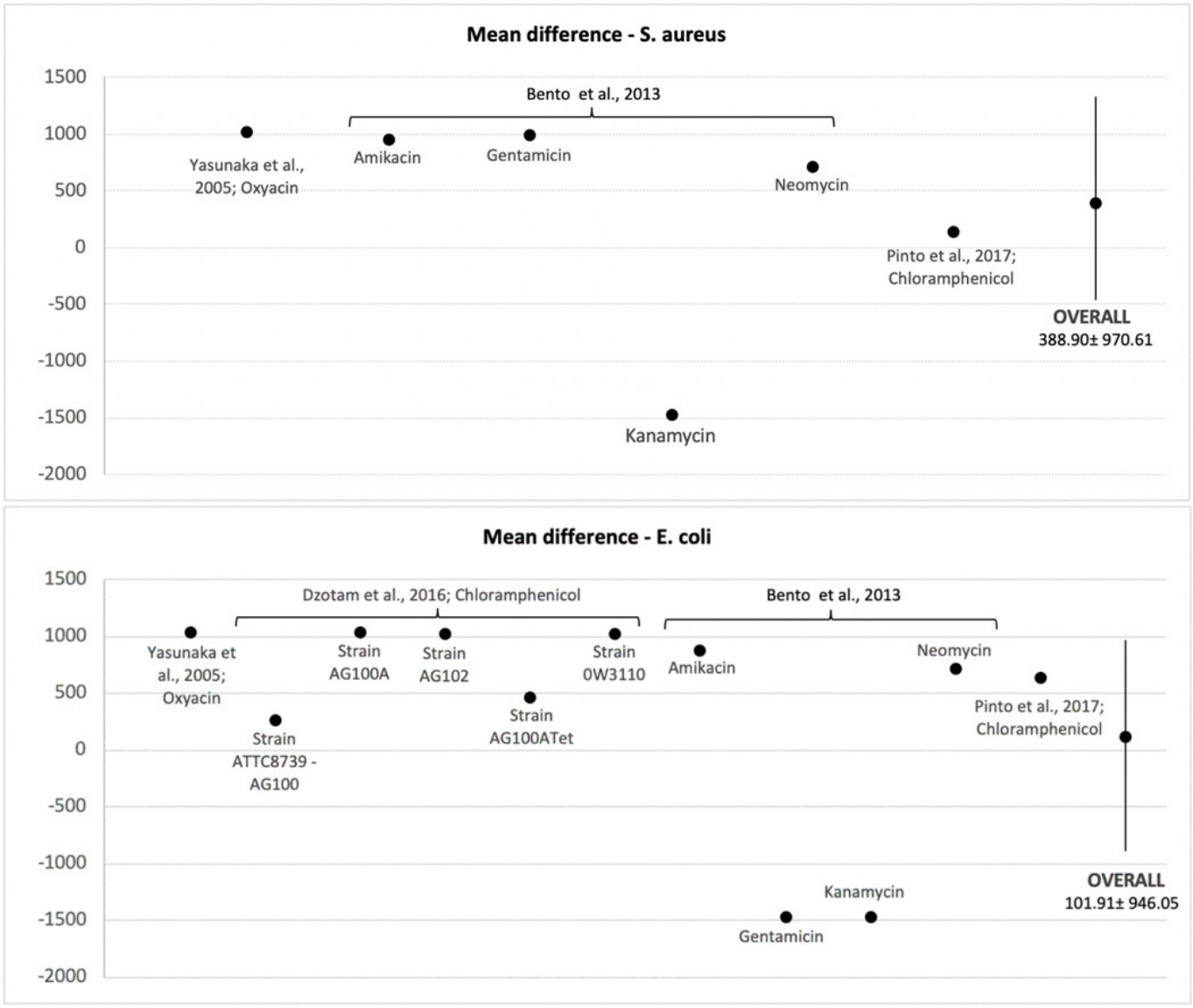

Figure 2. Difference between Minimum Inhibitory Concentration mean rates obtained for different antibiotics and *Annona muricata* L extracts against *Escherichia coli* and *Staphylococcus aureus* strains. Overall estimation (mean±standard deviation) is given on the right side.

in the Brazilian cultivated area, superseded only by sugar-apple, mainly in the northern region (Lemos, 2014).

The information about exsiccate deposit and taxonomic identification in a herbarium is very important and ensures that the researcher is working with the correct species (Peixoto & Maia, 2013).

Regarding the methodology for the preparation of the extract, most authors used methanol and ethanol (35.71%) as extraction solvents (n=5 each) currently used in the research of natural products, capable of extracting many phytochemicals. The use of non-aqueous solvent, such as dimethylsulfoxide (DMSO) is recommended by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards, NCCLS), because several compounds must be dissolved in solvents other than water. A series of dilutions with this solvent has been recommended for its preparation. Such procedure prevents artifacts resulting from the precipitation of products of low solubility in the aqueous medium (National Committee for Clinical Laboratory Standards, 2002).

Several different classes of metabolites were reported to exist in the extract of *A. muricata*, including tannins, alkaloids, flavonoids, polyphenols, saponins, diterpenoids, kaempferol and acetogenin compounds (Yang et al., 2015; George et al., 2015; Matsushige et al., 2012). The extract also contains triglycosides, megastigmans and more than 100 annonaceous acetogenin compounds (Moghadamtousi et al., 2015).

The extraction solvent concentration was reported only in one half of the studies. Further, lack of information on mass/volume ratio for extraction and re-dissolution solvent decreases the reproducibility of the research, highlighting the

need to increase scientific protocols when reporting results in basic science studies.

Most studies used the MAST method rather than DDT. Determination of MIC is more specific than growth inhibition halo, because same sample is tested at different concentrations. According to Klančnik et al. (2010), the disk diffusion method was appropriate only as a preliminary screening test prior to MIC determination with the dilution method.

S. aureus and *E. coli* were the most tested microorganisms. Chai et al. (2019) analyzed the incubation periods of enteric diseases in foodborne outbreaks in the United States from 1998 to 2013 and reported that *S. aureus* and Shiga-toxin producing *E. coli* were reported in 153 and 178 outbreaks, respectively, on the top of the list of five etiologic agents of Foodborne Disease outbreak. Further, methicillin-resistant *S. aureus* (MRSA) is an important healthcare-associated pathogen causing illnesses ranging from localized skin infections to systemic diseases, including toxic shock syndrome (Neyra et al., 2014; Nugraha et al., 2019).

Most studies used reference strains (71.4%) since they were actually the most efficacious for the quality control of dilution methods, with MIC rates close to mid-concentration range for all agents tested (National Committee for Clinical Laboratory Standards, 2016), allowing comparison between strains of clinical origin.

Moreover, 57.1% of publications analyzed used commercial antimicrobials, particularly chloramphenicol, for comparison with extract´s activities. Klančnik et al. (2010) observed that there is no standard procedure for comparing commercial antimicrobials.

Synergism, a positive interaction between two compounds, is one manner by which plant-derived compounds exert their antibiotic potential (Silva et al., 2019). For instance, Pinto et al. (2017) reported on the synergic action of *A. muricata* extract particularly interfering with the stability of cellular membranes that facilitates the activities of antimicrobial agents. Further, Bento et al. (2013) investigated the association between the ethanol extract of *A. muricata* and four antimicrobial agents (gentamicin, kanamycin, amikacin and neomycin) and reported that, in all cases, there was a reduction in MIC when compared to antimicrobial agents acting alone within the 75.0 - 99.9% range.

In other natural matrixes, Enemchukwu et al. (2019) studied *Vernonia amygdalina*, *Garcinia kola*, tetracycline and metronidazole and combinations and found that synergic activity exists against diarrheagenic bacteria. Mancarz et al. (2019) studied a synergic interaction of two extracts from *Liquidambar styraciflua* with ciprofloxacin and tetracycline and a synergistic interaction was observed against the gram-positive bacterial *Enterococcus faecalis* (ATCC 29212) and *S. aureus* (ATCC 2592).

In current systematic review, four articles reported the use of green nanotechnology. Ezealisiji et al. (2017) and Sánchez-Navarro et al. (2018) used silver as nanocarrier; Sebastiammal et al. (2019) used CeO2, and Aruan et al. (2017) employed polyvinyl with alcohol/soursop leaves extract to synthetize nanofibers and evaluate their potential as anti-bacterial wound dressing. This demonstrates the long path to explore the full potential of nanotechnology associated with *A. muricata* as an antimicrobial agent.

Drug delivery systems (DDS) are extensively studied and disseminated to improve the efficacy and administration of active pharmaceutical compounds (Anselmo & Mitragotri 2014). However, second generation (2G) DDS, mostly featuring green nanotechnology, emerged between 1980 and 2010. Third generation DDS (from 2010) will have to be much more advanced to overcome physiochemical and biological barriers through nontoxic excipients (Park, 2014). Current researches in the synthesis of nanoparticles (NPs) using plant extracts has opened a new era for the development of nontoxic methods for the preparation of NPs (Kanwar et al., 2019).

As far as it is known, this is the first systematic review on the anti-bacterial activities of *A. muricata* extracts. Bioprospection of tropical plants for antimicrobial activity is of growing interest as this evaluation may bring about novel active natural products to be used in chemotherapy and in the industry, such as food deterioration-retardant agents (Takahashi et al., 2006). Thus, a systematic review on the theme may summarize and disseminate research works to identify gaps, make recommendations for future research (Arksey & O'Malley, 2005) and bring faster results in basic research for its use in the pharmaceutical industry.

Among the limitations of current study, the heterogeneity of data is highlighted. In fact, different methodologies were used, namely, several studies did not include antibiotics as control; antibiotics used were very different in different studies; various concentrations of extracts were tested. In addition, the lack of comparison with standard strains may have biased results because, without these comparisons, they may not be reliable since they would be based on the absence of knowledge on the behavior of these clinical microorganisms. Klančnik et al. (2010) also observed that several methods for the plant extract´s MIC measurement are available.

Polled quantitative analysis has been employed to combine results from different studies and thus produce estimates that summarize all (Roever, 2017). This quantitative synthesis was influenced by the heterogeneity of the data, including differences in methodologies for assessing antibacterial activity and the lack of comparison with commercial antimicrobial agents in several studies. Indeed, only four articles tested the extracts of *A. muricata* and commercial antimicrobial agents against strains of *E. coli* and *S. aureus* using MAST (Yasunaka et al., 2005, Bento et al., 2013, Dzotam et al., 2016; Pinto et al., 2017). This fact alone makes it difficult to evaluate *A. muricata* extracts.

The development of systematic reviews and meta-analyses that provide data on therapeutic resources of plant biocompounds have to cope with difficulties related to the heterogeneity of results from research with methodological weaknesses that were also highlighted by Freitas et al. (2017) when they reviewed the use of medicinal plants in venous ulcers by Takooree et al. (2019) in their review on *Piper nigrum* L. and by Diefenbach et al. (2018) in their analysis on the effect of copaiba oil (*Copaifera* spp.) in oral pathogens.

Based on our findings and the difficulties reported by other authors, a flowchart on antimicrobial actions of plant extracts

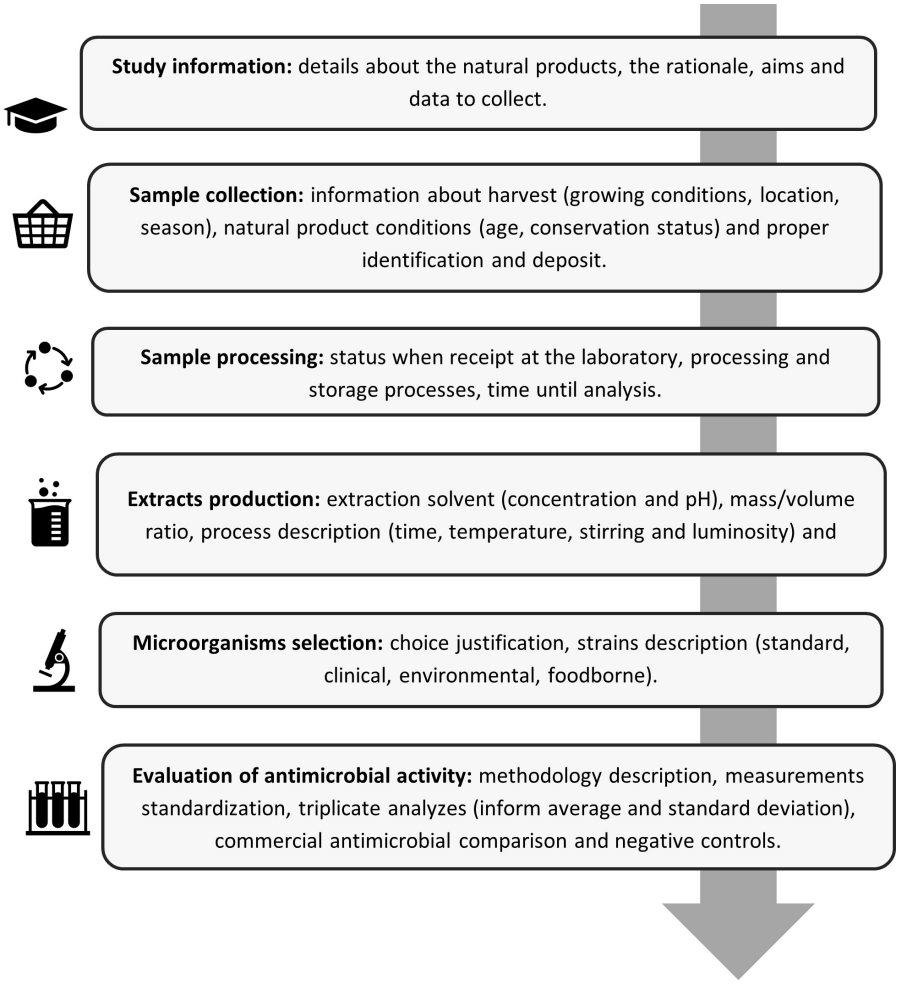

Figure 3. Research flowchart on antimicrobial action of natural products.

may be suggested which will help researchers to develop basic research in the area of biocompounds from vegetables (Figure 3). This flowchart includes (i) data collection; (ii) sample collection; (iii) sample processing; (iv) extracts production; (v) choice of microorganisms; (vi) evaluation of antimicrobial activities.

Although the methodology used in current analysis is commonly applied in clinical studies, its application in the setting of basic science is still fledging. It may, however, be a promising tool to increase statistical power and promote the translation of laboratory findings into practical applications.

5 Conclusion

The 14 publications selected for current study showed the several possibilities of the plant under analysis as a source of new phytochemicals against microorganisms. However, the study also recommended improvement of the methodological quality of the research on *A. muricata* extracts. Even though the antimicrobial action of *A. muricata* extracts was verified, the dispersion of results of articles with MAST was reported. Our findings may contribute to improve scientific standards and rigor of studies on this and other natural products, thereby increasing their reliability and importance while promoting the transition from basic to applied research.

In order to help in the production of new researches and accelerate the transition from basic research findings to therapeutic alternatives in human and veterinary medicine, the authors developed a research flowchart to guide future high-quality studies on the antimicrobial activity of natural products.

Further, besides the "isolated" study of phytochemicals, the assessment of the synergic action between the extract and commercial antimicrobial agents and the new possibilities of green nanotechnology is warranted, particularly taking into account the drug-resistance crisis that is expected in the future.

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Supplementary Material

Supplementary material accompanies this paper.

Supplementary Table 1. Characteristics of selected studies on the antibacterial action of Annona muricata L extracts against Staphylococcus aureus and Escherichia coli strains.

Suplemmentary Table 2. Assessment of quality of sellected studies on the antibacterial action of Annona muricata L extracts against Staphylococcus aureus and Escherichia coli strains.

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