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Original Article

Biological activities of endophytic fungi isolated from *Annona muricata* Linnaeus: a systematic review

Atividades biológicas de fungos endofíticos isolados de *Annona muricata* Linnaeus: uma revisão sistemática

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Abstract

This systematic review integrates the data available in the literature regarding the biological activities of the extracts of endophytic fungi isolated from *Annona muricata* and their secondary metabolites. The search was performed using four electronic databases, and studies' quality was evaluated using an adapted assessment tool. The initial database search yielded 436 results; ten studies were selected for inclusion. The leaf was the most studied part of the plant (in nine studies); *Periconia* sp. was the most tested fungus (n = 4); the most evaluated biological activity was anticancer (n = 6), followed by antiviral (n = 3). Antibacterial, antifungal, and antioxidant activities were also tested. Terpenoids or terpenoid hybrid compounds were the most abundant chemical metabolites. Phenolic compounds, esters, alkaloids, saturated and unsaturated fatty acids, aromatic compounds, and peptides were also reported. The selected studies highlighted the biotechnological potentiality of the endophytic fungi extracts from *A. muricata*. Consequently, it can be considered a promising source of biological compounds with antioxidant effects and active against different microorganisms and cancer cells. Further research is needed involving different plant tissues, other microorganisms, such as SARS-CoV-2, and different cancer cells.

Keywords: soursop, biotechnology, mycology, biological products.

Resumo

Esta revisão sistemática integra os dados disponíveis na literatura sobre as atividades biológicas dos extratos de fungos endofíticos isolados de *Annona muricata* e seus metabólitos secundários. A busca foi realizada em quatro bases de dados eletrônicas e a qualidade dos estudos foi avaliada por meio de instrumento de avaliação adaptado. A pesquisa inicial no banco de dados gerou 436 resultados; dez estudos foram selecionados para inclusão. A folha foi a parte mais estudada da planta (em nove estudos); *Periconia* sp. foi o fungo mais testado (n = 4); a atividade biológica mais avaliada foi anticâncer (n = 6), seguida de antiviral (n = 3). As atividades antibacteriana, antifúngica e antioxidante também foram testadas. Terpenóides ou compostos híbridos de terpenóides foram os metabólitos químicos mais abundantes. Compostos fenólicos, ésteres, alcalóides, ácidos graxos saturados e insaturados, compostos aromáticos e peptídeos também foram relatados. Os estudos selecionados destacaram a potencialidade biotecnológica dos extratos de fungos endofíticos de *A. muricata*. Por conseguinte, esta planta pode ser considerada uma fonte promissora de compostos biológicos com efeitos antioxidantes e ativos contra diversos micro-organismos e células cancerígenas. Mais pesquisas são necessárias envolvendo diferentes tecidos vegetais, outros microorganismos, como SARS-CoV-2, e diferentes células cancerosas.

Palavras-chave: graviola, biotecnologia, micologia, produtos naturais.

1. Introduction

Plants are hosts to communities of microorganisms, called endophytes, including fungi and bacteria that colonize plant tissues without causing any visible disease symptoms to host plants (Yan et al., 2019). Endophytic microorganisms live in association with plants for at least a part of their life cycle, creating symbiotic interactions, modulating both abiotic and biotic stresses, and playing a critical role in the plant defense system (Wu et al., 2015).

Endophytic fungi are a ubiquituos group that colonize all plant species on earth (Ramírez-Camejo, 2022). Endophytic fungi proved to be effective as biological control agents and a viable alternative to traditional chemical insecticides (Amatuzzi et al., 2018). Moreover, fungi from medicinal plants can be considered a source of bioactive metabolites. It is possible to include terpenoids, alkaloids, flavonoids, phenolic acids, quinones, steroids, tetralones,

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and xanthones (Tan and Zou, 2001; Diva et al., 2014). Moreover, due to their functional secondary metabolites' structural complexity and chemical diversity, endophytic fungi have proven useful for drug discovery (Kouipou Toghueo and Boyom, 2019).

The dicotyledonous plants *Annona muricata* L., commonly known as soursop, graviola, guanabana, or mullatha, is an evergreen plant primarily distributed in tropical areas and subtropical regions of the world. Since ancient times, this plant has been used to treat or alleviate several ailments like pain, fever, inflammation, respiratory and skin diseases, parasitic and bacterial infections, hypertension, diabetes, and cancer (Coria-Téllez et al., 2018). In the last decades, *in vitro* studies have characterized the extracts and phytochemicals from *A. muricata* as a valuable antimicrobial, anti-inflammatory, anti-protozoan, anti-neoplastic and antioxidant agent (Moghadamtousi et al., 2015; Coria-Téllez et al., 2018). *In vivo* studies in murine models of the extracts and isolated from *A. muricata* were shown to possess anxiolytic, ulcer-preventing, wound healing, hepato-protective, and hypoglycemic activities.

Furthermore, this plant has been studied for the bioactive compounds produced by its endophytic fungal populations (secondary metabolites) (Zhang et al., 2013). In association with the fact that microorganisms and natural products are the most productive source of "first-in-class" drugs, such properties prompted further investigations. In this line, a recent study has opened avenues for developing antimalarial compounds using the *A. muricata* with its endophytic fungi (Kouipou Toghueo and Boyom, 2019).

Considering the scarcity of systematic review of the biological activities of extracts of endophytic fungi isolated from *A. muricata*. this study aims to systematically review the data available in the literature on the bioactive properties of the endophytic fungi isolated from *A. muricata* and their secondary metabolites, with the final objective of making the practical application of such findings easier.

2. Material and Methods

2.1. Search strategy

The literature search was undertaken at four electronic databases: MEDLINE, via PubMed (NIH, 2021); Web of Science (2021); ScienceDirect (2021) and Scopus (2021), following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses [PRISMA] Guidelines, as proposed by Moher et al. (2009) and Higgins and Green (2011). The research was conducted from the collection of scientific articles published until June 2020, using the following terms: [("*Annona muricata*" or soursop or guanabana or Mullatha) and (endophyte* or fung* or mold*)]. No restriction on publication dates was applied, and the 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, inclusion, and exclusion criteria

Only basic research articles were eligible for the current systematic review. Inclusion criteria were: i) studies on the biological activity of endophytic fungi extracts from *A. muricata*. Exclusion criteria comprised: i) review articles; ii) studies that did not use extracts of the endophytic fungus *A. muricata*.

2.3. Study selection and data collection process

The studies identified through the electronic or manual search were independently screened by two authors (RMS and IMMS). In the first phase, titles and abstracts were carefully analyzed. Duplicates were excluded. Studies whose first screening clearly indicated that they failed to meet the inclusion criteria were immediately excluded. After, for all the remaining potentially relevant studies, the full text was evaluated to determine its inclusion or exclusion. Each author compared the lists of publications that met inclusion criteria, and disagreements were discussed until consensus. The following information was collected from the selected studies: authors' names, publication year, country, impact factor, plant's origin, plant material evaluated, plant sample authenticated by a plant taxonomist and voucher specimens number deposited in the herbarium, fungal specimen deposited in the Genbank database, methods of isolation and identification of the endophytic fungi, extraction solvent type and separation methods, methods used to assess the biological properties, type and chemical nature of metabolites, and significant results obtained. Whereas one author completed the evidence table, the second author verified the data's accuracy.

2.4. Assessment of the methodological quality

Two investigators independently assessed the methodological quality of the included studies. In basic science, unlike clinical studies, checklists and scores to assess prior literature in such a rigorous and quantitative manner are rare. Therefore, the Quality Assessment Tool for Studies with Diverse Designs (QATSDD) scale was adapted to research aims by the authors. This scale was used in the study to guide the critical appraisal and quality evaluation of clinical research described by Sirriyeh et al. (2012) and was adapted to the research aims by the authors. The adapted tool included 13 items, scored from 0 to 3, which reflected, among others, a clear definition of the research topic, purpose and hypothesis identification, study design, 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 the maximum possible score (39 points) to obtain the paper's overall quality score.

2.5. Statistical analysis

Data were extracted from each study, and descriptive statistics (mean and standard deviation) were calculated using Microsoft Excel 2018.

3. Results

3.1. Literature search and studies' selection

Figure 1 summarizes the studies' selection strategy. The initial electronic database search yielded 436 results

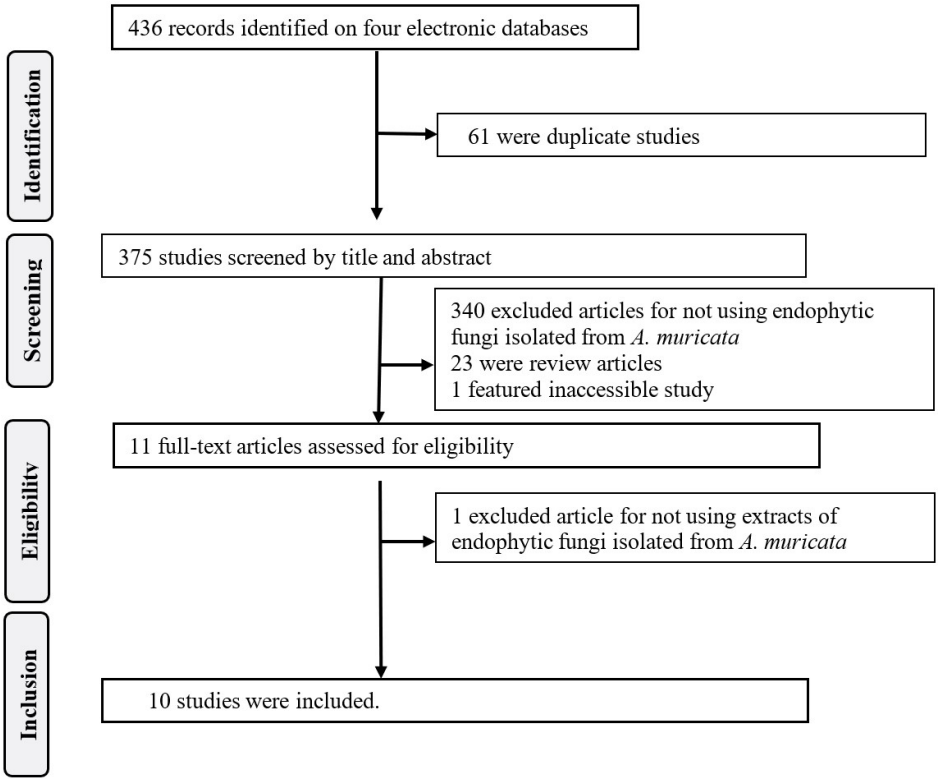

Figure 1. Flow diagram of the study's selection process.

(197 studies identified in PubMed; 117 in ScienceDirect; 79 in SCOPUS; 43 in Web of Science).

The manual bibliographic search did not retrieve any additional study. After removing duplicates (n = 61), 375 records were screened by title and abstract. By doing so, 364 studies were excluded (340 for not using *A. muricata* endophytic fungus and 23 for being review articles). In all, 11 studies (full texts) were carefully analyzed, and one was excluded for not assessing the endophytic fungus *A. muricata*. Therefore, ten studies were selected for inclusion in this systematic review.

3.2. Description of the included studies

Supplementary Material accompanies this paper. Supplementary Table 1 provides an overview of the key characteristics of the ten selected publications as full texts. The studies were published between 2011 and 2019, with the most significant number of articles (n = 4) published in 2017 (Arifni et al., 2017; Minarni et al., 2017; Asyura et al., 2017; Liu et al., 2017). The impact factor (IF), provided by the Journal Citation Reports (JCR), was absent in four studies (Arifni et al., 2017; Asyura et al., 2017; Abba et al., 2018; Abdel-Rahman et al., 2019); in those with IF, it ranged between 1.40 (Ge et al., 2011) and 6.55 (Zhang et al., 2016), using the year 2019 as a reference. Most tested plants (40%) hailed from China (Ge et al., 2011; Zhang et al., 2015, 2016; Liu et al., 2017). The leaf was the most studied morphological part of the plant, being evaluated in nine studies (Ge et al., 2011; Zhang et al., 2015, 2016; Arifni et al., 2017; Minarni et al., 2017; Asyura et al., 2017; Liu et al., 2017; Abba et al., 2018; Toghueo et al., 2019). Only in two studies, *A. muricata* was authenticated by a plant taxonomist. In these, the voucher specimen number was deposited on herbarium (Abba et al., 2018; Toghueo et al., 2019), and only three studies deposited the sequencing data of the fungus in the Genbank database (Zhang et al., 2016; Abba et al., 2018; Toghueo et al., 2019).

Periconia sp. was the most tested fungus (n = 4) (Ge et al., 2011; Zhang et al., 2015, 2016; Liu et al., 2017), and anticancer was the most activity evaluated (n = 6) (Ge et al., 2011; Zhang et al., 2016; Arifni et al., 2017; Minarni et al., 2017; Asyura et al., 2017, Liu et al., 2017) followed by antiviral activity - anti-human immunodeficiency virus (HIV), n = 3 (Zhang et al., 2015, 2016; Liu et al., 2017). Antibacterial and antifungal (Arifni et al., 2017; Abba et al., 2018) and antioxidant (Abba et al., 2018; Abdel-Rahman et al., 2019) activities were also tested.

Among the selected studies, seven used the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay method for cytotoxicity studies (Ge et al., 2011; Zhang et al., 2015, 2016; Arifni et al., 2017; Minarni et al., 2017; Liu et al., 2017; Toghueo et al., 2019), one used female rats and experimental design with mammary tumor induced by 7,12-dimethylbenz(α) anthracene (Asyura et al., 2017) and two did not use assay method for cytotoxicity studies (Abba et al., 2018; Abdel-Rahman et al., 2019).

Regarding the methods used to isolate the endophytic fungi, the Potato Dextrose Broth was the most used culture medium (n = 6) (Ge et al., 2011; Zhang et al., 2015, 2016; Liu et al., 2017; Abdel-Rahman et al., 2019; Toghueo et al., 2019). The incubation temperature ranged between 25°C (Ge et al., 2011; Zhang et al., 2015; Asyura et al., 2017; Liu et al., 2017; Abdel-Rahman et al., 2019; Toghueo et al., 2019;) and 29°C (Arifni et al., 2017) and the incubation time ranged between six (Toghueo et al., 2019) and 30 days (Abba et al., 2018), being ten days the most used incubation time (n=4) (Ge et al., 2011; Zhang et al., 2015, 2016; Liu et al., 2017). Identification of fungi isolates was carried out mainly using molecular-based analysis of Internal Transcribed Spacer (ITS) locus on the ribosomal RNA gene (n = 9) (Ge et al., 2011; Zhang et al., 2015, 2016; Arifni et al., 2017; Minarni et al., 2017; Asyura et al., 2017; Liu et al., 2017; Abba et al., 2018; Toghueo et al., 2019). In all studies, the fungal secondary metabolites were extracted with ethyl acetate (EtOAc). High-Performance Liquid Chromatography (HPLC) was the separation method used in half of the studies (Ge et al., 2011; Zhang et al., 2015, 2016; Liu et al., 2017; Abba et al., 2018).

Several different metabolites classes were reported to exist in the extract of *A. muricata* endophytes, including phenolic compounds, terpenoids or terpenoid hybrid compounds, ester group, alkaloids, fatty acids (saturated and unsaturated), aromatic compounds. Peptides, terpenoids, or terpenoid hybrid compounds were the predominant metabolites in six studies (Ge et al., 2011; Zhang et al., 2015, 2016; Liu et al., 2017; Abdel-Rahman et al., 2019; Toghueo et al., 2019).

The last column of the Supplementary Table 1 shows the methodological quality scores of the publications, whereas Supplementary Table 2 presents the classifications attributed to each quality criteria. Scores ranged between 71.79% and 100.00%, with a mean [±standard deviation] of 83.59 ± 9.06%. Overall, the highest scores were achieved for items "obtain valid and consistent data" and "draw consistent conclusions based on the evidence presented in the paper" and the lowest for "vegetal sample and microbial culture collection deposit process".

3.3. Biological activity - synthesis of results

Supplementary Table 3 presents the characteristics of selected studies on the antimicrobial activity of endophytic fungi isolated from *A. muricata.*

3.3.1 Antibacterial and antifungal activity

Abba et al. (2018) and Abdel-Rahman et al. (2019) tested the antimicrobial activity of endophytic fungi extracts. Abba et al. (2018) investigated the secondary metabolites of an endophytic fungus *Pseudofusicoccum* sp. isolated from the leaves of *A. muricata*. According to the authors, the fungal crude extract showed mild antimicrobial activity against *B. subtilis*, *S. typhi*, and *C. albicans*, with inhibition zone diameters (IZDs) of 2 ± 0.00, 3 ± 0.33, and 2 ± 0.33 mm, respectively. Abdel-Rahman et al. (2019) tested *Aspergillus niger* strain SH3 extract against *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans*. They observed that the extract had antimicrobial activity against *S. aureus* (IZD 12 mm ± 0.5), *P. aeruginosa* (IZD 15 mm ± 0.0), *E. coli* (IZD 14 mm ± 0.0), but *C. albicans* (0 mm ± 0.0) was resistant (Supplementary Table 3).

3.3.2. Antiviral activity

Supplementary Table 3 provides the characteristics of selected studies on the anti-HIV activity of endophytic fungi isolated from *A. muricata.*

Zhang et al. (2015, 2016), and Liu et al. (2017) tested the anti-HIV activity of endophytic fungi extracts from *A. muricata*. The inhibition rate was determined by using a firefly Luciferase Assay System. These studies identified Periconones, new polyketide–terpenoid hybrid molecules isolated from the endophytic fungus *Periconia* sp. F-31, and displayed anti-HIV activity.

Liu et al. (2017) tested Pericoannosin B, and this compound exhibited high anti-HIV activity with a half-maximal inhibitory concentration (IC50) value of 18.0μmol/L. Pericoannosin A and F were tested by Zhang et al. (2015), and these compounds presented high anti-HIV activity with IC50s of 69.6 and 29.2 μmol/L, respectively. Besides, Pericoannosin B was the metabolite identified and tested by Zhang et al. (2016), and it displayed low cytotoxic activity with the IC50 >100 μmol/L.

3.3.3. Anticancer activity

Supplementary Table 4 presents the characteristics of selected *in vitro* studies on the anticancer activity of endophytic fungi isolated from *A. muricata.*

The Bel-7402 human hepatocellular carcinoma cells (Ge et al., 2011; Zhang et al., 2016; Liu et al., 2017), HTC-8 human colon cancer (Ge et al., 2011; Zhang et al., 2016; Liu et al., 2017), and Michigan Cancer Foundation-7 (MCF-7), human breast adenocarcinoma cell line (Ge et al., 2011; Minarni et al., 2017; Liu et al., 2017) were the most cancer cells tested (in three studies each).

The result of the inhibition absorbance test of cytotoxic activity extract was then calculated to find the IC50 value. The IC50 value is the concentration of extract required to inhibit the growth of cancer cells by 50% (Boyd et al., 1992). A dose-response curve was plotted for each compound. Then, the IC50 value was calculated as the concentration of the test compound resulted in a 50% reduction of optical density compared with the positive control (Ge et al., 2011).

Arifni et al. (2017), Minarni et al. (2017), and Liu et al. (2017) observed high anticancer activity against the tested cells with IC50 cancer cell 20.80 μg/mL (Sir-SM2), 19.20 μg/mL (Sir-G5), and 1.20 μg/mL (*Periconia*), respectively (Supplementary Table 4). Arifni et al. (2017), Minarni et al. (2017) also demonstrated low toxicity to normal cells (Chang cell) compared with other fungal extracts with IC50 63.69 μg/mL (Sir-SM2) and 1,258.92 μg/mL (Sir-G5), respectively (Supplementary Table 4). Minarni et al. (2017) and Liu et al. (2017) confirmed cytotoxic activity against the breast adenocarcinoma MCF-7 cells. On the other hand, Ge et al. (2011) and Zhang et al. (2016) revealed low cytotoxic activity against human tumor cell lines. Asyura et al. (2017) were the only that performed *in vitro* studies. These authors tested the effectiveness of endophytic extract towards the growth of the mammary tumor and

showed that bodyweight in rats was not significantly different in each group (P>0.05). The quantity and tumor volume samples from Sir G5 isolate extract treatment groups were significantly lower (P <0.05) than negative control DMBA (7,12-dimethylbenz(α)anthracene) and positive control doxo (doxorubicin). They concluded that the administration of ethyl acetate extract of endophytic fungi in Sir G5 isolate from *A. muricata* leaves could inhibit the growth of rat breast tumors mainly using the effective treatment dose (20 mg/Kg body weight).

3.3.4. Antiprotozoal activity

Toghueo et al. (2019) evaluated endophytic fungi isolated from different organs of *A. muricata*. They were cultured, and the ethyl acetate extracts of conditioned media were screened for antiplasmodial activity using the 96-well microtiter plate format SYBR green fluorescence assay against Chloroquine-sensitive Pf3D7 and Chloroquine-resistant PfINDO/PfDd2 strains of *Plasmodium falciparum*. According to the authors, 17.76% (n = 27) of fungi tested were found to completely inhibit the growth of *Plasmodium* at 10 µg/mL. The highest infection frequencies were recorded in the trunk bark (60%) and root bark (50%).

The extracts from *Penicillium citrinum* AMrb11 (IC50 0.84–0.93 µg/mL) and *Neocosmospora rubicola* AMb22 (IC50 0.39–1.92 µg/mL) showed the highest promise against all three plasmodial strains with selectivity indices ranging from 34.71 to 180.97.

3.3.5 Antioxidant activity

Abba et al. (2018) evaluated the antioxidant activity of the crude ethyl acetate fungal extract. The extract showed good antioxidant activity. The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) antioxidant assay was performed, and DPPH crude extract produced an overall inhibition of 96% for *Pseudofusicoccum* sp. extract, which is higher than 93% recorded for the positive control, quercetin.

4. Discussion

Natural selection has been reported to be superior to combinatorial chemistry in discovering biologically active compounds that may lead to pharmaceutical products. In fact, more than one-third of all the Food and Drug Administration (FDA)-approved therapeutic agents over the past two decades are originated from or inspired by natural products (Li et al., 2019). Among these are some history-changing drugs, such as amphotericin, artemisinin, morphine, paclitaxel, and penicillin (Wright, 2019). In agreement with this, the interest in the biological activities of *A. muricata* has increased over the years. Several reports exist on its anticancer, anticonvulsant, anti-parasitic, antimalarial, hepato-protective, and anti-diabetic activities (Moghadamtousi et al., 2015).

Besides that, as reported in the ten studies included in this systematic review, *A. muricata* has been studied for the biological activities associated with its endophytic fungal populations (Ge et al., 2011; Zhang et al., 2015, 2016; Arifni et al., 2017; Minarni et al., 2017; Asyura et al., 2017; Liu et al., 2017; Abba et al., 2018; Abdel-Rahman et al., 2019; Toghueo et al., 2019). China was the country of origin of most of the plants tested in those studies. In this country, traditional markets are important places for the trading of medicinal plants harvested by rural villagers, which play a social role in exchanging traditional use of herbal medicine among different cultural and social groups at a local level (Lee et al., 2008). In a few studies, *A. muricata* was authenticated by a plant taxonomist; the registration of the specimen number and the deposition of the fungal sequence data in the Genbank deposit was also rare. The GenBank database incorporates DNA sequences, primarily through the direct submission of sequence data from authors and from large-scale sequencing projects (Benson et al., 2010). The information about the exsiccate deposit and the taxonomic identification in a herbarium is essential and warrants that the researcher is working with the correct species (Peixoto and Maia, 2013). Therefore, further efforts must be done to ensure that natural products are correctly identified and that their genetic sequence is submitted to a public repository as part of the publication process. The incubation conditions used by the most authors were Potato Dextrose Broth, at incubation temperature ranging from 25°C to 29°C for 10 days.

Frisvad (2012) showed that growth media and incubation conditions strongly influence secondary metabolite production. Thus, they recommended the standard incubation for seven days at 25°C in darkness.

All studies analyzed used ethyl acetate as an extraction solvent, which has been reported to extract many bioactive compounds. Actually, Hepsibah and Jothi (2016) performed a comparative analysis on the effect of the solvents on the phytochemical profile. They concluded that ethyl acetate was the best for extracting antifungal compounds, with 12 mm highest zone of inhibition against *Trichophyton mentagrophytes*. The sequential extraction was carried out using two sets of solvent systems: hexane, ethyl acetate, ethanol and chloroform, acetone, and methanol. In addition, Sharma et al. (2016) observed that crude extracts from ethyl acetate had the highest zone of inhibition against *E. coli* (15 mm) than methanol (14 mm).

Regarding extracts' composition, terpenoids or terpenoid hybrid compounds were the most abundant chemical metabolites identified. Souza et al. (2011) carried out a narrative review regarding the terpenoids isolated from endophytic fungi from several different plants and their biological activities. In this study, 127 terpenoids were isolated from endophytic fungi, mainly sesquiterpenes (n = 65) and diterpenes (n = 45), demonstrating the prevalence of those components in medicinal plants.

Concerning the biological activities of the endophytes isolated from *A. muricata*, the properties assessed in the ten included studies were anticancer, antiviral, antibacterial, antifungal, antiprotozoal, and antioxidant.

Anticancer activity was the most tested effect in the studies evaluated (n = 6), being used the MTT assay (methyl thiazolyl tetrazolium), as described by Mosmann (1983). A tetrazolium salt has been used to develop a quantitative colorimetric assay for mammalian cell survival, cytotoxicity, and proliferation. The assay detects living but not dead cells, and the signal generated depends on the

cells' activation degree. Based on the American National Cancer Institute (Itharat et al., 2004), a crude extract for assay has the criteria of cytotoxicity activity (capable of inhibiting 50% of the cancer cell population) if it has an IC50 value <30 µg/mL. For example, Arifni et al. (2017) and Minarni et al. (2017) evaluated the extract of endophytic fungi isolated from soursop leaf and observed that this plant had a high cytotoxic effect. They described an IC50 value of 20.80 µg/mL against WiDr colon cancer cells (Sir-SM2) and 19.20 µg/mL against human breast adenocarcinoma MCR cells (Sir-G5), respectively.

Regarding the antibacterial and antifungal activities, all the studies used the disk diffusion test (DDT). According to Jorgensen and Turnidge (2015), the DDT method is only appropriate as a preliminary screening test prior to minimum inhibitory concentration (MIC) analysis using the dilution method. MIC determination would be more specific than growth inhibition halo because the same sample is tested at different concentrations. Therefore, future studies must be performed using a more adequate and comprehensive methodology. Moreover, it would be essential to explore the synergistic interaction of endophytic fungi extracts from *A. muricata* with commercial antimicrobials. Bezerra dos Santos et al. (2015) evaluated the synergistic action of natural compounds from organic extracts of *Indigofera suffruticosa*. According to the authors, their extracts are promising for the development of new anti-*S. aureus* formulations due to their action in inhibiting methicillin-resistant *S. aureus* strains, which are maximized in combination with erythromycin. Notwithstanding, for the authors' knowledge, no studies have been carried out to evaluate the extracts of endophytic fungi from *A. muricata*.

Liu et al. (2017) tested pericoannosin B, and this compound exhibited high anti-HIV activity with a half-maximal inhibitory concentration (IC50) value of 18.0µmol/L. Pericoannosin A and F were tested by Zhang et al. (2015), and these compounds showed high anti-HIV activity with IC50s of 69.6 and 29.2 µmol/L, respectively. Pericoannosin B was the metabolite identified and tested by Zhang et al. (2016), and it showed low cytotoxic activity with IC50 values >100 µmol/L.

Zhang et al. (2015, 2016), and Liu et al. (2017) observed the anti-HIV activity of endophytic fungi extracts from *A. muricata*, and they exhibited anti-HIV activity with IC50 between 18.0 and 69.6 µmol/L. These results can serve as a starting point for research involving other viruses, for example, against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Indeed, in this setting, promising results were obtained using *A. muricata* seeds (Kolawole et al., 2020).

Toghueo et al. (2019) aimed to explore the endophytic fungi associated with some parts of *A. muricata* and their ability to produce antiplasmodial metabolites. According to the authors, the extracts from *Penicillium citrinum* AMrb11 (IC50 0.84–0.93 µg/mL) and *Neocosmospora rubicola* AMb22 (IC50 0.39–1.92 µg/mL) showed the highest promise against all three plasmodial strains. The authors tested many *A. muricata* tissue segments. The highest infection fungi frequencies were recorded in the trunk bark (60%) and root bark (50%), different from most studies that tested *A. muricata* leaves. Further studies are necessary to evaluate the antiprotozoal activity of endophytic fungi isolated from different *A. muricata* tissues.

There is little evidence regarding the antioxidant action of extracts of endophytic fungi of *A. muricata*, but other species of *Annona* have been evaluated. Sibanda et al. (2018) tested endophytic fungi in association with *A. senegalensis*. The preliminary results indicated that the isolated fungal endophytes from *A. senegalensis* belong to the phylum *Ascomycota* and have potential as natural antioxidants sources (593.46 ± 1.86 µM Copper Reducing Equivalents -CRE).

It is undeniable that scientific findings contribute to enhancing biodiversity value (Martinez-Klimova et al., 2017). Furthermore, ethnobotanical knowledge has been linked to the success of finding bioactive molecules, which may bring about novel compounds to be used in chemotherapy (Cragg and Newman, 2013). For the authors' knowledge, this is the first systematic review of the biological activities of extracts of endophytic fungi isolated from *A. muricata*. The scarcity of studies about this theme makes it challenging to select and isolate endophytic fungi from this plant, prevents the development of new bioactive compounds, and hampers the translation of the potential benefits into the pharmaceutical industries. Therefore, we aimed to summarize and disseminate the current evidence, identify gaps, and recommend future research.

5. Conclusion

The ten publications selected in this systematic review provide evidence regarding the biological properties of the endophytic fungi extract from *A. muricata* under analysis as a source of new bioactive compounds against different microorganisms (bacteria, fungi, virus, and protozoan) and cancer cells, in addition to antioxidant action (yet less evidence exists on the latter two effects).

Considering the biotechnological potential of *A. muricata* endophytic fungi, new researches are needed involving different plant tissues, other microorganisms, such as SARS-CoV-2, and different cancer cells. The evaluation of the synergic interaction of endophytic fungi extracts from *A. muricata* with commercial antimicrobials, and the new possibilities of green nanotechnology are warranted, considering the drug-resistance crisis that we face ahead.

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Supplementary Material

Supplementary material accompanies this paper.

Supplementary Table 1. Characteristics of selected studies on the biological activities of endophytic fungi isolated from Annona muricata L.

Supplementary Table 2. Assessment of quality of selected studies on the biological activities of endophytic fungi isolated from Annona muricata L.

Supplementary Table 3. Characteristics of selected studies on the antibacterial, antifungal and antiviral activities of endophytic fungi isolated from Annona muricata L.

Supplementary Table 4. Characteristics of selected studies in vitro on the anticancer activity of endophytic fungi isolated from Annona muricata L.

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